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RESEARCH ARTICLE

Development of Red Kidney Bean (Phaseolus *vulgaris L.*), Spinach, Green Gram Combine: A Protein and Mineral Rich Recipe

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Abstract

Coronary Heart diseases is one of the renowned leading killers in the world and most common reason of total-global deaths. Heart-attack victims are just the first wave of a swelling population of developing countries with heart problems. From the beginning, plant based foods play a prominent role and lie as the cornerstones in the prevention of coronary heart disease. Due to this reason, majority of civilizations have heavily relied on different types of vegetarian foodstuffs especially legumes- beans in the daily diet. Red Kidney beans (Phaseolus *vulgaris L.*) is intricately woven into fabric of human history due to their versatile health and nutritional benefits. Therefore, present study is planned to be conducted with the evaluation and enhancement of nutritional quality of red kidney beans through household adaptable processing method- hot water blanching (HWB) to explore the acceptability of nutritionally tenable food product – Dal-Sag (DS). Proximate analysis of nutrients, antioxidant and antinutrients was carried out in native and processed forms of the variety by using standardized AOAC procedure. Organoleptic evaluation was done on the basis of 9-point hedonic method. Data obtained was subjected to the analysis of mean, standard deviation and t-test. The conclusion drawn from the study is that developed recipe- Dal-Sag provides good nutrition with high index of protein, energy and iron. The sensory results indicated that BRKB obtained highest scores in terms of overall acceptability ratings.

Keywords

Blanched red kidney beans, Dal-Sag, Hot water blanching, Raw red kidney beans

Introduction

Coronary heart disease (CHD) refers to the failure of coronary circulation or to supply inadequate circulation to cardiac muscles and surrounding tissues. According to the survey of 2007, CHD is the leading cause of 25.4% total deaths of United States, England and Canada (Rosamond *et al*, 2007). In the United Kingdom, coronary heart diseases are responsible for 101,000 total deaths annually. Over 459,000 Americans died every year due to coronary heart diseases (Kaski, 2004). It has been estimated that almost all the regions of the world will be affected by 2020 (Boon *et al,* 2006).The epidemic of cardiovascular diseases in India is very rapidly. India is experiencing of an epidemiological health transition which is characterized by rapid decline in nutritional and parasitic diseases with an alarming rise in cardiac diseases, mainly coronary vascular disease and stroke (Yusuf *et al,* 2001). In 2000, there was an estimated 29.8 million people suffers from CHD in India, out of 1.03 billion total estimated population (Gupta *et al*, 2008). CHD may be associated with other diseases i.e. hypertension, diabetes mellitus, obesity, chronic kidney disease, heart failure, stroke and peripheral artery disease (Leeder *et al,* 2004). 52.2% of total deaths due to CVD below the age of 70 years in India as compared with 22.8% of the developed countries (Libby *et al*, 2007). Sedentary life style, consumption of atherogenic and thrombogenic diet, lack of physical activity and other related factors are responsible to the prevalence of degenerative diseases. Lifestyle modifications would be hugely effective in the treatment of coronary disease through the reduction of animal based diet and an inclusion of vegetarian diets may eventually mean an increase in the consumption of legumes, especially- beans in daily diet. Utilization of beans especially *kidney beans* can be further increased by popularizing its potential used as a substitute for other legumes in Indian Cuisine. They belong to the leguminous family and mostly consumed as whole beans by human beings throughout the world. One of the important economic varieties of genus *Phaseolus* are Red Kidney Beans, scientifically known as Phaseolus *vulgaris L.* and also referred as "*Common Beans*". It is also is also known as *Rajmah, Garden beans, Field beans and French beans*. It has

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a dark red skin with cream-colored flesh is named for its visual resemblance to a kidney. Kidney beans are now being correctly hailed as nutritional giants that pack a lot of health benefits in a tiny package. They are good and relatively inexpensive sources of protein (22.9 g), energy (846 kcal) and minerals (3.29 mg) for vegetarian diets in developing countries (Gopalan *et al*, 2004**).** Besides this, they have an excellent source of niacin, thiamine, fiber, complex carbohydrate, potassium, selenium, vitamin-B6, folic acid, and low in fat, sodium and cholesterol free in nature. They have often-overlooked source of incredible health benefits including reduction of cardiac and renal diseases, lowers the risk of diabetes, increased satiation and cancer prevention **(**Hangen and Bennink, 2002**).** A dietary intervention has been reported that diet supplemented with dry beans lowers the serum total cholesterol by as much as 19% and low density lipoprotein (LDL) cholesterol by 24% (Klevay, 2002). Red kidney bean consumption reduced total and LDL blood cholesterol concentrations in hypercholesterolemic subjects (Winham and Hutchins, 2007) because of richness of soluble fiber in beans which are fermented in the colon and generate short chain fatty acids (SCFAs) may hinder hepatic cholesterol synthesis. Therefore, in present scenario need to be focused on evaluation of nutrients, antioxidant and anti-nutritional factors of red kidney beans in raw form and after using appropriate household practices- hot water blanching **(**Granite *et al,* 2002**)** and to explore the acceptability of conventional recipe such- *Dal-Sag (DS)* was made in different proportion which could be beneficial for cardiac disease patients because of enriched nutritional patterns.

Materials and Method

In the present study, *Phaseolus* species of red kidney beans (*RSJ-178*) was selected on the basis of its wide cultivation and consumption in India. It was procured from authentic source, such as- Rajasthan Seed Corporation, Jaipur after due certification. After purchasing the beans, preliminary preparation like- cleaning was done and seeds were freed from any dirt or foreign matter. For optimizing nutrient yield and removal of antinutrients, raw kidney beans were processed by using household adaptable strategy such as- hot water blanching (HWB). Analysis for proximate principles and some minerals- iron, calcium and phosphorus were performed on raw and processed state of variety in triplicate sets. All chemicals were analytical grade; procured from credible concerns e.g. Merck, Loba, .Chemicals of higher purity and of scarce availability.

Processing techniques

Procured kidney beans were graded and good quality seeds were kept separately for analysis and rest discarded. Then, the beans were divided into two portions. One portion of the beans was ground into fine powder or flour and the other portion of beans was processed by giving the following treatments.

Hot-water blanching (HWB)

Another portion of beans was taken and soaked separately in boiled distilled water at 95°C into the ratio of 1:5w/v for ½ hour in beaker**. Mixed with spoon to 2-3 times and left for 30 minutes. After half an hour, the water was decanted.** Then, beans were washed with fresh water and soaked overnight at normal room temperature. Next day, water was drained and beans was dehulled manually, and then dried in direct sunlight properly so as to make them moisture free. Dried beans were ground into powdered form and **stored in air tight containers.** Flour of kidney beans was subjected to further treatment and analysis.

Chemical composition

Proximate composition: Protein, moisture, ether extract, ash, fiber and minerals like iron, calcium and phosphorus were determined by using standardized methods given by AOAC (2005)**.** Carbohydrates were determined by difference method.

Determination of phenol

Phenol was estimated as procedure suggested by Singleton and Slinkard (1977**).** The finely grounded sample **(**2g) was extracted with 5-10ml of 80% alcohol in a pestle mortar and the homogenate was boiled in water bath for 5 to 10 minutes, then centrifuged and supernatant was collected in other flask and volume made up. Then, samples (200 ml) were introduced in test tubes, and 1.0 ml of Folin-Ciocalteau's and 0.8 ml of sodium carbonate (7.5%) were added. The absorbance of sample was measured at 760nm after incubation at 30°C for 1.5 hrs. Result was expressed in milligram of gallic acid equivalent (GAE) as per gram of fresh weight of sample. Standard curve was drawn by plotting the absorbance against concentration of gallic acid.

Determination of tannin

Tannin was estimated as per the method given by Price *et al* (1978**).** 1gm sample was extracted with 10ml of 1% HCl in methanol at room temperature for 24 hours, and then centrifuged at 5,000 rpm. Vanillin HCl reagent was prepared by mixing equal volumes of 8% HCl and 2% vanillin in methanol. One ml of supernatant was mixed in 5ml of Vanillin-HCl. Catechin standard run along with

the sample. Absorbance was read at 500nm after 20 minutes incubated at room temperature.

Determination of phytic acid

Phytic acid was estimated as per method given by Davies and Reid (1979). One gram dried powdered sample was extracted with 3% TCA by continuous shaking, then filtered and made up to suitable volume with water. For 1.4ml filtrate, 1ml ferric ammonium sulfate solution (21.6mg in 100 ml water) was added, mixed and placed in boiling water bath for 20 minutes. Content was cooled, and then 5ml isoamyl alcohol was added and mixed. Into this, 0.1ml ammonia solution was added, shaken thoroughly and centrifuged at 3,000 rpm for 10 minutes. Alcoholic layer was separated and color intensity was read at 465nm against blank sample of amyl alcohol after 15 minutes. Standard Fe $(NO_3)^3$ was run along with the sample. Graph of standard was plotted and result was expressed as mg/100g.

Estimation of total cyanogens

Total Cyanogens were determined by Vogel (1996). 5 g sample was taken in glass stopper flask and 50ml of 1% HCL-methanol was added. It was stoppered and kept overnight. After filtration, 25ml of extract was taken in conical flask and 75ml distilled water was added. Then 5ml of ammonium chloride was added, and lastly, 2ml of 10% potassium iodide solution was added in a flask. The flask was kept on a sheet of black paper and titrated with standard 0.002M silver nitrate solution. When one drop gave permanent turbidity, end point was reached.

Determination of trypsin inhibitor activity

Trypsin inhibitor activity was estimated by Kakade *et al* (1969). 0.2-1.0 ml aliquot, trypsin solution (0.05mg/ml in 0.001M HCl) were pipette in separate triplicate sets of test tubes and final volume adjusted to 1 ml and 2 ml phosphate buffer (0.1M, pH 7.6) for aliquot and trypsin solution respectively. 1 ml trypsin solution was added into aliquot tubes and kept onto water bath at 37°C. One of the triplicate tubes of aliquot and trypsin solution, 6 ml of 5%TCA was added, marked as blank and in others, 2ml of 2% casein solution added and then kept at 37°C for exactly 20 minutes. 6 ml of 5% TCA was added and absorbance was measured at 280nm after 1hour against blank using a UV visible Elico spectrophotometer. Plot the absorbance against the volume of extract. Trypsin unit (TU) is defining as an increase 0.01 absorbance units at 280nm in 20 minutes per 10 ml of reaction mixture. Trypsin inhibitor activity is defined as number of trypsin unit inhibits (TIU).

Product development

Conventional recipe such as- Dal-Sag (20%) was developed by using the raw and processed forms of kidney beans. Standard (SD) of dal-sag was made with the using of washed moong dal (40g), spinach (30g), onion (10g), tomato (12g) and vegetable oil (8ml) used for frying. Test sample of recipe was made with the incorporation of kidney beans in raw (DSA1) and processed form (DSA2) at 20% level, washed moong dal (20g), spinach (30g), onion (10g), tomato (12g) and vegetable oil (8ml). This is healthy and nutritious recipe of north Indian cuisine. They have good source of fiber, protein, mineral, iron, vitamin-B, vitamin E and low in fat content which is considered as beneficial in reducing high blood pressure, arteriosclerosis, stroke and heart attacks (Bassano *et al*, 2001). Fiber content in recipe keeps helping in gastrointestinal tract functioning normally and prevents some serious problems such as colon cancer, and hemorrhoid. This recipe can be an excellent way to promote good cardiac health.

Organoleptic evaluation

Evaluation of sensory characteristics was conducted to evaluate the acceptability of the recipe on the basis of 9 point hedonic method by 15 semi-trained panel members (Jellinek, 1985) and to get the most acceptable level from the recipe.

Statistical analysis

Data was analyzed for mean, standard deviation and t-test (Gupta, 2004).

Results and Discussion

Result of nutrient analyses revealed the moisture content in processed form (*BRKB)* of red kidney beans as 13.2±0.36 g/100g and was higher than the value found in raw sample (*RRKB)* i.e. 12.3±0.43 g/100g. During blanching treatment, moisture content was found to be increased significantly which was similar to other study done by Aremu *et al,* 2010. This increase (dry weight basis or fresh weight basis) in moisture content is related to augmented water content during overnight soaking due to hydrolytic enzymes (Osman, 2007). Ash content in raw sample (*RRKB*) of kidney beans was 3.6±0.36g/100g. After processing, the ash content decreased significantly in blanched sample (*BRKB*) of red kidney beans to 3.2±0.79g/100g. Processing treatment i.e. blanching is reported to decrease the ash content significantly may be due to leaching out of macro as well as micro elements in soaking water. A similar result was reported by another study

conducted by Kazanas and Fields, 1981. The value of fat content in *RRKB* sample was 1.5±0.2g/100g. The results registered non-significant variation in the value of fat content after blanching treatment *in BRKB* sample was 1.3±0.1 g/100g. The decrease in fat content in soaked-blanched beans vis-à-vis raw ones can be corroborated with the findings of other study done by Ramakrishna *et al*, 2006**.** The reduction of fat content was probably due to break-down of the triglyceride into simple form due to high lipolytic enzyme activity during processing **(**Idouraine *et al,* 1980**).** Kidney beans having low fat content as compared to soybean, render it is a good food ingredient for diabetic, obese and hyperlipidemic people who are more vulnerable to metabolic disorders and degenerative diseases. Soaking of seeds followed by dehulling decreased significantly the fiber content of processed sample (*BRKB***)** as 3.6±0.38 g/100g as compared to raw sample (*RRKB).* Fiber content of red kidney beans was found to be in same range as quoted by Granite *et al,* 2002**.** Since legumes are good source of protein and, in the same vein, red kidney beans protein content stood at 23.6±1.18g/100g in raw sample (*RRKB*). Even after blanching, the difference in the protein content of the variety became negligible, standing at 21.7±1.50g/100g. Protein content indicates that kidney bean is a good protein food. The similar observation was reported by Ofuya and Akhidue, 2005. The reduction of protein content after processing may be attributed due to the hydrolysis of protein into simpler compounds or leaching of soluble proteins into soaking water **(**Sharma *et al*, 2002)**.** The carbohydrate content in *RRKB* sample (56.7±3.61g/100g) has been lower, while after blanching, the value of carbohydrate was increased significantly in *BRKB* i.e. 62.7±5.0g/100g. The values of carbohydrate of kidney bean variety was similar when compared to other varieties of sesbania seeds and jack beans (Hossain and Becker, 2001**)** but the values are higher than those of soybean (26.3%) and cranberry beans (31.5%) and lower than lima bean (66.9%) and pigeon pea (66.8%) reported by Aremu *et al*, 2006. Since carbohydrate content of plant food is calculated by difference, decrease in dietary fiber, protein, fat and moisture content of kidney beans after blanching will ultimately affect the value for carbohydrate content. It was found that calcium content of raw sample (*RRKB*) of kidney beans was 221±58.28mg/100g. Mineral analyses of processed form (*BRKB*) of kidney beans revealed that calcium content increased significantly to 235.6±55.51mg/100g. The calcium content of red kidney beans makes the beans fairly good source of calcium, as compared to other varieties of legumes. The similar findings was obtained by Ghavidel and Prakash, 2007**,** who reported that calcium content was found to 220mg/100 g in dry sample of kidney beans. Iron content of raw sample (*RRKB*) of RKB was 5.3±0.31mg/100g. The iron content of red kidney beans was found to be similar as stated by Towo *et al,* 2003**.** After the application of processing treatments, the iron content was found to be increased significantly in RKB to 6.0±0.1 mg/100g. The results showed that phosphorus content of raw sample (*RRKB*) of red kidney beans was 408±4.35 mg/100g. After processing, the phosphorus content of the blanched sample (*BRKB*) of the red variety stood at 415±5.29 mg/100g. It has higher phosphorus content comparatively than other legume seeds. The results indicated that the mineral content increased possibly due to the fact that the outer covering that got removed in the processing action culminating, in low calcium, iron and phosphorus content. The most abundant mineral in the kidney beans was calcium and phosphorus. Concentrated values of calcium and phosphorus make beans were more suitable for bone, blood formation and supportive structure of the body for children **(**Ogunlade *et al*, 2005)**.** The red kidney bean variety could become good source for providing minerals and helpful in overcome nutritional deficiencies of calcium, iron and phosphorus. (Table-1)

Table 1. Mean values of nutrient composition of RRKB and BRKB

Nutrients (Content per 100g)	**RRKB**	**BRKB**
Moisture (g/100g)	12.3 ±0.43	13.2 ±0.36*
Ash (g/100g)	3.6 ±0.36	3.2 ± 0.79*
Protein (g/100g)	23.63 ± 1.18	21.7 ±1.50*
Fat (g/100g)	1.5 ±0.2	1.3 ± 0.1**
Fiber (g/100g)	4.0 ±0.34	3.6 ± 0.38*
Carbohydrate (g/100g)	56.7 ±3.61	62.7 ± 5.0*
Calcium (mg/100g)	5.3 ±0.31	6.0 ±0.1*
Iron (mg/100g)	221 ±58.28	235.6 ± 55.51*
Phosphorus (mg/100g)	408 ±4.35	415 ± 5.29*

*Significant, **Non-Significant

Table 2. Mean values of antioxidant and antinutrients in RRKB and BRKB

Antioxidant and Antinutrients	RRKB	BRKB
Phenols (mg/100g)	5.19 ±1.12	4.12 ±0.87*
Tannins (mg/100g)	4533 ±251	2833 ± 602*
Phytic acid (mg/100g)	543.0 ± 6.1	343.0 ±9.7*
Total Cyanogens (mg/100g)	0.04 ±0.002	0.03 ± 0.002**
Trypsin Inhibitor Activity (U/g)*	12.2 ±0.81	12.0± 0.79*

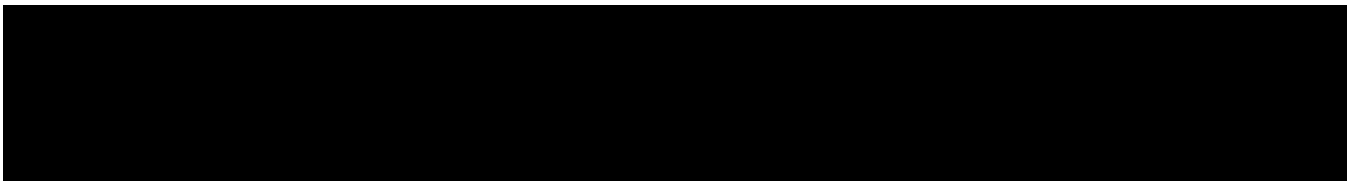
*One unit (U) of inhibitor activity is expressed as decrease by one unit of aborbance measured at 620nm in 20min.
*Significant, **Non-Significant

Antioxidant analysis of red kidney beans revealed that phenol content in *RRKB* stood at 5.19±1.12 mg/100g. The extractable total phenolic of raw kidney bean variety i. e. RKB has been found to be 5.19mg/100g. On blanching, it was seen that phenol content was decreased significantly in *BRKB* to 4.12±0.87 mg/100g which is validated from the study of Xu and Chan, 2009. Colored dry beans have an important source of antioxidants (Espinosa-Alonso *et al,* 2006). This decrease in phenols could result from the activation of polyphenol oxidase results of degradation and consequent loss of polyphenols during soaking prior to dehulling (Khandelwal *et al*, 2010). It was found that in *RRKB*, tannins content was 4533±251 mg/100g which was decreased significantly during blanching with the values coming down to 2833±602 mg/100g for *BRKB*. The processing treatment (hot-water blanching) significantly decreased tannins in kidney beans may be due to physical removal of seed coat of beans because most of the tannin is located in the testa of seeds (Reddy and Pierson, 1994). These results were in line with other study which stated that tannin content in black gram, red and white kidney beans were significantly reduced after soaking or other processing treatments (Rehman and Shah, 2005). Phytic acid content of *RRKB* was 543±6.1mg/100g. Phytic acid in the peas, cowpeas, lentil, kidney beans and chickpeas has been found to be 9.02, 6.83, 11.5, 10.99 and 8.40 mg/g respectively (Abd El-Hady and Habiba, 2003). After processing, the phytic acid decreased significantly in blanched sample of RKB to 343±9.7mg/100g. The results indicated that the reduction in the phytate content is due to their water- soluble property or leaching. This process also enhances the action of naturally occurring phytase in legumes (Kumar *et al*, 2010). Red kidney beans and pinto beans were soaked in distilled water for 18 hrs at room temperature reduced their phytate content by 51.7 and 52.7 % respectively (Akindahunsi, 2004). Total cyanogens content of raw sample of kidney beans viz. RKB was 0.04±0.002 mg/100g. In blanched samples it was found to be 0.03 ±.002 mg/100g for *BRKB*. Cyanogens content has found to be non-significantly reduced during processing i.e. blanching of red kidney beans. The trypsin inhibitor activity in raw kidney beans was found as 12.2±0.81 U/g for *RKB*. After the application of household processing i.e. blanching, the trypsin inhibitor activity was decreased significantly in *BRKB* to 12.0±0.79 U/g. Trypsin inhibitor activity in red kidney beans was found to be in agreement with previous data reported in another studies (Mejia *et al*, 2005). The trypsin inhibitor activity had significantly reduced (P>0.05) by various treatment methods, hot water blanching being the most effective. Similar observations were reported in *Dolichos lablab* bean (*Lablab purpureus* (*L*) *Sweet*), chickpea, winged beans, (Grewal and Jood, 2006) in green gram dal. It was observed that all antinutrients analyzed, i.e. tannins, phytic acid, total cyanogens and trypsin inhibitor activity, decreased during household processing treatment (*HWB*).(Table-2)

Organoleptic analysis

Dal-Sag was prepared by incorporating raw and blanched kidney bean flour in 20% proportion in both variants (*DSA1* and *DSA2*). Hedonic acceptability evaluation scores of *Dal-Sag* were shown in Table-3. From the scores of standard recipe and its variants for each attribute, it was clear that standard recipe was most acceptable with an overall acceptability score (8.1±0.99) and between both variants, DSA2 was more acceptable than DSA1 (was least acceptable) with an overall acceptability score 7.4±0.95 and 7.4±0.83 respectively. There was a negligible difference between the scores of VAR: DSA1 and VAR: DSA2. Appearance wise, standard stood out first followed by VAR: DSA1. Color of standard was best and VAR: DSA1 got higher mean scores than VAR: DSA2. The texture of VAR: DSA2 was good after standard but not much differ scores from VAR: DSA1. Taste wise, standard got first place. While, VAR: DSA2 was the second choice. Likeability of standard was best in terms of flavor too. VAR: DSA1 was best in flavor and got higher score among all variants. Mean score of after taste for standard was found high among all. VAR: DSA2 had much better mouth feel than other variants and also preferred by panel

Table 3. Mean Scores Obtained by Standardized Recipes Using Different Proportion of Red Kidney Beans in Raw and Processed forms



SD- Standard Dal Sag, DSA1 - Raw Sample, DSA2- Processed Sample

Table 4. Nutrient Composition of Dal-Sag prepared by incorporation of Kidney bean flour as per serving

Ingredients	Nutrient Content per serving (100g)										
Dal-Sag	**Energy**	**Protein**	**Fat**	**CHO**	**Fiber**	**Calcium**	**Iron**	**Phosphorus**	**β-Carotene**	**Folic acid**	**Vitamin-C**
MD (20g)	69.6	4.9	0.24	11.98	0.16	15	0.79	81	9.8	28	-
S (30g)	7.8	0.6	0.21	0.87	0.18	21.9	0.34	6.3	1674	36.9	8.4
ON (10g)	5.9	0.18	0.01	1.26	0.06	4	0.12	6	1.5	-	0.4
T (12g)	2.4	0.1	0.02	0.43	0.07	5.76	0.07	2.4	42.1	3.6	3.2
OIL (8g)	72	-	8	-	0.09	-	-	-	-	-	-
KBF (20g)	69.2	4.58	0.26	12.12	0.96	52	1.02	82	-	-	-
Total= 100g	226kcal	10.3g	8.74g	26.66g	1.52g	98.6mg	2.34mg	177mg	1727µg	68.5µg	12mg

As per values according to nutritive value of Indian foods given in ICMR *Gopalan et al, (2004)
MD- Moong dal (washed), S- Spinach, ON- Onion, T-Tomato, KBF- Kidney bean flour.

members. (Table- 3) The nutrient content of developed recipe- *Dal-Sag* was also calculated as per values given in the nutritive value of Indian foods by Gopalan *et al*, (2004) depicted in the following Table-4. This recipe was proving a good source of energy, protein, iron and calcium content.

Conclusion

With modernization, a large proportion of Asians are trading healthy traditional diets and improved nutritional patterns coupled with a decrease in cholesterol values. Hot water blanching has been pin-pointed very effective in reducing the estimated antinutrients. It also enhances the utilization of nutrients along with increasing the bioavailability of minerals after eliminating anti-nutrients. As the product developed- *Dal-Sag* has the potential to provide good nutrition with high protein, fiber and iron content and its acceptability ratings is also good, it is expected to fit the bill for a good food. Such product improvisation should work well in terms of satiating and nourishing masses at manageable price and availability.

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