

# Carbohydrate Composition of Apios Tubers Grown in Converted Paddy Fields and Common Fields in Aomori Prefecture

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In a previous study<sup>1)</sup>, for effective use of converted paddy fields (upland fields converted from paddy), we cultivated apios at three converted paddy fields and two common fields in Aomori and proved that apios tubers are a viable option for upland converted paddy fields. We also determined their carbohydrate composition, mono- and oligo-saccharide composition.

In this study, we compared the carbohydrate composition, mono- and oligo-saccharide composition, and starch grains of apios tubers harvested from the five fields in the previous study. In addition, we examined the effect of the 80% methanol-soluble fraction containing mono- and oligo-saccharides on the growth of DLD-1 human tumor and MRC5-30 cells.

We found there was no difference between apios harvested from upland converted paddy fields and apios harvested from common fields in Aomori.

Key words: upland field converted from paddy, *Apios Americana*, carbohydrate, mono- and oligo-saccharide, starch

## 1. INTRODUCTION

Today, Japan faces an important turning point in its 35 year rice policy, as upland crops (wheat, soybean, feed crops, buckwheat, etc.) are increasingly being raised in paddy fields instead of rice. However, there still exists a problem in that paddy fields are not used efficiently for rice production.

In previous studies<sup>1)2)</sup>, we chose apios (*Apios Americana* Medikes) as one paddy field alternative, and carried out a cultivation study comparing three upland fields converted from paddy and two common fields in Aomori. In addition, we compared the carbohydrate composition and mono- and oligo-saccharide composition, and analyzed the mono- and oligo-saccharide's effect on DLD-1 human tumor cell growth. The following conclusions were made:

- 1) Converted upland paddies and common fields produce similar yields of apios

tubers.

- 2) The carbohydrate composition of apios tuber is very similar to that of pulses.
- 3) DLD-1 human tumor cell growth is reduced by apios tuber oligosaccharides fractions.

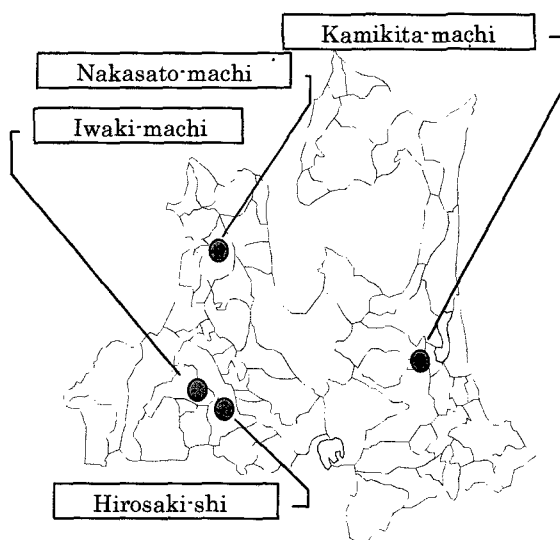
In this study, we compared carbohydrate composition, mono- and oligosaccharide and starch grains of apios tubers harvested in three upland converted paddy fields and two common fields in Aomori.

## 2. MATERIALS AND METHODS

### Materials:

Apios tubers harvested in 2003 were budded indoors in early April, grown to about 10 cm, and then planted in converted paddy fields in Hirosaki-shi, Nakasato-machi, and Kamikita-machi. In addition, apios tubers were planted in common fields in Hirosaki-shi and Iwaki-machi. These tubers were freeze-dried after

harvesting. The freeze-dried apios tubers were homogenized and triturated in a blender to give apios powder of Hirosaki-UFP (upland field converted from paddy), Nakasato-UFP, Kamikita-UFP, Hirosaki-CF (common field) and Iwaki-CF.



(Fig. 1 Cultivation sites in Aomori Prefecture)

#### Fractionation of carbohydrate in apios powder:

Carbohydrate of apios powder (1.0 g dry wt.) was fractionated into three fractions, mono- and oligo-saccharide fraction, starch fraction, and cell-wall polysaccharide fraction as described previously.<sup>1),2)</sup>

#### Fractionation of the 80% methanol soluble fraction by Bio-Gel P-2:

A portion of the 80% methanol soluble fraction was concentrated, freeze-dried, then dissolved in distilled water, and centrifuged. The supernatant was applied to a Bio-Gel P-2 column, and eluted with water. Fractions of 20ml each were collected and assayed for carbohydrates by the phenol-sulfuric acid method with glucose as a standard. Tubes 20-25, 26-36, 37-39, 40-44, and 45-60 were separately combined and concentrated to give fractions 1 to 5.

#### Mono- and oligo-saccharide analysis by HPAEC-PAD:

Mono- and oligo-saccharides in the 80% methanol soluble fraction were analyzed using HPAEC-PAD (High Performance Anion Exchange Chromatography with Pulsed

Amperometric Detection) on a DX-300 Dionex ion chromatograph, interfaced with an AI-450 workstation<sup>3)</sup>. The retention times of each sample were compared with those of standard sugars (glucose, fructose, sucrose, raffinose, stachyose).

#### Effects on DLD-1 human tumor and MRC5-30 cell growth:

The effects of 80% methanol soluble fractions, prepared from Hirosaki-UFP and Hirosaki-CF on the growth of DLD-1 human tumor cells, were examined according to the previous method.<sup>4)</sup>

#### Starch grains:

Apios powder (1.0 g dry wt.) was suspended in 30 ml of water, and then filtered through three layers of cheese cloths. The filtrate was re-suspended and filtered twice more. The final filtrate was centrifuged for 30 min at 9000rpm, and the precipitate was washed with acetone and dried to give starch.

The starch grains were examined with a scanning electron microscope and the size distribution was measured with a laser scattering particle size distribution analyzer (HORIBA LA-910).

### 3. RESULT AND DISCUSSION

#### Fractionation of carbohydrate in apios powder:

Apios powder obtained from Hirosaki-UFP, Nakasato-UFP, Kamikita-UFP, Hirosaki-CF and Iwaki-CF was separated into three fractions, mono- and oligo saccharide fraction, starch fraction and cell-wall polysaccharide fraction, and determined total carbohydrate contents. Table I shows the results.

(Table I Carbohydrate composition of apios tubers grown on the upland field converted from paddy and the common field.)

		Upland field converted from paddy			Common field	
		Hirosaki -UFP	Nakasato -UFP	Kamikita -UFP	Hirosaki -CF	Iwaki -CF
Mono- and oligo saccharides	mg	138.5	112.1	146.6	124.1	122.5
	(%)	(16.9)	(15.6)	(22.0)	(16.7)	(18.7)
Starch	mg	637.6	567.6	484.9	584.2	484.8
	(%)	(77.9)	(78.8)	(72.8)	(78.6)	(73.9)
Cell-wall	mg	42.3	40.4	34.7	35.4	48.8
Polysaccharides	(%)	(5.2)	(5.6)	(5.2)	(4.8)	(7.4)
Total	mg	818.4	720.1	666.2	743.7	656.1

The contents of total carbohydrate, mono- and oligo-saccharide, starch and cell-wall polysaccharide are different among Hirosaki-UFP, Nakasato-UFP, Kamikita-UFP, Hirosaki-CF and Iwaki-CF. However, the ratio of mono- and oligo- saccharides, starch and cell-wall polysaccharides was almost the same at all sites.

**Fractionation of the 80% methanol soluble fraction by Bio-Gel P-2:**

To compare the mono- and oligo-saccharide fractions among five apios powder, the 80% MeOH-soluble fraction were separated into 5 fractions (Fr), 1~5 by a gel-filtration chromatography on Bio-Gel P-2, on basis of degrees of polymerization (DP) of mono- and oligo-saccharides. Table II shows the results.

The ratio of fr.1 (DP>15), fr.2 (5-14), fr.3 (3-5), fr.4 (2-3), and fr.5 (1-2) of Hirosaki-UFP, Nakasato UFP, and Kamikita-UFP were 2.3~3.3:1.2~2.9:3.4~10.1:4.9~9.1:75.8~84.8.

Those of Hirosaki-CF and Iwaki-CF were 2.6~3.3:0.9~1.1:5.4~9.3:4.3~5.6:82.6~84.8.

There was no difference between converted paddy fields and common fields.

(Table II Sugar ratio of fractions separated by Bio-Gel P-2 of 80% methanol soluble fraction)

	Sugar Ratio (wt%)				
	Fr1	Fr2	Fr3	Fr4	Fr5
Hirosaki-UFP	3.3	2.1	8.4	4.9	81.3
Nakasato-UFP	3.1	2.9	10.1	7.5	76.4
Kamikita-UFP	2.3	1.2	3.4	9.1	84.0
Hirosaki-CF	2.6	1.1	9.3	4.3	82.7
Iwaki-CF	3.3	0.9	5.4	5.6	84.8

**Mono- and oligo-saccharide analysis by HPAEC-PAD:**

To know the constituent mono- and oligo-saccharide in the 80% methanol soluble fraction, the HPAEC-PAD analysis was done (Table III). Sucrose (Suc) was the major sugar component across all samples. Raffinose (Raf) and stachyose (Sta), which are peculiar to leguminous, were also detected in all samples

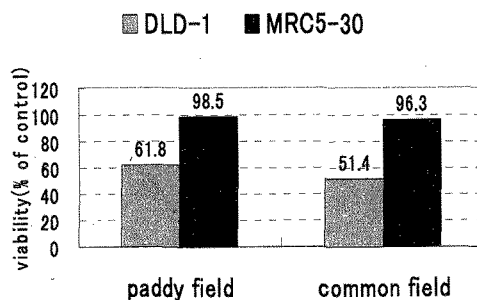
(Table III Ratio of Suc, Raf, Sta in each sample)

	Ratio (%)		
	Suc	Raf	Sta
Hirosaki-UFP	90.4	2.3	7.3
Nakasato-UFP	85.8	3.8	10.4
Kamikita-UFP	87.1	5.5	7.4
Hirosaki-CF	88.8	3.0	8.1
Iwaki-CF	89.2	2.9	7.9

**Effect on the Growth of DLD-1 human tumor and MRC5-30 cells:**

The 80% methanol-soluble fractions obtained from apios tubers in paddy field and common field were tested for their effect on the growth of DLD-1 human tumor and MRC5-30 cells. Results are shown in Fig. 1.

DLD-1 cell growth was significantly reduced by both samples. Neither sample inhibited the growth of human MRC5-30.



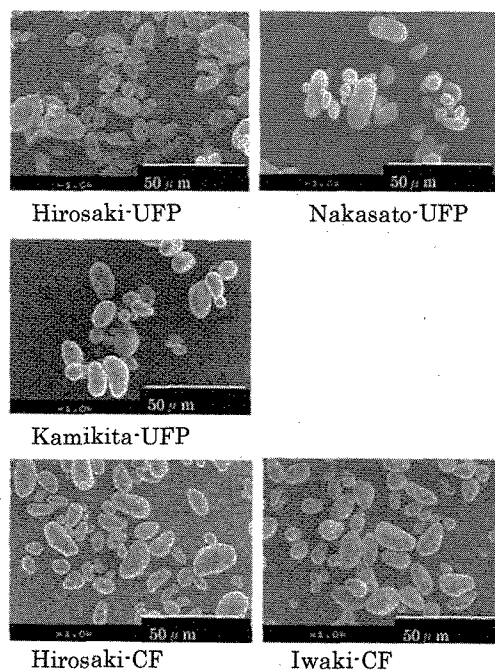
(Fig.1. Effect of 80% methanol soluble fractions on cell growth.)

**Starch grains:**

Scanning electron microscopy and Laser scanning particle size distribution analysis (HORIBA LA-910) revealed slightly oval grains with a particle size about 13 μm in all five samples (Table IV) (Fig.2).

(Table IV Average size of each starch)

	Average size(μm)
Hirosaki-UFP	13.2
Nakasato-UFP	13.5
Kamikita-UFP	13.6
Hirosaki-CF	14.3
Iwaki-CF	12.4



(Fig.2 Scanning electron microscopy of apios starch grains)

#### 4. CONCLUSION

We studied the carbohydrate composition, mono- and oligo-saccharide composition, and starch grain shape and size of Apios tubers grown at 5 different sites within Aomori prefecture.

There was no significant difference between apios cultivated in converted paddy fields and apios grown in common fields.

Therefore, apios cultivated in an upland field converted from paddy can be expected to be quite similar to that grown in common fields.

#### 5. ACKNOWLEDGEMENT

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#### 6. REFERENCES

- 1) Yasuo Ogasawara and Yoji Kato: *Trans. Mat. Res. Soc. Jpn.*, 1123-1126 Vol.30, No.4 (2004)
- 2) Yasuo Ogasawara, Yutaka Hidano and Yoji Kato: *Journal of the Japanese Society For Food Science and Technology* Vol.53, No2, 130-136(2006)
- 3) Teruko Konishi, Yasushi Mitsuishi, Yoji Kato: *Biosci. Biotechnol. Biochem.*, 62 (12), 2421-2424 (1998)
- 4) Yoji Kato, Junko Uchida, Seiko Ito, Yasushi Mitsuishi: *International Congress Series* 1223 161-164 (2001)

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