Int. J. Vitam. Nutr. Res., 73 (2), 2003, 152–159

Influence of Cooking Process on Phenolic Marker Compounds of Vegetables

Wilfried Andlauer, Claudia Stumpf, Maike Hubert, Andreas Rings and Peter Fürst

Institute for Biological Chemistry & Nutrition, University of Hohenheim, D-70593 Stuttgart, Germany

Received for publication: December 17, 2002

Abstract: Phenolic compounds are secondary plant metabolites which have long been associated with flavor and color characteristics of fruits and vegetables. These phenolic compounds attract great interest due to their postulated health protecting properties. However, adequate intakes and absorption rate of phenolic compounds are necessary for these beneficial effects. Until now, little is known about alterations of phenolic compounds content by the cooking process. In the present study, the influence of different volumes of cooking water on the amount of selected phenolic marker compounds resting in the vegetables was assessed. In zucchini, rutin was quantified as a marker for flavonoid glycosides. Chlorogenic acid, representative of phenolic acids was analyzed in carrots. In beans, rutin and quercitrin, both belonging to flavonoid glycosides, were investigated. In potatoes, chlorogenic and caffeic acid were determined. The cooking of zucchini, beans and carrots with smaller amounts of water resulted in significant higher content of phenolic phytochemicals in the vegetables compared to cooking with larger water volumes. For potatoes, which showed great variations in content of phenolic acids after cooking, no significant differences in phenolic acids was observed. It can be concluded from these observations, that real intakes of phenolic compounds from cooked vegetables are lower and that the amounts consumed are therefore overestimated.

Key words: bean, potatoe, zucchini, carrot, phenolic compounds, cooking, processing, leaching, HPLC

Introduction

Phenolic compounds are secondary plant metabolites which have long been associated with flavor and color characteristics of fruits and vegetables. These phenolic compounds attract great interest due to their postulated health protecting properties [1]. Foremost is their antioxidative effect, manifested by the ability to scavenge free radicals [2, 3] or to prevent oxidation of low-density lipoproteins [4]. However, adequate intakes and absorption rate of phenolic compounds would be necessary for these beneficial effects. Estimations based on phenolic contents of vegetables and human consumption [5–7] do not consider losses which might occur due to cooking process. While phenolic contents of vegetables have been investigated in numerous studies [8–11], few studies report the influence of different cooking processes on phenolic compounds content of vegetables [12–14], removal

Abbreviations used: LC-MS: liquid chromatography combined with mass spectrometric detection.

and degradation during cooking [15]. The aim of the present study was to assess the influence of different volumes of cooking water on the amount of selected phenolic marker compounds resting in the vegetables. Therefore, we analyzed vegetables before and after cooking with two different volumes of water. In order to obtain an overall balance of the marker compounds we also determined their content in the cooking water. For this purpose, we adopted and slightly modified a previously published HPLC method for simultaneous separation and quantification of polar and less polar phenolic compounds [16].

Material and Methods

Samples: Fresh carrots, zucchini and frozen princess beans were purchased at local supermarkets. Fresh potatoes (cultivar Christa), harvested in the first week of July 2001, were obtained from a local farm. Vegetables were stored light-protected, carrots and zucchini at 4°C, beans at –20°C and potatoes at room temperature.

Sample treatment prior to processing: The non-edible parts of the unwashed vegetables were removed. Both ends of the zucchini were cut off. The outer layer of the carrots was scraped off thinly (weight loss about 8%). Sliced carrots (pieces of 3–8 g) and zucchini (pieces of 15–20 g) were stored until extraction or cooking at 4°C under nitrogen to prevent oxidation. Potatoes were washed with cold water and air-dried. In each experiment, 500 g of tubers were cooked (eight tubers ranging from 50 g to 100 g). To reach the exact sample weight of 500 g, a small piece of one of the tubers was cut off.

Cooking Procedure with Small Amounts of Water: Cooking pots with a diameter of 24 cm and corresponding tops with the thermometer visiotherm 3000 and the timer audiotherm 3000 were used together with atmosphera 3000 automatic cooking plates (AMC International, Rotkreuz, Switzerland). The total cooking time was splitted into preheating and continued heating. During the preheating time, the pot filled with water (zucchini and carrots 30 mL, beans 40 mL, potatoes 50 mL) and vegetables (zucchini, carrots and beans 300 g, potatoes 500 g) was heated at medium level until the optimal cooking temperature was achieved. Then, during the continued heating time, the vegetables were cooked until they were done. Cooking parameters are given in Table I.

Cooking Procedure with Large Amounts of Water (Conventional Cooking): For this method, the same cooking pots without thermometer and timer were used. A water: vegetable-ratio of 1:1 was added to the pot. First, the water was heated, then the vegetables (zucchini, carrots and beans 300 g, potatoes 500 g) were added to the boiling water and cooked. Potatoes were heated together with the water.

Preparation of Raw Vegetables: Vegetables (40 g, 50 g in case of potatoes, respectively) were cut into pieces of 3–5 mm. For extraction, 40 mL methanol (potatoes 50 mL), ascorbic acid (1.8 g/L) and Naringin as internal standard (1 g/L, 450 µL, 500 µL in case of potatoes) were added. This mixture was homogenized with an ultra turrax (Janke & Kunkel, IKA-Werk Stauffen, Breisgau, Germany). Extraction was carried out by sonication for 20 min and subsequent centrifugation for 20 min at 2800 × g. The volume of the resulting clear supernatant was defined. The supernatant was evaporated to half the volume under reduced pressure (Rotavapor, Büchi, Switzerland). If the extract was turbid after evaporation, it was centrifuged again at 10 000 × g for 10 min. The resulting clear supernatant was analyzed by HPLC. The potatoe extract (200 µL) was diluted to 1 mL with water prior to analysis.

Preparation of Cooked Vegetables: After each cooking procedure, the vegetables were cooled in an ice bath for 5 min prior to weighing and extraction. The cooked vegetables were homogenized. Forty grams (50 g of potatoes) of the puree were extracted with methanol as described for the raw material.

Preparation of Cooking Water: The volume of the cooking water was defined and aliquots were centrifuged at 2800 × g at 4°C for 20 min. The resulting supernatant was analyzed by HPLC. For potatoes 10 mL of the supernatant were extracted two times with 2 mL acetic acid ethyl ester,

Table I: Times for cooking the vegetables with small and large volumes of water (min, MW ± SD, n = 5)

	Zucchini	Carrots	Beans	Potatoes
Large water volume	10.2 ± 1.2	10.8 ± 0.5	14.7 ± 1.6	32.3 ± 0.4
Small water volume				
Preheating	10.3 ± 0.7	11.5 ± 0.9	14.3 ± 2.1	8.1 ± 1.0
Continued heating	10.0 ± 0.0	10.0 ± 0.0	8.8 ± 1.8	30.0 ± 0.0
Cooking time	20.3 ± 0.7	21.5 ± 0.9	23.0 ± 2.1	38.1 ± 1.0

evaporated under nitrogen, dissolved in 250 µL methanol and diluted with solvent A (1750 µL in case of small or 750 µL in case of large amount of cooking water) prior to HPLC analysis.

All samples were analyzed in duplicate and data expressed as means.

Gradient HPLC-system with UV-detection: The HPLC-system (Sykam, Gilching, Germany) for analyses of beans, zucchini and carrots consisted of a micro solvent delivery system S 1100, a low pressure gradient mixer S 8110, a column oven S4110, an autoinjector Jasco 851 AS Intelligent Sampler (Jasco, Japan) with a 100 µL loop (injection volume = 20 µL) and an Axxiom Interface with Pyramid Chromatography Software (Axxiom Chromatography, Moorpark, USA). Separation was carried out on a Gromsil ODS-4 HE column (125 mm × 2.0 mm I.D., 3 µm, Grom Analytik & HPLC GmbH, Herrenberg, Germany) at 30°C. The analytes were monitored with a Merck-Hitachi 655 A variable wavelength monitor (Merck, Darmstadt, Germany) at 280 nm.

Analyses of potatoes were performed with a Merck-Hitachi L-6200 Intelligent Pump (Merck, Darmstadt, Germany), a low pressure gradient mix system, a T-6300 Column Thermostat (Merck, Darmstadt, Germany), an autosampler Triathlon Version 1.5 (Spark Holland, Emmen, The Netherlands) with a 20 µL loop and a data processor Chromatopac C-R6A (Shimadzu Corporation, Kyoto, Japan). Separation was carried out on a Hypersil ODS (125 mm × 4.0 mm I.D., 3 µm, Grom Analytik & HPLC GmbH, Herrenberg, Germany) at 40°C. The analytes were monitored with a Kratos Spectroflow 757 Absorbance Detector (Kratos Analytical, Manchester, UK) at 280 nm.

HPLC Conditions: All solvents were of HPLC grade and degassed with helium prior to use. For analyses of beans, zucchini and carrots, eluent A consisted of water/formic acid (95/5, v/v), eluent B was composed of acetonitrile/water/formic acid (53/42/5, v/v/v). Elution was performed at a flow rate of 0.24 mL/min using the following gradient conditions: 0–5 min, 0%B; 5–20 min, 0–25% B; 20–30 min, 25–50% B; 40–44 min, 50–60% B; 44–46 min, 60–0% B; 46–50 min 0% B.

For analyses of potatoes, eluent A consisted of acetonitrile/water/formic acid (2/93/5, v/v/v), eluent B of acetonitrile/water/formic acid (53/42/5, v/v/v). Flow rate was 0.8 mL/min, with the following gradient conditions: 0–10 min, 0% B; 10–30 min, 0–50% B; followed by flushing the column 10 min with acetonitrile/water (95/5, v/v) and reconditioning 15 min with eluent A.

Gradient HPLC-system with MS-detection (LC-MS): To confirm the identity of the marker compounds in the vegetables under investigation, we used a gradient HPLC system series 1100 (Hewlett Packard, Böblingen, Germany) combined with an autosampler ALS G1313A, a quat pump G1311A, a degasser G1322A, and a column oven Colcomp G1316A at 30°C. The mass spectrometric detector consisted of a Micro Mass Platform II (Mass Lynx 4.0, Manchster, U.K.) equipped with a cross-flow interface. We used electrospray ionization. Source temperature was maintained at 120°C, cone voltage at 50 kV, and acceleration lens potential at 0.5 kV. Negative ion characterization was performed in the m/e range of 150–800 at a scan rate of 2 scans/s and a multiplier voltage of 650 V. Capillary voltage was 3.0 kV for negative ionization. Elution was performed under the following conditions: 0–7 min, 0% B; 7–40 min, 0–55% B; 40–45 min, 55% B; 45–46 min, 55–0% B, 46–50 min, 0% B with 0,1 mol/L ammonium formiate buffer (pH 4) as eluent A and 100% acetonitrile as eluent B.

Reagents and Standards: Ultrapure water was generated with an Elga Maxima water purification system, including reverse osmosis, activated carbon, and ion-exchange cartridges (Elga, Lane End, U.K.). Quercitrin (quercetin-3-rhamnoside) was purchased from Sigma (Deisenhofen, Germany), chlorogenic and caffeic acid were obtained from Carl Roth GmbH (Karlsruhe, Germany), naringin (naringenin-7-rhamnoglucoside) from Fluka (Buchs, Switzerland) and rutin (quercetin-3-rhamnoglucoside) from Aldrich (Deisenhofen, Germany). All chemicals were of analytical or HPLC grade.

Standard Solutions for HPLC: For stock solutions 1 mg of the standard compound was dissolved in 1 mL of methanol. For preparation of a standard mixture, 100 µL of the stock solutions were used and further diluted 1:10 with ultrapure water. The standard mixture for analysis of bean extracts consisted of rutin, quercitrin and naringin (ISTD) with calibration curves in the range of 0.5–70 µmol/L. For analysis of zucchini extracts, a calibration curve of rutin with naringin (ISTD) was obtained in the range of 0.2–80 µmol/L. The standard mixture for the analysis of carrot and potatoe extracts consisted of caffeic acid, chlorogenic acid and naringin (ISTD). Calibration curves in the range of 0.4 µmol/L–0.1 mmol/L were acquired.

Calculations. Cooking procedures (contents of phenolic compounds in the cooked vegetables and in the cooking water) were compared using Student's t test for paired observations. In case of potatoes, which were cooked as whole tubers, Student's t test for unpaired observations was used. *P* values less than 0.05 were considered to indicate significant differences.

Results and Discussion

Numerous compounds were extracted from the vegetables and separated by our new HPLC-method. LC-MS enabled verification of selected marker compounds, representative for phenolic acids and flavonoids. In zucchini, we quantified rutin as a marker for flavonoid glycosides. Chlorogenic acid, representative of phenolic acids was analyzed in carrots. In beans, rutin and quercitrin, both belonging to flavonoid glycosides, were investigated. Major phenolic acids in potatoes are chlorogenic and caffeic acid [17], which were also determined in the present study.

Zucchini: Mean rutin content of raw zucchini was about 13.3 mg/kg (Table II; Fig. 1). Until now, there is no comprehensive report on phytochemical contents of zucchini. In a former study, small amounts of phenolic acids were observed, flavonoid content, however was not determined [18]. In cucumber, like zucchini, a member of the cucurbitaceae family, traces of quercetin and kaempferol have been reported [11].

Cooking with large amount of water decreased rutin content to 8.8 mg/kg. Cooking with small amounts of water resulted in a content of 10.1 mg/kg. As expected, losses due to the cooking process were significantly higher ($p < 0.05$) by cooking with large amount of water. Some rutin was lost due to degradation to non UV-active substances. The aglycon quercetin was not detected.

Carrots: Raw carrots showed a chlorogenic acid content of 82.4 mg/kg, which is somewhat higher than 65 mg/kg, reported by Herrmann *et al* [18]. Chlorogenic acid content was obviously higher ($p < 0.001$) after cooking with small amounts of water (60.3 mg/kg) compared to large amounts (29.6 mg/kg) (Table III; Fig. 2). High amounts of chlorogenic acid were lost during cooking, but not all was found in the cooking water. During cooking with large amounts of water, about 40% of chlorogenic acid was degraded or bound to the carrot matrix. Phenolic acids have been shown to bind covalently to plant proteins [19]. Using small amount of water, 19% of the chlorogenic acid loss cannot be explained by the cooking water. In the cooking waters, the hydrolysis product caffeic acid was detected and verified by LC-MS.

Beans: Mean rutin content of raw frozen beans was 47.7 mg/kg (Table IV; Fig. 3). For beans, a quercetin glycoside concentration of 39 mg/kg (calculated as quercetin) has

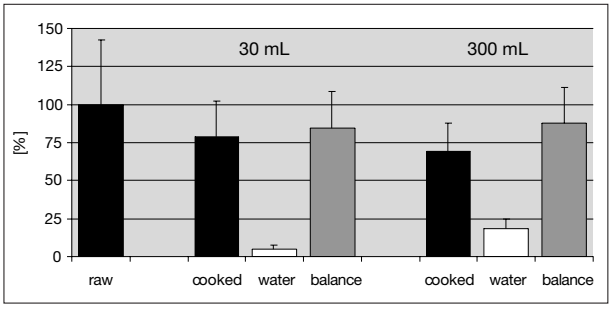

Figure 1: Rutin distribution and balance after cooking zucchini with 30 mL and 300 mL water, respectively. Values are means ± SD of five experiments and based on rutin content of raw zucchini.

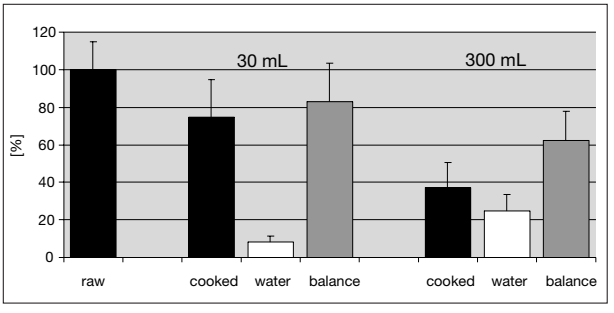

Figure 2: Chlorogenic acid distribution and balance after cooking carrots with 30 mL and 300 mL water, respectively. Values are means ± SD of five experiments and based on chlorogenic acid content of raw carrots.

Table II: Rutin content of raw and cooked zucchini and of the cooking water, respectively (mg in 300 g fresh weight)

		30 mL Water						300 mL Water					
Sample	Raw	Cooked		Water		Total		Cooked		Water		Total	
	mg	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]
1	4.3	4.4	102.9	0.2	4.3	4.6	107.2	3.6	83.4	0.9	21.9	4.5	105.3
2	6.8	3.9	58.0	0.5	7.2	4.4	65.2	3.5	52.2	1.1	16.8	4.7	69.0
3	3.0	1.6	51.6	0.1	2.8	1.6	54.4	1.5	49.7	0.5	15.4	2.0	65.1
4	2.4	2.4	100.0	0.2	7.5	2.6	107.5	2.2	91.4	0.7	27.4	2.8	118.9
5	3.4	2.8	82.1	0.2	5.1	2.9	87.2	2.4	70.1	0.3	10.3	2.7	80.5
MW	4.0	3.0	78.9	0.2	5.4	3.2	84.3	2.6	69.4	0.7	18.4	3.3	87.8
SD	1.7	1.2	23.5	0.2	2.0	1.3	24.2	0.9	18.5	0.3	6.5	1.2	23.4

[1] Based on rutin content of raw zucchini

Table III: Chlorogenic acid content of raw and cooked carots and of the cooking water, respectively (mg in 300 g fresh weight)

		30 mL Water						300 mL Water					
Sample	Raw	Cooked		Water		Total		Cooked		Water		Total	
	mg	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]
1	27.1	18.6	68.6	1.4	5.1	20.0	73.7	7.3	27.1	6.5	24.1	13.8	51.2
2	20.1	22.2	110.3	1.8	8.9	24.0	119.2	11.9	59.2	4.1	20.3	16.0	79.5
3	29.4	19.3	65.4	2.1	7.3	21.4	72.7	8.1	27.6	4.2	14.3	12.3	41.9
4	24.8	16.7	67.3	3.3	13.1	20.0	80.5	9.5	38.4	9.0	36.3	18.5	74.7
5	22.3	13.7	61.5	1.7	7.7	15.4	69.2	7.5	33.8	6.5	29.4	14.0	63.2
MW	24.7	18.1	74.6	2.1	8.4	20.1	83.0	8.9	37.2	6.1	24.9	14.9	62.1
SD	3.7	3.1	20.1	0.7	3.0	3.1	20.6	1.9	13.1	2.0	8.4	2.4	15.7

[1] Based on chlorogenic acid content of raw carrots

Table IV: Rutin content of raw and cooked beans and of the cooking water, respectively (mg in 300 g fresh weight)

		40 mL Water						300 mL Water					
Sample	Raw	Cooked		Water		Total		Cooked		Water		Total	
	mg	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]
1	12.5	7.7	61.8	0.6	5.1	8.3	66.9	6.4	51.0	2.2	17.8	8.6	68.8
2	11.8	12.9	109.5	0.5	4.0	13.4	113.5	9.1	76.6	3.9	33.0	13.0	109.7
3	14.5	14.7	101.7	0.6	4.4	15.3	106.1	8.2	56.3	3.0	20.4	11.2	76.7
4	20.0	17.1	85.2	0.6	3.1	17.7	88.3	12.7	63.5	2.5	12.3	15.2	75.9
5	12.8	17.0	132.9	0.6	4.7	17.6	137.6	13.3	104.0	2.2	17.3	15.5	121.3
MW	14.3	13.9	98.2	0.6	4.2	14.5	102.5	9.9	70.3	2.8	20.2	12.7	90.5
SD	3.3	3.9	26.6	0.1	0.8	3.9	26.6	3.0	21.2	0.7	7.8	2.9	23.4

[1] Based on rutin content of raw beans

been reported [11]. The rutin and quercitrin (see below) contents of the beans analyzed in the present study correspond to about 37 mg/kg quercetin, which is in good agreement with the former literature value. Generally, the rutin content of beans shows great variations, due to cultivar, seasonal variation, and storage (19 mg/kg to 184 mg/kg raw beans, [20]).

About 30% of the rutin was lost by cooking with large water volumes resulting in rutin contents of the cooked beans of 33.1 mg/kg (Table IV; Fig. 3). This high loss of rutin cannot be exclusively explained by the rutin in the cooking water. Rutin loss due to cooking with small amounts of water was significantly lower (about 2%, $p < 0.01$) than loss after cooking with large water volumes. Mean rutin content of beans after cooking with small amounts of water was 46.3 mg/kg.

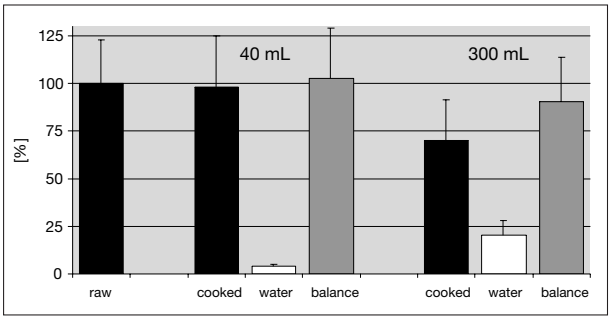

Figure 3: Rutin distribution and balance after cooking beans with 30 mL and 300 mL water, respectively. Values are means ± SD of five experiments and based on rutin content of raw beans.

Raw, frozen beans had a quercitrin content of 19.9 ± 10.7 mg/kg, which decreased due to cooking with high water volumes to 13.7 mg/kg (Table V; Fig. 4). Quercitrin loss after cooking with small water volumes was lower, yielding in a quercitrin content of 19.6 mg/kg (no significant difference).

We investigated two different bean charges, which showed markedly differences in quercitrin content. Differences in rutin content were not so obvious.

As in our experiments with large water volumes, in an earlier cooking study, a comparable decrease (23%) in quercetin glycoside content was observed [13]. Inline with our observations, this working group reported an increase in quercetin glycoside concentration (8%) after cooking with small volumes of water. In contrast to our experiments, they used microwave for cooking. Unfortunately, these authors give no explanation for this amazing observation. The quercetin glycosides might be released from the bean matrix during cooking.

Table V: Quercitrin content of raw and cooked beans and of the cooking water, respectively (mg in 300 g fresh weight)

		40 mL Water						300 mL Water					
Sample	Raw	Cooked		Water		Total		Cooked		Water		Total	
	mg	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]
1	3.2	2.1	66.2	0.1	4.55	2.3	70.7	2.0	63.0	0.7	20.2	2.7	83.2
2	2.8	2.6	94.1	0.1	3.69	2.7	97.8	2.5	91.1	0.8	28.05	3.3	119.2
3	7.6	8.8	116.5	0.4	4.86	9.2	121.3	4.4	58.5	1.8	23.27	6.2	81.8
4	10.5	6.9	65.3	0.3	3.24	7.2	68.5	6.0	56.6	1.3	12.78	7.3	69.4
5	5.8	8.9	153.4	0.4	7.09	9.4	160.6	5.7	97.4	1.2	19.98	6.8	117.3
MW	6.0	5.9	99.1	0.3	4.69	6.1	103.8	4.1	73.3	1.1	20.85	5.3	94.2
SD	3.2	3.3	37.1	0.1	1.49	3.4	38.4	1.8	19.4	0.4	5.57	2.1	22.6

[1] Based on quercitrin content of raw beans

Table VI: Caffeic acid content of raw and cooked potatoes and of the cooking water, respectively (mg in 500 g fresh weight). Note that different, whole tubers were used for experiments

		50 mL Water						500 mL Water					
	Raw	Cooked		Water		Total		Cooked		Water		Total	
	mg	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]
	4.5	1.6		0.1		1.7		1.9		0.1		2.0	
	4.2	2.6		0.9		3.5		2.8		0.5		3.3	
	4.9	4.3		0.3		4.6		3.7		1.2		4.9	
	5.3	4.0		0.4		4.4		3.2		0.1		3.3	
	5.4	2.5		0.2		2.7		2.3		0.3		2.5	
MW	4.9	3.0	61.4	0.4	8.5	3.4	69.9	2.8	57.7	0.4	8.6	3.2	66.2
SD	0.5	1.1	21.2	0.3	7.5	1.2	24.4	0.7	15.5	0.4	9.3	1.1	23.5

[1] Based on mean caffeic acid content of raw potatoes

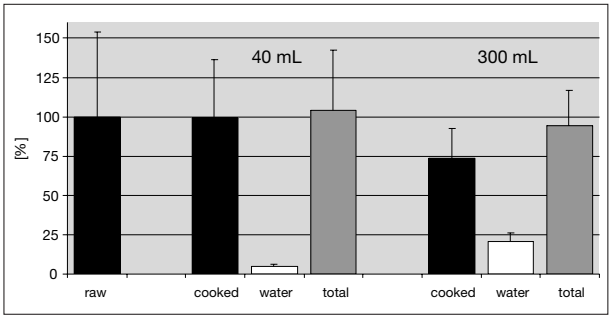

Figure 4: Quercitrin distribution and balance after cooking beans with 30 mL and 300 mL water, respectively. Values are means ± SD of five experiments and based on quercitrin content of raw beans.

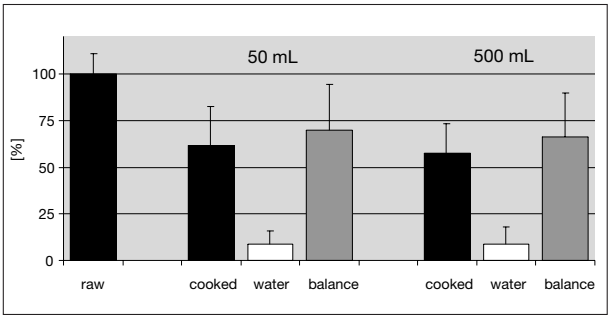

Figure 5: Caffeic acid distribution and balance after cooking potatoes with 50 mL and 500 mL water, respectively. Values are means ± SD of five experiments and based on caffeic acid content of raw potatoes.

The aglycon quercetin was not detectable in the cooking water and the beans. This result was confirmed by a cooking study with onions [14].

Potatoes: The potatoes (Christa cultivar) showed a mean caffeic acid content of 9.7 mg/kg (Table VI; Fig. 5), about half of the concentration (18 mg/kg) reported by Ramamurthy *et al* [21]. Both cooking processes yielded in noticeable losses of caffeic acid (42% and 39%, respectively). Larger volumes of cooking water, however, did not result in significant lower contents of caffeic acid in potatoes. These high losses might be explained by the fact that the concentration of phenolic acids is highest in the outer layers of the potatoes [17]. During the cooking process, these outer layers are extremely exposed to the water and are therefore well extracted.

The chlorogenic acid content of potatoes was determined to 261.5 mg/kg (Table VII; Fig. 6). This content is

Table VII: Chlorogenic acid content of raw and cooked potatoes and of the cooking water, respectively (mg in 500 g fresh weight). Notice that different, whole tubers were used for experiments

	50 mL Water							500 mL Water					
	Raw	Cooked		Water		Total		Cooked		Water		Total	
	mg	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]	mg	%[1]
	120.1	94.2		0.1		94.2		103.8		0.0		103.9	
	130.5	115.7		0.0		115.7		97.1		0.0		97.1	
	134.9	114.1		0.0		114.2		104.4		0.0		104.4	
	120.0	122.7		0.0		122.7		97.5		0.0		97.5	
	148.2	146.5		0.0		146.5		109.0		0.0		109.1	
MW	130.7	118.6	90.6	0.0	0.0	118.7	90.6	102.4	78.6	0.0	0.0	102.4	78.6
SD	11.8	18.8	9.9	0.0	0.0	18.8	9.9	5.1	5.3	0.0	0.0	5.1	5.3

[1] Based on mean chlorogenic acid content of raw potatoes

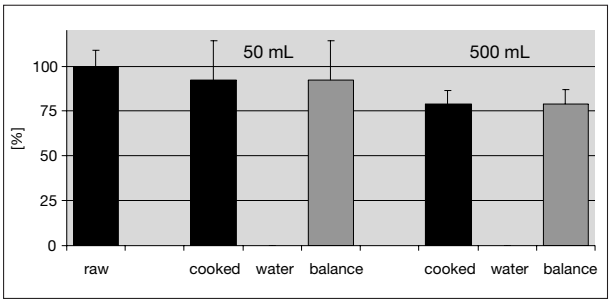

Figure 6: Chlorogenic acid distribution and balance after cooking potatoes with 50 mL and 500 mL water, respectively. Values are means ± SD of five experiments and based on chlorogenic acid content of raw potatoes.

higher than that reported by Dao and Friedmann (96 to 187 mg/kg raw material and which is highly dependant on cultivar, harvest-time and storage conditions [22, 23]. Losses due to cooking with large water volumes was about 21%; small water volumes resulted in a decrease of 9%. In experiments with potatoes, cooking experiments were performed with whole tubers, which might be different in phenolic acid concentrations. This might partly explain the observed different phenolic acid contents. Notwithstanding the high differences, the losses of chlorogenic acid were not significant, due to the highly variable phenolic acid contents of the potatoe tubers.

Additionally, chlorogenic acid was lost not only by the cooking water. The lacking chlorogenic acid might be hydrolyzed to caffeic and quinic acid or converted to chlorogenic acid isomers. A covalent binding of chlorogenic acid to potatoe proteins, as it was shown for phenolic acids, is also conceivable [19].

Conclusion

The present study clearly indicates that cooking of vegetables leads to losses of phenolic phytochemicals. The cooking of zucchini, beans and carrots with smaller amounts of water results in significant higher content of phenolic phytochemicals in these vegetables compared to cooking with larger water volumes. Keeping in mind that phenolic phytochemicals are highly potent chemopreventive substances, cooking with small volumes of water should be recommended, to keep most of these beneficial substances in the cooked vegetables. The more cooking water you use, the more phenolic phytochemicals you lose.

Estimations based on phenolic content of vegetables and human consumption [5–7] do not consider losses due to cooking process. Real intakes of phenolic compounds from cooked vegetables are lower and consumed amount is therefore overestimated.

Acknowledgments

We are indebted to W. Armbruster, Institute for Food Chemistry, University of Hohenheim, Stuttgart, Germany for performing the LC-MS-analyses. We like to thank the International Foundation for the Promotion of Nutrition Research and Nutrition Education (ISFE, Rotkreuz, Switzerland) and AMC International (Rotkreuz, Switzerland) for financial support.

References

1. Carbo, N., Costelli, P. and Baccino, F.M. *et al* (1999) Resveratrol, a natural product present in wine, decreases tumour growth in a rat tumour model. Biochem. Biophys. Res. Commun. 254, 739–743.

2. Soleas, G. J., Tomlinson, G., Diamandis, E. P. and Goldberg, D. M. (1997) Relative contributions of polyphenolic constituents to the antioxidant status of wines – development of a predictive model. J. Agric. Food Chem. 45, 3995–4003.
3. Frankel, E. N., Waterhouse, A. L. and Teissedre, P. L. (1995) Principal Phenolic Phytochemicals in Selected California Wines and their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoprotein. J. Agric. Food Chem. 43, 890–894.
4. Rifici, V. A., Stephan, E. M., Schneider, S. H. and Khachadurian, A. K. (1999) Red wine inhibits the cell-mediated oxidation of LDL and HDL. J. Am. Coll. Nutr. 18, 137–143.
5. Hertog, M. G. L., Hollman, P. C. H., Katan, M. B. and Kromhout, D. (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr. Cancer 20, 21–29.
6. Hertog, M. G. L., Kromhout, D. and Aravanis, C. *et al* (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch. Intern. Med. 155, 381–386.
7. Keli, S. O., Hertog, M. G. L., Feskens, E. J. M. and Kromhout, D. (1996) Dietary flavonoids, antioxidant vitamins, and incidence of stroke – The zutphen study. Arch. Intern. Med. 156, 637–642.
8. Herrmann, K. (1989) Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Crit. Rev. Food Sci. Nutr. 28, 315–347.
9. Justesen, U., Knuthsen, P. and Leth, T. (1998) Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. J. Chromatogr. A, 799, 101–110.
10. Paganga, G., Miller, N. and Rice-Evans, C. A. (1999) The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radic. Res. 30, 153–162.
11. Hertog, M. G. L., Hollman, P. C. and Katan, M. B. (1992) Contents of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. J. Agric. Food Chem. 40, 2379–2383.
12. Crozier, A., Lean, M. E., McDonald, M. S. and Black, C. (1997) Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. J. Agric. Food Chem. 45, 595.
13. Ewald, C., Fjelkner-Modig, S. and Johansson, K. *et al* (1999) Effect of processing on major flavonoids in processed onions, green beans, and peas. Food Chem. 64, 231–235.
14. Price, K. R., Bacon, J. R. and Rhodes, M. (1997) Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion *(Allium cepa).* J. Agric. Food Chem. 45, 938–942.
15. Hirota, S., Shimoda, T. and Takehama, U. (1998) Tissue ans spatial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. J. Agric. Food Chem. 46, 3497–3502.
16. Andlauer, W., Stumpf, C. and Fürst, P. (2000) Influence of the acetification process on phenolic compounds. J. Agric. Food Chem. 48, 3533–3536.
17. Friedman, M. (1997) Chemistry, biochemistry, and dietary role of potato polyphenols. A Review. J. Agric. Food Chem. 45, 1523–1540.
18. Herrmann, K. (1989) Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in food. Crit. Rev. Food Sci. Nutr. 28, 315–340.
19. Rawel, H. M., Rohn, S. and Kroll, J. (2000) Reactions of selected secondary plant metabolites (glucosinolates and phenols) with food proteins and enzymes – Influence on physico-chemical protein properties, enzyme activity and proteolytic degradation. Recent Res. Devel. Phytochem. 4, 115–142.
20. Hempel, J. and Böhm, H. (1996) Quality and quantity of prevailing flavonoid glycosides of yellow and green french beans *(Phaseolus vulgaris L.).* J. Agric. Food Chem. 44, 2114–2116.
21. Ramamurthy, M. S., Maiti, B., Thomas, P. and Nair, P. M. (1992) High performance liquid chromatographic determination of phenolic acids in potato tubers *(Solanum tuberosum)* wound healing. J. Agric. Food Chem. 40, 569–572.
22. Dao, L. and Friedman, M. (1992) Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. J. Agric. Food Chem. 40, 2152–2156.
23. Dao, L. and Friedman, M. (1994) Chlorophyll, chlorogenic acid, glycoalkaloid, and protease inhibitor content of fresh and greeen potatoes. J. Agric. Food Chem. 42, 633–639.

Wilfried Andlauer, Ph.D.

Haute Ecole Valaisanne
Route du Rawyl 47
CH-1950 Sion 2, Switzerland
Phone +41 276 068 652
Fax +41 276 068 515
E-mail: Wilfried.Andlauer@hevs.ch