農薬生産用農産食料製造-琉球諸島に在るクワズイモの茎の化学的組成(農芸化学科)

Pesticide-producing Agricultural Food Processing. Chemical composition of the stem of *Alocasia odora* C. KOCH (Araceae)

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I INTRODUCTION

In the course of investigation on developing safe pesticides from agricultural food products, anthocyan pigments were shown to be used as insect-vision inhibitors by interfering with color vision[2]. In order to formulate anthocyan vision-inhibitors in powder form, a proper adsorbent is required. One of the authors demonstrated that starch was an ideal adsorbent in view of the amount of dye adsorption[3]. This finding led us to explore a possibility of using starch from unused source plants. As the first starch source, *Alocasia odora* C. KOCH was selected and chemically analyzed.

There is no work on the chemical analysis of the alocasia habitates in the Ryukyu Islands. This work, at the first time, reports the basic chemical composition of the stem, the methods of removing raphides for obtaining pure starch, and the paper chromatographic identification of sugars.

Due to its abundance in a native state in the Ryukyu Islands, the use of alocasia biomass is undoughtedly significant in view of utilizing one of the natural resources unused at the present time.

II MATERIALS AND METHODS

1 Composition analysis

1) Sample

Alocasia was obtained at the edge area of a forest in Shuri, Naha, Okinawa.

2) Analytical Methods[5]

Stem moisture was determined after drying at a temperature of 100–110°C. Crude protein was evaluated by multiplying a factor of 6.25 to the nitrogen content obtained by Kheldarl method. Crude fat content was determined by ether extraction with Soxhlet fat extraction method. Crude fiber was evaluated from the weight difference between the sample boiled for 30 min in 0.248 N sulfuric acid and then in 0.322 N sodium hydroxide and followed by filtering

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and drying and the sample being ashed after the above treatments. Crude ash was determined by burning in an electric muffle oven. Nitrogen free extract was obtained by subtracting the all values mentioned above from a value of 100. Sugar content was determined by Bertland method after hydrolysis in 2.5% hydrochloric acid. Reducing sugar was evaluated by Bertland method on the water or 50%-alcohol extract of the sample. Starch content was obtained by multiplying a factor of 0.9 to the sugar content stated above. Calcium was determined by an oxidative titration method. All measurements were carried out five times and expressed by an average value.

2 Starch preparation

After peeling the skin, alocasia stem was macerated with a mixer (National Electric Co., Type MX-83, 50–60 c/s) and was filtered through five layers of gauze. Residues were put in water and separated again to residue and starch (I). The filterate was left for one day. The supernatant liquid was discarded while the precipitate was suspended in water and left for 4 hr. Then, the mixture was separated into the fraction of suspending starch (II) and that of precipitating starch (III) by a help of a siphon. The precipitate was put in water, mixed, left for 4 hr, and separated into starch (II) and starch (III). The above procedures were repeated six times. The fractions I, II, and III were centrifuged separately. The precipitates were dried and used for analysis. Since the starch fractions were contaminated with raphides, the starch content of these fractions was evaluated by the method stated in the previous section.

3 Paper chromatography of sugars

The solvents used were n-butanol-acetate-water (5 : 1 : 2) and phenolic ammonia, a mixture of 400 ml of worm phenol and 100 ml of 1% ammonia. Toyo filter paper No. 50 was used. The spots were visualized by aniline hydrogen phthalate. A diphenylamine reaction and an indol reaction were carried out for the distinction between aldose and ketose and for the identification of hexose, respectively.

4 Measurement of the size of starch granules and raphides

The size of starch granules and raphides was measured with the micrometer set in an optical microscope. The state of raphides during the acid or alkali treatments was also observed with the same microscopy.

III RESULTS AND DISCUSSION

1 Chemical composition

The basic chemical composition of alocasia is shown in Table 1. The stem moisture was extremely high, showing 95.5% in the sample used. This high stem moisture may be considered as one of hazards in utilizing alocasia stems. Nitrogen free extract, including sugar and starch, was

Table 1. The chemical composition of the stem of alocasia

Item analyzed	Air-dry sample	Fresh sample
Moisture	12.84	95.50
Crude protein	2.13	0.08
Crude fat	1.16	0.05
Crude fiber	7.56	0.30
Crude ash	12.44	0.49
Nitrogen free extract	63.87	3.58
Sugar	44.91	1.78
Reducing sugar	3.53	0.14
Starch	40.42	1.59
Calcium	2.24	0.09

the next to the stem moisture. Among them, starch was 1.59%, indicating an extremely low content in the fresh source material of alocasia. Crude ash was also high, having calcium in one-fifth of the total ash. Since the starch value of an air-dry sample was 40.42%, the alocasia stem may be regarded as one of the possible starch sources.

2 Starch preparation

Out of 14.81 kg of the fresh stem peeled, 191.9 g of starch II, 67.6 g of starch III, 108.0 g of starch I, and 4.5 g of residue were obtained. The total of 367.5 g of starch was corresponded to 2.48% of the total fresh weight. Since these starch fractions contained raphides and cell particles, the amount of starch was analytically determined. Starch II contained 83.46% pure starch and starch III had the value of 45.20%. From this, starch II and III gave 1.28% of starch within the fresh stem. Although starch I was excluded in obtaining the present value, the amount of the starch extracted was close to the analytical value shwon in Table 1.

The starch granule of alocasia stem was spherical with a diameter of 1.6–4.2 μ. The alocasia starch belongs to a smaller group in the starches known to the present time. Due to this small size, collecting the suspension in each washing processes would provide the starch having purity of higher than 80%.

3 Paper chromatographic identification of sugars

The Rf values of the sugar spots obtained in the present work are shown in Table 2. From the data glucose and fructose were identified. Due to the presence of other sugars in the samples, a diphenylamine reaction was carried out to distinguish ketose from aldose and an indole reaction was done to test hexose. As shown in Table 3, the presence of glucose and fructose was confirmed by the color test and the presence of pentoses was suggested by the green coloration in diphynlamine reaction.

Table 2. The Rf values of sugars in the stem of alocasia

Sample	n-Butanol, 5: acetate, 1: water, 2	Phenolic ammonia
Glucose	0.24	0.39
Fructose	0.27	0.52
50%-Ethanol extract	0.22, 0.26	0.15, 0.41, 0.52
Water extract	0.21, 0.25	0.15, 0.40, 0.51

Table 3. Coloring tests of the sugar spots on chromatographic paper

Sample	Diphenylamine	Indole
50%-Ethanol extract	Green	Brown
Water extract	Green	Brown

4 Raphides

Alocasia leaf and stem contained raphides within specialized cells. The raphides were in aggrigation in a bag and then scattered within tissues and the solutions prepared for starch extraction upon mechanical damage of the tissues and, eventually, the bag. Larger raphides precipitated alone or accompanying with tissues in a similar manner to starch while smaller raphides remained in the supernatant. The size of the raphides was about 40 μ in length and about 3.5 μ in width.

The stem sample dried as well as heated in an autoclave (100°C, 1 atm, 1 hr) gave the pungency quite comparable to the fresh stem sample untreated. This stability in pungency indicated that a proper use of the raphides could be considered as an attractive future research subject for a purpose of practical uses as biologically active substances, since the raphides were expected to function as irritants against noxious animals, protecting the plants having the raphides[1,4].

The treatments of the starch-extracting solutions with dilute acids could remove the raphides completely while a treatment with an alkali could not as shown in Table 4. This observation suggested that the mechanical removal of the raphides out of the starch extract, carried out in this

Table 4. The effect of acid or alkali treatments on the raphides in the starch extracts of alocasia

Treatment	Supernatant*	Precipitate*
Control	–	+
1% Sulfuric acid	–	–
3% Acetic acid	–	–
1% Hydrochloric acid	–	–
1% Sodium hydroxide	+	+

* +: Raphides were found, –: Raphides were not found.

work, was unsatisfactory while a dilute acid treatment could be effectively utilized as a purification procedure of the alocasia starch.

IV SUMMARY

The chemical composition, starch, and raphides of the stem of *Alocasia odola* C. KOCH (Arceae) were investigated in order to utilize the unused natural resource habitated in the Ryukyu Islands for the purpose of pesticide formulations. The stem moisture was as high as 95.5%. The starch content of a fresh sample was 1.59% while that of an air-dry sample was 40.42%. The starch granule of the stem was spherical with a diameter of 1.6–4.2 μ. The presence of glucose and fractose in water and 50%-ethanol extracts was shown by paper chloratographic methods. The irritant raphides which precipitated along with starch granules could be removed by solvilization with dilute acid treatments. The size of the raphides was about 40 μ in length and about 3.5 μ in width. Since the raphides did not lose irritant activities by drying and heating, the bioactive substances were suggested to be used as possible plant protectants against noxious animals.

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農薬生産用農産食料製造—琉球諸島に在るクワズイモの茎の化学的組成

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要 約

琉球諸島に在るクワズイモ，*Alocasia odora* C. Koch（Araceae），の農薬製剤化への利用を考え，茎の化学的組成，澱粉，及び針状刺激物質について調べた。クワズイモの茎は水分含量が高く，95.5%に達した。澱粉含量は，新鮮物では1.59%であったが，風乾物での澱粉価は40.42%であった。澱粉粒は球形で，その粒径は1.6～4.2 μであった。水及び50%エタノール水で抽出され得る糖の中で，グルコースとフラクトースがペーパークロマトグラフ法で検出された。クワズイモの茎から澱粉を調製する際に，刺激性を有する針状結晶が澱粉と共に沈澱したが，各種希酸で処理することにより除去できることが示された。針状結晶は，長さが約40 μで太さは約3.5 μであった。針状刺激性物質は，加熱及び乾燥によっても効力を失わなかったことから，加害性昆虫や他の動物に対する忌避性物質として利用され得る可能性が示された。

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