Bot. Bull. Academia Sinica 27: 175-186 (1986)

SOLUBLE LEAF PROTEINS OF SWEET POTATO CULTIVARS[1,2]

YAW-HUEI LIN and SHU-PENG HO

Institute of Botany, Academia Sinica
Nankang, Taipei, Taiwan 11529, Republic of China

(Received January 21, 1986; Accepted April 26, 1986)

Abstract

Patterns of seasonal variation of levels of leaf soluble protein of four sweet potato (*Ipomoea batatas* Lam.) cultivars Tainong 65, HP-4, Chang-hua, and Tainong 25 were similar. Extraction removed 57.8±9.2, 46.7±9.7, 40.3±5.9, and 35.9±5.1 g protein/kg fresh leaves (n=8) of four cultivars, respectively. Patterns of variation of apparent effector activities on trypsin of crude leaf extract from four cultivars were different from July to November 1978 but were similar from March to June 1979. Patterns of variation of apparent inhibitory activity on trypsin of heated crude leaf extract among Tainong 65, HP-4, and Changhua as well as Tainong 25 were different from March to June 1979. There were both trypsin inhibitor activity and activating activity on trypsin-casein in crude leaf extract of four cultivars. The trypsin inhibitor activity was more heat-stable than activating activity on trypsin-casein while the latter was more stable than the former at 7°C or in diluted solutions. The presence of activating activity on trypsin-casein in the leaf extract of four cultivars suggested the possible existence of a trypsin-like protease in leaves or germinating buds of sweet potato. In general, cultivars with higher levels of apparent trypsin inhibitor activity in leaves also had higher trypsin inhibitor activity in roots (cf. Lin and Chen, Bot. Bull. Academia Sinica 21: 1-13, 1980).

Key words: Leaf protein; trypsin inhibitor; activator of trypsin-casein; sweet potato.

Introduction

The first known plant protein inhibitors of protease were trypsin inhibitors isolated from soybeans (Kunitz, 1946). These inhibitors have since been investigated in many plants (Laskowski and Sealock, 1971; Leiner, 1951; Richardon, 1981; Ryan, 1981). Trypsin inhibitors in foods derived from plant materials may have an

[1] Paper No. 309 of the Scientific Journal Series, Institute of Botany, Academia Sinica, Taipei, Taiwan, Republic of China.

[2] Part of this work was supported by grant NSC-69B-0409-03(01) of the National Science Council, ROC.

adverse nutritional effect by inhibiting the proteolytic action of trypsin during the digestion process (Richardon, 1981).

The presence of trypsin inhibitors in sweet potato was first reported by Sohonnie and Bhandarker (1954). In feeding experiments of guinea pig, the results indicated that the low protease levels resulting from the ingestion of trypsin inhibitors in raw sweet potatos in conjunction with a low protein diet allows the B toxin of Clostridium welchii Type C to initiate the disease enteritis necroticans in animals. Under normal protease levels, this B toxin would be destroyed before causing any intestinal damage (Lawrence and Cooke, 1980; Lawrence and Walker, 1976). One of the major use of sweet potato roots in Taiwan is as feed for pigs. When we substituted sweet potato root for corn at low percentage in the feed, we observed satisfactory growth of pigs. However, when percentage of sweet potato roots increased, adverse effect on growth of pigs became obvious. In feeding experiments of rats, rats fed 1/3 (diet with dried fresh sweet potato chips: corn powder=8:2)+2/3 normal diet showed significantly lower ($p<0.05$) liveweight gain than rats fed control diets or ensiled sweet potato chips diets. They also showed abnormal enlargement of pancreas (Lin *et al.*, unpublished data). So, the continual consumption of raw sweet potatoes containing trypsin inhibitors is a nutritional concern. Since sweet potatoes are a main staple in some developing countries and are used as animal feed, the properties of these protease inhibitors warrant investigation.

Three different trypsin inhibitors were found in a sweet potato, i.e. *Ipomoea batatas* LAM. *var. edulis* Makino (Okinawa Kokei No. 14). The purification and some chemical and inhibitory properties of the trypsin inhibitors, named as inhibitors II and III, were described. Both inhibitors were fairly stable over a pH range from 2 to 11 at 37°C, and thermostable (Sugiura *et al.*, 1973). Modification of amino acid residues in inhibitor III has also been reported (Ogiso *et al.*, 1974). A brief report concerning the effect of varietal differences on the activity levels and the heat stability of the trypsin inhibitor of sweet potatoes has been published (AVRDC Sweet Potato Report, 1975). A further survey on levels and heat stability of trypsin inhibitor activity among 53 sweet potato varieties was reported by our laboratory (Lin and Chen, 1980; Lin, 1982). The presence of more than 3 molecular forms of typsin inhibitors in a Chinese sweet potato cultivar Tainong No. 65 was proposed (Lin *et al.*, 1983). Disc-gel electrophoresis revealed that the trypsin inhibitor fraction of 4 American sweet potato cultivars isolated via affinity chromatography was heterogenous. There were 7 different trypsin inhibitor bands after electrophoresis at pH 8.9 in 7.5% polyacrylamide gels (Dickey and Collins, 1984).

Trypsin inhibitor activity of sweet potato roots showed seasonal variation and was related to changes of climatic factors such as cumulative temperature and cumulative rainfall (our unpublished data). Our unpublished data also showed that

many agronomic characters of sweet potato were related to trypsin inhibitor activity. All these observations suggest that trypsin inhibitors of sweet potato roots may play important physiological roles.

In this study, seasonal variation of concentrations of soluble leaf protein, seasonal variation of activity levels of trypsin effectors of soluble leaf protein, and some properties of the activities of trypsin effectors were investigated.

Materials and Methods

Materials

Stems with leaves of sweet potato cultivars Tainong No. 65, Chang-hua and Tainong No. 25, and a breeding line HP-4 were planted on June 7, 1978 at Nankang without climatic controls and fertilizer application. During the experimental period almost no enlarged roots were found. The growth of leaves was divided into 4 stages: bud→folded small leaf→light green large leaf→dark green large leaf. Dark green large leaves were harvested year round. Trypsin (10,000–13,000 BAEE units per mg protein), N-benzoyl-L-arginine-4-nitroanilide hydrochloride (DL-BAPA), and hemoglobin were purchased from Sigma Co., USA. Casein and Folin phenol reagents were products of Wako Co., Japan. Trichloroacetic acid was a product of E. Merck, W. Germany.

Crude Extract

Fresh leaves of 5 g (2–4 leaves) were blended with 20 ml distilled water in a fruit juicer. The homogenate was filtered through 4 layers of cheesecloth twice and then centrifunged at 17,370 xg for 10 min. The supernatant was dialyzed twice against 100 volumes of distilled water for at least 4 h. The dialyzed sample was called "crude extract". Suitable dilution of crude extract was necessary for activity tests. All steps were carried out at 4°C. Slight variation of volumes of leaf extract of different sweet petato cultivars was found. There was no correlation between volumes of extract and amounts of soluble leaf protein among 4 cultivars. So, the slight variation reflected random errors during experiment. Difference of highest mean (21.29 ml, Tainong 65) and the lowest one (20.07, Chang-hua) was 1.22 with a coefficient of variance of 6%.

Determinations of Water Soluble Protein

The amount of protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Assay of Trypsin with Casein as Substrate

This was done mainly according to the procedure reported by Kunitz (1945,

1946). Standard assays were run by adding 0.5 ml double distilled water (DDW) and 1.0 ml trypsin solution (containing 20 μg trypsin in 0.25 mM HCl) to tubes containing 1.0 ml of 1% (or other concentrations specified in the text) activated (35°C, 5 min) casein solution (in Na_2HPO_4-NaH_2PO_4 buffer, pH 7.6). Proteolytic reaction was allowed to proceed at 37°C for 20 min. The solution was then poured into tubes containing 3.0 ml of 10% trichloroacetic acid. The precipitates formed were centrifuged off after standing for 1 hr or longer at about 25°C. The concentration of split products in the supernatant solution was determined by measuring the absorbance of the solution at 280 nm. Control tests were run by preincubating 0.3 ml samples containing 0.3–0.45 mg protein and 0.2 ml DDW with 1.0 ml of 1% (or other concentrations specified in the text) activated casein solution at 37°C for 15 min. Then 1.0 ml DDW was added and the mixture was allowed to stand at 37°C for another 20 min before being poured into 10% trichloroacetic acid. Sample tests were done by preincubating 0.3 ml sample and 0.2 ml DDW with 1.0 ml of 1% (or other concentrations specified in the text) activated casein solution at 37°C for 15 min. Then 1.0 ml trypsin solution was added and proteolytic reaction was proceeded as standard assays.

Trypsin assay with DL-BAPA as Substrate

This assay followed the procedure of Erlanger *et al.* (1961). Standard assays, control tests, and sample tests were run under the same conditions described in the preceeding section.

Calculation of Trypsin Inhibitor Activity

The percentage of inhibition was calculated by a formula as $100\% \times ((A_{280}$ of standard$+A_{280}$ of control$)-A_{280}$ of sample$)/(A_{280}$ of standard$)$, and the specific % inhibition was defined as % inhibition per mg soluble protein. Dilution of samples was made to get a % inhibition around 50%. The percentage of inhibition was converted to μg trypsin inhibited by multipling % inhibition by 20 μg.

Results

Seasonal Variation of Amounts of Soluble Leaf Protein of Sweet Potato Cultivars

Figure 1 shows the seasonal variation pattern of amounts of soluble leaf proteins of 4 sweet potato cultivars. No leaves were available in winter (December of 1978, and January and February of 1979). During the experimental period (July 1, 1978–June 23, 1979), amounts of soluble leaf proteins of all four cultivars fluctuated with almost the same pattern. The order of the amounts of soluble leaf proteins of the 4 cultivars was almost fixed as Tainong 65 (4.07 ± 0.65 mg/0.3 ml extract

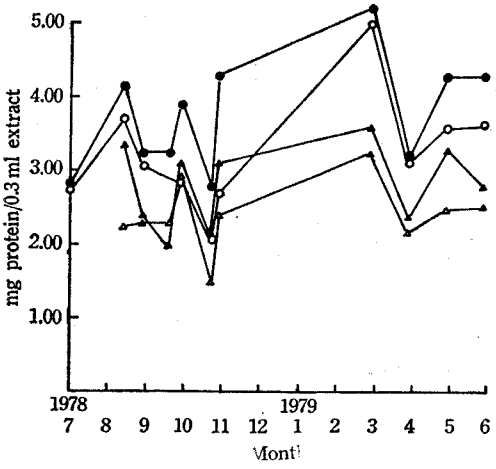

Fig. 1. Seasonal variation of concentrations of leaf soluble protein of sweet potato cultivars. Experimental conditions were described in "Materials and Methods". ●, Tainong 65; ○, HP-4; ▲, Chang-hua; △, Tainong 25.

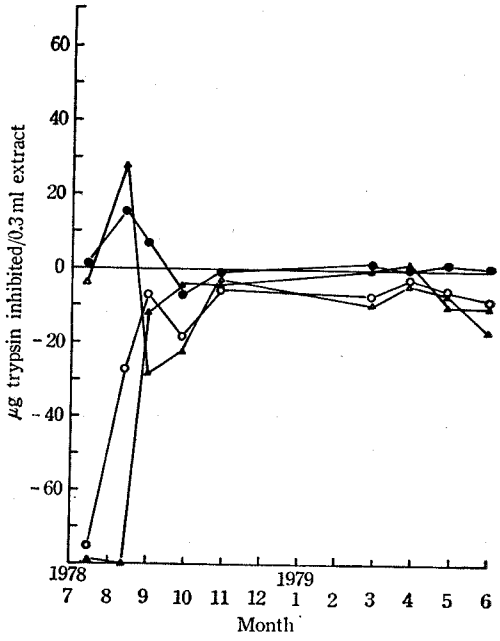

Fig. 2. Seasonal variation of effector activities on trypsin-casein of leaf soluble protein of sweet potato cultivars. ●, Tainong 65; ○, HP-4; ▲, Chang-hua; △, Tainong 25.

n=8)>HP-4 (3.48±0.72, n=8)>Chang-hua (3.01±0.44, n=8)>Tainong 25 (2.55±0.36, n=8). A few exceptions were: Tainong 65 (3.90 mg/0.3 ml)>Chang-hua (3.10)>Tainong 25 (2.92)>HP-4 (2.85) for samples of Oct. 23, 1978; Tainong 65 (4.29)>Chang-hua (3.10)>HP-4 (2.73)>Tainong 25 (2.39) for samples of Nov. 22, 1978.

Seasonal Variation of Effector Activities on Trypsin-Casein of Leaf Soluble Proteins of Sweet Potato Cultivars

Figure 2 shows the seasonal variation of combined effect on typsin-casein of inhibitor and activator activities of leaf soluble protein of sweet potato cultivars. Patterns of variation of all four cultivars were quite different. In average, leaf extracts of Tainong 65 exhibited the highest inhibitory activity while that of Chang-hua exhibited the highest activating activity. Leaf extracts of Tainong 25 showed the most drastic changes form being inhibitory to be activating with fluctuations. Leaf extract of HP-4 showed the least changes which fluctuated only with activating activity.

Seasonal Variation of Effector Activities on Trypsin-Casein of Heated Leaf Soluble Proteins of Sweet Potato Cultivars

Figure 3 shows variation of effector activities on trypsin casein of heated leaf soluble extract of sweet potato cultivars. Experiments were done with fresh leaves harvested on March 23, April 23, May 23, and June 25 1979. Comparing with Fig. 2, it is obvious that heating has differential inactivation toward inhibitory activity and activating activity of leaf extract on trypsin-casein. Heating reduced apparent activating activity while increased apparent inhibitory activity. In general, cultivars with higher apparent trypsin inhibitor activity also had higher trypsin inhibitor activity in roots (cf. Lin and Chen, 1980). The order was Tainong 65>Chang-hua >Tainong 25>HP-4 (results of May and June, 1979 were 2 exceptional data).

Responses of Trypsin Toward Effectors of Leaf Extract of Sweet Potato with Different Substrates

Activating activity of leaf extract was observed only when casein was used as substrate with a final concentration of 10 0 mg/2.5 ml. On the contrary inhibitory

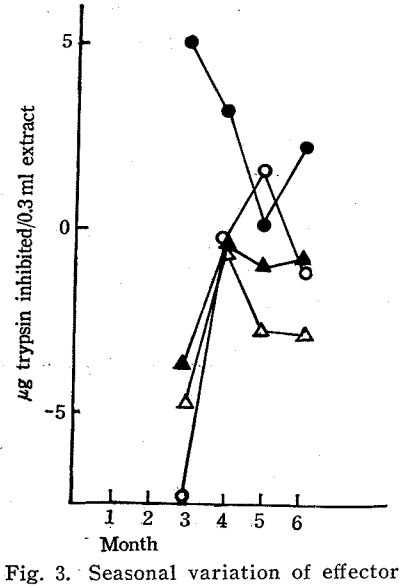

Fig. 3. Seasonal variation of effector activities on trypsin-casein of heated leaf soluble protein of sweet potato cultivars, 1979.
●, Tainong 65; ○, HP-4;
▲, Chang-hua; △, Tainong 25.

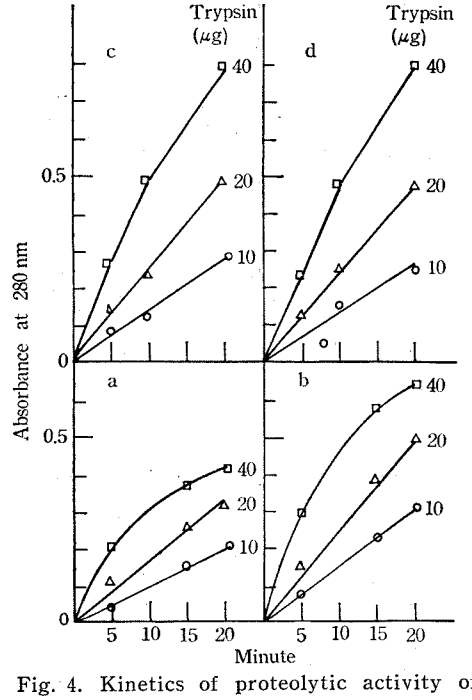

Fig. 4. Kinetics of proteolytic activity of trypsin on casein.
a. Final concentration of casein was 0.4%.
b. Final concentration of casein was 0.8%.
c. Final concentration of casein was 1.6%.
d. Final concentration of casein was 2.4%.

activity was found when hemoglobin was used as substrate with a final concentration of 10.7 mg/2.5 ml. When BAPA was used as substrate, an average of slight inhibition was observed (n=30). In order to further investigate this peculiar phenomenon, leaf extracts of four cultivars were used in a similar experiment. The results are presented in Table 1. Leaf extract of HP-4 showed a similar pattern of effect as that of Chang-hua. In the case of Tainong 65, although no apparent activating activity was observed when casein was used as substrate, the apparent inhibitory activity was much smaller than that observed with hemoglobin as substrate. This was actually a similar pattern as that of Chang-hua and HP-4. The only exception was Tainong 25. Inhibitory activity was observed no matter casein or hemoglobin was used as substrate. When hemoglobin was used as substrate higher inhibition was found with higher concentraton (32.0 mg/2.5 ml of reaction mixture) than that found with lower concentration (10.7 mg/2.5 ml) and this was true for all four cultivars used.

Table 1. *Responses of trypsin toward effectors of leaf extract of sweet potato with different substrates*

The concentrations of water-soluble protein of leaf extract of Chang-hua, HP-4, Tainong 65, and Tainong 25 were: 3.36, 3.72, 4.14, and 2.26 mg/0.3ml, respectively. Solutions of 8, 9, 9, and 6 times diluted extract of the 4 cultivars respectively were used for activity assays. Fresh leaf extract was prepared on Sep. 7, 1978. The concentration of casein used was 10 mg/2.5 ml of reaction mixture.

Cultivar	Casein		Hemoglobin	
	A	B	A	B
	mean±s. e.			
Chang-hua	−198.6±63.0 (n=4)	−59.1±27.9	32.2± 6.9 (n=4)[a]	9.6± 2.1
			84.2±51.0 (n=3)[b]	25.1+15.2
HP-4	− 27.2±25.0 (n=4)	− 7.3± 6.7	19.0± 9.7 (n=4)[a]	5.1± 2.6
			44.4± 6.1 (n=4)[b]	11.9± 1.6
Tainong 65	25.8±20.8 (n=3)	6.2± 5.0	41.4±11.4 (n=4)[a]	10.0± 2.8
			62.6±28.8 (n=6)[b]	15.1± 7.0
Tainong 25	28.0±11.3 (n=3)	12.4± 5.0	29.0±11.6 (n=4)[a]	12.8± 5.1
			44.0± 7.2 (n=4)[b]	19.5± 3.2

A: μg trypsin inhibited.
B: μg trypsin inhibited /mg protein of leaf extract.
[a]: The concentration of hemoglobin used was 10.7mg/2.5ml of reaction mixture.
[b]: The concentration of hemoglobin used was 32.0mg/2.5ml of reaction mixture.

In Table 1, the diluted leaf extract of Chang-hua and HP-4 contained 0.42 and 0.41 mg protein in 2.5 ml final reaction mixture which increased respectively 4.2% and 4.1% total soluble protein served as substrate. So it is obvious that the observed % activation of 993 and 136 for Chang-hua and HP-4 respectively can not be explained satisfactorily by increaseing of substrate protein. For the sake of certainty, we reexamined the kinetics of proteolytic activity of trypsin on casein. This was shown in Fig. 4. A final concentration of 0.4% casein equals to

Table 2. *Effect of heating on activating and inhibitory activities of leaf extract measured at different fold of dilution*

Concentrations of water-soluble protein of leaf extract of Chang-hua, HP-4, Tainong 65, and Tainong 25 were 3.60, 4.98, 5.22, and 3.24 mg/0.3ml, respectively. Fresh leaf extract was prepared on March 22, 1979. A and B are the same as those in Table 1.

Cultivar	Heating	Fold of dilution	A	B
			mean±s. e. (n=3)	
Chang-hua	no	8	−0.48±0.06	−0.13±0.02
		4	4.20±0.07	1.17±0.02
		2	5.20±0.07	1.44±0.02
	70°C, 10 min	8	−3.60±0.03	−1.00±0.01
		4	4.00±0.06	1.11±0.02
		2	13.80±0.07	3.83±0.02
HP-4	no	8	−7.20±0.02	−1.45±0.01
		6	−2.40±0.06	−0.48±0.01
		4	8.40±0.21	1.69±0.04
	70°C, 10 min	8	−7.20±0.05	−1.45±0.01
		6	−2.40±0.02	−0.48±0.003
		4	9.80±0.23	1.97±0.05
Tainong 65	no	8	1.82±0.01	0.35±0.001
		6	8.00±0.04	1.53±0.007
		4	8.00±0.08	1.53±0.02
	70°C, 10 min	8	5.02±0.02	0.96±0.005
		6	10.60±0.14	2.03±0.03
		4	10.20±0.13	1.95±0.02
Tainong 25	no	8	−8.60±0.01	−2.65±0.004
		4	−12.60±0.15	−3.89±0.05
		2	−33.00±2.20	−10.20±0.68
	70°C, 10 min	8	−4.60±0.06	−1.42±0.02
		4	−8.20±0.12	−2.53±0.04
		2	−1.05±0.05	−0.32±0.02

10.0 mg casein/2.5 ml reaction mixture. When 20 μg trypsin and 0.4% casein were included in the final reaction mixture A_{280} increased linearly with time up to 20 min but higher concentrations of casein up to 0.8%, raised the final level of A_{280} after 20 min. Increasing casein concentration 100% raised A_{280} less than 50%.

Effect of Heating on Activating and Inhibitory Activities of Leaf Extract Measured at Different Fold of Dilution

Table 2 shows that activating activity (or less inhibitory activity) was observed with more diluted samples and this was true for all cultivars except Tainong 25. In general, heating (70°C for 10 min) had higher degree of inactivation on activating activity than on inhibitory activity. This was found with samples of all four cultivars.

Discussion

Calculation based on data of Fig. 1 shows that water extraction removed an average of 57.8, 46.7, 40.3, and 35.9 g of protein from each kg of fresh leaves of Tainong 65, HP-4, Changhua, and Tainong 25 respectively. With leucerne (*Medicago sativa*), extraction removed an average of 95.9 g of protein from each kg of fresh herbage (Stockdale *et al.*, 1981). So, sweet potato seems to be a good source of leaf protein as far as the quantity is concerned.

Patterns of seasonal variation of soluble leaf protein of all four sweet potato cultivars are quite similar (Fig. 1). This suggests that the metabolic machinery of sweet potato leaves is well adaptive to climatic changes. There are some differences among patterns of seasonal variation in the activities of trypsin effectors of leaf extract from four sweet potato cultivars (Fig. 2 and Fig. 3). This suggests that biosynthesis and degradation of trypsin effectors do not synchronize with most soluble proteins.

The presence of activating activity on trypsin-casein in sweet potato leaves of some cultivars such as Chang-hua and HP-4 and possibly also in Tainong 65 and Tainong 25 is quite interesting. It is possible that there is trypsin-like proteinase in sweet potato leaves. The activity of the enzyme may be regulated by the activating activity which we have found.

Inhibitory activity on trypsin was observed when casein, hemoglobin, or BAPA was used as substrate although in the case of BAPA only slight inhibition was found. This suggests that the inhibitory activity aims directly toward trypsin instead of substrat molecules. Based on results of Table 1, activator(s) may convert the 3-D structures of casein molecules into conformations that will be hydrolyzed more easily by trypsin.

Two effector activities on trypsin could be observed when casein was used as substrate. Whether the apparent activity was inhibitory or activating depended on

the relative levels of both inhibitors and activators. Heating (70°C for 10 min) decreased primarily the activating activity (Table 2). So results obtained with heated crude extract represented mainly levels of inhibitor activity (Fig. 3). We have already found that crude root extract of two class III sweet potato cultivars (Tainong 62 and 54) expressd activating effect on trypsin-casein and crude root extract of all class III cultivars had higher inhibitory activity after heating at 70°C for 10 min than samples without heating (Lin and Chen, 1980). This suggests that in some cultivars the activators of trypsin-casein may be found in both leaves and roots.

The relationship between trypsin inhibitors in leaves and roots of sweet potato is not clear so far. As far as levels of TIA is concerned, a parallel relationship was found based on the results of Fig. 3 and our previous report (Lin and Chen, 1980). With exceptions of only two data points (May and June, 1979), cultivars with higher apparent trypsin inhibitor activity in leaves also had higher trypsin inhibitor activity in roots. The order is Tainong 65>Chang-hua>Tainong 25> HP-4.

Results of Table 2 together with our unpublished data show that leaf inhibitory activity was more heat stable than activating activity. But the latter was more stable when stored at 7°C than the former. Activating activity was observed with more diluted samples while inhibitory activity was observed with less diluted samples of the same preparation of the same cultivar (Table 2). This suggests either activator(s) on trypsin-casein may function at very low concentrations or activator(s) may be more stable than inhibitors at low concentrations. In case of Tainong 25 the opposite situation may be true. These properties are useful for future experiments of similar subject.

Literature Cited

AVRDC (Taiwan). 1975. The trypsin inhibitors of sweet potato. *In* Sweet Potato Report, p. 26.

Dickey, L. F. and W. W. Collins. 1984. Cultivar differences in trypsin inhibitors of sweet potato roots. J. Amer. Soc. Hort. Sci. **109**: 750-754.

Erlanger, B. F., N. Kokowsky, and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. **95**: 271-278.

Kunitz, M. 1945. Crystalline soybean trypsin inhibitor. Science **101**: 662.

Kunitz, M. 1946. Crystalline soybean trypsin inhibitor. J. Gen. Physiol. **29**: 149-154.

Laskowski, M., Jr. and R. W. Sealock. 1971. Protein proteinase inhibitors. *In* P. D. Boyor, H. Lardy, and K. Myrback (eds.), The Enzymes, Vol. 3. Academic Press, New York, pp. 375-473.

Lawrence, G. and R. Cooke. 1980. Experimental pigbel: the production and pathology of necrotizing enteritus due to *Clostridium welchii* Type C in the guinea pig. Brit. J. Expt. Pathol. **61**: 261-271.

Lawrence, G. and P. D. Walker. 1976. Pathogenesis of enteritis necroticans in Papua New Guinea. The Lancet, Jan. 17.

Lin, Y. H. and H. L. Chen. 1980. Level and heat stability of trypsin inhibitor activity among sweet potato varieties. Bot. Bul. Acad. Sinica **21**: 1-13.

Lin, Y. H. 1982. Levels and heat stability of trypsin inhibitor activity among sweet potato (*Ipomoea batatas* Lam.) varieties (including new data). Abstract of invited lecture given at "International Symposium on Medical and Biological Aspects of Proteinase Inhibitors" held at Tokushima, Japan, August 5th-8th, 1982.

Lin, Y. H., J. F. Cheng, and H. Y. Fu. 1983. Partial purification and properties of trypsin inhibitors of sweet potato (*Ipomoea batatas* Lam.) roots. Bot. Bull. Academia Sinica **24**: 103-113.

Lowry, O. H., A. L. Farr and J. J. Randally. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**: 265-275.

Ogiso, T., S. Tamura and Y. Kato. 1974. Studies on trypsin inhibitors in sweet potato. II. Modification of amino acid residues in inhibitor. III. J. Biochem. (Tokyo) **76**: 147-156.

Richardon, M. 1981. Protein inhibitors of enzymes. Food Chem. **6**: 235-253.

Ryan, C. A. 1981. Proteinase inhibitors. *In* P. K. Stumpf and E. E. Conn (eds.), The Biochemistry of Plants. Academic Press, New York, pp. 351-369.

Sohonie, K. and A. P. Bhandarker. 1954. Trypsin inhibitors in Indian foodstuffs: I. Trypsin inhibitors in vegetables. J. Sci. and Indust. Res. (India) **13B** (7): 500-503.

Stockdale, C. R., D. R. Kackenzie, and K. R. King. 1981. Nutritive value for lactating dairy cows of the pressed herbage remaining after the partial extraction of leaf juice. Aust. J. Exp. Agric. Anim. Husb. **21** (111): 376-381.

Sugiura, M., T. Ogiso, and K. Takeuchi. 1973. Studies on trypsin inhibitors in sweet potato I. Purification and some properties. Biochim. Biophys. Acta **328**: 407-417.

甘藷葉的水溶性蛋白質

林耀煇 何素鵬

中央研究院植物研究所

甘藷品種臺農65、HP-4，彰化和臺農25的葉子所含的水溶性蛋白質含量隨季節而變化的起伏樣式相當類似。從這4個品種每一公斤新鮮葉子可以分別抽出水溶性蛋白質的量依次爲57.8±9.2，46.7±9.7，40.3±5.9及35.9±5.1公克（8個月採集之結果，大約每個月採一次）。至於這4個品種葉子粗抽液的胰蛋白酵素（trypsin）的調節因子之活性高低隨季節變化的情形，從1978年8月到11月這段期間是不同的；然而從1979年3月到6月這段期間是類似的。粗抽液經加熱以後（70°C，10分鐘）所測得之胰蛋白酵素抑制因子的活性從1979年3月，到6月臺農65、HP-4以及彰化和臺農25分別表現出三種不同的樣式。這4種甘藷品種的葉子之水抽液中均含有胰蛋白酵素抑制因子，和胰蛋白酵素牛乳蛋白系統之致活因子。抑制因子比致活因子耐熱；但是在7°C儲藏或者在稀釋溶液中後者之活性都比前者耐久（這是以同一品種的同一次之葉子粗抽液比較得到的）。從這4個甘藷葉子粗抽液都含有致活因子之活性的事實來推論，甘藷葉或芽應該含有類似trypsin之蛋白質水解酵素。一般而言，在葉子內含高的trypsin抑制因子活性的品種它的塊根也含有高的抑制因子活性。