Studies on the pithiness of radish. I.

Relationship between the degree of pithiness and organic constituents in radish root

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INTRODUCTION

It is well known that pithiness in the radish root occurs as the plant ripens or grows old. Hagiya (1952) pointed out the difficulty of nutrients transport through the conducting tissue to the parenchyma on the occurrence of the pithy tissue. Fujimura (1957), based upon anatomical observations, stated that the process of maturation is particularly accompanied by the loss of pectate from the middle lamella and as dissolution of pectates continues, the parenchymatous cells become entirely separated from one another. These spaces with air bubbles gradually increase in size, and are finally recognized with the naked eyes. This process is so-called pithiness. Since these separated cells are no longer capable of translocating solutes, the cells become deficient in a nutrient.

In spite of the effort of many investigators, our knowledge on the pithiness is still limited to that obtained through anatomical observations. In order to elucidate the mechanism of pithy tissue formation and to prevent the pithiness, it is necessary to have data on organic constituents of the radish root having pithy tissue.

In the present study was attempted a gross analysis of the radish root to perceive the relationship between the degree of pithiness and organic constituents, and an attempt was also made to decide an appropriate method for the quantitative examination of vegetable products. In his recent papers Lehmann (1956, 1960) has thrown light on the method of their quantitative examination. As this method constituted a simple technique for the separation of organic constituents, the author applied it in his study.

MATERIAL AND METHOD

Seeds of *Rhaphanus sativus* LINN., variety Rapid Red, were sown in clay loams in the growing box (48×38×19 cm). Germinated plants were thinned to about 20 plants per box. When their roots were approximately 2.5 cm in diameter, they were sampled and graded by area of the pithy tissue in a cross section into 0, 0.5, 1, 2, 3, 4 (after Fujii 1941). Sap expressed from fresh sections was used for determination of soluble solids by Abbe's Refractometer.

The tissues were killed in an oven at 105°C and dried at approximately 75°C for about 48 hours in a forced draft oven. The dried material was ground and sieved through a small sieve having round holes, 0.5 mm in diameter, for analysis. The specific gravity of tissue powder was determined by the "powder method" (Koketsu 1924) and its values

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were expressed in gram per 1 cm³ powder volume of dry matter, because it has been proved by TAMAI and KÖKETSU (1933) that the specific gravity of tissue powder is directly proportional to the specific gravity of tissue. The former may be therefore used as a measure of firmness or solidness of tissue.

LEHMANN's method for fractionation of organic constituents is diagrammed in Fig. 1. In the first procedure one gram of the tissue powder is extracted with ether to remove crude fats, and the residue was then extracted with concentrated formic acid to remove protein, starch and low molecular substances. This procedure is primarily useful for preparation of cell wall substances. Separation of pure components from a mixture of protein and starch are as follows:

Preparation of starch: - 25 ml of formic acid solution is pipetted into a 500 ml ERLENMEYER flask. The starch is precipitated with acetic acid/benzene (125 : 2 v/v).

Preparation of protein: - The soluble part may now be fractionated with 150 ml of a mixture of ether/benzene/petroleum ether (1 : 1 : 1 v/v) to separate protein and low molecular substances from each other. In this procedure, the solution was centrifuged in a centrifuge tube for 10 minutes at 3,000 rpm.

Each of the precipitates, protein, starch and cell wall material, is washed with ether and dried in an oven at 90°C and weighed.

Separation of pectic components from cell wall material is accomplished by extraction with hot water, 1% oxalic acid and 0.5% ammonium oxalate. The extract is freed of oxalate by precipitation with acetone and alcohol. The precipitate is washed with
alcohol, dried and weighed. In the second method, separation of pectic substances and hemicellulose from cell wall material is accomplished by extraction with 0.5% ammonium oxalate. The residue then is extracted with 4% sodium hydroxide to separate hemicellulose. This method is diagrammed in Fig. 2.

RESULTS AND DISCUSSION

RELATIONSHIP BETWEEN PITHINESS AND THE CONCENTRATION OF SOLUBLE MATTER

It is a well established fact that the concentration of soluble matter can be used as a measure of physiological activity in the tissue. For example, the radish root shows, while enlarging, an increasing rate of translocation of assimilates and of mineral elements (MIYAZAKI). The root grown to its full size is richly stored with carbohydrates, especially with sugars. Therefore the concentration of soluble matter in the root is expected to increase during its enlarging growth.

Table 1 shows the concentration of soluble matter determined by a refractometric method. It reveals that the concentration of soluble matter tends to decrease when the tissue becomes pithy. This is in agreement with FUJI, HAGIYA, and MIYAZAKI's results. In fresh tissues, however, the concentration of soluble matters varies with the water content of individual roots. As shown in table 1, significant differences

<table>
<thead>
<tr>
<th>Degree of pithiness</th>
<th>Number of plants determined</th>
<th>Concentration of soluble matter %</th>
<th>L.S.D. (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>5.15</td>
<td>1.412</td>
</tr>
<tr>
<td>1</td>
<td>36</td>
<td>4.66</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>4.53</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3.93</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Relationship between the degree of pithiness and the concentration of soluble matter in radish root.
in the concentration of soluble matter were not found among the degrees of pithiness. Details on this point will appear elsewhere. It is therefore impossible to use the concentration of soluble matter as a measure of the degree of pithiness.

**RELATIONSHIP BETWEEN PITHINESS AND THE SPECIFIC GRAVITY OF TISSUE POWDER**

If the specific gravity of tissue powder is directly proportional to the true specific gravity of tissue, the former may be used as a measure of firmness of tissue. And there appears to be some relationship between the specific gravity of tissue powder and the degree of pithiness.

<table>
<thead>
<tr>
<th>Degree of pithiness</th>
<th>Specific gravity of tissue powder (g/cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.483</td>
</tr>
<tr>
<td>1</td>
<td>0.464</td>
</tr>
<tr>
<td>2</td>
<td>0.418</td>
</tr>
<tr>
<td>3</td>
<td>0.386</td>
</tr>
</tbody>
</table>

Table 2 clearly shows that the specific gravity of tissue powder is high in non-pithy tissue.

In another experiment, its strainal difference was investigated in Japanese radish variety Shōgoin. These values are as follows:

Early : 0.729 Mid-season : 0.730
Late : 0.910 Improved* : 0.864

As early strain of rapid growth is low and a late one of slow growth high in the specific gravity. Furthermore a strain resistant to the occurrence of pithiness has a high value. These results indicate there is a close relationship between pithiness or solidness of the root and the specific gravity of tissue powder. Although the author is not here concerned with the specific gravity of fresh roots, the above relationship must have existed in fresh roots. In fact, Watts (1960) stated that the difference between solid and pithy roots would be reflected by the specific gravity of the latter. And he produced such a variety by selecting plants with roots having the least pithiness.

**RELATIONSHIP BETWEEN PITHINESS AND ORGANIC CONSTITUENTS**

In view of the above results there is some relationship between the degree of pithiness and solidness of tissue. It may be suggested that the content of organic constituents in roots varies to the degree of pithiness. Of organic constituents, it is well known that starch content influences the specific gravity of radish root (Kumazawa and Nishimura 1939) and that the process of maturation is accompanied by the loss of pectate from middle lamella (Fujimura 1957). An experiment was designed to confirm this information. Table 3 gives the data obtained by the formic acid method for fractionation of organic constituents.

<table>
<thead>
<tr>
<th>Degree of pithiness</th>
<th>Dry matter</th>
<th>Crude fat</th>
<th>Starch</th>
<th>Protein</th>
<th>Cell wall material</th>
<th>Pectic substances</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.35</td>
<td>5.04</td>
<td>4.45</td>
<td>11.08</td>
<td>32.9</td>
<td>10.76</td>
</tr>
<tr>
<td>1</td>
<td>7.76</td>
<td>4.77</td>
<td>3.94</td>
<td>7.22</td>
<td>32.9</td>
<td>9.81</td>
</tr>
<tr>
<td>2</td>
<td>7.76</td>
<td>3.23</td>
<td>3.11</td>
<td>6.90</td>
<td>34.2</td>
<td>7.25</td>
</tr>
<tr>
<td>3</td>
<td>7.07</td>
<td>2.92</td>
<td>2.71</td>
<td>8.09</td>
<td>35.2</td>
<td>6.74</td>
</tr>
</tbody>
</table>

* Improved (Kairyo-Su-Irazu)—a strain resistant to the occurrence of pithiness.
It is shown that there is a fall in the content of dry matter on the occurrence of pithiness in root tissues. The contents of crude fat, starch, protein and pectic substances show the same tendency, but the content of total cell wall material is inversely relative to them. These findings support the view that there is a shortage of assimilate supply in vacuole and protoplasm in pithy tissues (Fujii 1941 and Hagiya 1952). Even if the cell contents were altered during maturation through extensive deposition of cell material, the change in cell wall material is even greater than that. This fact seems to suggest that a fall in cell content may be a reflection of a higher rate of respiration due to schizogenetic isolation of parenchymatous cells. In his unpublished data, the author found a higher rate of O$_2$ consumption in pithy tissues than in normal ones. This is in agreement with Fukushima and Masui's result (1953).

The author may now consider the change of cell material. Table 3 and 4 show that total cell wall materials increased during maturation, while pectic substances diminished in concentration in pithy tissues. The parallelism between the decrease in concentration of pectic substances and the increase in concentration of total cell wall material in pithy tissues has led to speculation that pectin itself might be transformed into lignin and non-cellulosic polysaccharide. But such a transformation is unlikely because of the basic difference in the nature of these substances.

<table>
<thead>
<tr>
<th>Degree of pithiness</th>
<th>Cell wall materials (on dry weight basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hemicellulose</td>
</tr>
<tr>
<td>0</td>
<td>5.75%</td>
</tr>
<tr>
<td>1</td>
<td>4.78%</td>
</tr>
<tr>
<td>2</td>
<td>4.23%</td>
</tr>
<tr>
<td>3</td>
<td>4.10%</td>
</tr>
</tbody>
</table>

*as Ca-pectate.

In another analysis, total pectic substances in the normal tissue and pithy one were 11.9 and 5.42%, respectively. From these results it may be concluded that the pectic substances actually disappear during the process of maturation. The results obtained in the analysis of cell wall material were essentially similar to those described by Fujimura for the process of pithiness.

Hemicellulose is also present in the root but is quantitatively of less importance. Skoog stated that the ratio of pectic substances to hemicellulose changed during maturation. In this experiment no relationship was found between pectic substances and hemicellulose. In other data, pectic substance/hemicellulose ratio in the normal tissue and pithy one were 3.17 and 1.36, respectively. At any rate, it seems that cell walls change greatly in composition during maturation of the root.

**SUMMARY**

In the present study was attempted a gross analysis of the radish root to perceive the relationship between the the degree of pithiness and organic constituents. In analysing them, the author repeated the formic acid method by Lehmann to separate organic constituents.

The root of *Rhaphanus sativus* Linn., variety Rapid Red, has been used as the material. The concentration of soluble matter tends to decreases when the tissue becomes pithy.
In the fresh tissue, however, its concentration varies with the water content of individual roots. Therefore the concentration of soluble matter cannot be used as a measure of the degree of pithiness.

The specific gravity of tissue powder is high in non-pithy root and in the root of a strain resistant to the occurrence of pithiness. Judging from these results, the differences in the contents of cell wall material must be significant between the non-pithy tissue and the pithy one.

The vacuolar and protoplasmic contents, such as crude fat, starch and protein, were low in the pithy tissue, but the content of total cell wall material was inversely relative to them. These findings support the view that there is a shortage of assimilate supply in vacuole and protoplasm of pithy tissues. On the other hand total cell wall material increased slightly and the pectic substances decreased markedly in content in the root showing a high degree of pithiness. The content of hemicellulose did not change greatly in the process of pithiness in the root. This was associated not only with deposition of woody elements, but also with decrease in content of pectic substances. In conclusion, then, the occurrence of schizogenous intercellular spaces among the parenchymatous cells is due to the disappearing of pectates in the middle lamella. This isolation of cells would result in the difficulty in assimilate supply from the conducting tissue to the parenchyma and in its shortage in vacuolar and protoplasmic contents.

I wish to express my thanks to Dr. T. TSUKAMOTO, Prof. of Kyoto University under whose guidance the original plan has been carried out. I also wish to express my thanks to Dr. M. SISA, Prof. of Nagoya University for his kind advice and encouragement.

REFERENCES

ダイコンのすいり現象に関する研究（第1報）

ダイコンのすいり程度と有機成分との関係

高野 泰吉
（名古屋大学農学部）

摘 要
脂肪、でんぶん、蛋白はすいり根で少なく、細胞膜はすいり根で多かった。細胞膜の構成分であるベクチン質はすいり程度の進んだ根で著しく少なくなるが、ヘミセルロースは著しい変化を認めなかった。

結論として、柔細胞の離生関係発生によるすいりは細胞膜中葉のベクチン質の消失によっておこる。その結果細胞が孤立して皮膚組織から柔組織への同化物供給が困難となり、細胞含有物の不足となるものと考えられる。

抄 録

リンゴ葉におけるNAAの吸収、転流、代謝

NAAはリンゴの摘果剤あるいは落果防止剤として研究されているが、品種および環境条件などにより、その効果が一定せず、まだ安全な使用法が確立していない。そこで著者らはNAAの効果の不安定の原因を究明するため\(^1\)CでラベルしたNAAを用いて、リンゴ葉でのNAAの吸収、転流、代謝について調査した。

Bramley's Seedlingの葉の上に20 ppmの\(^1\)C-NAAを1 ml滴下し、湿室内に4日間放置した後、その葉内吸収量を調査したところ、処理葉の約10%しか吸収されていなかった。そして残りの90%のうち、約10%は葉面に存在していたが、80%は消失していた。そこでその消失の原因を調べたところ、紫外線によりオキシシンとしては不活性な中性物質（紫外線吸収スペクトルではNAAに類似している）に変化したためと思われた。

Miller's Seedlingの花蕾の基葉に50 ppmの\(^1\)C-NAAを処理し、転流について調べたところ、葉内に入った\(^1\)C-NAAの17〜28%は4〜5日で果実の部分へ転流していることが認められた。しかしこの転流\(^1\)Cは\(^1\)C-NAAそのものから得られたものではなく、大部分は\(^1\)C-NAAの代謝産物から検出されたものであった。

次にBramley's SeedlingとCox's Orange Pippineの葉内の\(^1\)C-NAA代謝産物をペーパークロマトグラム法と電気泳動法で調査したところ、葉内に吸収された\(^1\)C-NAAの80〜80%は、ただちに水溶性のCompound I（Rf 0.6〜0.7，中性，オキシシンとして不活性）に変化した。さらに数日たとこのCompound IはCompound II（Rf 0.1〜0.2，酸性）へと移行していた。この移行の早さはCox's Orange Pippineの方がBramley's Seedlingより早かった。

以上のことから著者らはリンゴに対するNAAの摘果剤あるいは落果防止剤として効果が一定しないのは、紫外線によるNAAの分解現象や、品種特有のNAA代謝能力の差が関係しているのではないかと述べている。

（寺沼公士）


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