

Supplementary Materials

# Bioactive Compounds and Antioxidant Activity of Selected Pumpkin Cultivars: Impact of Cooking Treatments <sup>†</sup>

Roxana E. González <sup>1,2,\*</sup>, María B. Botella <sup>2,3</sup> and Pamela Y. Quintas <sup>2,3</sup>

<sup>1</sup> Estación Experimental Agropecuaria La Consulta, Instituto Nacional de Tecnología Agropecuaria (INTA), Ex ruta 40 km 96, La Consulta, Mendoza, 5567, Argentina

<sup>2</sup> Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, Mendoza, 5500, Argentina; marenas@mendoza-conicet.gob.ar (M.B.B.); pquintas@mendoza-conicet.gob.ar (P.Y.Q.)

<sup>3</sup> Laboratorio de Química Analítica para Investigación y Desarrollo (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo / Instituto Interdisciplinario de Ciencias Básicas (ICB), CONICET UNCUYO, Padre J. Contreras 1300, Mendoza, 5500, Argentina

\* Correspondence: gonzalez.roxana@inta.gob.ar or rgonzalezs@fcen.uncu.edu.ar

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**Table S1.** Temperature and time for each cooking treatment.

Cooking treatment	Conditions of processing	
	Temperature	Time
Boiling	100 °C	10 min.
Steaming	100 °C	20 min.
Baking	180 °C	10 min
Microwaving	800 W	5 min

**Table S2.** Phenolic compounds: flavanol, flavanones, flavonols and flavonones levels in samples of pumpkin exposed to different cooking treatments, expressed in  $\mu\text{g/g}$  fw (fresh weight). The values reported are the mean of three replicates along with their corresponding standard deviation.

Cultivar	Cooking methods	Phenolic compounds ( $\mu\text{g/g}$ fw)					
		Flavanol Catechin	Flavanones Naringenin	Apigenin	Flavonols Quercetin	Rutin	Flavonones Luteolin
Cuyano INTA	Raw	11.56 $\pm$ 0.20	0.28 $\pm$ 0.01	0.17 $\pm$ 0.00	1.08 $\pm$ 0.04	0.23 $\pm$ 0.01	0.77 $\pm$ 0.05
	Boiling	4.63 $\pm$ 0.38	0.20 $\pm$ 0.00	0.04 $\pm$ 0.00	1.35 $\pm$ 0.05	0.51 $\pm$ 0.03	0.34 $\pm$ 0.02
	Baking	0.69 $\pm$ 0.10	2.02 $\pm$ 0.02	0.37 $\pm$ 0.01	1.09 $\pm$ 0.12	0.34 $\pm$ 0.00	0.23 $\pm$ 0.01
	Steaming	1.58 $\pm$ 0.05	0.10 $\pm$ 0.02	0.18 $\pm$ 0.01	1.66 $\pm$ 0.20	0.34 $\pm$ 0.01	0.06 $\pm$ 0.01
	Microwav- ing	2.16 $\pm$ 0.11	0.16 $\pm$ 0.02	1.46 $\pm$ 0.11	0.98 $\pm$ 0.01	0.47 $\pm$ 0.03	0.57 $\pm$ 0.10
Cokena INTA	Raw	10.81 $\pm$ 0.36	8.81 $\pm$ 0.18	0.77 $\pm$ 0.02	1.02 $\pm$ 0.16	0.44 $\pm$ 0.00	15.04 $\pm$ 0.64
	Boiling	11.04 $\pm$ 0.23	0.62 $\pm$ 0.07	2.16 $\pm$ 0.06	0.99 $\pm$ 0.04	0.36 $\pm$ 0.01	5.16 $\pm$ 0.01
	Baking	2.73 $\pm$ 0.14	0.48 $\pm$ 0.05	1.41 $\pm$ 0.18	1.39 $\pm$ 0.05	0.36 $\pm$ 0.01	1.01 $\pm$ 0.06
	Steaming	8.20 $\pm$ 0.01	1.48 $\pm$ 0.17	1.44 $\pm$ 0.09	0.98 $\pm$ 0.01	0.64 $\pm$ 0.09	1.44 $\pm$ 0.12
	Microwav- ing	8.20 $\pm$ 0.01	1.48 $\pm$ 0.17	1.44 $\pm$ 0.09	0.98 $\pm$ 0.01	0.64 $\pm$ 0.09	1.44 $\pm$ 0.12
Dorado INTA	Raw	1.01 $\pm$ 0.17	1.02 $\pm$ 0.03	1.19 $\pm$ 0.03	1.07 $\pm$ 0.00	0.51 $\pm$ 0.04	0.28 $\pm$ 0.02
	Boiling	3.98 $\pm$ 0.21	0.85 $\pm$ 0.04	1.07 $\pm$ 0.19	< LOD <sup>a</sup>	0.31 $\pm$ 0.01	0.49 $\pm$ 0.03
	Baking	2.74 $\pm$ 0.30	3.76 $\pm$ 0.71	0.48 $\pm$ 0.02	0.42 $\pm$ 0.08	0.52 $\pm$ 0.07	2.65 $\pm$ 0.35
	Steaming	0.79 $\pm$ 0.02	2.32 $\pm$ 0.17	1.13 $\pm$ 0.06	0.54 $\pm$ 0.09	0.35 $\pm$ 0.00	2.15 $\pm$ 0.00
	Microwav- ing	2.10 $\pm$ 0.12	0.03 $\pm$ 0.00	0.42 $\pm$ 0.05	0.81 $\pm$ 0.11	0.37 $\pm$ 0.08	1.12 $\pm$ 0.11
Paquito INTA	Raw	0.14 $\pm$ 0.02	0.58 $\pm$ 0.13	2.27 $\pm$ 0.06	0.42 $\pm$ 0.00	0.42 $\pm$ 0.01	0.95 $\pm$ 0.08
	Boiling	12.43 $\pm$ 0.05	65.59 $\pm$ 0.22	0.14 $\pm$ 0.01	0.82 $\pm$ 0.01	0.35 $\pm$ 0.01	90.83 $\pm$ 0.65
	Baking	4.37 $\pm$ 0.71	1.05 $\pm$ 0.03	1.23 $\pm$ 0.19	0.74 $\pm$ 0.07	0.57 $\pm$ 0.04	0.10 $\pm$ 0.02
	Steaming	3.29 $\pm$ 0.07	2.15 $\pm$ 0.06	0.62 $\pm$ 0.00	1.36 $\pm$ 0.03	0.46 $\pm$ 0.01	1.88 $\pm$ 0.05
	Microwav- ing	15.08 $\pm$ 1.32	1.04 $\pm$ 0.16	1.06 $\pm$ 0.04	1.48 $\pm$ 0.01	0.37 $\pm$ 0.00	1.18 $\pm$ 0.04

<sup>a</sup>LOD: Limits of detection.

**Table S3.** Phenolic compounds: hydrocinnamic acid levels in samples of pumpkin exposed to different cooking treatments, expressed in  $\mu\text{g/g}$  fw (fresh weight). The values reported are the mean of three replicates along with their corresponding standard deviation.

Cultivar	Cooking methods	Phenolic compounds ( $\mu\text{g/g}$ fw)						
		Hydrocinnamic acids					Cinnamic acid	Chlorogenic acid
		Caffeic acid	Ferulic acid	Sinapic acid	Cumaric acid			
Cuyano INTA	Raw	0.23 ± 0.01	0.33 ± 0.02	6.32 ± 0.74	0.22 ± 0.00	0.05 ± 0.00	2.77 ± 0.15	0.00 ± 0.00
	Boiling	0.27 ± 0.01	0.35 ± 0.02	0.35 ± 0.01	0.28 ± 0.02	0.42 ± 0.00	0.19 ± 0.02	22.02 ± 1.15
	Baking	0.19 ± 0.00	1.59 ± 0.10	2.79 ± 0.42	0.22 ± 0.00	0.42 ± 0.01	0.45 ± 0.07	0.00 ± 0.00
	Steaming	0.20 ± 0.01	1.37 ± 0.00	2.21 ± 0.31	0.33 ± 0.00	0.07 ± 0.00	0.52 ± 0.10	0.00 ± 0.00
	Microwaving	1.53 ± 0.18	0.19 ± 0.02	5.62 ± 0.21	0.31 ± 0.00	0.45 ± 0.02	0.27 ± 0.03	1.09 ± 0.11
Cokena INTA	Raw	0.14 ± 0.02	0.08 ± 0.00	6.40 ± 0.19	0.15 ± 0.02	0.28 ± 0.05	0.20 ± 0.00	5.07 ± 0.16
	Boiling	0.14 ± 0.01	3.65 ± 0.04	3.10 ± 0.13	0.24 ± 0.00	1.34 ± 0.02	0.09 ± 0.00	10.85 ± 0.85
	Baking	0.16 ± 0.01	0.02 ± 0.00	6.60 ± 0.05	0.15 ± 0.02	0.24 ± 0.02	0.19 ± 0.00	< LOD <sup>a</sup>
	Steaming	0.14 ± 0.02	0.07 ± 0.01	5.91 ± 0.04	5.08 ± 0.04	0.50 ± 0.15	0.27 ± 0.01	6.35 ± 0.00
	Microwaving	0.14 ± 0.02	0.07 ± 0.01	5.91 ± 0.04	5.08 ± 0.04	0.50 ± 0.15	0.27 ± 0.01	6.35 ± 0.00
Dorado INTA	Raw	0.26 ± 0.01	3.93 ± 0.00	6.01 ± 0.00	0.19 ± 0.01	0.55 ± 0.08	0.96 ± 0.03	3.80 ± 0.29
	Boiling	0.16 ± 0.04	0.21 ± 0.01	2.50 ± 0.05	0.19 ± 0.00	0.47 ± 0.00	0.41 ± 0.02	< LOD <sup>a</sup>
	Baking	0.56 ± 0.06	0.68 ± 0.30	1.39 ± 0.32	0.33 ± 0.05	0.37 ± 0.06	0.11 ± 0.03	< LOD <sup>a</sup>
	Steaming	0.21 ± 0.00	0.28 ± 0.00	2.70 ± 0.01	0.13 ± 0.00	0.48 ± 0.00	0.29 ± 0.00	< LOD <sup>a</sup>
	Microwaving	0.17 ± 0.03	0.31 ± 0.03	1.68 ± 0.03	0.92 ± 0.14	0.20 ± 0.02	0.47 ± 0.03	< LOD <sup>a</sup>
Paquito INTA	Raw	1.79 ± 0.04	1.94 ± 0.00	3.06 ± 0.01	0.17 ± 0.01	0.43 ± 0.00	0.14 ± 0.01	0.57 ± 0.02
	Boiling	0.53 ± 0.04	0.17 ± 0.01	2.06 ± 0.13	0.27 ± 0.01	0.57 ± 0.08	0.25 ± 0.03	0.13 ± 0.00
	Baking	0.26 ± 0.02	0.21 ± 0.01	0.51 ± 0.00	0.30 ± 0.02	0.51 ± 0.03	0.12 ± 0.01	< LOD <sup>a</sup>
	Steaming	0.17 ± 0.00	0.18 ± 0.01	1.09 ± 0.10	0.17 ± 0.01	0.44 ± 0.01	0.12 ± 0.00	< LOD <sup>a</sup>
	Microwaving	0.08 ± 0.01	0.25 ± 0.01	2.08 ± 0.02	4.16 ± 0.05	0.37 ± 0.00	0.26 ± 0.01	0.19 ± 0.01

<sup>a</sup>LOD: Limits of detection.

**Table S4.** Phenolic compounds: hydroxybenzoic and phenyl alcohol levels in samples of pumpkin exposed to different cooking treatments, expressed in  $\mu\text{g/g}$  fw (fresh weight). The values reported are the mean of three replicates along with their corresponding standard deviation.

Cultivar	Phenolic compounds ( $\mu\text{g/g}$ fw)		
	Cooking methods	Hydroxybenzoic acids	Phenyl alcohol
		Vanillic acid	Tyrosol
Cuyano INTA	Raw	< LOQ <sup>b</sup>	0.28 $\pm$ 0.01
	Boiling	0.35 $\pm$ 0.02	0.20 $\pm$ 0.00
	Baking	0.06 $\pm$ 0.03	1.16 $\pm$ 0.03
	Steaming	< LOQ <sup>b</sup>	0.10 $\pm$ 0.02
	Microwaving	5.16 $\pm$ 0.13	0.16 $\pm$ 0.02
Cokena INTA	Raw	< LOQ <sup>b</sup>	0.09 $\pm$ 0.03
	Boiling	6.29 $\pm$ 0.05	0.62 $\pm$ 0.07
	Baking	1.04 $\pm$ 0.01	0.48 $\pm$ 0.05
	Steaming	< LOQ <sup>b</sup>	1.06 $\pm$ 0.16
	Microwaving	< LOQ <sup>b</sup>	1.06 $\pm$ 0.16
Dorado INTA	Raw	0.66 $\pm$ 0.00	1.02 $\pm$ 0.03
	Boiling	< LOQ <sup>b</sup>	0.85 $\pm$ 0.04
	Baking	0.47 $\pm$ 0.01	2.27 $\pm$ 0.47
	Steaming	4.04 $\pm$ 0.04	0.65 $\pm$ 0.15
	Microwaving	< LOQ <sup>b</sup>	0.03 $\pm$ 0.00
Paquito INTA	Raw	0.02 $\pm$ 0.00	0.58 $\pm$ 0.13
	Boiling	0.26 $\pm$ 0.01	0.82 $\pm$ 0.01
	Baking	0.06 $\pm$ 0.01	1.05 $\pm$ 0.03
	Steaming	< LOQ <sup>b</sup>	0.50 $\pm$ 0.03
	Microwaving	< LOQ <sup>b</sup>	1.04 $\pm$ 0.16

<sup>b</sup>LOQ: Limits of quantification.

**Table S5.** Enantiomers D and L (Trp and Tyr) levels in samples of pumpkin exposed to different cooking treatments, expressed in  $\mu\text{g/g}$  fw (fresh weight). The values reported are the mean of three replicates along with their corresponding standard deviation.

Cultivars	Cooking treatment	L-Tyr	D-Tyr	D-Trp	L-Trp
Cuyano INTA	Raw	196 $\pm$ 8	< LOD <sup>a</sup>	2.02 $\pm$ 5.28E-3	15.1 $\pm$ 0.1
	Boiling	121 $\pm$ 1	< LOD <sup>a</sup>	2.06 $\pm$ 3.33E-2	8.88 $\pm$ 0.2
	Steaming	216 $\pm$ 6	< LOD <sup>a</sup>	1.04 $\pm$ 1.77E-2	24.6 $\pm$ 6.6E-2
	Baking	224 $\pm$ 5	< LOD <sup>a</sup>	2.03 $\pm$ 5.38E-2	18.7 $\pm$ 7.2E-2
	Microwaving	77.9 $\pm$ 2.7	< LOD <sup>a</sup>	< LOQ <sup>b</sup>	5.11 $\pm$ 5.3E-2
Cokena INTA	Raw	224 $\pm$ 5	< LOD <sup>a</sup>	< LOD <sup>a</sup>	19.1 $\pm$ 0.1
	Boiling	177 $\pm$ 5	< LOD <sup>a</sup>	1.65 $\pm$ 3.41E-2	12.4 $\pm$ 4.6E-2
	Steaming	333 $\pm$ 2	< LOD <sup>a</sup>	2.54 $\pm$ 4.01E-2	30.0 $\pm$ 2.8E-2
	Baking	337 $\pm$ 5	< LOD <sup>a</sup>	2.00 $\pm$ 4.67E-2	< LOQ <sup>b</sup>
	Microwaving	339 $\pm$ 5	< LOD <sup>a</sup>	3.58 $\pm$ 7.34E-2	26.0 $\pm$ 4.4E-2
Dorado INTA	Raw	259 $\pm$ 2	< LOD <sup>a</sup>	4.08 $\pm$ 3.22E-2	14.0 $\pm$ 0.2
	Boiling	119 $\pm$ 4	< LOD <sup>a</sup>	0.98 $\pm$ 3.81E-2	11.9 $\pm$ 5.9E-2
	Steaming	204 $\pm$ 1	< LOD <sup>a</sup>	< LOD <sup>a</sup>	22.3 $\pm$ 9.9E-2
	Baking	95.5 $\pm$ 0.2	< LOD <sup>a</sup>	0.99 $\pm$ 2.79E-2	6.21 $\pm$ 7.69E-2
	Microwaving	445 $\pm$ 4	< LOD <sup>a</sup>	13.7 $\pm$ 9.5E-2	45.6 $\pm$ 0.2
Paquito INTA	Raw	291 $\pm$ 11	< LOD <sup>a</sup>	< LOQ <sup>b</sup>	67.0 $\pm$ 1.0
	Boiling	414 $\pm$ 2	< LOD <sup>a</sup>	1.07 $\pm$ 6.59E-2	49.5 $\pm$ 3.7E-2
	Steaming	586 $\pm$ 5	< LOD <sup>a</sup>	< LOD <sup>a</sup>	64.1 $\pm$ 0.2
	Baking	583 $\pm$ 5	< LOD <sup>a</sup>	1.54 $\pm$ 2.19E-2	79.2 $\pm$ 4.0
	Microwaving	729 $\pm$ 29	< LOD <sup>a</sup>	1.21 $\pm$ 6.44E-2	86.8 $\pm$ 1.7

<sup>a</sup>LOD: Limits of detection; <sup>b</sup>LOQ: Limits of quantification.

**Table S6.** ANOVA table of Try and Trp variation due to cultivar, cooking treatment, and their interaction.

<b>L-Tyr</b>					
Source	d.f	Sum of square		F value	p-value
Cooking treatment	4	229555.46	(11.1%)	877.22	<0.0001
Cultivar	3	1578183.62	(76.2%)	8041.1	<0.0001
Cooking treatment*Cultivar	12	261154.04	(12.6%)	332.66	<0.0001
Error	40	2616.86	(0.1%)		
Total	59	2071509.98	(100%)		
<b>L-Trp</b>					
Source	d.f	Sum of square		F value	p-value
Cooking treatment	4	2985.51	(7.8%)	1970.48	<0.0001
Cultivar	3	30643.01	(79.7%)	727.99	<0.0001
Cooking treatment*Cultivar	12	4756.05	(12.4%)	9962.76	<0.0001
Error	40	41.01	(0.1%)	386.58	<0.0001
Total	59	38425.57	(100%)		
<b>D-Trp</b>					
Source	d.f	Sum of square		F value	p-value
Cooking treatment	4	508.09	(20.5%)	17039.32	<0.0001
Cultivar	3	104.28	(16.7%)	18502.62	<0.0001
Cooking treatment*Cultivar	12	84.93	(62.8%)	17368.07	<0.0001
Error	40	318.88	(0.01%)	17039.32	<0.0001
Total	59	0.06	(100%)		