

Application of Traditional Cooking Methods in Chestnut Processing: Effects of Roasting and Boiling on Secondary Metabolites and Antioxidant Capacity in *Castanea* spp. Fruits

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SUPPLEMENTARY MATERIALS

Solvents, Chemicals, and Standards

Analytical HPLC grade solvents (acetonitrile, methanol, and formic acid), reagents for HPLC buffer (potassium dihydrogen phosphate and phosphoric acid) were purchased from Fluka Biochemika (Buchs, Switzerland) and Sigma–Aldrich (St Louis, MO, USA).

All polyphenolic standards (caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, hyperoside, isoquercitrin, quercetin, quercitrin, rutin, ellagic acid, gallic acid, catechin, epicatechin, castalagin, vescalagin) were purchased from Sigma–Aldrich. Milli-Q ultrapure water was produced by Sartorius Stedim Biotech mod. Arium (Sartorius, Göttingen, Germany).

Stock solutions of cinnamic acids and flavonols with a concentration of 1.0 mg mL⁻¹ were prepared in methanol. From these solutions, four calibration standards (1000 ppm, 50 ppm, 250 ppm, 125 ppm) were prepared by dilution with methanol; stock solutions of benzoic acids, tannins, and catechins with a concentration of 1.0 mg mL⁻¹ were prepared in a solution of 95% methanol and 5% water. From these solutions, four calibration standards were prepared by dilution with 50% methanol-water.

Table S1. Chromatographic conditions of the used methods.

Method	Compounds of Interest	Stationary Phase	Mobile Phase	Flow (mL min ⁻¹)	Wavelength (nm)
A	cinnamic acids, flavonols	KINETEX—C18 column (4.6 × 150 mm, 5 μm)	A: 10 mM KH ₂ PO ₄ /H ₃ PO ₄ , pH = 2.8 B: CH ₃ CN	1.5	330
B	benzoic acids, catechins, Tannins	KINETEX—C18 column (4.6 × 150 mm, 5 μm)	A: H ₂ O/CH ₃ OH/HCOOH (5:95:0.1 v/v/v), pH = 2.5 B: CH ₃ OH/HCOOH (100:0.1 v/v)	0.6	280

Method A—gradient analysis: 5% B to 21% B in 17 min + 21% B in 3 min (2 min conditioning time).

Method B—gradient analysis: 3% B to 85% B in 22 min + 85% B in 1 min (2 min conditioning time).

Table S2. The chromatographic fingerprint of the analysed chestnut groups.

Sample	Cinnamic acids							
	caffeic acid (mg/100 g DW)		chlorogenic acid (mg/100 g DW)		coumaric acid (mg/100 g DW)		ferulic acid (mg/100 g DW)	
CB	0.53	0.08	0.15	0.03	3.17	0.68	1.20	0.04
CC	1.70	0.27	0.18	0.04	9.29	0.86	1.62	0.25
CF	1.05	0.12	0.53	0.12	6.13	0.73	0.81	0.17
IB	3.71	0.05	2.24	0.12	4.38	0.56	3.29	0.26
IC	4.42	0.60	2.56	0.07	12.19	0.64	4.60	1.15
IF	4.10	0.39	2.06	0.03	7.98	0.85	3.63	0.14
MB	4.77	0.01	4.11	0.55	7.07	0.49	5.73	0.52
MC	7.68	0.51	3.70	0.35	13.62	0.83	6.98	0.41
MF	6.03	0.81	3.41	0.60	10.90	1.31	5.85	1.06
SB	2.32	0.33	4.34	0.16	7.68	0.79	5.90	0.36
SC	4.40	0.62	4.48	0.50	13.91	0.77	7.30	0.78
SF	3.56	0.95	3.68	0.12	10.89	0.55	5.89	0.61

Sample	Flavonols									
	hyperoside (mg/100 g DW)		isoquercitrin (mg/100 g DW)		quercetin (mg/100 g DW)		quercitrin (mg/100 g DW)		rutin (mg/100 g DW)	
CB	2.59	0.56	2.16	0.21	0.81	0.04	1.20	0.05	1.15	0.05
CC	2.44	0.31	2.19	0.03	0.45	0.09	1.46	0.12	0.78	0.09
CF	2.14	0.15	2.64	0.22	0.60	0.04	1.21	0.03	1.37	0.06
IB	5.23	0.57	4.82	0.49	2.38	0.08	2.88	0.08	3.34	0.11
IC	4.85	0.46	4.01	0.74	2.47	0.18	3.56	0.20	3.25	0.06
IF	4.24	0.64	4.33	0.42	2.72	0.06	2.25	0.09	3.04	0.27
MB	7.51	0.27	5.96	0.48	3.88	0.73	4.45	0.50	5.42	0.60
MC	6.39	0.04	5.54	1.16	3.67	0.75	4.81	0.31	5.23	0.58
MF	6.84	0.29	6.60	0.47	3.36	0.36	4.55	0.51	5.37	0.54
SB	7.13	0.50	6.74	0.25	3.98	0.18	4.70	0.07	5.56	0.15
SC	6.80	0.68	5.97	0.68	3.78	0.16	4.81	0.17	5.09	0.24
SF	6.66	0.11	6.67	0.42	3.65	0.16	4.66	0.04	5.45	0.21

Sample	Benzoic acids				Catechins				Tannins			
	ellagic acid (mg/100 g DW)		gallic acid (mg/100 g DW)		catechin (mg/100 g DW)		epicatechin (mg/100 g DW)		castalagin (mg/100 g DW)		vescalagin (mg/100 g DW)	
CB	12.51	1.03	41.06	2.19	128.96	1.30	7.51	1.12	13.44	2.30	84.05	5.52
CC	10.16	0.61	21.12	1.25	63.73	0.73	3.92	0.55	20.23	0.62	104.08	1.83
CF	43.48	2.23	57.88	2.70	98.75	0.74	1.09	0.19	36.71	1.55	118.86	3.57
IB	15.06	1.79	47.72	1.41	134.99	2.25	9.94	0.68	19.79	1.65	93.75	2.88
IC	12.67	0.84	24.03	0.56	70.94	1.15	6.74	0.77	29.17	1.96	112.28	0.66
IF	44.38	1.54	63.67	1.13	105.29	1.36	3.12	0.46	41.61	2.29	127.89	2.32
MB	16.98	0.68	53.24	0.20	142.95	1.24	12.04	1.38	26.29	1.25	101.59	1.04
MC	14.97	0.56	27.67	0.13	78.13	1.11	8.36	1.40	34.47	0.27	121.12	1.09
MF	46.65	0.93	69.75	1.02	113.29	1.40	4.84	0.90	48.65	1.14	136.48	0.73
SB	16.93	0.69	53.43	0.26	143.23	1.15	12.07	0.99	26.70	1.02	102.42	0.75
SC	14.83	0.64	27.69	0.62	78.38	0.83	8.55	0.65	34.71	0.66	121.16	0.55
SF	46.74	1.00	70.44	0.76	113.16	0.91	5.38	0.36	49.36	0.19	137.24	0.49

The mean value and standard deviation (SD) of each sample are given (n = 3).

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