Oxalic Acid and Calcium Determination in Wild Edible Plants

José Luis Guil,*,† María Esperanza Torija,† Juan José Giménez,‡ Ignacio Rodríguez-García,‡ and Antonio Giménez§

Departamento de Nutrición y Bromatología II, Facultad de Farmacia de la Universidad Complutense de Madrid, 28040 Madrid, Spain, and Departamento de Química Orgánica and Ingeniería Química, Facultad de Ciencias Experimentales, Universidad de Almería, 04120 Almería, Spain

The contents of oxalic acid and calcium in several wild edible plants were analyzed. Oxalic acid was determined by an espectrophotometric method based on the decrease of absorbance at 335 nm due to the dissociation of the zirconium(IV)−chloranilate complex, consecutive to the addition of oxalic acid. Calcium was determined by atomic absorption spectroscopy. The results show the absence of this acid in two of the studied plants: hoary cress (*Cardaria draba* (L.) Desv.) and vervain (*Verbena officinalis* L.). Also noticeable is the high content of oxalic acid in goosefoot (*Chenopodium album* L.), with a range of values from 360 to 2000 mg/100 g of fresh weight, and the high ratio (oxalic acid/calcium) in curly dock (*Rumex crispus* L.), with a mean value of 32.

Keywords: *Oxalic acid; calcium; antinutritive substance; wild edible plants*

INTRODUCTION

Oxalic acid (HOOC−COOH) is a white crystalline solid, approximately 10% soluble in water at 20 °C. It is a strong acid (pK_1 = 1.46; pK_2 = 4.40) which forms water-soluble Na^+ and K^+ salts but less soluble salts with alkaline earth and other bivalent metals. Calcium oxalate is particularly insoluble at neutral or alkaline pH but readily dissolves in acid medium.

The toxicity threshold of oxalic acid is quite low; minimal lethal dose for humans is considered to be ca. 5 g for an adult. Therefore the safety margin is low. Thus, a high consumption of rhubarb can represent one-tenth of the minimal lethal dose. From a strictly nutritional point of view, the main problem which arises is the bioavailability of calcium in the alimentary ration. This bioavailability is determined by the ratio (oxalic acid g kg^{-1})/(calcium g kg^{-1}). Some nourishments have high ratios: rhubarb (25.0), spinach (10.0), potato (5.0), cocoa (6.7), and tea (2.6) (Derache, 1990). Nourishments with ratios higher than 2.5 are poor calcium sources and also can be considered as decalcifiers (Belitz and Grosh, 1988; Concon, 1988; Gontzea and Sutzescu, 1968; Derache, 1990; Linder, 1978). As the human consumption of wild edible plants is an alimentary option increasingly widespread and since the available guides on the subject do not offer information about nutritional data, we decided to study the contents of oxalic acid and calcium in the most representative vegetables which are collected for that purpose and for livestock consumption.

The oxalic acid content in some of these plants appears sometimes in the literature. Thus, the presence of crystals of oxalic acid in amaranth (*Amaranthus viridis* L.) has been reported (Bharadwaj, 1988). Several articles show a high content of this antinutritive substance in goosefoot (*Chenopodium album* L.), with figures of ca. 1 g/100 g and higher, referred to fresh weight (Gontzea and Sutzescu, 1968; Prakask and Pal, 1991; Singh and Saxena, 1972; Singh, 1973; Tabekhia, 1980). One of these plants, curly dock (*Rumex crispus* L.), caused fatal poisoning due to ingestion of the plant material. Among the pathological findings were centrolobular hepatic necrosis and birefringent crystals in the liver and kidneys (Panciera et al., 1990; Reig et al., 1991).

The main target of our research was to study the concentrations of oxalic acid and calcium in wild edible plants from the southeast of Spain, in order to know the viability of its consumption, in connection to the mentioned poisoning effects to which this antinutritive substance could give rise.

EXPERIMENTAL PROCEDURES

Samples. Ten species of wild edible plants were harvested (Table 1). Only edible parts by humans (tender leaves) of the plants were used. The harvested plants were washed before performing the analysis, dried (T < 50 °C), and stored in a dessicator when required to room temperature. All the plants were collected from five different places for oxalic acid and calcium determination, in several physiological edible states, and analyzed separately. Size area for gathering purposes in all the places was very reduced, with a maximum of 1 ha. All the soils were in a very modified state by human cultivation, except in the case of *Crithmum maritimum* L., gathered from beaches and cliffs. Nevertheless, the soils of these places were also very modified by human activities.

Oxalic Acid Determination. Oxalic acid extraction was achieved by heating the samples (1−5 g of the plant) in distilled water with HCl and *n*-octanol. It was then filtered, cooled to room temperature, and diluted to a known volume, 250 mL (AOAC, 1990). The concentration of the acid was determined using 0.1−0.5 mL of this solution.

Oxalic acid concentrations were measured spectrophotometrically by dissociation of the complex zirconium(IV)−chloranilate (Dona and Verchère, 1991). Stock solutions of known concentrations of chloranilic acid (Fluka, puriss), zirconyl chloride octahydrate (Fluka, puriss), and oxalic acid were prepared by exact weighing and dilution in purified (Millipore) water. The initial absorbance of a solution of the zirconium(IV)−chloranilate complex was 0.89, for a wavelength of 335 nm.

The addition of oxalic acid gives rise to a decrease in the absorbance of the complex. The calibration curve is lineal for additions of oxalic acid up to 0.1 mg (0.1 mL of a solution of c = 1 g L^{-1}) to 50 mL of solution of complex. The correlation coefficient is r = 0.999. The concentration of oxalic acid was determined by the decrease in the absorbance of the complex

† Departamento de Nutrición y Bromatología II.
‡ Departamento de Química Orgánica.
§ Departamento de Ingeniería Química.

S0021-8561(95)00472-9 CCC: $12.00

Table 1. Moisture, Oxalic Acid, and Calcium Contents in 100 g of Wild Edible Plant Material and Oxalic Acid/Calcium Ratio

species	moisture (g)		oxalic acid (mg)		calcium (mg)		oxalic acid/calcium	
English name/Spanish name	mean and range	σ_{n-1}	mean and range	σ_{n-1}	mean and range	σ_{n-1}	mean and range	σ_{n-1}
Amaranthus viridis L.	80.11	2.87	960	220	150	29	7.1	3.3
amaranth/bledo	75.81−83.58		810−1353		106−174		5.0−12.8	
Cardaria draba (L.) Desv.	85.58	1.65	traces		170	44		
hoary cress/antoñico	83.72−87.92				128−220			
Chenopodium album L.	71.09	0.40	1100	604	310	54	3.3	3.6
goosefoot/cenizo	70.62−71.65		361−2027		236−371		3.1−5.5	
Crithmum maritimum L.	86.79	1.83	53	25	97	11	0.6	0.3
rock samphire/hinojo de mar	84.08−88.78		16−77		86−111		0.2−0.9	
Plantago major L.	87.69	1.14	67	40	108	19	1.1	1.0
plantain/llantén	86.44−89.47		34−136		86−134		0.4−2.7	
Rumex crispus L.	89.68	2.88	620	80	21	6	32.0	5.7
curly dock/alaveza crespa	86.35−93.12		517−697		15−29		24.0−40.0	
Sisymbrium irio L.	88.71	0.86	140	93	130	10	1.1	0.7
hedge mustard/matacandil	87.94−90.02		60−292		114−142		0.5−2.4	
Sonchus tenerrimus L.	87.73	1.88	64	20	90	16	0.8	0.3
sow-tistle-of-the-wall/cerraja	85.08−90.00		41−86		74−112		0.4−1.2	
Stellaria media Villars.	93.26	0.91	367	55	65	27	6.3	2.2
chickweed/pamplina	91.91−94.02		295−433		40−111		3.7−8.7	
Verbena officinalis L.	77.24	3.26	traces		300	58		
vervain/verbena	72.98−81.64				241−361			

when no more than 0.5 mL of sample solution was added to 50 mL of the complex.

A Milton Roy Spectronic 3000 ARRAY spectrophotometer with UV lamps and quartz cells was used. pH values were measured with a Crison 20002 pH meter. As it is new when applied to vegetables, recovery experiments were performed, in order to know to which extent the oxalic acid present in the samples was being measured.

Calcium Determination. Calcium was determined in ashes from incineration of leaves at $T \leq 450$ °C. The ashes were dissolved in mixtures of HNO_3 and HCl and diluted in water. Calcium concentration was determined with an atomic absorption spectrophotometer (Perkin-Elmer 2280). In order to avoid phosphorus interferences, lanthanum salt was added (Torija Isasa, 1981).

All samples were digested and analyzed at least in triplicate. Mean values are shown in the table.

RESULTS AND DISCUSSION

Recoveries. Recoveries were performed by addition of known quantities of solid oxalic acid to the plant material at the beginning of the process. The method for the determination of oxalic acid is very accessible and efficient. Fresh and dried plants were analyzed for comparison purposes. Recoveries were performed for all the plants studied, with the result that the method is more efficient for fresh plants (mean recoveries $X = 101\%$; variation coefficent 3.4%) than for dried plants ($X = 109\%$; variation coefficient 7.1%), possibly because some substances originating in the drying process could interfere with analysis. Therefore, analyses were performed with freshly harvested plants.

Oxalic Acid Content. Table 1 shows the concentrations of oxalic acid and calcium present in the studied species. The large standard deviations are inherent with diversity of plant material, due to the fact that the differences in the cultivated soil conditions have a large influence in the oxalic acid and calcium contents. A range of values for oxalic acid from absence to 1.1 g of oxalic acid/100 g of plant material was found.

It was not found in hoary cress and vervain and was scarce in rock samphire (53 mg/100 g), sow-tistle-of-the-wall (64 mg/100 g), and plantain (67 mg/100 g). At the top of the range are goosefoot (1100 mg/100 g), amaranth (960 mg/100 g), and curly dock (620 mg/100 g). Values for goosefoot are in agreement with those found in the literature.

Calcium Content. The calcium content found in the plants of our study is similar to that of the vegetables of common consumption (Table 1). It is high in goosefoot (310 mg/100 g) and vervain (300 mg/100 g). The lowest values are for curly dock (21 mg/100 g) and chickweed (65 mg/100 g).

Oxalic Acid/Calcium Ratio. The values of these ratios are in good accordance with those for usually consumed vegetables (Table 1). In addition to the two plants that showed no content of oxalic acid, it is low in rock samphire (0.6) and sow-tistle-of-the-wall (0.8). On the other hand, it is high in chickweed (6.3), amaranth (7.1), and curly dock (32.0). This latter one could have the highest adverse impact on dietary calcium bioavailability due to the value of the ratio oxalic acid/calcium, although it is not the one with the highest value of oxalic acid. Therefore we do not recommend ingesting the cooking water of the plant and, due to the reports of poisoning with this plant, also do not recommend consuming it. In relation to chickweed and amaranth, it is necessary to advise consuming these vegetables only occasionally because of the large variation in oxalic acid and calcium. For example, the consumption of 400 g of amaranth could surpass the minimal lethal dose for an adult.

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Received for review July 25, 1995. Revised manuscript received February 20, 1996. Accepted April 23, 1996.⊗

JF950472A

⊗ Abstract published in *Advance ACS Abstracts,* June 1, 1996.