In vitro evaluation of short duration cassava varieties as livestock feed

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ABSTRACT

This study was taken up to assess the nutritional value of short duration cassava varieties, Sree Jaya (V1), Sree Vijaya (V2) and Vellayani Hraswa (V3). The tuber, stem and leaves of above varieties were evaluated by in vitro gas production technique in a 3×3 factorial design. The tuber yield was the highest in V3 variety, followed by that in V1 and the lowest yield was observed in V2 variety. Irrespective of cassava variety, the total ash, CP, EE and ADL content was highest in leaves. The other cell wall contents were highest in stem portion. Irrespective of the part of plant, the CP and hemicellulose contents were highest in V1. The NDF and ADF content was highest in V3. The protein fractionation revealed that the albumin in V1 was higher than that in V2 but comparable with that of V3. The globulin in V1 was higher than V3. Reverse trend was observed in case of prolamins. Irrespective of cassava variety, the net gas production (NGP), NDF and true OM digestibility, ME content, methane emission, total and individual VFAs production, fermentation efficiency and efficiency of conversion of fermented hexose energy to VFA energy were highest in cassava tubers followed by stem and lowest was observed in leaves. These parameters were not affected by cassava variety, except that ME was the highest and methane emission was the lowest in V3. It was concluded that Vellayani Hraswa variety and amongst different parts of cassava plant, tubers irrespective of cassava variety were observed to be highly nutritious.

Keywords: Cassava varieties, In vitro evaluation, Methane emission, Protein fractions

Cassava (Manihot esculenta Crantz) is a long duration crop that can tolerate extreme weather conditions like high temperature, heat waves and moisture stress (Nedunchezhiyan and Mohanty 2005). It is cultivated worldwide for its starchy tuberous roots, which are used as a staple food (Heuzé et al. 2016). For every tonne of roots that are harvested, there are an additional 600 kg of stems and leaves which also have a high potential feeding value for cattle (Ffoulkes and Preston 1978, Wanapat et al. 1997), and goats (Ho Quang Do et al. 2002). Cassava tubers are also used for ethanol production (Kuiper et al. 2007). Other cassava products include the finger-like leaves, which are consumed as vegetables or used as feed (Heuzé and Tran 2016). By-products from cassava processing industries like cassava flour, peels, pomace, sievate, stumps and whey are used as potential animal feeds (Boscolo et al. 2002, Aro et al. 2010).

Cassava roots for animal feeding are commonly harvested from the 9th to 12th month after cultivation (Kuiper et al. 2007, Gomes 1991). Suja et al. (2010) reported that short duration cassava (7–8 months) can be grown in rice based cropping system for crop diversification, intensification and profit maximization. However, little information is available on the cultivation of short duration cassava varieties in the Trans-Gangetic plain region of North India which experiences long cold season. Therefore, three short duration varieties like Sree Jaya (V1), Sree Vijaya (V2) and Vellayani Hraswa (V3) were cultivated in Punjab Agricultural University. After the stipulated period, the crop was harvested and yield was recorded. But no reports are available regarding the nutritive value of different parts of short duration varieties of cassava. Therefore, leaves, stem and tubers of short duration varieties were evaluated as livestock feed by in vitro gas production technique.

MATERIALS AND METHODS

The short duration cassava varieties, Sree Jaya (V1), Sree Vijaya (V2) and Vellayani Hraswa (V3) were cultivated at Punjab Agricultural University, Ludhiana with seven replicates. Setts were planted on ridges at a spacing of 75×75 cm in sandy loam soil. Fertilizers to supply N, P2O5 and K2O @ 75:50:75 kg/ha were applied, half N and K2O and full P2O5 was applied at the time of planting and the remaining half N and K2O at 60 days after planting (DAP). The crop was irrigated as and when required. The crop was harvested after 8 months of cultivation. The soil was characterized by pH 7.4, low organic C (0.5%) and available N (216 kg/ha) and medium available P (18.3 kg/ha) and

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available K (155 kg/ha).

**Chemical composition:** The leaves, stem and tubers were dried in a hot air oven and ground through a 1mm sieve. The samples were analyzed for proximate principles (AOAC 2007), cellulose (Crampton and Maynard 1938) and other cell wall constituents (Van Soest et al. 1991). The protein in the leaves was fractionated into four protein fractions (Globulin, albumin, prolamin and glutelin) based on the solubility (Monteiro et al. 1982).

In *vitro* studies: The nutritional value of cassava tuber, stem and leaves were evaluated by *in vitro* gas production technique (IVGPT, Menke et al. 1979, Menke and Steingass 1988). Three rumen fistulated male buffalo calves used as donor for rumen contents were maintained on 2 kg conventional concentrate mixture (Maize 32, barley 20, soybean meal 15, groundnut extraction 15, rice bran 15, mineral mixture 2 and common salt 1% each), 2 kg green fodder and *ad lib.* wheat straw. About 375±5 mg finely ground cassava tuber, stem or leaves (on DM basis) was incubated with buffered rumen fluid in triplicate in a water bath at 39°C for 24 h in 100 ml calibrated glass syringes (Haberle Labortechnik, Germany). After 24 h, the volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spout-less beaker, boiled with neutral detergent solution for assessing the true OM and NDF digestibility.

**Estimation of volatile fatty acids:** After 24 h of incubation, a 5 mL aliquot of fluid from each syringe was mixed with 1 mL of 25% meta-phosphoric acid and kept for 1h at ambient temperature (Erwin et al. 1961). Thereafter, it was centrifuged at 5500 rpm for 10 min and clear supernatant was collected and stored at –20°C until analyzed. The volatile fatty acids were estimated using Netchrom 9100 gas chromatograph (Cottyn and Boucque 1968).

**Hydrogen balance:** Hydrogen recovery, hydrogen consumed via CH₄/VFA (Demeyer 1991), VFA utilization index which represents non-glucogenic VFAs to glucogenic VFAs ratio (NGGR) and microbial biomass synthesis and methane produced during fermentation were calculated from VFA concentration (Widiawati and Thalib 2009). The energetic efficiency of rumen fermentation (E: Orskov et al. 1968), efficiency of conversion of fermented hexose energy to VFA energy (E₁: Czerkawski 1986) and efficiency of conversion of fermented hexose energy to CH₄ energy (E₂: IAEA 1985) were calculated from the molar proportion of VFAs cited by Baran and *Titan* (2002).

**Statistical analysis:** The impact of different cassava varieties and parts of plant on different parameters was analyzed by 3 × 3 factorial design (Snedecor and Cochran 1994) by using SPSS (2009) version 16.0 and the means were tested for the significant differences by using Duncan’s multiple range test. The interactions were worked out between cassava varieties and parts of plant in all possible combinations (Systat 1996).

### RESULTS AND DISCUSSION

The tuber yield was the highest (P<0.05) in *Vellayani Hraswa* variety (40.8 t/ha), followed by that in *Sree Jaya* (33.6 t/ha) and the lowest yield was observed in *Sree Vijaya* variety (25.2 t/ha). The yield of tubers in all the short duration varieties (Hira Singh et al. 2013) was much higher than the average worldwide tuber yield of 13 t/ha in 2009 (FAO 2011). Khang et al. (2005) have reported the fresh tuber yield of 34.5 t/ha.

Irrespective of cassava variety, the total ash, CP, EE and ADL content was highest (P<0.001) in leaves followed by stem and lowest was observed in cassava tubers, but reverse trend (P<0.001) was observed in case of OM (Table 1). The NDF, ADF, cellulose and hemicellulose content was highest (P<0.001) in stem portion followed by leaf and lowest was observed in cassava tubers, except hemicellulose which was lowest (P<0.001) in cassava leaves. The CP and EE content in leaves obtained in the present study was lower, but the cell wall constituents of leaves were much higher than those reported by Heuzé and Tran (2016), it may be due to varietal difference. Besides protein, the cassava leaves have a good amino acid profile except for methionine. They are good sources of minerals (Ca and trace elements) although P and Na contents are rather low.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Part of plant (PP)</th>
<th>PSE</th>
<th>Variety (V)</th>
<th>PSE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Tuber</td>
<td>V₂</td>
<td>V₃</td>
</tr>
<tr>
<td>Total ash</td>
<td>7.88c</td>
<td>4.83b</td>
<td>3.45a</td>
<td>0.20</td>
<td>5.33</td>
</tr>
<tr>
<td>OM</td>
<td>92.12a</td>
<td>95.17b</td>
<td>96.55c</td>
<td>0.20</td>
<td>94.67</td>
</tr>
<tr>
<td>CP</td>
<td>18.15c</td>
<td>5.32b</td>
<td>3.20a</td>
<td>0.12</td>
<td>8.31A</td>
</tr>
<tr>
<td>EE</td>
<td>6.07c</td>
<td>1.05b</td>
<td>0.68a</td>
<td>0.05</td>
<td>2.67</td>
</tr>
<tr>
<td>NDF</td>
<td>53.03b</td>
<td>64.90b</td>
<td>16.60a</td>
<td>0.26</td>
<td>47.90C</td>
</tr>
<tr>
<td>ADF</td>
<td>44.58b</td>
<td>53.85c</td>
<td>6.48a</td>
<td>0.16</td>
<td>40.28B</td>
</tr>
<tr>
<td>Cellulose</td>
<td>20.73b</td>
<td>35.63c</td>
<td>1.33a</td>
<td>0.18</td>
<td>19.13B</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>8.45a</td>
<td>11.05c</td>
<td>10.12b</td>
<td>0.28</td>
<td>7.62A</td>
</tr>
<tr>
<td>ADL</td>
<td>19.25c</td>
<td>16.82b</td>
<td>6.73a</td>
<td>0.20</td>
<td>15.05B</td>
</tr>
</tbody>
</table>

1Irrespective of the cassava variety; 2Irrespective of the part of cassava plant; V₁, Sree Jaya; V₂, Sree Vijaya; V₃, Vellayani Hraswa; Mean with superscriptsabc for different parts of plant in a row differ significantly; Mean with superscriptsABC for different short term cassava varieties in a row differ significantly; PSE, Pooled standard error.

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The chemical composition of tubers was comparable to that reported earlier (Heuzé et al. 2016), except that the NDF content was higher in the present study. Cassava roots are considered as an excellent energy feed mainly because of high starch (70–85%) content (Ly 1998, Régnier 2011). However, crude protein content of tubers is lower than that of cereal grains. Irrespective of the part of plant, the total ash, OM and EE content were similar in all the three cassava varieties. The CP and hemicellulose contents were highest in varieties. The CP and hemicellulose contents were highest in stem and lowest in tubers. While amongst the insoluble category, prolamins were the highest in leaves followed by that in stem and lowest in tubers. The soluble proteins and insoluble proteins as percent of total proteins were highest in leaves and lowest in stem portion. Reverse trend (P<0.001) was observed in case of prolamins. The glutelin content was similar in all the three varieties. The soluble protein content and SP as per cent of total proteins was highest in leaves followed by that in stem and lowest was in V3 variety. Reverse trend (P<0.005, P<0.001) was observed in insoluble protein content and IP as per cent of TP. The SP to IP ratio was similar in V1 and V2 varieties, but higher (P<0.001) than V3 variety.

Irrespective of cassava variety, the NGP was highest (P<0.001) in cassava tubers followed by stem and lowest was observed in leaves (Table 3). The NDF and true OM digestibility and ME content was also highest (P<0.001) in tubers followed by leaves and lowest in stem portion. Similar trend (P<0.001) was observed in methane emission. But reverse trend (P<0.001) was observed in partitioning factor and ammonia production. Roza et al. (2013) revealed that cassava leaves flour (CLF) is the source of carbon frame and bypass protein increased (P<0.05) dry matter and organic matter digestibility in vitro. Irrespective of the part of plant, NDF, NDF and true OM digestibility; and PF were not affected by the cassava variety. The ME in V3 was higher (P<0.001) than V1 but comparable with V2. The CH4 emission from V3 was lower (P<0.005) than V1 and V2, which were statistically comparable. Phanthavong et al. (2015) studied the effect of biochar and leaves from sweet or bitter cassava on gas and methane production in an in vitro rumen incubation using cassava root pulp as source of energy. The percentage of methane in the gas was lower for: (i) bitter compared with sweet cassava; (ii) fresh versus dried leaves; and (iii) from substrates with biochar than for those without biochar.

Irrespective of cassava variety, the total and individual VFAs production was the highest (P<0.001) from cassava tubers followed by that from leaves and the lowest production was observed from stem portion (Table 4). However, the valerate production was the highest (P<0.001) from the leaves followed by tubers and the lowest was observed from the stem portion. The acetate to propionate ratio was also the best (P<0.001) in tubers followed by that from leaves and stem and lowest was observed in V3 variety.
from stem and leaves. Similar trend (P<0.001) was observed in relative proportion of propionate, isobutyrate (P<0.005), butyrate and isovalerate, but reverse trend (P<0.001) was observed in relative proportion of acetate and valerate. Roza et al. (2013) revealed that cassava leaves flour (CLF) as the source of carbon frame and bypass protein increased (P<0.05) bacteria count and VFA production in vitro.

Irrespective of the part of plant, the total and individual VFA production was the highest (P<0.001) from V1 and lowest production was observed from V3, except that of isovalerate production where reverse trend (P<0.001) was observed. The acetate to propionate ratio was also the best (P<0.001) in V1 followed by V3 and lowest was in V2 variety. The relative proportion of acetate was highest (P<0.001) in V2 and that of propionate and valerate was observed in V1. The relative proportion of these VFAs was lowest (P<0.001) in V3.

Irrespective of cassava variety, the highest (P<0.001) hydrogen recovery was from tubers followed by that from stem and the lowest was observed from leaves (Table 5). Reverse trend (P<0.001) was observed in hydrogen consumption and VFA utilization index. The fermentation efficiency (E) and efficiency of conversion of fermented hexose energy to VFA energy (E1) was highest (P<0.001) from cassava tubers followed by stem and the lowest from leaves. The microbial biomass synthesis was highest (P<0.001) in cassava tubers, while the lowest was observed in stem. Irrespective of the part of plant, the hydrogen recovery in V3 was higher (P<0.001) than V2 but comparable with that of V1. Reverse trend (P<0.001) was observed in

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**Table 3. In vitro evaluation of leaf, stem and tuber of different short term varieties of cassava**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Part of plant (PP)</th>
<th>PSE</th>
<th>Variety (V)</th>
<th>PSE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Tuber</td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>NGP</td>
<td>129.18a</td>
<td>142.37b</td>
<td>338.67c</td>
<td>0.58</td>
<td>203.11</td>
</tr>
<tr>
<td>NDFD</td>
<td>26.57b</td>
<td>13.03a</td>
<td>59.54c</td>
<td>2.48</td>
<td>32.71</td>
</tr>
<tr>
<td>TOMD</td>
<td>59.24b</td>
<td>43.15a</td>
<td>93.07c</td>
<td>0.66</td>
<td>64.23</td>
</tr>
<tr>
<td>PF</td>
<td>2.79c</td>
<td>2.55b</td>
<td>1.10a</td>
<td>0.02</td>
<td>2.14</td>
</tr>
<tr>
<td>ME</td>
<td>7.79b</td>
<td>6.20a</td>
<td>11.06c</td>
<td>0.01</td>
<td>8.42A</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.031c</td>
<td>0.018b</td>
<td>0.003a</td>
<td>–</td>
<td>0.016A</td>
</tr>
<tr>
<td>CH₄ (mL/100mg DM)</td>
<td>0.74b</td>
<td>0.57a</td>
<td>1.30c</td>
<td>0.04</td>
<td>0.86B</td>
</tr>
</tbody>
</table>

V₁, Sree Jaya; V₂, Sree Vijaya; V₃, Vellayani Hraswa; NGP, Net gas production (ml/24h/g DM); TOMD, True OM digestibility (%); NDFD, Neutral detergent fibre digestibility (%); PF, Partitioning factor (mg/ml); ME, Metabolizable energy (MJ/kg DM); Mean with superscripts a,b,c for different parts of plant in a row differ significantly; Mean with superscripts A,B,C for different short term cassava varieties in a row differ significantly; PSE, Pooled standard error.

**Table 4. Total and individual volatile fatty acid production (mM/DL) from leaf, stem and tuber of different short term varieties of cassava**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Part of plant (PP)</th>
<th>PSE</th>
<th>Variety (V)</th>
<th>PSE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
<td>Tuber</td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>TVFA</td>
<td>2.12b</td>
<td>1.66a</td>
<td>3.44c</td>
<td>0.01</td>
<td>2.46C</td>
</tr>
<tr>
<td>Acetate (A)</td>
<td>1.49b</td>
<td>1.13a</td>
<td>2.20a</td>
<td>0.004</td>
<td>1.63B</td>
</tr>
<tr>
<td>Propionate (P)</td>
<td>0.336b</td>
<td>0.286a</td>
<td>0.622c</td>
<td>–</td>
<td>0.423B</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>0.024b</td>
<td>0.012a</td>
<td>0.039c</td>
<td>0.002</td>
<td>0.023</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.201b</td>
<td>0.191a</td>
<td>0.493c</td>
<td>–</td>
<td>0.310C</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>0.032b</td>
<td>0.020a</td>
<td>0.063c</td>
<td>–</td>
<td>0.037B</td>
</tr>
<tr>
<td>Valerate</td>
<td>0.038b</td>
<td>0.017a</td>
<td>0.030b</td>
<td>0.00</td>
<td>0.031B</td>
</tr>
<tr>
<td>A:P</td>
<td>4.45c</td>
<td>3.97b</td>
<td>3.55c</td>
<td>0.01</td>
<td>3.93A</td>
</tr>
</tbody>
</table>

Relative proportion, %

| Acetate         | 70.18c | 68.24b | 64.05a | 0.08 | 67.20B | 68.52C | 66.76A | 0.08 | <0.001 | <0.001 | <0.001 |
| Propionate      | 15.77a | 17.21b | 18.04c | 0.03 | 17.18C | 17.01B | 16.84A | 0.03 | <0.001 | <0.001 | <0.001 |
| Isobutyrate     | 1.13b | 0.78a | 1.20b | 0.08 | 0.91 | 1.10 | 1.10 | 0.08 | <0.013 | 0.219 | 0.001 |
| Butyrate        | 1.14b | 0.74a | 1.10b | 0.06 | 0.91A | 1.10B | 1.10B | 0.08 | <0.001 | <0.001 | <0.001 |
| Isovalerate     | 1.51b | 1.22a | 1.81c | 0.02 | 1.46A | 1.42A | 1.64B | 0.02 | <0.001 | <0.001 | <0.001 |
| Valerate        | 1.73c | 1.06b | 0.87a | 0.01 | 1.30B | 1.27B | 1.09A | 0.01 | <0.001 | <0.001 | <0.001 |

V₁, Sree Jaya; V₂, Sree Vijaya; V₃, Vellayani Hraswa; TVFA, Total volatile fatty acids; Figures with different superscripts a,b,c in a row differ significantly; Mean with superscripts a,b,c for different parts of plant in a row differ significantly; Mean with superscripts A,B,C for different short term cassava varieties in a row differ significantly; PSE, Pooled standard error.
Table 5. Hydrogen balance and efficiency of energy utilization from leaf, stem and tuber of different short term varieties of cassava (% DM basis)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Part of plant (PP)</th>
<th>PSE</th>
<th>Variety (V)</th>
<th>PSE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Stem</td>
<td>Tuber</td>
<td>V1</td>
<td>V2</td>
<td>V3</td>
</tr>
<tr>
<td>HR</td>
<td>29.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05</td>
<td>32.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HC</td>
<td>0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>–</td>
<td>0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>VFA UI</td>
<td>4.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02</td>
<td>4.54&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>E</td>
<td>72.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.02</td>
<td>73.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E1</td>
<td>78.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.02</td>
<td>80.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E2</td>
<td>20.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01</td>
<td>20.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MBM</td>
<td>53.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11</td>
<td>63.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>V<sub>1</sub>, Sree Jaya; V<sub>2</sub>, Sree Vijaya; V<sub>3</sub>, Vellayani Hraswa; HR, Hydrogen recovery; HC, Hydrogen consumed via CH₄/VFA; E, Efficiency of rumen fermentation; E₁, Efficiency of fermented hexose energy to VFA energy; E₂, Efficiency of fermented hexose to methane; VFA UI, VFA utilization index; MB, Microbial biomass (g/day); Mean with superscripts<sup>a,b,c</sup> for different parts of plant in a row differ significantly; Mean with superscripts<sup>A,B,C</sup> for different short term cassava varieties in a row differ significantly; PSE, Pooled standard error.

case of hydrogen consumed. The fermentation efficiency (E) in V<sub>1</sub> and V<sub>3</sub> was comparable but higher (P<0.001) than V<sub>2</sub>. But efficiency of fermented hexose energy to VFA energy (E₁) in V<sub>3</sub> was the highest (P<0.001), followed by V<sub>1</sub> and the lowest was observed in case of V<sub>2</sub>. The microbial biomass synthesis was highest (P<0.001) in V<sub>1</sub> followed by V<sub>2</sub> and the lowest was observed in case of V<sub>3</sub>. It was concluded that with respect to tuber yield and nutritional worth Vellayani Hraswa (V<sub>3</sub>) variety and amongst different parts of cassava plant, tubers irrespective of cassava variety were observed to be highly nutritious.

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