ELSEVIER

Food Chemistry 85 (2004) 281–287

Food Chemistry

www.elsevier.com/locate/foodchem

Assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam *(Dioscorea spp.)* tubers of Nepal

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Received 4 April 2003; accepted 1 July 2003

Abstract

The purpose of this study was to determine the antinutritional factors in four wild yam species (*Dioscorea bulbifera, D. deltoidea, D. versicolor and D. triphylla)* of Nepal. The ranges of antinutrient contents were found to be: oxalates (Ox) 67–197 mg/100 g fresh weight (FW), phytate (Phy) 184–363 mg/100 g dry matter (DM), cyanogens 3.2–6.0 mg of HCN/kg FW, trypsin inhibitor activity 4.1–20.9 mg of pure trypsin inhibited/g DM, and α-amylase inhibitory activity 78-147 IU/g DM. The ranges of Ox:Ca, Phy:Zn, Ca:Phy and [Ca] [Phy]/[Zn] molar ratios for yam tubers studied were: 1.1–2.2, 10.4–32.3, 5.0–14.1 and 0.27–1.9, respectively. The molar ratios indicated that the bioavailability of Ca and Zn in these tubers could be low. In general, the results tend to imply that the Nepalese wild yams may present a health-hazard potential, which in turn demands proper processing before consumption to eliminate the effects of the antinutrients.

Keywords: Wild yam; *Dioscorea*; Antinutritional factors; Oxalate; Phytate; Molar ratio

1. Introduction

Wild yam tubers (*Dioscorea spp.*) are freely available in the wild habits and are consumed in several parts of Nepal, especially in the Mid-hills and Terai region (Malla, Rajbhandari, Shrestha, Adhikari, & Adhikari, 1982). Generally, wild yams are eaten as such, after boiling or baking and mixing with other vegetables (Gurung, 1995). The tubers were found to have high amounts of protein with a good proportion of essential amino acids and appeared as fairly good sources of many dietary minerals (Bhandari, Kawabata, & Kasai, 2003). Historical reports of these yam tubers suggest that tubers are acrid, causing irritation and inflammation; consumption can result in gastrointestinal disturbances, vomiting and diarrhea when large amounts are ingested in the human body. Although, these wild yam tubers constitute one of the least expensive sources of food, their utilization limited by the presence of some toxic antinutrients responsible for several health complications (Singh, 1960).

The underground and aerial tubers of yam (genus *Dioscorea*) are one of the important sources of carbohydrate in some regions of the tropics (Wanasundera & Ravindran, 1994). Several antinutritional factors are present in root and tuber crops. Enzyme inhibitors, e.g. against amylase and protease, occur in many tubers. The presence of these inhibitors could impair the digestion of starch and protein, thereby reducing the nutritional value of tubers and limiting their utilization as food (Prathibha, Bala, & Leelama, 1995). Oxalate is a common constituent of plants and several species, including some crop plants, accumulate high levels of this dicarboxylic acid anion (Libert & Franceschi, 1987). Oxalates in tubers may either be a cause or a contributor as to the acridity, which causes irritation, and swelling of mouth and throat (Holloway, Argall, Jealous, Lee, & Bradbury, 1989). Phytate is widespread in roots and tubers (McCance & Widdowson, 1935). Oxalates and phytate are well known antinutrients of plant food, and they are associated with a decrease in bioavailability of nutritionally significant mineral elements. These organic substances can bind essential minerals to form insoluble or indigestible complexes in the lumen of intestinal tracts, thereby preventing their absorption (Davis & Olpin, 1979).

Bhandari et al. (2003) have lately reported a nutritional evaluation of these less-known wild yam tubers. However, information about the antinutritional and

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0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.07.006

toxic factors in these yam tubers appears to be lacking. Hence, this study was carried out to determine levels of potential toxic and antinutritional factors in these tubers. This paper attempts to examine antinutrient contents (oxalates, phytates, cyanogens, trypsin inhibitor activity and α-amylase inhibitor activity) in four species (*Dioscorea)* of Nepali wild yam. The effects of oxalate and phytate on the bioavailability of Ca and Zn were also studied by calculating respective Ox:Ca, Phy:Zn, Ca:Phy and [Ca] [Phy]/[Zn] molar ratios.

2. Materials and methods

2.1. Materials

The four species of wild yam (*D. bulbifera, D. versicolor, D. deltoidea and D. triphylla*) were collected from the central region (Narayani Zone) of Nepal. They were stored at 15 °C until processed. Dried powder of yam sample was used for the determination of oxalate and phytate. Yam tubers were weighed, peeled, fractionated into little pieces and dried at 40 °C in a hot air oven to constant weight. The dried samples were ground to a fine powder by using an electric grinder and the analyses were carried out on the flours (Holloway et al., 1989). Fresh tubers were used for assay of trypsin and α-amylase inhibitory activities (Prathibha et al., 1995).

Trypsin was purchased from Wako Pure Chemicals Industries Ltd. (Japan) and α-amylase was from Sigma Chemical Company (USA). All other chemicals and reagents used were of analytical grade and purchased from Wako Pure Chemicals Industries Ltd. (Japan).

2.2. Analysis of oxalate

The oxalate was determined by HPLC, using the extraction and analysis method developed by Holloway et al. (1989). With 30-ml capacity glass-stoppered test tubes, 1 g of the dried powder was added to either 25 ml of distilled water (for soluble oxalate) or to 25 ml of 0.25 M H_2SO_4 (for total oxalate), and 1 ml of internal standard (10 g glutaric acid in 100 ml of water) was added. The mixture was placed in a boiling water bath for 10 min, cooled, and made up to volume in a 100-ml standard flask. A sample volume of solution was filtered and this filtered solution was then filtered through a 0.45 μm Millipore (Nihon Millipore Kogyo K. K., Yonezawa, Japan) membrane before separation by HPLC.

HPLC analysis of extracted sample was carried out on 300×7.8 mm ion exclusion column (HPX-87H, Bio-Rad) using 0.0125 M H_2SO_4 as mobile phase at a flow rate of 0.5 ml/min and UV detector operating at 214 nm. For calibration of the HPLC system, a standard mixture of sodium oxalate (0.02 g) and glutaric acid (0.08 g) in 100 ml of 0.0125 M H_2SO_4 was used. Extraction with 0.25 M H_2SO_4 and distilled water gave total oxalate and soluble oxalate, respectively. The difference between acid (total) and water (soluble) extractions is assumed to be insoluble calcium oxalate. The calculated results were expressed as milligrammes per 100 gramme fresh weight.

2.3. Analysis of phytate

The phytate was determined according to Latta and Eskin (1980) and later modified by Vaintraub and Lapteva (1988). The absorbance of supernatant was measured at 500 nm using a UV-1600 spectrophotometer (UV-Visible spectrophotometer, Shimadzu, Japan). The phytate concentration was calculated from the difference between the control absorbance and that of the assayed sample. A series of standard solutions containing 5-40 μg/ml of phytic acid in distilled water was prepared and a standard curve was prepared (Latta & Eskin, 1980). The concentration of phytate was calculated from the standard curve, and results were expressed as phytic acid in mg per 100 g dry matter.

2.4. Analysis of cyanogens

Cyanogens were determined by the method of Bradbury, Bradbury, and Egan (1994), using acid hydrolysis and the isonicotinic acid/barbituric acid colour reagent. The cyanogens were calculated and presented as HCN equivalents in mg per kg of fresh sample.

2.5. Analysis of trypsin inhibitor

Trypsin inhibitor activity (TIA) was determined essentially according to the method described by Smith, Megen, Twaalfhoven, and Hitchcock (1980). TIA values were estimated in terms of weight of pure trypsin inhibited per g dry matter and calculated by using the following formula derived by Smith et al. (1980).

$$\text{TIA} = (2.632 \times \text{Dilution factor} \times A_i/S)$$
$$\text{mg pure trypsin inhibited per g sample,}$$

where, A_i = the change in absorbance due to trypsin inhibition per ml sample extract, and S = sample weight in grammes.

2.6. Analysis of α-amylase inhibitor

Yam tubers were homogenized with 0.02 M phosphate buffer (1:4 w/v). After standing for about 15 min the homogenates were centrifuged at 5000 rpm for 20 min (Prathibha et al., 1995). The supernatants were tested for α-amylase enzyme inhibitory activity. α-Amylase inhibitory activity was evaluated according to the

method reported by Alonso, Orue, and Marzo (1998). The absorbance of reaction mixture was measured at 540 nm. One unit of enzyme activity was defined as that which liberates, from soluble starch, one micromole of reducing groups (calculated as maltose) per min at 37 °C and pH 7.0, under the specified conditions. One unit of α-amylase enzyme activity inhibited was defined as one α-amylase enzyme inhibitory unit (IU), and expressed as IU per g of dry matter.

2.7. Statistical analysis

All the results for oxalates, phytates, cyanogens, trypsin inhibitor activity and α-amylase inhibitor activity were reported as mean values with their respective standard deviations.

3. Results and discussion

3.1. Oxalates

Table 1 shows the results of oxalate estimation in the wild yam tubers. The levels of total oxalate varied greatly between species and ranged from 67 to 197 mg per 100 g FW. The oxalate content was lowest (67 mg/100 g FW)) for *D. bulbifera* and highest (197 mg/100 g FW) for *D. deltoidea*. The oxalate levels were lower than reported values for giant swamp taro and elephant foot yam and were higher than reported values for other common root crops (Holloway et al., 1989). But the obtained values for oxalates were comparable to the reported values for Sri Lankan yams (Wanasundera & Ravindran, 1994) and New Zealand yams (Ross, Savage, Martin, & Vanhanen, 1999). The oxalate contents were about 4–10 times higher than reported values for tropical cultivated yam species (Holloway et al., 1989). The improved varieties are low or free from the chemicals (oxalates). The wild varieties develop the chemical in plenty (Dey, 1985). The high oxalate content in these wild yam tubers may largely be due to this. These results imply that the observed acridity and inflammation effects in these wild yam tubers might be due to this high level of oxalate. In tropical root crops, calcium oxalate is present as fine needle-like crystals or raphides. The occurrence of these crystals has been considered as either the cause of or a contributor to the acridity, which initiates irritation and swelling of mouth and throat (Holloway et al., 1989). Table 1 shows that the contents of soluble oxalate and calcium oxalate ranged from 37 to 85 mg/100 g FW and from 10 to 112 mg/100 g FW, respectively. The obtained values for calcium oxalate were comparable to reported values for taro, but were lower than reported values for giant taro and elephant foot yam (Holloway et al., 1989). The yam tuber studied contained substantial amounts of both soluble and calcium oxalates. This is in contrast to the New Zealand yam analyzed by Ross et al. (1999), which contained only soluble oxalate.

Table 1
Total, soluble and calcium oxalate contents of wild yam (mg/100 g FW)[a]

Yam species	Total oxalate	Soluble oxalate	Calcium oxalate
Dioscorea bulbifera	67±9	44±3	23±6
Dioscorea versicolor	70±15	60±9	10±7
Dioscorea deltoidea	197±25	85±2	112±25
Dioscorea triphylla	104±24	37±2	67±24

[a] Values are means ±SD, ($n=6$).

Oxalic acid and its salts can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones (Noonan & Savage, 1999). High-oxalate diets can increase the risk of renal calcium oxalate formation in certain groups of people (Libert & Franceschi, 1987). The majority of urinary stones formed in humans are calcium oxalate stones (Hodgkinson, 1977). Wild yam tubers analyzed in this study varied widely in oxalate levels, but all with high levels compared to the recommendations for patients with calcium oxalate-kidney stones. Currently, patients are advised to limit their intake of foods with a total intake of oxalate not exceeding 50–60 mg per day (Massey, Palmer, & Horner, 2001). Under these guidelines, no yam tubers analyzed could be recommended for consumption for patients with a history of calcium oxalate kidney stones. However, the levels of oxalate in these wild yam tubers are not a major concern for normal healthy people, as about 1 kg of fresh *D. deltoidea* (high oxalate-containing yams) would be necessary for consumption (at once) to ingest 2 g of oxalate, which is thought to be the fatal dose for humans (Libert et al., 1987).

3.2. Phytate

The phytate contents obtained in wild yam tubers are given in Table 2. The levels of phytate in yam tubers ranged from 184 to 363 mg/100 g DM. Results showed that *D. bulbifera* and *D. deltoidea* recorded lowest (184 mg/100 g DM) and highest (363 mg/100 g DM) level of phytate, respectively. A comparison of this result with the previous one showed that these values were higher than those of Sri Lankan yams (Wanasundera & Ravindran, 1994) and some tropical tubers (Oladimeji, Akindahunsi, & Okafor, 2000) but were close to the phytate levels reported for Nigerian yam species (Adeyeye, Arogundade, Akintayo, Aisida, & Alao, 2000). Results of our study showed that Nepalese wild yam tubers have fairly high contents of phytate and contents among the species varied significantly. In many cases, phytic acid content may vary depending on the

Table 2
Phytate (as phytic acid, mg/100 g DM) and cyanogens (as mg HCN/kg FW) contents of wild yam tubers[a]

Yam species	Phytate content	Cyanogens content
Dioscorea bulbifera	184±14	3.3±0.9
Dioscorea versicolor	231±29	6.0±4.0
Dioscorea deltoidea	363±12	3.2±0.6
Dioscorea triphylla	201±14	3.3±1.3

[a] Values are means ±SD, (for phytate, $n=6$, and for cyanogens, $n=9$).

variety, climatic conditions, location, irrigation conditions, type of soil, and year during which they are grown (Deshpande, Sathe, Salunkhe, & Cornforth, 1982). The high phytic acid content of wild yam tubers in the present study may largely be due to these factors. High phytate contents indicate that the consumption of these yam tubers could decrease the bioavailability of minerals, especially Ca and Zn. Phytic acid markedly decreases calcium bioavailability and forms Ca-phytate complexes which inhibit the absorption of Fe and Zn (Sirkka, 1997). Phytic acid intake of 4–9 mg/100 g DM is said to decrease iron absorption by 4–5 fold in humans (Hurrel, Juillert, Reddy, Lynch, Dassenko, & Cook, 1992). Phytates have also been implicated in decreasing protein digestibility by forming complexes and also by interfering with enzymes, such as trypsin and pepsin (Reddy & Pierson, 1994). Phytic acids may affect the digestibility of starch. Phytic acid and starches are structurally capable of combining via phosphate linkages. It can also affect starch digestion by combining with digestive enzymes or bind minerals such as Ca, which are known as catalysts or cofactors of enzyme activity (Sirkka, 1997). Our results indicate that high phytate contents in these yam tubers not only reduce the bioavailability of essential dietary minerals but might also have adverse effects on the digestion of starches and proteins.

3.3. Cyanogens

The results of cyanogen determination are presented in Table 2. The cyanogen contents of wild yam tubers ranged from 3.2 to 6.0 mg HCN equivalents per kg fresh weight of tubers. The results showed that *D. versicolor* had the highest content of cyanogens (6.0 mg HCN/kg FW) and all other species had almost half of this value, ranging from 3.2 to 3.3 mg HCN/kg FW. The cyanogen contents of studied yam tubers were found to be notably lower than reported cyanogen levels for wild cassava (Nassar & Fichtner, 1978) and reported levels for various food sources (Rezaul & Bradbury, 2002). The results indicated that the cyanogens levels found in these yam tubers studied were satisfactorily below the safety level for cyanide poisoning. The lethal dose range for humans, of HCN taken by mouth, is estimated to be only 0.5 to 3.5 mg/kg body weight (Bradbury, 1991). However, the presence of this smaller amount of cyanogen may have some long-term adverse effects on human health. The evidence is now strong that cyanogen ingestion can give rise to chronic neurological disease in humans (Montgomery, 1980).

3.4. Trypsin inhibitor activity (TIA) and α-amylase inhibitor activity (AIA)

The results of trypsin inhibitor activity (TIA) and α-amylase inhibitor activity (AIA) are presented in Table 3. TIA contents ranged from 4.1 to 20.9 mg pure trypsin inhibited per g dry matter. The TIA content was found to be lowest (4.1 mg pure trypsin inhibited/g DM) for *D. deltoidea* and highest (20.9 mg pure trypsin inhibited/g DM) for *D. triphylla*. The large variation in TIA of yam tubers may be due to species differences as well as other environmental factors. The handling conditions, degree of maturity and physiological conditions are very important for the production of these antinutritional factors (Sotelo, Contreras, Sousa, & Hernandez, 1998). The values obtained for TIA were lower than the reported TIA content for wild potato tubers (Sotelo et al., 1998), and soybean (Smith et al., 1980). The presence of protease inhibitors in the diet leads to the formation of an irreversible trypsin enzyme-trypsin inhibitor complex, causing a trypsin drop in the intestine and decrease in the diet protein digestibility, leading to slower animal growth (Siddhuraju & Becker, 2001). However, the heat-labile nature of these inhibitors suggests that they can be inactivated during cooking (Prathibha et al., 1995). This indicates that these trypsin inhibitors may not interfere with digestion, if tubers are properly cooked before consumption. The results of the present investigation show that inhibitors of α-amylase were present in varying amounts, ranging from 78 to 147 IU/g DM (Table 3). Among the yam tubers studied, *D. versicolor* and *D. triphylla* have highest (147 IU/g DM) and lowest (78 IU/g DM) α-amylase inhibitory activities, respectively. It is difficult to compare the enzyme inhibitory activities of yam tubers as reported

Table 3
Trypsin inhibitor activity (mg pure trypsin inhibited/g DM) and α-amylase inhibitor activity (inhibitory units/g DM) of wild yam tubers[a]

Yam species	Trypsin inhibitor activity	α-amylase inhibitor activity
Dioscorea bulbifera	4.3±0.2	139±2
Dioscorea versicolor	6.9±0.5	147±0.2
Dioscorea deltoidea	4.1±0.2	81±3
Dioscorea triphylla	20.9±1.0	78±6

[a] Values are means ±SD (for Trypsin inhibitor, $n=6$, and for α-amylase inhibitor, $n=4$).

by different investigators, primarily because of the differences in method used. Prathibha et al. (1995) reported that the amylase inhibitor in *Dioscorea* tuber was heat-stable and that the amylase inhibitor did not show any reduction in activity even at 100 °C. This implies that the presence of α-amylase inhibitors in these tubers could interfere with digestion.

3.5. Interrelationship among Ca, Zn, oxalate (Ox), and phytate (Phy) in wild yam tubers

The molar ratios for oxalate, calcium, zinc and phytate were calculated to evaluate the effects of elevated levels of oxalate and phytate in the bioavailability of dietary minerals (Table 4). The calculated values are also compared with the reported critical values for these ratios, which are shown in Fig. 1.

The importance of oxalate contents of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio of Ox:Ca is greater than one. Under this circumstance, the oxalate has potential to complex, not only the Ca contained in the plant, but also that derived from other food sources (Davis, 1979). Yam tubers showed that the Ox:Ca ratio ranged from 1.1 to 2.2. A high ratio of Ox:Ca in the diet may cause chronic calcium deficiency (Kelsay, 1985). According to this investigation, all yam tubers had Ox:Ca values higher than the reported critical value (1.0), which implies that a high level of oxalate could have adverse effects on bioavailability of dietary calcium in these tubers.

The importance of foodstuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Zinc deficiency has been shown to be the cause of dwarfism and hypogonadism among adolescents from the lowest classes of Egypt (Prasad, 1984). Phytate may reduce the bioavailabity of dietary zinc by

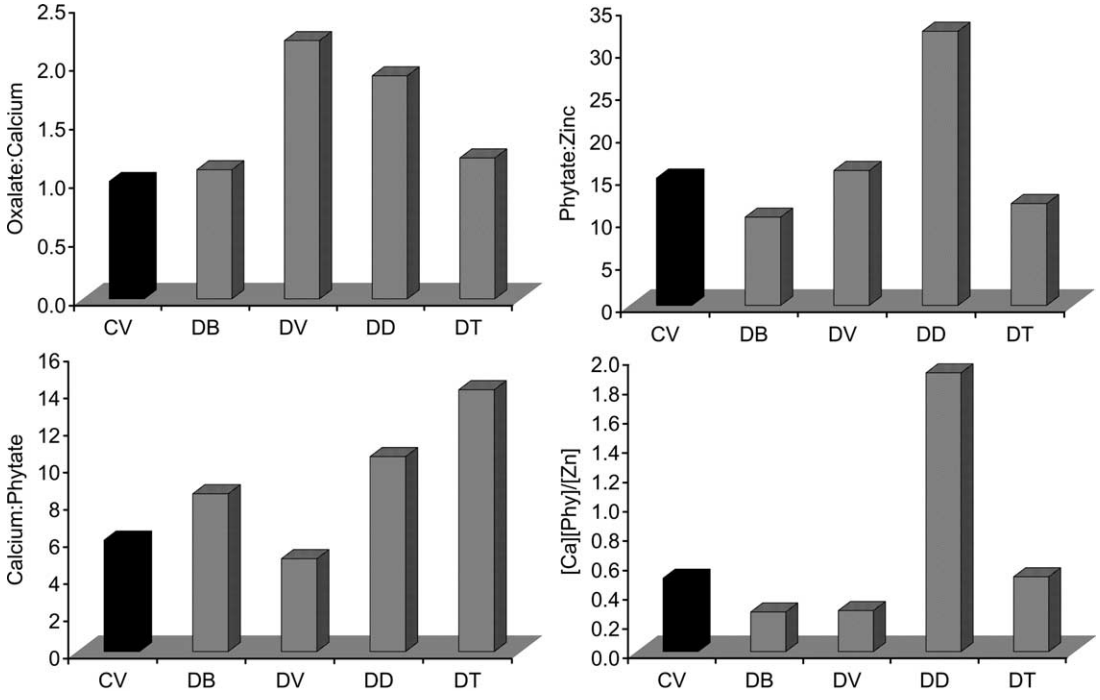

Fig. 1. Comparison of oxalate:calcium, phytate:zinc, calcium:phytate and [Ca][Phy]/[Zn] molar ratios with the recommended critical values for these ratios; critical value (CV), *D. bulbifera* (DB), *D. versicolor* (DV), *D. deltoidea* (DD) and *D. triphylla* (DT).

Table 4
Concentrations of Zn, Ca (mg/100 g DM)[a] and calculated Ox:Ca, Phy:Zn, Ca:Phy and [Ca][Phy]/[Zn] molar ratios (mol/kg) of yam tubers

Yam species	Zn	Ca	Ox:Ca	Phy:Zn	Ca:Phy	[Ca][Phy]/[Zn]
Dioscorea bulbifera	1.76	96.4	1.1	10.4	8.5	0.27
Dioscorea versicolor	1.50	72.3	2.2	15.9	5.0	0.28
Dioscorea deltoidea	1.13	238	1.9	32.3	10.6	1.90
Dioscorea triphylla	1.69	173	1.2	12.2	14.1	0.51

[a] Values are taken from Bhandari et al. (2003).

forming insoluble mineral chelates at a physiological pH (Oberleas, 1983). Zinc has been described as the essential mineral most adversely affected by phytate and the phytate-to-zinc molar ratio has been proposed as an indicator of zinc bioavailability (Sirkka, 1997). In human studies, Phy:Zn molar ratios of 15:1 have been associated with reduced zinc bioavailability (Turnlund, King, Keyes, Gong, & Michel, 1984). In our study, the Phy:Zn ratios of *D. versicolor* and *D. deltoidea* were 15.9 and 32.3, respectively. These values were comparable to reported values for Nigerian yam species (Adeyeye et al., 2000), but the obtained figures were higher than the critical molar ratios of Phy:Zn, which indicates low zinc availability in these yam species. *D. bulbifera* and *D. triphylla* have low Phy:Zn ratio values, indicating that zinc is probably more available in these species.

Phytic acids markedly decrease Ca bioavailability and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Ca:Phy is reported to be 6:1 (Oladimeji et al., 2000). The molar ratios of Ca:Phy obtained in *D. bulbifera*, *D. deltoidea* and *D. triphylla* were 8.5, 10.6, and 14.1, respectively. These values were higher than reported values for Nigerian yam species (Adeyeye et al., 2000) and were also higher than the reported critical molar ratio of Ca:Phy, indicating that absorption of calcium may be adversely affected by phytate in these yam tubers.

The values of [Ca][Phy]/[Zn], i.e. (Ca×Phy:Zn) are given in Table 4. The values of the molar ratios, [Ca][Phy]/[Zn], ranged from 0.27 to 1.9. Davies and Warrington (1986) and Ellis, Kelsay, Reynolds, Morris, Moser, and Frazier (1987) indicated that the ratio [Ca][Phy]/[Zn] is a better predictor of zinc availability and, if the values were greater than 0.5 mol/kg, there would be interference with the availability of zinc. In our study, the values for the molar ratios of [Ca][Phy]/[Zn] were found less than, or equal to, 0.5 mol/kg in *D. bulbifera, D. versicolor and D. triphylla*, whereas *D. deltoidea* showed a higher value for this ratio. That is to say, using this indicator, Zn availability could be negatively affected in *D. deltoidea*.

Ekpedeme, Bassey, and Ekaete (2000) reported that high levels of anti-nutrients, such as oxalate, phytic acid and HCN, are known to be very poisonous to humans. Our study revealed that the high level of oxalates could be partly responsible for the acrid taste and for causing the inflammation observed in Nepalese wild yam tubers. It appears that these tubers are unsuitable for consumption by those suffering from oxalate kidney stones. The results also indicate that these yam tubers have substantial amounts of oxalates and phytates, which could have adverse effects on the bioavailabiltiy of essential dietary minerals, especially Ca and Zn. In order to reduce the effects of antinutrients, which may have health-hazard potential, proper processing before consumption is recommended.

Acknowledgements

The authors thank Prof. T. KASAI of Fuji Women's University, Sapporo, Japan and Asst. Prof. K. SONAYAMA of Laboratory of Food Biochemistry, Hokkaido University, Japan for enthusiastic comments and discussion. The authors also wish to thank local people of Nepal for their invaluable assistance with sample collection.

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