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A Comparative study on the nutritional quality of horse gram and other selected pulses

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A B S T R A C T

Pulses are considered to be the prior source of protein, dietary fibre and energy vital for human health. The rationale of the present study is to analyze the nutrient profile of horse gram, an underutilized pulse, and to compare it with that of the very common pulses- green gram and cowpea. Biochemical attributes (total protein, total carbohydrate, reducing sugar and phenol) and enzymatic assays (amylase and invertase) of selected pulses- cow pea, green gram and horse gram during different soaking periods up to germination is determined. Nutritional properties of pulses are known to be affected by several factors and processes. Soaking caused significant increase in total protein in all the pulses studied with the highest amount in horse gram. Total carbohydrate content decreased and reducing sugar has increased with germination.SDS PAGE profiling of horse gram showed comparable protein bands with green gram and cowpea and the primary storage protein is found to be albumin in all the three pulses.

Keywords: Total protein, horse gram, soaking, germination, pulses, SDS PAGE.

A R T I C L E I N F O

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1. Introduction

Pulses are rich in protein and are the second important constituent of Indian diet after cereals. The crop legumes grounds for 27% of the world's primary agricultural crop production, with grain legumes bestowing 33% of daily

protein nitrogen intake of humans[1]. Pulses are the chief source of protein in the primarily vegetarian based Indian diet[2]. India accounts for 33% of the world area and 22% of the world production of pulses. The major pulse crops grown in India includes chickpea, pigeon pea, lentil, mungbean, urdbean field pea and many more [3].The Food and Agricultural Organization of the United Nations (FAO) define pulses as "annual leguminous crops yielding from one to 12 grains or seeds of variable size, shape and color within a pod"[4].

Pulses have a handful of attractive attributes as a food group. They are relatively low in energy density and are a good source of digestible protein. Other appealing features include high dietary fiber and low lipid content as well as presence of many biologically active compounds[5]. Pulse carbohydrates are slowly digested, which earmark some of the lowest glycemic index (GI) among carbohydrate-containing foods[6, 7]. This property of pulses has multiple positive implications in human health and nutrition. Considering the nutritional importance of pulses and the vast variety of leguminous crops available, their exploitation and potential use in our daily diet is very less. The current increased interest in utilization of legume grains is not only from the fact that they typically possess two to four times the quantity of protein than traditional cereal grains, but that their protein are surprisingly of higher nutritional quality[8,9].

Pulses are said to be especially nutritious when they are sprouted than dry grains. Germination improves the nutritive value of cereals and legumes by decrease the level of anti nutrients present in cereal and maximize the level of utilizable nutrients[10, 11]. Germination depends on various factors including temperature, moisture, light etc. Sprouting also makes pulses more digestible and rich in bioavailable phyto-chemicals as these are crucial for the growth of germinating plants. Soaking of pulses is believed to activate their enzyme system and influence the nutritional value[12].

In the current scenario of changed lifestyle where obesity, protein deficiency and malnutrition are increasing at an exponential rate, pulse production and consumption should be given more emphasis. And we utilize only a less percent of available pulses while a greater percent remain as underutilized. In view of this, current study is aimed to examine the nutritional properties of an underutilized pulse and to compare with two commonly used pulses in Indian diet. The pulses selected for the study are green gram (*Vigna radiata* (L.) Wilezek*)*, cowpea (*Vigna unguiculata subsps. Unguiculata* (L.) Walp.) and horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.).

In the present study the biochemical profile of the selected pulses were studied by quantifying total protein, total carbohydrate, reducing sugar and total phenol during different soaking periods. The enzymatic assay of amylase and invertase were performed with the selected pulses. SDS PAGE profiling of the selected pulses were carried out to compare the storage proteins present.

2. Materials and Methods

2.1 Collection of material

Dry seeds of green gram, cowpea and horse gram were purchased from grocery store in Alwaye, Ernakulam. They were thoroughly washed and 5 g of each pulse were soaked in distilled water. Seeds were collected for investigation at different soaking intervals viz., 1h, 6h, 12h, 18h and 24h.

2.2 Preparation of Extract

From each soaking period, samples of all the pulses collected and seed coat is removed.1g of tissue is measured and homogenized in 10ml of distilled water using a mortar and pestle. For enzymatic assays, the extracts were taken in appropriate buffers according to the procedure and kept in ice.

2.3 Biochemical Analysis

2.3.1 Quantification of total Carbohydrate

Total carbohydrate was quantified using conc. H_2SO_4 – UV spectrophotometer method[13]. The extracts were diluted by taking 0.5ml and making it to 20ml. An aliquot of 1ml is taken from this, 3ml conc. H2SO4 is added with rapid mixing. Keep it in ice bath for 2min and read the absorbance in a UV spectrophotometer at 315nm. Standard graph prepared.

2.3.2 Quantification of reducing sugar

Reducing sugar was determined according to the Dinitrosalicyclic acid method[14]. 3, 5 – dinitrosalicylic acid in alkaline medium is reduced to 3-amino 5- nitro salicylic acid by reducing sugars present in the sample. Absorbance was measured using a visible spectrophotometer at 540nm. Standard graph prepared using glucose.

2.3.3 Quantification of Total Protein

Total protein was quantified by using Lowry's protocol[15]. In this method the protein sample is allowed to react with Folin's reagent and copper sulphate to get a blue colored complex. The color is read in a spectrophotometer at 660nm. Standard graph prepared using bovine serum albumin as protein sample.

2.3.4 Estimation of Total Phenol

Total phenol was estimated using the Folin's Ciocalteau reagent. Phenols react with the phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce a blue colored complex. Its absorbance was measured against a reagent blank at 650nm in a visible spectrophotometer[16.]

2.4 Enzymatic Assays

2.4.1 Amylase assay

Amylase activity was assayed using starch as substrate and dinitrosalicyclic acid[17.] The reaction mixture was heated at boiling water bath for 5-10 minutes to develop the red-brown color. Add 8ml distilled water to the test tubes. After cooling to room temperature in a cold water bath, the absorbance was recorded against a reagent blank with a spectrophotometer at 540nm.

2.4.2 Invertase assay

The invertase assay was performed with Dinitrosalicyclic acid using sucrose as substrate[18]. Absorbance was measured at 650nm. Standaard graph prepared with sucrose as substrate.

2.5 SDS PAGE

SDS gel electrophoresis of seed storage proteins in cowpea, green bean and horse gram were performed using 5% stacking and 12.5% separating gels according to the method of Laemmli[19].

3. Results and Discussion

3.1 Total Carbohydrate: Starch is the main carbohydrate component of legume grains, with higher amylose content than amylopectin[20]. Total carbohydrate is found to be decreasing on soaking and germination in the pulses studied. Total carbohydrate content in horse gram is comparable with cowpea and green gram. Mobilization of carbohydrate in horse gram upon germination is observed to be lesser than that of other two. The total carbohydrate content in horse gram in 12h-24h duration decreased from 28.14 mg/g – 18.286mg/g, while that of cow pea and green gram decreased from approx 36mg/g to 10 mg/g.

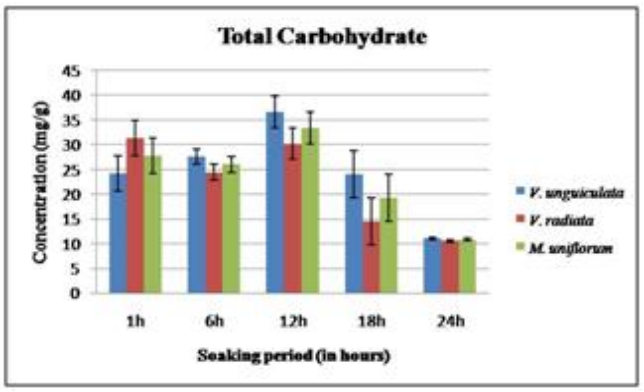

Figure 1: Total carbohydrate present in selected pulses.

3.2 Reducing Sugar: Reducing sugars are the main source of energy from food. Reducing sugar content has shown an increase with germination in selected pulses and the values were comparable in all the three pulses studied. This can be related with the decrease in total carbohydrate content upon germination. During the period of soaking reducing sugar content in all pulses were found to be less which suggest that the sugars are used up for growth and development. Upon germination reducing sugar content enhances indicating the mobilization of stored complex sugars. Enzymatic profile of amylase and invertase in this study is also in accordance with this data.

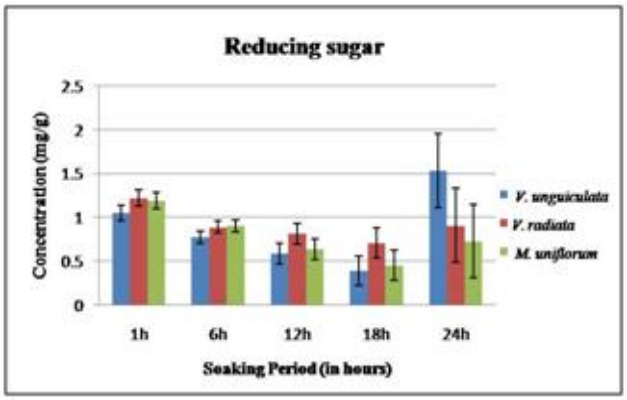

Figure 2: Reducing Sugar content in pulses studied.

3.3 Total Protein

Pulses are said to be the primary source of protein in a vegetarian diet[2]. The total protein content in horse gram, cowpea and green gram increases drastically during soaking and germination. The graph shows an exponential rate in protein content upon germination in all selected pulses. This data supports previous studies on protein content in pulses. An increase in total protein implies an increase in total available protein. Thus soaking improves the nutritional profile of pulses by increasing protein content and this rewards the use of pulses as functional food in daily diet.

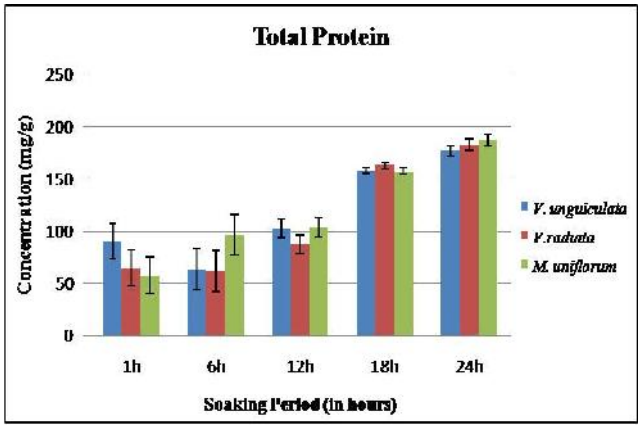

Figure 2: Total Protein Content in Pulses studied.

3.4 Amylase Activity

α-Amylase activity, as a function of germination time, is shown in graph. Amylase activity increased significantly in selected pulses on germination. In this study amylase activity was higher in horse gram. During soaking period the activity was around a constant value and increased after germination. Activity of amylase in previous studies of green gram and cowpea also supports this data[21]. The amylase activity can be correlated with the carbohydrate and reducing sugar amount observed in this study.

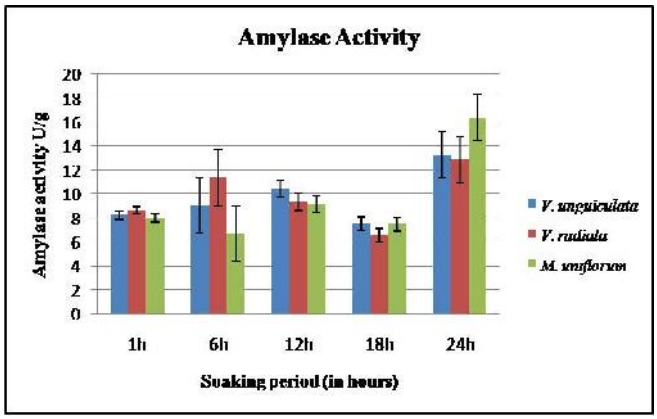

Figure 3: Amylase activity

3.5 Invertase Activity

The activity of invertase during soaking and germination is depicted in the graph. The invertase activity is comparatively less significant than amylase activity in this study. The invertase activity is found to have an increase

upon germination in cowpea and green gram. In horse gram there is no significant increase in invertase activity on soaking and germination.

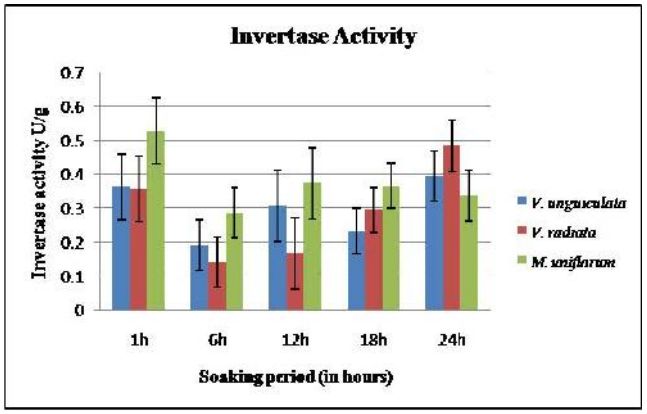

Figure 4: Invertase Activity

3.6 Total Phenol

The phenolic content in selected pulses during soaking and germination is studied. The total phenol content in horse gram is higher than other pulses in this study. The phenolic content in horse gram decreases after 1h soaking and maintains a constant rate till germination. During germination it decreases but not significantly. In this study green gram has lowest phenolic content. Phenolic compounds have both nutritional and anti nutritional aspects. The polyphenols in pulses give antioxidant properties[22]. On the negative aspect they hinder the availability of proteins and other nutritional factors in seeds.

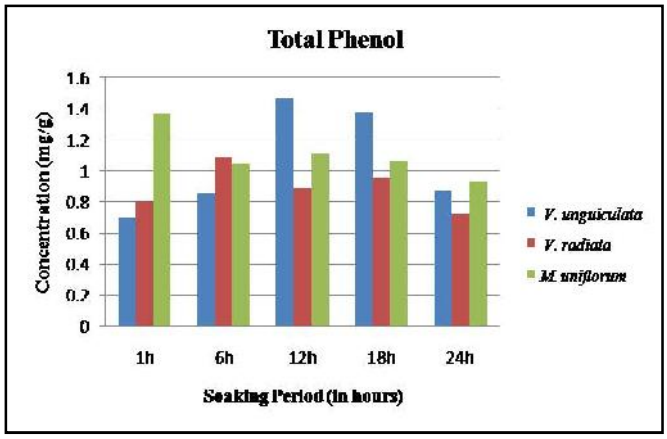

Figure 5: Total Phenol content in pulses studied.

3.7 SDS PAGE

SDS – PAGE profile of all the pulses studied showed sharp comparable bands in all soaking period. The predominant proteins in selected pulses resolved into two groups of bands 52 – 66kDa and 24 – 46kDa. This suggests that the predominant protein present in all the selected pulses is the same and show slight increase during soaking and germination. Previous studies have reported similar bands in horse gram23. In this study all the three pulses have prominent band at 66kDa which indicates albumin is one of the major seed storage proteins in pulses.

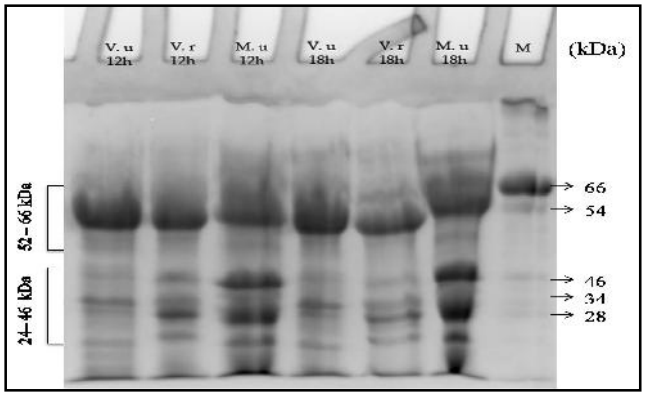

Figure 6: SDS PAGE profile of pulses studied at 12hours and 18 hours of soaking. (V.u-*Vigna unguiculata*, V.r-*Vigna radiata*, M.u-*Macrotyloma uniflorum*, M- protein marker.)

4. Conclusion

Grain legumes are predominant sources of food proteins and indispensable from human diet. Food proteins are not only a source of constructive and energetic compounds as the amino acids, but also they may play a bio-active role by themselves and/or can be the precursors of biologically active peptides with various physiological functions. From the above discussion it can be concluded that the studied pulses have similar nutritional profile and are extremely rich in proteins. Horse gram which is an underutilized pulse has comparable nutritional attributes similar to that of commonly used pulses cowpea and green gram. Soaking and germination has shown to improve the nutritional factors in selected pulses. SDS PAGE profiling of the selected pulses also depicted comparable bands in all the three pulses and albumin was found to be the major storage protein present in these pulses. This study clearly unveils the nutritional potential of the underutilized pulse horse gram which has shown to have high protein content than the other two pulses studied. Though the anti-nutrient factors in horse gram has to be further analyzed to get a solid depiction of the nutrient value, it is unquestionable that horse gram can be included in diet habits of individuals for better nutritional benefits.

5. Acknowledgement

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