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FORTIFICATION OF WHITE FLAT BREAD WITH SPROUTED RED KIDNEY BEAN (*PHASEOLUS VULGARIS*)

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ABSTRACT

Background. Protein quantity in diet including the digestibility and bioavailability of protein is of importance to eradicate undernutrition in developing countries. Bread protein is an incomplete source as it lacks an essential amino acid lysine. When they are combined with other plant foods like pulses and legumes, they become a complete source of protein. Since bread is most common staple food the objective of this study is to fortify bread with legumes in order to increase the total protein content of bread to 13-15% which is required to meet at least 1/3rd of protein requirement of an adult recommended daily allowance.
Material and methods. Fortification of flat bread was done by adding sprouted red kidney bean flour (*Phaseolus vulgaris*) at 5, 15 and 25% to white flour. The composite bread was analysed for crude protein and in vitro protein digestibility using the Kjeldahl and pepsin-pancreatin method.
Results. The protein content of raw beans showed trivial increase on soaking for 17 h and sprouting for 3 days. On the other hand, a remarkable increase was observed in protein digestibility i.e., 8% and 11% respectively. The protein content of control and composite breads increased gradually at 1% and protein digestibility decreased by 12% from control. This is due to the presence of dietary fibers which bind with protein and inhibit its digestibility.
Conclusion. The study infers that sprouting the beans for 72 h did not show any remarkable increase in protein content but a significant increase in invitro protein digestibility was observed. Overall, breads made using 15% legume flour was comparatively equal in protein content, with overall acceptable quality.

Key words: red kidney beans, sprouting, protein, in-vitro protein digestibility, flat bread

INTRODUCTION

Food technologists are constantly working forward for identifying the right food combinations which can satisfy consumers growing needs and demands for more natural and highly nutritious food at affordable cost [Siro et al. 2008]. In order to develop a food product which is highly nutritious with acceptable sensory characteristics provided it is not deviated from the original has been analysed by the ancestral researchers [Giannou et al. 2003].

Kidney beans (*Phaseolus vulgaris* L.) are one of the most widely cultivated and consumed food legumes and are a vital source of plant proteins, B-vitamins and minerals [Yasmin et al. 2008, Prodanov et al. 1997]. In addition, presence of complex carbohydrates and dietary fibre contributes to its low glycemic index, which has been proved to be associated to reduce the risk of heart disease, diabetes and obesity [Anderson et al. 2009]. In spite of its high nutritive

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value and health benefits, raw kidney beans contains large amount of anti-nutritional factors such as phytic acid, phytoheamagglutinins, trypsin inhibitors, saponins, and tannins which can affect absorption of protein, carbohydrates and certain minerals [Shimelis and Rakshit 2007, Weder et al. 1997]. Therefore, removal or inactivation of these anti-nutritional factors is imperative [Prodanov et al. 1997].

Traditional processing methods such as hydration, germination, thermal processing such as cooking, autoclaving and fermentation have been proved to be effective in eliminating the anti-nutritional factors present in the legumes [Khattab et al. 2009, Nergiz and Gokgoz 2007, Khalil 2001]. Out of which, soaking and germination has been acknowledged as a cost-effective technology and as it causes considerable changes in the nutritional and sensory characteristics of legumes [Vidal-Valverde et al. 2002, Chang and Harrold 1988, Martín-Cabrejas et al. 2008] which may be due to "breakdown of complex macromolecular structures of starch and proteins into reducing sugars and amino acids as a result of increased metabolic activity" as described by Mwikya et al. [2001]. Hence, sprouted legumes either by themselves or combined with cereal flours, can produce highly nutritious and digestible foods [Prodanov et al. 1997].

White bread contains 8 to 9% protein and lacks an essential amino acid called lysine [Anjum et al. 2005]. As this indicates as an issue an extensive work is being carried for the past few decades, to fortify white bread with high protein and high lysine materials [Deshpande et al. 1983]. According to the previous studies, cereal and legume proteins are nutritionally complementary to each other in terms of sulphur containing amino acids namely, methionine and lysine [Obatolu 2002]. At the same time, Bloksma and Bushuk [1988] reported that "Addition of wheat flour with non-wheat flours can cause dough and bread quality to diminish". It was also observed that loaf volume and other physical attributes of the bread such as crust and crumb texture would change undesirably in yeast-leavened composite breads [Fenn et al. 2010]. Further, a study by Abdelaal et al. [1993], conveyed that "in order to maintain acceptable bread structures and organoleptic properties, the maximum levels of non-wheat flours in the blends had to be restricted to 15-20%". Therefore, the most ideal way for supplementation of legume flour is un-leavened flat breads at a ratio of 15-20% [Abdelaal et al. 1993]. For instance, flat breads such as pizza, pita, and recently tortillas are turning out to be trendy among the countries like United States, Canada, Europe, Australia, and others which signifies its future prospects [Flat... 1996]. These breads do not need high quantity of gluten so supplementation can be done up to 25% without affecting its physical properties.

Considering all the above revealed evidences given, this study focuses to provide a wholesome food for people by developing an existing product, yeast leavened flat bread supplemented by the sprouted red kidney bean flour at 5, 15 and 25% respectively, so that the protein content per serving (100 g) would be at most to 13-15% without massive variation from the original product. 10 and 20% supplementation was skipped in this study due to time constrains.

MATERIAL AND METHODS

Materials

Dry red kidney beans (*Phaseolus vulgaris*), purchased from a local supermarket were screened and washed to remove any broken seeds, weed seeds and other foreign matters adhering on their surface for not more than 1 min [Mwikya et al. 2001].

Sprouting trays were purchased online. Chemicals required for the analysis were either previously available or prepared according to the usage. Some were purchased from Sigma chemicals. The white flour used in this study was commercially available strong white flour used for bread making. The protein and ash content was estimated to be 12.1 and 0.003 g respectively.

Sprouting

Germination procedure was carried out according to Vidal-Valverde et al. [2002] and good manufacturing practices (GMP) were followed to avoid the growth of *Staphylococcus aureus* and *Bacillus cereus* [Kimanya et al. 2003]. Initially, 3 kg of red kidney beans were weighed accurately and soaked into three large containers each containing 5000 ml water and 0.07% sodium hypochlorite solution (bleach), and left for 30 min at room temperature for surface disinfection. Some broken and floating legumes were removed at this stage and separated from rest of the beans. The hydrated beans were then removed and washed

thoroughly in running water for 1 min and soaked in tap water for 17 h (over-night). The following day, about 500 g of the soaked beans were shifted to cold storage room for freezing followed by lyophilization for further analysis. The remaining hydrated seeds were located in 6 trays by layering them one over the other above moist filter paper and cotton wool. The trays were then covered using perforated aluminium foil and left undisturbed for sprouting under normal room temperature. After 72 h, the sprouted seeds which were about 2" long were sorted and left in freezer bags for freeze-drying. Raw beans, which served as a control, were also freeze-dried without further processing.

Flour preparation. Raw, soaked and sprouted beans was freeze-dried and ground to pass through 310 µm sieve. The flour was subsequently stored in labelled freezer bags in room temperature for further analysis. The schematic representations of various processing and analysis of the samples was according to Sangronis et al. [2006], but the cooking method followed by this author, was not analysed in this study.

Blends

Whole, germinated red kidney bean flour was supplemented with strong white flour at three levels namely 5, 15 and 25% respectively. Our proportions was designed to achieve 15% of protein through one serving (100 g) of our formulation.

Test baking for flat bread. The test baking for flat bread was according to Abdelaal et al. [1993] with some modifications. Firstly, instead of adding all the ingredients at a time, dry and liquid ingredients was separated and added one by one in the Do-Corder, in order to achieve a homogenous blend without any lump formation. Secondly, the test baking was done at 220°C, for 15 mins instead of 325°C as, the maximum temperature setting of the Simon rotary oven was almost 220°C.

The baking formula is listed in Table 1. Dry ingredients was weighed accurately and pre-mixed in Do--Corder (Brabender) at 41 rpm for 30 s 100% water was equally divided between yeast and salt in order to prepare their respective solutions. The so-formed liquid ingredients was later added to the dry ingredients and mixed again in Brabender for 3 min at same

Table 1. Baking formula for flat bread preparation

Ingredient	Percentage (% flour basis)
Flour (strong white)	100
Water	50
Yeast (fresh)	2
Salt	2
Fat	4

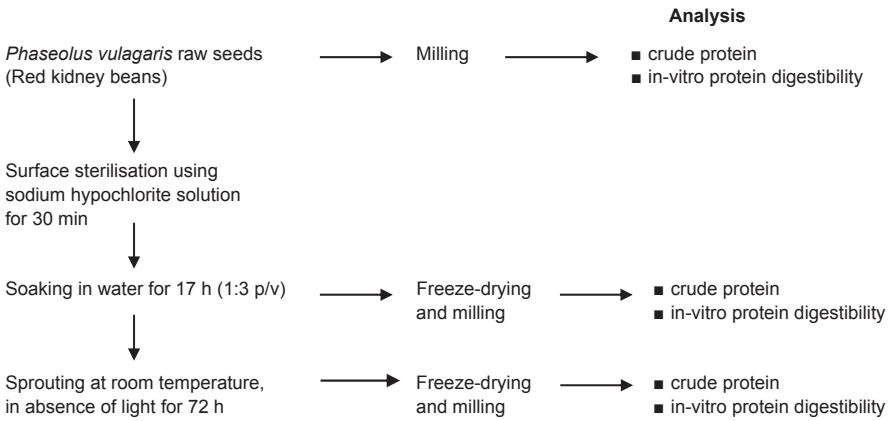

Fig. 1. Various processing steps and analysis carried out for the red kidney beans (*Phaseolus vulgaris*) are schematically represented in this figure. Figure modified from Sangronis et al. [2006], according to our study

speed. The obtained dough was weighed and approximately cut into 100 g pieces to get the triplicates. The cut pieces were then passed through dough moulder and flattened into 2 mm thickness followed by dough fermentation for 1 hr at 29°C and 85% relative humidity. The fermented flat dough pieces was punched in order to avoid air pockets forming during baking and the punched flat breads was again held in proofing cabinet for 15 min at 30°C and 85% relative humidity and baked for 15 min at 220°C in Simon rotary oven. All test bakes were performed in triplicate. The schematic representation of different processes involved at each stage of test baking is given in Figure 2.

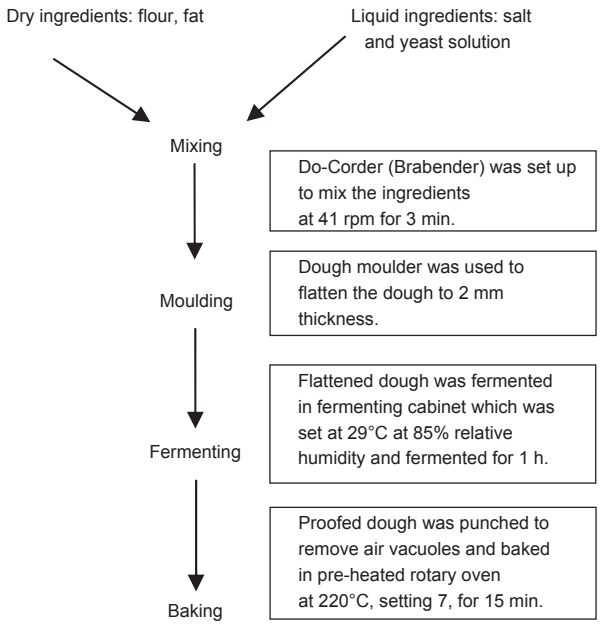

Fig. 2. Flat bread making procedure according to Abdelaal et al. [1993]. Fat was homogenised with the flour initially to prevent lump formation. Salt solution was added to yeast solution to prevent death of yeast

Methods of analysis

Analysis of samples was carried out according to the published standard methods with some modifications depending upon the lab requirements.

Determination of crude proteins. Crude protein contents was determined using the Kjeldahl method according to [Official... 1995] with slight alterations. Mercury catalyst was replaced by potassium sulphate and copper sulphate tablets.

About 2 g of dried sample was transferred into digestion tube containing 5 catalyst tablets. 25 ml of concentric sulfuric acid was added through a measuring cylinder with care. Digestion of the sample was conducted on an electro thermal mantle in the fume cupboard till a clear liquid was obtained. After that, the digest was allowed to cool and 400 ml of distilled water was added immediately. The content was shaken and carefully transferred to a distillation flask using a funnel. Then, 10 drops of phenolphthalein indicator and about 1 g of anti-bumping granules using powder funnel was added. The distillation apparatus was set up and the condensing delivery tube dipped into 400 ml boric acid solution containing one drop of methyl red indicator. Sodium hydroxide (40%) was added via dropping funnel until test solution became alkaline (purple in colour). The scaffolding was gently shaken to ensure uniform mixing, followed by heated the distillation flask at a constant rate until a minimum of 400 ml of distillate was collected. The obtained solution was then titrated with 0.25 M sulphuric acid to calculate the amount of ammonia collected in the boric acid solution. The obtained nitrogen value was multiplied by a conversion factor of 5.70 to obtain the percentage crude protein of flour and bread samples [Official... (190.87) 1995].

Determination of in-vitro protein digestibility (IVPD) by pepsin-pancreatin method. In-vitro protein digestibility was measured according to Saunders et al. [1973] with some adjustments. 250 mg of the sample was precisely weighed and placed into a 50-ml centrifuge tube. 15 ml of 0.1 N HCl containing 1.5 mg of pepsin, was added and the tube was incubated at 37°C for 3 h. The suspension was then neutralized with 3.3 ml of 0.5 M NaOH and treated with 4 mg of pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 7.9). The mixture was then gently shaken and incubated at 37°C for 24 h. After incubation, the sample was treated with 10 ml of 10% trichloroacetic acid and centrifuged at 14,000 rpm in Beckman centrifuge for 5 min at 11°C. The supernatant was discarded and the pellet was air dried over-night and used to analyse the indigested protein fragment. The octuplicate was combined together, in order to obtain ~2 g

of sample. The nitrogen in the indigested fragments was estimated using the Kjeldahl method as described before. The following calculation used for protein digestibility is as given below.

Digestibility was calculated using the formula:

$$\text{IVPD \%} = \frac{\text{total protein content} - \text{protein in pellet} \cdot 100}{\text{total protein content}}$$

The total protein content was obtained from the crude protein analysis.

Calculations and statistical analysis

Mean and standard deviations was calculated from the results of the analysis performed. The data was subjected to analysis of variance (ANOVA) and the comparison of means was performed using Tukey's Honesty Significant difference Test at significance $p \leq 0.05$ and standard deviation was calculated using spreadsheet. M.S. Excel and R script for Windows Vista computer software was used for analysing the data. All experiments were performed in triplicate.

RESULTS

One set of triplicate for each sample type was analysed for protein and in-vitro protein digestibility. The mean and standard deviation among the triplicates are given under Table 2. On observing the statistics, the variance was significant at 95% confidence interval. 1 outlier was observed in each case of white flour, soaked and sprouted kidney bean flour in crude protein analysis and in raw and soaked bean flour in case of protein digestibility which didn't show much higher significant difference. With low variances for all parameters, all the values were within the limits which indicated a high precision of the analysis performed.

Effects of soaking and sprouting on protein and IVPD of red kidney bean

In order to study the changes in protein and protein digestibility of raw kidney bean on soaking for 17 h and sprouting for 3 days without light and to determine the effectiveness of blending sprouted bean flour with white flour for making fat bread and the analsysis for the same was done using the standard official method for crude protein determination namely the Kjeldahl and pepsin-pancreatin index method for invitro protein digestibility. The conversion factor used for finding the crude protein was set to 5.70 for all type of samples.

Table 2. Effect of soaking and sprouting on protein and in-vitro protein digestibility of red kidney bean

Flour type	Protein content* g/100 g	In-vitro protein digestibility, %
White flour	9.62 ±0.57[a]	100
Raw red kidney bean flour	22.88 ±0.21[b]	52.03 ±1[a]
Soaked red kidney bean flour (17 h)	23.47 ±0.45[c]	55.22 ±1.21[b]
Sprouted red kidney bean flour (3 days)	23.48 ±0.37[c]	63.28 ±0.54[c]

*N × 5.70.
Values are mean ±SD. Means followed by different letters denoted in superscript within the same column are significantly different from each other ($p \leq 0.05$) except for the protein content of soaked and sprouted beans.

The obtained values are summarised under Table 1. From the table we can infer that the protein content of white flour was 9.62 g which is 13.26% lower than that of the raw kidney bean flour and the protein digestibility of white flour was not detectable due to small sample size obtained after invitro digestibility and centrifugation of the sample. Suprisingly, there was only a mere increment observed in protein content on sprouting the raw beans for 3 days and at the same time, there was no significant difference observed between soaked and sprouted kidney bean flour in terms of their protein content. The increment in protein digestibility of raw kidney bean on either soaking or sprouting was notable. Chiefly, the protein digestibility increased by 11.25% on sprouting the raw kidney beans for 3 days in absence of light. Soaking also showed some remarkable increase in digestibility by 3%, but significantly less 8% compared to the sprouted ones. The samples left for protein digestion for crude protein determination took a long time for digestion compared to digestion for the in vitro protein analysis. Overall, sprouting observed to increase the in vitro protein digestibility and not the protein content.

Quality control was taken into consideration while performing this analysis. Flat bread, prepared by

Table 3. Blending sprouted kidney bean flour with white flour to enhance protein content

Composite bread (white flour : soaked red kidney bean flour)	Protein content* g/100 g	In vitro protein digestibility %
100 : 0	10.11 ±0.23[a]	92.74 ±1.29[a]
95 : 5	11.26 ±0.17[b]	91.67 ±1.05[b]
85 : 15	13.26 ±0.19[c]	82.86 ±1.32[c]
75 : 25	14.85 ±0.22[d]	80.27 ±1.41[d]

*N × 5.70.
Values are mean ±SD. Means followed by different letters denoted in superscript within the same column are significantly different from each other ($p < 0.05$).

adding all the ingredients, including white flour apart from the sample served as an control for comparison with other composite breads. Triplicate copies for each bread type were analysed for protein and protein digestibility and the mean and standard deviations among the copies are given under Table 3. The statistical analysis showed the values are significant at 95% confidence interval. 1 outlier was observed in each of control, composite bread with 15 and 25% sprouted legume flour in terms of both protein and its digestibility analysis. However, the values was within the limits which again confirms that no significant problems occurred during the analytical procedures.

Composition of bread

The sprouted bean flour was supplemented with white flour at different ratios to analyse the increase in protein content and protein digestibility. The obatined results are listed in Table 2.

According to the table shown, we can perceive that the protein content has not shown much increase from control to the composite bread made with 5% sprouted bean flour. Notably a significant increase i.e., by 2% was observed with 15% flour composition. The protein digestibility of bread types has decreased gradually starting from the control to the composite bread with maximum bean flour. Particularly, a 10% decrease in protein digestibility was observed between control and 15% bean flour blend and the maximum decline in in-vitro protein digestibility i.e., 12% was observed with the composite bread containing maximum bean flour. When considering the protein content of white flour and comparing it with the control bread, we can infer that the protein availability has been increased on the sequence of baking process. Each sample varies from each other significantly at 0.05% confidence interval. The composite breads with 15 and 25% samples took an extended period of time for digestion for protein analysis. Whereas, the digestion for in vitro nitrogen analysis was quicker.

DISCUSSION

The crude protein and the protein digested by in-vitro method of our samples was analysed using the Kjeldahl method. This method was preferred over others as its the standard official method, which is applied internationally to determine protein content of foods [McGorrin 2009]. Its accuracy was proved in this study, when the raw kidney beans and white flour was analysed for crude protein it appeared to be in well coherence with Yasmin et al. [2008] and Ribotta et al. [2005] findings. One of the major drawbacks of this method is that it determines the total nitrogen of the sample in spite of true protein and the conversion factor varies based on the type of protein present in each food [Wang et al. 2010]. One of the possible errors occurred during the calculation, was the conversion factor for the legume flour was set to 5.70 instead of 6.25. This is predicted to cause, under-estimation of the actual values.

On soaking the raw beans for 17 h the proteins content have increased in trivial. This may be due to leaching loss of some soluble anti-nutritional factors and protein into the soaking water [Vidal-Valverde et al. 1994, Khalil 2001]. Sprouting of red kidney bean has been proved as an effective means of increasing the protein content [Sangronis et al. 2006]. But our result was in contrast, which was also observed by Mwikya et al. [2001]. This may be due to short period of germination. If the germination time was increased, increase in protein content could be observed [Khattak et al. 2008].

Protein digestibility of the food samples was determined by using in vitro method rather than in-vivo methods as they cannot be performed at short period of time and it is more expensive [Buchanan 1969]

and involves live animals for analysis, which cannot be performed in our laboratory. Moreover, the results of vitro studies correlates well with in-vivo method [Saunders et al. 1973], which makes it reliable. The digested protein was later determined by macro-Kjeldahl method instead of micro. This was due to lack of availability of micro-Kjeldahl apparatus. Moreover, a study conducted by Jung et al. [2003], showed that there was no significant difference between both methods.

The in-vitro protein digestibility of the sprouted beans was high compared to raw and soaked. This was also noted by Mwikya et al. [2001, Mubarak [2005]. This can be due to either leaching loss of the anti-nutritional factors or destruction of complex structures such as carbohydrates and fibre, which has exposed the proteins binding to it, eventually making it vulnerable to proteolytic activity [Sangronis et al. 2006].

The supplementation of sprouted bean flour at 5% did not show much variation from the standard bread, In terms of both nutrition and physical characteristics. This may be due to less composition of the sprouted bean flour [Abdelaal et al. 1993]. As the dilution increased from 15 to 25%, significant changes was observed in nutrition, physical and sensory characteristics of the product which varied much from the standard as described by Fenn et al. [2010]. Therefore, blending at 15% can be considered to be ideal as stated by Abdelaal et al. [1993]. Interestingly, the protein digestibility of flat bread decreased with increasing concentration of bean flour. This is because, addition of bean flour to the white flour, which contains complex macro-molecular structures such as dietary fibre and starch can inhibit protein absorption [Mwikya et al. 2001].

This study gives light for future research on the effect of dough rheology, bread texture and shelf-life stability with the same composite flour. This is because, the composite bread results in interesting crumb texture and showed to have longer shelf-life. From our results we conclude that composite bread made using 25% legume flour showed highest protein content but bread made using 15% legume flour was comparatively equal in protein content, with acceptable qualities.

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