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Direct analysis of plant minerals and comparison of extraction processes using ICP-AES

Chin Choon Ong

Department of Chemical Process Technology, Singapore Polytechnic, 500 Dover Road, Singapore 0513, Singapore

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The mineral contents of two plants, *Chrysanthemum indicum* flowers and *Rhoeo spathacea* leaves, were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). This technique allows metallic and non-metallic minerals, like phosphorus and sulphur, to be analysed directly. The calcium concentration was found to be high in both plants, compared to other local vegetables and plants. In *R. spathacea*, the calcium level in the leaves is not affected by the level of calcium in the soil in which they are grown. Four different methods of extracting calcium from three different plants, (*Clitorea ternatea, R. spathacea* and *C. indicum*) were examined, and the best method is by using a pressure cooker followed by direct boiling and possibly, double boiling and crockpot. There is no significant difference between the crockpot and double-boiling system. Using the same method of extraction, the percentage of extraction is dependent upon the type of plant. The high calcium concentration in these plants shows that they can be significant sources of calcium when brewed as herbal drinks for human consumption.

INTRODUCTION

Although minerals make up only 4% of the human body, they are essential, as they control water balance, regulate acid–base balance, provide structure, catalyse vital reactions and serve as constituents of enzymes and hormones (Ensminger *et al.*, 1983). They are classified as either macrominerals or microminerals, according to their levels of occurrence in the body. An important macromineral is calcium, whose intake is very important for women who have had several pregnancies or suffer from osteoporosis (Heaney & Recker, 1986). Furthermore, the possibility of dietary calcium supplementation lowering the blood pressure in hypertensive individuals has resulted in several processed foods being fortified with calcium to enhance its uptake (Grobbee & Hofman, 1986).

In Asian countries, a principal source of minerals comes from native plants that for decades have been commonly used as colourings, food, flavourings and medicine (Wagner *et al.*, 1988). One such plant is *Chrysanthemum indicum*, the dried flowers and whole plant of which are used as a tonic for prevention of colds, influenza and anti-pyretic purposes (Juang, 1988). The Chinese patent medicine name for this plant is *Mistura Wu-Wei-Xiao-Du*. Although it has a bitter and acrid taste, commercially produced sweetened chrysanthemum drinks, in packets as well as bottles, have gained wide acceptance in several countries.

Another important plant is *Rhoeo discolour* Hance or *Rhoeo spathacea*. It is also known by its two common names, oyster plant and 'Moses in the cradle'. Its attractive, two-coloured, flat, pointed leaves, with the upper layer green and purple beneath, made the plant ideal for ornamental purposes. These leaves are believed to possess anti-inflammatory and anti-diarrhoeal properties. When boiled, they give a sweet and bland taste.

A third type is *Clitorea ternatea*,a legume and climbing woody plant, originally from South America, (Polunin, 1987). The blue hue of the flowers are used to dye food, especially rice. The plant is also called by its native name, 'bunga biru', meaning blue flowers, and, in other cases, the pea plant.

Extracts of these three plants are usually obtained by directly boiling them in water, but little is known about the effectiveness of the extraction of minerals by this process. Hence, the initial part of this study was to determine some essential mineral contents of chrysanthemum flowers and oyster-plant leaves using inductively coupled plasma atomic emission spectroscopy (ICP-AES). ICP-AES has several advantages over conventional methods, as it can analyse nonmetallic elements such as sulphur and phosphorus and is free from interference by refractory compounds (Pritchard & Lee, 1984). The next part of the study compared their calcium contents with those found in other local fresh vegetables. Finally, comparisons of the different types of extraction processes used and the effect of their boiling times were made.

MATERIALS AND METHODS

All reagents used were analytical grade and deionized water was used to prepare all solutions.

Preparation of sample

Fresh leaves of *Rhoeo spathacea* and the flowers of *Clitorea ternatea* were collected from different areas of Singapore. Local fresh vegetables were purchased from different markets and the dried flowers of *Chrysanthemum indicum* were purchased from different medical shops. They were carefully washed with deionized water and wiped with a cloth. The samples were then dried in an oven at 40–60°C until constant weight. They were cut into small pieces and passed through a 1-mm sieve to ensure homogeneity. Samples were used as freshly prepared or stored within glass bottles at temperature between 0 and 4°C for less than one day. A similar method was used for the soil sample.

Wet ashing

Exactly 1 g of the sample was placed in a Teflon polyfluoroacetal (PFA) vessel. Concentrated nitric acid (10 ml) and 50% hydrogen peroxide (10 ml) were then added and the vessel capped. The vessel was placed into a digestion turntable microwave for 12 min (De Boer & Maessen, 1983). This microwave instrument consisted of five major components:

(i) a microwave generator which had an output frequency of 2450 ± 13 MHz to give an effective output of 600 W;
(ii) a wave guide, which channels the microwave generated to the microwave cavity;
(iii) a microwave cavity, where samples absorb the microwave;
(iv) a mode stirrer, which reflects and mixes the energy entering the microwave so that heating will be independent of position; and
(v) a turntable, which alternates back and forth 180° so that the vessels are uniformly heated. The minerals in the digested solution, which was diluted to 100 ml, were not significantly effected by the acidic matrix (Yoshimura *et al.*, 1990).

Dry ashing

The filtered plant material was placed in an oven for 3 h at 200–250°C. Subsequently, the dried samples were ashed at 475–500°C for at least 24 h in platinum crucibles. Concentrated nitric acid was then added to dissolve the remaining white ash (Hoenig & De Borger, 1983).

Anion chromatography

A HPLC AG4A-AS4A column was used together with an AMMS-1 suppressor. A pressure of 5451 kPa (790 psi) was maintained with a flow rate of 2·0 ml min^{-1}. The eluent contained 0·0012 M sodium carbonate and 0·0011 M sodium bicarbonate dissolved in 18 MΩ of deionized water. A conductivity detector with a range of 30 μs was used to monitor the anions in the column eluate.

ICP-AES

The test solutions were subjected to wavelength scanning prior to actual quantification of the analytes. A frequency of 27·12 MHz and a power of 1·1 kW were used. For sulphur and phosphorus, a vacuum was applied and argon gas was used to purge the optical path of the ICP-AES to avoid any interference from oxygen in the environment.

Determination of extracted calcium

Since there was a small difference in the calcium concentration between the same species of a plant, the total calcium in each sample was obtained by combining the amount of calcium dissolved in the filtrate with the amount of calcium remaining in the undissolved plant material. The amount of calcium in the undissolved plant material was determined by dry ashing followed by dissolution using nitric acid and then ICP-AES.

$$\text{Calcium extracted (\%)} = \frac{\text{Amount of calcium in filtrate}}{\text{Total amount of calcium}} \times 100$$

Extraction processes

Four different processes commonly used in domestic cooking were studied. In all cases, 10 g of uncut sample and 150 ml of water were used.

(i) *Crockpot*. Boiling water was added to the sample. At the first indication of boiling, the sample was added and allowed to boil for 30, 60 and 90 min.

(ii) *Double boiler*. The double boiler consisted of two pots. The external pot contained boiling water and was in direct contact with the flame. The internal pot also contained water but was placed inside the external pot. When the water started to boil in the internal pot, the sample was added and the time recorded.

(ii) *Direct boiling*. The sample was added to boiling water in a glass beaker and the boiling time specified.

(iv) *High pressure*. The sample and water were transferred into a 250-ml bottle, capped and placed into a pressure cooker. When steam escaped from the small outlet of the pressure cooker, the pressure load was added and the timing started. This system is equivalent to autoclaving the sample at 103·5 kPa (15 psi) at 121°C.

RESULTS AND DISCUSSION

The essential minerals of oyster-plant leaves and chrysanthemum flowers, as determined by ICP-AES, are shown in Table 1. In both cases, there were significant amounts of calcium, while the magnesium content was comparable to common leafy vegetables like artichoke, asparagus, chicory and lettuce (Willis *et al.*, 1986). The chrysanthemum flowers had higher phosphorus and sulphur contents than the oyster-plant leaves. This sulphur is likely to exist mainly as the sulphate ion, because analysis by anion chromatography of the water boiled with chrysanthemum flowers gave the sulphate-ion concentration as 440 mg per 100 g. By using a conversion factor of 0·3333 to calculate the sulphur in sulphate ion, the amount is 146·6 mg per 100 g, which is very close to that found by ICP-AES on the whole chrysanthemum flowers. This direct analysis of non-metallic minerals by ICP-AES is advantageous, as it does not require further preparations and other reagents, which may lead to contamination.

The ratio of calcium to phosphorus influences the dynamic equilibrium state of bone minerals. Generally, a calcium to phosphorus ratio of 2 : 1 is desirable for dietary calcium absorption (Food and Nutrition Board, 1980). The calcium to phosphorus ratio of chrysanthemum flowers is favoured at 1·5 : 1; therefore, it is a more suitable source of mineral drink than the oyster plant leaves, although the latter has a higher calcium concentration.

Although the calcium levels in the soil fluctuated between 2502 and 88 mg per 100 g, the calcium contents in the leaves of the oyster plant grown in these soils had a consistent value (see Table 2). No statistical correlation between the calcium levels in soil and oyster plant could be found. This absence of any relationship between the metal ions found in plants and the soil is also true for several other plants (Suzuki & Iwao, 1982). Although calcium-poor soil may retard the growth rate of oyster plants, they can still grow in this media and serve as control plants for soil erosion. The plant concentrates calcium from the soil and there is little variation in their quantities. This ability to concentrate metal ions from the environment is also common in other plants (Zurera-Cosano, 1987). Therefore, it can be a good source of calcium for feeding grazing animals like the cow, provided that the oxalate concentration is not too high to affect its bioavailability (Poneros-Schneier & Erdman, 1989).

By comparison, Table 1 also shows that the calcium concentrations in the oyster-plant leaves and chrysanthemum flowers are high relative to those found in some common local vegetables and plants. All these vegetables are cooked and eaten whole, as they provide a source of calcium to the Asian population (Herklots, 1972). By boiling these two plants in water, it is possible to obtain an alternative source of calcium as a drink, like milk. This drink will be suitable to individuals who are lactose intolerant, cholesterol conscious or dieting, which may result in submarginal intake of calcium (Hourigan & Mittal, 1984). The calcium content of broccoli reported in this study is comparable to that found in other countries (Wills *et al.*, 1984). The pea-plant flowers, unlike the chrysanthemum flowers, provide little calcium. Therefore, their primary function when added to food by the local inhabitants is as colourings (Polunin, 1987).

Table 1. Mineral analysis (mg per 100 g) using ICP-AES[a]

Plant	Al	Ca	Fe	Mg	P	S
Chrysanthemum	11·8±0·8	230±12	12·8±0·8	15·7±0·2	158±4	146±3
Oyster plant	ND[b]	332±15	ND	15·8±0·6	18·3±0·4	18·8±0·3
Pea plant (*Clitorea ternatea*)		1·9±0·1				
Broccoli (*Brassica oleracea*)		34·4±1				
Ts'oi Sum (*Brassica chinensis*)		96±1				
Lettuce (*Lactuca sativa*)		76·6±1				

[a] Average of at least four determinations.
[b] ND—Not detected.

Table 2. Calcium content (mg per 100 g) in the leaves of the oyster plant and in the corresponding dried soil in which it was grown

Ca in the plant	Ca in the soil
314±10	88±4
319±12	2 502±20
304±13	224±9

Table 3 shows the percentage of calcium extracted when the components of the three different plants are boiled in water. Their values ranged from 22·9 to 55·1 for chrysanthemum flowers, 11·5 to 21·8 for pea-plant flowers and 12·9 to 59·1 for oyster-plant leaves. The overall percentage of calcium extracted was dependent upon the type of boiling process. The best method was by pressure cooker, and a short period of less than 30 min is sufficient. This percentage of extraction is similar to that for direct boiling for 90 min and suggests that a long period of direct boiling is equivalent to a shorter period in the pressure cooker. The reduction in boiling time to produce the same effect is comparable to that reported in the cooking of cowpea (Uzogara *et al.*, 1988). Boiling in a crockpot or a double-boiling system gave lower extraction percentages. By comparison, there was no significant difference between their extraction efficiencies when the same species of sample is used. Therefore, it can be concluded that these two methods are the same. Another important factor that affects the extraction efficiency is the type of sample used. Among the flowers, the calcium in chrysanthemum is more easily extracted than the pea plant. Hence, the type of plant selected for boiling is critical and separate experiments must be conducted on each individual plant to determine their absolute extraction efficiencies.

CONCLUSION

ICP-AES is a fast method to directly analyse metallic and non-metallic ions in oyster-plant leaves and chrysanthemum flowers. For both plants, the calcium concentrations were high relative to other local vegetables. No particular relationship was found between the calcium content in the oyster-plant leaves and the metal ions in the soil in which the sampled plant was grown. The best way to maximize calcium extraction from these plants for mineral drinks is by boiling with a pressure cooker for a short period. The effect is the same as direct boiling for a long period of time. The percentage extracted is affected by the type of plant used. Crockpot and the double-boiling system have lower extraction percentages and gave the same results.

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Table 3. Extraction processes of calcium in the oyster-plant leaves, pea-plant flowers and chrysanthemum flowers[a]

Plant	Process	Percentage extracted		
		30 min	60 min	90 min
Chrysanthemum	Crockpot	22·9±2·5	27·6±2·7	30·3±3·2
	Double boiling	23·1±2·7	28·6±2·9	30·2±3·2
	Direct boiling	41·0±4·1	46·9±4·2	48·8±4·1
	Pressure cooker	49·8±3·8	53·7±3·8	55·1±4·3
Pea plant	Crockpot	11·5±1·2	13·6±1·3	14·6±1·7
	Double boiling	12·6±2·3	13·4±1·8	14·4±1·4
	Direct boiling	13·9±1·5	18·4±1·9	20·6±2·0
	Pressure cooker	20·7±2·1	21·3±2·3	21·8±2·0
Oyster plant	Crockpot	12·9±1·3	17·9±1·9	18·8±2·0
	Double boiling	14·8±1·4	19·0±1·8	20·1±2·2
	Direct boiling	40·4±3·8	47·1±4·6	49·9±5·0
	Pressure cooker	55·9±5·7	57·1±6·0	59·1±6·0

[a] Average of three determinations.

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