Enhancement of nutritional value of finger millet-based food (Indian dosa) by co-fermentation with horse gram flour

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Abstract
Co-fermentation of finger millet with horse gram was carried out to produce inexpensive protein-rich food (dosa-an Indian breakfast food). Natural fermentation of finger millet–horse gram flour blend in different proportions (2:1, 3:1, 4:1 and 5:1) was performed for 24 h. Biochemical analysis showed a reasonable drop in pH (6.6–4.2) and starch content (25.52%) with considerable augment in titratable acidity (0.168–1.046%), soluble proteins (1.1-fold) and free amino acids (2.6-fold) at 16 h. Lactic acid bacteria dominated yeast counts throughout the fermentation accompanied by a decrease in total soluble and reducing sugars. Total essential amino acids increased 1.1-fold at 16-h fermentation with protein containing 48.68% of essential amino acids over total amino acids. Lysine increased from 5.87 to 6.73 g of amino acid/100 g of total amino acids. Dosa, prepared from 16-h fermented batter, showed better sensory attributes for 4:1 ratio. The formulated new product might be used to overcome the protein–energy malnutrition problems.

Keywords: finger millet, horse gram, co-fermentation, traditional fermented food, dosa

Introduction
Cereals such as wheat, rice, maize, sorghum and minor millets like pearl millet and finger millet are important sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for the people residing in Asian and African sub-continents (Blandino et al. 2003). However, they offer several challenges from the nutritional point of view, especially swelling of their starch on cooking and limited bioavailability of amino acids and minerals due to the presence of antinutritional compounds that tend to reduce bioavailability by 5–15% (Nout 2009). Several processing methods such as cooking, germination, milling and fermentation are routinely employed to improve the nutritional properties of cereals. Among these, fermentation has proved to be the best processing method (Blandino et al. 2003). Fermentation not only improves the sensory characteristics of a product, but also eliminates certain undesirable constituents, makes the nutrients more accessible while preserving and enhances the levels of many bioactive compounds (Hubert et al. 2008). Co-fermentation of cereals with legumes has often been proposed to produce inexpensive protein-rich foods for improving the macro- and micronutrient balance of cereal-based fermented food products. Generally, cereals are deficient in lysine, but are rich in cysteine and methionine, whereas legumes are rich in lysine, but deficient in sulphur-containing amino acids. Therefore, by combining cereals with legumes, the overall protein quality can be improved. The most popular traditionally consumed cereal–legume-based fermented foods in India include adai, dhokla, dosa, idli and vadai (Chavan and Kadam 1989).

Finger millet (Eleusine coracana) constitutes the staple food of people belonging to the low socio-economic group. It has a carbohydrate content of

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81.5%, protein 9.8%, crude fibre 4.3% and mineral 2.7%. Nowadays, it is recognized as nutraceuticals due to its high content of polyphenols (0.3–3%) and dietary fibre (19%), which offer health benefits such as hypoglycaemic, hypocholesterolaemic, anti-microbial and anti-oxidant properties. However, maximum utilization of the nutrient potential of the millet is limited by the presence of phytates (0.48%), tannins (0.61%) and enzyme inhibitors (Ravindran 1992). While phytates bind essential minerals and proteins, tannins complex with proteins and enzyme inhibitors reduce digestibility (Reddy and Pierson 1994) and fermentation enhances the biological value, net protein utilization, thiamin, riboflavin and niacin contents in finger millet (Aliya and Geervani 1981).

Auto-fermentation of finger millet significantly reduces the anti-nutritional components with simultaneous increase in mineral extractability, in vitro protein and starch digestibility. Traditionally, it is consumed in the form of thick porridge (mudde or dumpling), thin fermented porridge (ambali), fried or baked pancake (roti, dosa) and beverages (chang/jnard) (Mallesh and Hadimani 1993), and most of these are prepared by fermentation. Since it is consumed whole, fibre, minerals, phenolics and vitamins present in the outer layer of the grain or the seed coat are retained, which offer health benefits such as prevention of many chronic diseases (Antony et al. 1996).

However, studies on the co-fermentation of finger millet with legumes and their fermented food products are scarce. Hence, our present study mainly focuses and incorporates co-fermentation of finger millet with a cost-effective protein source such as horse gram (Macrotyloma uniflorum L.), which is also known as poor man’s pulse in Southern India. It is an excellent source of proteins (17.9–25.3%), carbohydrates (51.9–50.9%), essential amino acids, energy, low content of lipid (0.58–2.06%), iron and molybdenum (Bravo et al. 1999). It possesses hypoglycaemic and hypolipidaemic activity. Traditionally, it is consumed either as a whole seed, sprouts or cooked with rice, sorghum, pearl millet by soaking or dry heating the seeds. The use of dry seeds as human food in large population is limited due to its poor cooking quality, the presence of high levels of enzyme inhibitors and haemagglutinin activities (Ray 1969). Utilization can be made significant by implementation of economically viable diverse processing strategies. Although soaking, germination, dry heating, popping and microwave cooking methods have been adopted for processing horse gram, the extent of fermentation to which they enhance the nutritional values was not carried out till date. Fermentation may improve the texture and palatability, and inactivates the anti-nutritional factors in legume-based products.

Usually, idli and dosa products are based on rice-black gram blends; this study reports the alternative of this with finger millet–horse gram. This study has been carried out to determine the biochemical, nutritional and microbial population changes during co-fermentation of finger millet and horse gram flour along with the evaluation of sensory attributes of fermented food product (Indian dosa) prepared from the blended batter. This is expected to provide more insight on the co-fermentation of cereals with legumes, and improves the overall nutritional status of fermented foods. Additionally, it is envisaged that the development of a new product might prove to be useful to overcome the protein-energy malnutrition problems in developing countries.

Methods

Materials

Finger millet Co. (Ra)-14 brown variety was purchased in bulk from the Millet Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India, and horse gram was purchased from the local market as a single batch. The grains were cleaned, washed, dried and stored in airtight containers. All the chemicals and organic solvents used in this study were of analytical grade (Merck, Mumbai, India). The growth media for microbial analysis were procured from HiMedia laboratories, Mumbai, India.

Proximate analysis of the raw materials

The proximal analysis of finger millet and horse gram was determined. The moisture content was measured using Moisture Analyser MA30 (Sartorius AG, Goettingen, Germany), crude protein, crude fibre, crude fat and total ash content by AOAC method (2000), and carbohydrate content was determined as the weight difference (Total carbohydrate = 100 – (Moisture + crude protein + crude fibre + lipid + ash)). The energy value was determined by multiplying the percentage of crude protein, crude fat and carbohydrate by factors 4, 9 and 4, respectively, and the estimation was recorded as kcal/100 g (Gopalan et al. 2000).

Preparation of batter

The whole finger millet grains were milled in a flour miller (100 mesh size). Horse gram seeds were dehulled by soaking in water for 2 h followed by sun drying (after draining water). The dried seeds were dehulled in a traditional dehuller made of stone. Winnowing was done to separate the cotyledons from hulls and then milled in the flour miller. Further, the flour was mixed in different proportions viz. (A) 2:1, (B) 3:1, (C) 4:1 and (D) 5:1w/w (Finger millet: Horse gram), and batter (consistency of dosa batter) was prepared by mixing blended flour (100 g) of each with 120 ml of water and 0.9% w/w salt in a 500 ml beaker. The beakers were covered with perforated aluminium foil and fermented at 30°C for 24 h. For stimulating
household conditions of fermentation, neither the containers nor the grains were sterilized. Aliquots of the fermenting batter for analyses were drawn up to 24 h with 4 h intervals under aseptic conditions and stored at −70°C until analyses. Fresh batter samples were used for the microbial analysis, pH and titratable acidity. The fermentations were carried out in triplicates.

Analysis

Microbial analysis. The microbial analysis of blended batters was performed by determining the colony counts of aerobic mesophilic bacteria, lactic acid bacteria (LAB) and yeasts by pour plate technique on plate count agar, lactic agar and yeast glucose chloramphenicol agar, respectively. One gram of fresh fermented batter was homogenized with 9 ml of sterile saline solution to obtain a uniform suspension and plated in duplicates after serial dilution. Plate count agar plates were incubated at 37°C for 48 h, lactic agar plates at 30°C for 48 h and yeast glucose chloramphenicol agar plates at 30°C for 3 days. The colonies that appeared after the incubation time were counted as colony-forming units (cfu) per gram of dried batter. Characteristics of the colony were observed and representative single colonies were isolated, subcultured and stored at 4°C for further analysis.

pH and titratable acidity

The pH of the different sets of batter was measured directly at specific time intervals (4 h) by a digital pH meter (Elico LI617, India).

Titratable acidity of fermented batter was determined by titrating 5 g of fermented batter in 50 ml of distilled water against freshly prepared 0.1 N NaOH, using 1% phenolphthalein as an indicator. The values were expressed in g lactic acid equivalents/100 g of fermented batter.

Determination of total soluble, reducing and non-reducing sugars

About 100 mg of the fermented batter was extracted four times with 20 ml of 80% hot ethanol, pooled extracts were centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The supernatant was concentrated, and the volume was made up to 10 ml. Hundred microlitres of the aliquot was assayed for total soluble sugars by phenol–sulphuric acid method (Dubois et al. 1956), reducing sugars by Somogyi method (Somogyi 1952) and non-reducing sugars by calculating the difference between total soluble and reducing sugars. Glucose was used as a standard, and values were expressed in g/100 g of dried batter.

Determination of total starch content

The total starch was determined from alcohol insoluble residue of fermented batter based on the method described by McCreary et al. (1990). The residue was solubilized with 52% perchloric acid to estimate the sugar content by phenol–sulphuric acid method (Dubois et al. 1956), and values were multiplied by a factor of 0.9 to arrive at the starch content. The percentage decrease in starch content was calculated by the difference between initial and final content at different time intervals.

Determination of soluble proteins

Soluble proteins were determined by centrifuging the fermented slurry at 12,000 rpm for 15 min, and the supernatant was estimated for protein content by Folin–Ciocalteu reagent as per Lowry’s method (Lowry et al. 1951). Bovine serum albumin was used as a standard, and the values were expressed in mg protein/100 g of dried batter.

Determination of total free amino acids and total amino acids composition

Free amino acids were determined spectrophotometrically by reaction with ninhydrin in the ethanolic extract prepared as above (Magne and Larher 1992). A suitable standard curve was prepared with leucine, and values were expressed in g leucine equivalents/100 g of dried batter.

Determination of soluble proteins

Individual amino acid content in the fermented batter was analysed as per the protocol described by Mbithi-Mwikya et al. (2000) and Onyango et al. (2004) with some modification. Samples (100 mg) were hydrolyzed by 6 N HCL in sealed ampoules in an oven at 110°C for 23 h. The hydrolysate was rapidly cooled in an ice-water bath and then made up to 1 L and filtered. An aliquot of the filtrate was evaporated to dryness at 40°C in a rotavapour system. The residue was redissolved in 0.2 M sodium citrate buffer (pH 2.2), kept in a shaking water bath for 1 h and filtered through nylon syringe filter (0.2 μM). The filtrate was then injected into the automated amino acid analyser (Model 4151, Pharmacia LKB, Alpha Plus, England). The analyser is a single column ion-exchange chromatograph, which uses five lithium acetate buffer solutions of increasing pH and ionic strength, a regenerating agent and an ion-exchange column. Separated amino acids were detected by the ninhydrin colour reaction and photometric detection at 570 nm for α-amino acids and at 440 nm for the imino acids in a post-column derivatization step.

Batter volume

Twenty millilitres aliquot of batter from each proportion was placed in 100 ml measuring cylinders,
and rise in the volume during fermentation was noted at specific intervals (4 h) (Steinkraus et al. 1967). The increased volume was measured in millilitre, and results were expressed in % increase of batter volume.

Sensory analysis

Sensory evaluation of the dosa (pancake) prepared from each blend of 16 h fermented batter was carried out to determine their organoleptic characteristics. Dosa was prepared by cooking the thin spread batter (1–5 mm thickness) on a flat heated plate for 2–3 min smeared with little cooking oil. Each product was coded with a number, and was served to the panellists. A panel of 10 pre-trained judges comprising research scholars, postgraduate students and technical assistants of the laboratory was constituted to evaluate the products overall acceptability. To determine the acceptability of the samples, a seven-point hedonic rating scale, 7: excellent, 6: very good, 5: good, 4: satisfactory, 3: fair, 2: poor, 1: very poor, was used.

Statistical analysis

All the experiments were carried out in triplicates, and the results were represented as mean ± SD. Data were assessed by analysis of variance and the significant differences were found, the mean was separated by Duncan’s multiple range tests with a probability of \( p \leq 0.05 \) (Duncan 1955). This analysis was done using the SPSS 13.0 for Windows (2000) computer software.

### Results

#### Proximate composition

The proximate composition of raw materials (finger millets and horse gram) used in this work is given in Table I.

#### Microbial population

The microbial analysis of all the four batter samples revealed the presence of both bacteria and yeasts throughout the fermentation process (Table II). The total aerobic bacterial count in the four different batter combinations increased from 0 to 16 h, and thereafter counts stabilized with a slight decrease up to 24 h. In A (2:1) and C (4:1) samples, no yeast population was detected till 4 and 0 h, respectively, whereas in B (3:1) and D (5:1), yeast count increased from 0 to 20 h. The lactic acid bacterial count increased steadily from 0 to 24 h in all the blended batter samples with counts of 6.04–7.99 log cfu/g. Initially, LAB constituted 10, 27.8, 25 and 12.5% and gradually increased to 67.9, 55.2, 50.9 and 63.6% in A, B, C and D, respectively.

#### Biochemical changes occurring during fermentation

**pH and titratable acidity.** The fermentation of finger millet–horse gram flour blend resulted in considerable drop in pH with a corresponding increase in the titratable acidity in all four combinations. The initial of pH 6.6 dropped significantly (\( p < 0.05 \)) to 4.2 at the end of fermentation (24 h) (Figure 1(a)). The titratable acidity increased significantly (\( p < 0.05 \)) with the increase in fermentation time in all batter combinations from 0.17 to 1.05% (Figure 1(b)). There is no significant decrease and increase in the pH and titratable acidity between the batter combinations. However, the sample C (4:1) showed higher acid

### Table I. Proximate composition of finger millet and horse gram seeds (g/100 g).

<table>
<thead>
<tr>
<th>Nutrients†</th>
<th>Finger millet</th>
<th>Horse gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.46 ± 0.05</td>
<td>8.95 ± 0.03</td>
</tr>
<tr>
<td>Crude protein</td>
<td>7.2 ± 0.07</td>
<td>22.3 ± 1.30</td>
</tr>
<tr>
<td>Total ash</td>
<td>2.93 ± 0.03</td>
<td>3.79 ± 0.01</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>2.29 ± 0.02</td>
<td>1.88 ± 1.83</td>
</tr>
<tr>
<td>Fat</td>
<td>1.77 ± 0.12</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>77.35 ± 0.32</td>
<td>62.41 ± 1.52</td>
</tr>
<tr>
<td>Energy (kcal/100 g)</td>
<td>375.13 ± 1.23</td>
<td>304.03 ± 1.16</td>
</tr>
</tbody>
</table>

*The above values are mean ± SD of triplicate values.

### Table II. Microbial profile of finger millet–horse gram blended fermentation.

<table>
<thead>
<tr>
<th>Batter combination</th>
<th>Total aerobic bacteria (log cfu/g batter)</th>
<th>Lactic bacteria (log cfu/g batter)</th>
<th>Yeast (log cfu/g batter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Fermentation time (h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.04</td>
<td>7.29</td>
<td>7.12</td>
</tr>
<tr>
<td>4</td>
<td>7.38</td>
<td>7.60</td>
<td>7.76</td>
</tr>
<tr>
<td>8</td>
<td>7.94</td>
<td>8.02</td>
<td>7.99</td>
</tr>
<tr>
<td>12</td>
<td>8.21</td>
<td>8.24</td>
<td>8.00</td>
</tr>
<tr>
<td>16</td>
<td>8.23</td>
<td>8.36</td>
<td>8.32</td>
</tr>
<tr>
<td>20</td>
<td>8.20</td>
<td>8.19</td>
<td>8.24</td>
</tr>
<tr>
<td>24</td>
<td>8.15</td>
<td>8.20</td>
<td>8.23</td>
</tr>
</tbody>
</table>

Note: Batter combinations (Finger millet: Horse gram) – A (2:1); B (3:1); C (4:1) and D (5:1).
proteins from 0 to 12 h in all blended proportions (Figure 4(a)). However, after 12 h, the soluble protein content declined significantly \((p < 0.05)\). There is a significant \((p < 0.05)\) difference in the soluble protein content between the batter combinations (A, B, C and D). A significant change in total free amino acids was observed in all batter combinations (Figure 4(b)). In B and C, amino acids increased till 16 h, whereas in A and D, free amino acids increase was observed till 24 h fermentation. A high amount of free amino acids 1.1 g/100 g was observed in C (4:1) at 16-h fermentation.

Results on the total free amino acids indicated that the sample C (4:1 ratio blended batter) is found to be optimum for dosa preparation. Hence, further analysis on changes in the amino acids composition during fermentation was analysed at 8 h intervals with sample C. Amino acid profile and essential amino acid content of 1.05 g lactic acid equivalents/100 g at the end of fermentation as compared to other proportions.

**Total soluble, reducing and non-reducing sugars**

Co-fermentation of finger millet and horse gram resulted in significant \((p < 0.05)\) changes in the profile of available sugars between fermentation time and batter combinations. The total soluble sugar, reducing sugar and non-reducing sugars increased substantially at 4 h followed by a significant \((p < 0.05)\) decrease up to 20 h, which was further stabilized insignificantly at 24 h in all the four batter combinations (Figure 2).

**Starch content**

Starch content of the batter decreased significantly \((P < 0.05)\) from 0 to 24 h fermentation in all combinations (Figure 3). Sample D (5:1) showed higher decrease in the starch content (25.52%) followed by B (23.93%), C (23.26%) and A (21.30%) at the end of fermentation.

**Soluble proteins, total free amino acids and amino acid profile during fermentation of batter**

Finger millet–horse gram batter fermentation led to a significant \((p < 0.05)\) increase in soluble fraction of proteins from 0 to 12 h in all blended proportions (Figure 4(a)). However, after 12 h, the soluble protein content declined significantly \((p < 0.05)\). There is a significant \((p < 0.05)\) difference in the soluble protein content between the batter combinations (A, B, C and D). A significant change in total free amino acids was observed in all batter combinations (Figure 4(b)). In B and C, amino acids increased till 16 h, whereas in A and D, free amino acids increase was observed till 24 h fermentation. A high amount of free amino acids 1.1 g/100 g was observed in C (4:1) at 16-h fermentation.

Results on the total free amino acids indicated that the sample C (4:1 ratio blended batter) is found to be optimum for dosa preparation. Hence, further analysis on changes in the amino acids composition during fermentation was analysed at 8 h intervals with sample C. Amino acid profile and essential amino acid

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**Figure 1.** (a) pH and (b) titratable acidity profile of co-fermenting finger millet and horse gram. Batter combinations (Finger millet: Horse gram) – A (2:1); B (3:1); C (4:1) and D (5:1). Each values represent mean ± SE of the mean \((n = 3)\).

**Figure 2.** Effect of co-fermentation on sugar content in finger millet–horse gram blend. (a) Total soluble sugars; (b) reducing sugars and (c) non-reducing sugars. Batter combinations (Finger millet: Horse gram) – A (2:1); B (3:1); C (4:1) and D (5:1). Results are mean of three determinations ± SD. Bars having different letters a–f (within fermentation time) and w–z (within batter combination) in particular sugar content are significantly different at \(p < 0.05\).
chemical scores calculated from relative amino acid amount in sample/amino acid content in FAO (1991) reference protein are presented in Table III. Fermentation resulted in an increase in most of the individual amino acids, while a decrease was also observed in some amino acids. Total essential amino acids increased from 48.25 to 48.73 g/100 g total amino acids at the end of fermentation. Among the essential amino acids, lysine increased significantly from 5.87 to 6.73 g of amino acid/100 g of total amino acids. Threonine, cysteine and isoleucine increased notably, whereas other essential amino acid contents are more or less stabilized or decreased. In case of non-essential amino acids, serine and arginine increased significantly with a slight increase in alanine and other amino acids decreased drastically. Essential amino acid score of the batter was higher than the FAO amino acid scoring pattern for all amino acids and scored higher than 1.

Batter volume (%)

Fermentation of batter invariably leads to the increased volume. As observed, there was no apparent increase in batter volume in all proportions till 4 h of fermentation. After 4 h, there was a little increase till 12 h. At the end of 16 h, batter volume increased around 50% in 3:1, 4:1 and 5:1 proportions but there was only 12.7% increase in 2:1 proportion. After that, the batter volume started to decline in all blended proportions, and a foul smell was noted on prolonged fermentation above 24 h.

Sensory analysis

The scores of the sensory evaluation of dosa prepared from different ratio blends of finger millet and horse gram flour are given in Table IV. Sixteen hours fermented batter was considered for dosa preparation due to the sour aroma development and increased batter volume. The consumer acceptability of the dosa produced from the different blends showed that all the samples were rated above average. However, the sample C had the highest preference rating followed by D, B and A. Appearance, texture and colour of the product prepared from the four different proportions scored more or less equal but their score values differed in rating of taste, flavour and mouth feel.

Discussion

The proximate composition of finger millet and horse gram used in this study is comparable to the already reported varieties. Crude fibre of finger millet is distinctly higher than that of wheat and rice (Sripriya et al. 1997), and the fat content of horse gram was found to be low.

From the above results, it can be seen that LAB and yeast play a significant role throughout the fermentation process of finger millet–horse gram blend. Microbiological studies of the batter showed the predominance of LAB, which is on par with the previous report of Chavan and Kadam (1989) on cereal fermentation. The low yeast count indicates that the fermentation was non-alcoholic and bubbles formed during the fermentation of batter indicated heterolactic fermentation. According to Steinkraus et al. (1967), initial bacterial counts in rice–black gram mixture ranged from $10^3$ to $10^5$ cfu g$^{-1}$, rising to $10^8$–$10^9$ cfu g$^{-1}$ after 20–24 h of fermentation.

Figure 3. Decrease in starch content during co-fermentation of finger millet–horse gram blend. Batter combinations (Finger millet: Horse gram) – A (2:1); B (3:1); C (4:1) and D (5:1). Values are mean ± SE of three independent determinations.

Figure 4. Changes in soluble proteins (a) and total free amino acids (b) during co-fermentation of finger millet–horse gram flour. Batter combinations (Finger millet: Horse gram) – A (2:1); B (3:1); C (4:1) and D (5:1). Each values represent mean ± SE of the mean ($n = 3$). Bars having different letters a–f (within fermentation time) and w–z (within batter combination) in soluble protein content are significantly different at $p < 0.05$.
Table III. Amino acid profile and amino acid chemical score of finger millet–horse gram co-fermented batter (C (4:1 ratio blended)).

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Fermented batter</th>
<th>Essential amino acids</th>
<th>FAO ref protein†</th>
<th>0h</th>
<th>8h</th>
<th>16h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>8h</td>
<td>16h</td>
<td>24h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>4.27</td>
<td>4.29</td>
<td>4.33</td>
<td>4.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>5.97</td>
<td>5.94</td>
<td>5.85</td>
<td>5.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.92</td>
<td>1.9</td>
<td>1.93</td>
<td>1.95</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Methionine</td>
<td>2.78</td>
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<td>2.72</td>
<td>2.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.21</td>
<td>4.25</td>
<td>4.37</td>
<td>4.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>9.82</td>
<td>9.76</td>
<td>9.67</td>
<td>9.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.47</td>
<td>5.42</td>
<td>5.39</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>2.57</td>
<td>2.56</td>
<td>2.56</td>
<td>2.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>5.87</td>
<td>6.23</td>
<td>6.66</td>
<td>6.73</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tryptophan</td>
<td>1.23</td>
<td>1.12</td>
<td>1.08</td>
<td>1.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total essential amino acids§</td>
<td>48.25</td>
<td>48.29</td>
<td>48.68</td>
<td>48.73</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Aspartic acid</td>
<td>7.15</td>
<td>6.89</td>
<td>5.92</td>
<td>4.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>5.64</td>
<td>5.87</td>
<td>5.96</td>
<td>6.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>22.59</td>
<td>20.45</td>
<td>19.78</td>
<td>17.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>5.78</td>
<td>5.45</td>
<td>4.23</td>
<td>4.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>5.80</td>
<td>5.80</td>
<td>5.82</td>
<td>5.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>4.82</td>
<td>4.89</td>
<td>5.12</td>
<td>5.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total amino acids‡</td>
<td>11.42</td>
<td>11.41</td>
<td>11.46</td>
<td>11.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Amino acid composition expressed in g of amino acid/100 g of total amino acids; † Amino acid chemical scores calculated from relative amino acid amounts in sample/amino acid content in FAO reference protein; ‡ FAO reference protein pattern for preschool-age child (2–5 years) (FAO 1991); †† Pairs of amino acids considered physiologically substitute to one another (FAO 1991); § Total of essential amino acids (g/100 g of total amino acids); ‖ Total amino acids in g amino acids/100 g of dry matter calculated from protein content.
Acidification and leavening are the two important changes that occur due to microbial activities in the *idli* and *dosa* batters, which are involved in the production of acid and gas from various carbohydrates. Interactions between yeasts and lactobacilli are vital for the metabolic activity of the batter. The acid and gas required for leavening are produced exclusively by heterofermentative LAB such as *Leucconostoc mesenteroides*, *Streptococcus faecalis* and *Pediococcus cerevisiae* (Steinkraus et al. 1967). During *dosa* batter fermentation, *L. mesenteroides*, *S. faecalis*, *Lactococcus fermentum* and *Bacillus amylovorans* are the predominant bacteria that are responsible for souring and leavening. In addition, yeasts such as *Saccharomyces cerevisiae*, *Debaryomyces Hansenii* and *Trichosporon beigelli* produced flavour along with the help that enzyme in the saccharification of starch (Son et al. 1986). In this study, 10 different predominant lactic cultures were found which are gram-positive cocci and rods and all were catalase negative. Three strains were identified by 16S rRNA sequencing as *Enterococcus durans* (GenBank accession no. JF920299), *Enterococcus faecium* (JF920300) and *Lactococcus plantarum* (JF920301). Identification of remaining strains is in progress.

Biochemical changes such as drop in pH and increase in acidity, sugars, soluble proteins and amino acids observed in this study are mainly attributed by the activities of fermenting microbes. They secrete a number of enzymes, which catalyze the hydrolysis of carbohydrates, lipids, proteins and anti-nutritional factors (Rolle 1998). A drop in pH and increase in titratable acidity during co-fermentation of finger millet and horse gram are in accordance with the results of autofermentation of finger millet alone (Sripriya et al. 1997). A pH range of 3.6–4.1 is evidently favourable for eliminating undesirable microbial flora (coliforms) in fermented foods (Chavan and Kadam 1989).

Decrease in sugars in all batter combinations could be attributed to the utilization of sugars by fermenting microorganisms that are generally highly active, and this exploitation is higher than the rate of production. After 20 h of fermentation, sugar content is stabilized as the growth of microorganisms ceases due to acid accumulation. Acids, especially lactic acids accumulate due to the conversion of sugars to acids. Similarly, Khetarpaul and Chauhan (1991) have reported a significant reduction in the amount of total soluble, reducing and non-reducing sugars and starch during sequential fermentation of pearl millet (*Pennisetum typhoidenum*) using yeasts, lactobacilli. Reducing sugars (as glucose) showed a steady decrease from 3.3 mg/g in dry ingredients to 0.8 mg/g in 20 h in *idli* batter fermentation (Desikachar et al. 1960). Hydrolysis of starch by fermenting microbes that possess alpha amylase reduces starch content in the fermented product. In this study, starch content was decreased nearly by 25% at the end of fermentation and hydrolyzed to sugars and in turn converted to acids and gas, which is evidenced by an increase in acidity and microbial population. Due to the availability of the excess of reducing sugars, the microbes grew faster and multiplied in large numbers and brought about an increase in acid levels. Sripriya et al. (1997) reported that the decrease in starch content by 7.4% during germination and fermentation of finger millets may be due to fermenting carbohydrates (starch).

Increase in the soluble protein content may be ascribed by an increase in the microbial enzyme activity, protein hydrolysis and breakdown of tannins and phytates (Chavan and Kadam 1989). Phytates form a strong insoluble complex with proteins, and their interaction is pH dependent and resists proteolysis. Low pH in the fermentation medium solubilizes the complex, and lactic and acetic acids enhance the phytase activities, resulting in lowering of phytate and thereby increasing the level of soluble proteins (Lopez et al. 1983). Tannins form complex with enzymes of digestive tract and also bind with proteins thereby adversely affecting the utilization of proteins. Fermentation reduces the tannin content, which might be due to the action of microbial enzymes on tannin–protein complex resulting in the release of proteins. Soluble proteins and free amino acids increase is also due to the hydrolysis of insoluble proteins by bacterial proteases. The microorganisms are able to hydrolyze proteins into usable amino acids and peptides. These amino acids can be readily utilized by the microflora for their metabolic activity. Additionally, during their growth cycle, they can synthesize amino acids from metabolic intermediates (Au and Fields 1981). When the amino acid increment (promoted by the referred

### Table IV. Acceptability and sensory evaluation scores of *dosa* (fermented finger millet–horse gram flour).

<table>
<thead>
<tr>
<th>Samples†</th>
<th>Appearance</th>
<th>Texture</th>
<th>Colour</th>
<th>Taste</th>
<th>Flavour</th>
<th>Mouth feel</th>
<th>Overall, acceptability‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.3 ± 0.95</td>
<td>5.1 ± 0.88</td>
<td>5.9 ± 0.74</td>
<td>4.6 ± 1.17</td>
<td>4.8 ± 0.92</td>
<td>4.9 ± 0.99</td>
<td>5.08 ± 1.00*</td>
</tr>
<tr>
<td>B</td>
<td>5.1 ± 1.10</td>
<td>4.8 ± 0.92</td>
<td>5.3 ± 0.95</td>
<td>5.1 ± 0.74</td>
<td>5.3 ± 0.67</td>
<td>5.4 ± 0.84</td>
<td>5.16 ± 0.86*</td>
</tr>
<tr>
<td>C</td>
<td>5.1 ± 0.88</td>
<td>5.7 ± 0.95</td>
<td>5.0 ± 0.82</td>
<td>6.5 ± 0.71</td>
<td>5.7 ± 0.82</td>
<td>6.1 ± 0.88</td>
<td>5.70 ± 0.97*</td>
</tr>
<tr>
<td>D</td>
<td>4.8 ± 0.79</td>
<td>5.4 ± 1.07</td>
<td>4.8 ± 1.03</td>
<td>6.0 ± 0.82</td>
<td>5.9 ± 0.74</td>
<td>5.9 ± 0.57</td>
<td>5.48 ± 0.96*</td>
</tr>
</tbody>
</table>

* 7-point hedonic scale is as follows: 7-excellent, 6-very good, 5-good, 4-satisfactory, 3-fair, 2-poor, 1-very poor; † Batter combinations (Finger millet: Horse gram) – A (2:1); B (3:1); C (4:1); D (5:1); ‡ Mean ± S.D. score of 10 determinations (10 panellists) of each three sets of batter; † Mean values bearing different letters a, b and c within the same column are significantly different (p < 0.05) on the application of Duncan’s multiple range test.
production and/or by protein hydrolysis) is superior to
the amino acid utilization, a final increase in free
amino acid levels is observed (Correia et al. 2010).
The total free amino acids were observed to increase
rapidly for about 4–5-fold during germination and
doubled at 18 h fermentation of finger millets (Sripriya
et al. 1997).

Significant changes in amino acids are due to the net
effect of LAB, yeast and fermenting conditions, which
determines the direction of changes in individual
amino acids. Methionine, the limiting amino acid in
legume and lysine, the limiting amino acid in cereals
are increased by co-fermentation. On mixing finger
millets and horse gram, the proteins complement one
another to produce protein of a better quality by
providing each other significant amounts of the
respective limiting amino acids. In this study, essential
amino acids such as cysteine, threonine and isoleucine
increased nearly one fold, whereas lysine increased 1.2-
fold. The degradation of prolamins into lower peptides
and free amino acids supplies amino groups, which
may be used through transamination to synthesize
lysine. Glutamic acid and proline have been implicated
in providing nitrogen for the synthesis of lysine and
other essential amino acids (Mbithi-Mwikya et al.
2000). These results are in accordance with cereal–
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to the bean flavour of horse gram which was present in high proportion and 5:1 ratio blended product rated second due to high ragi floury taste and mouth feel. Thus, the proportion of ingredients (finger millet and horse gram) used for the preparation of fermented food product is essential. The success of fermentation depends on the acceptability of the products. Fermentation leads to rapid acidification of the raw material through the production of organic acids, mainly lactic acid; in addition, certain aroma compounds, bacteriocins, exopolysaccharides and several enzymes are produced. As a result, they yield non-toxic substrates (within limits) that often have desirable spin-offs, such as enhanced taste, aroma, shelf life, texture and nutritional value, which are attractive and palatable to the human consumer (Steinkraus 2002). According to Chavan and Kadam (1989), during cereal fermentations several volatile compounds were formed, which contribute to a complex blend of flavours in the products. It has been reported by Soni and Arora (2000) that yeasts involved in fermentation not only contribute towards gas production that results in good texture, but also towards the sensory qualities of the idlis. Generally, over-fermentation gives way to more acid accumulation resulting in sour taste and undesirable odour, which is not preferred for household purposes.

Conclusion
This work was conducted to study the biochemical and microbial changes during co-fermentation of finger millet and horse gram in different proportions for the preparation of dosa, which plays a significant role in maintaining the final product quality. On the basis of the results, 16-h fermentation of 4:1 ratio blended batter was found to be better compared to other proportions. This fermented product (dosa) is expected to contribute immensely as a protein source and dietary supplement in developing countries like India. However, further research works are warranted to evaluate the availability of nutrients and other nutritional requisites like vitamins and minerals by in vitro and in vivo studies. Identification and characterization of inhabiting microorganisms and development of starter cultures for improved product quality are being carried out. Efforts will be made further to transfer this product to household purposes.

Declarations of interest: Authors do not have any conflict of interest.

References


