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Effect of pretreatments to field bean for enhancement of protein content

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Abstract
The field bean is one of the common annual legume available across the tropics. This crop is underutilized in spite of its high nutritional value, ease of availability, and affordability. This study was done to substantiate that field bean can be a good source of nutrition. The study revealed that the pre-treatments like soaking, germination, and boiling had an effect on the nutritional quality of the field beans. The protein content of the raw, soaked, germinated and boiled field bean flour was 21.45%, 24.79%, 26.36% and 21.79%, respectively. Thus the germination treatment proved to be effective to increase the protein content of field beans. Protein was extracted from pre-treated field bean flour and maximum protein extraction yield was found for the proteins extracted from germinated field bean flour. The extraction yield of proteins extracted from raw, soaked, germinated, and boiled field bean flour were 31.61%, 33.02%, 34.33%, and 30.84% respectively. Thus, germination is proved to be best pre-treatment process for enhancement of amount and yield of protein amount. Likewise, colour attribute was also best for the germinated, raw and soaked field bean flour against boiled sample. Hence, germination can be considered as a better pretreatment process for extraction of proteins from field bean.

Keywords: Field bean, pretreatment, soaking, germination, protein, extraction

1. Introduction
Alternative food sources that are easily accessible, economical, and yet abundant in nutrients and energy are urgently needed in emerging and underdeveloped nations (Hossain *et al*., 2016) [8]. Legumes are significant providers of dietary fibre, minerals, carbs, and proteins. Very less number of the legume species that are known are widely utilized and endorsed. This plant's young pods and immature seeds are consumed as vegetables, while mature seeds are used as pulses. It is a good source of calories, dietary fibre, protein, and carbohydrates (Myrene, 2013) [10]. Despite having excellent nutritious value, the globe is seeing a decline in the use of legumes.

Field beans, often referred to as lablab beans or hyacinth beans, are common beans that are members of the Leguminosae (Fabaceae) family. They are grown for their edible beans across the tropics and originated in Africa (Ademola, Olaleye Abdul. Abioye, 2017) [1]. Field beans are mostly grown in the peninsular area of India, particularly in Maharashtra, Tamil Nadu, Karnataka, and Andhra Pradesh. In India, distinct varieties of hyacinth beans are produced in various regions under a variety of well-known regional names.

A little-known legume called the field bean has the potential to help emerging, protein-deficient countries. Field bean seeds that have been dried and immature pods both include approximately 4.5 and 25% protein, respectively (Myrene, 2013) [10]. The bean is a valuable source of carbohydrates, energy, and proteins according to studies on nutritional content. They go by the name "poor man's meat," that says it is in line with the idea that bean consumption and earnings are inversely related. The seeds of lablab are not much famous in spite of knowing the information that, even if it has the ability to offer a variety of nutritional supports and may thus be advantageous, its full potential is not being utilized in many underdeveloped regions of the world (Hossain *et al*., 2016) [8].

In light of this, our current study focused on a crop that has been underutilized but has the potential to be a valuable supplier of key nutrients. The aim of this study was to look at the nutritious value i.e., to clarify the proximate chemical composition of the pre-treated field bean flour as well as to study the impact of germination, soaking, and boiling on the proteins extracted from them.

2. Materials and Methods

The raw material i.e., the dried field beans required for this work were purchased from the local market of Thanjavur, Tamil Nadu. All the chemicals and reagents used for the analysis purpose were of analytical grade purchased from Thermo Fisher Scientific India Pvt. Ltd., HiMedia Laboratories Pvt. Ltd.

2.1 Preparation of flour from raw field beans

For the isolation of proteins from field beans, the field beans should be in the form of flour. The field beans were cleaned. The stones, chaff, and sticks were removed. The cleaned field beans were dried using a tray drier where the temperature was maintained at 45 ºC. The drying was continued till moisture content was obtained to a constant value. The dried raw beans were then converted into flour using pulverizer.

2.2 Preparation of flour from soaked field beans

The raw field beans were cleaned for chaffs and stones, and washed with clean water. The cleaned field beans were soaked in potable water for 12 hrs. After 12 hrs, the water was removed from the beans and beans were again washed with potable water. The soaked field beans were then kept for drying in tray drier at 45 ºC. The soaked field beans were dried for a certain time period and removed when it attained the constant weight. The dried beans were then converted into flour in pulverizer (Myrene, 2013) [10].

2.3 Preparation of flour from boiled field beans

For preparing the flour of boiled field beans, the dry field beans were first cleaned, and washed with potable water. The cleaned field beans were soaked for 3-4 hrs and the water was then changed for boiling. The field beans were boiled until the beans attained a mushy texture. The boiled beans were dried in a tray drier at 45 ºC till a constant reading was obtained for moisture content. The beans were then converted into flour using pulverizer (Myrene, 2013) [10].

2.4 Preparation of flour from germinated field beans

The method used for the preparation of germinated field bean flour was similar to that used for preparing flour from soaked field beans (section 2.2). The only step added in this was the germination of field beans. The soaked field beans were tied in a clean muslin cloth and kept for germination for 24 hrs. The germinated beans were dried in a tray drier at 45 ºC for constant weight and the dried beans were grinded into flour using pulverizer (Borijindakul & Phimolsiripol, 2013) [4].

2.5 Proximate analysis of pre-treated field bean flour

The obtained flour was analysed for protein, fat, carbohydrate, moisture and ash content according to the AOAC (Association of Official Analytical Chemists) methods (William, 2000) [17]. The moisture content was determined using the hot air oven method where the flour was kept inside a hot air oven and temperature was maintained at 105 ºC until constant mass, first reading of which was taken after 2 hrs. The fat content of field bean flour was estimated (before defatting) using Soxtron with hexane as the solvent. The protein content of field bean flour was estimated by the Kjeldahl method and the conversion factor is 6.25. The ash content was measured by calcination using muffle furnace at 650 ºC. The sample was kept in a desiccator to bring it to room temperature before taking the reading for moisture content and ash content so that it does not absorb moisture. The carbohydrate content was calculated by elimination method (Quintero-Quiroz *et al*., 2022) [12].

2.6 Defatting of pre-treated field bean flour

The fat content of the field bean flour was removed using the soxhlet method. The solvent used in this process was hexane. The field bean flour was packed inside filter paper and the solvent was added to it. The soxhlet assembly was run for 6 hrs and the field bean flour was completely defatted. All the four samples *viz*., raw, soaked, germinated, and boiled field bean flour were defatted in the same manner (Cao *et al*., 2021) [5].

2.7 Extraction of proteins from field bean flour

The proteins were extracted from the field bean flour using acid-alkali pH shift method (Figure 1). Briefly, 10.0 g of defatted field bean flour was dissolved in 100 mL of distilled water. The sample containing field bean flour and distilled water was adjusted to pH 11 using 1 N NaOH. This mixture was then stirred using magnetic stirrer for 2 hrs and then centrifuged in refrigerated centrifuge at 4 ºC for 20 min at 5000g. After extraction, the pH of the supernatant was adjusted to 4.5 so that the precipitation of proteins will occur. This mixture was put in a refrigerated centrifuge at 5000g for 20 min to collect the precipitate. The proteins i.e., the precipitate was then washed using distilled water. Finally, the field bean proteins were freeze dried and stored at 4 °C for further analysis (Hadnadjev *et al*., 2017) [7].

2.8 Extraction yield

The extraction level of field bean flour was measured using the Kjeldahl method according to the given following equation (Wang *et al*., 2020) [16]:

$$\text{Protein extraction level (\%)} = \frac{W1 \times C1}{W2 \times C2} \times 100$$

where W1 is the weight of field bean protein; W2 is the weight of field bean flour; C1 is the protein content of field bean; C2 is the field bean flour protein content

2.9 Protein content of the proteins extracted from pre-treated field bean flour

In the digestion tube of the Kjeldahl digestion unit, 0.1 g of the sample was taken and 6 g catalyst mixture was added, to which 10 mL of conc. H_2SO_4 was included. This combination was digested for 3 hrs. The flask was cooled to room temperature and then 30 mL distilled water was added. The digestion tube was transferred into the distillation apparatus. After distillation, four drops of methyl red indicator were added to the sample collected in conical flask. This was followed by the titration of the solution received in the conical flask with 0.1 M HCl (William, 2000) [17].

2.10 Colour

The method given by (Dabbour *et al*., 2018) [6] was used to check the colour of field bean flour by using the CIE L^* a^* b^*

colour system, where L* = lightness, –b* = blueness, +b* = yellowness, –a* = greenness, +a* = redness.

2.11 Statistical analysis
All the experimental data was obtained in triplicate. The results were expressed as mean±standard deviation. Statistical data was analysed using the Minitab 18 software following the analysis of variance (ANOVA) with significance tested at $p<0.05$.

3. Result and discussion
3.1 Proximate analysis
The proximate chemical composition of the pre-treated field bean flour is mentioned in the figure 2. The field bean flour contains noticeably higher values of proteins, carbohydrates, and low amount of fat, moisture.

The moisture content of the raw, soaked, germinated, and boiled field bean flour was 7.10%, 7.63%, 8.04%, and 101% respectively. This was found to fell between the range of moisture levels other legumes, which were found in other research to vary between 5.0% and 11.0% (Aremu *et al*., 2006) [2]. The seeds' detected moisture content is a sign that the microbes' activity will likely be decreased, potentially lengthening the flour's shelf life. With the applied treatments, the moisture content increased because legumes quickly absorb water from their environment to start their metabolic activities. The increased water intake over time is the result of the seed's growing number of hydrated cells (Myrene, 2013) [10]. Similar results were found for Bengal gram and green gram (Khatoon & Prakash, 2006) [9].

The fat content of the raw field bean flour was found to be the highest value of all (1.19%) while the boiled field bean flour had the lowest fat content (0.21%). This has the inverse relation with the moisture content. The sample with high fat content has the least moisture content and vice versa. Overall, the field bean flour has a low fat content. The field bean flour had a fat content which was found to be suggesting that consumption of this legume can also act as a source of oil (in context to essential fatty acids) for the weaker section of society who cannot afford other edible oil in their regular meals (Hossain *et al*., 2016) [8].

The total protein content of germinated field bean flour was highest (26.36%), followed by soaked (24.31%), raw (21.63%), and boiled (22.12%) field bean flour. The considerable rise in protein content found in germinated field bean flour is ascribed to altered hormonal activity (Nonogaki *et al*., 2010) [11], increased water activity as a consequence of hydrolytic enzyme induction (Bau *et al*., 1997) [3], or a compositional change as a result of the breakdown of other components. The field bean flour has a higher protein content in comparison with the other legume i.e., chickpea which is consumed usually (Khatoon & Prakash, 2006; Srivastava & Ali, 2004) [9, 12]. The high protein content of the field bean flour highlights how important it is as a source of this essential ingredient for addressing the protein deficiency among the undernourished population.

With the applied treatments, it was discovered that the total carbohydrate content decreased. The least carbohydrate content was found in the germinated field bean flour. There was a slight increase with other processing techniques. During the process of germination, the use of carbohydrates as a source of energy for the development of embryo may be responsible for this decrease (Vidal-Valverde *et al*., 2002) [15]. When the seeds develop and expand, there is the breakdown of the starch in the cotyledon into smaller molecules like fructose and glucose. They serve as source of energy for cell division (Nonogaki *et al*., 2010; Vidal-Valverde *et al*., 2002) [11, 15]. The activity of the enzyme β-amylase, which hydrolyzes starch into simple carbohydrates, was also elevated in a variety of germinating legumes (Suda *et al*., 1986) [14].

The field beans had an ash content ranged from 2.56%-3.32%. High amounts of macro- and micro-minerals are indicated by a high total ash content. With the given treatments the ash content was found to be decreased. This reduction in ash content may be due to the leaching out of macro and micro minerals into the water used during soaking and boiling (Myrene, 2013) [10].

3.2 Protein content of the extracted proteins
The protein content of the proteins extracted from the pre-treated field bean flour as well as the untreated field bean flour is mentioned in the figure 3. The proteins obtained from germinated field bean flour had highest protein content, followed by the proteins obtained from field beans that are soaked and boiled, respectively. It was seen that the pre-treatments also affected the protein content of the proteins extracted from the field beans when compared with the proteins obtained from the untreated field bean flour. The pre-treatments have increased the protein content of the proteins from the field beans.

3.3 Extraction yield
The extraction yield of the field bean proteins was influenced by the different pre-treatments like soaking, germination, and boiling. The extraction yield of all the samples is mentioned in figure 4. The germination pre-treatment has given the highest protein yield i.e., 34.33% after extraction, while the extraction yield of proteins from soaked field bean flour was greater than the proteins from raw field bean flour. The boiled field bean flour gave least protein extraction yield of 30.84%. The boiled field bean flour had the highest moisture content of all the samples which may have attributed to its low extraction yield.

3.4 Colour
The colour values of the proteins extracted from field bean flour which was given different pre-treatments like soaking, germination, boiling, and raw sample are mentioned in the table 1. The L* value ranges from 0 to 100 corresponding to black and white. Results showed that the proteins obtained from boiled field bean flour was dark amongst all. The L* value of the proteins from boiled field bean flour was significantly different from the other samples which remained the same. Colour value is important as may affect the colour the product in which these proteins can be used.

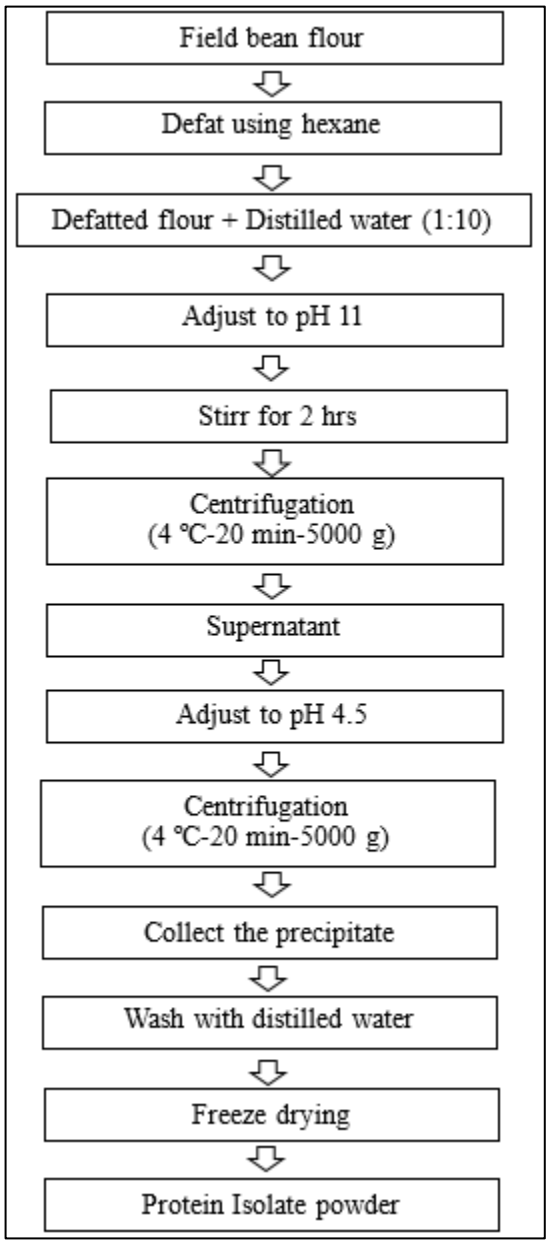

Fig 1: Preparation of protein isolate powder

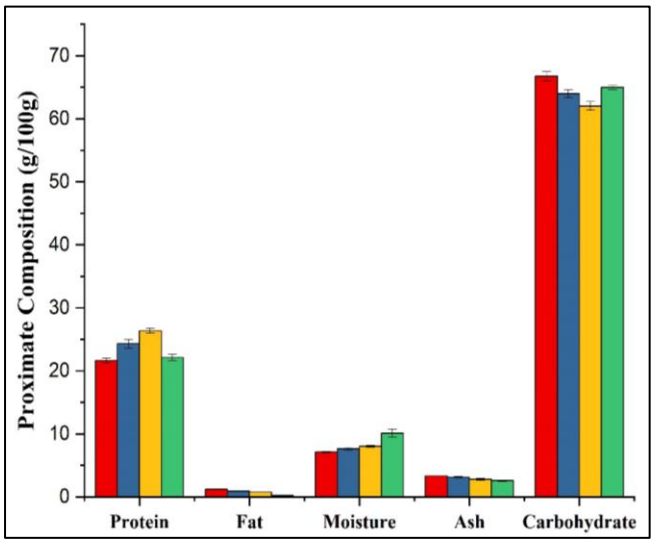

Fig 2: Proximate analysis of the field bean flour from ■ Raw ■ Soaked ■ Germinated ■ Boiled field beans

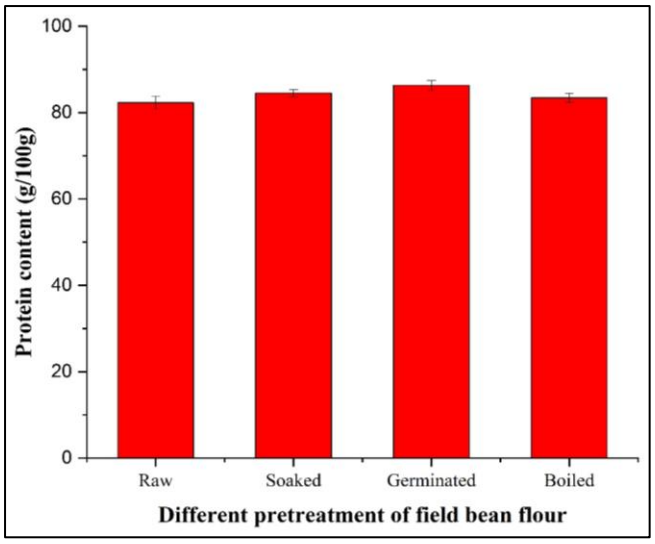

Fig 3: Protein content of the isolates extracted from field bean flour upon various treatments like untreated (raw), soaked, germinated, boiled field beans

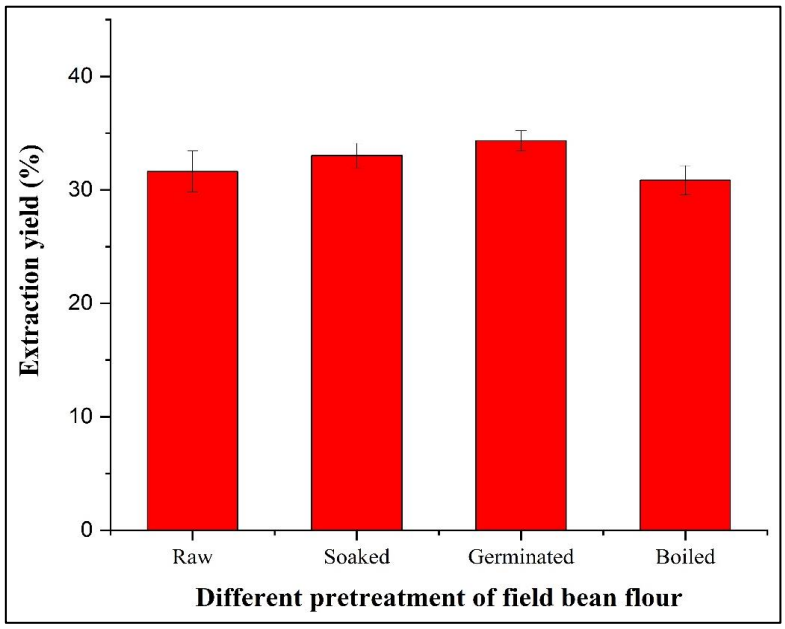

Fig 4: Extraction yield of the proteins from field bean flour upon various treatments like untreated (raw), soaked, germinated, boiled field beans

Table 1: Colour values of the protein isolate powder of field bean flour after various treatments like untreated (raw), soaked, germinated, boiled field beans

S. no.	Different pretreatment to field bean flour	L*	a*	b*
1	Raw	64.18±1.09[a]	2.35±0.48[a]	12.18±0.82[a]
2	Soaked	65.00±1.28[a]	3.22±0.54[a]	13.63±0.73[ab]
3	Germinated	64.95±0.23[a]	2.87±0.12[a]	12.44±0.41[b]
4	Boiled	61.57±0.88b[b]	3.34±0.53[a]	14.91±0.39[b]

4. Conclusion

It can be concluded from the results of proximate analysis that the germinated field beans are rich in proteins than the untreated (raw), soaked and boiled field beans. The high protein content of germinated field bean makes it suitable as a protein supplement and can be taken as a vegan protein source. The sample had low moisture and fat content, thus it can be stored for longer period of time. Since the proteins are extracted from legumes, i.e., a plant source, field bean proteins can be used as a vegan protein supplement.

5. Author contribution statement

Bhakti Anand Narale: Data collection, Review and research work, Analysis, Writing - original draft and Editing.
Akalya Shanmugam: Conceptualization, Reviewing, Editing and Supervision. Ashish Rawson: Reviewing.

5.1 Declaration of interest

The authors declare no conflict of interest.

6. Acknowledgement

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