Determination of Cyanogenic Compounds in Edible Plants by Ion Chromatography

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Cyanogenic glycosides are HCN-producing phytotoxins; HCN is a powerful and a rapidly acting poison. It is not difficult to find plants containing these compounds in the food supply and/or in medicinal herb collections. The objective of this study was to investigate the distribution of total cyanide in nine genera (*Dolichos*, *Ginkgo*, *Hordeum*, *Linum*, *Phaseolus*, *Prunus*, *Phyllostachys*, *Phytolacca*, and *Portulaca*) of edible plants and the effect of the processing on cyanide concentration. Total cyanide content was measured by ion chromatography following acid hydrolysis and distillation. Kernels of *Prunus* genus are used medicinally, but they possess the highest level of total cyanide of up to 2259.81 CN⁻/g dry weight. Trace amounts of cyanogenic compounds were detected in foodstuffs such as mungbeans and bamboo shoots. Currently, except for the WHO guideline for cassava, there is no global standard for the allowed amount of cyanogenic compounds in foodstuffs. However, our data emphasize the need for the guidelines if plants containing cyanogenic glycosidesare to be developed as dietary supplements.

Key words: Cyanogenic compounds, Herbal medicines, Ion chromatography

INTRODUCTION

There are various forms of cyanogenic compounds that release hydrogen cyanide upon breakdown. The cyanogenic compound is present mainly as glycoside in more than 2650 plant species. Apricot kernel, peach kernel, cassava, almond, bamboo shoot, sorghum, Japanese apricot, flaxseed among others have been consumed by human worldwide either as food or as herbal medicine (1,2). About ten cyanogenic glycosides including amygdalin, prunasin, dhurrin, linamarin, and taxiphyllin have been reported in edible plants (3). Hydrogen cyanide derived from cyanogenic glycoside can cause health concerns including cell death by blocking cytochrome oxidase and the arrest of the ATP production.

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Several symptoms have been related to the consumption of cyanogen containing foodstuff including vomiting, nausea, dizziness, weakness, konzo, and occasional death. Chronic intake has been linked to goiter especially in iodine deficiency cases (4,5).

Various methods have been used for the quantitative analysis of cyanogenic compounds of edible plants. The colorimetric method via König reaction after acid hydrolysis, picrate method, and the chromatographic method are the most common ones (6-13). The analysis using the colorimetric method involves three steps: (1) extraction of cyanogenic compounds from the plants material, (2) acid hydrolysis of cyanogenic glycoside, and (3) the color development and detection of cyanide (14). The König reaction used in the color development step detects not only cyanide but also thiocyanate, other plant secondary metabolites, making the method less specific to cyanide. Also during the acid hydrolysis, HCN gas may dissipate, resulting in an underestimation. The colorimetric method may employ enzymatic hydrolysis either intrinsic or extrinsic, however, tannin from the plant tissue may inhibit the enzyme giving rise to another source of underestimation (14,15). A gas chromatographic measurement of hydrogen cyanide released

by the hydrolysis has been tried but this method could not prevent the loss of the HCN gas (12). Liquid chromatography has been used to quantify cyanogenic glycosides. But it can only be used for the known cynogenic glycoside with standard materials and it is difficult to estimate the total cyanogenic compounds in plant. Moreover tannin, flavonoid and chlorophyll in plant tissue have been shown to interfere with the liquid chromatographic determination of the cyanogenic glycosides (11,13). The hydrolysis step seems to be inevitable to measure total cyanogen content. In this study, ion chromatogryphy following acid hydrolysis was employed to prevent thiocyanate interference of the colorimetric methods.

There has been no concern for the cyan toxicity from Korean staple. However, since there have been attempts to develop dietary supplements with some of the plants containing cyanogenic glycoside, it was imperative to determine cyanogens in plants consumed in Korea.

MATERIALS AND METHODS

Food materials and Herbal medicines reported to contain cyanogenic compounds were purchased from the various markets in Seoul. Edible plants from 14 genera, *Dolichos*, *Ginkgo*, *Hordeum*, *Linum*, *Malus*, *Manihot*, *Phaseolus*, *Phyllostachys*, *Phytolacca*, *Portulaca*, *Prunus*, *Sorghum*, *Vicia*, and *Vitis*, have been included in the study (4,7,11,16). The origins of the plants were Korea (both South and North) and China. Cyanide standard for the ion chromatography (1000 ppm) was obtained from Fluka Co, USA. Phosphoric acid, barbituric acid, isonicotinic acid and chloramine-T were obtained from Sigma-Aldrich Chemical Co, USA. Micro Dist, from Lachat Co (USA) and Dionex (USA) ion chromatograph, DX-2500, were used for the cyanide distillation and quantification, respectively.

Detection of total cyanide in edible plants. Acid hydrolysis of Brandbury (8) and distillation used by Hong *et al*. (6) has been combined and used for the pre-chromatographic step. Each sample (about 1 g) was homogenized

**Table 1.** Ion Chromatographic Condition to analyze CN⁻ anion

Item	Conditions
IC	Dionex DX-2500 system
Column	Guard: IonPac AG7 (40 mm × 50 mm)
	Analytical: IonPac AS7 (40 mm × 250 mm)
Detector	ED40, DC Amperometry
Flow rate	1.0 ml/min (isocratic), 200 µl injection
Electrode cell	Silver working electrode
	0.00 V vs Ag/AgCl reference
Mobile phase	0.5 M Sodium Acetate/0.1 M Sodium Hydroxide/ 0.5% (v/v) Ethylenediamine

with 30 ml of 0.1 M phosphoric acid and the solution was centrifuged for 20 min at 8000 rpm. 3 ml of the supernatant was transferred to the tight capped vial and the equal amount of 4 M sulfuric acid was added. Hydrolysis was started by heating to 100°C and continued for 50 mins. The reaction mixture was allowed to be cooled in ice. The hydrolysis mixture was transferred to the Micro Dist tube (Lachat, USA) with 0.75 ml of 0.79 M MgCl₂ and heated for 45 min. After cooling at ambient temperature, cyanide collected in sodium hydroxide solution was analyzed by ion chromatography. The chromatographic condition was summarized in Table 1. Calibration curve was constructed with ion chromatographic cyanide standard. The working standards were diluted with 0.2 M sodium hydroxide solution.

RESULTS AND DISCUSSION

Cyanide analysis using ion chromatography. The ion chromatograms of cyanide standard (a) and cyanide in apricot kernel (b) are given in Fig. 1. Unlike colorimetric method, thiocyanate ion did not interfere with the detection (data not shown). Limit of detection, determined as signal to noise ratio(S/N) of 3 was 0.005 mg CN⁻/L.

Total cyanide in herbal medicines. Herbal medicines used in Korea, which belong to the genera known to con-
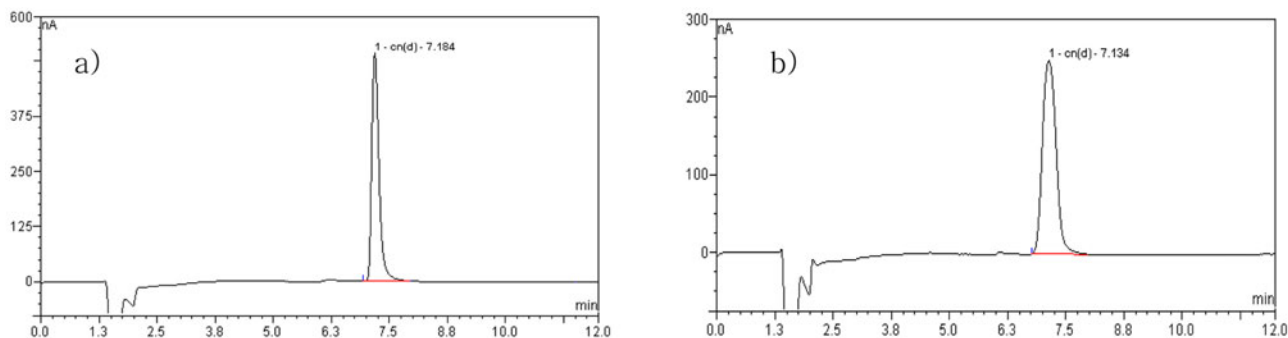
Fig. 1. Ion chromatogram of (a) cyanide standard and (b) cyanide from apricot kernel.

Table 2. Cyanogenic Compounds in Medicinal Plants Sold in Korea

Scientific name		Sample name	N	Total Cyan Liberated by Acid Hydrolysis (µg CN⁻/g dry wt.)*	
				Range	Mean ± SD†
Dolichos	*D. lablab*	Hyacinth bean	3	n.d. - trace	
Ginkgo	*G. biloba*	Ginko semen	4	trace - 1.00	0.25 ± 0.5
		Dried Ginko	1		trace
Hordeum	*H. vulgare*	Hordei germinates	1		2.65
Linum	*L. usitatissimum*	Flaxseed	4	261.9 - 345.4	299.82 ± 37.58
Phyllostachys	*P. nigra*	Bamboo inner skin	3	n.d. - trace	trace
	P. bambusoides	Bamboo leaf	3		trace
Phytolacca	*P. esculenta*	Phytolacca radix	1		149.55
Portulaca	*P. grandiflora*	Rose moss	1		14.81
	P. oleraceae	Pulselane	3	1013.39 - 1371.85	1152.25 ± 192.39
Prunus	*P. armeniaca*	Apricot kernel	10	6.42 - 2259.81	1679.27 ± 697.41
		Apricot kernel, skinned	13	n.d. - 152.70	22.93 ± 41.98
	P. nakii	*P. nakaii* semem	9	226.64 - 1305.34	872.38 ± 419.41
	P. mume	Mume fruits	4	22.63 - 88.27	45.87 ± 30.45
	P. persica	Peach kernel	14	442.72 - 1044.45	884.89 ± 159.42
	P. tomentosa	Cherry, dried	1		trace

* N: The number of samples; n.d.: Not Detected. trace: a peak can be seen but its area was less than limit of detection.
† Where there was a single sample, the value represent the mid-point of duplicates for the sample. In such cases C.V. was less than 10%.

tain cyanogenic glycosides, were purchased from the professional market in Seoul. All of the samples were originated from either Korea (South and North) or China. The results are shown in Table 2. Each analysis was duplicated and a mid-point value was reported for each sample. Coefficient of variance was lower than 10% for each of the samples. Since the cyanogen contents varied greatly from sample to sample, ranges of the values as well as the mean are reported. In cases where both domestic and imported samples were analyzed, there was no difference in cyanogens contents by origin.

The highest amount was found in apricot kernels (*P. armeniaca*), followed by *P. domestica* and *P. nakii*. Peach kernels (*P. persica*) also showed high amount of high amount of cyanogens. In case of apricot kernel, both peeled and intact dried kernels were available in the market. The peeled kernel showed average 22.9 µg CN⁻/g, while intact kernel showed average 1679 µg CN⁻/g . It seemed that most of cynogenic compounds were localized in the skin. In Korea, these medicinal plants can be purchased without prescription in the open market. Since apricot kernel is said to have cosmetic effect, the consumption of the apricot kernel as folk medicine both in topical and oral use is not rare among middle aged Korean women. Moreover, some of the plants listed in Table 2, though the cyanogens contents were low, are permitted as foods as well. Ever since the phytoestrogenic activities of the flaxseed lignin were reported, the consumption of flaxseed has been increasing.

The cyanogenic compounds were also detected in other

herbal medicines. *Prunus mume* was dried form of a Japanese apricot, and showed 45.87 µg CN⁻/g dry weight. Cyanogenic compounds were detected in the kernels of all of the tested species of *prunus*.

The difference between cyanogens contents in the samples of the single species purchased in different locations ranged greatly, up to thousand fold, probably reflecting the variations of cultivation, harvest and/or storage.

Total cyanide analysis in dietary plants. Including *Prunus* genera, many other plants known to contain cyanogens are consumed as food as well as herbal medicines. Food originated from *Hordeum*, *Marlus*, *Manihut*, *Phaseolus*, *Prunus*, *Sorghum*, and *Vitis* are analyzed for the total cyanogens and shown in Table 3. While kernels from the *Prunus* genera, which are generally consumed as medicine, contained large amount of cyan, the edible parts including peel and flesh showed much smaller amount. However seeds from the fruits, apple, grape, peach, plum, and apricot, showed larger amount of cyanogens than flesh (data not shown), necessitating caution in food preparation. Cassava and the starch derived from it are not typical food items in Korea, but gaining popularity through the globalization of the food supply.

As mentioned earlier, some of the medicinal foods are also consumed as food. Purslane, though not cultivated, are gathered and eaten as cooked, and showed average 1150 µg CN⁻/g. Also flaxseed showed about 300 µg CN⁻/g. Though cyanogens were detected in other edible plants, the amount

Table 3. Cyanogenic compounds in foodstuffs

| Scientific name | Common name | N | Amount of cyanide (ug CN⁻/g) * | |
			Range	Mean ± SD †
Hordeum	*H. vulgare*	Barley	11		trace
Malus	*M. pumila*	Apple, edible parts	6		trace
		Apple, skin	6	trace - 1.27	0.42 ± 0.73
		Apple, seed	6	104.39 - 178.19	124.4 ± 31.4
Manihot	*M. esculenta*	Cassava	1		365.44
		Tapioka (starch)	1		0.81
Phaseolus	*P. angularis*	Red bean	5	n.d. - trace	trace
	P. limensis	Lima bean	1		trace
	P. radiatus	Mungbean, whole	2		trace
		Mungbean, skinned	4	trace - 2.23	0.56 ± 1.12
		Mungbean powder	2		trace
	P. vulgaris	Kidney bean	2		trace
Prunus	*P. armeniaca*	Apricot, flesh	2	trace - 1.01	0.51
		Apricot, skin	2	trace	trace
	P. aviun	Cherry, flesh	1	trace	trace
		Cherry, skin	1	trace	trace
		Cherry, seed	1		522.78
	P. communis	Almond	2		n.d
	P. domestica	Plum, flesh	8	n.d. - trace	trace
		Plum, skin	8	n.d. - 13.26	2.65 ± 5.93
		Plum, seed	5	505.14 - 1032.96	659.1 ± 321.16
	P. mume	Japanese apricot, flesh	2	trace - 0.82	0.41
		Japanese apricot, skin	2		trace
		Japanese apricot, seed	2	1026.59 - 1155.45	1091.02
	P. persica	Peach, flesh	11	n.d. - trace	trace
		Peach, skin	11	n.d. - trace	trace
		Nectarine, flesh	4	n.d. - trace	trace
		Nectarine, skin	4	trace	trace
Sorghum	*S. bicolor*	Millet	7	n.d. - 1.39	0.20 ± 0.53
Vitis	*V. vinifera*	Grape, flesh	6	n.d. - trace	trace
		Grape, skin	6	n.d. - trace	trace
		Grape, seed	5	n.d. - trace	trace

* N : The number of samples; n.d. : Not Detected. trace : a peak can be seen but its area was less than limit of detection.
† Where there was a single sample, the value represent the mid-point of duplicates for the sample. In such cases C.V. was less than 10%.

was too low to pose health concern.

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