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Antinutrients and total cyanide content of taro corms

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ANTINUTRIENTS AND TOTAL CYANIDE CONTENT OF TARO CORMS

A Thesis

Presented to

The Faculty of the Department of

Nutrition and Food Science

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Sirapa Oudomugsorn

May 2003

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ABSTRACT

ANTINUTRIENTS AND TOTAL CYANIDE CONTENT OF TARO CORMS

by Sirapa Oudomugsorn

The purpose of this study was to determine the levels of trypsin inhibitor, phytic acid, oxalic acid, and total cyanide in taro corms. Taro corm samples were obtained from a local oriental grocery store. The corms were cleaned, peeled, freeze-dried, and ground. The resulting freeze-dried powder was used for subsequent analyses. The proximate composition for the taro corms was determined according to AOAC (2000) methods. Trypsin inhibitor activity was analyzed by the TAME method. Phytic acid and oxalic acid were analyzed by AOAC (2000) methods. Total cyanide content was analyzed using a macro diffusion apparatus and the pyridine-barbituric colorimetric method. Taro corm samples contained approximately 71% moisture, 1.66% protein, 0.2% fat, 1.05% ash, 0.63% fiber (ADF), and 25.41% carbohydrate. On fresh weight basis, taro corms used in this study contained 384 trypsin inhibitor unit (TIU)/g, 0.3 mg phytic acid/ 100g, and 5.88 mg oxalic acid/ 100g. No cyanide was detected. Trypsin inhibitor activity levels of the taro samples were higher than those published values previously reported, but oxalic acid and cyanide values were lower. Based on the levels of antinutrients and total cyanide found in this study, there is a very little risk of toxicological effects from the consumption of taro corms.

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PREFACE

This thesis is written in a publication style. The second chapter is written in journal format and will be submitted to Journal of Agricultural and Food Chemistry. Chapters 1 and 3 are written according to the guidelines outlined in the Publication Manual of the American Psychological Association, 4 th. edition, 1998.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Taro (*Colocasia esculenta*) belongs to the Araceae family. It is grown widely throughout humid tropical regions for its edible corms and leaves (Wang, 1983). This plant originated in India and South-East Asia, and spread to China, Japan, Egypt, and Africa. Later, it became an important crop in Central and South America, as well as the Pacific Islands (Davidson, 1999). Since ancient times, taro has been consumed as a culture food and a staple food in the areas where it has been grown, especially in developing countries (Chandra, 1984). In the US, the increasing demand for taro is attributed to a massive number of immigrants from the tropics (O'Hair, 1990). According to Food and Agriculture Organization of the United Nations (FAO) summary (1997), "Agriculture: Towards 2010", the production of taro is expected to increased in the future due to an increase in planting and an increase in crop yields.

Taro corm, an enlarged starchy underground stem, is consumed for dietary energy. The patterns of its utilization vary considerably among countries (FAO, 1990; Moy & Nip, 1983). When eaten alone, it can be steamed or boiled, and peeled. It can also be boiled in soup along with other vegetables. Taro corms can be served in various ways from main dishes to desserts. Boiling, baking, steaming, and frying are the most common methods of preparation (Davidson, 1999; Moy & Nip, 1983). Besides these simple methods, they can be processed to make flour, paste, beverage powder, and extruded products. In Hawaii, taro corms are used mainly to make a cooked purple-gray

paste, packed in plastic bags, jars, or cans, called poi. Poi is sold commercially and consumed widely in the Pacific Islands (Moy & Nip, 1983). Taro corms are not only used to make food for adults, but are also used to make food for babies. The FAO (1990) suggests that taro and other root crops can be used in baby food; however, they should be appropriately supplemented. Hence, taro corms can be an alternative food for babies born with wheat or grain allergies.

Antinutrients and toxic compounds are major concern in underdeveloped countries where the major staple foods are derived from plant sources. Antinutrients and plant toxins are known to interfere with the bioavailability of some nutrients, or to cause harm to the biological system (Whitney & Rolfes, 1999; De Vries, 1997). Therefore, antinutrients and plant toxins have been investigated in various foods, both raw and processed (Isong & Essien, 1996; Badifu, 2001; Almasan, 1995; Wanasundera & Ravindran, 1994; Akpanabiatu, Bassey, Udosen, & Eyoung, 1998; Siddhuraju, Vijayakumari, & Janardhanan, 1995).

Due to increasing health concerns and food safety awareness, researchers have increased their interest in antinutrients and plant toxins (De Vries, 1997; Akwaowo, Ndon, & Etuk, 2000; Atony & Chandra, 1998; Badifu, 2001). Among various antinutrients and natural plant toxins, trypsin inhibitor (TI), phytic acid, oxalic acid, and cyanide are found in root crops (Wanasundera & Ravindran, 1994; Peng, Bradbury, Hammer, & Shaw, 1993; Bradbury & Hammer, 1990). The presence of these antinutrients limits the nutritive value of these crops. In animal studies, trypsin inhibitors are reported to interfere with protein absorption, limit the growth of several animals, and

cause pancreatic hypertrophy (Chernick, Lepkovsky & Chaikoff, 1948; Green & Lyman, 1972; Kakade, Hoffa, & Liener, 1973; Ge & Morgan, 1993). Phytic acid and oxalic acid are able to bind minerals (e.g. calcium, iron, zinc), and make them unavailable for absorption (Zhou & Erdman, 1995; Noonan & Savage, 1999; Heaney, Weaver, & Recker, 1988). Moreover, oxalic acid in calcium oxalate form is a major component of renal stones. High oxalate intake leads to hyperoxaluria, resulting in an increasing risk of kidney stone formation (Ogawa, Miyazato, & Hatano, 2000; Noonan & Savage, 1999). On the other hand, cyanogenic glycosides, toxic compounds found in cassava, are known to liberate cyanide. This cyanide causes inhibition of respiratory processes at the cellular level. The detoxification of cyanide in the body yields thiocyanate, which can cause goiter, due to its interference with iodine absorption (Oke, 1969; Conn, 1969).

Although the nutritive value of taro has been studied and reported by several sources (United States Department of Agriculture [USDA], 2002; FAO 1998a; Huang, Tichenal, & Meilleur, 2000), data in nutritional factors and cyanide content are still limited. As a carbohydrate staple food for millions of people, there is a pressing need to evaluate taro corms for these compounds.

Objective

The objective of this study was to determine the levels of trypsin inhibitor, phytic acid, oxalic acid, and total cyanide in taro corms.

Significance of the Study

Since taro is a staple food in developing countries and is an alternative source of carbohydrate in many areas of the world, its antinutrient and toxic content may affect the

health of many consumers. In order to conduct risk assessment and risk management of taro consumption, a measurement of taro antinutrients and its natural toxin levels is needed. The results of this study will contribute information on antinutrients and cyanide content of taro corms both for consumers and for further studies in this area.

Review of Literature

Taro

Taro (*Colocasia esculenta*) is an ancient crop grown throughout the humid tropics mainly for its edible corms. It is classified under the Araceae family. The plant is thought to have originated in India and South-East Asia. It later became a staple food in Asia, Africa, Latin America, and the Pacific Islands (Wang, 1983; Chandra, 1984). The production and consumption of taro seemed to be mostly within the areas where it is grown (FAO, 1997). Worldwide production of taro is reported ranging from 9.7 to 13.5 million tons/year during 1990-1995. In Africa, where taro corms are consumed as a staple food, the production during 1990-2000 is reported within the range of 2.5-6.6 million tons/year (FAO, 1998b). According to the FAO (1997) taro production is expected to increase toward the year 2010. In the United States, taro consumption is expected to increase due to a demand by increasing numbers of immigrants, mostly from the Caribbean and South-East Asia (O'Hair, 1990). The majority of taro is used for human food (FAO, 1997). The pattern of taro preparation varies considerably from region to region. The part of the taro plant that is used as a carbohydrate food source is the corm.

The taro corm is a starchy underground stem. Its skin is brown and hairy with concentric rings of leaf scars and scales, and its flesh can be white, pink, or purple (Strauss, 1983; Davidson, 1999). Most taro corms normally mature in six to ten months after planting (Valenzuela & Sato, 2002). Basically, mature corms are prepared for consumption by boiling, steaming, baking, or frying. In Japan, they are boiled with other vegetables to make a soup. Similarly, in India, they are boiled with mixed vegetables to make curries. In the South Pacific, baked corms are served as a major portion of the meal. In Hawaii, poi, a cooked taro paste, is very popular. Taro corms are also currently utilized as animal feeds (Moy & Nip, 1983; Carpenter & Steinke, 1983).

Food Potential and Nutritional Value of Taro

The potential food value of taro corm, either fresh or processed, is considerable. Besides simple cooking in the household, the corms can be processed and made into various products such as taro flour, taro flakes, beverage powder, and frozen desserts. The potential of taro flour as a food ingredient has been studied extensively. It can be partially substituted in cereal flour to make extruded and bakery products. It has been used as an ingredient in a variety of food products including infant food (Moy & Nip, 1983). The FAO (1990) recommended; however, that composite flours prepared from root crops and cereals have to be appropriately supplemented in order to be used as infant food. The nutritive value of taro has been investigated and reported (Standal, 1983; FAO, 1998a; USDA, 2002). Recently, Huang et al. (2002) reported the nutrient composition of two major taro varieties cultivated in Hawaii.

The nutrient composition of taro corms is shown in Table 1. Like other root crops, taro corm is low in protein and low in fat, but high in carbohydrate. It is a good source of potassium, and provides a moderate level of phosphorus. It is low in vitamin C and completely deficient in vitamin A (see Table1) (Standal, 1983; USDA, 2002).

Although taro corm is not a good source of vitamins and minerals, other than potassium, the small granule size of its starch helps increase the bioavailability of its nutrients due to efficiency of digestion and absorption (Standal, 1983). The granule size of taro starch ranges from 0.5 to 5 μm . This is smaller than the granule of yam which is reported to be 1-10 μm (Wang, Wang, & Chang, 1997), and is ten times smaller than the potato starch granule (Parkinson, 1984). The digestibility of raw taro starch in humans has been reported to be 97 percent (Langworthy & Deuel, 1922).

Antinutrients and Natural Plant Toxins

In addition to nutrient composition, antinutrients and natural plant toxins are also considered important compounds affecting human health (De Vries, 1997). In order to conduct risk assessment of food consumption, the determination of their levels (Akwaowo et al., 2000; Dipietro & Liener, 1989; Isong & Essien, 1996; Siddhuraju et al., 1995; Zvauya & Muzondo, 1995; Hollway, Agall, Jealous, Lee, & Bradbury, 1989), and the evaluation of their adverse effects on humans and animals (Nyman & Bjorck, 1989; McLaughlin, Peikin, & Baile, 1983; Libert & Franceschi, 1987; Oke 1969), have been extensively studied. In taro, the trypsin inhibitor activity (TIA) and oxalate contents of its corm have been determined and reported by several researchers (Bradbury & Hammer, 1990; Hollway et al., 1989; Huang & Tanudjaja, 1992). The cyanide content

Table 1

Nutrient Composition of Taro Corm

Nutrient	Unit	Value per 100 g of Edible Portion
Water	g	70.64
Protein	g	1.50
Fat	g	0.2
Carbohydrate	g	26.46
Fiber	g	4.1
Ash	g	1.2
Minerals		
Calcium	mg	43
Iron	mg	0.55
Phosphorus	mg	84
Potassium	mg	591
Zinc	mg	0.23
Vitamins		
Vitamin C	mg	4.5
Vitamin A	mg	N/A

Note From “USDA Nutrient Database for Standard Reference, Release 14,” by USDA Agricultural Research Service, 2002. Home page, <http://www.nal.USDA.gov/fnic/foodcomp/>

of taro has been reported by Osuntokun (1980). However, the study of antinutrients and toxic compounds in taro corms seems to be limited. In fact, in other root crops such as yam, the presence of phytic acid has been reported (Wanasundera & Ravindran, 1994; Cooke, 1978). The adverse effects of trypsin inhibitor, phytic acid, oxalic acid, and cyanide compounds lead to an interest in determining their levels in food (Bradbury & Hammer, 1990; Wanasundera & Ravindran, 1994; Libert & Franceschi, 1987; Oke, 1969).

Trypsin Inhibitor

Trypsin inhibitors (TIs) are mostly proteins that inhibit proteolytic activity of trypsin, a digestive protease secreted in the pancreas. They are present in legumes, vegetables, cereals, and potatoes (Van Genderen, 1997). Plant TIs from different sources vary in structure, molecular weight, and chemical composition, but are quite similar in the amino acid sequence of the active site (Liener, 1994; Richardson, 1977). The molecular weight of TIs ranges from 4,000 to 60,000 daltons. Typically, they are low molecular weight polypeptides of L-amino acids. The polypeptides consist of high percentages of half cystine residues that form disulfide crosslinks. Plant TIs are rich in aspartic acid, glutamic acid, serine, and lysine residues, but low or devoid in methionine, histidine, and tryptophan (Ryan, 1981).

Trypsin Inhibitors in Taro

Trypsin inhibitors (TIs) from taro have been purified and characterized by Sumathi and Pattabiarman (1979), and Hammer, Shaw, and Bradbury (1989). The results of these two studies showed that the TIs of taro had a molecular weight of 40,000 daltons.

The study of Hammer et al. (1989) showed that taro TIs contained two identical subunits having molecular weights of about 20,000 each. The N-terminal sequences demonstrated homology with Kunitz soybean TIs, giant taro (*Alocasia macrorrhiza*) TIs, and giant swamp taro (*Cyrtosperma chamissonis*) TIs. Sumathi and Pattabiraman (1979) reported that the purified TIs from taro corms inhibited bovine trypsin, human trypsin, and weakly inhibited bovine chymotrypsin. The purified inhibitor was stable over a pH range of 2.0-12.

In 1993, Peng et al. compared the amino acid sequences of TIs of ordinary taro, giant taro, and giant swamp taro. They found that the inhibitors of taro and giant taro were more related to each other than giant swamp taro. They also suggested that this evidence could be used to support the taxonomic classification of taro into different tribes (Bradbury & Hammer, 1990). Values of trypsin inhibitor activity (TIA) of different taro tribes are shown in Table 2.

Adverse Effects of Trypsin Inhibitor

It is known that trypsin inhibitor (TI) interferes with the digestion of protein (Liener, 1979). Normally, when protein reaches the duodenum, it stimulates the pancreas to secrete the digestive proteases, trypsin and chymotrypsin. The secretory activity of the pancreas is regulated by the activity of proteases needed to digest protein. When the activity of these proteases in the gut increases, the secretory activity of the pancreas decreases (Whitney & Rolfes, 1999).

The alteration of pancreatic activity due to the presence of TI is attributed to Cholecystokinin (CCK), a hormone released from the intestinal wall. When the inhibitor

Table 2

Trypsin Inhibitor Activities of Taro, Giant Taro, and Giant Swamp Taro in Edible Corms

Source	Trypsin inhibitor activity (TIU/g fresh)
Taro (<i>Colocasia esculenta</i>)	5.5-27
Giant taro (<i>Alocasia macrorrhiza</i>)	200-340
Giant Swamp taro (<i>Crytosperma chamissonis</i>)	2.5

Note Modified from "Comparative Study of Proteinase Inhibitors in Tropical Root Crops and Survey of Allelochemicals in the Edible Aroids," by J. H. Bradbury and B. C. Hammer, 1990, Journal of Agricultural and Food Chemistry, 38, p. 1450.

combines with trypsin and forms an inactive complex, this inactive complex effectively lowers the concentration of active trypsin. Consequently, CCK is released to stimulate trypsin production in order to compensate for inactive trypsin. Prolonged exposure to TI leads to overstimulation of the pancreas, causing pancreatic hypertrophy. The mechanism of TI action involving its interference with protein digestion is shown in Figure 1 (Liener, 1979).

Figure 1. Regulation secretion of trypsin by pancreas

From “Significance for Humans of Biologically Active Factors in Soy Bean and Other Food Legumes” by I. E. Liener, 1979, *Journal of American Oil Chemists’ Society*, 56, p. 122.

The nutritional significance of plant TIs has been extensively investigated (Liener and Kakade, 1980). The high level of TIs in legumes caused pancreatic hypertrophy in chicks, mice, and rats fed on those feeds (Chernick et al., 1948; Rackis, 1965, Kakade et al., 1973). The TIs in raw soybean flour caused not only pancreatic hypertrophy, but also growth suppression in rats and mice (Ge & Morgan, 1993). Chan and De Lumen (1982) showed the same results in rats fed casein and isolated winged bean TIs. The lower TI

activity obtained by cooking faba beans led to improvement of the protein efficiency ratio (PER) and food transformation index in the rats (Vidal-Valverde et al., 1997).

Determination of Trypsin Inhibitor

The methods used in TI determination are based on the reduction in the rate of hydrolysis of trypsin substrate caused by the addition of TI extract. The hydrolysis rate is determined by the spectrophotometer (Tan & Wong, 1982; Dipietro & Liener, 1989; Vidal-Valverde et al., 1997). Both natural and synthetic substrates have been used in the determination of TIA.

Casein has been used as a natural substrate in TIA determination. However, due to unreproducible and inaccurate results from using casein as a substrate, synthetic substrates have been used in recent years (Knuitz, 1947; Bradbury, Baines, Hammer, Anders, & Millar, 1984). The commonly used synthetic substrates are benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) and p-toluene-sulfonyl-L-arginine methyl ester (TAME) (Samar, 1992; Tan, Wong, & De Lumen, 1984; Vidal-Valverde et al., 1997; Bradbury et al., 1984). In root crops, TAME has been used for the determination of TIs in sweet potato, edible aroids, and taro (Bradbury et al., 1984; Bradbury & Hammer 1990).

Cyanogenic Glycosides and Cyanide

Cyanogenic glycosides are natural toxins found in a variety of plants. In general, cyanogenic glycosides consist of a saccharide moiety and an aglycone, β -hydroxynitrile. The saccharide group can be synthesized by proteins which are mostly tyrosine, phenylalanine, valine, leucine and isoleucine (Poulton, 1990). Upon hydrolysis with

diluted acid or an appropriate hydrolytic enzyme, cyanogenic glycosides liberate hydrogen cyanide (HCN), sugar, and aldehyde or ketone. The ability of plants to release HCN is known as cyanogenesis (Conn, 1969).

More than 1000 plant species have been reported to exhibit the phenomenon of cyanogenesis. These cyanophoric plants are capable of liberating HCN when plant tissues are damaged. Because the cyanogenic glycosides are largely localized within the vacuoles of cyanophoric plant cells, and their hydrolytic enzymes are in cytoplasm, damaging cells lead to the disruption of cellular integrity resulting in the contact of the hydrolytic enzymes, β -glucosidases and hydroxynitrilelyase, and the cyanogenic compounds. Consequently, HCN is released. The degree of cyanogenesis in these plants depends on the synthesis and accumulation of cyanogenic compounds in their organs such as leaves, stems, fruits, seeds, and roots as well as the presence of specific hydrolytic enzyme, such as linamarase in cassava (Nartey, 1981). Cyanogens can be formed in almost any part of the plant; however, the leaves often contain the highest concentration (Conn, 1969). Moreover, the cyanogenic glycosides in plants vary depending on plant cultivars, planting season, stage of growth, weather, and soil condition. The most familiar cyanogenic glycosides presenting in edible plants are amygdalin, dhurrin, linamarin, and lotaustalin (Oke, 1969). Amygdalin is found in almonds, apple, apricot, cherry, plum, peach, pear, and quince. Dhurrin presents in sorghums and kaffir corn. Linamarin and lotaustalin coexist in cassava, lima beans and flax seed (De Vris, 1997; Nartey, 1981).

Because the toxicity of cyanogenic glycosides depends on the liberation of HCN, the cyanide content of foods have been investigated and reported, see Table 3 (De Vris, 1997). Among root crops, only cassava has been well investigated for cyanide content, due to evidence of cyanide toxicity in areas where cassava is consumed as a staple food (Cooke, 1978; Fukuba & Madoza, 1984).

Adverse Effects of Cyanide

Neither cyanogenic glycosides nor their hydrolytic enzymes are toxic. The toxicity of cyanogenic glycosides is due to cyanogenesis when the hydrolytic enzymes come into contact with these compounds, and HCN is released (Oke, 1969). This degradation process is illustrated in Figure 2. Linamarin is hydrolyzed by a β -glucosidase to yield cyanohydrin (2-cyano-2-propanol) and glucose. The cyanohydrin is acted upon by hydroxynitrile lyase forming the corresponding ketone and HCN (Conn, 1969).

Figure 2. Degradation of the cyanogenic glycoside linamarin

Table 3

Hydrogen Cyanide Contents of Some Common Foods

Source	HCN (mg/100g)
Lima Beans	210-310
Almonds	250
Sorghums	250
Cassava	110
Peas	2.3
Beans	2.0

Note. From "Food Safety and Toxicology," (p.13) by J. De Vries, 1997, CRC Press New York.

Hydrogen cyanide is a colorless, poisonous compound which evaporates at 26° C. It can cause both acute and chronic effects to humans and animals consuming the cyanophoric plant foods. Its acute effects are due to its affinity to metal ions. It can bind with the copper of cytochrome oxidase, which inhibits the oxidative functions of this enzyme, resulting in oxygen starvation at the cellular level (Oke, 1969). Symptoms occurring a few hours after ingestion include hyperventilation, headache, vertigo, vomiting, and collapse. If a lethal dose is ingested, death occurs within 15 min to a few hours. The minimum lethal dose of cyanide is approximately 0.5-3.5 mg/ kg of human body weight depending on the rate of absorption into the body (Mongomery, 1969). Intake of plant tissue containing over 20 mg HCN/ 100g is considered potentially toxic (Cotgageorge, 1978).

On the other hand, the chronic effects of cyanide are attributed to the detoxification process within the human body which yields thiocyanate as a product. Since thiocyanate has the same molecular size as iodine, it is able to interfere with iodine uptake by the thyroid gland (Bourdoux et al., 1978). The increase of thiocyanate in serum causes an increase of iodine excretion and a reduction of iodine uptake. This condition contributes to the occurrence of endemic goiter in areas where cassava is a staple food (FAO, 1990).

Determination of Cyanide or HCN

Due to the toxicity of cyanide, several methods have been developed to measure cyanide content in foods such as cassava (O'Brien, Taylor, & Pouter, 1991; Cooke, 1978), soybean as well as soybean products (Honig, Hockridge, Gould, & Rackis, 1983),

passion fruit (Chassagne, Crouzet, Claude, Bayonove, & Baumes, 1996), and animal feeds (Harris, Merson, Hardy, & Curtis, 1980). The procedure for quantitative estimation of cyanide liberated from cyanophoric plant materials involves 3 steps: 1) hydrolysis of cyanogenic glycosides and liberation of cyanide from plant materials by acid, endogenous enzyme; or exogenous enzyme, 2) isolation of cyanide liberated into a trap; 3) estimation of the liberated cyanide using chemical reactivity or physical behavior of the cyanide ion (Nahrstedt, Erb, & Zinsmeister, 1981).

The isolation of liberated cyanide has been performed in several ways. Nashida, Shirashi, and Uda (1988) and Rubio, Sanz, and Rauret (1987) used Conway's micro diffusion apparatus (Figure 3) to isolate cyanide released from hydrolysis of cyanogenic glycosides. In this apparatus, cyanide released from the sample was trapped by the cyanide absorbing solution in a different compartment. Haskins (1988) incubated the food sample extract with exogenous enzyme for 1.25 hr in a capped tube. After incubation, the cyanide absorbing solution (1 M NaOH) is added immediately to trap cyanide. Zitnak (1973) used acid hydrolysis to liberate cyanide followed by steam distillation into a diluted NaOH solution.

Figure 3. Conway's micro diffusion apparatus

The colorimetric method has been used widely to estimate or determine the amount of cyanide liberated. Mendoza et al. (1984) compared three colorimetric methods for cyanide analysis in cassava root extract including pyridine-barbiturate, pyridine-pyrazolone, and alkali picrate methods. Although alkali picrate method yielded the highest value of cyanide, pyridine barbiturate method appeared to be the best method due to the specificity, sensitivity, and simplicity of that analysis. In addition, this method required a short time and the color developed was stable.

Phytic Acid

Phytic acid or myo-inositol hexakisphosphate is widely distributed in plants. This compound and its salts, phytates, are particularly abundant in legumes and cereals ranging from 1 to 5% (Schlemmer, Muller, & Jany, 1995). Phytic acid is thought to be a major storage form of phosphorus to provide phosphate for the synthesis of nucleic acid during germination (Plaami & Kumpulainen, 1995). Since a molecule of phytic acid contains six highly charged phosphate groups, it is an excellent chelator of all polyvalent trace elements and minerals. The structure of phytic acid is shown in Figure 4 (De Vries, 1997).

Figure 4. Phytic acid

In plant food, phytic acid commonly occurs in the forms of phytates, which are mixed metal complexes. Phytic acid is capable of binding polyvalent ions such as Cu^{2+} , Zn^{2+} , $\text{Fe}^{2+}/\text{Fe}^{3+}$, and Ca^{2+} (Schlemmer et al., 1995). This property is the basis of the concern that a high intake of phytic acid may interfere with the bioavailability of essential minerals (Zhou & Erdman, 1995). Various studies have been done to investigate the concentration of phytic acid and ways to reduce it in staple foods, for example wheat (Nahapetian & Bassiri, 1976), rye, barley, oats (Fredlund, Asp, Larsson, Marklinder, & Sandberg, 1997) and beans (Ockenden, Falk, & Lott, 1997). The concentration of phytic acid presented in some cereals and legumes is shown in Table 4.

Adverse Effects of Phytic Acid

The adverse nutritional effect of phytic acid refers to the insolubility of phytates. In the gut, phytic acid is liberated from food during digestion. Trace elements and minerals are bound with high affinity to phytic acid. Phytate complexes are formed depending on the metal available in the chyme. These phytate complexes can be soluble or insoluble, depending on type of metal ions, phytic acid: metal ratio, and pH of the environment. Under the physiological conditions of the intestine, the affinity of phytate-metal complexes is too high to allow metal ions to be released for absorption (Schlemmer et al., 1995; Cheryan, 1980).

Phytates have been reported to interfere with essential mineral absorption, especially iron, calcium, and zinc. Hurrell et al. (1992) reported that reducing the amount of phytate from 4.9-8.4 to < 0.01 mg/g of soy protein isolate increased iron absorption by humans subjects four to five times. Heaney, Weaver, and Fitzsimmons (1991) found that

Table 4

Phytic Acid Contents of Some Cereals and Legumes.

Source	Phytic acid (mg/100 g dry matter)
Wheat ^a	916.8
Rye ^a	829.7
Barley ^a	910.2
Oat ^a	876.5
Soybean ^b	1400
Green pea ^b	880
Lentil ^b	710

Note ^a Values adapted from “ Phytate Production in Whole Grains of Wheat, Rye, Barley and Oats after Hydrothermal Treatment,” by K. Fredlund, N. G. Asp, M. Larsson, I. Marklinder, and A. S. Sandberg, 1997, Journal of Cereal Science, 25, p. 86.

^b Values adapted from “ The Degradation of Phytic Acid in Legumes Prepared by Different Methods,” by U. Schlemmer, H. Muller, and K L-D. Jany, 1995, European Journal of Clinical Nutrition, 49 (suppl 3), p. 209.

calcium absorption by humans from high phytate soybean was significantly lower than that of low phytate soybean. Hotz, Gibson, and Temple (2001) reported that reducing the phytate content of unrefined maize to 49% of its initial value increased the bioavailability of zinc for children consuming this meal from 24% to 33%.

Determination of Phytic Acid

There are several methods available for the determination of phytic acid content of various foods. The anion-exchange method has been used to determine phytic acid in some African foods composed of cereals, legumes, and some root crops such as cassava and sweet potato (Ferguson et al., 1993), in cereal-based vegetarian meals (Agte, Tarwadi, & Chiplonkar, 1999), and in some beans (Ockeden et al., 1997). Ferric precipitation has been used in the determination of phytate in flaxseed (Oomah, Kenaschuk, & Mazza, 1996) and cereal products (Haug & Lantzsch, 1983). High performance liquid chromatography (HPLC) has been used to determine different forms of phytic acid such as inositol hexa, penta, tetra phosphate in brown beans (Gustafsson & Sandberg, 1995), and a variety of cereals (Fredlund et al., 1997).

Oxalic Acid

Oxalic acid occurs in plants as an end product of tissue metabolism. It is present in various forms depending on the pH of plant tissues and available metal ions (Libert & Franceschi, 1987). Oxalic acid forms water-soluble salts with Na^+ , K^+ , and NH^+ ; it can also bind with Ca^+ , Fe^{2+} , and Mg^{2+} , rendering insoluble oxalate salts. In plants with a cell sap at pH 2, oxalate exists as the acid oxalate (HC_2O_4^-), usually as acid potassium oxalate, such as some species of *Oxalis* and *Rumex*. In other plants with cell sap at pH 6,

such as some species of the goose foot family, it exists as the oxalate ion ($C_2O_4^-$) usually as sodium oxalate, magnesium oxalate, and calcium oxalate (Noonan & Savage, 1999).

Most of the high-oxalate plants (more than 5% oxalate by dry weight) belong to the Caryophyllidae, Chenopodiaceae, or Polygonaceae family (Libert & Franceschi, 1987). The most common oxalate-rich foods are spinach, rhubarb, beets, and tea (Massey, Smith, & Sutton, 1993). Although oxalate-rich foods are usually a minor component of the human diet, sometimes they become important as seasonal diets in certain areas of the world (Noonan & Savage, 1999). Due to concern about health risks caused by high oxalate intake, various studies have been done to evaluate the oxalate content of many foods such as tomato, sweet corn, lettuce, rice, potato (Munro & Bassir, 1969), rhubarb broccoli, carrot, spinach (Savage, Vanhanen, Mason, & Ross, 2000), cabbage, peanut, sweet potato, and collard (Mosha, Gaga, Pace, Laswai, & Mtebe, 1995). The concentration of oxalate in some foods is shown in Table 5.

According to Noonan and Savage (1999), taro (*Colocasia esculenta*) is a member of the ariod family which, in general, contain high levels of oxalate. Hollway et al. (1989) reported that the levels of oxalate in taro leaves and corms were 278-574 mg/100 g fresh weight and 65 mg/100 g fresh weight respectively. The study of Huang and Tanudjaja (1992) showed that taro corms contained 43-156 mg oxalate/100 g fresh weight.

Adverse Effects of Oxalic Acid

Adverse effects of oxalic acid are attributed to its property as a chelator. It is able to bind with trace elements and minerals, and make them unavailable for absorption

Table 5

Oxalate Contents of Some Foods

Food source	Oxalate level
	(mg/ 100 g fresh weight)
Spinach ^a	970
Rhubarb stalks ^b	986.7
Sweet potato ^c	59-94
Cassava ^c	29-48
Yam ^c	9-20
Potato ^a	50

Note ^a From “Oxalic Content of Selected Vegetable,” by U.S. Department of Agriculture, Agricultural Research Service, 2002, USDA Nutrient Database for Standard Reference, Release 14. Home page, <http://www.nal.USDA.gov/fnic/foodcomp/>

^b From “Effect of Cooking on the Soluble and Insoluble Oxalate Content of Some New Zealand Foods,” by G. P. Savage, L. Vanhanen, S. M. Mason, and A. B. Ross, 2000, Journal of Food Composition and Analysis, 13, p. 205.

^c From “Organic Acid and Calcium Oxalate in Tropical Root Crops,” by W. D. Hollway, M. E. Argall, W. T. Jealous, J. A. Lee, and J. H. Bradbury, 1989, Journal of Agricultural and Food Chemistry, 37, p. 339.

(Libert & Franceschi, 1987). Scholfield et al. (1990) found that a high oxalate diet (450mg/day) significantly decreased phosphorus absorption in humans consuming this diet for 6 weeks and 12 weeks. The study of Weaver, Martin, Ebner, and Krueger (1987) demonstrated that calcium bioavailability in low oxalate kale, in CaCO_3 , and in CaCl_2 was nearly 10 times greater than in calcium oxalate and in high oxalate spinach fed to rats.

Apart from interference with mineral absorption, a high oxalate intake may lead to kidney stone formation (Massey et al., 1993; Ogawa et al., 2000) because calcium oxalate is known as a major component of renal stones. Increase in oxalate intake has been one of several factors that caused an increase in urinary oxalate excretion, resulting in stone formation (Ogawa et al., 2000). A review by Hughes and Norman (1992) indicated that dietary oxalate accounted for 10% to 15% of urinary oxalate. However, a recent study of Holmes, Goodman and Assimos (2001) showed that oxalate containing diets contributed to urinary oxalate excretion much more than previously estimated. The results of their experiment indicated that urinary oxalate excretion increased from 24 to 41% in human subjects when the oxalate content of the diet increased from 10 mg to 250 mg oxalate/day. Moreover, people with hyperoxaluria were sensitive to an increase of urinary oxalate excretion (Hughes & Norman, 1992).

Determination of Oxalic Acid

Three major methods have been used to estimate the amount of oxalic acid in foodstuffs. They are the calcium oxalate precipitation method, the enzymatic method, and the HPLC method (Munro & Bassir, 1969; Ross, Savage, Martin, & Vanhanen, 1999;

Mosha et al., 1995). The first method involves extraction of total oxalic acid by HCl, precipitation of calcium oxalate, and finally determination of the amount of oxalate indirectly by titration or by using the atomic absorption spectrophotometer (Munro & Bassir, 1969). The principle of the enzymatic method is based on the specificity of enzyme oxalate decarboxylase to hydrolyze oxalic acid to release CO₂ and formic acid. Consequently, the amount of oxalic acid can be calculated from CO₂ produced in the reaction (Jarcoby, 1974). The last approach, HPLC method, is less time consuming and more precise than the previous methods because oxalic acid can be extracted from the sample and determined directly by HPLC (Libert & Franceschi, 1987).

All of the above methods have been used in the determination of oxalate in various foods. The calcium oxalate precipitation method has been used to determine the oxalic acid content of various Nigerian vegetables (e.g., spinach, yams, lettuce, and carrots) (Munro & Bassir, 1969), and tropical root crops (Hollway et al., 1989). The oxalate decarboxylase method has been used to evaluate the oxalic acid value in oca, a New Zealand yam (Ross et al., 1999). The HPLC method has been used in the determination of oxalic acid in collard, cabbage, peanuts, sweet potatoes (Mosha et al., 1995), and taro (Huang & Tanudjaja, 1992).

Chapter 2

JOURNAL ARTICLE

ANTINUTRIENTS AND TOTAL CYANIDE CONTENT OF TARO CORMS

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ABSTRACT

The purpose of this study was to determine the levels of trypsin inhibitor, phytic acid, oxalic acid, and total cyanide in taro corms. Taro corm samples were obtained from a local oriental grocery store. The corms were cleaned, peeled, freeze-dried, and ground. The resulting freeze-dried powder was used for subsequent analyses. The proximate composition for the taro corms was determined according to AOAC (2000) methods. Trypsin inhibitor activity levels were analyzed by the TAME method. Phytic acid and oxalic acid were analyzed by AOAC (2000) methods. Total cyanide content was analyzed using a macro diffusion apparatus and the pyridine-barbituric colorimetric method. Taro corm samples contained approximately 71% moisture, 1.66% protein, 0.2% fat, 1.05% ash, 0.63% fiber (ADF), and 25.41% carbohydrate. On fresh weight basis, taro corms used in this study contained 384 trypsin inhibitor unit (TIU)/g, 0.3 mg phytic acid/ 100g, and 5.88 mg oxalic acid/ 100g. No cyanide was detected. Trypsin inhibitor activity levels of the taro samples were higher than those published values previously reported, but oxalic acid and cyanide values were lower. Based on the levels of antinutrients and total cyanide found in this study, there is a very little risk of toxicological effects from the consumption of taro corms.

INTRODUCTION

Taro (*Colocasia esculenta*) belongs to the Araceae family. It is grown widely throughout humid tropical regions for its edible corms and leaves (Wang, 1983). This plant originated in India and South-East Asia, and spread to China, Japan, Egypt, and Africa. Later, it became an important crop in Central and South America, as well as the Pacific Islands (Davidson, 1999). Since ancient times, taro has been consumed as a culture food and a staple food in the areas where it has been grown, especially in developing countries (Chandra, 1984). In the US, the increasing demand for taro is attributed to a massive number of immigrants from the tropics (O'Hair, 1990). According to Food and Agriculture Organization of the United Nations (FAO) summary (1997), "Agriculture: Towards 2010", the production of taro is expected to increased in the future due to an increase in planting and an increase of crop yields.

Taro corm, an enlarged starchy underground stem, is consumed for dietary energy. The patterns of its utilization vary considerably among countries (FAO, 1990; Moy and Nip, 1983). When eaten alone, it can be steamed or boiled, and peeled. It can also be boiled in soup along with other vegetables. Taro corms can be served in various ways from main dishes to desserts. Boiling, baking, steaming, and frying are the most common methods of preparation (Davidson, 1999; Moy and Nip, 1983). Besides these simple methods, taro corms can be processed to make flour, paste, beverage powder, and extruded products. In Hawaii, they are used mainly to make a cooked purple-gray paste, packed in plastic bags, jars, or cans, called poi. Poi is sold commercially and consumed widely in the Pacific islands (Moy and Nip, 1983). Taro corms are not only used to make

food for adults, but are also used to make food for babies. The FAO (1990) suggests that taro and other root crops can be used in baby food; however, they should be appropriately supplemented. Hence, taro can be an alternative food for babies born with wheat or grain allergies.

Antinutrients and toxic compounds are major concern in underdeveloped countries where the major staple foods are derived from plant sources. Antinutrients and plant toxins are known to interfere with the bioavailability of some nutrients, or to cause harm to the biological system (Whitney and Rolfes, 1999; De Vries, 1997). Therefore, antinutrients and plant toxins have been investigated in various foods, both raw and processed (Isong and Essien, 1996; Badifu, 2001; Almasan, 1995; Wanasundera and Ravindran, 1994; Akpanabiatu et al., 1998; Siddhuraju et al., 1995).

Due to increasing health concerns and food safety awareness, researchers have increased their interest in antinutrients and plant toxins (De Vries, 1997; Akwaowo et al., 2000; Atony and Chandra, 1998; Badifu, 2001). Among various antinutrients and natural plant toxins, trypsin inhibitor (TI), phytic acid, oxalic acid, and cyanide are found in root crops (Wanasundera and Ravindran, 1994; Peng et al., 1993; Bradbury and Hammer, 1990). The presence of these antinutrients limits the nutritive value of these crops. In animal studies, trypsin inhibitors are reported to interfere with protein absorption, limit the growth of several animals, and cause pancreatic hypertrophy (Chernick et al., 1948; Green and Lyman, 1972; Kakade et al., 1973; Ge and Morgan, 1993). Phytic acid and oxalic acid are able to bind minerals (e.g. calcium, iron, zinc), and make them unavailable for absorption (Zhou and Erdman, 1995; Noonan and Savage, 1999; Heaney

et al., 1988). Moreover, oxalic acid in calcium oxalate form is a major component of renal stones. High oxalate intake leads to the hyperoxaluria resulting in the increasing risk of kidney stone formation (Ogawa et al., 2000; Noonan and Savage, 1999). On the other hand, cyanogenic glycosides, toxic compounds found in cassava, are known to liberate cyanide. This cyanide causes inhibition of respiratory processes at the cellular level. The detoxification of cyanide in the body yields thiocyanate, which can cause goiter, due to its interference with iodine absorption (Oke, 1969; Conn, 1969).

Although the nutritive value of taro has been studied and reported by several sources (United State Department of Agriculture [USDA], 2002; FAO 1998; Huang et al., 2000), studies of its antinutrient and cyanide content were still limited. As a carbohydrate staple food for millions of people, there is a pressing need to evaluate taro corms for these compounds. The objective of this study was to determine the levels of trypsin inhibitor, phytic acid, oxalic acid and total cyanide in taro corms.

MATERIALS AND METHODS

Sample and Sample Preparation. Fresh taro corms were purchased from a local oriental grocery market. The corms were washed, dried with paper towels and peeled immediately. The flesh was cut into approximately 2x1x1 cm³ pieces, and then freeze-dried. The freeze-dried flesh pieces were ground with a Scienceware coffee grinder (Bel-art, NJ), blended together, stored in tightly capped bottles, and frozen until use. All subsequent analyses were conducted in triplicate.

Proximate Analyses. Analyses of moisture, crude protein, crude fat, ash, and fiber (acid detergent fiber, ADF) were conducted according to the methods of analysis of

the Association of Official Analytical Chemists (AOAC, 2000). Total carbohydrate was determined by subtracting the percentages of moisture, crude protein, crude fat, ash, and fiber from 100.

Determination of Trypsin Inhibitor Activity. A one gram sample was homogenized with 19 ml of distilled water for 2 min using a mechanical homogenizer (PowerGer 700, Fisher Scientific). The homogenate was allowed to stand 5 min before 30 min centrifugation at 15,500 rpm. Supernatant aliquots of 0.1, 0.5, and 1 ml were diluted to 10 ml with tris buffer (0.05 M, pH 8.2). Their trypsin inhibitor activity (TIA) was determined by the TAME (p-toluene-sulfonyl-L-arginine methyl ester) method described by Worthington Biochemical Corporation (1972). The assay buffer was a 0.046 M tris HCl buffer (pH 8.1) with 0.0115 M CaCl_2 . A stock trypsin solution was prepared by dissolving 25 mg of bovine trypsin (type II, Sigma Chem Co) into 25 ml of 0.001 M HCl, then the stock enzyme was stored at 5° C. To prepare the working trypsin solution, 0.5 ml of stock solution was diluted with 0.001 M HCl to a total volume 10 ml. The TAME solution was prepared by dissolving 37.9 mg of p-toluene-sulfonyl-L arginine methyl ester hydrochloride in 10 ml distilled water. The rate of hydrolysis of TAME, both in the presence and absence of the inhibitor, was observed with Shimadzu UV visible recording spectrophotometer at 247 nm.

In the determination step, the first cuvette (blank) was filled with a mixture of 0.1 ml 0.001 M HCl, 0.3 ml TAME substrate, and 2.6 ml tris buffer. In the second cuvette, 0.1 ml extracted inhibitor, 2.5 ml buffer, and 0.1 ml working trypsin were added. After 5 min incubation at room temperature, 0.3 ml of substrate was added. The mixture was

mixed rapidly, and absorbance change was monitored every 30 sec for 3 min. The reaction was also conducted in the absence of the inhibitor by filling 0.3 ml substrate with 2.6 ml buffer in the second cuvet. After mixing 0.1 ml of working enzyme into the mixture of substrate, the absorbance was read against the blank. Trypsin inhibitor activity was expressed as trypsin inhibitory unit per mg of protein or per gram of sample. One inhibitory unit of trypsin inhibitors (TIU) is defined as the amount of inhibitor that inhibited 1.0 μ g of pure trypsin (Tan et al., 1984).

Determination of Total Cyanide. The preliminary testing for liberation of hydrocyanic acid from taro sample was carried out in Conway's semimicro diffusion apparatus (Figure 6). Because of the very limited amount of sample used, cyanide was not detected using the semimicro diffusion system. A modified macro diffusion apparatus (Figure 7) was used in the determination of cyanide.

In the macro diffusion apparatus, a 15 g of sample was dispersed in 50 ml of H_2O , and 10 ml of 1.0 N NaOH was used to absorb the liberated cyanide. The sample was incubated at room temperature for 24 and 48 hr. The amount of cyanide absorbed was determined by the pyridine-barbituric acid method as described by Haskin et al.(1988). Aliquot 1 ml of NaOH was treated with 0.5 ml 1.0 M acetic acid followed by 5 ml of succinimide -N-chlorosuccinimide solution prepared by dissolving 2.5 g of succinimide and 0.25g of N-chlorosuccinimide in 1 L of H_2O . Then, 1 ml of barbituric acid solution, prepared by dissolving 6g barbituric acid and adding 30 ml of pyridine in 70 ml of water, was added. The mixture was allowed to stand for 20 min to develop a pink color, and the absorbance was read at 580 nm against a blank containing 1.0 N NaOH plus color reagent

using the Shimadzu recording spectrophotometer. The amount of cyanide liberated was determined from a standard curve prepared from KCN solution containing 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 μg cyanide /ml.

The results from the macro diffusion method were further confirmed by the alkali-heat treatment method of Patel (1992). One gram of sample was homogenized with 19 ml of distilled water using the PowerGer 700 homogenizer for 2 min at room temperature. The suspension was filtered through Whatman No.1 filter paper. One ml of filtrate was treated with 9.0 ml of 1.0 N NaOH and heated in boiling water. Aliquot was taken at various time intervals, and the cyanide content was analyzed colorimetrically as describe above.

Determination of Phytic Acid. Phytic acid was determined using the AOAC (2000) method with minor modifications. The steps involved were as follows:

a) Extraction – An eight gram freeze-dried sample was extracted with 40 ml of 2.4 % HCl. Extraction was carried out using a mechanical shaker (Thermolyn Maxi-Max Type 65800) at 600 rpm for 3 hr. This mixture was vacuum filtered through Whatman No.1 filter paper. Ten ml aliquot of the filtrate were transferred into a 25 ml glass stoppered volumetric flask. Ten ml of Na_2EDTA (0.11 M)-NaOH (0.75 M) solution were added into the flask containing aliquot. This mixture was diluted to the volume and mixed thoroughly. The resulting mixture was passed through a pre-prepared anion exchange column.

b) Preparation of anion exchange column – To prepare the column, 3 ml of H_2O was poured into the empty mounted column, followed by H_2O slurry of 0.5 g anion

exchange resin (Dowex, 100-200 mesh, chloride form, Sigma Chem Co.). After resin bed formed, the column was washed with 15 ml of 0.7 M NaCl and 15 ml of H₂O respectively, and set aside until used.

c) Elution of phytic acid from anion exchange column – After passing the mixture of extract from step a) into the column, the elute was discarded. The column then was eluted with 15 ml 0.1 M NaCl, 15 ml H₂O, and 15 ml 0.7 M NaCl respectively. Only the last elute was collected into micro-Kjeldahl flask. Before adding the next test extract, the column was eluted with 15 ml H₂O. After 1 week or 3 test extracts, the old resin was replaced with a fresh one.

d) Digestion– To the elute in micro-Kjeldahl flask, 0.5 ml H₂SO₄, 3 ml HNO₃, and 3 glass beads were added respectively. The flask was heated on micro-Kjeldahl rack over medium heat until a cloud of thick yellow vapor filled the neck of the flask. The content was kept on medium heat for 5 min followed by 5 min on low heat, and left at room temperature until cool.

e) Analysis – Phytic acid was analyzed indirectly by measuring the amount of phosphorous released after digestion. The digested solution was diluted with 10 ml H₂O, swirled, and quantitatively transferred to 50 ml volumetric flask. Two ml of molybdate solution (2.5% ammonium molybdate in 0.5 M H₂SO₄) and one ml of sulfonic acid reagent (0.04 g of 1 – amino-2- naphthol-4-sulfonic acid, 0.48 g Na₂SO₃, and 2.4 g NaHSO₃ diluted to 25 ml in volumetric flask with H₂O) were added to the content. The mixture was shaken after adding each reagent. Finally, the mixture was made up to the

volume with H₂O, shaken, left at room temperature for 15 min, and read absorbance at 640 nm using Shimadzu UV 160 spectrophotometer.

Before reading the absorbance, the spectrophotometer was adjusted to 640 nm and equilibrated ≥ 15 min. The standard curve was prepared by phosphate standard solution containing 80, 240, and 400 μg phosphate/ml. The typical standard curve is shown in Figure 5.

Determination of Oxalic Acid. Analysis of oxalic acid was conducted as follows:

a) Extraction – The method described by Savage et al. (2000) was used. A ten gram freeze-dried sample was mixed with 50 ml 2 M HCl, incubated in water bath (80 °C) for 15 min, cooled and quantitatively transferred into 100 ml volumetric flask. This mixture was made up to the volume, mixed thoroughly and vacuum filtered through quantitative filter paper. The first 10 ml of filtrate were discarded and the rest was collected.

b) Precipitation – To a 25 ml aliquot of the filtrate was added 5.0 ml of tungstophosphoric acid reagent (2.5 g Na₂WO₄·2H₂O + 4 ml H₃PO₄ + 50 ml H₂O mixture diluted to 100 ml), mixed thoroughly and let stand for at least 5 hr. The mixture was then filtered through quantitative filter paper. A 20 ml aliquot of the filtrate was transferred in a conical centrifuge tube and the pH adjusted to 4 – 4.5 with NH₄OH. Five ml of acetate-CaCl₂ buffer were added to precipitate calcium oxalate. The mixture was stirred and left at room temperature overnight. Acetate-CaCl₂ buffer solution was prepared by adding

33 g $\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$ to CaCl_2 solution, containing 2.5 g of anhydrous CaCl_2 in 20 ml acetic acid solution (1+1), and diluting the resulting mixture to 50 ml with acetic acid. The next day, the mixture was centrifuged for 15 min at 1700 rpm to compact the precipitate. The supernatant was decanted and the centrifuge tube was held upside down on a clean filter to let the remaining supernate drain completely. The calcium oxalate precipitate was then washed with 20 ml of a cold pre-filtered wash solution. Wash solution was prepared by diluting 12.5 ml acetic acid to 250 ml with distilled water and the resulting mixture saturated with calcium oxalate powder. The solution was filtered and stored under refrigeration prior to use. Washing, centrifugation and decantation were repeated three times.

c) Analysis – Oxalic acid in the precipitate was analyzed indirectly. The calcium oxalate precipitate was dissolved in 5 ml of H_2SO_4 (1+1) solution, transferred quantitatively into 25 ml volumetric flask and diluted to volume with H_2SO_4 (1+1) solution. A five ml aliquot was transferred into 50 ml volumetric flask containing 10.0 ml of 5.0% lanthanum solution. The amount of calcium in the solution was determined using the atomic absorption spectrophotometer (Perkin-Elmer2380). The setting of the atomic absorption spectrophotometer is shown in Table I.

RESULTS AND DISCUSSION

Proximate Analyses. The freeze-dried sample contained 1.94% moisture. This value was used to calculate the proximate and antinutrient composition as well as the total cyanide content of taro corms in fresh basis. The results of proximate composition are shown in Table II. The taro corm samples contained typically high moisture and high

carbohydrate, but they were low in protein and low in fat. The results, except fiber content, are comparable to published USDA data (USDA, 2002). Fiber content of the taro samples was determined as insoluble fiber (ADF) while USDA reported total fiber. Furthermore, the results of crude protein, crude fat, ash, and moisture content are within the range of two major cultivars, Lehau and Bunlong, grown in Hawaii (Huang et al., 2000). However, the insoluble fiber content of the taro samples is lower than that of those two cultivars. Compared to other root crops (USDA, 2002), the proximate composition of taro is comparable to those of sweet potato and yam, although, sweet potato contains more fat. The moisture and protein contents of taro are higher than that of cassava, but lower than those values of potato. In contrast, the carbohydrate content and the fat content of taro are much lower than that of cassava, but much higher than that of potato.

Trypsin Inhibitor. On dry basis, taro corms contained 1326.77 TIU/ g dry matter. Based on 71% moisture, this is equivalent to 384 TIU/ g fresh taro corm (Table III). This value is relatively higher than the value reported by Bradbury and Hammer (1990). Compared to other root crops, the average level of trypsin inhibitor obtained in this study is higher than those reported in sweet potato, yam, and cassava, but comparable to giant taro (see Figure 8) (Bradbury and Hammer, 1990).

High levels of trypsin inhibitor in plant foods are of major concern because it is known that trypsin inhibitor can cause nutritional disorders, growth depression, and pancreatic hypertrophy and hyperplasia (Liener and Kakade, 1980). The trypsin inhibitor contents of soybean and winged bean were reported as 18,900 and 15,200-32,400 TIU/g

fresh weight respectively (Tan and Wong, 1982). The trypsin inhibitor content of faba bean, field pea, and grass pea ranged from 2,620 to 30,790 TIU/g dry matter (Vidal-Valverde et al., 1997; Wang et al., 1998). Compared to these legumes, taro corms contain a much lower trypsin inhibitor level. Typically, taro is consumed after cooking, which is reported to reduce trypsin inhibitor activity substantially (Tan and Wang, 1982; Tan et al., 1984; Vidal-Valverde et al., 1997). With relatively low trypsin inhibitor content, and cooking before consumption, taro consumption should be safe from the adverse effects of trypsin inhibitor.

Total Cyanide. Total cyanide was not found in our taro corm samples (Table III). In the literature, Osuntokun (1980) cited that Nigerian taro contained 2.7 mg HCN/100 g fresh weight. The differences in these cyanide values may be due to the variation in variety and environmental conditions such as plant cultivars, planting season, stage of growth, and soil condition (Oke, 1969).

Cyanide causes the adverse effects on humans. The minimum lethal dose of cyanide for a man was estimated to be 0.5 mg/kg body weight (Oke, 1969). Bolhuis (1954) estimated a lethal dose of 50-60 mg HCN/day for a European adult. One chronic effect of cyanide, endemic goiter, occurs in the areas where cassava is consumed (FAO, 1990). The cyanide content of cassava ranged from 18 to 113 mg per 100 g fresh weight (Mendoza et al., 1984). From the results of this experiment, since taro corms contained non-detectable total cyanide, the consumption of taro corms should not cause any harms to human because of the adverse effects of cyanides.

Phytic Acid. Phytic acid content of taro samples was found in trace amounts (Table III). No comparable data on phytic acid content of taro was available in the literature. Among root crops, phytic acid levels in only yam and potato were investigated and reported (Wanasundera and Ravindran, 1994; De Vries, 1997). The level in taro is far lower than that in yam and potato. Furthermore, the phytic acid content of taro is drastically lower than the values of 400-2060 mg /100 mg dry matter reported for cereals and legumes (Reddy et al., 1982).

Phytic acid is a human health concern due to its adverse effects on mineral bioavailability. High intake of phytic acid has been known to interfere with absorption of minerals, such as calcium, iron, and zinc (Zhou and Erdman, 1995; Hurrell et al., 1992; Heaney et al., 1991). For high phytate foods, it was suggested that phytic acid should be reduced to <10 mg/meal in order to allow a significant increase in iron absorption (Hurrell et al., 1992). Since taro corm samples contained very low amounts of phytic acid, the consumption of taro corms should not cause any problem to the consumer regarding the adverse effects of phytic acid.

Oxalic Acid. The average value of oxalic acid content of the taro corm samples is shown in Table III. This value is much lower than the value of 45-150 mg oxalic acid/100 g fresh weight found in taro grown and consumed in Hawaii and the South Pacific (Huang and Tanudjaja, 1992; Hollway et al., 1989). The variation in oxalic acid content may be due to both cultivars and environmental factors. Wanasundera and Ravindran(1994) reported a wide variation in the oxalic acid content among different cultivars of yam grown in Sri Lanka. Also the oxalic acid values of yams from their

study are much higher than those values of yams grown in the South Pacific (Hollway et al., 1989).

The oxalic acid contents of other root crops including cassava, sweet potato, and potato were reported in the literature. The values were 1260, 240, and 50 mg/100g in cassava, sweet potato, and potato respectively (USDA, 2002). The average level of oxalic acid of the taro samples is far lower than all of those 3 root crops (Figure 9). Compared to food containing high oxalate, the average oxalic acid value of taro samples is much lower than that of spinach and rhubarb which were reported to be 970 and 805 mg/100 g respectively (Figure 9) (USDA 2002, De Vries, 1997).

Oxalic acid has been known to affect human nutrition (Noonan and Savage, 1999). High oxalic acid intake has a possibility of reducing absorption of minerals such as calcium, iron, and zinc (Noonan and Savage, 1999; Weaver et al., 1987). An intake of 450 mg oxalic acid/ day is reported to decrease phosphorus absorption and interfere with metabolism (Scholfield, 1990). Munro and Bassir (1969) estimated a toxic dose of oxalic acid as 2-5 g intake. From the results of this study (Table III), the taro corm samples contained approximately 5.88 mg oxalic acid/100g fresh weight. This value is extremely below the toxic dose. To reach a toxic dose of oxalic acid (2-5 g), or even 450 mg, more than 7 kg of taro would need to be consumed.

Apart from interfering with mineral absorption, consumption of oxalic acid and its oxalates, is considered one of the factors promoting kidney stone formation (Ogawa et al., 2000). Holmes et al. (2001) reported that urinary oxalate excretion increased as dietary oxalate was increased. Ten to fifty mg of oxalate intake caused to 24.4-26.9%

urinary oxalate excretion; however, the percentage increased to 41.5% when 250 mg oxalate was consumed (Holmes et al., 2001). Taro contained a very low amount of oxalic acid (Table III); therefore, taro consumption should have a low contribution to urinary oxalate excretion.

Figure 5. Typical standard curve for the determination of phytic acid

Figure 6. Conway's semimicro diffusion apparatus

(1) Lid with silicon grease; (2) Diffusion chamber; (3) Absorbing solution (1.0 N NaOH) in outer chamber; (4) Sample in inner chamber.

Figure 7. Modified macro diffusion apparatus

(1) Lid with silicon grease; (2) Diffusion chamber; (3) Absorbing solution (1.0 N NaOH) in center well; (4) Glass rod for center well; (5) Sample.

Note.: sources of values: Taro from our experimental data; Sweet potato, Yam, and Cassava from “Comparative study of protease inhibitors in tropical root crops and survey of allelochemicals in the edible aroids,” by J.H. Bradbury and B.C. Hammer, 1990, Journal of Agricultural and Food Chemistry, 38, p.1450.

Figure 8. Comparison of trypsin inhibitor activity levels

Note: Sources of values: Taro from our experimental data; Yam adapted from “Nutritional assessment of yam (*Discorea altata*) tubers,” by J.P.D. Wanasundera and G. Ravindran, 1994, Plant Foods for Human Nutrition, 46, p. 38; Sweet potato, Cassava, Potato, Tea, Spinach, and Rhubarb from “USDA Nutrient database for standard reference, Release 14,” by USDA, 2002, Home page, <http://www.nal.usda.gov/fnic/foodcomp/>

Figure 9. Comparison of oxalic acid levels

Table I. Atomic Absorption Spectrophotometer Operating Parameters for Oxalic Acid

Determination

Site	Setting
Wavelength, nm	422.7
Slit width, mm	1-setting 4
Hollow Zn-Ca cathode	15-20
Air, aspirating	9.0 (scale divisions)
Acetylene fuel	9.5 (scale divisions)
Flame	Reducing
Height, burner to light path, in	ca 0.5
Sample uptake, ml/ min	5
Optimum concentration range, $\mu\text{g/ml}$	0-10

Table II. Proximate Composition of Taro Corms

Constituent	Value ^a
	(% fresh basis)
Moisture	71±0.90
Protein	1.66±0.13
Fat	0.2±0.04
Ash	1.05±0.12
Fiber(ADF)	0.63±0.05
Total Carbohydrate	25.41

^a mean± standard deviation of three determinations

Table III. Trypsin Inhibitor, Phytic Acid, Oxalic Acid, and Cyanide Content of Taro Corms

Compounds	Value ^a
Trypsin Inhibitor (TIU/ g sample)	384.10±91.02
Phytic Acid (mg/100g)	0.3±0.07
Oxalic Acid (mg/100g)	5.88±0.6
HCN (mg/100g)	n.d

^a means± standard deviation of three determinations

n.d = not detected

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CHAPTER 3

SUMMMARY AND RECOMMENDATIONS

Summary

The objective of this study was to determine the amount of trypsin inhibitor, phytic acid, oxalic acid, and total cyanide in taro (*Colocasia esculenta*) corms. Fresh taro corms obtained from a local oriental grocery store were cleaned, peeled, and freeze-dried. The resulting freeze-dried sample was analyzed for proximate composition, trypsin inhibitor, phytic acid, oxalic acid, and total cyanide contents. The proximate analysis that was conducted followed the methods of AOAC (2000). Trypsin inhibitor activity was determined by the TAME method (Worthington Biochemical Corporation, 1972). Phytic acid and oxalic acid were analyzed using AOAC (2000) methods. Total cyanide was measured by a modified macro diffusion apparatus and pyridine-barbituric colorimetric method (Patel, 1992). The results were reported as the mean of three determinations $\pm$ standard deviation.

The results of this study demonstrated that taro corms contain low amounts of trypsin inhibitors and oxalic acid, negligible amounts of phytic acid, and non-detectable total cyanide. Although the amounts of trypsin inhibitors of taro corms in the present study are much higher than the values reported in the literature (Bradbury & Hammer, 1990), the values of trypsin inhibitors in this study are still much lower than the values in those foods of concern that contained high amounts of trypsin inhibitors such as legumes. Also, taro corms are typically consumed after cooking, which is reported to significantly reduce the amounts of antinutrients including trypsin inhibitor (Savage et al., 2000, Tan

& Wong, 1982, Vidal-Valverde et al., 1997). Hence, taro could be an important source of carbohydrate; however, they are low in protein. There is a very little risk of toxicological effects from the consumption of taro corms.

Recommendations

The results of the present study suggest that taro corm is a good source of carbohydrate with low antinutrients and non-detectable total cyanide. Therefore, it is recommended that taro corms be used as an alternate carbohydrate source in composite diets for people who have wheat and grain allergies.

In this experiment, the methods used for the analysis of phytic acid and oxalic acid (AOAC, 2000) were time consuming and tedious. The AOAC 2000 methods are not recommended for use in the experiments which require the analyses of numerous samples. Although HPLC is considered a rapid, alternative method, it is still expensive and not available everywhere. Hence, there is a need to develop a simple rapid method to determine the two compounds, phytic acid and oxalic acid, in order to analyze more samples in less time.

Standard methods for analyses of food antinutrients and natural toxins need to be determined and agreed upon in order to construct comparative data, and build up the collaborative work in this area. The collaborative study of antinutrients and plant natural toxins is needed in order to conduct risk assessment of plant food consumption, search for alternative food sources, and breed the low antinutrient and natural toxin plant species.

In specific areas of the world where nutrients are limited, antinutrients and plant food natural toxins need to be evaluated, especially in staple foods which are consumed

in large quantities because the consumption of these foods will have a great effect on consumers. Like taro, other major root crops such as sweet potato, yam and, cassava have had limited studies of antinutrients and plant natural toxins. Hence, it is recommended that such studies should be conducted on major root crops.

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