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Full Length Research Paper

Amino Acids Profiling and Functional Properties of Non-Biofortified Hybrid and Biofortified Pearl Millet Varieties

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Abstract

Indian pearl millet varieties which include non-biofortified hybrid HHB-234, biofortified HHB-299 and HHB-311 were selected and subjected to some processing methods like the samples were dried and milled into fine flour for the study of Proximate composition like ash content, moisture content, crude fiber, carbohydrates, crude protein. Also, quantitative analysis of amino acids was done using HPLC which revealed that HHB-234 have (0.61%,0.64%,0.70%, 0.30%) Leucine, Isoleucine, valine, and Threonine. HHB-299 (0.44%.0.61%,0.09%,0.82%) Leucine, Isoleucine, valine and Threonine. HHB-311 (0.49%,0.44%,0.59%,0.32%) Leucine, Isoleucine, valine and Threonine. Result of the current study would help people to consume these pearl millet varieties to fulfill their daily amino acid requirements to a better extent.

Keywords: Functional Properties; Pearl Millet; Proximate Analysis; Amino Acid Profiling; Non-Biofortified

Introduction

Pearl Millet is one of the popular crops which is consumed throughout the Indian as well as African continents of the world. These are the cereal grains that are consumed by humans as well as domestic animals. The seed of pearl millet is small and round and belongs to the Poaceae family [1]. Pearl millet is strong to- ward weather change due to its inherent capacity for dry spells and high temperatures. Pearl millet has a high capacity to toler- ate the salt water and acid present in soils, and it is well adapted to surplus with low productivity [2]. Pearl Millet was shipped to the United States in 1860 and began breeding in 1936. Tift23A has developed a cytoplasmic male sterility (CMS) line at USDA-ARS in Tifton, Georgia, with the help of Glenn Burton, converting the production of pearl millet hybrids in India. Entering the 1960s, late in the United States. In Tifton, Georgia, USDA-ARS was used to create a predominant gene store, precocial, and other valuable records of pearl millet reproduction and genetics. The success of the pearl millet and double millet hybrid breeding package began in India in the 1960s. Proximate analysis revealed the presence of (01.4%,01.6%,01.4%in HHB-234, HHB-299, HHB-311) total ash, (4.87%,5.09%, 7.81%) crude fat, (22.8%,12.8%, 20.8%in HHB- 234, HHB-299, HHB-311) moisture, (02.80%, 01.20%,02.00% in-HHB-234, HHB-299, HHB-311) crude fiber,(70.00%, 73.37%, 68.46% HHB-234, HHB-299, HHB-311) carbohydrates, (12.39%,9.38%, 10.20%) HHB-234, HHB-299, HHB-311 crude protein. These pearl millet varieties also have better water and oil absorption capacity, foaming capacity, metal chelating activity, emulsion activity which are analyzed during their study. After the quantitative analysis we found that these pearl millet varieties contains a good amount of amino acids. After the consumption of certain amount of these varieties a person can fulfill their daily requirement of amino acids like Valine, Threonine, Isolucine, lucine.

Materials and Methods

Sample collection

The procurement and further processing of pearl millet, a total of three Pearl millet varieties, non-biofortified (HHB-234) and bio-for- tified hybrids, HHB-299 and HHB-311, are taken in this experiment. All three Pearl millet varieties (HHB-234, HHB-311, HHB-299) were purchased from the Bajra Section of CCS Haryana Agricultural Univer- sity, Hisar, India. We gathered each kind in weights ranging from 1 to 1.5 kg. The experiments were performed in the Department of Nutri- tion Biology, Central University of Haryana, Mahendergarh, Haryana,

India, and the Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management, Kundli, Sonepat, Haryana, India.

Sample preparation

After the collection, samples were prepared by washing with normal tap water followed by sun drying for two days; after sun drying the, 100gram of each sample was ground in fine flour and stored in a vacuum-tight container for experimental use, and the rest of the samples (pearl millet grains) was stored till further use.

Extract preparation

In a 50 ml test tube, one gram of the sample was taken, and 10 ml of methanol was added and kept in a shaking incubator for 5 hours at 37°C, and the extract was filtered after five hours of incuba- tion using a Whatman filter paper, and the filtrate was kept at -80°C for further use.

Chemical used

• Ethanol • Methanol • Ferrous chloride • Ferrozine • Ground nut oil

Instrument used

• UV-VIS Spectrophotometer • Hot air oven • Muffle furnace • Centrifuge • Shaking incubator • Weighing balance • -800°C Refrigerator • Homogenizer

Bulk density

10 ml measuring cylinder was taken and filled with the grains of pearl millet; after filling it once, to fill the empty space inside the cylinder, the bottom of the cylinder was repeatedly tapped. After tapping the cylinder again, it was filled up to the 10 ml mark. Then Bulk density (BD) of pearl millet was calculated according to the weight of sample in grams per unit volume of sample (g/ml). All the measurements were taken in triplicates [3].

Proximate analysis

The moisture content of pearl millet an empty glass dish was oven dried at 105 degrees Celsius for an hour, and was cooled using a desiccator, and weight (W1). The glass dishes were dried at 105°C for 1 hour before being cooled in a desiccator [3]. Then 5 grams of sample were weighed and poured into a dish. After six hours at 105 °C in a hot air oven, the dish was weighed after cooling in a desiccator. A formula and the same procedure to calculate moisture content were repeated for other samples:

$$\text{Moisture (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

As earlier stated, the content of ash was calculated as referred [4]. The crucibles were washed and oven dried at 105°C for 1 hour before being kept in a desiccator for cooling (W1). After that, 2 grams of dried material was placed in the wt. crucible, and new weight was calculated (W2). Contents of the crucible were then placed in the kiln for 1 hour at 250°C, followed by another 5 hours at 550°C to ensure full combustion. Following a desiccator's cool- ing process, the samples were weighed to determine their ultimate weight (W3). The amount of ash in a given volume can be calculated using the formula below:

$$\text{Ash content (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

Crude fiber

The crude fiber of pearl millet flour was done by [5], 200 mil- lilitres of 1.25% H_2SO_4 were used to treat 5 gram of sample. The mixture was then heated for 30 minutes, filtered under pressure, and the leftover material was rinsed with the aid of boiling water. After that, this leftover is cooked in 100 millilitres of 1.25% NaOH solution. The final residue was weighed after drying at 100°C and cooled in a desiccator (W1). The final residue that was obtained was then calcined for 5 hrs at 550°C in a furnace, transferred to a desicator, and weighed again (W2). This is how the given formula calculated the crude fiber:

$$\text{Crude fiber (\%)} = \frac{w2-w1}{weight\ of\ original\ sample}$$

Carbohydrate content

The total carbohydrate content is determined by;

% Total carbohydrate=100 − (% moisture + % total ash + % crude fat + % crude fiber + % crude protein)

Crude fat

The crude fat content was calculated using the AOAC method [4]. A five-gram sample was ground up and extracted in 100 millilitres of diethyl ether for a full day. The extract was filtered into an accurately weighed beaker (W1). Diethyl ether was added, and the volume was measured to be 100 ml. After six hours of stirring, W1 collects all the filtrate. The ether was concentrated in a water bath until dry, then dried at 40 to 60°C before being reweighed in the beaker (W2). The crude fat content is estimated to be:

$$Crude\ fiber(\%) = \frac{w2 - w1}{original\ weight\ of\ sample}$$

Crude protein content

As stated in AOAC (2005), the Kjeldahl method was utilised to calculate the protein content [6]. Weigh 2 grammes of the sample precisely and add it to a 250 ml digestion flask, 20 ml of concentrated H2So4, and one digester. After cooling and diluting with 250 ml of distilled water, the mixture was heated and brought to a boil (until a clear or transparent residue was achieved). It was then put in a 500 ml Kjeldahl flask and treated with 50 ml of a 40 percent NaOH solution before distillation. Then, pour 100 ml of 0.1 N HCl and 150 ml of distillate into a flask. Then, using methyl orange as an indicator, the material was titrated with 2.0 mol/L of NaOH. Yellow is used as the new color to indicate the endpoint. The nitrogen content percentage is determined as follows:

$$\frac{[(ml\ standard\ acid \times N\ of\ acid) - (ml\ blank \times N\ of\ base)] - (ml\ std\ base \times N\ of\ base)]}{Weight\ of\ sample\ (g)} \times 1.4007$$

According to Siroha., *et al.* [4], the percentage of crude protein was calculated by multiplying the nitrogen value by a constant 6.25. %; crude protein = Nitrogen in sample × 6.25.

Foaming capacity

For the approximate estimation of flour's foaming capacity (FC), the method was followed [7]. For twenty-three minutes, the selected samples (50 ml; 3% w/v) were thoroughly blended in distilled water using a homogenizer. The graduated cylinder was filled with the homogenizer beaker after it had been quickly rinsed with 10 ml of distilled water. I took notes on the volume before and after the whip. Because of ongoing birching, foaming capacity was measured as a volume raised (%).

Emulsion activity

The emulsification features were estimated using the method of [8]. Next, using a homogenizer set to a higher revolutions per minute (RPM), the sample flour was homogenised for 30 seconds in 50 millilitres of water. Then 25 ml of Groundnut oil was added, and the mixed sample was homogenized for 30 s once again. After that, add again 25 ml of groundnut oil, then homogenize the mixture for 90 s again. After that, the emulsion solution was divided equally between two distinct 50 ml centrifuge tubes and centrifuged once more for five minutes at 1100 g. Before centrifugation × 100, the volume of the emulsified layer was evenly separated and divided by the emulsion volume to estimate the emulsification activity.

Water and oil absorption capacity

The water and oil absorption capacity of pearl millet flour was estimated by using the procedure of Sathe., *et al*. [9], with numerous changes. 2 grams of each pearl millet flour (sample) was mixed with 20-20 ml of oil, and DW in centrifuge tubes. Then, the samples were kept steady at 30°C for 30 min then centrifuged at 3000 revolutions per minute (RPM) for 10 minutes. After that, it was taken out and measured using a measuring cylinder. Then, the mean of triplicate was reported.

Metal chelating activity

Dinis., *et al.* [10] reported a technique for determining the metal chelating activity of samples. The extract was 0.5 ml mixed with 1.6 ml of 80% methanol and 50 microliters of $FeCl_2$ (2 mM/l). Five minutes later, 100 microliters of five mM/l ferrozines were added, and the mixture was vortexed and shaken to start the reaction. For 10 minutes, the mixture was incubated at RT (25°C) on a Spectrophotometer, the solution's absorbance was measured at 562 nm. The extract's Fe2+ chelating activity was determined using the formula below:=

$$\text{Iron } (Fe^{2+}) \text{ chelating activity } (\%) = 1 - \frac{(Absorbance\ of\ samples)}{Absorbance\ of\ control} \times 100$$

Amino acids profiling

The amino acid composition was quantified and analyzed by using HPLC method. 100 grams of all three samples were subjected to amino acid profiling using high-performance liquid chromatography (HPLC) method (SGS, India). The amino acid profiles for the selected varieties were HHB-234 (0.61%,0.64%,0.70%, 0.30%) Leucine, Isoleucine, valine, and Threonine. HHB-299(0.44%.0.61%,0.09%,0.82%) Leucine, Isoleucine, valine and Threonine.HHB-311(0.49%,0.44%,0.59%,0.32%) Leucine, Isoleucine, valine and Threonine.

Results and Discussion Bulk density

The bulk density of pearl millet grains is shown in (Figure 1) HHB 234 showed the highest value (8.15g/10 ml), where- as

HHB299 and HHB311 had lower values with a difference of (0.35g/10ml and 0.4g/10ml). Bulk density reflects the load that can be supported when a sample is placed directly on top of an- other sample [11].

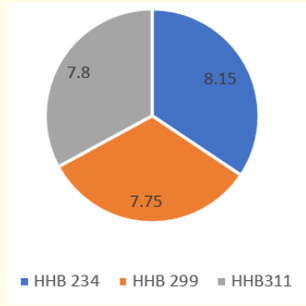


Figure 1: Bulk density of pearl millet (gm/10ml).

Proximate analysis

Table 3 shows the proximate composition of all three PM vari- ants. The carbohydrate and moisture content of PM cultivars were found to be in the ranges of 68.46 ± 0.51 to 73.37.40 ± 0.28% and 12.8.13 ± 0.22 to 20.8.54 ± 0.15%, respectively. The carbohydrate level of HHB 299 was the highest of the three varieties. Our samples had crude fiber and ash contents ranging from 1.2 ± 0.02 to 2.606 ± 0.02% and 1.4 ± 0.21 to 1.608 ± 0.60%, respectively. Our findings are consistent with those of Samtiya., *et al*. [1]; the HHB 234 type had the highest protein concentration, whereas HHB-299 had the lowest. The HHB-311 has the highest fat content of the three kinds. Corroborating our findings, Siroha and colleagues have demon- strated that various types of pearl millet found in India had fiber, ash, protein, and fat contents ranging from 2.9-3.8%, 1.65-1.90%, 9.7-11.3%, and 5.1-7.2%, respectively [4]. The aforementioned ranges indicate that pearl millet is a good source of protein and that, by producing various ready-to-cook/eat (RTC/RTE) items, it may help reduce protein deficiency.

Water absorption capacity

Water absorption capacity (WAC) [12], different pearl millet flours ranged from 4% to 7.5% (Table 3). The flour variants HHB 234 and HHB 311 showed higher WAC. Among these three variet- ies, HHB 299 has the lesser water-absorbing capacity, as shown in figure 3.

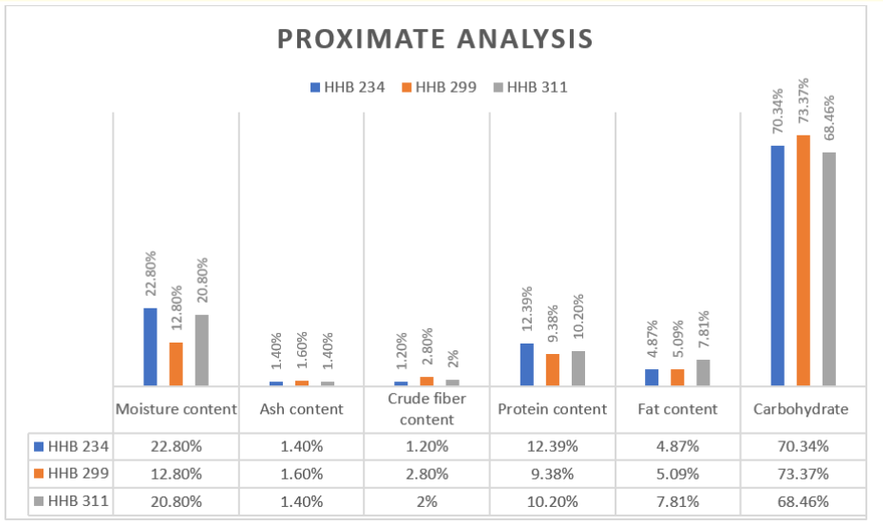


Figure 2: Proximate analysis.

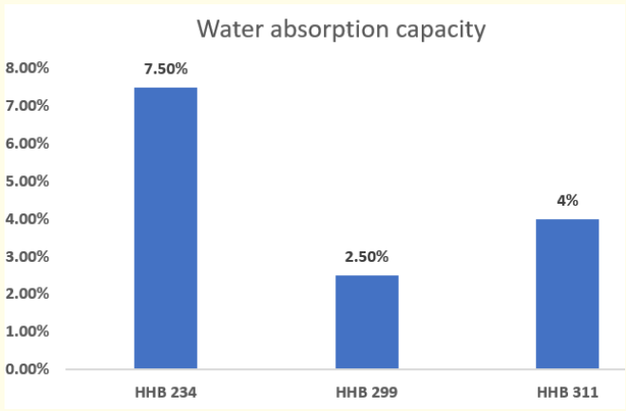


Figure 3: Water absorption capacity.

S. No	Name of Variety	Before whipping	After whipping	Foam
1.	HHB 234	50 ml	56 ml	6 ml
2.	HHB 299	50 ml	52 ml	2 ml
3.	HHB 311	50 ml	55 ml	4 ml

Table 1: Foaming capacity.

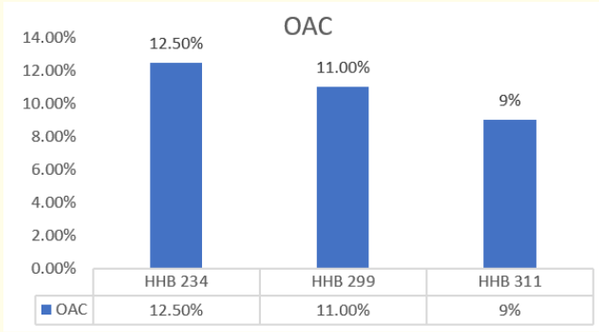


Figure 4: Oil absorption capacity.

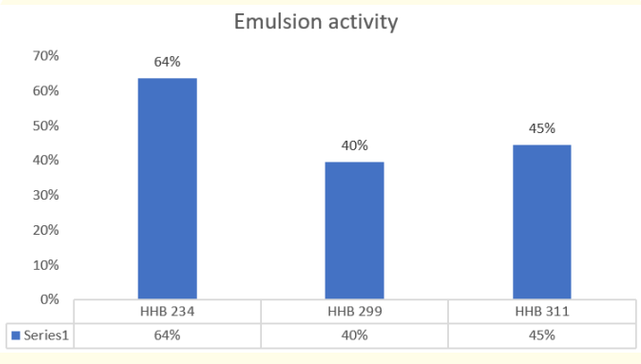


Figure 5: Emulsion activity.

Oil absorption capacity

For HHB 234 flour, the highest amount of oil absorption capacity (OAC) was observed, whereas the minimum oil absorption capacity was detected for HHB 299 and HHB-311 flours. Analyzing those flours with high (OAC) oil absorption capacity can help with 22 flavor retention, palatability, and shelf-life extension, especially in bread and meat products requiring fat absorption [13].

Foaming capacity pearl millet

The interfacial membrane generated by the protein influences foaming capability and stability. This makes the bubbles float and slowly retarded the rate of coal essence [14]. Flour foaming capacity ranged from 6 to 12%, highest in HHB-234 and lowest in HHB-299. Those fluctuations in foam volume may be due to dissimilarity in protein quality [15].

Emulsion activity

Emulsion activity (EA) [16], which measures a flour's ability to emulsify oil, varied considerably ($p > 0.05$) between flours and ranged from 60 to 70%. Compared to other flours, HHB-311 and HHB 234 have an excellent ability to emulsify the oil.

Metal chelating activity

HHB 234 has the highest absorbance, which is 63.6%, while HHB 299 and HHB311 (39.6 and 44.6) have low absorbance compared to HHB234 as shown in fig 6. HHB 234 has a high quantity of metal chelating activity in Pearl Millet flour, which could be attributed to heat-induced chemicals such as maillard reaction products, which could have AOA [17].

HHB 311

In this variety of pearl millet valine amino acid is present in the highest amount i.e. 590 mg/100gram as shown in table 4, whereas isoleucine and leucine is present 440 mg/100gram and 490 mg/100gram and threonine is present in the lowest amount i.e. 320 mg/100gram as compared with other amino acids of HHB 234 variety. At the time increases the retention time changes and many peaks are observed as shown in the chromatogram 3 give below. Valine and Leucine has a critical role in regulation of blood sugar. Increased dietary leucine has been found in animal studies to lower dietary adiposity, hyperglycemia, and cholesterol levels [22,23]. Evidence suggests that this amino acid might help prevent diabetes as well [24].

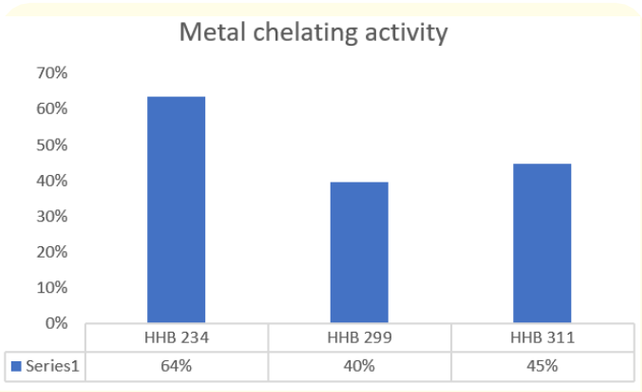


Figure 6: Metal chelating activity.

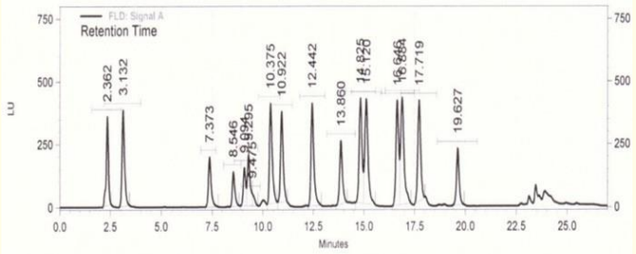
Amino acid profiling.

HHB 234

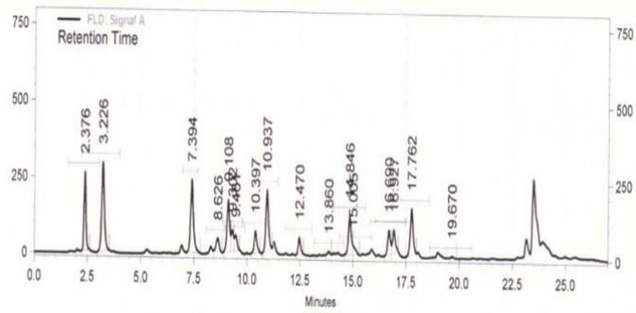
In this variety of pearl millet, valine was present in the highest amount i.e. 700mg/100gram, as shown in Table 4, whereas isoleucine and leucine were present at 640mg/100gram and610 mg/100gram, and threonine is present at in the lowest amount i.e. 310 mg/100gram as compared with other amino acids of HHB 234 variety. Valine is necessary for cerebral clarity, physical coordination, and emotional stability. Valine deficiency, valine metabolism problems, and Leucine and Isoleucine cause maple syrup urine disease. The disease is named after the fact that the urine of those infected smells like maple syrup. In many cases, valine insufficiency might affect the myelin sheath that protects nerves [18]. This disease can be cured naturally after consuming HHB 234 variety of pearl millet.

HHB 299

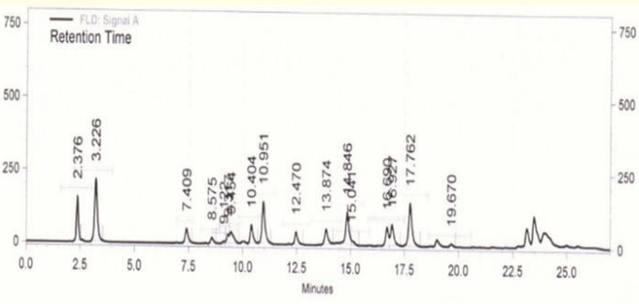
In this variety of pearl millet, threonine was present in the highest amount i.e. 820 mg/100gram as shown in table 4 whereas isoleucine and leucine are present 610 mg/100gram and 440 mg/100gram and valine is present in the lowest amount i.e. 90 mg/100gram as compared with other amino acids of HHB 234 variety. The central nervous system has large levels of threonine. The use of this amino acid in the treatment of Amyotrophic Lateral Sclerosis (Lou Gherigs Disease) has aroused the curiosity of researchers [19,20]. However, more research is required. In addition, a recent study has suggested that threonine may be useful in minimizing certain Multiple Sclerosis (MS) symptoms, such as spasticity [21].



Chromatogram 1



Chromatogram 2



Chromatogram 3

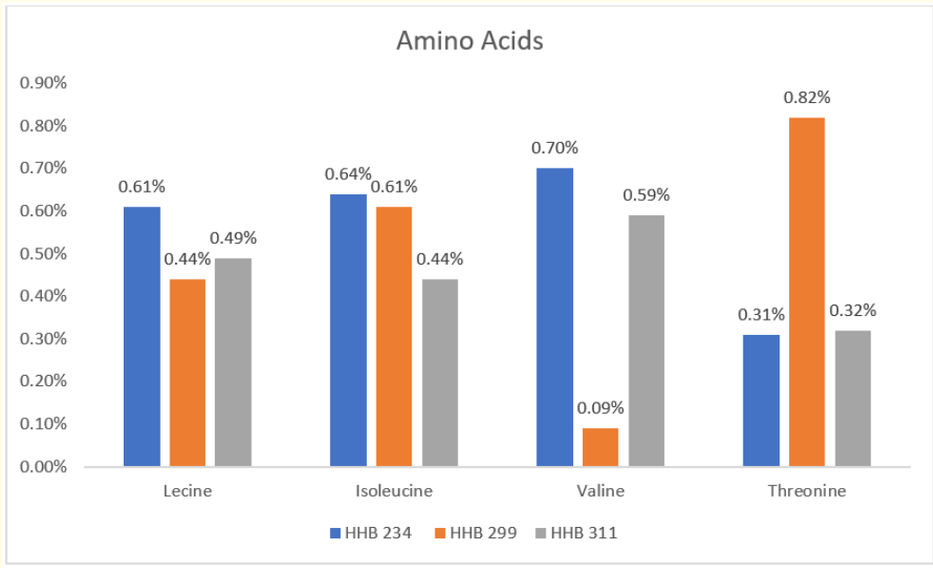


Figure 7: Amino acids comparison in Pearl millet varieties.

Conclusion

The Indian varieties of pearl millet had shown that all the three samples had a good proximate composition, Metal chelating activ- ity, Bulk density, WAC, OAC, Foaming capacity and emulsion activ- ity. The pearl millet are a good source of amino acid and different cultivars of pearl millet have different quantity of amino acid and the highest amount threonine that is 820mg/100gram present in HHB 299 and valine and isoleucine is 700 mg/100 g 640mg/100gr and HHB 311 had 440mg/100g. Therefor by checking the emul- sion activity of the samples, we came to know what is the rate of the phase separation in water and oil during the storage of the samples. Pearl millet is very reasonable for maximum of population and had a good amount of amino acids, and by consuming these pearl millet varieties on daily bases can cure many disease naturally which was caused due to lack of amino acid intake. As a result, more research on various amino acids is needed.

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Conflict of Interest

The authors have declared that they have no potential conflicts of interest.

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