Effect of Storage and Heat Treatments on the Sugar Constituents in Cassava and Yambean Roots

Akiko KAWABATA*, Shigeru SAWAYAMA*, Ricardo R. Del ROSARIO**
and Marissa G. NOEL**

* Department of Nutrition, Faculty of Agriculture, Tokyo University of Agriculture, 1-1-1, Sakuragaoka, Setagaya-ku, Tokyo 156
** Institute of Food Science and Technology (IFST), College of Agriculture, University of the Philippines at Los Baños (UPLB) College, Laguna 3720, Philippines

The effect of storage and heat treatments on the sugar constituents of cassava and yambean roots were investigated using a high performance liquid chromatography (HPLC). Essentially, the total sugar content increased during the first one or two weeks of any 18~28 days storage period. Also, the ratios of fructose and glucose to total sugar content increased, while the ratios of sucrose and inositol decreased. The linamarin content increased markedly in the first 7 days, and continued to increase for several additional days; then it gradually declined and almost disappeared before decay. In yambean root, the three sugars of glucose, fructose and sucrose were found. Although heat treatments by boiling, roasting, and drying all tended to increase the total sugar content of these roots, the linamarin content of cassava roots and of finely-chopped cassava leaves significantly showed a tendency to decrease by such treatments. Investigated at the same time were changes in the constituent pectic substances of these roots. Finally, the changes in appearance occurring in cassava root tissue during storage and heat treatment were observed by a scanning electron microscope (SEM).

There are various root crops grown in tropical areas, such as the Philippines, where cassava and yambean are among the most important, and their roots are usually consumed directly as food. The changes that occur to these starchy roots during storage are important concerning their subsequent use. During storage, there is known to occur a rapid accumulation of total sugars accompanied by a small decline in starch content.

Booth et al.1) and Richard and Coursey2) have reported that early in cassava root storage there appears an increase in reducing sugar content, which is later followed by a decrease as storage is prolonged. Sucrose, on the other hand, constantly increases. The effect on cyanogenic glycoside (linamarin), remains unclear, and there are several reports, such as by Wood3), Cook et al.4), and Fukuba et al.5) using the enzymatic method, and Conn6) using paper chromatography for determining linamarin content.

The main aim of this study has been to elucidate in more detail the changes that occur in the sugar constituents of cassava and yambean roots during storage, and as the result of heat treatments. At the same time, the pectic substances in these roots were determined, and the tissue structure of these roots was observed by a scanning electron microscope (SEM).

Materials and Methods

Materials
Cassava (Manihot esculenta Crantz) : Three cultivars of cassava, grown at the Institute of Plant Breeding, University of the Philippines at Los Baños, were used. These included cv. ‘Lakan’ and cv. ‘Vassourinha’ as sweet types, and cv. ‘Datu’ as a bitter type.

Yambean (Pachyrhizus erosus Urban) : One cultivar of yambean was used in the study. It was obtained from a market at Los Baños, Laguna.

The roots were stored under ambient con-
Methods

Determination of sugar content using a high performance liquid chromatography (HPLC): The determination of sugar content was done by use of an HPLC with a TSK-GEL, LS-450 NH2 (4 mm i.d. x 300 mm) column made by Toyo Soda Co., Ltd. It was eluted at a flow rate of 0.8 ml/min with 75% acetonitrile in water. The sugar contents of the cassava were determined from the proximal, middle, and distal parts of the parenchymal tissues of each sample. Samples of yambean used in the study were taken from the middle parts of the roots. These roots were stored for a period of 18 to 28 days and samples were taken for analysis every second or third day.

Samples for heat treatment were taken from the middle parts of the roots and cut into slices 5 cm thick. They were heat-treated by boiling at 100°C for 18 min., roasting at 200°C for 1 h., and drying at 60°C for 19 h. The method of sample preparation for HPLC is shown in Fig. 1.

Determination of pectic substances: Test samples were taken from the same parts of the cassava roots used for determining the sugar constituents. About 25 g of each sample was grated into ethyl alcohol, then heated repeatedly in warm 70% ethyl alcohol solution until no sugar could be detected by the Molisch reaction. The residues were treated with absolute ethyl alcohol and then with ethyl ether, dried at room temperature, and pulverized to pass through a 10 to 20 mesh screen. These processed residues, referred to as alcohol insoluble solids (AIS), were used to determine pectic substances. The pectic substances were fractionally extracted by the method of SAWAYAMA et al. into four fractions: the water soluble fraction (W-S); the hexametaphosphate soluble fraction (P-S); the hydrochloric acid soluble fraction (H-S); and the potassium hydroxide soluble fraction (K-S). By using the meta-hydroxydiphenyl method, the pectic substances in these four fractions were determined as anhydrogalacturonic acid.

Observation of cassava root tissue using a scanning electron microscope (SEM): Observation and photography of the cassava root tissue was carried out with a Nippon Denshi JSM-35 SEM after fixation of the tissue by glutaraldehyde and osmic acid; the tissue had been dried by the critical point drying method.
Results and discussion

Effect of storage on the sugar constituents of roots

The results concerning changes in the sugar constituents of cv. ‘Lakan’ cassava roots during storage are shown in Table 1. The daily sampling results reflect the averaging of six measurements, each taken individually from the three parts (proximal, middle, and distal) of two roots. The six samples had remained healthy during the first two days of storage, but after 4 days some parts showed a minimal deterioration due to vascular streaking, i.e., physiological deterioration.

Moisture content in the roots decreased from 66.6% to about 60.0%, and total sugar content increased from about 500 to 3900 mg per 100 g fresh weight; the increase was essentially during the first two weeks of storage. This rapid increase of sugar content was considered to be a reflection of the metabolic change from starch to sugar that occurs during storage.

The ratio of fructose and glucose to total sugar content also began increasing from the 7th day of storage; this was similar to results from the study by Booth et al., which showed an increase in reducing sugar during a 2-4 week storage period. The total amount of inositol was not large but there was a slight tendency for inositol to increase during storage. No literature concerning the amount of inositol in cassava root was found, but the existence of inositol in sweet potato roots is reported by Yoshimura and Nagahara.

The existence of linamarin (cyanogenic glucoside) found in cassava presents a serious problem concerning the use of cassava as a foodstuff; it has been customary to use a sweet type cassava which contains less linamarin. The bitter type cassava, those which contain large amounts of limamarin, are usually used as raw materials to produce starch.

Though cv. ‘Lakan’ is of the sweet type cassava, it did initially contain 17 mg of limamarin per 100 g fresh weight. After 7 days storage this increased to 35 mg; yet by the 14th day the linamarin content had reversed itself and decreased to nearly one third its former value. This trend continued and at the end
of three weeks, the limamarin content had diminished to but a trace. At the same time, decay had increased to affect, at least partially, up to two-thirds of the cassava roots. This gradual increase of cyanide over several days of storage had likewise been noted by KOJIMA et al.15) who concluded this phenomenon was closely related with linamarase activity, and suggested that linamarase had played a primary role in regulating the levels of cyanogenic glucoside in cassava roots.

For cv. ‘Vassourinha’ cassava roots, the changes in sugar constituents during storage are shown in Table 2. The moisture content of cv. ‘Vassourinha’ root was 10% higher than that of cv. ‘Lakan’. This may explain why the cv. ‘Lakan’ kept a healthy condition throughout all 28 days of storage, while the cv. ‘Vassourinha’ could maintain its health for only 18 days; the total sugar content of cv. ‘Vassourinha’ also was higher than that of cv. ‘Lakan’. Concerning the ratios of various sugars to total sugar content, cv. ‘Vassourinha’ showed the same tendency as cv. ‘Lakan’ up to the 14th day; but by the 18th day, the glucose ratio for cv. ‘Vassourinha’ became the highest.

<table>
<thead>
<tr>
<th>Duration of storage (days)</th>
<th>Moisture content (%)</th>
<th>Total sugar (mg/100 g fresh weight)</th>
<th>Sugar content [mg/100 g fresh weight, (%) total sugar]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sucrose</td>
</tr>
<tr>
<td>0 (fresh)</td>
<td>75.6</td>
<td>1388</td>
<td>1008 (72.6)</td>
</tr>
<tr>
<td>2</td>
<td>75.1</td>
<td>1284</td>
<td>885 (68.9)</td>
</tr>
<tr>
<td>4</td>
<td>73.5</td>
<td>1420</td>
<td>496 (35.1)</td>
</tr>
<tr>
<td>7</td>
<td>72.6</td>
<td>3797</td>
<td>2366 (62.3)</td>
</tr>
<tr>
<td>9</td>
<td>71.0</td>
<td>3874</td>
<td>2315 (59.7)</td>
</tr>
<tr>
<td>11</td>
<td>70.3</td>
<td>4046</td>
<td>2491 (61.6)</td>
</tr>
<tr>
<td>14</td>
<td>70.4</td>
<td>3916</td>
<td>2091 (53.4)</td>
</tr>
<tr>
<td>16</td>
<td>72.5</td>
<td>3938</td>
<td>1385 (34.5)</td>
</tr>
<tr>
<td>18</td>
<td>73.0</td>
<td>4498</td>
<td>774 (17.2)</td>
</tr>
</tbody>
</table>

Table 2 Effect of storage on the sugar constituents of cassava root (cv. ‘Vassourinha’)

<sup>a</sup>, **: The same as the footnote to Table 1.
Storage condition: See the footnote to Table 1.

<table>
<thead>
<tr>
<th>Duration of storage (days)</th>
<th>Moisture content (%)</th>
<th>Total sugar (mg/100 g fresh weight)</th>
<th>Sugar content [mg/100 g fresh weight, (%) total sugar]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sucrose</td>
</tr>
<tr>
<td>0 (fresh)</td>
<td>88.5</td>
<td>2817</td>
<td>485 (17.2)</td>
</tr>
<tr>
<td>3</td>
<td>88.1</td>
<td>3 617</td>
<td>463 (12.8)</td>
</tr>
<tr>
<td>5</td>
<td>87.7</td>
<td>4 120</td>
<td>461 (11.2)</td>
</tr>
<tr>
<td>7</td>
<td>87.3</td>
<td>4 102</td>
<td>714 (17.4)</td>
</tr>
<tr>
<td>10</td>
<td>86.1</td>
<td>5 060</td>
<td>789 (15.6)</td>
</tr>
<tr>
<td>12</td>
<td>87.7</td>
<td>4 379</td>
<td>858 (19.6)</td>
</tr>
<tr>
<td>14</td>
<td>87.0</td>
<td>4 745</td>
<td>859 (18.1)</td>
</tr>
<tr>
<td>17</td>
<td>85.7</td>
<td>5 291</td>
<td>1 106 (20.9)</td>
</tr>
<tr>
<td>19</td>
<td>87.0</td>
<td>4 745</td>
<td>1 110 (23.4)</td>
</tr>
<tr>
<td>21</td>
<td>86.5</td>
<td>4 361</td>
<td>1 073 (24.6)</td>
</tr>
</tbody>
</table>

Table 3 Effect of storage on the sugar constituents of yambean root

Storage condition: See the footnote to Table 1.
The results are the average of two measurements.
The increase of limamarin content in the first 7 days were also shown, but as with cv. "Lakan" it almost disappeared prior to decay.

The changes in sugar constituents of yambean root during storage are shown in Table 3.

The moisture content decreased slightly from 88.5% to 86% during storage, and even though the moisture content is high, the moisture-loss was less shown in yambean than in cassava. The total amount of sugars varied from about 1,800 to 5,300 mg per 100 g of root. The ratio of sucrose content to total sugar was lower than that of glucose or fructose. Glucose content was the highest, in a range of about 41 ~51% of total sugar. No other sugars than these three were found in yambean root.

**Effect of heat treatment on sugar constituents of roots and leaves**

Both cassava roots and cassava leaves are commonly eaten after subjection to one or several methods of pre-cooking and/or cooking. In this study, three types of heat treatment were used: a) boiling, b) roasting, and c) drying. The sugar constituents of the heat-treated samples were then determined and compared with the raw samples. As shown in Table 4, the total sugar content (dry-weight basis) of the roots tended to increase regardless of the treatment method. It was only limamarin that showed a marked decrease because of heat treatment. On average, 40% of the original linamarin content of these roots was removed by the various treatments. This is consistent with Wood3), Cook et al.4) and Fukuba et al.5) who considered in their reports that cyanogenic glucosides decomposed and diminished as a result of heat treatment.

In cassava leaves there is a much higher concentration of linamarin than found in the roots. Therefore, the changes in linamarin content of these leaves caused by boiling in water was studied and is shown in Table 5. After 5 minutes of boiling, the linamarin content of whole leaves did not change so much. However when the leaves were first finely-chopped, boiling caused total sugar content to decrease by about 75%, and about 73% of the linamarin content was removed. It was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture content (%)</th>
<th>Total sugar (mg/100 g dry weight)</th>
<th>Sugar content [mg/100 g dry weight]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh (raw)</td>
<td>67.0</td>
<td>2,463</td>
<td>Sucrose 2,004 Fructose 91 Glucose 98 Inositol 161 Linamarin 109</td>
</tr>
<tr>
<td>Boiled</td>
<td>62.5</td>
<td>3,289</td>
<td>Sucrose 2,800 Fructose 128 Glucose 108 Inositol 189 Linamarin 64</td>
</tr>
<tr>
<td>Fresh (raw)</td>
<td>62.0</td>
<td>2,646</td>
<td>Sucrose 2,026 Fructose 181 Glucose 170 Inositol 171 Linamarin 98</td>
</tr>
<tr>
<td>Roasted</td>
<td>51.0</td>
<td>3,443</td>
<td>Sucrose 2,714 Fructose 244 Glucose 231 Inositol 193 Linamarin 61</td>
</tr>
<tr>
<td>Fresh (raw)</td>
<td>62.0</td>
<td>1,396</td>
<td>Sucrose 1,194 Fructose 44 Glucose 43 Inositol 55 Linamarin 56</td>
</tr>
<tr>
<td>Dried</td>
<td>10.0</td>
<td>1,506</td>
<td>Sucrose 1,166 Fructose 58 Glucose 167 Inositol 82 Linamarin 33</td>
</tr>
</tbody>
</table>

a) 100 g of round-sliced root was boiled in 500 ml water at 100°C for 18 min.
b) Oven-roasted at 200°C for 1 h.
c) Oven-dried at 60°C for 19 h.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture content (%)</th>
<th>Total sugar (mg/100 g dry weight)</th>
<th>Sugar content [mg/100 g dry weight]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh (raw)</td>
<td>52.0</td>
<td>3,538</td>
<td>Sucrose 1,649 Fructose 493 Glucose 868 Inositol 286 Linamarin 242</td>
</tr>
<tr>
<td>Boiled</td>
<td>83.0</td>
<td>2,210</td>
<td>Sucrose 1,252 Fructose 143 Glucose 327 Inositol 261 Linamarin 238</td>
</tr>
<tr>
<td>Boiled</td>
<td>80.0</td>
<td>892</td>
<td>Sucrose 545 Fructose 55 Glucose 125 Inositol 105 Linamarin 65</td>
</tr>
</tbody>
</table>

A 10 g mixture of whole leaves and finely-chopped leaves were boiled in 300 ml of water at 100°C for 5 min.
observed that in using young leaves as foodstuffs, expansion of the cut surface area is an important technique for dissolving linamarin in boiling water.

**Effect of storage and heat treatment on the pectic substances of roots**

Pectic substances are the main constituents of the middle lamella of root-cell walls and, as such, give dynamic properties like hardness, elasticity, plasticity, and so forth to the cell structure. It is known that during storage cassava root tissue, becomes soft from physiological deterioration, but reports on changes in those pectic substances which contribute to the tissue structure cannot be found.

Changes in the galacturonic acid content of cv. ‘Lakan’ cassava roots during storage are shown in Fig. 2. The total content of galacturonic acid ranged from about 1570 to 2150 mg per 100 g fresh weight. After two days storage, the amounts of total galacturonic acid and of H-S fraction had sharply increased, but thereafter, held to a roughly constant level. Among the fractions, W-S tended to increase only over the last 12 days. P-S and K-S declined slightly. It was considered\[16\] that the W-S fraction was water soluble pectic acid and pectic acid; the P-S fraction had water insoluble salts of pectinic and pectic acids; and the H-S and K-S fractions mainly included protopectin in which pectic substances were combined with cellulose. The increase of W-S fraction after 21 days is related to the softening phenomenon of root tissue.

In Fig. 3 is shown the content of galacturonic acid in four fractions from cv. ‘Vassourinha’ cassava roots during storage. The total galacturonic acid content of cv. ‘Vassourinha’ is lower than that of cv. ‘Lakan’, and the H-S ratio is also somewhat lower. This is considered to be related to the dynamic properties of root tissue structure which, together with amounts of moisture and sugar content, produce a profound effect upon the deterioration of stored roots.

Changes in the galacturonic acid content of cassava and yambean roots, as caused by roasting, are shown in Table 6. All samples showed a tendency for increasing ratios of W-S after roasting. FUCHIGAMI and OKAMOTO\[37\] noted that changes in the firmness of vegetable roots during cooking is influenced by the quantity
Table 6 Effect of heat treatment on the galacturonic acid of cassava and yam bean roots

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water soluble fraction (W-S)</td>
<td>29 (1.4)</td>
<td>263 (9.8)</td>
<td>10 (0.7)</td>
<td>158 (8.9)</td>
<td>2 (0.2)</td>
<td>21 (1.8)</td>
</tr>
<tr>
<td>Hexametaphosphate soluble fraction (P-S)</td>
<td>231 (11.0)</td>
<td>289 (10.8)</td>
<td>252 (17.8)</td>
<td>347 (19.6)</td>
<td>125 (12.6)</td>
<td>122 (10.4)</td>
</tr>
<tr>
<td>Hydrochloric acid soluble fraction (H-S)</td>
<td>1,583 (75.2)</td>
<td>1,956 (73.1)</td>
<td>907 (64.1)</td>
<td>892 (50.3)</td>
<td>407 (41.2)</td>
<td>363 (31.0)</td>
</tr>
<tr>
<td>Potassium hydroxide solution fraction (K-S)</td>
<td>262 (12.4)</td>
<td>168 (6.3)</td>
<td>247 (17.4)</td>
<td>377 (21.2)</td>
<td>454 (46.0)</td>
<td>665 (56.8)</td>
</tr>
<tr>
<td>Total</td>
<td>2,105</td>
<td>2,676</td>
<td>1,416</td>
<td>1,774</td>
<td>988</td>
<td>1,171</td>
</tr>
</tbody>
</table>

( ) : % total galacturonic acid

Fig. 4 Scanning electron photomicrographs of cassava roots
1) Cross-section of fresh (raw) cassava roots (cv. ‘Lakan’)
2) Cassava root (cv. ‘Lakan’) after storage for 28 days
3) Raw cassava root (cv. ‘Datu’)
4) Cassava root (cv. ‘Datu’) boiled at 100°C for 1 h.
(Primary magnification: ×600)
and quality of pectic substance. The results in Table 6 show that water soluble pectic substances increased by pyrolyzer during cooking.

**Scanning electron microscopy (SEM) of root tissue**

The changes in appearance occurring in cassava (cv. 'Lakan') root tissue during storage were observed through SEM; typical photographs of these observations are shown in Fig. 4-1 and 4-2. Destruction of the root-cell walls was observed to have occurred after 28 days of storage. The SEM appearance of raw cassava (cv. 'Datu') root is shown in Fig. 4-3. The appearance of the same root after boiling for 1 hour is shown in Fig. 4-4, where it can be seen how starch granules in the tissue became gelatinized and swollen, and also how some of the granules have ruptured.

**Summary**

Roots of two cassava cultivars (cv. 'Lakan' and cv. 'Vassourinha'), and one cultivar of yambean were stored under ambient conditions for 18 to 28 days. The following results were obtained:

1. Sugar content was determined by a high performance liquid chromatography (HPLC). The total sugar contents essentially increased during the first one or two weeks, whereas linamarin content of the cassava roots increased markedly in 7 days, continued to increase for several days, then decreased gradually and almost disappeared before decay. Root deterioration was observed to occur faster in cv. 'Vassourinha' than in cv. 'Lakan'. In yambean root moisture content was more than 80% and the amount of total sugar was higher than that of cassava roots.

2. The total sugar content of cassava roots was increased by heat treatment. Linamarin content, however, decreased significantly, as was empirically foreseen.

3. The pectic substances were extracted fractionally into four fractions from the roots. The water soluble pectin (W-S) tended to increase, while protopectin showed a slight decrease at the end of storage. The changes in appearance that occurred in cassava root tissue during storage were observed under a scanning electron microscope.

**Acknowledgements:** The authors would like to acknowledge the continuing guidance and encouragement of Dr. Hiroyasu Fukuba, Professor of Ochanomizu Women's University, and to express their thanks to Ms. Naomy Sabiniano, Virgie Almenteros, Lucile Abad, and the other staff of the Food Chemistry Laboratory, Institute of Food Science and Technology of the Philippines at Los Baños, for their assistance in the experiments.

**References**

キャッサバおよびヤムビーン根茎の構成糖に及ぼす
貯蔵と加熱処理の影響

川崎晶子*・澤山 茂・リカドー R. デル
ロザリオ**、マリサ G. ソエル**

(* 東京農業大学農学部栄養学科 〒156 東京都世田谷区坂丘1-1-1)
(** フィリピン大学ロスバニオス校食品工学研究所)

キャッサバおよびヤムビーン根茎の収穫直後、貯蔵中
および加熱処理による構成糖の変化を高速液体クロマト
グラフを用いて定量し、検討した。根茎類は室温（28～
32℃、相対湿度66〜88％）で、18〜28日間貯蔵された。
キャッサバ根茎中の合計糖含量は、貯蔵初期2週間に顕
著な増加を示した。合計糖含量に対する比率では、シュ
クロースの含量が最も高かった。貯蔵期間中、フラクト
ースとグルコースが増加するのに対し、シュクロースは
減少した。リナビリン（クエン酸糖体）は、貯蔵1週間目
に顕著な増加を示したが、それ以後減少し、貯蔵終期には
殆ど消失していた。ヤムビーン根茎中からは、シュク
ロース、フラクトース、グルコースの3種の糖のみが定
量された。4 画分に分別定量したペクチン質では、少量
ながら、貯蔵期間において水溶性ペクチンの増加の傾向
が認められた。走査型電子顕微鏡によって組織を観察し
た結果、収穫直後と比べて、貯蔵28日後の細胞組織は
崩壊し、また、加熱によるデンプンの変化などをとらえ
ることができた。