Risk Assessment Studies
Report No. 27

Natural Toxins in Food Plants

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Table of Contents

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Abstract

Natural toxins are present in a wide variety of plants. Some of these plants are commonly consumed as food. These toxic substances when ingested in significant amount or when they are not processed appropriately can be potentially harmful to human health causing food poisoning.

This study conducted by the Centre for Food Safety of the Food and Environmental Hygiene Department aimed to review natural toxins in food plants commonly consumed in Hong Kong and the measures that can be employed to prevent poisoning from consumption of these food plants. Laboratory study of two natural toxins, glycoalkaloids and cyanogenic glycosides, was carried out to determine the levels of these toxins in food plants commonly consumed in Hong Kong. The effects of preparation and cooking on the reduction of the toxin levels were also studied.

Results showed that glycoalkaloid contents varied among the different types of the fresh potatoes tested which ranged from 26-88 mg/kg (average 56 mg/kg). This was within the normal range of glycoalkaloid contents in potatoes of 20 - 100 mg/kg, which JECFA considered that consumptions on a daily basis were not of concern. The highest concentrations of glycoalkaloids were found in potato sprouts. Cyanide was detected in bitter apricot seed, bamboo shoot, cassava, and flaxseed samples in their raw state at levels of 9.3 mg/kg to 330 mg/kg. Cyanide contents were found to be higher in bitter cassava than sweet cassava. Cyanide concentration was found to be highest at the tip portion of bamboo shoot, followed by the middle portion, then the base portion. Cutting cyanogenic food plants into small pieces and cooking them in boiling water reduced cyanide contents of the food commodities by over 90%. Dry heat could not reduce cyanide contents effectively and only reduced around 10% of the cyanide contents in flaxseeds following oven-heating for 15 minutes.

Consumers should avoid buying or eating potatoes that show signs of sprouting, greening, physical damage or rotting since glycoalkaloids are not decomposed by cooking. Cutting the cyanogenic plants into smaller pieces and cooking thoroughly in boiling water help release toxic hydrogen cyanide before consumption. When the cooking method chosen is heating under dry-heat or at low moisture contents, limit the intake of the cyanogenic plants to only small amounts.

Risk Assessment Studies –

Natural Toxins in Food Plants

OBJECTIVES

This study aimed to review natural toxins in food plants commonly consumed in Hong Kong and the measures that can be employed to prevent poisoning from consumption of these food plants. A laboratory study was conducted to determine the levels of toxins in some food plants and the effect of preparation and cooking on the toxin levels. Health advice concerning food selection, methods of preparation and processing of toxigenic food plants for safe consumption was formulated.

INTRODUCTION

2. Natural plant toxins may be present inherently in plants such as fruits and vegetables which are common food sources. They are usually metabolites produced by plants to defend themselves against various threats such as bacteria, fungi, insects and predators.[1] Natural toxins may also be present in food plants as a result of natural selection and new breeding methods that enhance these protective mechanisms.

Common fruits and vegetables containing natural toxins

3. Different types of natural toxins may be found in different crop plants and in different parts of a plant. The parts of a crop plant which may be used as food sources include the foliage, buds, stems, roots, fruits and tubers. Common examples of natural toxins in food plants include lectins (植物血球凝集素) in beans such as green beans (四季豆、扁豆、刀豆), red kidney beans (紅腰豆) and white kidney beans (白腰豆); cyanogenic glycosides (氰甙) in bitter apricot seed (北杏), bamboo shoots (竹筍), the edible roots of cassava plants (木薯), and flaxseeds (亞麻籽); glycoalkaloids (甙生物鹼) in potatoes (馬鈴薯); and muscarine (毒蕈鹼) in wild mushrooms.

Methods of reduction of plant toxins

4. For some types of natural toxins, post-harvest processing treatments and cooking of the plant result in destroying the endogenous toxic substances or reduction of its toxicity. Toxin reduction may be made more effective by certain preparation or processing methods such as cutting into smaller pieces prior to cooking. Other natural toxins, however, remain unchanged after usual preparation and cooking. In these cases, special care has to be exercised in selecting the food plants and in limiting the amount of intake.

Toxicological effects of plant toxins

5. Toxicological effects following ingestion of plant toxins may range from acute effects of gastroenteritis to more severe toxicities in the central nervous system leading to death, as are seen in cases of poisoning due to cyanide

or certain alkaloids. In addition to acute toxicities, some plant toxins such as pyrrolizidine alkaloids may also cause chronic systemic effects, organ toxicities or teratogenic effects.

Food poisoning due to natural plant toxins

6. Poisonings induced by plant toxins have long been known through consumption of foods such as beans that are not fully cooked, some cultivars of potatoes, and ingestion of plants picked from the wild not intended for human consumption such as poisonous berries and mushrooms. Acute poisoning cases caused by plant toxins are sometimes underestimated due to the fact that the toxicity symptoms can be rather non-specific. In the past, acute poisoning from a high intake of glycoalkaloid, such as solanine, from potatoes has been mis-diagnosed as microbial food poisoning.[2]

7. In Mainland China, according to the statistics of the Ministry of Health, a total of 816 cases of food poisoning (involving 6804 number of persons with 109 deaths) caused by poisonous animals and plants were reported in the year 2005.[3] The Ministry of Health reported an increase of 43.1% of food poisoning cases caused by poisonous animals and plants in the last quarter of 2006 compared with the third quarter. This increase was mainly caused by inadequate cooking of beans (which constitutes as much as 54% of all cases of food poisoning from poisonous animals and plants).[4]

8. These data indicate that food poisoning from natural plant toxins can be a significant public health concern. Better education in the proper cooking and preparation of these toxigenic food plants is therefore important in the

prevention of human poisoning. In Hong Kong, the Centre for Food Safety received enquiries from time to time regarding the safe consumption for foods containing natural toxins. Hence, it is considered necessary to conduct a study in natural toxins in some food plants available in the Hong Kong market and commonly consumed by the local population so that advice to the public can be formulated for the prevention of food poisoning.

SCOPE OF STUDY

9. The types of natural toxins and the plants containing them are numerous. This study focuses on some toxins in food plants commonly consumed in Hong Kong where acute poisoning may be a concern. In particular, attention is given to those groups where measures can be employed to prevent poisoning so that advice for intake or methods of reduction can be formulated. The group of plants from the wild cultivar not intended for human consumption but causes food poisoning from time to time due to wild-picking or to mis-identification is outside the scope of this study. Therefore, acute poisonings from wild mushroom or other wild plants such as giant elephant's ear is not the subject of study here.

10. This study is comprised of the following components:

a) Review of a number of natural toxins in food plants commonly consumed in Hong Kong, including the toxicological effects associated with the ingestion of these toxins, the ways in which toxin levels may be reduced and the measures for the prevention of poisoning from the consumption of these food plants.

b) Laboratory study of two natural toxins, glycoalkaloids and cyanogenic glycosides, to determine the levels of these toxins in the food plants available locally and the effect of preparation and cooking in the reduction of the toxin levels.

Review on Some Natural Toxins in Food Plants

Background

11. Toxic food components of plant origin may be low-molecular-weight endogenous toxins or products of secondary metabolism. Products of primary metabolism are involved in energy metabolism, such as photosynthesis, growth, and reproduction. The macronutrients and micronutrients in plants are the products of primary metabolism. Products of secondary metabolism are species specific and give the plant its particular characteristics. They include plant pigments, flavours, and compounds that serve to protect the plants. Some of these secondary metabolic products impart toxicity to the individual when taken orally. These substances may be growth inhibitors, neurotoxins, carcinogens, and teratogens.[5]

12. Common classes of plant toxins include alkaloids such as pyrrolizidine alkaloids and glycoalkaloids, cyanogenic glycosides, lectins, saponins, and antinutrients. Some of these toxins will be discussed in the following sections.

ALKALOIDS

13. Alkaloids are the bitter components of plants found widely in nature and frequently have pharmacological properties. Mostly acting as

secondary plant metabolites, alkaloids are often basic nitrogen–containing compounds able to form salts with acid.

14. Alkaloids have been isolated from the roots, seeds, leaves, or bark of some members of at least 40% of plant families. Families being particularly rich in alkaloids include Amaryllidaceae, Compositae, Leguminosae, Liliaceae, Papaveraceae, and Solanaceae.[6]

Alkaloids Found in Plants and in Common Foods

15. One type of alkaloid widely found in the plant kingdom is the pyrrolizidine alkaloids. These are chemicals found in as many as 6000 plant species or 3% of flowering plants. Plants containing these compounds are distributed in all climatic regions of the world.[7,8]

16. Ingestion of plants containing pyrrolizidine alkaloids are usually through contaminated crops since plants containing these alkaloids may grow as weeds in food crops such as wheat or corn and may be harvested with the grain. Another source of oral exposure to this group of toxins may be via intake of herbal foods and preparations containing these toxins. Prevention of poisoning can be achieved by reducing ingestion of alkaloid-containing foods and herbal preparations, and by applying effective measures in agriculture to reduce contamination of pyrrolizidine containing plants in food crops.

17. Other plant alkaloids such as the glycoalkaloids are found in common food plants acting like a natural pesticide against common pests. Common examples include solanine and tomatine. Solanine is an alkaloid present in small amounts in potatoes while tomatine is found in tomatoes.

Glycoalkaloids

18. Glycoalkaloids consist of a steroidal alkaloid coupled to one or more monosaccharides. All members of the botanical family solanacea produce glycoalkaloid toxins. In potatoes, the major glycoalkaloids are α-solanine and α-chaconine. They are formed in the parenchyma cells of the periderm and the cortex of the tubers. These chemicals are toxic to insects and animals and serve to protect the plant from predators. They are normally present in all potato tubers in small amounts and are concentrated in the skin of the potato and also in the areas of high metabolic activity such as the eye regions. Its level is increased in greened potatoes or blighted potatoes, and can reach very high levels in the sprouts. This toxin is not decreased by any of washing, soaking or cooking. Cooked potatoes that contain high level of solanine have a bitter taste and cause a burning sensation in the throat.

Toxicity

19. The amount of glycoalkaloids usually found in edible plants that are fresh and undamaged do not normally cause toxicity. However, glycoalkaloids can produce toxic effects at higher doses.

Acute Toxicity

20. The greatest concern for glycoalkaloid toxicity is its acute toxicity. There have been many reported cases of human poisonings (sometimes fatal) due to the ingestion of greened, damaged or sprouted potatoes as a consequence of high levels of glycoalkaloids.[9,10] Acute toxicity syndromes in humans have been observed at glycoalkaloid levels of more than 2.8 mg/kg body

weight.[9,10] Onset of symptoms has ranged from minutes to 2 days after ingestion of toxic potatoes, with longer incubation periods generally associated with the more severe cases.[11]

21. For low grade poisoning, symptoms are acute gastrointestinal upset with diarrhoea, vomiting and severe abdominal pain. The *solanum* alkaloids have strong anticholinesterase activity on the central nervous system.[12] Many alkaloids cause acute toxicity by mimicking or blocking the action of nerve transmitters. In more severe cases, neurological symptoms may be observed including drowsiness and apathy, confusion, weakness, and vision disturbances followed by unconsciousness and in some cases death.[2]

22. Glycoalkaloids also have saponin–like properties and can disrupt membrane function in the gastrointestinal tract leading to haemorrhagic damage. This damage can be severe enough to cause death with the extent of necrosis far outweighing the inhibitory effects on acetylcholinesterase activity.[13]

Chronic Toxicity

23. Because of the lack of chronic toxicity data, an adequate no-observed adverse effect level (NOAEL) for potato glycoalkaloids has not been assessed, and a tolerable daily intake (TDI) for humans has not been determined.[11]

<u>JECFA Evaluation</u>

24. JECFA considered that, despite the long history of consumption of plants containing glycoalkaloids, the available epidemiological and experimental data from human and laboratory animal studies did not permit the

determination of a safe level of intake.

25. Based on the available human data, an intake of total glycoalkaloids of 3-6 mg per kg body weight (bw) is considered by JECFA a potentially lethal dose for humans, and total glycoalkaloids of more than 1 to 3 mg per kg bw is considered a toxic dose for humans.[11,14] Children may be more sensitive than adults. Other factors may be present in suspect potatoes and modulate the toxicity of the steroid glycoalkaloids.

26. The JECFA Committee recognised that the development of empirical data to support a safe level of intake would require considerable effort. Nevertheless, it felt that the large body of experience with the consumption of potatoes, frequently on a daily basis, indicated that normal glycoalkaloid levels (20-100 mg/kg) found in properly grown and handled tubers were not of concern. [11]

CYANOGENIC GLYCOSIDES

27. Cyanogenic glycosides occur in at least 2000 plant species, of which a number of species are used as food. They are amino-acid-derived constituents of plants produced as secondary metabolites. There are approximately 25 cyanogenic glycosides known. Different kinds of cyanogenic glycosides may be found in different cyanogenic food plants, e.g. taxiphyllin in bamboo shoots, linamarin in cassava.[15]

Occurrence

28. Important staple foods for some parts of the world (such as

cassava and sorghum) contain cyanogenic glycosides. Other edible plants containing cyanogenic glycosides include bamboo shoot, flaxseeds, and seeds of stone fruits such as apricot and peach, seeds of peas and beans such as lima beans, and shell of soya beans. Other food products that may contain cyanogenic glycosides include some food ingredients with flavouring properties such as ground almonds powder or paste, marzipan, stone fruit preserves (cherry, plum, apricot, peach), and stone fruit juices, and alcoholic drinks made from stone fruits. These foods therefore represent potential sources of hydrogen cyanide.

<u>Toxicity</u>

29. Toxicity of cyanogenic glycosides-containing plant is due to the cyanide produced on ingestion. The plant species that produce cyanogenic glycosides usually also has a corresponding hydrolytic enzyme (β-glucosidase). In the presence of water, the non-toxic cyanogenic glycosides are hydrolysed by the enzyme producing cyanohydrins which quickly decompose to the toxic hydrogen cyanide. In this way, cyanogenic plants are protected against predators. Cyanogenic glycosides, cyanohydrins and hydrogen cyanide are collectively known as cyanogens.

<u>Acute toxicity</u>

30. In humans, the clinical signs of acute cyanide intoxication can include: rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhoea, mental confusion, stupor, cyanosis with twitching and convulsions followed by terminal coma. [15]

31. Death due to cyanide poisoning can occur when the cyanide level

exceeds the limit an individual is able to detoxify. The likelihood of cyanide intoxication from consumption of cyanide-containing food is dependent on body weight. For example, it is possible that a child or person of smaller body weight would not be able to detoxify the cyanide resultant from a meal of inadequately prepared cassava or bamboo shoots. The acute lethal dose of hydrogen cyanide for humans is reported to be 0.5-3.5 mg/kg bw. Approximately 50-60 mg of free cyanide constitutes a lethal dose for an adult man.[15]

Chronic toxicity

32. It is uncommon for dietary cyanide intake to cause chronic diseases. Adverse effects may, however, accompany individuals who have underlying dietary deficiency such as inadequate protein and/or iodine intake. For example, individuals already suffering from goiter and cretinism due to iodine deficiency may sometimes experience exacerbation of the condition following continuous dietary cyanide exposure.

33. Neurological diseases such as konzo and tropical ataxic neuropathy (TAN) have been reported in individuals with inadequate dietary intake of protein and/or iodine where cassava is consumed as a staple food. Konzo is an upper motor neuron disease characterised by irreversible but non-progressive symmetric spastic paraparesis with an abrupt onset, whereas tropical ataxic neuropathy (TAN) is a severe neurological syndrome with clinical symptoms of optical atrophy, angular stomatitis, sensory gait ataxia, and neurosensory deafness.[15]

Safety of cyanogenic glycosides

34. The safety of cyanogenic glycosides has been evaluated by JECFA in 1992. However, safe level of intake could not be estimated due to lack of quantitative data.[15]

LECTINS

35. Lectins (phytohaemagglutinins) are proteins or glycoproteins of non-immune origin which have multiple highly specific carbohydrate binding sites. They were originally identified in the castor bean but are now known to be widespread in the plant kingdom including grain products. Lectins are particularly concentrated in legume seeds and have been shown to cause gastroenteritis, nausea, and diarrhoea in men. Many types of beans contain lectins including green beans, red kidney beans and white kidney beans.

Toxicity

36. Symptoms of acute toxicity include severe stomachache, vomiting and diarrhoea. Lectins can destroy the epithelia of the gastrointestinal tract, interfere with cell mitosis, cause local haemorrhages, damage kidney, liver and heart and agglutinate red blood cells.

Methods of reduction

37. Cooking with moist heat can reduce the toxicity of lectins. Therefore the use of lectin-containing plants as food in the human diets is not a cause for concern after adequate cooking. Special attention, however, should be paid when the lectin-containing food is prepared at high altitudes where the boiling point is reduced, when low heat cooking methods are employed, or in

situations where heat transfer is uneven.

38. To destroy the toxins, beans should be soaked and boiled thoroughly in fresh water. Beans should not be cooked at a low temperature, for example in a crock pot, since it may not destroy the toxin.

SAPONINS

39. Saponins are water-soluble plant constituents, which can form soapy foam even at low concentrations. They are glycosides with a non-sugar aglycone portion which is termed a sapogenin. Saponins are distinguished by their bitter taste, and ability to haemolyse red blood cells. They are classified according to the chemical nature of the sapogenin into two major groups: steroidal and triterpenoid saponins.

40. Saponins are widely distributed in the plant kingdom and can occur in all parts of plants, although the concentration is affected by variety and stage of growth. They are found in soybeans, sugar beets, peanuts, spinach, asparagus, broccoli, potatoes, apples, eggplants, alfalfa and ginseng root.

Toxicity

41. Saponins are capable of disrupting red blood cells and producing diarrhoea and vomiting. Their toxic effects are related to the reduction of surface tension. Saponins are generally harmless to mammals and other warm-blooded animals except at large doses. The body is able to detoxify when saponin is taken in a small amount since intestinal microflora can destroy them and blood plasma can inhibit their action. However, in large quantities they can be irritating to the

gastrointestinal tract causing vomiting and diarrhoea.

Consumption

42. Consumption of saponin-containing plants should be limited to a moderate amount.

Food item containing other toxins associated with poisoning

The Seed of *Ginkgo Biloba*

43. The seeds of ginkgo biloba (ginkgo nuts) are commonly eaten as an ordinary food throughout China, Japan and Korea. Ginkgo biloba contains a number of plant toxins including cyanogenic glycosides and 4'-methoxypyridoxine (4'MPN). However, 4'MPN has been thought to be the incriminated active ingredient. Ginkgo seeds have dangerous poisoning effects due to the presence of toxins that are mainly neurotoxic.

Toxicity

44. Acute toxicity is the main concern of ginkgo biloba poisoning. Vomiting, irritability, and tonic and clonic convulsions are the classic symptoms in ginkgo seed poisoning which usually begin 1 to 12 hours after ingestion. Children are especially susceptible to this type of food poisoning. In severe cases where large amounts have been taken or in susceptible individuals, loss of consciousness and deaths may occur.

45. It has been reported that ingestion of 10-50 of the cooked seeds at

one time can cause acute poisoning in humans.[16] Deaths have been reported following ingestion of ginkgo biloba seeds ranging from 15 to 574 seeds.[17]

46. The pathophysiological mechanisms underlying the convulsion-inducing effect of ginkgo nuts still remained undetermined. 4'MPN has been thought to be the candidate agent due to its pharmacological competition with vitamin B6, which is a cofactor of glutamate decarboxylase.[18, 19] 4'MPN has been considered to indirectly inhibit the enzyme activity of glutamate decarboxylase, resulting in a decrease of the γ-aminobutyric acid (GABA) level in the brain, inducing the convulsion.

47. Unripe and uncooked seeds are reported to be more toxic. Although 4'MPN is a heat-stable substance that cannot be inactivated by cooking,[20] cooking ginkgo seeds may reduce the toxicity by inactivating other toxins in the seed that are heat-labile such as cyanogenic glycosides in the presence of water.

Advice to consumers

48. Consumers, especially children, and other susceptible individuals are advised not to consume more than a few seeds at one time. Consumers are advised to cook ginkgo biloba seeds before consumption to reduce the toxicity.

LABORATORY STUDY

OBJECTIVES

49. The laboratory study was carried out for the analyses of two groups of natural toxins, glycoalkaloids and cyanogenic glycosides. The followings were investigated:

(1) the levels of these toxins in some food plants which are locally available and commonly consumed in Hong Kong and,

(2) the effects of preparation and cooking on the reduction of the toxin levels.

I. GLYCOALKALOIDS

BACKGROUND

50. Glycoalkaloids are natural toxins found in greened potatoes, sprouted potatoes and at lower levels in fresh potatoes (*Solanum tuberosum*). These toxins are rather heat stable, and are not decomposed during cooking, steaming, baking, frying or microwaving.[21] They have a bitter taste and are not easily soluble in water.

51. Factors such as light exposure and improper storage conditions, mechanical damages, and rotting caused by fungi or bacteria may lead to rapid formation of these toxins.[5, 11]

Greening of Potatoes

52. On exposure to light, chlorophyll is produced in the potato so that photosynthesis can take place ready for the new plant to grow. This is the greening effect. Through a separate process, light exposure also causes elevated levels of glycoalkaloids to be produced in the same region where chlorophyll is formed. The glycoalkaloids are concentrated in and directly under the skin acting as protective chemicals for the plant. Although the amount of greening is not a direct measure of the glycoalkaloid content, greened potatoes are often higher in glycoalkaloids than those not greened.

Sprouting of Potatoes

53. Once potato tubers have broken dormancy, they are capable of growth. Immediate sprouting begins if environmental conditions are favourable for growth such as warm temperatures. Sprouts develop from the eye regions of tubers and grow upwards. During this process, large amount of glycoalkaloids is produced at the eye regions and concentrated in the sprouts. The dormancy or rest period can be prolonged if steady, cool storage conditions are maintained.

METHODS

54. The following experiments aimed to determine the amount and distribution of glycoalkaloids, α-solanine and α-chaconine, present in common varieties of potatoes available in the Hong Kong market, as well as in sprouted potatoes.

Sampling and Processing

55. Samples of common varieties of potatoes available in the Hong Kong market were obtained from Cheung Sha Wan Wholesale Food Market and Western Wholesale Food Market in August 2006. Five varieties were analysed : (a) new potato (新品馬鈴薯/新薯) (b) russet potato (褐色馬鈴薯/焗薯) (c) red-skinned variety (紅皮馬鈴薯) (d) yellow-skinned variety 1 (黃皮馬鈴薯) (e) yellow-skinned variety 2 (黃皮腰薯).

56. Samples were sent to the Food Research Laboratory (FRL) for analyses. All potatoes were washed before analyses. Ten samples were pooled for each testing for each variety. Contents for the two glycoalkaloids, α-solanine and α-chaconine, were determined for the peel (occupies around 20% weight of the whole potato tuber) and the flesh portions of potatoes (the remaining portion with the peel removed).

57. Potatoes of the red-skinned variety (紅皮馬鈴薯) were left to be sprouted to determine the levels of the glycoalkaloids, α-solanine and α-chaconine, in potato sprouts and flesh.

Laboratory Analysis

Determination of potato toxins – α-solanine and α-chaconine

58. Laboratory analysis was carried out according to the AOAC Official Method 997.13.[22] Ten potato tubers were peeled. The peel and flesh were ground and homogenised in liquid nitrogen. The homogeneous sample was extracted with dilute acetic acid, followed by purification by solid phase extraction

(SPE). Finally, α-solanine and α-chaconine were quantified by HPLC with ultraviolet detection at 202 nm. The limit of detection (LOD) was 10 mg/kg for both α-solanine and α-chaconine.

RESULTS

1. Fresh Potatoes

59. Glycoalkaloid contents varied among the different types of the potatoes tested. Glycoalkaloid contents in whole potato for the different varieties ranged from 26-88 mg/kg (average 56 mg/kg) (see Table 1).

60. Glycoalkaloids were concentrated in the peel portion of the potatoes. Glycoalkaloids were not detected in the flesh of the potatoes. The average α-solanine and α-chaconine contents in the potato peels for the different types of potatoes were 66 mg/kg (range of 20 - 120 mg/kg) and 176 mg/kg (range of 70 - 300 mg/kg) respectively (see Table 1).

Table 1: Contents of α-solanine and α-chaconine in Flesh and Peel of Various Potatoes (mg/kg)

Variety	α–solanine		α–chaconine		Glyco-alkaloids*
	Flesh	Peel	Flesh	Peel	
New Potato	ND	120	ND	200	72
Russet Potato	ND	20	ND	70	26
Red-skinned Potato	ND	30	ND	110	36
Yellow-skinned Potato 1	ND	100	ND	300	88
Yellow-skinned Potato 2	ND	60	ND	200	60

ND: not detected; Limit of Detection (LOD) = 10 mg/kg

*Glycoalkaloid content for the whole potato was calculated as the sum of α-solanine content and α-chaconine content in the peel (20% by weight) and the content in the flesh (80% by weight). The content in the flesh was calculated using the value of 1/2 LOD for non-detects.

2. Sprouted Potatoes

61. High levels of α-solanine and α-chaconine were found in the sprouts of the red-skinned potato samples and were 3500 and 4100 mg/kg respectively (see Table 2).

Table 2: Contents of α-solanine and α-chaconine in sprouts and flesh of red-skinned potatoes (mg/kg)

Variety	α–solanine		α–chaconine	
	Flesh	Sprout	Flesh	Sprout
Sprouted red-skinned potato	ND	3500	ND	4100

ND: not detected; Limit of Detection (LOD) = 10 mg/kg

II. CYANOGENIC GLYCOSIDES

BACKGROUND

62. Cyanogenic glycosides are present in a number of food commodities commonly consumed in Hong Kong. Bitter apricot seed is commonly used in Chinese soups. Bamboo shoot is a common ingredient in

stir-fry and other dishes in Chinese and oriental cooking. Although cassava is not part of the staple diet in Hong Kong, bitter cassava is still found in the market. Moreover, sweet cassava is commonly eaten as a dessert in restaurants serving Thai and South-east Asian cuisines. Processed cassava-derived products such as tapioca flour and tapioca pearls are the common forms of cassava consumed by the Hong Kong population. Tapioca flour may be used in daily cooking and tapioca pearls are commonly found in Chinese desserts and also in drinks served in Taiwanese style tea houses. Flaxseed has been marketed as a health food in recent years due to its high contents of lignan and omega-3 fatty acids and is growing in consumption. The cyanide contents of these food commodities were therefore analysed.

63. Cyanogenic glycoside is not toxic on its own. However, when cell structures of a plant are disrupted, cyanogenic glycoside will be brought together with the corresponding β-glucosidase enzyme. It will be subsequently broken down to sugar and a cyanohydrin which rapidly decomposes to an aldehyde or a ketone and releases the toxic hydrogen cyanide. This process takes place in the presence of water. It happens when the plant is chewed releasing the toxic cyanide to the predator. In the same way, toxic cyanide is released when the plant is cut into small pieces during food preparation, and the resulting hydrogen cyanide is easily removed by cooking in water since it is volatile.

METHODS

64. The following experiments aimed to determine the amount of releasable cyanide present in various food plants and their products and the

amounts of reduction of the cyanide content resulting from different processing methods.

Sampling and Processing

65. The following food commodities derived from food plant that are known to contain cyanogenic glycoside were studied: 1) bitter apricot seed, 2) fresh bamboo shoot and the processed products, 3) fresh cassava and the processed products including tapioca flour and tapioca pearl, and 4) flaxseed.

66. Samples of each food commodities were sent to the Food Research Laboratory (FRL) for analyses of the amount of releasable cyanide present. All samples were washed before analyses. The samples were either analysed raw or following certain preparations or cooking as below. At least 3 samples were pooled to obtain the average free cyanide content.

Commodity 1: Bitter apricot seed

67. Determinations of free cyanide content were made after cooking in boiling water for 0, 15, 30, 60 and 120 minutes.

Commodity 2: Bamboo shoot

(1) Determination of cyanide contents of raw bamboo shoots from different countries of origin

68. The fibrous tissue at the base of the bamboo shoot was trimmed and the outer leaves peeled away before processing. Free cyanide contents for the whole raw bamboo shoots were determined.

(2) Comparison of cyanide contents for base, middle and tip of bamboo shoots

69. The fibrous tissue at the base of the bamboo shoot was trimmed and the outer leaves peeled away before processing. Free cyanide contents for the base one-third, middle one-third and tip one-third were determined.

(3) The effect of cooking bamboo shoot in water

70. The bamboo shoots were prepared by slicing prior to cooking. They were then cooked in boiling water for 0, 15, 30 and 60 minutes. Free cyanide contents were determined at given time points.

(4) Determination of free cyanide levels in processed bamboo shoot products

71. Free cyanide levels for processed bamboo shoot products (canned, packaged and dried) were determined individually without further processing.

Commodity 3: Cassava

(1) Determination of free cyanide contents for bitter and sweet cassava samples

72. Cassava samples were peeled before analyses. Free cyanide contents of the raw cassava samples for the bitter variety and the sweet variety were determined.

(2) The effect of cooking cassava in water

73. Bitter cassava samples were peeled, and cut in cubes before cooking. They were cooked in boiling water for 0, 20 and 35 minutes. Free cyanide contents were determined for each cooking duration.

(3) Determination of free cyanide levels in cassava products

74. Free cyanide levels for the tapioca flour and tapioca pearls were determined individually without further processing.

Commodity 4: Flaxseed

(1) Determination of free cyanide contents for flaxseed samples

75. Cyanide contents were determined for whole flaxseeds and flaxseed meal samples as obtained from the retail market without further processing.

(2) The effect of cooking flaxseed in dry heat

76. Free cyanide contents were determined for whole flaxseeds and flaxseed meal samples following dry-heating in oven at 177˚C for 15 minutes.

Laboratory Analysis

77. The test sample was grounded and homogenised by mill and was immediately subject to undergo enzymatic hydrolysis in the presence of β-glucosidase inside a close system. The hydrogen cyanide formed from cyanogenic glycosides during the reaction was trapped by 0.1M sodium hydroxide solution.

Finally, free cyanide content in sample was quantified by barbituric acid – pyridine colorimetric method. The limit of detection (LOD) for the free cyanide analysis was 0.1 mg/kg.

RESULTS

78. Cyanide was detected in bitter apricot seed, bamboo shoot, cassava, and flaxseed samples in their raw state.

Commodity 1: Bitter apricot seed

79. Raw bitter apricot seed samples contained an average content of 330 mg/kg free cyanide. A 98% reduction of free cyanide content was observed after cooking in boiling water for 15 minutes (see Table 3).

Table 3: Cyanide Contents in Bitter Apricot Seed after Cooking in Boiling Water

Bitter apricot seed	Cooking in boiling water (min)				
	0	15	30	60	120
Cyanide (mg/kg)	330	7.3	6.8	3.4	1.0

Commodity 2: Bamboo shoot

(1) Bamboo shoots grown in different geographical regions

80. Average cyanide contents for 2 different sources of bamboo

shoots grown in different geographical regions were 25 mg/kg and 40 mg/kg respectively.

(2) Cyanide contents in different parts of bamboo shoots

81. Cyanide contents were different among the base, middle and tip portions. The highest concentration was found at the tip portion of bamboo shoot (120 mg/kg), followed by the middle portion, then the base portion (see Table 4).

Table 4: Cyanide Contents in Different Parts of Bamboo Shoots

Bamboo shoot	Base 1/3	Middle 1/3	Tip 1/3
Cyanide (mg/kg)	1.1	12	120

(3) Effect of cooking on cyanide contents in bamboo shoots

82. A 91% reduction of cyanide content was observed following slicing and cooking bamboo shoot in boiling water for 15 minutes (see Table 5).

Table 5: Effect of Cooking on Cyanide Contents in Bamboo Shoots

Bamboo shoot	Cooking in boiling water (min)			
	0	15	30	60
Cyanide (mg/kg)	40	3.7	1.9	ND

ND: not detected; (LOD=0.1mg/kg)

(4) Cyanide contents in processed bamboo shoots

83. Cyanide levels for canned and packaged bamboo shoots samples ranged from non-detected to 5.3 mg/kg. Cyanide was not detected in any of the dried bamboo shoot samples tested.

Commodity 3: Cassava

(1) Cyanide content in different varieties of fresh cassava

84. Different amounts of cyanide were detected in the two different varieties of cassava – bitter cassava and sweet cassava. The contents of cyanide in bitter cassava and sweet cassava were 120 mg/kg and 9.3 mg/kg respectively.

(2) Effects of cooking on cyanide levels in bitter cassava

85. Cyanide levels in bitter cassava were reduced by cooking in boiling water. Cyanide content was reduced by 97% after cooking in boiling water for 20 minutes (see Table 6).

Table 6: **Effect of Cooking on Cyanide Content of Bitter Cassava**

Bitter cassava	Duration of cooking in boiling water (min)		
	0	20	35
Cyanide (mg/kg)	120	3.3	0.5

(3) Cyanide contents in processed cassava products

86. Of the 8 processed cassava products including tapioca flour and tapioca pearl, cyanide was detected in only 1 tapioca pearl product at 0.1 mg/kg.

Commodity 4: Flaxseed

(1) Cyanide content in whole flaxseeds and flaxseed meal

87. Average cyanide contents in the samples of whole flaxseeds and flaxseed meal were 200 and 230 mg/kg respectively.

(2) Effects of dry-heating on cyanide levels in whole flaxseeds and flaxseed meal

88. Dry-heating only reduced around 10 % of cyanide contents of flaxseeds and flaxseed meal samples after heating at 177˚C for 15 min (see Table 7).

Table 7: **Effect of Dry Heat on Cyanide Content of Flaxseed**

Flaxseed	Before heating	177˚C for 15 min
Whole flaxseed (cyanide, mg/kg)	200	180
Flaxseed meal (cyanide, mg/kg)	230	210

DISCUSSION

I. GLYCOALKALOIDS IN POTATOES

89. The levels of glycoalkaloids present in potatoes are determined by the particular variety or cultivar and are affected by the growing conditions.[11] Glycoalkaloid contents (sum of α-solanine and α-chaconine) for the five lots of potatoes tested in this study ranged between 26 - 88 mg/kg. These values were within the normal glycoalkaloid levels of 20-100 mg/kg found in properly grown and handled potato tubers. JECFA considered that consumptions of potatoes within this range of glycoalkaloid contents on a daily basis were not of concern. The potatoes sampled from the market in this study were therefore safe for regular consumption.

90. The glycoalkaloids, α-solanine and α-chaconine, are distributed mainly in the peels, with little glycoalkaloids in the flesh of the potatoes. This was evident for all five lots of potatoes tested. The highest concentrations of glycoalkaloids were found in potato sprouts.

91. In order to reduce the risk of glycoalkaloid toxicity, consumers are advised to discard sprouted potatoes. Potatoes that show signs of greening, rotting or are physically damaged should not be consumed.

92. Solanine at high concentrations contributes to the bitter taste in potatoes.

II. CYANOGENIC GLYCOSIDES IN FOOD PLANTS

Potential toxicity of cyanogenic plant

93. The potential toxicity of a cyanogenic plant depends primarily on the potential that its consumption will produce a concentration of hydrogen cyanide (HCN) that is toxic to the exposed animals or humans. The potential for toxicity of cyanogenic plants has been expressed as mg of releasable cyanide/ kg of the food commodity. However, factors such as bioavailability, susceptibility and nutritional status of the individual, and preparations and processing of the food plant will determine the final amount of the cyanide toxin presented to the consumer.

Free cyanide levels in the food commodities

94. The free or releasable cyanide levels in the raw commodities determined in this study were consistent with published reports. In this study, average cyanide levels in the cassava were 120 mg/kg for the bitter variety and 9.3 mg/kg for the sweet variety. These were in the similar range with cyanide contents for cassava reported in other countries (15-400 mg/kg for bitter cassava; and 15-50 mg/kg for sweet cassava).[23] It is generally considered that bitter cassava can reach levels as high as 1 g/kg whereas sweet cassava is mostly in the range of more than 20 mg/kg.

95. In our study different levels of cyanide contents were found in bamboo shoots grown in two different geographical regions. This is expected since cyanide content is known to vary widely according to the different types of bamboo shoots and growing conditions in different countries. Cyanide content is

also reported to decrease substantially following harvesting. Our results that cyanide contents were concentrated in the bamboo shoot tips are consistent with published findings although levels may be lower than some reports. This may be due to the proportion of the tip analysed.

Reduction of cyanide content by cooking and processing

96. Our results showed that cyanide contents of food commodities could be reduced by cooking in the presence of water. Among the different commodities tested, bitter apricot seed has the highest cyanide content in the uncooked state. Cooking in boiling water was able to reduce cyanide content by more than 90% in all the commodities studied.

97. Processing prior to cooking facilitates the reduction of cyanide content in food plants. For bamboo shoots, cutting and slicing liberates hydrogen cyanide, which is removed by boiling. This also applies to cassava, where peeling and cutting into smaller pieces before cooking disrupts the cell structure of the plant, with subsequent liberation of hydrogen cyanide.

98. For processed commodities such as canned and dried bamboo shoots, tapioca flour and tapioca pearls, cyanide levels are generally low since hydrogen cyanide are also liberated during the manufacturing processes.

99. It is important to note that great variabilities exist between cyanide contents of even the same cyanogenic plants species (depending on a combination of endogenous factors and exogenous factors e.g. environment), the durations of cooking for optimum reduction may be longer for some varieties. Particular care and attention need to be paid to cassava and bamboo shoots where cyanide

contents can be as high as 1000 mg/kg. Cutting cassava and bamboo shoots into smaller pieces before cooking and use longer durations of cooking helps reduce the level of the toxin.

100. Flaxseeds are often eaten with salads or with warm water alone without further cooking or it may be oven-baked before consumption like being made into muffins or wafers with little or no water. In this study, the effectiveness in the reduction of cyanide content using dry heat was investigated. Dry-heating of flaxseeds in the absence of water did not reduce cyanide content effectively. Dry-heating only reduced around 10% of cyanide contents of the flaxseeds and flaxseed meal samples. This is consistent with previous studies where dry-heating produced a percentage reduction of cyanide of around 16-18%, much less effective than cooking in the presence of water after a similar duration.[24,25]

III. RISK CHARACTERISATION

101. In the food commodities tested in this study, potato is the most commonly consumed food within the population. In general, when potato tubers are grown in appropriate conditions, and have not been stored for a prolonged period, the levels of glycoalkaloids are generally low, and normal intake should not pose significant health risk. Our results showed that the glycoalkaloid contents in the common varieties of potatoes tested were all within the range at which JECFA considered safe for daily consumption. Therefore it can be concluded that there is no indication of specific risk of these commodities to the Hong Kong population provided that they are consumed fresh and care is taken to

avoid eating greened, damaged or sprouted potatoes.

102. In Hong Kong, cyanogenic plants are generally not consumed as a staple food. Although fresh bamboo shoots and bitter apricot seeds are common ingredients in Chinese cuisines, they are mostly consumed in small to moderate amounts in the local population. Flaxseed, although growing in consumption, is generally consumed in small amounts. Since cyanide contents can be reduced significantly by the effect of cooking in boiling water, the health risk is low even for high consumers since adequate boiling can reduce cyanide to as much as over 90%. The situation, however, is different for those who consume a larger amount of flaxseed without boiling treatment. Processed products like canned and dried bamboo shoots, tapioca flour and tapioca pearls do not contain significant amount of cyanide and therefore do not pose a risk to the population.

103. Since it is not possible to bring down levels of inherent natural toxicants by a code of practice or through good agricultural practice, reduction of the risk posed by ingestion of natural plant toxins depends on methods of preparation of the food before ingestion or limiting the amount of consumption of these foods where toxin reduction is not possible through proper processing.

104. For a number of natural toxins reviewed in this study, the level of toxins can be reduced by cooking. These included cyanogenic glycosides in bitter apricot seed, cassava, bamboo shoot. Cutting plants such as cassava and bamboo shoot into smaller pieces facilitated the release of hydrogen cyanide before it was cooked in boiling water, where it could be easily removed due to its volatility.

For cyanogenic plants that were processed with minimal amount of water such as flaxseed, the toxin could only be removed by 10% based on the cooking instruction on the menu (i.e. oven-baking for 15 minutes). This ineffective reduction of cyanide contents in dry-heat indicated that intake should be limited to only small amounts at any one time.

105. Cassava, bamboo shoots, bitter apricot seeds and flaxseeds contain cyanogenic glycosides that break down under the action of the enzyme β-glucosidase to produce hydrogen cyanide, which can cause both acute and chronic toxicity in humans. Since these food commodities are not normally consumed in large amounts in Hong Kong, the risk of acute toxicity is considered low in the local population provided that these food items are thoroughly cooked before consumption. The chronic effects of cyanide intoxication are linked to a regular long-term consumption of cyanide-containing foods in individuals with poor nutrition (e.g. when cassava is used as the staple food in malnourished population). However, this situation is not common in Hong Kong.

106. It was not possible to estimate dietary exposure to the natural plant toxins in the population due to the inherent nature of these toxins, e.g. large variations in toxins levels as a result of their species, growth conditions and geographical factors. Exposures to the plant toxins are also dependent on the food matrices as well as the cooking procedures employed which may vary widely.

107. In addition to the amount of the toxin presented to the individual through ingestion, factors including bioavailability, individual susceptibility and sensitivity to a natural toxin will determine whether or not the consumer will

experience adverse health effects. Therefore the level of intake that may pose health risk may vary widely between individuals. It is considered that the benefits of normal consumption of the food plants containing glycoalkaloids and cyanogenic glycosides far outweighs the health risk that may pose to the consumer after following the necessary safety precautions such as careful selection, preparation and appropriate cooking conditions.

LIMITATION

108. The sample size was limited by the available resources. A more precise estimate could be obtained by increasing the number of samples taken for each food commodities.

CONCLUSION & RECOMMENDATIONS

109. Natural toxins are found widely in edible plants which are otherwise nutritious and beneficial to health. These food plants can be safely consumed if suitable measures are taken, such as careful selection, adequate processing and cooking, and limitation of intake.

110. Natural toxins were detected in food plant samples obtained from the Hong Kong market for this study, namely glycoalkaloids in potatoes and cyanogenic glycosides in bitter apricot seeds, bamboo shoots, cassava and flaxseeds.

111. Glycoalkaloid contents of the five different varieties of potatoes

obtained from the Hong Kong market were within the level of 100 mg/kg at which JECFA considered not of concern for daily consumption. Glycoalkaloids were concentrated in the peels of the potatoes. The highest level was found in the potato sprouts.

112. Higher cyanide contents were found in bitter cassava than sweet cassava. Cyanide concentration was found to be highest at the tip portion of bamboo shoot, followed by the middle portion, then the base portion. Cutting them into small pieces and cooking in boiling water reduced cyanide contents of food commodities by more than 90%. Dry heat could not reduce cyanide content effectively in flaxseeds, hence intake should be limited to small amounts.

113. The amount of ingestion of food plants containing natural toxins that will cause food poisoning depends on many factors such as individual susceptibility, the cooking methods and the levels of toxin in the plant which may vary according to the species and geographical environment. The public is advised to take precautions in limiting the amount of intake and observing safety measures for toxin reduction prior to consumption especially for children and the elderly. For individuals with illness or with poor health conditions, they may wish to consult their doctors for further advice.

114. The public is reminded to follow the health advice of maintaining a balanced and varied diet, including a wide variety of fresh fruits and vegetables, as they are nutritious and safe to eat after observing the above advice.

ADVICE TO TRADE

1. Store potatoes in a cool, dry and dark environment. Avoid keeping stocks for prolonged periods.

2. Display a smaller stock at any one time.

3. Discard stocks that show signs of sprouting, greening, physical damage or rotting.

4. Do not use sprouting, greened or damaged potatoes for making food products.

ADVICE TO PUBLIC

Purchase

1. Avoid buying potatoes that show signs of sprouting, greening, physical damage or rotting.

Storage

1. Remove potatoes from plastic bags and place them in a cool, dry, and dark place at home.

2. Store only small amounts of potatoes at home.

3. Discard potatoes that show signs of sprouting, greening, physical damage or rotting.

Preparation and consumption

Potatoes

1. Avoid eating potatoes that show signs of sprouting, greening, physical damage or rotting.

Cyanogenic plants

1. Cutting the cyanogenic plants into smaller pieces and cook thoroughly in boiling water to release toxic hydrogen cyanide before consumption helps reduce the level of the toxin. Since hydrogen cyanide is volatile, it is easily removed by open-lid cooking.

2. When the cooking method chosen is heating under dry-heat or at low moisture contents, limit the intake of the cyanogenic plants to only small amounts.

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