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Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC-enzyme-reactor

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Abstract

Food oxalate analysis in foods is problematic due to the wide range of interfering substances. To prevent oxalate generation during sample preparation mild and rapid extraction methods were evaluated. Soluble oxalate was extracted with distilled water and total oxalate was extracted with 2 N hydrochloric acid. Filtrates were analysed using the HPLC enzyme reactor method. Evaluation yielded a high level of precision and recovery. Glyoxylic acid, D/L malic acid, isocitric acid, oxaloacetic acid, pyruvate, mesoxalic acid, ascorbic acid, D(+) glucose, D(−) fructose, chlorogenic acid, caffeic acid could be excluded as a source of oxalate generation during extraction with hot acids. The soluble and total oxalate content of about 150 food samples were investigated.

Keywords: Food; Total oxalate; Soluble oxalate; Oxalate-generation

1. Introduction

The main sources of dietary oxalate are plants and plant products. Oxalate occurs in plant tissue as soluble and insoluble salts. Soluble oxalate includes sodium hydrogen oxalate, potassium hydrogen oxalate and free oxalic acid, poorly soluble oxalate salts are magnesium salts. Insoluble salts are calcium oxalate (CaOx) crystals. These crystals consist of one of two hydration forms, monohydrates or dihydrates. The shapes of CaOx crystals vary considerably and are described as raphides, druses and crystal sand (Franceschi & Horner, 1980) These crystals are located within cells (most oxalates were found within the vacuoles of the cells), or in or on their cell walls (Ilarson, Palmer, Imsande, & Horner, 1997).

Consumption of large amounts of oxalate can cause oxalosis and an elevated urinary oxalate excretion. An increase in urinary oxalate concentration has much greater effects on calcium oxalate crystallization than an increase in calcium concentration (Finlayson, 1974). Urinary oxalate is derived from endogenous production and from intestinal absorption. Holmes and Kennedy (2000) found that the contribution of dietary oxalate to urinary oxalate is between 10 and 72%. Hesse, Schneeberger, Engfeld, von Unruh, and Sauerbruch (1999) found that oxalate absorption correlates with oxalate excretion and that intestinal oxalate absorption of subjects with a history of stone formation was significantly higher than the absorption rate of healthy subjects (9.2% vs 6.7%).

The bioavailability and the intestinal absorption of oxalate may depend on the ratio of soluble and unsoluble oxalate. Different sample preparations for the determination of soluble and total oxalate contents were only used by Herrmann (1972), Ohkawa (1985) and Libert and Franceschi (1987), but the number of investigated samples is too small for constructing low oxalate diets.

The accurate determination of oxalate in plants appears to be problematic because of its extraction from plant tissue and its generation from ascorbic acid during extraction. In previous studies dissolution of CaOx was frequently carried out by using hot acids to avoid incomplete extraction and any oxalate generation due to oxidation of ascorbic acid (Andrews & Viser, 1951;

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AOAC, 1990; Baker, 1952; Fretzdorff & Betsche, 1998; Libert & Franceschi, 1987; Mahlmann, Flohr, Chen, Kleinschmidt, & Hautmann, 1994). On the other hand, Zarembski and Hodgkinson (1962a) observed oxalate generation from unknown sources during extraction with hot hydrochloric acid. They supposed that carbohydrates may be responsible for the elevated values. They minimized errors by using cold acid extraction. But they used a colorimetric determination that demands precipitation of CaOx to remove disturbing coloured matrix substances. Precipitation of CaOx always involves loss of oxalate and is a very time-wasting step. Kasidas and Rose (1980) tried to obviate any preliminary isolation of oxalate by using enzymatical determination with oxalate decarboxylase, but this method may lead to elevated values due to an incomplete removal of dissolved carbon dioxide from the sample solution.

To overcome these problems we chose a very sensitive and selective HPLC-enzyme reactor method (HPLC-ER) (Hönow, Bongartz, & Hesse 1997). This method combined enzymatic conversion and amperometrical detection with chromatographic separation of oxalate. The high selectivity enabled us to develop rapid and simple sample preparations for the determination of soluble and total oxalate. For this, we tested different extraction times and temperatures to exclude any oxalate generation during sample preparation. To enable us to construct a low oxalate diet for stone formers and standardized diets for studies, we investigated the oxalate contents of a large number of common foods.

2. Materials and methods

2.1. Samples for method-development

Homogenized samples were investigated raw, boiled or prepared for normal consumption. To check for complete dissolution of different crystal shapes, carrots (crystal sand: *Daucus carottus*), grapes (raphides: *Vitis vinifera*), cherries (druses: *Prunus avium*) were observed by microscopy during treatment with 2 N hydrochloric acid at room temperature. For microscopy, samples were prepared as for normal consumption, with no previous treatment in order to ensure that the cells were complete and not lysed. Complete cells with crystals inside were observed by polarisation technique. During observation 2 N HCl was dropped on the slides, so that the acid slowly penetrated the samples.

Cherry juice (free of parts from plant tissue) was produced from the fruits by mincing and subsequently centrifugation. It could be used to see whether the raise in oxalic acid after HCl treatment is really due to a generation of oxalic acid or due to a more complete extraction.

2.2. Sample preparation

Extraction of total oxalate was carried out by suspending 2.0 g of the homogenized samples with 4.0 ml HCl (p.a.; Merck, Darmstadt, Germany):

A: 2 N hydrochloric acid at 80 °C (water bath) or
B: 25% hydrochloric acid at 80 °C (water bath) or
C: 25% hydrochloric acid at 100 °C (reflux)

After 30 and 180 min 1 ml of the solutions were filtrated (folder filter; Ø 8 mm, Schleicher & Schüll, Germany) and analysed by HPLC-ER. These methods (A–C) were tested for carrots, cherries, strawberries and cherry juice. Each sample was analysed in double. Because cherries yielded the highest elevations, the oxalate generation in cherries was further investigated under mild conditions (2 N HCl). Four different mild extraction methods (D–G) were tested. Each was repeated 6-fold to detect any significant differences. Portions of 2.0 g of homogenised cherries were suspended with 4.0 ml 2 N hydrochloric acid (p.a.; Merck, Darmstadt, Germany) and subsequently stirred for:

D: 15 min at 21 °C or
E: 30 min at 21 °C or
F: 15 min at 80 °C (water bath) or
G: 30 min at 80 °C (water bath)

Filtrates were immediately analysed by HPLC-ER.

These conditions were also tested for the extraction of soluble oxalate. Extraction of soluble oxalate was carried out by suspending 2.0 g of the homogenized cherries with 4.0 ml distilled water (J.T. Baker Water; HPLC-reagent, Deventer, The Netherlands).

To stabilize ascorbic acid the filtrates were acidified by adding hydrochloric acid (50 μl 2 N HCl/ml).

2.3. HPLC-enzyme reactor method (all equipment Gynkotek, Germering, Germany)

Oxalate was separated from matrix substances by an anion exchange column (AS4A-DIONEX, Sunnyvale, CA) and a mobile phase of 2 g EDTA/L (Merck, p.a.) distilled water was adjusted to pH 5.0 (by adding 15 μl 0.3% NaOH suprapur; Merck, Darmstadt, Germany). The enzyme reactor contained 5 units of immobilized oxalate oxidase (E.C. 1.2.3.4.; obtained by Sigma diagnostics; St. Luis; USA carrier: VA Epoxy Biosynth, Riedel-de-Häen, Seelze, Germany) which converted oxalate to hydrogen peroxide and carbon dioxide. Resulting hydrogen peroxide was detected amperometrically (Hönow et al., 1997).

2.4. Investigations of oxalate generation during extraction with hot acids

The very high amounts of generated oxalate during hot acid extraction of cherries led us to investigate the source for this oxaloneogenesis. Zarembski and Hodgkinson (1962b) suggested carbohydrates as source-substances. Therefore we tested aqueous solutions of D(+) glucose, D(−) fructose and pectine (galacturone methylester). Additional substances and the tested concentrations are given in Table 1. As a catalyst for conversion, each solution was spiked with cherry juice (1 ml cherry juice/10 ml).

2.5. Precision and detection limit

Between-batch precision was investigated for extraction and subsequently analysed by HPLC-ER. Minced cherries (12×2.0 g) were prepared and analysed.

Within-run precision and the detection limit were calculated for aqueous solutions in a previous publication for the determination of oxalate by HPLC-ER (Hönow et al., 1997). The same applies to the filtrates of prepared food samples. Detection limits for solid samples depend on the water content of each sample, since the different water contents have to be taken into account for the calculation of the oxalate concentration after analysing.

2.6. Recovery rate

An aqueous sodium oxalate solution (20 μl, 10mM) was added to 2 g of homogenized sample. After mixing thoroughly samples were extracted with distilled water for 15 min at 21 °C. For the determination of total oxalate, 20 μl of a suspension of CaOx H_2O were added to 2 g of homogenized sample. These samples were extracted with 2 N HCl acid at 21 °C. Because it is impossible to introduce CaOx into the cells, it should be taken into account that this is only an external addition. Observations by microscopy showed that 2 N HCl dissolved CaOx crystals even if they were located inside the cells.

Statistical significance was tested by two-tailed *t*-test (Statistical package for social science version 1.12.01) Outliers were determined according to Shapiro–Wilks test.

2.7. Samples for construction of a low oxalate diet

Samples were shreded and mixed before preparation. Each sample was analysed in double. A lot of samples of different origin or sort were analysed (this is marked in the result-tables as *n*.

3. Results

3.1. Total oxalate A–C

The extraction with 25% HCl (B + C) resulted in an increase in total oxalate of up to 280% for cherry juice (examples are given in Table 2). The highest elevation was found for cherries and cherry juice. Because even

Table 1
Substances investigated for oxalate generation during treatment with hot 25% HCl (100 °C; 180 min; reflux, spiked with cherry juice)

Substances	Concentration in cherries* (mg/100 g)	Concentration (mg/10 ml)	Conversion to oxalate (%)
glyoxylic acid	Unknown	1300	0.17
sec. Alcohols			
D/L malic acid	90	D(+): 649	0
		L(−): 636	0
Isocitric acid	Unknown	208	0
Oxocarboxylic acids			
Oxaloacetic acid	Unknown	205	0.07
Pyruvate	Unknown	103	0
Mesoxalic acid	Unknown	138	1.2
Ascorbic acid	1.5	150	1.6
Literature			
D(+) glucose	610	5419	0
D(-) fructose	550	423	0
Pectine (galacturone Methylester)	40	80	0.4
Other contents of cherries			
Chlorogenic acid	0.61	10	0
Caffeic acid	0.70	10	0

* Values according to Souci et al. (1986)

cherry juice also yielded increased values after treatment with hot hydrochloric acid, the source of the elevation must be attributed to oxalate generation and not to improved removal or dissolution of CaOx crystals from the plant tissue (s.a. material and methods: preparation of cherry juice—it does nor contain plant tissue). The elevation was higher when treated at 100 °C (reflux) than at 80 °C (water bath). Treatment with 2 N HCl yielded a slight elevation for cherry juice and strawberries, but not for carrots and cherries. So also a treatment at 80 °C seemed to be inaccurate.

To verify complete dissolution of CaOx crystals from plant tissue, several samples with different crystal shapes were treated with 2 N HCl under observation by microscope. Because 2 N HCl was strong enough to dissolve all crystal shapes in 5 min at room temperature, different extraction times and temperatures were tested (D–G). As cherries showed the highest potential for oxalate generation, the different conditions were tested by preparing cherries. The results yielded for the extraction with 2 N HCl at 21 °C and 80 °C for 15 and 30 min are shown in Table 3. There were no significant differences between the extraction times of 15 and 30 min or between 21 and 80 °C. But the extraction at 80 °C yielded a slight elevation. Hence we chose an extraction with 2 N HCl for 15 min at 21 °C for further investigations.

Table 2
Examples of oxalate generation (mg/100 g) after treatment with hot hydrochloric acid (means of $n=2$)

	30 min	180 min	Increase 30 min vs 180 min (%)
Carrots			
A: 2 N HCl 80 °C	44.4	45.0	1.35
B: 25% HCl 80 °C	40.3	42.8	6.2
C: 25% HCl 100 °C	42.9	69.6	62.2
Cherries			
A: 2 N HCl 80 °C	3.0	3.0	0
B: 25% HCl 80 °C	2.2	3.2	45.5
C: 25% HCl 100 °C	6.8	16.2	138.2
Cherry juice			
A: 2 N HCl 80 °C	5.3	7.7	45.3
B: 25% HCl 80 °C	5.7	8.3	45.6
C: 25% HCl 100 °C	18.3	70.2	283.6
Strawberries			
A: 2 N HCl 80 °C	2.1	3.1	47.6
B: 25% HCl 80 °C	–	–	
C: 25% HCl 100 °C	3.1	5.2	67.7

3.2. Soluble oxalate

To investigate the conditions of oxalate generation from ascorbic acid during the sample preparation for the determination of soluble oxalate, different extraction times and temperatures were tested. The results are reported in Table 4.

Soluble oxalate values increased significantly after extraction at 80 °C compared to extraction at 21 °C ($P<0.05$). No statistically significant differences were detected between extraction within 15 min and within 30 min. On the basis of these results, we propose that soluble oxalate should be extracted with distilled water for 15 min at room temperature.

The calculation of the between-batch precision yielded a relative coefficient of variation (rCV) of 4.76% for the determination of soluble oxalate, and a rCV = 3.87% for the determination of total oxalate. The detection limit for aqueous solutions was 0.68 μM. The recovery rates calculated for each of the analysed samples yielded mean values of 100.6% (rCV 1.9%) for soluble oxalate, and 97.4% (rCV 2.97%) for total oxalate.

3.3. Oxalate generation during extraction with hot acids

None of the investigated substances (see Table 1) could be the source of the oxalate generation during extraction with 25% HCl at 100 °C (reflux) because the amounts yielded are too small in comparison to the amounts yielded for cherries. Slight generation of oxalate was only detected with glyoxylic acid, oxaloacetic

Table 3
Total oxalate (mg/100 g) in cherries extracted with 2 N HCl for 15 min and 30 min at 21 °C and for 15 min and 30 min at 80 °C (water bath)

15 min 21 °C	30 min 21 °C	15 min 80 °C	30 min 80 °C
2.93	2.96	3.81	3.92
3.14	2.36	3.41	4.63
3.24	2.50	4.09	3.73
2.91	0.35[a]	2.69	3.18
2.59	3.35	4.95	1.95[a]
2.69	3.60	5.70[a]	3.56
Ø 2.92±0.25	Ø 2.95±0.53	Ø 3.79±0.84	Ø 3.49±0.54

Six-fold analysis from one homogenated sample. Bottom line: mean±S.E.M.

[a] Outliers according to Shapiro–Wilks test.

Table 4
Soluble oxalate (mg/100 g) in cherries extracted with dist. water for 15 and 30 min at 21 °C and for 15 and 30 min at 80 °C (water bath)

15 min 21 °C	30 min 21 °C	15 min 80 °C	30 min 80 °C
0.46	0.64	0.75	1.06
0.82	0.70	0.99	0.87
0.72	0.78	0.84	1.25
0.71	0.62	0.88	1.24
0.73	0.93	1.24	1.43
1.24	0.98	1.29	1.31
Ø 0.78±0.10	Ø 0.78±0.24	Ø 0.99±0.37*	Ø 1.19±0.24*

Six-fold analysis from one homogenated sample. Bottom line: mean±S.E.M.

* Significantly different to 21 °C ($P<0.05$).

Table 5
Oxalate in fruits (mg/100 g)

Sample	Kind of samples	Our values oxalate content (mg/100 g)						Values from literature oxalate mg/100 g (soluble/total)							
		Range				Mean		Ogawa et al. (1984)	Zarembski and Hodgkinson (1962b)	Kasidas and Rose (1980)	Andrews and Viser (1951)	Elmadfa et al. (1992/1993)	Hodgkinson (1977)	Awadalia et al. (1985)	Herrmann (1972)
		n	soluble	*n*	total	Soluble	Total								
Apple *Malus sylvestris*	Cox Kent, Raw	26	0.3–1.8	28	0.4–5.8	0.4	1.0	<d.l.	1.5	1.0	30.0	–	–	–	–
Apple	Granny Smith, roh	1	1.8	1	3.5	1.8	3.5	–	–	–	–	–	–	–	–
Apple puree	With ascorbic acid	1	0.7	1	4.6	1.7	4.6	–	–	–	–	–	–	–	–
Apricot *Prunus armeniaca*	Raw	2	1.4–2.3	2	5.2–8.4	1.9	6.8	–	–	–	–	6.8	–	–	3.4/6.8
Banana *Musa paradisiaca*	Raw	7	0.1–2.2	7	0.5–23.9	0.7	6.8	8.9	0.7	–	–	–	0.7	–	–
Bilberry *Vaccinium myrtillus*	Raw	3	<d.l.-0.5	3	0.6–2.1	0.1	1.5	–	–	–	–	–	–	–	–
Bilberry	Preserved	1	1.1	1	8.2	1.1	8.2	–	–	–	–	–	–	–	–
Black current *Ribes nigrum*	Raw	1	3.0	1	19	3.0	19	–	–	–	–	–	–	–	–
Bramble (black berry) *Rubus fruticosus*	Raw	4	0.4–2.5	4	24.9–39.4	0.9	29.2	–	–	–	–	12.4	–	–	6.8/12.4
Carambola *Averrhoa carambola*	Raw	4	81.4–185.6	4	212.6–345.7	138.9	295.4	–	–	–	–	–	–	–	–
Cherry *Prunus avium*	Sweet, raw	5	0.3–3.1	5	2.1–3.1	1.5	2.4	–	–	–	–	–	7.2	–	4.3/7.2
Elderberry *Sambucus nigra*	Raw, black	4	5.0–8.0	4	35.1–86.6	7.1	72.1	–	–	–	–	–	–	–	–
Fig *Ficus carica*	Raw	3	3.5–3.8	3	7.8–32.9	3.3	20.5	–	–	–	–	–	–	–	–
Fig *Ficus carica*	Dried	2	8.6–11.2	1	95.1	9.9	95.1	–	–	–	–	–	–	–	–
Goosberry *Grossularia uva cispa*	Red	1	3.2	1	21.6	3.2	21.6	–	–	–	–	–	–	–	–
Gooseberry *Grossularia uva crispa*	Green, Raw	1	3.1	1	27	3.1	27	–	–	–	–	–	–	–	–
Gooseberry	Mixed	1	4.3	1	18	4.3	18	–	–	–	–	19.3	–	–	10/19
Granadilla *Passiflora edulis*	Raw	1	0.6	1	1.0	0.6	1.0	–	–	–	–	–	–	–	–
Grape *Vitis vinifera*	Green, Raw	9	0.3–1.1	9	0.4–5.2	0.6	1.7	–	–	–	–	7.9	–	–	3.3/7.8
Kiwi fruit *Actinida chinesis*	Raw	6	0.8–3.4	6	0.8–47.3	2.4	23.0	19.7	–	–	–	–	–	–	–
Lemon *Citrus medica*	Raw	2	0.3–0.6	2	1.5–4.7	0.5	3.1	47	–	–	–	–	–	–	–
Lime *Citrus auranttifolia*	Raw	1	0.5	1	7.5	0.5	7.5	–	–	–	–	–	–	–	–
Litchi Litchi chinensis	Raw	1	<d.l.	1	<d.l.	<d.l.	<d.l.	–	–	–	–	–	–	–	–
Mandarin *Citrus nobilis*	Raw	2	0.3	2	3–14.1	0.3	8.5	–	–	–	–	–	–	–	–
Mango *Magnifera indica*	Raw	4	0.1–1.1	5	0.6–2.7	0.5	1.6	–	–	–	–	–	–	–	–
Mirabelle *Prunus domestica syriaca*	Raw	4	<d.l.–0.5	4	<d.l.–28.0	0.2	8.1	–	–	–	–	–	–	–	–
Muskmelon *Cucumis melo*	Raw	6	0.6–1.1	7	0.6–1.5	0.9	1.0	–	–	–	–	–	–	–	–
Orange *Citrus sinsensis*	Raw	1	0.2	1	1.8	0.2	1.8	–	6.2	4.0	–	6.2	–	–	–
Oval Kumquat *Fortunella Margarita*	Raw	1	0.8	1	3.5	0.8	3.5	–	–	–	–	–	–	–	–
Papaya *Carica papaya*	Raw	3	0.5–0.6	3	0.5–1.7	0.5	1.3	–	–	–	–	–	–	–	–
Peach *Prunus persica*	Raw	3	<d.l.–0.4	3	1.0–5.0	0.2	2.5	–	1.2	–	–	–	–	–	<d.l.
Pear Pyrus communis	Raw	10	0.1–7.1	11	0.7–9.0	0.9	2.7	<d.l.	1.7	–	–	6.2	1.3–1.7	–	4.3–6.7
Pear	paeled	2	0.5–7.1	3	1.0–9.0	3.8	3.7	<d.l.	–	–	3.0	–	–	–	–
Pinapple *Ananas comosus*	Preserved, without sugar	2	0.3–1.6	3	3.0–4.3	0.9	4.9	–	<d.l.	1.0	–	–	0–3.7	–	–
Plum *Prunus domestica*	Raw	8	<d.l.–0.5	8	0.7–2.5	0.5	1.7	–	1.1	–	–	11.9	1.1–3.4	–	5.1–7.5
Raspberry Rubus idaeus	Raw	4	2.7–5.9	4	11.3–25.7	3.4	18.9	–	2.2	–	–	16.4	–	–	11.3/16
Red current *Ribes rubrum*	Raw,red	4	2.0–9.3	4	4.9–25.5	4.9	19.8	–	–	–	–	9.9	–	–	2.2/9.9
Strawberry *Fragaria*	Raw	8	0.6–1.9	8	1.5–4.3	0.9	2.9	23.4	1.9	15	47	15.8	1.9–11.5	49	9.9/15.8
Sultana	Dried	1	3.2	1	8.5	3.2	8.5	–	–	–	–	–	–	–	–
Tamarillo *Tamarindus indica*	Raw	1	3.7	1	19.9	3.7	19.9	–	–	–	–	–	–	–	–
Watermelon *Citrullus lanatus*	Raw	1	0.3	1	0.3	0.3	0.3	–	–	–	–	–	–	–	–
Yellow plum *Prunus domestica* ssp *syriaca*	Raw	2	<d.l.–0.4	2	0.7–2.0	0.4	1.4	–	–	–	–	–	–	–	6.6/10.7
								–	–	–	–	–	–	–	–

n = number of different fruits, <d.l. = below detection limit; – = not analysed.

Table 6
Oxalate in vegetables and salad (mg/100 g)

Food	Kind of samples	Our values oxalate contents (mg/100 g)						Values from literature[a] Oxalate mg/100 g (soluble/total)										
		Range				Mean		Ogawa et al. (1984)	Zarembski and Hodgkinson (1962b)	Suvachittanont et al. (1973)	Massey et al. (1993)	Kasidas and Rose (1980)	Souci et al. (1986)	Andrews and Viser (1951)	Elmadfa et al. (1992/1993)	Hodgkinson (1977)	Ciba-Geigy (1977)	Herrmann (1972)
		n	soluble	*n*	total	Soluble	Total											
Artichoke *Cynara scolymus*	Boiled	1	6.8	1	6.8	6.8	6.8	–	–	–	–	–	8.3/ 8.8	–	8.8	–	–	8.3/ 8.8
Asparagus *Asparagus officinalis*	Boiled	3	0.5–1.1	3	1.8–3.1	0.9	2.6	14.6	1.7	–	–	–	–	18/15	–	1.7	–	–
Aubergine *Solanum melogena*	Raw	2	8.3–23.1	2	8.3–24.0	15.7	16.2		–	3.9	190	–	9.5	–	9.5	–	9.5	6.7
Aubergine *Solanum melongena*	Boiled	1	4.8	1	12.8	4.8	12.8	15.3	–	–	–	–	9.5	18.0	9.5	–	–	6.7/ 9.5
Avocado *Persea gratissima*	Raw	2	0.4–2.1	2	0.4–2.1	1.3	1.3	8.6	–	–	–	–	–	–	–	–	–	–
Bean *Phaseolus vulgaris*	Preserved white	2	1.9	2	52.1–56.3	1.9	54.2	4.7	30.2	<d.l.	359	15	–	–	43.7	–	43.7	9.6/40–46
Bean	Kidney-red	1	1.5	1	13.9	1.5	13.9	45.6	–	–	–	–	–	–	–	7.2/61.8	–	–
Bean *Phaseolus vulgaris* ssp *vulgaris var nanus*	Blue, boiled	1	1.5	1	16.7	1.5	16.7	–	–	–	–	–	–	–	–	–	–	–
Beet root *Beta vulgaris*	Boiled	2	15.1–17.4	2	32–41.7	16.3	36.9	2.2	124	–	675	675	116/181	–	72.2	968	–	28-115
Broccoli *Brassica oleracea*	Boiled	2	0.5–1.7	2	0.8–1.9	1.1	1.4	–	–	–	190	–	–	21	–	–	–	–
Brussel sprout Brassica oleracea	Boiled	3	0.7–0.8	3	0.8–1.6	0.8	1.2	–	–	4.6	360	–	5.8/6.1	–	6.1	2.1–3.6	–	–
Cauli flower *Brassica oleacea*	Raw	1	0.3	2	0.4	0.3	0.4	4.5	–	1.0	–	–	4.3	25.0	6.6	–	–	4.3/ 5.4
Carrot *Daucus carota*	Roh	24	4.1–17.7	24	9.1–33.6	9.0	17.8	48.5	22.7	–	4.0	1,5/6.1	60	–	6.1	7.4–22.7	1.5/5.3–6.9	–
Carrot	Boiled	1	2.3	1	4.9	2.3	4.9	–	–	–	–	–	–	–	–	–	–	–
Celeriac *Apium graveolens*	Canned	1	3.5	1	6.7	3.5	6.7	–	–	–	–	–	–	2.1	6.8	–	15.2	–
Cucumber *Cucumis sativus*	Raw	1	0.3	1	0.4	0.3	0.4	1.4	–	–	20	1.0	<d.l.	4	–	–	<d.l.	<d.l.
Fennel *Foeniculum vulgare*	Raw	1	17.2	1	19.7			–	–	–	–	–	5.0	–	–	–	–	–
Fennel *Foeniculum vulgare*	Boiled	2	2.7–3.8	2	3.5–7.0	3.3	5.3	–	–	–	–	–	–	–	–	–	–	–
Kohlrabi *Brassica oleacea* onvar. *acephala* var. *gongylodes*	Boiled	1	0.7	1	0.7			–	–	–	–	–	2.2/ 2.8	–	2.8	–	–	2.2/ 2.8
Leek *Allium porrum*	Raw	2	1.1–17.7	2	1.2–32.7	9.4	17.0	–	–	–	–	–	–	–	–	–	<d.l.	<d.l.
Lentil *Lens esculenta*	Dried	1	1.9	1	13.3	1.9	13.3	–	–	–	–	–	–	–	–	–	–	–
Lettuce *Valeriana locusta*	Raw	1	0.9	1	1.3			–	–	–	–	–	<d.l.	–	<d.l.	–	–	<d.l.
Lettuce *Lactuca sativa*	Raw	2	0.3	2	0.3	0.3	0.3	2.0	–	105	330	–	11.0	–	–	–	2.2	<d.l.
Linseed *Linium usitatissimum*	Grinded coarsely	1	7.2	1	7.7	7.2	7.7	–	–	–	–	–	–	–	–	–	–	–
Mangold *Beta vulgaris*	Raw	6	134–501	6	436–1614	327	874	–	–	–	–	–	650	–	–	–	–	–
Olive *Olea europaea*	Green, canned	2	0.8–1.5	2	28.6–62.7	1.2	45.7	–	–	–	–	–	–	–	–	–	–	–
Olive *Olea europaea*	Black, canned	3	1.1–2.7	3	12.0–17.2	1.6	13..9	–	–	–	–	–	–	–	–	–	–	–
Pea *Pisum sativum*	Green, dried	2	<d.l.	2	<d.l.	<d.l.	<d.l.	–	–	–	–	–	–	–	–	–	–	–
Pea	Boiled	1	0.2	1	0.2	0.2	0.2	–	1.3	–	–	–	<d.l.	–	<d.l.	–	–	<d.l.
Pea	Canned	1	6.2	1	6.2	6.2	6.2	41.3	–	–	50	–	–	6.0	–	0.8–1.3	<d.l.	–
Potato *Solanum tuberosum*	Raw	2	13–16	2	13–21.1	13.0	17.1	–	2.3	–	50	–	<d.l.	40	–	–	–	–
Potato	Boiled	3	8.8–18.9	3	8.8–35.3	12.8	24.3	–	–	–	–	<d.l.	–	–	–	2.3–7.1	5.2	–
Potato	Deep fried	1	17.0	1	26.9	17.0	26.9	–	–	–	–	–	–	–	–	–	–	–
Potato	Baked	1	11.7	1	13.0	11.7	13.0	–	–	–	–	–	–	–	–	–	–	–
Potato	Chips	1	45.8	1	47.0	45.8	47.0	–	–	–	–	–	–	–	–	–	–	–
Pumpkin *Cucurbita pepo*	Raw	1	<d.l.	1	<d.l.			–	–	–	–	–	–	–	–	–	–	–
Radish Raphanus sativus	Raw, red	1	1.4	1	1.7	1.4	1.7	4.6	0.3	–	480	–	–	15	–	0.3	0.3	<d.l.
Radish *Raphanus sativus*	Raw, white	1	<d.l.	1	<d.l.	<d.l.	<d.l.	–	–	–	–	–	–	–	–	–	–	–
Rhabarb *Rheum rhabarbarum*	Raw	1	380	2	570–1900	380	1235	–	460	–	860	850	270/460	438	537	260/620	537	507–569
Rice *Oryza sativa*	Raw	1	12.8	1	12.8	12.8	12.8	82	<d.l.	112	–	<d.l.	<d.l.	186	–	–	–	–

(continued)

Table 6 (*continued*)

Food	Kind of samples	Our values oxalate contents (mg/100 g)						Values from literature[a] Oxalate mg/100 g (soluble/total)										
		Range				Mean		Ogawa et al. (1984)	Zarembski and Hodgkinson (1962b)	Suvachittanont et al. (1973)	Massey et al. (1993)	Kasidas and Rose (1980)	Souci et al. (1986)	Andrews and Viser (1951)	Elmadfa et al. (1992/1993)	Hodgkinson (1977)	Ciba-Geigy (1977)	Herrmann (1972)
		n	soluble	*n*	total	Soluble	Total											
Sauerkraut *Brassica oleracea*	Natur	1	7.1	1	7.1	7.1	7.1	–	–	–	–	–	–	–	–	–	–	–
Savoy cabbage *Brassica oleracea* var. *Sabauda*	Boiled	1	1.3	1	3.5	1.3	3.5	–	–	–	–	–	–	–	–	–	–	–
Spinach *Spinacia oleracea*	Boiled	2	33.3–168	2	100–627	101	364	–	–	–	–	–	126/442	–	–	–	–	–
Spinach	Boiled with cream	1	123	1	412	123	412	–	–	–	–	–	–	–	–	–	–	–
Tomato *Lycopersicum esculentum*	Raw	3	2.5–4.5	3	3.7–13.7	3.6	8.5	–	5.3	–	50	2.0	<d.l.	13	–	5.3	3.9	<d.l.
Tomato	Canned, peeled	1	3.1	1	12.7	3.1	12.7	–	–	–	–	–	–	–	–	–	–	–
Tomato-ketchup		1	3.3	2	7.1–8.9	3.3	7.7	–	–	–	–	–	–	–	–	–	–	–
Vipergrass-*Salsify Scorzonera hispanica*	Canned	1	6.5	1	9.1	6.5	9.1	–	–	–	–	–	–	–	–	–	<d.l.	–

<d.l. = below detection limit; – = not analysed.

Table 7
Oxalate in beverages (mg/100 ml)

Food	Kind of samples	Our values oxalate content (mg/100 g)						Values from literature					
		Range				Mean		Oxalate mg/100 ml (soluble/total)					
		n	Soluble	*n*	Total	Soluble	Total	Kasidas and Rose (1980)	Souci-Fachman-Kraut (1986)	Andrews and Viser (1951)	Elmadfa et al. (1992/1993)	Hodgkinson (1977)	Ciba-Geigy (1977)
Juices													
Apple juice *Malus sylvestris*	100%	16	0.07–0.35	3	0.8–0.9	0.19	0.9						
Apricot juice *Prunus armeniaca*	55%	1	0.5	1	1.8	0.5	1.8						
Black current juice *Ribes nigrum*	100%	1	1.0			1.0	–						
Carrot juice *Daucus carotus*	100%	1	2.8	3	1.1–6.5	2.8	4.6						
Carrot juice	20%	2	1.0–1.4	2	2.3–4.5	1.2	2.9						
Cherry juice	100%	1	1.17			1.17							
Cranberry juice *Vaccinium vitis idaea*	100%	1	0.4	1	0.4	0.4	0.4						
Grapefruit juice	100%	2	0.04–0.2			0.12	–						
Grape juice *Vitis vinifera*	Red 100%	5	0.9–1.6	2	1.0–3.1	1.2	2.1						
Grape juice	Green 100% + ascorbic acid	2	1.3–1.5	1	1.5	1.4	1.5						
Orange juice *Citrus sinensis*	100%	4	0.03–0.3	2	0.1–0.2	0.1	0.2						1.2
Pineapple juice *Ananas comosus*	100%	3	1.0–2.1			1.4	–						
Plum juice *Prunus domestica*	100%	2	3.1–3.7			3.4	–						
Red current juice *Ribes rubrum*	28% fruit	3	0.95–1.0			1.0	–						
Tomato juice *Lycopersicum esculentum*	Salted, fitrated	1	3.6	4	2.6–8.0	3.6	4.1						
Lemon juice *Citrus medica*	100%	3	0.16–0.64	4	0.6–0.7	0.3	0.6						

(continued)

Table 7 (*continued*)

Food	Kind of samples	Our values oxalate content (mg/100 g)						Values from literature					
		Range				Mean		Oxalate mg/100 ml (soluble/total)					
		n	Soluble	*n*	Total	Soluble	Total	Kasidas and Rose (1980)	Souci-Fachman-Kraut (1986)	Andrews and Viser (1951)	Elmadfa et al. (1992/1993)	Hodgkinson (1977)	Ciba-Geigy (1977)
Tea													
Camomille Tea *Matricaria chamomilla*	1.5 g/200 ml 5 min 70 °C	4	0.2–0.7			0.3	–						
Chinese tea Camellia sinensis	1.75 g/ 200 ml 5 min 70 °C	4	0.9–2.8			1.7	–						
Fennel tea *Foeniculum vulgare*	3.5 g/200 ml 5 min 70 °C	8	1.0–1.6			1.3	–						
Fruit tea	3 g/200 ml 5 min 70 °C	8	0.3–0.8			0.6	–						
Green tea *Camellia sinesis*	1.75 g/200ml 5 min 70 °C	53	0.9–19.6			6.3	–						
Matétea *Ilex paraguariensis*	Green 1.8 g/200 ml 5 min 70 °C	2	3.5–3.6			3.6	–						
Matétea	Roasted 1.8 g /200 ml	2	3.1–3.2			3.2	–						
Peppermint tea *Mentha piperita*	1.25 g/200 ml 5 min 70 °C	3	0.5–0.7			0.6	–						
Stinging nettle tea	2 g/200 ml 5 min 70 °C	5	0.4–0.5			0.4	–						
Tea, black *Camellia sinensis*	1.75 g/200 ml 5 min 70 °C	8	2.5–6.2			4.0	–	4.6–12.6			12.5	4.6–17.2	
Others													
Beer	Hefe-Weizen			2	1.7–1.8	–	1.8	0/4.0			1.7	0.8–3.9	1.7
Beer	Kölsch	1	1.0	1	1.1	1.0	1.1						
Cacao powder *Theobroma cacao*	oil removed			2	154–980	–	567		470	908			623
Coke	Coca–Cola			2	0.05	–	0.05	n.d.				1.12	
Coffee *Coffea*	30 g/L	2	0.5–0.7			0.6	–				1.0	1.0–7.3	1.0
Milk	1.5% fat homogen.	2	0.4	1	0.4	0.4	0.4	0.3		1.8	0.7		0.7
Milk	1.5% fat pasteur.	2	0.1	2	0.1	0.1	0.1						

Table 8
Oxalate content of spices and herbs (mg/100 g)

Food	Kind of sample	Our values oxalate content (mg/100 g)						Values from literature					
		Range				Mean		Oxalate mg/100 g (soluble/total)					
		n	Soluble	*n*	Total	Soluble	Total	Ogawa et al. (1984)	Zarembski and Hodgkinson (1962b)	Massey et al. (1993)	Souci et al. (1986)	Elmadfa et al. (1992/1993)	Herrmann (1972)
Savory *Satureja hortensis*	Raw	1	12.9	1	55.0	12.9	55.0						
Dill *Anethum graveolens*	Raw	1	60.0	1	158.6	60.0	159						7.0/ 30.0
Pepper *Piper nigrum*	Black, grind	1	90.8	1	623.0	90.8	623						
Pepper *Piper nigrum*	White, grind	1	2.4	1	28.5	2.4	28.5						
Peppermint *Mentha piperita*	Leaves	1	26.8	1	55.7	26.8	55.7						
Sage *Salvia officinalis*	Leaves	1	41.2	1	85.2	41.2	85.2						
Mustard *Sinapis albe*		1	1.2	1	3.5	1.2	3.5	5.6					
Thyme *Thymus vulgaris*	Dried	1	22.3	1	182	22.3	182						
Lemon balm *Melissan officinalis*	Leaves	1	26.5	1	27	26.5	27						
Onion *Allium cepa*	Raw	2	0.3–2.8	2	0.4–2.9	1.6	1.7	7.3	3.0		3.9/ 5.5		4.3/ 3.5
Chives *Allium achoenoprasum*	Raw	1	4.0	1	4.0	4.0	4.0		1.1	1480			<d.l.
Parsley *Petroselinum sativum*		1	75.9	2	125–146	76	136		166	1700	<d.l.	5.7	<d.l.
Cress *Nasturtium officinale*		1	<d.l.	1	<d.l.	<d.l.	<d.l.						

n = number of samples; <d.l. = below detection limit;– = not analysed.

Table 9
Oxalate in nuts and cereals (mg/100 g)

Samples	Kind of samples	Our values oxalate contents (mg/100 g)						Values from literature					
		Range				Mean		Oxalate mg/100 g (soluble/total)					
		n	Soluble	*n*	Total	Soluble	Total	Ogawa et al. (1984)	Zarembski and Hodgkinson (1962b)	Suvachittantont et al. (1973)	Kasidas and Rose (1980)	Andrews and Viser (1951)	Hodgkinson (1977)
Almond *Prunus amygdalus*	Slices	1	89.9	1	383.3	89.9	383.3						
Buckwheat *Fagopyrum esculentum*		1	56.7	1	133.0	56.7	133.0						
Cornflakes	Frosties	2	1.9–2.3	2	3.0–3.1	2.1	3.0		5.6		2.0	30	4.4–5.6
Crispbread		1	26.0	1	90.8	26.0	90.8						
Flour	Wheat Typ 550	1	2.6	1	14.8	2.6	14.8						
Hazel nut *Corylus avellana*	Chopped	1	35.9	1	167.4	35.9	167.4						
Maronen *Castanea vesca*	Canned	1	<d.l.	1	<d.l.	<d.l.	<d.l.						
Pistachio *Pistacia vera*	Chopped	1	36.8	1	56.5	36.8	56.5						
Rice *Oryza sativa*	Raw	1	12.8	1	12.8	12.8	12.8	82	<d.l.	112	<d.l.	186	
Rice *Oryza sativa*	Boiled	1	0.4	1	1.8	0.4	1.8						
Rolled oats *Avena sativa*	Whole corn	2	2.9–18.1	2	24.0–28.7	10.5	26.4						
Sesame *Sesamus indicum*	Seed dry	1	123	1	3800	123	3800						
Sunflower seed *Helianthus annuus*	Peeled	2	7.9–15.6	2	22.2–26.7	11.8	24.5						
Sweetcorn *Zea mays*	Canned	4	1.5–2.1	4	1.6–2.1	1.6	1.8						
Sweetcorn	Raw	1	0.9	1	0.9	0.9	0.9						
Wholemeal bar	Without chocolate	1	0.8	1	3.9	0.8	3.9						
Wholemeal bar	With chocolate	1	7.1	1	37.9	7.1	37.9						

n = number of samples; <d.l. = <detection limit.

Table 10
Oxalate in alga and mushrooms (mg/100 g)

Samples	Kind of samples	Our values oxalate content [mg/100 g]						Values from literature	
		Range				Mean		Oxalate mg/100 g	
		n	Soluble	*n*	Total	Soluble	Total	Ogawa et al. (1984)	Hodgkinson (1977)
Algae *Porphyra umbilicalis*	dried	1	66.5	1	108.6	66.5	109		
Chanterelles *Cantharellus cibarius*	canned	2	0.4–0.6	2	0.5	0.5	0.5		
Mushroom *Agaricus bisporus*	canned	3	0.1–0.7	3	0.7	0.4	0.7	2.0	2.0
Mushrooms	boiled	1	0.1	1	0.5	0.1	0.5		

n = number of samples

acid, mesoxalic acid, ascorbic acid and pectine. The rates of oxalate generation from the investigated substances are given in Table 1.

Oxalate contents of fruits, vegetables, herbs and spices, nuts and cereals, beverages and mushrooms are given in Tables 5–10. High soluble oxalate contents were found for (> 50 mg/100 g): sesame, rhubarb, mangold, black pepper, carambola, spinach, almond, dill, parsley and buckwheat. High total oxalate contents were found for (> 50 mg/100 g): sesame, rhubarb, mangold, black pepper, spinach, almond, carambola, hazel nut, elderberry, pistachio, bean and for the most part of herbs and spices. Lower values compared to previous publications were found for strawberry, tomato and cauliflower. Higher values were found for red currant, bramble and kiwi. Their oxalate contents were found to be about 50% higher as reported by Herrmann (1972) and Ogawa, Takahashi and Kitagawa (1984).

4. Discussion

As had been the case with Zarembski and Hodgkinson (1962), we also detected an oxalate generation during extraction with hot acids. This would appear to indicate that sample preparation plays an important role in the determination of total oxalate and soluble oxalate. Kasidas and Rose (1980) showed that ultrasonic cell disruption is not necessary for complete extraction of CaOx from the cells. They compared oxalate contents before and after cell breakdown and did not find any differences. In line with their results, we achieved complete dissolution of CaOx crystals from the cells with 2 N HCl at room temperature. This was checked by microscopy (polarisation technique). The main problems seem to be the prevention of oxalate losses and the prevention of oxalate generation. The sources of oxalate generation appear to be different for the determination of soluble and total oxalate. Oxalate generation due to oxidation of ascorbic acid occurs only at pH values above 5.0 (Chalmers, Cowley, & McWhinney, 1985), whereas the sources of oxalate generation during extraction with strong hot acids are still unknown. Although ascorbic acid is described by other authors as being stable at pH values below 5.0, in our investigations it yielded small amounts of oxalate after treatment with hot 25% HCl. Besides ascorbic acid, pectine, mesoxalic acid and glyoxylic acid also exhibited conversion to oxalate. But the occurrence of these substances in cherries is too small to account for the elevated oxalate values (see Table 1).

Many previously employed methods failed to consider these sources of error, because these methods included non-specific detection that needed complex and time-wasting sample preparations. By using modern chromatographic methods these preparatory steps are not longer necessary.

With the exception of Herrmann (1972) and Frank (1969), all previous studies confined their investigations to the total oxalate content alone. But the soluble oxalate content may influence the amount of intestinal absorption much more than the insoluble part. Therefore it is of great interest to investigate the soluble oxalate content of foods in addition to their total oxalate content.

Our investigations enabled us to determine soluble and total oxalate in foodstuffs. Due to the mild conditions an oxalate generation during sample preparation could be excluded. We are using the method described above, for the determination of about 150 foods. Many food samples were prepared the first time. There oxalate contents were still unknown until we analysed them. The differences between our values and the values reported in literature may be caused by a sample preparation that was not tested on a possible oxalate generation, a loss of oxalate or an incomplete extraction from cells.

But differences may also be caused by the use of different plants from different sources. Oxalate content may vary according to soil quality, climate or different state of fruit ripeness (Austenfeld & Leder, 1978; Libert & Franceschi, 1987; Zindler-Frank, 1974, 1975).

Our values corresponded to those of Zarembski and Hodgkinson (1962b), because they used a similar preparation-method.

Some food tables like Souci, Fachmann, and Kraut (1986) only collected values from other authors. It is a big weakness of these tables that they do not declare the source of their data or the analysis-method.

Our results showed that previous dietary advices to avoid strawberries and tomatoes is not longer tenable.

To make use of the result-tables it is important to take into account the usual amount of consumption, for example, the most part of herbs and spices yielded high oxalate values but the daily amount of consumption will be very low. On the other hand green and black tea yielded only moderate amounts of oxalate, but a daily consumption of perhaps 2.5 l may lead to an elevated urinary oxalate concentration.

5. Conclusion

The advantages of the HPLC-ER method are low costs (in comparison with an ionchromatographic system like DIONEX or an enzymatic kit like SIGMA, when the number of analysed samples is high) and very high sensitivity and selectivity. The high selectivity enabled us to develop mild and rapid extraction methods. These are necessary to avoid oxalate generation during extraction. Additionally, the combination of

rapid sample preparation and cheap analysis by HPLC-ER enabled us to investigate a great number of food samples.

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