Quality characteristics, amino acid composition, and bioactive potential of wheat cookies protein-enriched with unconventional legume protein isolates

Prashant Sahni[1]*, Savita Sharma[2]

[1]College of Dairy & Food Technology, Agriculture University, Jodhpur, Rajasthan, India; [2]Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, Punjab, India

***Corresponding author:** Prashant Sahni, College of Dairy & Food Technology, Agriculture University, Jodhpur, Rajasthan, India. Email: ftech.sahni@gmail.com

Received: 30 July 2022; Accepted: 28 December 2022; Published: 1 April 2023

RESEARCH ARTICLE

Abstract

The present investigation was intended to utilize protein isolates from forage legumes as unconventional protein ingredients for the development of protein-enriched wheat-based cookies. Alfalfa and dhaincha protein isolates (API and DPI) were supplemented at levels of 2.5, 5, 7.5, and 10%, and the effect of supplementation was evaluated on the quality attributes, nutritional composition, amino acid profile, and bioactive potential of cookies. The baking loss, water activity, and spreading (except for 10% API and 5% DPI) decreased, whereas the hardness increased with the increase in supplementation level and the effect was more pronounced with the supplementation of DPI. The non-enzymatic browning index showed that it was not affected by the supplementation. DPI-supplemented cookies showed a color change, whereas no change in the color was observed in API-supplemented cookies. Cookies maintained good sensorial characteristics up to the supplementation of 10% API and 5% DPI and higher supplementation of DPI comprised all the sensorial attributes. Supplementation with protein isolates also enhanced the protein and essential amino acid content, total phenols, flavonoids, and antioxidant activity of the cookies.

Keywords: alfalfa; antioxidant activity; cookies; dhaincha; protein isolate

Introduction

Protein-based ingredients are gaining momentum in the formulation of a variety of convenience food products. Unlike carbohydrates and fats, which are often utilized in the formulation of these products and have gained a bad reputation in nutrition circles; protein is considered a healthy ingredient for supplementation in food products (Sahni *et al*., 2018). Furthermore, protein-enriched products are often positioned as healthy food products in the market and used as a marketing tool to attract a segment of the population targeting adequate nutrition, muscle building, and weight loss (Sahni *et al*., 2018, 2022). Particularly, there is a paradigm shift in the consumption of meat and conventional plant proteins to unconventional plant-based protein ingredients. Furthermore, plant-based alternative protein sources have shown exponential growth in their market share (Bashi *et al*., 2019).

Particularly, protein from forage legumes can be a valuable resource for its utilization as protein ingredient in food formulations owing to the ease of maintenance of forage legumes and their resistance to climatic stresses, diseases, and insect pests (Bhat and Karim, 2009). Alfalfa (*Medicago sativa*) and dhaincha (*Sesbania aculeata*) protein isolates present huge prospects for their utilization as unconventional protein ingredients due to their well-balanced combination of good essential amino acid composition, high bioactive potential, and

techno-functionality in the food system. Particularly, their good hydration and surface-active properties alter the food matrix to confer good quality characteristics (Sahni, 2020; Sahni *et al*., 2020, 2022), making them a potential ingredient for protein enrichment in bakery products.

The bakery segment is a sunrise sector of the food processing industry. Particularly, cookies have become an indispensable part of our lives and can be a good carrier for protein enrichment due to their convenience, palatability, long shelf life, ease of storage, and likability among wide demographics (Sahni and Shere 2017). Furthermore, supplementing wheat-based cookies with legume-based ingredients can be a good approach to allow the legume–pulse combination in the food formulation to enhance its nutritional value. Studies have reported that the supplementation of cookies with different protein isolates from watermelon seed, soy, whey, and Bambara groundnut have conferred different effects on the quality attributes of the cookies (Arise *et al*., 2021; Sarabhai *et al*., 2015; Tang and Liu, 2017; Wani *et al*., 2012). Cookie formation involves the development of a matrix where starch and protein form a network in the presence of sugar syrup and confer desirable structure and texture to the cookies (Slade and Levine, 1994). Furthermore, during the formation of cookies, development of gluten is detrimental to the cookie's quality. However, the type and concentration of protein may alter the development of the cookie matrix and final quality characteristics depending on the interaction of protein with other constituents of the dough, where it may promote or inhibit the development of gluten (Tang and Liu, 2017). Therefore, the present investigation was carried out to utilize alfalfa and dhaincha protein isolates (API and DPI) as unconventional protein ingredients for the development of wheat-based protein-enriched cookies and to evaluate the effect of their supplementation on the quality characteristics, nutritional and amino acid composition, bioactive constituents, and antioxidant activity of the cookies.

Materials and Methods

Unconventional legume protein isolates

Protein isolates from forage legumes (alfalfa and dhaincha) were used as unconventional legume protein ingredients for the protein enrichment of the cookies. Protein isolates were prepared by pH-based solubilization and precipitation of alfalfa and dhaincha flour at solubilization and precipitation pH of 10.0 and 4.0, respectively. The isolated protein was neutralized with 0.1 M NaOH and freeze dried using a lyophilizer (Sahni, 2020; Sahni *et al*., 2020). Lyophilized API and DPI had water absorption capacity of 1.288 and 1.774 g/g and least gelation concentrations (LGC) of 25 and 14%, respectively.

Preparation of cookies

Cookies were prepared by creamery method using refined wheat flour (100 g), bakery shortening (45 g), powdered sugar (60 g), baking powder (1.5 g), baking soda (1.5 g) and ammonium bicarbonate (1.5 g) and water as per requirement to make crumbly dough. The kneaded dough was sheeted on a wooden plank (0.5 cm thickness), cut into a circular shape using a cookie cutter, and placed on a tray smeared with the shortening. Baking was done at 160°C for 20 min. Baked cookies were cooled, packaged in polypropylene jars, and stored at ambient conditions (Sahni *et al*., 2019). Blends were prepared for the formulation of cookies by replacing refined wheat flour (w/w) with protein isolates at 2.5, 5, 7.5, and 10% levels of supplementation.

Physical properties of the cookies

Cooled cookies and cut cookie dough prior to baking were weighed on an electronic weighing balance, and baking loss was evaluated in percentage by equation (1). The diameter and thickness of cookies were recorded in millimeter by using a digital vernier caliper. The spread ratio (SR) was calculated by dividing the diameter of the cookie with its thickness (AACC, 2000). SR was calculated by comparing the spread factor of cookies supplemented with protein isolates with that of the control cookie and considering the value of SR 100% for the control cookie. Top grain development was noted as the number of cracks formed on the surface of the cookie and was recorded as most, moderate, rare, and absent (Sahni *et al*., 2019). The water activity of the cookies was evaluated by a digital water activity meter at 28°C. The non-enzymatic browning index was determined by the procedure of Hwang *et al*. (2001) by extracting 1 g sample in 50 mM $CaCl_2$/50 mM Tris buffer (pH 7.0) and obtaining the supernatant, followed by centrifugation at 2000 × g for 15 min. The optical density of the supernatant was noted at 420 and 550 nm. The non-enzymatic browning index was calculated as per equation (2)

$$\text{Baking loss (\%)} = \frac{\text{Weight of dough} - \text{Weight of cookie}}{\text{Weight of dough}} \times 100 \quad (1)$$

$$\text{Non-enzymatic browning index} = \text{Absorbance}_{420\,\text{nm}} - \text{Absorbance}_{550\,\text{nm}} \quad (2)$$

Texture

The texture of the cookies was evaluated on a TA.HD*plus* Texture Analyzer (Stable Micro System Ltd.). The texture

was evaluated by single bite test using Warner Bratzler Blade and noting the maximum force (N) required to break the cookies as hardness. The pre-test and post-test speeds of 20 mm/sec and 75% compression were employed for the testing (Sahni *et al*., 2019).

Color measurement

The external and internal colors of cookies were determined using hunter color lab (CR-300 Minolta Camera, Japan). External color was noted by measuring the surface color of the cookies, whereas internal color was noted by breaking the cookie from the center and noting the color of the cookie matrix. Color characteristics were recorded in terms of L* value (Lightness: 0 (black) to 100 (white), a* value (+a* (redness) to -a*(greenness) and b* value (+*b (yellowness) to -*b (blueness).

Sensory evaluation

Sensory evaluation was carried out on a 9-point hedonic scale by evaluating three-digit coded cookie samples by 100 semi-trained panelists (50 males and 50 females, 20–57 years old) from Punjab Agriculture University, Ludhiana for sensory attributes like color and appearance, texture, taste, flavor, and overall acceptability. The evaluation was carried out at 27 ± 5°C in a well-lit room and panelists were given water to rinse the mouth before the evaluation of the next sample. Based on the sensory evaluation, the control sample, and the samples that scored highest among cookies supplemented with alfalfa and dhaincha protein isolate were selected for evaluation of proximate composition, bioactive constituents, antioxidant activity, and amino acid profile.

Proximate analysis

Moisture, crude protein (using the factor 6.25 × N), crude fat, crude fiber, and ash were evaluated using AACC (2000) procedures. Nitrogen free extract (NFE) was estimated by subtracting the sum of moisture, crude protein, crude fat, crude fiber, and ash from 100. The values were expressed on a dry-matter basis.

Bioactive constituents

Samples were extracted with 80% (v/v) methanol for the extraction of total phenols and flavonoids and were evaluated colorimetrically using the procedures of Flores *et al*. (2014) and Kiranmai *et al*. (2011), respectively. Total phenols and flavonoids were expressed in terms of gallic acid equivalent (GAE mg/g) and quercetin equivalent (QE mg/g), respectively. The values were expressed on a dry matter basis.

Antioxidant activity

DPPH· Radical Scavenging Activity (Kiranmai *et al*., 2011) and ABTS·+ Radical Scavenging Activity (Thaipong *et al*., 2006) were evaluated and expressed as trolox equivalent antioxidant capacity (TEAC µmol/100 g). Ferric-ion reducing antioxidant power (FRAP) was evaluated by the method of Thaipong *et al*. (2006) and results were expressed as TEAC µmol/g. Reducing power was estimated as described by Sharma and Sahni (2021) and expressed as ascorbic acid equivalent (AAE mg/g). Metal chelating activity was determined as per Chew *et al.* (2009) and results were expressed as mmol Ethylenediamine tetraacetic acid (EDTA) equivalent/100 g. Results were expressed on a dry matter basis.

Amino acid analysis

Amino acids were determined by HPLC by performing hydrolysis with 6 M HCl containing 0.1% phenol at 110°C for 24 h. Cystine and methionine were evaluated pre-hydrolysis with performic acid oxidation. Tryptophan was determined by alkaline hydrolysis. Corrections were applied to Thr and Ser values for the extrapolation to time zero. Values were expressed as g/100 g sample on a dry weight basis.

Statistical analysis

The data were analyzed for statistical significance at $P < 0.05$ using SPSS software (Version 22, IBM Corporation). Data were analyzed using ANOVA followed by post-hoc Tukey's test and represented as mean ± standard deviation. Sensory evaluation data were analyzed by Friedman bilateral variance rank analysis. Principle component analysis (PCA) for the quality characteristics of cookies was done using Statistica v.12.

Results and Discussion

Physical characteristics

Physical characteristics of cookies are an important indicator of cookie quality, as their evaluation predicts the influence of supplementation of non-conventional protein isolates on the baking performance of cookies. Baking loss is an important parameter, as reduced baking loss is manifested with a higher yield of the product. The addition of API and DPI resulted in reduction

in baking loss with the increase in the level of supplementation (Table 1). Cookies supplemented with DPI exhibited a higher reduction in the baking loss in comparison to API at the same level of supplementation. A higher reduction of baking loss with supplementation of DPI can be manifested with the higher water absorption capacity of DPI as compared to API. The cookies showed a reduction in diameter with the supplementation of 2.5% API, followed by an increase in diameter at the 5% level. However, further supplementation resulted in a reduction in diameter. Supplementation of DPI showed an increase in the diameter at a 2.5% concentration, followed by a linear decrease in the diameter. However, higher spreading was observed in the case of cookies supplemented with API in comparison to DPI. The spreading of cookies is dictated by dough consistency, which is a cumulative function of the formation of syrup during baking as a result of different ingredients (particle size of the flour, type and concentration of protein/fiber, and the type of fat) utilized in the cookie formulation (Mamat and Hill, 2018; Slade and Levine, 1994). The supplementation of proteins in the cookie formulation can alter the spreading of the dough due to modulation in the dough thickness during baking ascribed to the hydration properties of the protein (Sahni *et al*., 2018). The increase in the spread factor of cookies at the lower level of supplementation with protein isolates can be ascribed to the dilution of gluten, whereas the reduction in the spread factor at the higher level of supplementation can be attributed to the high water binding capacity of protein isolates that resulted in a poor increment of syrup formation during baking and resulted in thicker dough and consequently reduced spreading (Slade and Levine, 1994; Sahni *et al*., 2018). Wani *et al*. (2012) observed a similar trend of an increase in the spread factor of cookies up to 7.5% level of supplementation of watermelon seed protein isolates, followed by a reduced spread factor at supplementation of 10%.

Water activity is an important quality characteristic that influences the shelf life of the cookies. The low water activity of the cookies is manifested in their longer shelf life and crisp texture. Supplementation of API and DPI resulted in the concomitant decrease of the water activity of the cookies with the increase in the level of supplementation. The decrease in the water activity of cookies due to supplementation of protein isolates can be ascribed to the water binding capacity of proteins. Furthermore, proteins have a tendency to undergo gelation, which results in the entrapment of water in the gel matrix and a decrease in the water activity (Sahni *et al*., 2018). Similar reduction in the water activity of soy protein–enriched cookies was observed with the increase in the supplementation of protein level (Singh and Mohamed, 2007). Reduced water activity values were also observed by using unconventional flours of buckwheat, rye, and spelt in the biscuit formulation instead of conventional wheat flour (Hercegová et al., 2019). Higher reduction in the water activity was observed for the supplementation of dhaincha protein isolates in comparison to alfalfa protein isolates due to better gelation capacity (LGC 14%) and consequently higher water binding of denatured proteins for DPI. Sahni *et al*. (2022) reported a similar trend of higher reduction in the water activity of cereal bars incorporated with DPI in comparison to API. The non-enzymatic browning index of the cookies represents the degree of Maillard browning in the cookies. The development of non-enzymatic browning in cookies is dictated by a number of factors, including moisture content, baking time and temperature, and the formulation of the cookies (the concentration of proteins and reducing sugars) (Leiva-Valenzuela *et al*., 2018). Supplementation of API and DPI showed no significant change in the non-enzymatic browning index of the cookies as sucrose was used solely as a source of sugar in the cookie formulation. Sahni *et al*. (2022) reported an increase in the non-enzymatic browning index of cereal bars supplemented with API and DPI due to the utilization of honey as a source of sugar.

Textural characteristics

The crispness of the cookies is regarded as an important quality attribute that directly influences the eating quality of the cookies. However, changes in the formulation influence the hardness of cookies as a result of modulated viscosity of the dough, the development of gluten, and protein-protein/starch association (Sahni and Shere, 2017; Sahni *et al*., 2018; Sahagún and Gómez, 2018; Slade and Levine, 1994). Particularly protein-rich formulations can show variation in the hardness of cookies based on the source of the protein, where hydration and gelation properties can influence the stiffness of the dough and their resultant behavior during baking (Sahagún and Gómez, 2018). The incorporation of API and DPI up to 5% and 2.5%, respectively, showed no significant variation in the hardness of the cookies. However, at a higher level of incorporation, a linear increase was observed in the hardness of the cookies. Furthermore, cookies incorporated with DPI were harder in comparison to cookies incorporated with API. This is due to the better gelation of DPI (LGC 14%) in comparison to API (LGC 25%) which resulted in stronger protein-protein association in the cookies matrix and resulted in harder cookies. Wani *et al*. (2012) also reported an increase in the hardness of the cookies with the supplementation of watermelon seed protein isolate, with significantly higher hardness values at the 10% supplementation level. Jayasena and Nasar-Abbas (2011) observed the similar increase in the hardness of the biscuits with the supplementation of lupin flour.

Table 1. Physical and textural characteristics of cookies.

Supplementation (%)	Physical characteristics								Hardness (N)
	Baking loss (%)	Diameter (mm)	Thickness (mm)	Spread ratio	Spread factor (%)	Top grain development	a_w	Non-enzymatic browning index (OD/g sample)	
Control	11.34 ± 0.14^{a}	72.48 ± 0.21^{e}	9.15 ± 0.19^{d}	7.92 ± 0.11^{c}	100^{c}	Most	0.203 ± 0.002^{a}	0.202 ± 0.01^{a}	53.57 ± 3.11^{f}
API									
2.5	11.26 ± 0.21^{a}	70.52 ± 0.11^{g}	9.57 ± 0.09^{c}	7.36 ± 0.05^{e}	92.92^{e}	Most	0.198 ± 0.001^{b}	0.189 ± 0.007^{a}	57.42 ± 2.56^{f}
5	10.49 ± 0.24^{b}	81.48 ± 0.24^{a}	9.21 ± 0.11^{d}	8.84 ± 0.07^{a}	111.61^{a}	Most	0.187 ± 0.002^{c}	0.203 ± 0.009^{a}	56.49 ± 2.63^{f}
7.5	10.03 ± 0.19^{c}	73.52 ± 0.19^{d}	10.21 ± 0.16^{b}	7.20 ± 0.13^{ef}	90.90^{ef}	Most	0.172 ± 0.002^{e}	0.198 ± 0.01^{a}	63.76 ± 3.90^{e}
10	9.57 ± 0.11^{d}	71.82 ± 0.16^{f}	9.42 ± 0.12^{cd}	7.62 ± 0.15^{d}	96.21^{d}	Moderate	0.164 ± 0.004^{f}	0.198 ± 0.008^{a}	73.04 ± 6.50^{d}
DPI									
2.5	10.08 ± 0.23^{c}	76.48 ± 0.18^{b}	9.13 ± 0.06^{d}	8.37 ± 0.08^{b}	105.68^{b}	Most	0.179 ± 0.001^{d}	0.196 ± 0.01^{a}	55.87 ± 2.23^{f}
5	9.22 ± 0.10^{e}	74.16 ± 0.22^{c}	10.77 ± 0.09^{a}	6.88 ± 0.17^{g}	86.86^{g}	Most	0.164 ± 0.002^{f}	0.203 ± 0.007^{a}	83.43 ± 2.48^{c}
7.5	8.86 ± 0.13^{f}	74.45 ± 0.13^{c}	10.15 ± 0.07^{b}	7.33 ± 0.09^{e}	92.92^{e}	Most	0.153 ± 0.002^{g}	0.199 ± 0.01^{a}	93.79 ± 4.53^{b}
10	8.47 ± 0.20^{g}	71.75 ± 0.16^{f}	10.23 ± 0.10^{b}	7.01 ± 0.11^{f}	88.51^{f}	Moderate	0.141 ± 0.001^{h}	0.201 ± 0.01^{a}	129.95 ± 9.24^{a}

Values are expressed as mean ± standard deviation (n = 5). API, Alfalfa protein isolate; DPI, Dhaincha protein isolate.
The means within columns having different superscript are significantly different at $P < 0.05$.

Color characteristics

The color development in cookies is a primary function of Maillard browning, and variation in the color can be associated with changes in the formulation of the cookies. Particularly, high-protein formulations manifest darker products due to browning reactions (Leiva-Valenzuela *et al.*, 2018; Sahagún and Gómez, 2018; Sahni *et al.*, 2022). However, as aforesaid, no change was observed in the non-enzymatic browning cookies as sucrose was used solely as a source of sugar in the cookie formulation. The external and internal color values of the API-supplemented cookies also exhibited non-significant variation in the color values ascribed to no change in the non-enzymatic browning index (Table 2). However, a higher L* value and lower a* and b* values were observed for internal color in contrast to the external color attributed to higher browning at the surface during baking. A similar trend was also observed for the higher L* and lower a* and b* values for the internal and external color of DPI-supplemented cookies. However, the L* and b* values showed a linear reduction with the increase in the level of supplementation. The change in the color values can be ascribed to the brown color of DPI that imparted a brown tint to the cookie dough.

Sensory characteristics

The sensory characteristics of API- and DPI-supplemented cookies are presented in Figure 1. Color and appearance of cookies are important parameters for evaluating the baking quality of cookies and well-baked cookies have a characteristic brown color and top grain development. Supplementation of API showed no significant effect on the color and appearance of cookies. However, cookies supplemented with 5% API showed higher scores due to better spreading and top grain development. Supplementation of DPI showed a reduction in the color and appearance score of cookies. Cookies supplemented with brown-colored DPI rendered the cookie dough brown and the resultant cookies dark. Alruqaie and Al-Ghamidi (2015) also linked the darker appearance of the cookies with the addition of sama flour and date powder. Cookies maintained good texture up to 10% API supplementation, but cookies supplemented with DPI showed more decline in the texture scores due to excessive hardness in the cookies and was in agreement with the hardness values of cookies (Table 1). Sarabhai *et al.* (2015) also observed the variation in appearance and texture of cookies incorporated with whey protein and soy protein isolate (WPI and SPI) and reported the large cracks with the incorporation of WPI whereas SPI decreased the crispness of the cookies. Taste and flavor scores also showed marked decline at the 7.5 and 10% levels of DPI supplementation due to the peculiar strong

Table 2. Color characteristics of cookies.

Supplementation (%)	Color characteristics									
	External color					Internal color				
	L*	a*	b*	Chroma	Hue (°)	L*	a*	b*	Chroma	Hue (°)
Control	56.81 ± 1.91^{a}	4.78 ± 0.08^{b}	18.57 ± 0.28^{a}	19.17 ± 0.34^{a}	1.31 ± 0.02^{a}	65.48 ± 0.28^{a}	3.35 ± 0.10^{d}	14.81 ± 0.20^{b}	15.18 ± 0.22^{b}	1.35 ± 0.0^{a}
API										
2.5	57.49 ± 1.01^{a}	4.64 ± 0.11^{b}	18.49 ± 0.14^{a}	19.06 ± 0.13^{a}	1.32 ± 0.01^{a}	65.49 ± 0.12^{a}	3.48 ± 0.10^{d}	15.72 ± 0.27^{a}	16.10 ± 0.27^{a}	1.35 ± 0.0^{a}
5	59.84 ± 1.21^{a}	4.32 ± 0.16^{b}	18.25 ± 0.18^{a}	18.75± 0.25^{a}	1.33 ± 0.02^{a}	65.48 ± 0.32^{a}	3.32 ± 0.12^{d}	15.46 ± 0.24^{a}	15.91 ± 0.14^{a}	1.35 ± 0.0^{a}
7.5	57.57 ± 1.43^{a}	4.70 ± 0.22^{b}	18.20 ± 0.21^{a}	18.70 ± 0.24^{a}	1.31 ± 0.02^{a}	64.24 ± 0.21^{a}	3.45 ± 0.13^{d}	15.78 ± 0.23^{a}	16.15 ± 0.20^{a}	1.35 ± 0.0^{a}
10	59.01 ± 1.51^{a}	4.46 ± 0.21^{b}	18.24 ± 0.32^{a}	18.58 ± 0.27^{a}	1.32 ± 0.03^{a}	64.39 ± 0.26^{a}	3.21 ± 0.09^{d}	15.83 ± 0.22^{b}	16.17 ± 0.21^{a}	1.35 ± 0.0^{a}
DPI										
2.5	51.31 ± 1.11^{b}	5.76 ± 0.28^{a}	16.87± 0.13^{b}	17.82 ± 0.23^{b}	1.24 ± 0.03^{b}	57.45 ± 0.36^{b}	4.47 ± 0.21^{c}	14.54 ± 0.26^{b}	15.21 ± 0.19^{b}	1.27 ± 0.02^{b}
5	50.62 ± 0.84^{b}	5.59 ± 0.23^{a}	16.54 ± 0.09^{b}	17.45 ± 0.21^{b}	1.24 ± 0.03^{b}	53.32 ± 0.41^{c}	4.96 ± 0.32^{c}	13.82 ± 0.17^{c}	14.68 ± 0.18^{c}	1.22 ± 0.02^{c}
7.5	47.48 ± 2.21^{c}	5.96 ± 0.33^{a}	14.73± 0.18^{c}	15.89 ± 0.56^{c}	1.18 ± 0.03^{c}	48.21 ± 0.32^{d}	5.24 ± 0.18^{b}	10.21 ± 0.09^{d}	11.47 ± 0.22^{d}	1.09 ± 0.01^{d}
10	45.32 ± 1.36^{c}	5.84 ± 0.39^{a}	14.45 ± 0.24^{c}	15.58 ± 0.26^{c}	1.18 ± 0.04^{c}	43.22 ± 0.22^{e}	5.76 ± 0.12^{a}	9.27 ± 0.13^{e}	10.91 ± 0.19^{e}	1.01 ± 0.02^{e}

Values are expressed as mean (n = 10) API, Alfalfa protein isolate; DPI, Dhaincha protein isolate.
The means within column having different superscript are significantly different at P < 0.05.

taste and aroma of DPI. Overall, the cookies supplemented with 10% API and 5% DPI showed good overall acceptability. Wani *et al.* (2012) reported the acceptable sensory scores for cookies incorporated with watermelon seed protein isolate up to 7.5% level of supplementation.

Nutritional composition and bioactive potential

The incorporation of API and DPI showed an increase in the moisture content of cookies, even though a higher moisture content was observed for cookies incorporated with 5% DPI (3.12%) in comparison to supplementation at 10% API (2.89%) (Table 3). Higher moisture levels in DPI-incorporated cookies can be attributed with the higher water absorption capacity of DPI in comparison to API. Furthermore, proteins undergo amplified water-binding after processing owing to the entrapment of water in the gel matrix (Sahni *et al*., 2018). Better gelling ability of DPI also contributed to more retention of moisture during the baking of the cookies. The crude protein content of the cookies increased significantly, justifying the use of protein isolates for protein enrichment. Crude fat, crude fiber, and ash content showed no significance since protein isolates and refined wheat flour majorly contain protein and starch. The NFE content of the cookies decreased with the incorporation of protein isolates. Wani *et al.* (2012) also observed a similar trend for the composition of watermelon seed protein isolate supplemented wheat-based cookies.

The incorporation of protein isolates enhanced the bioactive potential due to enhancement in bioactive constituents and antioxidant activity of the cookies (Table 3). Total phenols showed a significant increase with the addition of protein isolates, whereas flavonoids showed a much larger increase with the incorporation of API. Free radical scavenging also showed enhancement with the supplementation of protein isolates. However, ABTS·+ radical scavenging activity was higher in comparison to DPPH· radical scavenging activity. FRAP, reducing power, and metal chelating activity were also increased with the incorporation of protein isolates. The increase in the bioactive potential of cookies supplemented with API and DPI is due to the associated bioactive constituents and their resultant antioxidant activity (Sahni, 2020; Sahni *et al*., 2020). Cereal bars supplemented with API and DPI also showed a similar increase in the total phenols, flavonoids, reducing power, and DPPH· radical scavenging activity (Sahni *et al*., 2022).

Amino acid profile

The incorporation of API and DPI improved the amino acid profile of the cookies (Table 4). However, a slight

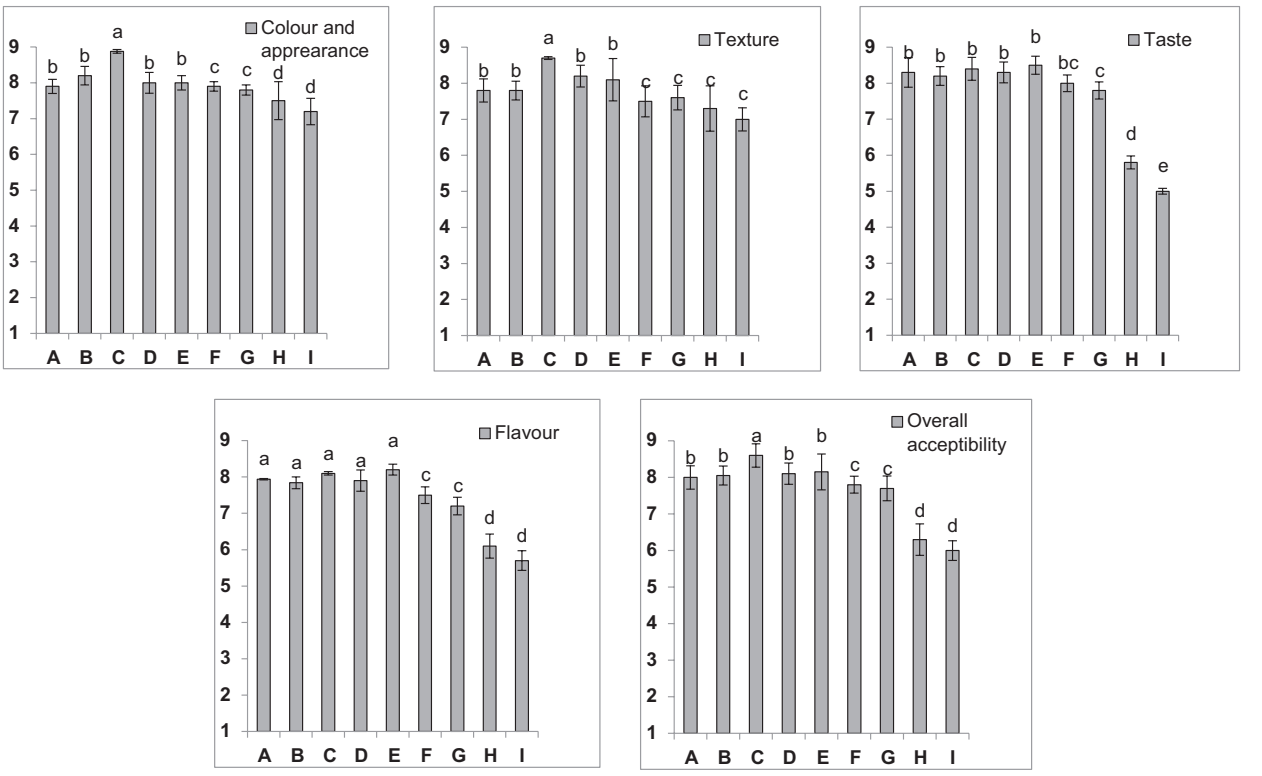


Figure 1. Sensory characteristics of cookies. *A (Control), B (AP1 2.5%), C (AP15%), D (AP1 7.5%), E (AP1 10%), F (DP1 2.5%), G (DP15%), H (DP1 7.5%), I (DP1 10%). API, Alfalfa protein isolate; DPI, Dhaincha protein isolate. *Values are expressed as mean and error bars represent standard deviation (n = 100). * The means with different superscripts are significantly different at P < 0.05.

Table 3. Nutritional composition and bioactive potential of cookies.

	Control	API $_{(10\ \%)}$	DPI $_{(5\%)}$
Proximate composition			
Moisture (%)	1.43 ± 0.04^{c}	2.89 ± 0.04^{b}	3.12 ± 0.09^{a}
Crude Protein (%)	5.26 ± 0.08^{c}	9.04 ± 0.12^{a}	7.13 ± 0.06^{b}
Crude Fat (%)	23.24 ± 0.64^{a}	24.46 ± 0.77^{a}	23.98 ± 1.04^{a}
Crude Fiber (%)	0.36 ± 0.04^{a}	0.32 ± 0.02^{a}	0.36 ± 0.02^{a}
Ash (%)	0.83 ± 0.12^{a}	0.73 ± 0.09^{a}	0.86 ± 0.10^{a}
NFE (%)	68.88	62.56	64.55
Bioactive constituents			
Total Phenols (μg GAE/g)	189.24 ± 5.3^{c}	1005.36 ± 7.5^{a}	536.24 ± 12.9^{b}
Flavonoids (μg QE/g)	26.27 ± 1.2^{c}	395.92 ± 3.8^{a}	89.28 ± 5.3^{b}
Antioxidant activity			
DPPH· RSA (μmol TE/100 g)	3.56 ± 0.04^{c}	10.97 ± 0.12^{a}	6.43 ± 0.07^{b}
ABTS·+ RSA (μmol TE/100 g)	2.49 ± 0.17^{c}	21.39 ± 0.14^{a}	9.73 ± 0.27^{b}
FRAP (μmol TE/g)	3.42 ± 0.04^{c}	6.85 ± 0.05^{a}	5.34 ± 0.04^{b}
Reducing Power (μg AAE/g)	773.2 ± 4.3^{c}	1446 ± 7.6^{a}	1164 ± 5.4^{b}
Metal Chelating Activity (μmol EDTAE/g)	18.39 ± 1.70^{c}	56.32 ± 1.23^{a}	35.48 ± 2.89^{b}

Values are expressed on % dry weight basis as mean ± standard deviation (n = 3).
Nitrogen Free Extract: 100 – % (Moisture + crude protein + crude lipid + crude fiber + ash).
The means within row followed by different superscripts are significantly different at P < 0.05.
API (10%): Cookies supplemented with 10% alfalfa protein isolate; DPI (5%): Cookies supplemented with 5% dhaincha protein isolate.
GAE, Gallic acid equivalent; QE, Quercetin equivalent; TE, Trolox equivalent; AAE, Ascorbic acid equivalent; EDTAE, Ethylenediamine tetraacetic acid equivalent; NFE, Nitrogen free extract.

Table 4. Amino acid profile of cookies.

	Control	API (10%)	DPI (5%)
Alanine	0.10 ± 0.01^{c}	0.30 ± 0.0^{a}	0.13 ± 0.0^{b}
Arginine	0.20 ± 0.02^{c}	0.34 ± 0.01^{a}	0.25± 0.0^{b}
Aspartic acid	0.17 ± 0.01^{c}	0.60 ± 0.02^{a}	0.42 ± 0.0^{b}
Cystine	0.09 ± 0.01^{c}	0.13 ± 0.01^{b}	0.20± 0.0^{a}
Glutamic acid	1.97 ± 0.01^{a}	1.91 ± 0.01^{b}	1.91 ± 0.02^{b}
Glycine	0.18 ± 0.0^{c}	0.36 ± 0.01^{a}	0.28 ± 0.0^{b}
Histidine	0.10 ± 0.01^{c}	0.36 ± 0.02^{a}	0.25 ± 0.01^{b}
Isoleucine	0.17 ± 0.0^{c}	0.43 ± 0.0^{a}	0.23 ± 0.0^{b}
Leucine	0.41 ± 0.0^{c}	0.75 ± 0.02^{a}	0.47 ± 0.0^{b}
Lysine	0.08 ± 0.0^{c}	0.88 ± 0.01^{a}	0.17 ± 0.0^{b}
Methionine	0.09 ± 0.0^{c}	0.26 ± 0.01^{a}	0.19 ± 0.01^{b}
Phenylalanine	0.24 ± 0.0^{c}	0.33 ± 0.0^{a}	0.31 ± 0.0^{b}
Proline	0.60 ± 0.01^{c}	0.76 ± 0.01^{a}	0.63 ± 0.0^{b}
Serine	0.26 ± 0.02^{c}	0.43 ± 0.01^{a}	0.31 ± 0.02^{b}
Threonine	0.17 ± 0.0^{c}	0.31 ± 0.0^{a}	0.24 ± 0.01^{b}
Tryptophan	0.08 ± 0.0^{c}	0.24 ± 0.0^{a}	0.18 ± 0.0^{b}
Tyrosine	0.11 ± 0.01^{c}	0.22 ± 0.01^{a}	0.15 ± 0.0^{b}
Valine	0.14 ± 0.0^{c}	0.31 ± 0.01^{a}	0.23 ± 0.0^{b}

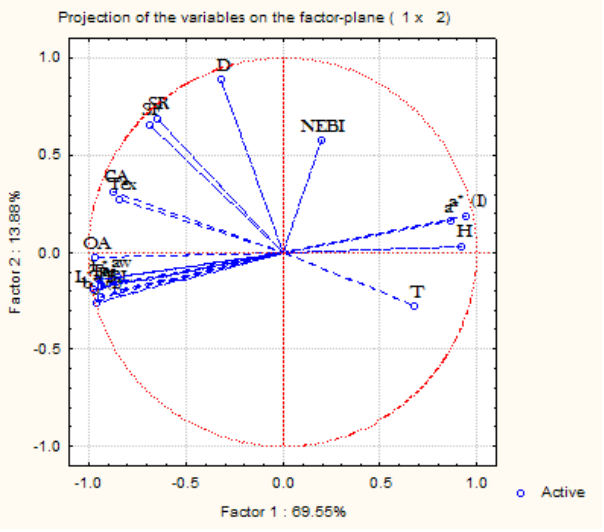
Values are expressed as g/100 g sample on dry weight basis as mean ± standard deviation (n = 3).
The means within column having different superscript are significantly different at P < 0.05.
API (10%): Cookies supplemented with 10% alfalfa protein isolate; DPI (5%): Cookies with supplemented with 5% dhaincha protein isolate.

reduction in glutamic acid was observed with the supplementation of protein isolates. Glutamic acid is the most abundant amino acid (30.53–37.18 g/100 g protein) in wheat flour, whereas API and DPI have lower concentrations of glutamic acid (Sahni, 2020; Sahni *et al*., 2020; Siddiqi *et al*., 2020). All the essential amino acids showed a significant increase with the increase in supplementation. Particularly, a remarkable increase in the leucine and lysine content of the cookies. Even though the majority of the amino acids showed an incremental increase in concentration as per the level of supplementation, resulting in higher values for cookies supplemented with API. However, lysine content showed a much higher increase for API-supplemented cookies (0.88 g/100 sample) in comparison to DPI-supplemented cookies (0.17 g/100 sample). Arise *et al*. (2021) also reported improvements in the amino acid profile of cookies with the supplementation of Bambara groundnut protein isolate.

Principal component analysis

The principal component analysis for the quality characteristics of cookies is presented in Figure 2. The quality characteristics in the same quadrants of loading plot (Figure 2A) are positively correlated whereas the quality characteristics in the opposite quadrants represent a negative correlation. The thickness (T) was negatively correlated with SR whereas diameter (D) was positively correlated. The non-enzymatic browning index (NEBI)

(A)



(B)

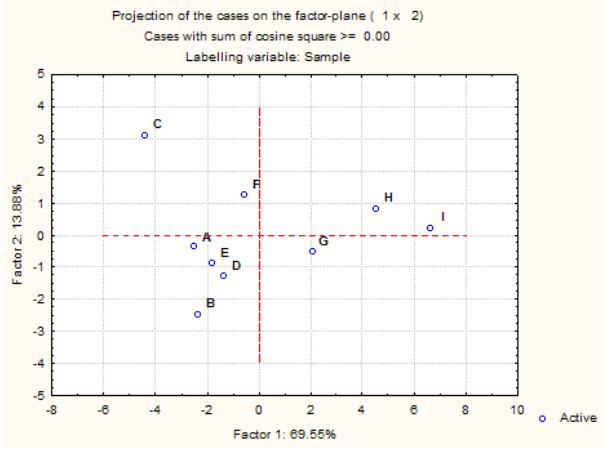


Figure 2. Principle component analysis (PCA) showing loading (A) and score plot (B) for quality characteristics of cookies. **BK: Baking loss, D: Diameter, T: Thickness, SR: Spread Ratio, SF: Spread factor, aw: Water activity, NEBI: Non-Enzymatic Browning Index, H: Hardness, L*, a*, b* (External color values), L*(I), a*(I), b*(I) (Internal Color Values), CA: Color and appearance, Tex: Texture, Tas: Taste, F: Flavor, 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.**

was positively correlated with a* value of the cookies. Hardness (H) and non-enzymatic browning index (NEBI) also showed a negative correlation with the overall acceptability score (OA). The score plot (Figure 2B) represents the variation in the cookies sample as a result of incorporation with API and DPI. Sample E (API 10%), D (API 7.5%), B (API 2.5%) showed high similarity to the control sample (A) whereas variation in sample C (API 5%) was attributed to higher spreading in comparison to the aforesaid samples. The samples incorporated with DPI showed high variability between them and sample G (5% DPI) was most similar to the control sample (A).

Conclusion

API and DPI exhibited good potential for their utilization as unconventional legume protein ingredients for protein-enrichment in wheat-based cookies. The quality characteristics of the cookies were not adversely affected up to a supplementation level of 10 and 5% for alfalfa and dhaincha protein isolate, respectively. Cookies showed appropriate spreading, top grain development, and color and textural attributes at aforesaid level of supplementation. Supplementation with protein isolates improved the nutritional profile and bioactive potential of the cookies by enhancing their protein and essential amino acid content and total phenol, flavonoid, and antioxidant capacity, respectively. Utilization of unconventional legume protein ingredients from forage legumes in the development of cookies opens new avenues for the development of protein-enriched convenience foods with high bioactive potential.

References

AACC, 2000. Approved methods of the American association of cereal chemists. 10th ed. American Association of Cereal Chemists, St. Paul.

Alruqaie, I.M. and Al-Ghamidi, F.A., 2015. Sensory and nutritional attributes of samh flour and dates powder supplemented cookies. Quality Assurance and Safety of Crops & Foods 7(3): 261–270. http://dx.doi.org/10.3920/QAS2013.0350

Arise, A.K., Akeem, S.A., Olagunju, O.F., Opaleke, O.D. and Adeyemi, D.T., 2021. Development and quality evaluation of wheat cookies enriched with Bambara groundnut protein isolate alone or in combination with ripe banana mash. Applied Food Research 1(1): 100003. http://dx.doi.org/10.1016/j.afres.2021.100003

Bashi, Z., McCullough, R., Ong, L. and Ramirez, M., 2019. Alternative proteins: the race for market share is on. McKinsey and Company, India.

Bhat, R. and Karim, A.A., 2009. Exploring the nutritional potential of wild and underutilized legumes. Comprehensive Reviews in Food Science and Food Safety 8(4): 305–331. http://dx.doi.org/10.1111/j.1541-4337.2009.00084.x

Chew, Y.L., Goh, J.K. and Lim, Y.Y., 2009. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chemistry 116(1): 13–18. http://dx.doi.org/10.1016/j.foodchem.2009.01.091

Flores, F.P., Singh, R.K., Kerr, W.L., Pegg, R.B. and Kong, F., 2014. Total phenolics content and antioxidant capacities of microencapsulated blueberry anthocyanins during in vitro digestion. Food Chemistry 153: 272–278. http://dx.doi.org/10.1016/j.foodchem.2013.12.063

Hercegová, D., Ivanišová, E., Zagula, G., Terentjeva, M., Kročko, M., Tvrdá, E. and Kačániová, M., 2019. Technological, phytochemical and sensory profile of honey biscuits made from buckwheat, rye, spelt and wheat flour. Quality Assurance and Safety of Crops & Foods 11(4): 333–340. https://dx.doi.org/10.3920/QAS2018.1376

Hwang, J.Y., Shue, Y.S. and Chang, H.M., 2001. Antioxidative activity of roasted and defatted peanut kernels. Food Research International 34(7): 639–647. http://dx.doi.org/10.1016/S0963-9969(01)00083-7

Jayasena, V. and Nasar-Abbas, S.M., 2011. Effect of lupin flour incorporation on the physical characteristics of dough and biscuits. Quality Assurance and Safety of Crops & Foods 3(3): 140–147. http://dx.doi.org/10.1111/j.1757-837X.2011.00100.x

Kiranmai, M., Kumar, C.M. and Mohammed, I., 2011. Comparison of total flavanoid content of Azadirachta indica root bark extracts prepared by different methods of extraction. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2(3): 254–261.

Leiva-Valenzuela, G.A., Quilaqueo, M., Lagos, D., Estay D. and Pedreschi, F., 2018. Effect of formulation and baking conditions on the structure and development of non-enzymatic browning in biscuit models using images. Journal of Food Science and Technology 55(4), 1234–1243. http://dx.doi.org/10.1007/s13197-017-3008-7

Mamat, H. and Hill, S.E., 2018. Structural and functional properties of major ingredients of biscuit. International Food Research Journal 25(2): 462–471.

Sahagún, M. and Gómez, M., 2018. Influence of protein source on characteristics and quality of gluten-free cookies. Journal of Food Science and Technology 55(10): 4131–4138. http://dx.doi.org/10.1007/s13197-018-3339-z

Sahni, P., 2020. Quality assessment, characterisation and functionality of forage legumes for food use. Doctoral dissertation, Punjab Agricultural University, Ludhiana.

Sahni, P., Sharma, S. and Singh, B., 2019. Evaluation and quality assessment of defatted microalgae meal of *Chlorella* as an alternative food ingredient in cookies. Nutrition & Food Science 49(2): 221–231. https://dx.doi.org/10.1108/NFS-06-2018-0171

Sahni, P., Sharma, S., Singh, B. and Bobade, H., 2022. Cereal bar functionalised with non-conventional alfalfa and dhaincha protein isolates: quality characteristics, nutritional composition and antioxidant activity. Journal of Food Science and Technology 53: 3827–3835. http://dx.doi.org/10.1007/s13197-022-05404-5

Sahni, P., Sharma, S. and Surasani, V.K.R., 2020. Influence of processing and pH on amino acid profile, morphology, electrophoretic pattern, bioactive potential and functional characteristics

of alfalfa protein isolates. Food Chemistry 333: 127503. http://dx.doi.org/10.1016/j.foodchem.2020.127503

Sahni, P. and Shere, D.M., 2017. Physico-chemical and sensory characteristics of carrot pomace powder incorporated fibre rich cookies. Asian Journal of Dairy and Food Research 36(4): 327–331. http://dx.doi.org/10.18805/ajdfr.DR-1268

Sahni, P., Singh, B. and Sharma, S., 2018. Functionality of proteins and its interventions in food. IFI Mag 37(3): 41–52.

Sarabhai, S., Indrani, D., Vijaykrishnaraj, M., Arun Kumar, V. and Prabhasankar, P., 2015. Effect of protein concentrates, emulsifiers on textural and sensory characteristics of gluten free cookies and its immunochemical validation. Journal of Food Science and Technology 52(6): 3763–3772. http://dx.doi.org/10.1007/s13197-014-1432-5

Sharma, S. and Sahni, P., 2021. Dynamics of germination behaviour, protein secondary structure, technofunctional properties, antinutrients, antioxidant capacity and mineral elements in germinated dhaincha. Food Technology and Biotechnology 59(2): 238–250. http://dx.doi.org/10.17113/ftb.59.02.21.6922

Siddiqi, R.A., Singh, T.P., Rani, M., Sogi, D.S. and Bhat, M.A., 2020. Diversity in grain, flour, amino acid composition, protein profiling, and proportion of total flour proteins of different wheat cultivars of North India. Frontiers Nutrition 7(141):1–16. http://dx.doi.org/10.3389/fnut.2020.00141

Singh, M. and Mohamed, A., 2007. Influence of gluten–soy protein blends on the quality of reduced carbohydrates cookies. LWT-Food Science and Technology, 40(2): 353–360. http://dx.doi.org/10.1016/j.lwt.2005.09.013

Slade, L. and Levine, H., 1994. Structure-function relationships of cookie and cracker ingredients. The Science of Cookie and Cracker Production 9: 23–141.

Tang, X. and Liu, J., 2017. A comparative study of partial replacement of wheat flour with whey and soy protein on rheological properties of dough and cookie quality. Journal of Food Quality 2017: 1–10. https://dx.doi.org/10.1155/2017/2618020

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19(6–7): 669–675. http://dx.doi.org/10.1155/2017/2618020

Wani, A.A., Sogi, D.S., Singh, P., Sharma, P. and Pangal, A., 2012. Dough-handling and cookie-making properties of wheat flour–watermelon protein isolate blends. Food and Bioprocess Technology 5(5): 1612–1621.