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Quality attributes of cookies enriched with functional protein isolate from red kidney beans

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Abstract: Red kidney bean protein isolate was prepared and incorporated into wheat flour at levels of 0, 5, 10, 15, and 20% to prepare protein-enriched cookies which were then evaluated for various quality attributes. Nutritional evaluation of cookies showed that the supplementation of bean protein isolate increased the protein content of the cookies from 7.87% in T_1 (control or wheat flour cookies) to 16.92% in T_6 (cookies supplemented with 20% bean protein isolate), which indicated a 115% increase in protein content. The physical characteristics of cookies such as width, thickness and spread factors differed significantly. Sensory attributes of all the supplemented cookies were within the acceptable range but cookies supplemented with 20% level of protein isolate were less preferred due to slightly darker colour and harder texture. Conclusively, the red kidney bean protein isolate can be successfully utilised for the production of high-protein cookies. Such high-protein products can be advantageously utilised to cope with the problem of protein-energy malnutrition. Moreover, such nutritious products can also be used as emergency foods during natural disasters as well as during war times.

Keywords: supplementation; malnutrition; beans protein; sensory analysis; nutritional evaluation

Proteins play an imperative role in human nutrition primarily for their amino acid profile. Besides providing energy, they have a vital role in the building and repairing of body tissues, maintenance of acid-base balance as well as a synthesis of important substances like hormones and enzymes (Friedman 1996; Awan 2007). Consumption of high-quality protein is essential in a diet containing all the essential amino acids in balanced proportion. Lower intake or consumption of poor-quality protein is associated with infectious and parasitic diseases due to immune system weakness. This type of malnutrition is common in infants and children resulting in weight loss, wasting and stunting. Long-term deficiency of protein is associated with Kwashiorkor and Marasmus which are common in the third world countries (Harding et al. 2018; Wali et al. 2019).

Inadequate intake of protein is a common phenomenon in nations relying on cereal based diets that is generally observed in developing countries such as Asian and Sub-Saharan regions (Arif et al. 2012; Rahman et al. 2016). This practice provides neither a sufficient amount of protein nor a balanced ratio of amino

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acids. Consequently, the proportion of underweight, wasted and stunted children in developing countries is 31, 38, and 9%, respectively (Akhtar 2016). This type of malnutrition mainly prevails in the South Asian region where the proportion of malnourished children is higher than even in Sub-Saharan Africa (Akombi et al. 2017). In Pakistan, about 28.9% of children under the age of five years are underweight, 40.2% are stunted, while 17.7% are wasted (GOP 2018).

Large segments of the population in developing countries have limited access to animal proteins due to their increased cost and limited supplies. Hence, the quest for alternative sources of proteins has prompted researchers towards legume proteins because of their comparative cheapness, nutritive value and variety of sources (Boye et al. 2010; VazPatto et al. 2015). Among the legumes, red kidney bean (*Phaseolus vulgaris* L.) is widely produced in Asia, South America and Africa and is also used as a staple food in Mexico, South American and African countries (Yin et al. 2010; Mundi and Aluko 2012). Although beans contain high amounts of starch, dietary fibre, minerals, vitamins as well as an extensive array of phenolic compounds, the most important component of nutritional significance is their high protein content which is more than twice as compared to cereal grains. Kidney beans contain protein in the range of 20–30%, also containing elevated levels of indispensable amino acids, particularly lysine, which is deficient in cereal grains (Siddiq et al. 2010; Hayat et al. 2014). Thus, the combined consumption of kidney beans and cereals can ensure a balanced protein diet due to the complementation of essential amino acids.

The best strategy for more efficient utilisation of kidney bean proteins is to produce protein isolates or concentrates. Such high protein products exhibit industrial important functionalities and can also be utilised as an ingredient in different food products (Yin et al. 2008; Sparvoli et al. 2016). Bakery products including cookies are the ideal tool to incorporate these plant based proteins as these are liked among all segments of the population. The development of high protein products can be the ideal strategy to enhance the nutritional status of the diets and such products can also be used as emergency foods (Yousaf et al. 2013). This could be of particular interest for the people of low income group in developing countries who are mostly suffering from protein deficiency problems. The prime purpose of this study was to prepare a protein isolate from red kidney beans for the development of high-protein cookies and to evaluate various quality attributes of the cookies.

MATERIAL AND METHODS

Raw materials and chemicals. The seeds of an indigenous cultivar of red kidney beans (Accession No. 027076) were obtained from the National Agriculture Research Centre, Islamabad, Pakistan. The seeds were screened and rinsed in deionised water to remove the extraneous material. After manual dehulling and drying the seeds at room temperature (28 ± 2 °C), they were ground (Brabender Rotory Mill; Brabender GmbH & Co. KG, Germany) and passed through a screen to get uniform size flour which was stored in air-tight polyethylene bags at 10 °C until use. White wheat flour and other ingredients for the preparation of cookies were purchased from the local market. The analytical grade chemicals were used during the analysis.

Preparation of red kidney bean protein isolate. Red kidney bean seed flour was defatted with hexane under continuous shaking for 12 h (Max Q 4000 model SHK4000-ICE shaking incubator; Barnstead Lab-Line, US), while the removal of hexane was carried out by the decantation process. The defatted bean flour was then dried in air and used for the preparation of protein isolate. Different steps involved in the preparation of protein isolate are depicted in Figure 1.

Chemical analysis of wheat flour and kidney bean protein isolate. Red kidney bean protein isolate and wheat flour were analysed for moisture (Method No. 44-19), crude fibre (Method No. 32-10.01), crude protein (Method No. 46-10), crude fat (Method No. 30-10), ash (Method No. 08-01) as described in AACC (2000) while carbohydrate contents were determined by difference.

Preparation of blends. The red kidney bean protein isolate was incorporated into wheat flour at different levels as given in Table 1.

Preparation of cookies. The cookies with 0, 5, 10, 15 and 20% supplementation levels of red kidney bean protein isolate were prepared by following the procedure of AACC (2000) Method No. 10-50D with slight modifications. The recipe used for making cookies is shown in Table 2.

Nutritional evaluation of cookies. Cookies were evaluated for carbohydrate, crude fibre, crude protein, ash, and moisture contents following the standard procedures of AACC (2000). Moisture content was determined by drying the disintegrated cookie samples in a hot air oven at 130 °C (Memmert Schutzart DIN 40050-IP20; Memmert GmbH Co., Germany) untill constant weight was attained (Method No. 44-19). Protein content was estimated by MicroKjeldahl method using 6.25 as a fac-

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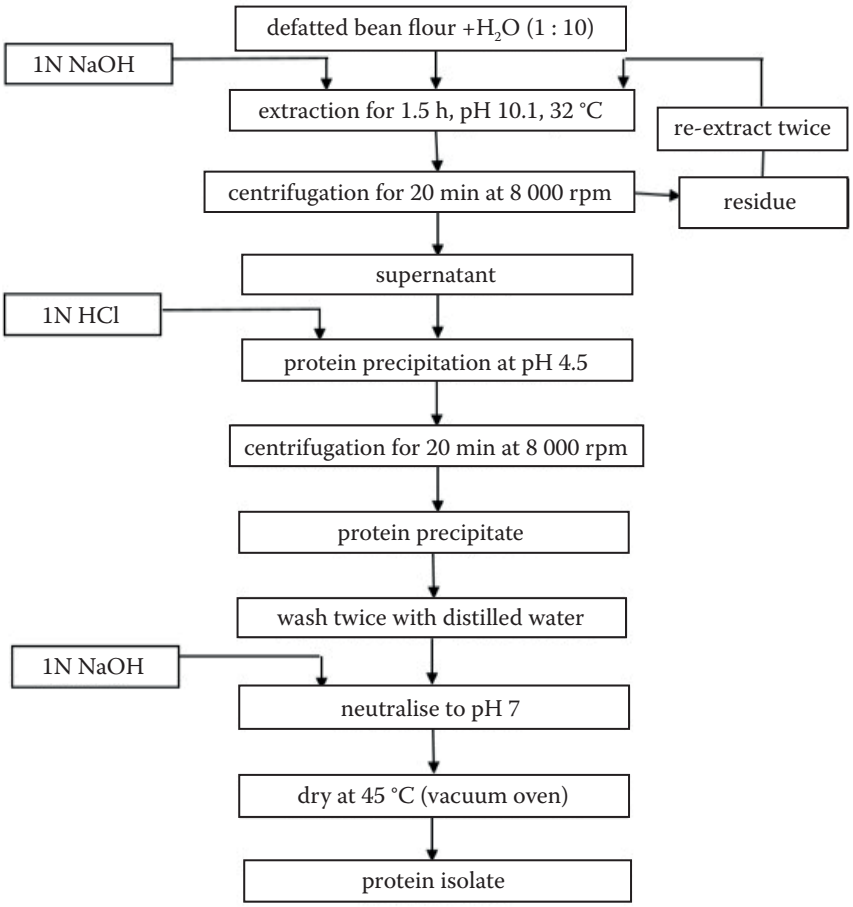

Figure 1. Steps involved in the preparation of *Phaseolus vulgaris* L. protein isolate

tor (Method No. 46-10). The estimation of the fat content of the samples was carried out in Soxhlet apparatus using petroleum ether (Method No. 30-10) while the ash content was determined using a muffle furnace (box--type resistance furnace No. SX2-4-17TP, Meditry Instrument Co., Ltd, China) at a temperature of 550 °C for 8 h to 10 h (Method No. 08-01). Carbohydrate content of cookies was calculated by difference while the gross energy was calculated by multiplying percentages of fat, protein and carbohydrates by their Atwater calorie conversion factors such as 9, 4, 4, respectively.

Table 1. Percentage composition of flour blends (red kidney bean protein isolate + wheat flour) (%)

Treatment	Red kidney bean protein isolate	Wheat flour
T_1	0	100
T_2	5	95
T_3	10	90
T_4	15	85
T_5	20	80

Physical evaluation of cookies. The physical characteristics such as thickness, width and spread factor of the cookies were evaluated by following Method No. 10-53 of AACC (2000).

Sensory evaluation of cookies. Sensory attributes of cookies like colour, texture, taste, flavour and overall acceptability were evaluated by a panel of seven trained judges on a 9-point hedonic scale by following the guidelines (Meilgard et al. 1991). A three-digit code was assigned to each cookie sample and a briefing session was also conducted with all judges before the evaluation process. The samples were randomly presented to the panel while maintaining uniform conditions during

Table 2. Recipe followed for the cookie preparation

Ingredients	Quantity
Flour/flour blends	100 g
Hydrogenated vegetable ghee	50 g
Sugar	45 g
Baking powder	1.0 g
Beaten eggs	7.5 mL

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the evaluation process. The hedonic scale was arranged such that 9 – like extremely, 8 – like very much, 7 – like moderately, 6 – like highly, 5 – neither like nor dislike, 4 – dislike slightly, 3 – dislike moderately, 2 – dislike very much, and 1 – dislike extremely. Sensory studies evaluated the degree of cookie acceptability on the basis of pleasurable and unpleasurable experience of judges by marking the above-mentioned score scale.

Statistical analysis. The analyses were performed in triplicate and the average values were calculated. The data was subjected to the analysis of variance (ANOVA) technique using SPSS 16 statistical software. The means were separated by the Duncan's multiple range (DMR) test by setting $P < 0.05$ (Steel et al. 1997).

RESULTS AND DISCUSSION

Chemical composition of wheat flour and red kidney bean protein isolate. The chemical compositions of wheat flour and red kidney bean protein isolate used for the cookie preparation are presented in Table 3. The composition of wheat flour was in close proximity with the previously reported values for different wheat varieties grown in Pakistan. The protein content of red kidney bean protein isolate was found to be 90.23% while minor quantities of other components like carbohydrates, crude fibre, crude fat and ash contents were also observed. The protein content is an important criterion to assess the suitability of wheat varieties for different products. The flours obtained through the milling of soft wheat cultivars have the protein content in the range of 7–9% and such flours are suitable for the preparation of cookies and cakes. The comparatively low amounts of fat and fibre contents in wheat flour may be ascribed to the removal of the bran and aleurone layer during the milling process. The purity of bean protein isolate was higher than the previously reported values for cowpea protein isolates (Butt and Batool 2010) and broad bean protein isolates (Qayyum et al. 2012), which indicates the suitability of the method and conditions used for protein extraction. The higher protein purity of red kidney bean protein isolate indicates its favourable potential as an ingredient to develop protein-based food products.

Table 3. Proximate composition of wheat flour and red kidney bean protein isolate (%) (mean ± SD; $n = 3$)

Parameter	Wheat flour	Red kidney bean protein isolate
Moisture	12.71 ± 0.57	4.11 ± 0.01
Protein	9.12 ± 0.06	90.23 ± 1.66
Crude fat	0.92 ± 0.02	0.12 ± 0.02
Crude fiber	0.90 ± 0.02	0.34 ± 0.01
Ash	0.57 ± 0.01	1.86 ± 0.03
Carbohydrates	76.11 ± 0.14	3.32 ± 1.72

Nutritional evaluation of cookies. The results regarding the nutritional evaluation of cookies are depicted in Table 4. These results clearly manifested that the supplementation of red kidney bean protein isolate to wheat flour exhibited significant variations ($P < 0.05$) in moisture, protein, carbohydrate, fibre and energy contents of the cookies. An increasing trend in moisture, crude protein and ash contents, while a decreasing trend in crude fibre, fat and carbohydrate contents, were observed with increasing the supplementation level of red kidney bean protein isolate in the cookies. In comparison with wheat flour cookies, an increase of 115.00% and 85.38% in protein content was observed in cookies supplemented with 20% and 15% levels of bean protein isolate, respectively. It is evident from Table 5 that gross energy values decreased with increasing the level of protein isolate in the supplemented cookies, which was mainly due to low fat content in the protein isolate as compared to wheat flour. The minimum recommended value of the energy content of the supplementary food is 393.88 kcal $(100\ g)^{-1}$ for young children (FAO/WHO 1994), so despite the decrease in the energy values in supplemented cookies these values are still within the recommended range. The main purpose of supplementation of red kidney bean protein isolate was to enhance the protein content of supplemented cookies which was improved up to 115% as compared to control samples. In comparison with the huge improvement of protein content in the supplemented cookies, the decrease in gross energy values of supplemented cookies was negligible. The energy contents of the supplemented cookies were decreased but this decrease was within the narrow range in comparison with the enormous improvement in their protein contents.

The high moisture content in the supplemented cookies was attributed to the presence of polar amino acids as well as the better hydration capacity of red kidney bean protein. Previously, Korean cookies developed through the supplementation of soy protein isolate had higher moisture content as compared to the control samples (Lee and Brennand 2005). The percentage increase in the protein content of supplemented cookies observed in this study was higher when compared with the results of cookies supplemented with soy protein iso-

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Table 4. Nutritional evaluation of cookies as influenced by different supplementation levels of red kidney bean protein isolate (mean ± SD; n = 3)

Treatment	Protein isolate level	Moisture	Crude protein	Crude fiber	Crude fat	Ash	Carbohydrates	Gross energy
		(%)						[kcal (100g)$^{-1}$]
T_1	0	3.14 ± 0.12^{e}	7.87 ± 0.26^{e}	0.50 ± 0.02^{a}	26.48 ± 1.21	0.39 ± 0.01	61.61 ± 1.12^{a}	516.28 ± 4.79^{a}
T_2	5	3.59 ± 0.08^{d}	10.13 ± 0.18^{d}	0.48 ± 0.01^{ab}	25.56 ± 1.10	0.40 ± 0.02	59.84 ± 0.94^{b}	509.92 ± 3.63^{b}
T_3	10	4.15 ± 0.24^{c}	12.34 ± 0.44^{c}	0.46 ± 0.03^{abc}	25.43 ± 1.21	0.41 ± 0.01	57.20 ± 1.25^{c}	507.03 ± 4.47^{c}
T_4	15	4.73 ± 0.16^{b}	14.59 ± 0.12^{b}	0.44 ± 0.02^{bc}	25.29 ± 0.88	0.43 ± 0.01	54.50 ± 1.33^{d}	504.01 ± 4.76^{d}
T_5	20	5.49 ± 0.31^{a}	16.92 ± 0.38^{a}	0.43 ± 0.01^{c}	25.11 ± 0.98	0.45 ± 0.02	51.59 ± 1.16^{e}	500.04 ± 5.04^{e}

$^{a–e}$Means containing same letters within the columns are statistically non-significant ($P \leq 0.05$)

late (Mohsen et al. 2009) or a combination of broad bean, chickpea and soy protein isolate (Rababah et al. 2006). This increase in the protein content of supplemented cookies was attributed to the highest protein content of the red kidney bean protein isolate. The strategy of preparing protein-enriched cookies by utilising such high protein sources can be valuable to counter the menace of protein-energy malnutrition. Variations in the energy content of cookies were associated with their fat content, thus the slightly lower fat content of supplemented cookies resulted in a decrease in their energy content. The minimum recommended value of the energy content of supplementary food is 393.88 kcal (100 g)$^{-1}$ for young children (FAO/WHO 1994). The energy contents of the protein enriched cookies were also within the recommended range for young children.

Physical characteristics of cookies. The dimension of the cookies is one of the important attributes not only for their aesthetic perspectives but also it is helpful to determine the packaging requirements. The results regarding the physical characteristics of cookies are depicted in Table 5. A decreasing trend in the width and spread factor while an increasing trend in thickness was observed with increasing the supplementation level of bean protein isolate in the cookies.

The spread ratio depends on the width and thickness values of the cookies and is usually influenced by the competition of flour and other ingredients for water during dough mixing. Thus the ingredients with more hydrophilic sites will absorb more water as well as increase the dough viscosity resulting in a decrease in the spread factor (Yousaf et al. 2013). As the red kidney bean protein isolate has a higher water retention capacity so supplementation of the isolate reduced the spread factor of cookies. Although supplementation of bean protein isolate resulted in a decreased spread factor of cookies, that decrease was negligible and cookies with acceptable physical characteristics can be produced by supplementation of kidney bean protein isolate to wheat flour.

Sensory evaluation of cookies. The sensory characteristics such as colour, texture, flavour, taste and overall acceptability of the cookies were significantly ($P \leq 0.05$) affected among different treatments (Table 6). It is evident from the results that the panellists accepted the sensory characteristics of all the cookie samples but T_3 (cookies with 10% protein isolate supplementation) was ranked best with maximum scores while T_5 (cookies with 20% protein isolate supplementation) got the minimum scores. The co-

Table 5. Physical attributes of cookies as influenced by different supplementation levels of red kidney bean protein isolate (mean ± SD; n = 6)

Treatment	Protein isolate level (%)	Width (mm)	Thickness (mm)	Spread factor
T_1	0	56.50 ± 0.5^{a}	7.00 ± 0.5^{c}	8.09 ± 0.65^{a}
T_2	5	55.16 ± 0.28^{b}	7.50 ± 0.5^{bc}	7.37 ± 0.46^{ab}
T_3	10	54.33 ± 0.23^{bc}	7.83 ± 0.28^{b}	6.94 ± 0.27^{bc}
T_4	15	53.50 ± 0.5^{cd}	8.16 ± 0.28^{ab}	6.55 ± 0.28^{cd}
T_5	20	52.83 ± 0.76^{d}	8.66 ± 0.28^{a}	6.09 ± 0.20^{d}

$^{a–d}$Means containing same letters within the columns are statistically non-significant ($P \leq 0.05$)

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Table 6. Sensory attributes of cookies as influenced by different supplementation levels of red kidney bean protein isolate (mean ± SD; n = 3) (9-point hedonic scale)

Treatment	Protein isolate level (%)	Colour	Flavour	Taste	Texture	Overall acceptability
T_1	0	7.57 ± 0.53^{ab}	7.42 ± 0.53^{bc}	7.71 ± 0.75^{ab}	7.57 ± 0.53^{a}	7.42 ± 0.78^{a}
T_2	5	7.85 ± 0.37^{ab}	7.71 ± 0.75^{ab}	7.85 ± 0.69^{a}	7.71 ± 0.75^{a}	7.71 ± 0.75^{a}
T_3	10	8.14 ± 0.69^{a}	8.28 ± 0.48^{a}	8.14 ± 0.69^{a}	7.85 ± 0.89^{a}	8.00 ± 0.81^{a}
T_4	15	7.28 ± 0.75^{b}	7.57 ± 0.53^{bc}	7.42 ± 0.53^{ab}	7.14 ± 0.69^{ab}	7.28 ± 0.75^{a}
T_5	20	6.14 ± 0.69^{c}	7.00 ± 0.57^{c}	7.00 ± 0.81^{b}	6.71 ± 0.75^{b}	6.14 ± 0.69^{b}

$^{a–c}$Means containing same letters within the columns are statistically non-significant ($P \leq 0.05$)

lour as well as other sensory attributes were evaluated by the panel of trained judges by the hedonic scale. The judges assigned the colour scores on the basis of visual observation. In comparison with control samples, the colour of cookies supplemented with higher levels of red kidney bean protein isolate was found slightly darker, due to which they assigned lower scores to those cookie samples.

The colour of the cookies not only indicates the suitability of the raw materials used for preparation but also provides a cue about the formulation as well as the quality of the end product. This darker appearance in cookies was attributed to the Maillard reaction between the amino acids and sugars of the cookies (Zucco et al. 2011). These brown pigments are also produced due to the degradation of Amadori products as well as sugar caramelisation during the baking process (Ait-Ameur et al. 2008). The flavour of the cookies may be influenced by different factors. Different volatile compounds are generated during baking as a result of the Maillard reaction. The higher levels of protein may have an indirect effect on the rate of Maillard reaction either due to deamination of bound amino acids or by hydrolysis. The presence of higher levels of protein during the baking process promotes the generation of pyrazines which impart a roasted flavour impression. Moreover, thermal degradation of lipids during the baking process also produces different volatile compounds which may influence the flavour of the cookies (Whitfield 1992; Mohsen et al. 2009). The acceptable flavour and taste scores for all the cookie samples elucidated that the supplementation of *P. vulgaris* L. protein isolate even at higher concentration did not exhibit any larger effect on cookies. Due to this favourable attribute, bean protein isolate can be advantageously utilised in different food formulations without affecting their original tastes and flavours. The sensory attributes of cookies improved up to 15% supplementation level of *P. vulgaris* L. protein isolate as compared to the control treatment while higher levels resulted in sensorily slightly darker colour as well as harder and crumbly texture. This may be due to the fact that bean protein exhibits low viscoelastic properties, so when incorporated at higher levels into wheat flour, it disrupted the gluten network and formed new interactions with other components, which resulted in a relatively harder cookie texture (Lee and Brennand 2005). Acceptability of a product presents an idea of consumer satisfaction with the product. The judges accepted the cookies from all treatments, however, the cookies supplemented with a 20% level of the *P. vulgaris* L. protein isolate (T_5) were less preferred by the judges, mainly due to their darker colour and harder texture.

CONCLUSION

Protein supplementation is the best way to replace some portion of wheat flour with kidney bean protein to cope with the issues of protein energy malnutrition all around the world. Hence the supplemented baked products are of immense importance to the malnourished population.

Being a rich source of protein, red kidney beans have a vast potential to be transformed into high-protein value-added products such as isolates or concentrates. These high protein products can be successfully used as promising food ingredients into wheat flour up to 15% level to prepare nutritious baked products (cookies). The increased substitution of protein beyond this level has an impact on the colour and texture of cookies, so the response of organoleptic evaluation was less preference because of exceeding the concentration of kidney bean protein although the nutritional quality was acceptable. Such nutritious products can be advantageously utilised to combat the problems of malnutrition, especially in developing countries.

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