Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 383–388
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Determination of oxalates in Japanese taro corms using an *in vitro* digestion assay

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Received 25 August 2006; received in revised form 14 December 2006; accepted 16 December 2006

Abstract

An *in vitro* assay was used to extract gastric soluble and intestinal soluble oxalates from the corms of four different Japanese taro (*Colocasia esculenta* (L.) var. Schott) cultivars (Akame, Ishikawa-wase, Yamato-wase and an unnamed cultivar). The oxalate contents were measured in the raw tissue and after boiling and baking the corms.

Akame contained the highest levels of gastric soluble oxalates in the raw tissue (168.9 ± 12.0 mg/100 g fresh weight (FW)) and was significantly higher ($P < 0.001$) than the other three cultivars (mean 86.5 ± 8.1 mg/100 g FW). There was no significant difference in gastric soluble oxalates between Ishikawa-wase, Yamato-wase and the unnamed cultivar. Raw Akame corms contained the highest levels of soluble oxalate after intestinal incubation 134.8 ± 9.8 mg/100 g FW. This was significantly different ($P < 0.001$) from the other three cultivars, which contained a mean of 66.4 mg intestinal soluble oxalates/100 g FW.

Boiling the Japanese taro corms for 40 min reduced the level of intestinal soluble oxalates in the cooked tissue to a low level (mean for all the cultivars, 17.7 mg/100 g FW) as the soluble oxalates were leached into the cooking water. In addition, the mean moisture content of the boiled corms increased to 83.2% compared to the mean moisture content of raw tissue (75.7%).

Baking the four cultivars at 180 °C for 40 min led to a significant reduction in the moisture content of the tissue (from a mean of 75.7% moisture content in the fresh tissue to a mean of 50.7% when baked) and an effective concentration of gastric soluble oxalates in the cooked tissue (overall mean 276.1 mg/100 g FW) while the mean intestinal soluble oxalates rose to 187.2 mg/100 g FW. The results confirm that baked taro corms contain moderate amounts of soluble oxalates.

The total gastric soluble oxalates of the four different taro corms were similar to the total oxalate contents determined using hot acid method, however the *in vitro* method used to extract intestinal soluble oxalate appeared to extract more oxalates when compared to the hot water extract method.

Baked taro corms should be avoided by people who have an increased risk of renal calcium oxalate stone formation because they contain high levels of intestinal soluble oxalates.

Keywords: Taro corms *in vitro* assay; Oxalates; Boiling; Baking

1. Introduction

Oxalic acid is a common and wide spread constituent of plants, being found in almost all plant families usually at low levels. It occurs as the free acid, as soluble salts of potassium and sodium and as insoluble salts of calcium, magnesium and iron (Noonan & Savage, 1999). High oxalate concentrations in the leaves and corms of plants consumed daily are of concern because of the harmful health effects associated with the intake of high amounts of oxalates. A number of foods such as spinach, rhubarb, beets, nuts, chocolate, wheat bran and strawberries are known to contain high oxalate levels (Noonan & Savage, 1999; Savage, 2002; Savage, Vanhanen, Mason, & Ross, 2000). The oxalate contents of some tropical foods have been investigated by Holloway, Argall, Jealous, Lee, and

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0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.12.023

Bradbury (1989) who reported on the oxalate content of the tubers of four different cultivars of taro grown in Fiji. In their study the total oxalates ranged from 65 mg/100 g fresh weight (FW) for taro (*Colocasia esculenta*) to 319 mg/100 g FW for giant swamp taro (*Cyrtosperma merkussii*). The oxalate levels of more unusual foods such as oca (Albihn & Savage, 2001; Dubois, Savage, Deo, & Martin, 2007; Ross, Savage, Martin & Vanhanen, 1999), taro corms imported into New Zealand (Busch, Vanhanen, & Savage, 2003) and taro leaves grown in New Zealand (Savage & Dubois, 2006) have also been investigated over recent years.

Soluble (oxalic acid and soluble salts) and insoluble oxalates (predominantly the calcium salt) can be extracted from foods, using hot water to extract soluble oxalates and dilute acids to extract total oxalates which include the insoluble oxalate fraction (Hodgkinson, 1977; Holloway et al., 1989). Wet chemistry techniques to determine oxalates have been used by Zarembski and Hodgkinson (1962) and Hodgkinson (1977). High performance liquid chromatography (HPLC) (Holloway et al., 1989; Savage et al., 2000) and enzymic methods (Sigma oxalate kit), GLC (Ohkawa, 1985) or capillary electrophoresis (Chai & Liebman, 2005) can be used to quantify the extracted oxalates. In some papers only total oxalates are reported while in most recent papers water soluble and acid soluble oxalates are reported.

It is commonly assumed that soluble oxalates are absorbed in the small intestine while the insoluble oxalate fraction is not available for absorption. Holmes, Goodman, and Assimos (1995) showed that only a fraction of the total oxalates ingested were absorbed in normal individuals. Whether this is all of the soluble oxalates presented to the intestine is unknown. The remainder of the soluble oxalates is likely to be broken down by oxalate degrading bacteria in the colon (Stewart, Duncan, & Cave, 2004) or is passed out in the faeces. pH changes along the gastrointestinal tract may have a large effect on the absorption of dietary oxalate.

It has been suggested that the proximal small intestine is the major site of oxalate absorption (Daugherty & Mrsny, 1999; Hanes, Weaver, Heaney, & Wastney, 1999a; Holmes et al., 1995) as many studies have shown that a peak in oxalate absorption occurs after 1–6 h following ingestion of oxalate containing food in normal subjects (Brinkley, MgGuire, Gregory, & Pac, 1981; Barilla, Notz, Kennedy, & Pak, 1978; Liebman & Chai, 1997; Marangella, Fruttero, Bruno, & Linari, 1982). However, Hanes, Weaver, and Wastney (1999b) suggest that the ileum is the main site of absorption of soluble oxalates.

The proportion of ingested oxalates absorbed in the intestine was reported to range from 5 and 15% depending on the co-ingestion of calcium (calcium oxalate is insoluble), magnesium and fibre (Holmes et al., 1995) while von Unruh, Voss, Sauerbruch, and Hess (2003) reported that the oxalate absorption in the gastrointestinal tract ranged between 2.2 and 18.5%.

The effect of the acid conditions in the stomach should not be overlooked. The stomach pH ranges from 1.5 to 2 is likely to solublize the insoluble oxalate in plant foods. The stomach digesta, containing all the solublised oxalates, then passes into the alkaline environment of the small intestine. At this point some of the oxalates solubilised in the stomach will reform insoluble complexes with calcium, magnesium and iron, which are also present in the intestinal contents. Calcium oxalate is not soluble at the mean pH 6.5 of the small intestine but the intestinal soluble oxalate will be available for absorption through the mucosa.

A more reliable indication of the amount of soluble oxalates that will be available for absorption in the small intestine could be determined by using an *in vitro* digestion method that attempts to follow the natural digestion process by taking account of the changing pH occurring in the gastrointestinal tract. This will give a clearer picture of the amount of oxalates available for absorption from foods passing down the gastrointestinal tract.

Recently, a number of *in vitro* assays have been proposed (Beer, Wood, Weisz, & Fillion, 1997; Chidambaram, Reddy, Thompson, & Bates, 1989; Glahn, Wien, Van Campen, & Miller, 1996; Glahn, Lee, Yeung, Goldman, & Miller, 1998; Glahn, Lee, & Miller, 1999; Kennefick & Cashman, 2000) but the protocol outlined by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005) appears to follow the human digestive process most accurately and this method has been modified by Catherwood (2005) to give a measure of oxalates solubilized in the stomach and the small intestine.

The *in vitro* digestion method proposed by Versantvoort et al. (2005) is based around the transit times, temperatures, constituents, concentrations and pH of normal physiological values reported in the literature. It is a static gastrointestinal model based around the addition of four solutions, saliva, gastric and duodenal juices and bile solution to the test food incubated at 37 °C.

The objective of this study was to use an *in vitro* method to determine the gastric and intestinal soluble oxalate content of raw and cooked taro corms and to compare these values with the soluble and insoluble oxalate contents determined on the same samples and reported in an earlier paper by Catherwood, Savage, Mason, and Scheffer (2007). The effect of cooking on the oxalate content of the tissue needs to be investigated so that reliable advice can be given to all sectors of the population about any possible adverse effects of consuming this food.

2. Materials and methods

2.1. Sample collection and cooking

Four lateral corm producing cultivars of Japanese taro (*Colocasia esculenta* (L) Schott), Akame, Ishikawa-wase, Yamato-wase and "Unnamed" were grown on Patumahoe clay loam in a randomised trial with four replications at Crop & Food's Research Centre at Pukekohe in the

Auckland region of New Zealand. Dormant corms were planted in October 2003 and the crop was harvested between late September and October 2004. After a preliminary two-week drying period, the corms were stored in open trays in ambient conditions in a closed shed until early November 2004 when a random sample of about 10 corms was taken from each of the 16 plots for oxalate analysis. Analysis of the taro was carried out on both raw and cooked corms. Prior to cooking approximately 5 mm of the outer skin was removed using a knife and the remaining inner flesh was cut into 20 mm^3 cubes. These cubes were either boiled in water for 40 min or baked at 180 °C for 40 min.

2.2. Dry matter

Dry matter of all samples was determined in duplicate by drying them to constant weight in an oven at 105 °C for 24 h (AOAC (2002) method 925.10).

2.3. Determination of gastric and intestinal soluble oxalates

2.3.1. Preparation of the digestion solutions

All chemicals for the digestive fluids were obtained from BDH (BDH Chemicals Ltd., Pool, UK), except for glucuronic acid, lipase, mucin and bile (Sigma–Aldrich Co. St Louis, USA). One litre of the following solutions was prepared fresh each day by dissolving the following chemicals and reagents in nanopure H_2O (Barnstead nanopure II). Each solution was warmed to 37 °C before use.

Saliva solution (pH 6.5 ± 0.2), KCl 8.96 mg, KSCN 200 mg, NaH_2PO_4 888 mg, Na H_2PO_4 570 mg, NaCl 298 mg, NaOH 72 mg, urea 200 mg, α-amylase 145 mg, uric acid 15 mg, mucin 5 mg. Gastric juice (pH 1.07 ± 0.07), NaCl 2.75 g, NaH_2PO_4 266.4 mg, KCl 824.2 mg, $CaCl_2 \cdot 2H_2O$ 399.6 mg, conc. HCl 99.6 μl, NH_4Cl 550.8 mg, glucose 650 mg, glucuronic acid 20 mg, urea 7.4 mg, glucosamine hydrochloride 5.5 mg, BSA 1.2 g, pepsin 1.2 g, mucin 3.6 g. Duodenal juice (pH 7.8 ± 0.2), NaCl 7.01 g, $NaHCO_3$ 3.39 g, KH_2PO_4 80 mg, KCl 564.5 mg, $MgCl_2$ 50 mg, conc. HCl 43.2 μl, urea 100 mg, $CaCl_2 \cdot 2H_2O$ 479.5 mg, BSA 2.45 g, pancreatin 7.2 g. Bile solution (pH 8.0 ± 0.2), KCl 376.3 mg, NaCl 5.26 g, $NaHCO_3$ 5.78 mg, conc. HCl 20 μl, urea 250 mg, $CaCl_2 \cdot 2H_2O$ 222 mg, BSA1.8 g, Bile 6 g.

2.3.1.1. Gastric soluble oxalate. The method for the determination of gastric soluble oxalate in Japanese taro has been described in detail by Catherwood (2005) and is based on the method proposed by Versantvoort et al. (2005). Briefly, 5 g of Japanese taro was accurately weighed into a 125 mL conical flask and 9 mL of the saliva solution was added. The flask was then incubated at 37 °C for 5 min then 13.5 mL of gastric juice was added and the flask was incubated at 37 °C for a further 2 h. The contents of the flask was then quantitatively transferred to a 250 mL volumetric flask and made up to volume with 0.2 mol/L HCl. An aliquot was centrifuged at 2889 rcf for 15 min. The supernatant was then filtered through a 0.45 μm cellulose acetate filter (Sartonius, Goettingen, Germany) prior to injection into the HPLC. This analysis was carried out in triplicate.

2.3.1.2. Intestinal soluble oxalate. The initial gastric digestion as outlined above was followed by the addition of 27 mL of duodenal juice and 9 mL of bile solution to the flask. The flask was then incubated for a further 2 h at 37 °C. The contents of the flask were then quantitatively transferred to a 250 mL volumetric flask and made up to volume with nanopure water. An aliquot was centrifuged at 2889 rcf for 15 min. The supernatant was then filtered through a 0.45 μm cellulose acetate filter (Sartonius, Goettingen, Germany) prior to injection into the HPLC.

2.3.1.3. HPLC analysis. Each sample was analysed in triplicate as described in detail by Savage et al. (2000). A recovery study was also carried out by adding 10 mg oxalic acid to 5 g samples of the raw tubers. The mean recovery of oxalate ±SD after gastric extraction was $97.7 \pm 1.2\%$. The recovery following the intestinal extraction was $96.7 \pm 1.3\%$.

2.4. Statistical analysis

Minitab version 14 was used to determine the descriptive statistics and GenStat for Windows (Version 7, Laws Agricultural Trust, UK) was used to test for the significant differences between cultivar and treatment means.

3. Results

The results of the oxalate analysis of the four Japanese taro cultivars are shown in Table 1. Each analysis is the mean of four plots, and the values for each plot were determined in triplicate. The dry weight determinations for each cultivar and treatment are also shown in Table 1. There were significant differences ($P < 0.001$) between raw cultivars for total oxalate content.

There was a significant difference ($P < 0.001$) between cultivars and between cooking methods for gastric and intestinal soluble oxalates. Akame had the highest values of gastric soluble oxalates and intestinal soluble oxalates for all cooking treatments.

Boiling significantly ($P < 0.001$) reduced the concentration of gastric soluble and intestinal soluble oxalates in all four cultivars. The mean reductions in gastric soluble oxalates following boiling were 67.8%, 66.6%, 61.4% and 74.1% for Akame, Ishikawa-wase, Yamato-wase and Unnamed, respectively. The mean reductions in intestinal soluble oxalates following boiling were 78.9%, 78.6%, 74.2% and 81.7% for Akame, Ishikawa-wase, Yamato-wase and Unnamed, respectively.

For all four cultivars, baking the corms gave the highest overall values, on a wet matter basis, for gastric and intestinal soluble oxalates. This was due to loss of moisture during baking. The gastric soluble values ranged from 172.6 ± 23.2 mg/100 g FW for Yamato-wase to 333.4 ± 7.5 mg/

Table 1
Mean gastric and intestinal soluble oxalates compared with total and soluble oxalates of the four different cultivars of Japanese taro corms (mg/100 g FW ± SE)

Cultivar/cooking method	Dry matter (mg/100 g FW)	Gastric soluble oxalate (mg/100 g FW)	Intestinal soluble oxalate (mg/100 g FW)	Total oxalate (mg/100 g FW) (Catherwood et al., 2007)	Soluble oxalate (mg/100 g FW) (Catherwood et al., 2007)
Akame					
Raw	24.7	168.9 ± 12.0	134.8 ± 9.8	171.4 ± 11.4	108.6 ± 12.8
Boiled	17.3	54.5 ± 6.9	28.4 ± 5.2	50.6 ± 6.6	nd
Baked	46.6	333.4 ± 7.5	277.1 ± 13.6	328.3 ± 7.5	216.2 ± 16.8
Ishikawa-wase					
Raw	29.9	94.1 ± 9.1	70.0 ± 5.5	94.1 ± 4.4	55.0 ± 11.0
Boiled	17.9	31.4 ±10.1	15.0 ± 3.5	29.7 ± 9.7	nd
Baked	59.3	226.4 ± 9.5	181.2 ± 6.4	218.3 ± 6.3	135.9 ± 23.5
Yamato-wase					
Raw	24.9	70.2 ± 4.48	49.6 ± 6.6	73.2 ± 2.6	35.7 ± 6.6
Boiled	17.9	27.1 ± 4.93	12.8 ± 2.2	26.2 ± 5.4	nd
Baked	53.4	172.6 ± 23.2	125.4 ± 25.2	171.6 ± 20.9	97.1 ± 25.3
Unnamed					
Raw	18.6	95.1 ± 10.7	79.6 ± 11.4	95.5 ± 8.8	63.7 ± 8.5
Boiled	13.8	24.6 ± 8.1	14.6 ± 4.6	22.2 ± 6.7	nd
Baked	37.7	203.0 ± 19.6	164.9 ± 18.0	197.9 ± 19.9	136.2 ± 18.3
Source of variation	df				
Cultivar	3	***	***	***	**
Cooking	2	***	***	***	***
Cultivar × cooking	6	***	***	***	**
l.s.d (5%) between cultivars		35.4	34.5	32.3	38.9
l.s.d. (5%) within cultivars		31.9	31.6	28.7	32.4

l.s.d.: least significant difference. Significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; nd: not detected.

100 g FW for Akame, while the intestinal soluble values ranged from 125.4 ± 6.4 mg/100 g FW for Yamato-wase to 277.1 ± 13.6 mg/100 g FW for Akame.

Overall, for both the gastric and intestinal soluble oxalates the differences between cultivars were dependant upon the cooking method, as indicated by a significant ($P < 0.001$) cultivar/cooking interaction shown in Table 1.

The total and soluble oxalate content of the four cultivars of Japanese taro corms are also presented in Table 1. These values were determined on the same samples using the hot water and hot dilute acid method to determine total and soluble oxalates of the raw, boiled and baked tissue (Savage et al., 2000). These values have been published by Catherwood et al. (2007).

4. Discussion

Japanese taro are an important traditional vegetable in Japan, but they are virtually unknown in New Zealand where they are only sold as a frozen, processed product in a few specialty shops (Scheffer, Douglas, & Trigs, 2005). As the consumption of taro is likely to increase in New Zealand it is important to measure the oxalate levels in both boiled and baked tissue to determine whether the oxalate content constitutes a negative feature of this newly introduced food.

The gastric soluble oxalate content of the corms in this study showed significant variation between cultivars. The gastric soluble oxalate content of the taro corms is the total oxalates in the taro tissue while the intestinal soluble oxalate represents the soluble oxalate potentially available for absorption in the alkaline medium of the small intestine as the insoluble oxalates reform. The difference between these two values is the amount of insoluble oxalate (oxalate predominantly bound to calcium). The mean intestinal insoluble oxalate in the raw corms of the four cultivars is 23.6 ± 3.93 mg/100 g FW which is 23% of the mean of the total oxalates.

Cooking Japanese taro had a significant effect on the concentration of oxalates in the tissue. Boiling reduced the intestinal soluble oxalate in the tissue to a mean for the cultivars of 17.7 ± 3.60 mg/100 g FW as the majority of the intestinal soluble oxalates (soluble oxalates) was leached into the cooking water. During boiling the taro corms absorbed a mean of 10.1%, water which had the effect of diluting the gastric and intestinal oxalates remaining in the tissue when the data is considered on a wet matter basis.

Baking the taro had the effect of concentrating both soluble and insoluble oxalates in the tissue as a mean of 33% moisture was removed during baking. However, if the data is compared on a dry weight basis, the concentrations of

oxalates in the tissue after baking was very similar to the uncooked tissue for all of the cultivars.

Comparison of the gastric soluble oxalate values for raw, boiled and baked tissue of each of the four cultivars investigated with the total oxalate values (Table 1) reported by Catherwood et al. (2007) showed that there was no significant difference between the two sets of results. Regression analysis showed that the r^2 was 0.99 and the intercept was not significantly different from zero ($P = 0.999$). The total oxalate values reported by Catherwood et al. (2007) were extracted using the method of Savage et al. (2000) which used 0.2 mol/L HCL at 80 °C.

Regression analysis of the soluble oxalate values with the intestinal soluble oxalate contents of the four cultivars of taro (Table 1) gave an r^2 of 0.991 but the intercept was significantly different from zero ($P < 0.001$). The intercept value in the regression equation was 12.70 which showed that more intestinal soluble oxalate was determined in the taro compared to the soluble oxalate content determined using the method of Savage et al. (2000). In the Savage et al. (2000) method soluble oxalates are extracted from food materials using hot water at 80 °C. Overall, the data obtained in this experiment suggests that more soluble oxalate is available for absorption in the small intestine than previously suggested by the results determined by the water soluble extraction method used by Savage et al. (2000).

5. Conclusions

This is the first experiment that describes the extraction of different forms of oxalate from a food using an *in vitro* extraction technique. While the *in vitro* method gives similar results to the hot acid method used to extract total oxalates from foods (Savage et al., 2000), the *in vitro* method used to extract intestinal soluble oxalate from foods appears to extract more soluble oxalate from foods when compared to the hot water extract used by (Savage et al., 2000). The *in vitro* method may represent more accurately the processes that actually occur in the small intestine.

This study has shown that boiled taro corms contain low levels of intestinal soluble oxalate while baking the corms had the effect of concentrating oxalates in the cooked tissue. The effective concentration of oxalates in baked tissue observed in this present experiment may have some significance for human nutrition as baked foods tend to be more readily consumed. These results suggest that Japanese taro corms should be included in the moderate oxalate food group as defined by Noonan and Savage (1999).

Acknowledgements

The authors wish to thank J.J.C. Scheffer of Crop & Food Research, Pukekohe for the supply of Japanese taro and Leo Vanhanen for his assistance with the HPLC analysis.

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