EXCRETION OF VITAMIN C DURING GERMINATION OF LEGUMINOUS SEEDS

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Germination of seeds is generally preceded by a process of steeping in flowing water which serves to soften the cell walls and equalise the moisture of the tissues. It is familiar knowledge that the process of germination is attended by a variety of changes, mostly enzymic in nature; in fact, germinating seeds show increase in the activity of most enzymes. As a result, there is a considerable breakdown of the proteins into simpler peptides and amino acids, a partial saccharification of the starch and a release of inorganic phosphates from the phytin. The biochemical changes that occur during germination are typified by the studies on the chemistry of malting.

Taking into account the high solubility as well as extreme lability of ascorbic acid and considering that during the steeping and subsequent sprouting of seeds, their moisture content varies from 60–90 per cent., it appeared worthwhile to study whether, in addition to the elaboration of the vitamin in the germinated seed, a part of it was also excreted into the surrounding medium. It is possible that such an observation has not been recorded by earlier workers because of the high susceptibility of the vitamin to rapid oxidation to dehydro-ascorbic acid and, irreversibly, to diketogulonic acid.

In studies on the excretion of amino acids by legumes, Virtanen and co-workers have shown that a considerable part of the nitrogen fixed by root nodule organisms is secreted chiefly as aspartic acid and as degraded β-alanine; this secretion of nitrogen is favoured in presence of media which could absorb the excreted nitrogen. The old established fact that non-legumes benefit by an association with legumes, is explained on this basis; in fact these authors observed an increased excretion of nitrogen in associated cultures of inoculated legumes and non-legumes capable of utilizing the excreted nitrogen compounds.

EXPERIMENTAL

Preliminary overnight soaking of the seeds in water was done either in beakers or in Petri-dishes; subsequent germination was allowed to proceed by spreading out the soaked seeds in Petri-dishes lined with moistened...
cotton wads. In order to provide for prevention of auto-oxidation of any ascorbic acid that might be excreted either in the steep water or during germination, recourse was had to the use of thiourea and sulphur dioxide, the latter as potassium meta-bisulphite. These compounds are known to stabilize aqueous ascorbic acid against oxidation.\textsuperscript{11-12} It was observed, however, that thiourea, even in concentrations as low as 500 p.p.m., inhibited germination; hence, the studies reported here relate only to the use of potassium meta-bi-sulphite in a concentration of 300 p.p.m.; at this concentration, germination was apparently normal and comparable to control lots steeped in or treated with water alone.

Ascorbic acid and total ascorbic acid (ascorbic acid plus dehydro-ascorbic acid) determinations were carried out both in the steep water used during preliminary soaking and in the moist cotton wads at different intervals during germination. Mapson's procedure\textsuperscript{13} for the determination of the vitamin in presence of sulphites, sulphides and thiol compounds was adopted; from recovery experiments, it was always possible to secure an accuracy within 2-3 per cent.

For germination in the dishes, the cotton wads were kept moistened with water containing 300 p.p.m. of potassium meta-bi-sulphite. In the examination of cotton wads for the presence of ascorbic acid during various stages of germination, the wads were first drained out of the absorbed meta-bi-sulphite solution, extracted at least twice with the least quantity of water containing potassium meta-bi-sulphite and made up to volume. An aliquot of the total extract was made to 4 per cent. strength of oxalic acid for the determination of ascorbic acid and total ascorbic acid.\textsuperscript{4} Results represent averages of triplicate determinations.

**Results**

When mung (\textit{Phaseolus radiatus}) seeds were soaked overnight in 5-6 times water, it was observed that the steep water did not contain any ascorbic acid or dehydro-ascorbic acid, although the ascorbic acid content of the soaked seeds had risen from nil to 40 mgm. per cent. on dry basis. On the other hand, if instead of water, a solution of potassium meta-bi-sulphite (300 p.p.m.) was used for steeping, ascorbic acid could always be detected in the steep water. Thus, using 10 gm. lots of mung seeds, the results obtained after varying periods of soaking are given in Table I together with similar results for Masoor (\textit{Lens esculenta}) and black pea (\textit{Pisum arvense}); with the latter sets, the solutions in which the seeds were soaked turned pink on acidification and hence a modified procedure for coloured extracts\textsuperscript{14} was followed.
Excretion of Vitamin C during Germination of Leguminous Seeds

TABLE I

Ascorbic acid excretion during steeping

<table>
<thead>
<tr>
<th>Legume</th>
<th>Period of soaking (in hours)</th>
<th>Excreted ascorbic acid (in mg.)</th>
<th>Excreted total ascorbic acid (in mg.)</th>
<th>Total ascorbic acid in 10 g. of dry seeds (in mg.)</th>
<th>Per cent. excreted total ascorbic acid (with mean deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mung</td>
<td>4</td>
<td>0.15</td>
<td>0.17</td>
<td>1.63</td>
<td>10.4±1.61</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.33</td>
<td>0.42</td>
<td>3.11</td>
<td>13.5±0.53</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.54</td>
<td>0.72</td>
<td>4.80</td>
<td>15.0±0.83</td>
</tr>
<tr>
<td>Masoor</td>
<td>4</td>
<td>0.11</td>
<td>0.15</td>
<td>1.91</td>
<td>13.0±1.48</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.32</td>
<td>0.34</td>
<td>1.85</td>
<td>18.4±1.33</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.51</td>
<td>0.69</td>
<td>3.20</td>
<td>21.6±1.35</td>
</tr>
<tr>
<td>Black pea</td>
<td>4</td>
<td>0.09</td>
<td>0.12</td>
<td>1.01</td>
<td>11.9±0.82</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.21</td>
<td>0.27</td>
<td>1.87</td>
<td>14.4±0.54</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.62</td>
<td>0.72</td>
<td>3.60</td>
<td>20.0±0.88</td>
</tr>
</tbody>
</table>

As reported earlier, no dehydro-ascorbic acid was present in the seeds either after overnight soaking or during the initial phases of subsequent germination. The steady increase in its proportion with steeping would suggest increased oxidation of the excreted ascorbic acid; it is possible that there has also been some partial destruction of the oxidized form of the vitamin.

When the seeds after preliminary overnight soaking were allowed to germinate in dishes with cotton wads moistened with 300 p.p.m. solution of potassium meta-bi-sulphite, ascorbic acid could be detected in the cotton wads only during the first 24 hours (i.e.), on the 2nd day of germination, this period being counted from the commencement of steeping. Results are given in Table II.

TABLE II

Excretion of ascorbic acid during germination

<table>
<thead>
<tr>
<th>Legume</th>
<th>Excreted ascorbic acid in mg.</th>
<th>Excreted total ascorbic acid in mg.</th>
<th>Total ascorbic acid in 10 g. of seeds in mg.</th>
<th>Per cent. excreted total ascorbic acid (with mean deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mung</td>
<td>0.42</td>
<td>0.55</td>
<td>8.9</td>
<td>6.9±1.27</td>
</tr>
<tr>
<td>Masoor</td>
<td>0.42</td>
<td>0.52</td>
<td>4.2</td>
<td>12.4±1.07</td>
</tr>
<tr>
<td>Black pea</td>
<td>0.50</td>
<td>0.66</td>
<td>4.7</td>
<td>14.0±0.81</td>
</tr>
</tbody>
</table>

It was ascertained that there was negligible excretion during subsequent germination over a period of 8 days.
In his experiments on amino acid excretion, Virtanen (loc. cit.) had observed that excretion occurred only in media capable of absorbing the excreted amino nitrogen. To find out whether ascorbic acid excretion was modified by the presence of an absorbing medium, 10 gm. lots of mung were soaked overnight in 300 p.p.m. solution of potassium meta-bi-sulphite in beakers and in petri-dishes lined with cotton wad (Table III). The former was merely a water medium, while the latter provided a medium capable of absorbing excreted vitamin.

**TABLE III**

*Excretion of ascorbic acid in relation to absorbing medium*

<table>
<thead>
<tr>
<th>Ascorbic acid content of mung (10 g.) in beaker mg.</th>
<th>Excretion in beaker</th>
<th>Ascorbic acid content of mung (10 g.) in dish mg.</th>
<th>Excretion in dish with cotton wads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid content of mung (10 g.) in mg.</td>
<td>4.8</td>
<td>0.69</td>
<td>4.7</td>
</tr>
<tr>
<td>Total ascorbic acid in mg.</td>
<td>0.81</td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>Total ascorbic acid in mg.</td>
<td>6.02</td>
<td></td>
<td>0.56</td>
</tr>
</tbody>
</table>

Excretion was higher in the beaker than in the dish by \(42.1 \pm 6.02\) per cent. It is obvious that the low figures of free and total ascorbic acid in the dish have been due to pronounced irreversible oxidation of the vitamin on account of the larger surface area of exposure.

**DISCUSSION**

In the germinating seed, the leaf is the seat of maximum ascorbic acid elaboration, while the radical and stalk has a significantly low value.\(^4\) The excretion observed had taken place when the radical had not pierced through the seed coat. This would mean that the ascorbic acid was secreted not from the radical but probably through the seed coat by osmosis. The absence of excretion at later stages of germination must be due to the fact that the ascorbic acid content of the radical which is then the only part in contact with the medium is very low so that, even if a part of the vitamin is secreted into the surrounding medium, the quantity would be very small.

Though excretion of ascorbic acid is appreciable as compared to the ascorbic acid contents of the seed on 1st day of steeping, the total excretion is negligible when compared to the ascorbic acid potency of fully germinated seed. Thus, in mung seeds, the ascorbic acid excreted during soaking is about 0.7 gm. while the ascorbic acid content of the seeds that cause this excretion is about 5 mgm., but the same quantity of seeds would have elaborated about 15 mgm. of ascorbic acid after germination for 5 days.
**Excretion of Vitamin C during Germination of Leguminous Seeds**

**SUMMARY**

Excretion of small but definite and measurable amounts of ascorbic acid takes place during the preliminary period of soaking prior to germination of leguminous seeds. There is no secretion of the vitamin into the surrounding medium during the subsequent normal germination period.

**REFERENCES**

1. Giri and Sreenivasan
2. ————
3. Hopkins and Krause
4. Sreenivasan and Wandrekar
5. Virtanen
   .. *Biochem. Zeit.*, 1933, 258, 106.
7. ——— and v. Hausen
8. ——— and Laine
9. ——— and Tormainen
10. Sisakyan and Vasileva
    .. *Biokhimiya*, 1945, 10, 117.
11. Anon
    .. *Nature*, 1944, 153, 384.
12. Kendrick and Downer
13. Mapson
14. Harris and Olliver