Some Properties of Starches of Job's Tears†

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Distribution of isoamylase-debranched starch components, X-ray diffraction patterns, gelatinization characteristics by photopastegraphy and differential scanning calorimetry, and starch-granule susceptibility to amylases were investigated for starches of Job's tears (hatomugi, Coix lacryma-jobi L. mayuen (Roman) Stapf. and juzudama, Coix lacryma-jobi L.). Starches were prepared from seeds of hatomugi and juzudama cultivated or collected in Japan and Korea. There were two types of grain among hatomugi open-cultivated at Nakazato and Okayama. Therefore, the waxy-type grains were separated from the nonwaxy-type ones by iodine staining of endosperms.

The results obtained were as follows; (1) Fundamentally, endosperms of hatomugi had waxy-type starch and those of juzudama had nonwaxy-type starch. Starch of nonwaxy-type Nakazato was resemble to that of juzudama. (2) Starches of hatomugi and juzudama were close resemblance in distribution of starch components and gelatinization characteristics to those of waxy and nonwaxy-type of maize, respectively. (3) All starches had A-type pattern of X-ray diffractograms. (4) Starch-granules of juzudama showed a wide range of susceptibility values to amylases. The phenomenon indicated that the starches had a wider range of characteristics than those of domesticated plants. (5) There was a great resemblance in properties of endosperm starches between Job's tears and maize.

Juzudama (Coix lacryma-jobi L.), one of Job's tears, belongs to the same family (Gramineae) and tribe (Maydeae) as those of maize (Zea mays L.) on botanical classification. In the case of hatomugi, the other Job's tears, there are two opinions in the classification, which have not been settled yet. One is that hatomugi (Coix lacryma-jobi L. ma-yuen (Roman) Stapf.) is a variety of juzudama, and the other is that hatomugi belongs to Coix ma-yuen Roman., and a different species from juzudama. But generally, the former opinion has been more acceptable.

Hatomugi was domesticated first in the India-Burma area for shifting cultivation before upland rice was established. However, hatomugi was accepted as a medicinal herb rather than foods in Japan. Their seeds had several pharmacological effects for some diseases and was also used for nourishing food. Juzudama has been believed to be native to Asia and was utilized for similar purpose to hatomugi. Thus, hatomugi and juzudama have been considered as materials of the herb medicine or foods for keeping health. Recently, both plants have been brought to attention as forage crops and materials of food industry because they are able to grow on lowland fields in place of paddy rice.

Grains (hulled seeds) of Job's tears contain 50-60% of carbohydrates, of which starch is the main component. Several reports suggested that...
endosperm starch of juzudama was nonwaxy-type and that of hatomugi was waxy-type. Several cereals; rice, barley, foxtail millet, maize, proso millet and sorghum, have both waxy mutants and their normal or nonwaxy counterparts in each one of the same species. Kempton reported that he found nonwaxy-type grains among the seeds classified as ma-yuen. It is very interesting point in connection with botanical classification whether hatomugi has waxy mutant only.

There are only few reports on starches of Job's tears in these years. Sato and Miyata reported properties of starches from hatomugi, juzudama and hybrid between hatomugi and juzudama. But there is no work in which properties of several kinds of hatomugi and juzudama starches were investigated at the same time. We already reported some properties of starches from several cereals which have both waxy and nonwaxy endosperms in each one of the species. In this study, fundamental properties of starches are investigated for several kinds of hatomugi and juzudama cultivated or collected in Japan and Korea.

MATERIALS AND METHODS

Seeds. Places of cultivation or collection and sample numbers of the seeds which were used for preparation of starch-granules are shown in Table 1. Seeds of Okayama, Nakazato and Toyama of hatomugi and those of Kanagawa, Tokyo and Miyazaki of juzudama were cultivated or collected in Japan, and others were collected in Korea.

There were two types of grain in Okayama and Nakazato, one was stained reddish-brown with iodine solution and the other blue. Therefore, the waxy-type grains were separated from the nonwaxy-type ones by iodine staining of endosperms. Seeds of Okayama and Nakazato had 2.4% and 40.5% of nonwaxy-type, respectively. Then the waxy-type grains of Okayama and Nakazato, and the nonwaxy-type grains of Nakazato were used for preparation of starch-granules. The samples were named as Okayama, Nakazato (waxy) and Nakazato (nonwaxy), and others were listed by sample numbers as shown in Table 1. Symbols of large letters (A–F) were given to hatomugi and those of small letters (a–f) to juzudama (see Table 1), and these symbols were used for Figs. 1–7.

Table 1. Places of collection and sample numbers of Job's tears.

<table>
<thead>
<tr>
<th>Hatomugi, Coix lacryma-jobi L. ma-yuen (Roman.) Stapf</th>
<th>Juzudama, Coix lacryma-jobi L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Toyama (76505)</td>
<td>a. Kanagawa (76501)</td>
</tr>
<tr>
<td>B. Korea (79517)</td>
<td>b. Tokyo (76502)</td>
</tr>
<tr>
<td>C. Korea (79519)</td>
<td>c. Miyazaki (76503)</td>
</tr>
<tr>
<td>D. Okayama</td>
<td>d. Kanagawa (77509-2)</td>
</tr>
<tr>
<td>E. Nakazato (waxy)</td>
<td>e. Tokyo (79513)</td>
</tr>
<tr>
<td>F. Nakazato (nonwaxy)</td>
<td>f. Korea (79514)</td>
</tr>
</tbody>
</table>

Preparation of starch-granules. Modified Watson's method was used for preparation of starch-granules. Briefly, hulled seeds were soaked over-night in 0.2% potassium pyrosulfite solution adjusted in pH 5 with potassium hydroxide. The grains were ground in a mortar and a pestle, and passed through nylon cloth screen, then 200 or 300 meshed sieves. Cold sodium hydroxide solution adjusted in pH 11 (approx. 0.1% NaOH) was added to the sieved granules and the mixture was kept in a cold room over-night. After the granules were washed with cold water several times, protein was removed from the granules shaking with isoamylalcohol or toluene. Then the granules were washed with water several times and ethanol, and dried at room temperature. The recovery of starch-granules based on weight of hulled seeds were 36.0–49.2%, and the starch-granules contained not more than 1% of protein by Lowry's method. It was observed that the starch-granules were polyhedral or round in shape and had various size (6–29 µm) by scanning electron microscopy.

Absorption spectra of starch-iodine complexes. Absorption curves of starch-iodine complexes were recorded by the method reported previously.

Gel filtration of isoamylase-debranched starches. Procedure for debranching of starches by Pseudomonas isoamylase and fractionation of debranched starches on a Sephadex G-75 column were described earlier. Total carbohydrate contents of each tube were determined by the phenolsulfuric acid method, and reducing ends of carbohydrates were measured with a modified Park-Johnson's method reported by Hizukuri and Takeda.

X-ray diffractometry. X-ray diffraction diagrams were recorded by the method reported.
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Previously.\(^{13}\)

**Photopastegraphy and differential scanning calorimetry.** Photopastegrams and DSC thermograms of the starches recorded as reported previously.\(^{13}\)

**Degradation of starch-granules by amylases.** These methods and source of amylases were reported previously.\(^{20}\)

**RESULTS AND DISCUSSION**

**Absorption spectra of starch-iodine complexes**

Figure 1 shows absorption curves of starch-iodine complexes from several Job's tears and non-waxy maize starch. Wavelengths at maximum absorbance (\(\lambda_{max}\)) and absorption intensities at 680 nm, that is "blue value" (BV), are shown in Table 2.

Iodine-absorption curves of hatomugi starches except Nakazato (nonwaxy) were drawn between C, 79519 and B, 79517 curves. In juzudama starches, curves were between b, 76502 and d, 77509-2 curves (Fig. 1).

Since \(\lambda_{max}\) of absorption curves were at 520–530 nm and BV showed low level for hatomugi except Nakazato (nonwaxy), these results suggested that the starches were waxy-type (Table 2). On the other hand, juzudama starches had 580–590 nm of \(\lambda_{max}\) and 0.36–0.40 of BV (Table 2). These values indicated that the starches were nonwaxy-type same as nonwaxy maize starch.

There was little difference in \(\lambda_{max}\) of iodine staining curves between Nakazato (nonwaxy) and juzudama starches. But absorption intensity of Nakazato (nonwaxy) was lower at each wavelength, and the starch might be a different type.

**Elution profiles of debranched starches by gel filtration**

Elution patterns of isoamylase-debranched starches of several Job's tears are shown in Fig. 2. Two peaks appeared for the waxy-type starches (Fig. 2A, 2E) and three peaks for the nonwaxy-type starches (Fig. 2F, 2b, 2d). The first peak, fraction I (Fr. I), comprised higher molecular-weight materials and regarded as amylose because absorption maxima of the iodine-carbohydrate complexes of the fraction were over 620 nm. The second and third peaks, fraction II (Fr. II) and fraction III (Fr. III), arose from amylopectin because chain-lengths of apices of their peaks were about 40 and 12–16, respectively.

Table 3 represents contents of each fraction, ratios of Fr. III to Fr. II (Fr. III/Fr. II) and average chain-lengths (\(\bar{C}\)) of each apices of Frs. II and III of all samples. Hatomugi starches except Nakazato (nonwaxy) had 0% of Fr. I and 3–4 of Fr. III/Fr. II. These values were similar
to those of other waxy-type starches. It is concluded that these hatomugi starches are waxy-type by the results. Hatomugi starches of #79517 and #79519 cultivated in Korea had somewhat higher contents of intermediate fraction. But contents of other fractions were resemble to those of hatomugi cultivated in Japan, and there was statistically insignificant difference for CL each other.

Starches of juzudama and Nakazato (nonwaxy) had 22–26% of Fr. I, no more than 5% of intermediate fraction and 3.4–3.8 of Fr. III/Fr. II (Table 3). These results were similar to those of nonwaxy maize, and indicated that these starches were nonwaxy-type.

X-ray diffractograms

X-ray diffraction diagrams of hatomugi starches are shown in Fig. 3 and those of juzudama starches in Fig. 4. These patterns indicated that starches of Job's tears had A-type crystalline structure according to Katz's classification. Diagrams of #79517, #79519 and #79514 (data were not shown here) were also A-type structure.

Photopastegrans of starches

Photopastegrans obtained from several starches are shown in Figs. 5 and 6. Concentrations of the starch suspension were 0.2% for Fig. 5 and 0.1% for Fig. 6. After one-step alterations, decrease of transmittance were observed in waxy-type starches of other job's tears. The elution patterns on a Sephadex G-75 column of isoamylase-debranched starches of Job's tears are shown in Fig. 2. Elution patterns on a Sephadex G-75 column of isoamylase-debranched starches of Job's tears.

Table 3. Summary of some properties of isoamylase-debranched components of starches of Job's tears.

<table>
<thead>
<tr>
<th></th>
<th>Fr. I (%)</th>
<th>Intermediate Fr. (%)</th>
<th>Fr. II (%)</th>
<th>Fr. III (%)</th>
<th>Fr. III/Fr. II</th>
<th>Fr. III (CL)</th>
<th>Fr. II (CL)</th>
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<tr>
<td>Hatomugi</td>
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<td>76505</td>
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<td>1.2</td>
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<td>4.2</td>
<td>42</td>
<td>12</td>
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<tr>
<td>79517</td>
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<td>71.9</td>
<td>3.1</td>
<td>36</td>
<td>16</td>
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<tr>
<td>79519</td>
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<td>3.0</td>
<td>21.8</td>
<td>75.2</td>
<td>3.5</td>
<td>36</td>
<td>12</td>
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<tr>
<td>Okayama</td>
<td>0</td>
<td>0.7</td>
<td>21.0</td>
<td>78.3</td>
<td>3.7</td>
<td>38</td>
<td>14</td>
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<tr>
<td>Nakazato (waxy)</td>
<td>0</td>
<td>0.6</td>
<td>21.4</td>
<td>78.1</td>
<td>3.6</td>
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<td>13</td>
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<tr>
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<td>59.8</td>
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<td>39</td>
<td>14</td>
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<tr>
<td>Juzudama</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>3.3</td>
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<td>15</td>
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<td>21.8</td>
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<td>57.7</td>
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<td>57.1</td>
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<td>3.1</td>
<td>15.9</td>
<td>56.9</td>
<td>3.6</td>
<td>39</td>
<td>14</td>
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</table>

Each fraction (Fr.) was divided according to $\lambda_{\text{max}}$ of iodine-carbohydrate complexes as follows: Fr. I, $\lambda_{\text{max}} \geq 620$ nm; Intermediate Fr., 620 nm > $\lambda_{\text{max}}$ > 600 nm; Fr. II, 600 nm > $\lambda_{\text{max}}$ > 540 nm; Fr. III, 540 nm > $\lambda_{\text{max}}$. 

Fig. 2. Elution patterns on a Sephadex G-75 column of isoamylase-debranched starches of Job's tears.
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Fig. 3. X-ray diffractograms of starches of hatomugi.
A, 76505; D, Okayama; E, Nakazato (waxy); F, Nakazato (nonwaxy).

Fig. 4. X-ray diffractograms of starches of juzudama.
a, 76501; b, 76502; c, 76503; d, 77509-2; e, 79513.

hatomugi (Figs. 5D, 5E, 6A) as shown for those of waxy maize and rice. Juzudama and Nakazato (nonwaxy) showed typical two-step gelatination curves (Figs. 5F, 6a–6e) as shown in non-waxy-type cereal starches. Initiation tempera-

Fig. 5. Photopastegrams of several starches of Job’s tears.
D, Okayama; E, Nakazato (waxy); F, Nakazato (nonwaxy). Concentrations of the starch suspension were 0.2%.

Fig. 6. Photopastegrams of several starches of Job’s tears.
A, 76505; a, 76501; b, 76502; c, 76503; d, 77509-2; e, 79513. Concentrations of the starch suspension were 0.1%.
ture of gelatinization for juzudama starches were somewhat higher than those of hatomugi.

**DSC thermograms**

Thermograms of several starches are shown in Fig. 7. Onset temperature ($T_o$), peak temperature ($T_p$) and recovery temperature ($T_r$) were obtained by extrapolation as shown in thermogram (Fig. 7e), and heat of gelatinization were calculated by measuring the areas surrounded by thermogram curves and base lines. These values were summarised in Table 4 for all samples.

Waxy-type starches of maize have a wider range of gelatinization temperature than those of non-waxy-type. Similar phenomena were observed in the waxy-type hatomugi starches (Fig. 7). Namely, their $T_o$ values were lower than those of juzudama and heat of gelatinization was higher than those of juzudama (Table 4).

**Starch-granule susceptibility to amylases**

Figure 8 shows starch-granule susceptibility to hog pancreas $\alpha$-amylase and Fig. 9 shows those to a crude preparation of *Rhizopus amagasakiense* glucoamylase. These figures are represented by the ratio of percent degradation of starch-granule to percent degradation of nonwaxy maize starch which is equal to 100. Starch-granules of the waxy-type hatomugi were degraded 2-3 times faster than those of nonwaxy maize for both amylases. Degradation patterns of #79517 and #79519 (data were not shown here) were similar to those of waxy-type hatomugi. The phenomena were similar in the other cereals.

Starch-granule susceptibility of juzudama showed a wide variation, that was, #76502 showed similar susceptibility to the nonwaxy maize, while #77509-2, #79513 (Figs. 8 and 9). In #77509-2, #79513 (Figs. 8 and 9).

**Table 4.** DSC characteristics of starches of Job's tears.

<table>
<thead>
<tr>
<th>Starch</th>
<th>$T_o$ (°C)</th>
<th>$T_p$ (°C)</th>
<th>$T_r$ (°C)</th>
<th>Heat of gelatinization (cal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hatomugi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76505</td>
<td>61</td>
<td>70</td>
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<td>79517</td>
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<td>73</td>
<td>81</td>
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<td>79519</td>
<td>63</td>
<td>74</td>
<td>82</td>
<td>3.2</td>
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<tr>
<td>Okayama</td>
<td>64</td>
<td>69</td>
<td>79</td>
<td>2.8</td>
</tr>
<tr>
<td>Nakazato(waxy)</td>
<td>64</td>
<td>68</td>
<td>79</td>
<td>2.9</td>
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<tr>
<td>Nakazato(nonwaxy)</td>
<td>63</td>
<td>67</td>
<td>73</td>
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<tr>
<td><strong>Juzudama</strong></td>
<td></td>
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<td></td>
</tr>
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<td>76501</td>
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<td>75</td>
<td>83</td>
<td>3.4</td>
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</table>
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From the results, we confirmed that juzudama had nonwaxy-type starches and hatomugi was waxy-type ones except Nakazato (nonwaxy). Starches of Nakazato (nonwaxy) gave similar properties to those of juzudama. However, it is not clear whether the result means existence of nonwaxy-type of hatomugi nor xenia between hatomugi and juzudama because they easily cross each other.  

Few percents of nonglutinous (nonwaxy) rice grain mix among the glutinous (waxy) rice in open pollination because of xenia. Such 2–3 % of contamination as shown in Okayama is presumed xenia from the case of rice. But it is difficult to explain as the result of rarely occurred natural crossing that such large quantity of nonwaxy-type grains exist in Nakazato. Anyway the further study is in progress on the point.

There was insignificant difference between properties of starches of Job's tears cultivated in Japan and Korea. Differences for properties of starch were not observed according to their cultivated or collected places.

Finally, there is a great resemblance in properties of endosperm starches between Job's tears and maize.

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