Protein and Starch in Tubers of Winged Bean, Psophocarpus tetragonolobus(L.) DC., and Yam Bean, Pachyrrhizus erosus(L.)Urban

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Protein and Starch in Tubers of Winged Bean, *Psophocarpus tetragonolobus* (L.) DC., and Yam Bean, *Pachyrrhizus erosus* (L.) Urban

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Introduction

The seeds of winged bean, *Psophocarpus tetragonolobus* (L.) DC., one of the tropical legumes, have attracted much attention as a protein food source in recent years. The plant produces large edible tubers, which contain a high level of protein and are used widely as food in tropical areas[18]. Yam bean or Kuzuimo, *Pachyrrhizus erosus* (L.) Urban, is another tropical legume. The grown tuber of this species is rich in starch and used as starch resources[15]. The immature tuber is also served as salad or cooked food, but the seeds containing toxic substance(s) are not utilized for human consumption[16].

Ishihata, one of the present authors, has introduced and cultivated experimentally many strains of winged bean and yam bean at Ibusuki Experimental Botanic Garden, Kagoshima University[8]. In the preceding paper, we evaluated the nutritive value of young pods collected from these strains of winged bean[14]. Some strains of winged bean and yam bean among them produced, in a plastic house, tubers large enough to be used as food. We have made analytical works on these tubers, because detailed reports on nutrients of tubers of winged bean and yam bean are scarce.

The present paper describes the proximate composition of tubers of winged bean and yam bean; the fractionation and amino acid composition of tuber proteins; and properties of tuber starches.

Materials and Methods

1. Tuber samples of winged bean and yam bean

At the first year cultivation, no tubers of winged bean grew enough in Ibusuki Experimental Botanic Garden. Per stem, about ten small tubers were formed in branched or bunched forms, and the total weight of these ranged from 100 to 200 g. From the plant, however, which passed the winter in a plastic house, mature tubers weighing from 300 to 400 g in total weight were obtained (Fig. 1). The yam bean plant grown in a plastic house produced one block tuber of 400 to 500 g

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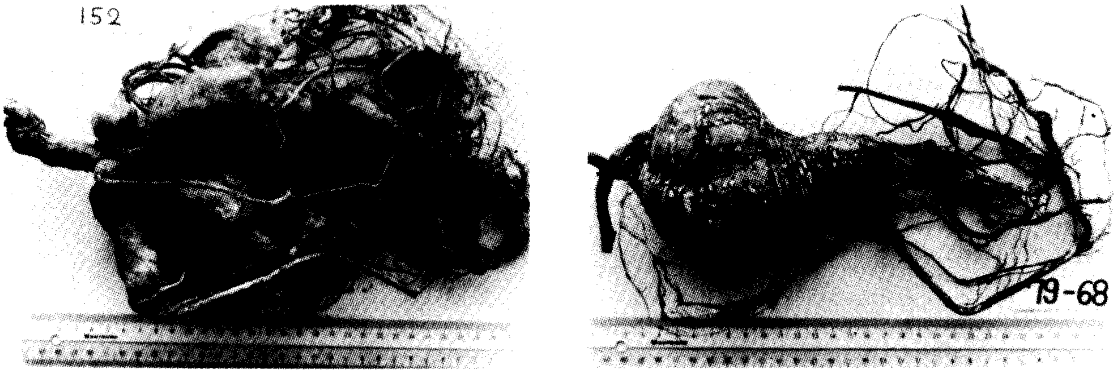

Fig. 1. Photographs of tubers of winged bean (left) and yam bean (right).

(Fig. 1). As both tubers can not grow well in the field, the winged bean and yam bean do not seem promising ones as root crops in Kagoshima Prefecture.

A part of the harvested tubers were cut out with 1-cm thickness, and the slices were freeze-dried and were ground into fine powder. Fresh tubers (the strains of winged bean, KaS–8, –12 and –19*, harvested in January 1982) and powdered ones (the strains of winged bean, KaS–20 and –29, harvested in May 1981; the strain of yam bean, 79–66*, harvested in October 1980) were applied for the analytical works and isolation of starches. Powdered tubers of yam bean, 79–68 harvested in June 1981, were also used for isolation of starch.

2. Reagents

Glucoamylase of *Rhizopus niveus* was purchased from Seikagaku Kogyo Co., cellulose tube (27/32) from Visking Co., Diaflo membrane, PM–10, from Amicon Co. and other reagents from Nakarai Co. Sweet potato and potato starch samples were those prepared in Laboratory of Applied Starch Chemistry.

3. Determination of protein, sugar and starch

Crude protein was determined by multiplying %N by 6.25, %N being estimated by a semi-micro Kjeldahl method.

Sugar substances were analyzed as follows. A sample was extracted with water, and the residue was hydrolyzed with dilute hydrochloric acid according to the method of AOAC[1]. The water extract and hydrolyzate were analyzed for reducing sugars and starch, respectively, by the Somogyi-Nelson method[13]. The water extract was applied also for the determination of non-reducing sugars by the method of Nagahara *et al*[12].

4. Paper chromatographic analysis of sugars in tubers

The water extract was concentrated and subjected to paper chromatography with the solvent systems of *n*-butanol/acetic acid/water (4/1/2, v/v) and *n*-butanol/pyridine/water (6/4/3, v/v). Spots of the reducing and non-reducing sugars were revealed with the silver nitrate-sodium hydroxide[15] and the resorcinol-sulfuric acid reagents[2], respectively.

5. Fractionation and amino acid composition of tuber proteins

Fractionation and amino acid analyses of tuber proteins were performed as described in the preceding paper[14].

6. Preparation of starch samples

The material was soaked in water, and the slurry was stirred with a magnetic stirrer and allowed

* The strain numbers of winged bean and yam bean were given at Ibusuki Experimental Botanic Garden, Kagoshima University[7].

to stand. The collected precipitate of starch was repeatedly suspended in water and finally passed through a 400-mesh sieve. Crude starch preparation was then well suspended in a sodium chloride solution of 0.1 M containing 1/10 volume of toluene, and the suspension was shaken, allowed to stand and centrifuged. Repetition of this procedure was effective in removing the impurities. The precipitate was washed successively with methanol and ether and then dried at room temperature. The recoveries of tuber starches of winged bean and yam bean were 5.0 and 1.1%, respectively, on a fresh root material basis.

7. *Properties of starch*

Properties of tuber starches were investigated according to the methods of Fujimoto *et al*[5].

(*1*) *Determination of total phosphorus*

Total phosphorus was determined by the method of Fukui[6].

(*2*) *Morphological observation*

A suspension of starch granules was viewed under Olympus microscope, BH-2.

(*3*) *X-ray diffraction*

X-ray diffraction patterns were recorded on a Rigaku Denki X-ray diffractometer D-3F under the following conditions: voltage, 30 kV; current, 15 mA; scanning speed, 1° (2θ)/min.

(*4*) *Amylography*

Amylograms were taken on a Brabender Amylograph DC-8. A starch suspension (6%) was heated from 35°C to 95°C at a rate of 1.5°C/min, kept at 95°C for 10 min, and then cooled to 50°C at a rate of 1.5°C/min.

(*5*) *Absorption spectra of starch-iodine complexes*

The reaction mixture consisted of starch (2 mg), iodine (4 mg) and potassium iodide (40 mg) in water (50 ml). Absorption spectra of starch-iodine complexes were measured on a Hitachi spectrometer EPS-3T at 500–700 nm.

(*6*) *Swelling power and solubility*

Starch (500 mg) was suspended in distilled water (25 ml) and the suspension was heated at 60°, 70°, 80° or 90°C for 30 min. The amount of starch of the supernatant solution was determined by the phenol-sulfuric acid method[3].

(*7*) *Digestibility with glucoamylase*

Starch (100 mg) was incubated at 30°C stirring with glucoamylase (168 units) in a 1/10 M acetate buffer of pH 4.2 (5 ml) and the amount of solubilized sugar was periodically determined by the phenol-sulfuric acid method[3].

Results and Discussion

1. Proximate composition of tubers

Table 1 shows the proximate composition of tubers of winged bean and yam bean. The high moisture and the extremely low starch contents of tubers of yam bean indicate that the tubers used in this work were still immature. The protein content of tubers of winged bean, 15–22% on a dry weight basis, is much higher than that of many root crops. The values are, however, still 2/3–1/2 of the reported ones[4,18], and so it is possible that these tubers of winged bean may be still immature.

Paper chromatographic analyses of sugars in tubers showed that glucose and sucrose were the main reducing and non-reducing sugars, respectively. Tubers of yam bean contained larger amount of fructose than those of winged bean.

Table 1. Proximate composition of tubers of winged bean and yam bean (%, on a fresh weight basis)

Component	Winged bean*				Yam bean*
	KaS–8	KaS–12	KaS–19	KaS–20	79–66
Moisture	72.41	74.47	63.76	68.69	87.49
Reducing sugar (as glucose)	2.30	2.29	1.75	0.78	3.39
Non-reducing sugar (as sucrose)	0.91	1.52	2.99	2.62	1.02
Starch	9.54	10.52	19.60	13.57	2.01
Crude protein	6.13	4.25	6.00	4.72	0.83

* The strain numbers were given at Ibusuki Experimental Botanic Garden.

2. Fractionation and amino acid composition of tuber proteins

Proteins of tubers of winged bean and yam bean were fractionated into the 2% sodium chloride, 30% *iso*-propanol, 4% lactic acid and 0.5% potassium hydroxide fractions according to the method of Maes[10], as modified by Kanamori *et al*[9]. The sodium chloride fraction was further separated into the water-soluble and -insoluble fractions by dialysis against water. These five fractions were desalted and concentrated by ultra-filtration with Amicon PM–10 Diaflo membrane, and analyzed for crude protein. The main proteins are albumins, globulins and glutelins, as shown in Table 2. Much crude protein of the sodium chloride fraction was lost at the step of dialysis to separate the water-soluble and -insoluble fractions, as observed in young pods of winged bean[14]. No reasonable explanation would be found for this except that such lower recovery is in part due to the occurrence of non-protein nitrogen in the sodium chloride fraction.

Table 2. Relative amounts of proteins fractionated from tubers of winged bean and yam bean

Fraction	Winged bean		Yam bean
	KaS–20	KaS–27	79–66
2% NaCl (%)	76.83	68.84	34.02
Water-soluble (%)	35.61	8.50	5.60
Water-insoluble (%)	9.61	14.17	16.81
30% *iso*-PrOH (%)	0.97	3.02	1.95
4% Lactic acid (%)	3.50	0.58	0.97
0.5% KOH (%)	8.80	14.12	12.70
Total (%)	90.10	86.56	49.64

Tables 3 and 4 show the amino acid composition of the fractionated proteins and whole tubers. There are significant differences between winged bean and yam bean. The contents of acidic amino acids and lysine are high in winged bean and these values are in fairly good agreement with the reported ones[4,11].

3. Properties of starch samples

The proximate composition of the isolated starch samples is summarized in Table 5. The crude protein and total phosphorus are low in both the samples.

Table 3. Amino acid composition of proteins fractionated from tubers of winged bean (weight percent of each amino acid in total ones)

Amino acid	Whole tuber		Water-soluble		Water-insoluble		*iso*-PrOH		Lactic acid		KOH	
	KaS-20	KaS-27	KaS-20	KaS-27	KaS-20	KaS-27	KaS-20	KaS-27	KaS-20	KaS-27	KaS-20	KaS-27
Lys	7.4	7.8	6.1	6.7	6.0	6.5	5.7	—	7.9	9.2	5.4	6.9
His	3.6	2.6	2.2	2.1	2.4	trace	1.7	—	2.9	2.0	2.5	2.8
Arg	4.6	5.2	5.1	5.0	5.3	6.2	4.6	—	6.5	7.4	5.0	6.8
Asp	20.1	17.7	13.1	12.5	11.4	10.6	13.8	—	15.0	13.3	13.4	14.4
Thr	2.8	5.9	7.0	5.8	6.3	5.8	5.8	—	5.6	6.0	3.9	4.4
Ser	3.6	6.6	7.3	6.0	5.9	5.1	3.3	—	3.9	6.0	3.9	5.5
Glu	10.7	8.4	10.6	10.5	10.7	10.8	12.2	—	11.6	10.3	12.3	6.8
Pro	7.1	7.1	7.0	8.8	11.1	7.7	8.6	—	3.3	8.1	6.1	12.0
Gly	4.8	4.4	5.3	5.0	4.7	5.0	6.8	—	6.3	5.3	6.1	6.0
Ala	4.3	4.2	3.9	3.8	3.8	6.2	4.5	—	5.2	4.0	5.9	4.9
Cys	0.6	3.0	1.6	1.3	1.0	0.8	2.5	—	trace	trace	trace	trace
Val	6.3	6.3	4.6	6.5	6.0	6.3	6.7	—	7.5	6.7	7.8	7.0
Met	0.5	0.5	1.0	1.2	0.7	1.3	trace	—	trace	0.9	1.3	1.2
Ile	4.6	4.1	4.8	4.9	4.1	4.7	5.8	—	5.3	5.0	5.3	4.6
Leu	8.2	5.5	6.8	7.7	7.6	11.7	6.8	—	8.3	7.0	9.0	7.6
Tyr	4.8	4.7	4.9	4.6	5.0	5.0	4.4	—	4.5	3.3	4.6	3.5
Phe	6.0	6.0	6.7	7.6	5.7	6.3	6.8	—	6.2	5.5	6.4	5.6

Table 4. Amino acid composition of proteins fractionated from tubers of yam bean (weight percent of each amino acid in total ones)

Amino acid	Whole tuber	Water-soluble	Water-insoluble	*iso*-PrOH	Lactic acid	KOH
Lys	5.9	5.5	5.4	4.0	6.0	1.4
His	4.6	2.0	1.8	1.2	2.5	0.7
Arg	2.7	4.5	5.4	3.1	3.7	1.1
Asp	9.6	12.6	10.1	12.8	10.5	12.2
Thr	8.4	7.6	5.7	6.5	5.7	6.0
Ser	11.6	10.8	7.4	9.3	8.7	7.5
Glu	7.4	0.1	12.4	11.0	9.4	10.1
Pro	11.1	6.8	4.2	4.5	8.4	8.6
Gly	7.6	12.2	10.7	10.7	12.4	10.7
Ala	7.3	13.3	11.0	9.9	9.4	9.5
Cys	0.4	0.9	trace	trace	trace	trace
Val	6.4	7.1	9.6	6.3	6.7	8.9
Met	0.6	2.2	0.8	1.2	0.8	1.6
Ile	3.8	0.4	4.4	4.6	4.9	5.8
Leu	6.1	7.2	7.8	7.9	7.5	8.2
Tyr	3.4	3.5	trace	3.4	trace	2.8
Phe	3.2	3.3	3.5	3.7	3.5	4.9

Table 5. Analyses of starches isolated from tubers of winged bean and yam bean (%, on a fresh weight basis)

Component	Tuber starch from	
	Winged bean	Yam bean
Moisture	7.6	11.1
Crude protein	0.11	—
Total phosphorus	0.006	0.003
Amylose	20	13

Figure 2 shows absorption spectra of starch-iodine complexes. The blue values for winged bean, yam bean and sweet potato starches are 0.400, 0.329 and 0.415, respectively. The amylose content calculated from these values is presented in Table 5. Here it is assumed that amylose and amylopectin of winged bean and yam bean starches give the same blue values as amylose (1.200) and amylopectin (0.200), respectively, of sweet potato starch as control[5]. The amylose content of winged bean starch is similar to that of sweet potato starch (21%).

Figure 3 shows the photomicrographs (×400) of starch granules of winged bean and yam bean. Winged bean starch is composed of spherical, compound and semi-spherical granules. Yam bean starch granules are spherical and compound, and small granules are mixed with comparatively larger ones.

Distribution of granular size is shown in Fig. 4. The average particle sizes for winged bean and yam bean are 4.59 and 3.82 μm, respectively. Granules of sweet potato starch used here as

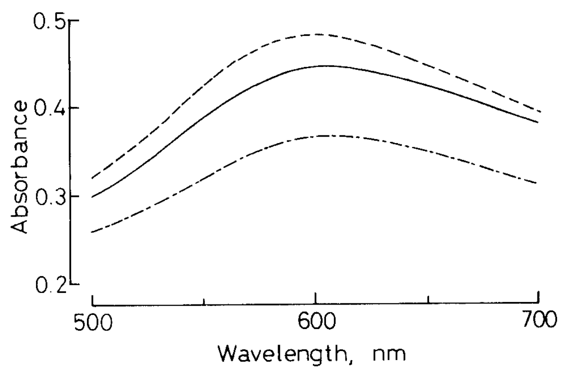

Fig. 2. Absorption spectra of starch-iodine complexes.
——, Winged bean; —·—, Yam bean; ----, Sweet potato.

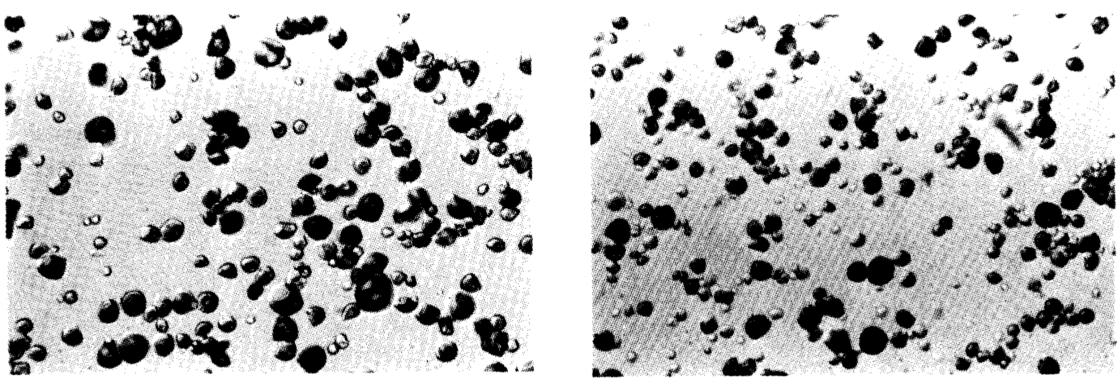

Fig. 3. Photomicrographs of starches isolated from tubers of winged bean (left) and yam bean (right).

control were smaller than those of the usual preparation.

X-ray diffraction patterns of both tuber starches are shown in Fig. 5, together with those of sweet potato and potato starches as controls. The pattern of winged bean starch is similar to that of potato starch, namely the B type. The pattern of yam bean starch seems to be the C_A type.

Figure 6 shows the time course of digestion of winged bean and yam bean starches by glucoamylase. Winged bean and sweet potato starches gave a similar result. On the other hand, yam bean starch was easily digested to 80% in 24 hours. The high digestibility of yam bean starch is compatible with its small particle size (Fig. 4) and its low blue value (Fig. 2).

Swelling power and solubility of winged bean starch are a little larger than those of sweet potato starch (Fig. 7).

Figure 8 shows the Brabender amylograms at 6% starch concentration of winged bean and

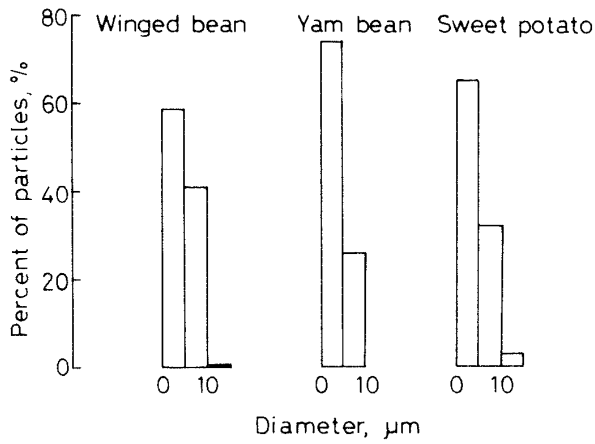

Fig. 4. Distribution of granular size of starch samples.

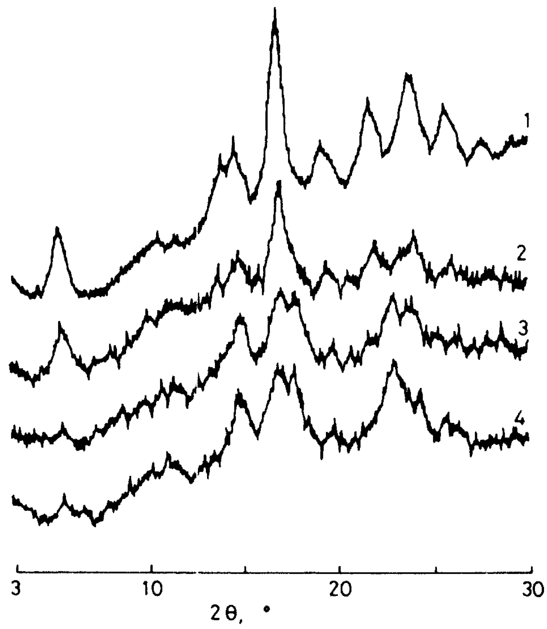

Fig. 5. X-ray diffraction patterns of starches. 1, Potato; 2, Winged bean; 3, Yam bean; 4, Sweet potato.

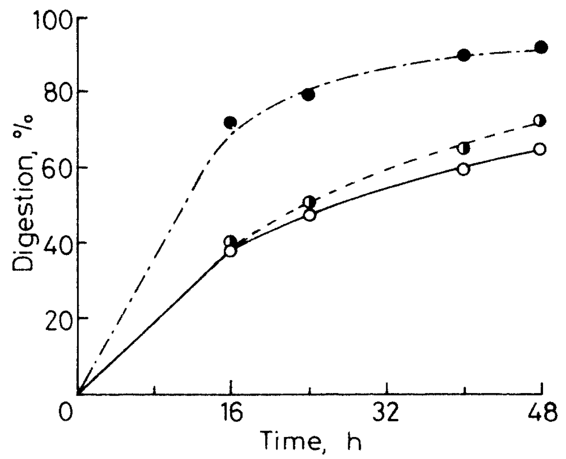

Fig. 6. Time course of digestion of starches with glucoamylase.

—O—, Winged bean, ---●---, Yam bean; --◑--, Sweet potato.

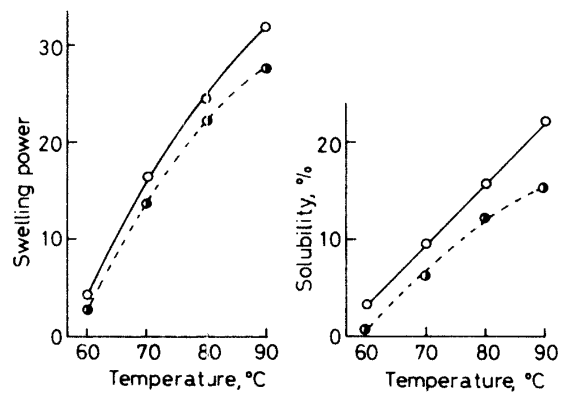

Fig. 7. Swelling power (left) and solubility (right) of starches.

—O—, Winged bean; --◑--, Sweet potato.

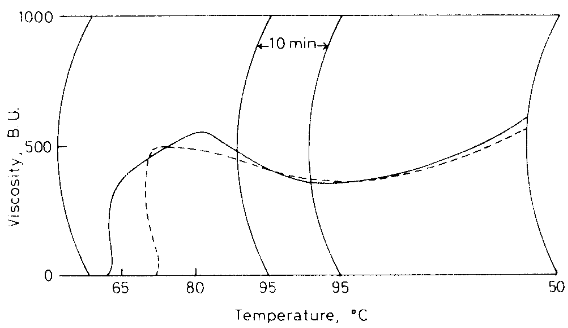

Fig. 8. Brabender amylograms of starches.
——, Winged bean; ----, Sweet potato.

Table 6. The data obtained from Brabender amylograms

Sample	Gelatinization temp* (°C)	Maximum viscosity		Breakdown (B. U.)	Viscosity at 50°C (B. U.)
		(B. U.)	(°C)		
Winged bean	63.0	550	88.0	200	610
Sweet potato	73.0	500	78.0	120	590
Potato	62.5	<1,000	70.0	—	—

* Temperature giving a viscosity of 20 B. U.

sweet potato starches. The data are presented in Table 6. A wide temperature range necessary to reach maximum viscosity is observed in winged bean starch, suggesting that starch granules are highly heterogenous, not swelling uniformly.

Umadevi *et al.* reported some properties of starch isolated from seeds of winged bean[17]. From our results described above, tuber starch evidently differed from seed starch in the amylose content, particle size and in some physicochemical proterties.

Summary

The protein content of tubers of winged bean (*Psophocarpus tetragonolobus* (L.) DC) was much higher than that of many root crops. The protein and starch contents of tubers of yam bean (*Pachyrrhizus erosus* (L.) Urban) which were immature were extremely low. The proteins of both the tubers mainly consisted of albumins, globulins and glutelins.

Starch samples were prepared from tubers of winged bean and yam bean. The X-ray diffraction pattern of winged bean starch was similar to that of potato starch, namely the B type. The amylose content and digestibility resembled those of sweet potato starch. Gelatinization temperature was 63.0°C and maximum viscosity was 550 B. U. The X-ray diffraction pattern of yam bean starch seemed to be C_A type. The starch sample showed high digestibility, small particle size and low blue value.

As the plants of winged bean and yam bean can not pass the winter in the field in Kagoshima Prefecture, we can not obtain grown tubers. Therefore, the winged bean and yam bean do not seem promising ones as root crops in Kagoshima Prefecture.

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