Supplementary material

Broccoli glucosinolates degradation is reduced when thermal treatment is performed in binary systems with other food ingredients

Materials and methods

Statistics

Experiment 1: four factor factorial, including the effects of: mixture (5 levels: broccoli only control, and the four combination of broccoli and added ingredient), heating temperature (two levels: 90 and 100 °C), ingredient ratio (two levels: 1/1 and 1/9 broccoli/added ingredient), heating time (two levels: Time zero, after 300 minutes). The data of total GLs and glucoraphanin were also transformed into relative amounts with respect to the initial content (Time zero): in this case the data were processed as a three-factor factorial, missing the time of heating effect.

Experiment 2: two factor factorial, including the effects of: mixture (four levels: broccoli only control and 1/1, 3/7, 1/9 broccoli/onion ratio) and heating time (six levels: 0, 15, 30, 60, 120, 300 min.)

Experiment 3: two factor factorial, including the effects of: mixture (four levels: broccoli only control and 1/9 ratio with three different onion varieties) and heating time (six levels: as in experiment 2).

In experiment 1, the differences between the broccoli only control and the mixtures, within each of the other combinations of factors, have been tested by means of the Dunnett test. The differences between the other treatments have been tested by means of the Tukey HSD test. The kinetics of relative GLs and glucoraphanin variation have been analyzed and modeled according to the following procedure.

The data for which a series of time points was available, were used to preliminary explore the kind of kinetic model better fitting the data: a) the broccoli control and the three broccoli/onion weight ratios for experiment 2, and b) the broccoli control and the 1/9 ratio with three different onion varieties for experiment 3. Two procedures were adopted:

a) the trends of log-transformed or reciprocal-transformed relative (to Time zero) GLs and glucoraphanin content against time were tested for deviation from linearity, to test the better fit of a first order and a second order kinetic equation, respectively; higher order models were not considered, since data showed that they would not have added any further improvement, besides being empirical;

b) the untransformed data relative (to Time zero) GLs and glucoraphanin content were fitted by means of a) a first order; b) a second order; c) a n order kinetic equation, according to:

(1) $RC = RCi * e^{-k * t}$, first order;

(2) $RC = RCi / (1 + k^2 * t * RCi)$, second order;

(3) $RC = (RCi^{(1-n)} + (n-1) * k^n * t)^{(1 / (1-n))}$, n-order, where:

RC: relative (to Time zero) GL content (either total GLs or glucoraphanin); RCi: initial (at Time zero) RC; k, k^2, k^n: kinetic degradation constants for order 1, 2 and n relations, respectively; t: time from Time zero; n: order of the n-order equation.

In equation 3, therefore, the order n was estimated as a parameter. As a consequence, equations 1 and 2 had two parameters, whereas equation 3, had 3 parameters.

The n-order model was compared to both the order 1 and 2 order kinetic equations by means of the corrected Akaike information criterion (Burnham & Anderson, 2002).

The calculated rate constants from the best kinetic model resulting from the selection procedure (a second order equation, as it will be further illustrated) were related to the concentration of the broccoli/onion systems, to check the nature and significance of this relation. The experiment 2 data were used for model building, by fitting them by means of a generalized second order kinetic model in which the rate constant was a linear function of onion relative concentration, according to the relation:

(4) $RC = RCi / (1 + ((Ik^2 + Dk * RCONC) * t * RCi))$,

in which RC, RCi, t, have the same meaning as in (2);

the second order rate constant of (2): k^2, is here expressed as a linear function of onion relative concentration:

(5) $k^2 = Ik^2 + Dk * RCONC$, where:

Ik^2, value of the rate constant, at 0 onion concentration (broccoli only);

Dk, rate of k^2 variation, function of onion relative concentration;

RCONC, relative onion concentration in the model system.

In this form, all the three parameters (RCi, RCONC and Ik^2) have a functional meaning.

Finally, data of experiments 1 and 3 were used as validation set for the relation obtained, for a total of 84 validation points, including individual replications. In particular: a) the data of the broccoli control and the broccoli/onion systems at 1/1 and 1/9 ratio, at 100 °C heating temperatures, were used from experiment 1 (24 points); b) the data of the broccoli control and all combinations with three onion varieties (1/9 weight ratio), at different heating times, were used from experiment 3 (60 points).

The degradation kinetics of onion FLA was also assessed, including a zero-order kinetic, as suggested by the data pattern, in the form:

(6) $RCF = RCFi + k^0 * t$, where:

RCF: relative flavonoid content; RCFi: relative flavonoid content at Time zero; k^0: zero-order kinetic degradation constant.

Table 1S

Total FLA content, relative FLA content with respect to Time zero, and percentage individual FLA, in broccoli/onion systems and their trends during heating.

ratio**	Time of heating (min)	FLA (mg kg^{-1})	relative FLA	Flavonoid components* Q 4 GLC	Q 3,4′ DGLC	Q	I 4 GLC	others
				%				
1/9	0	3835 ± 248	1.00	62.5 ± 0.6	26.4 ± 0.7	3.1 ± 0.4	4.5 ± 0.1	3.5 ± 0.2
	15	3713 ± 272	0.97 ± 0.01	66.0 ± 0.4	21.1 ± 0.3	4.9 ± 0.2	4.2 ± 0.1	3.8 ± 0.2
	30	3876 ± 570	1.00 ± 0.10	71.6 ± 2.2	15.9 ± 2.8	6.2 ± 0.7	4.1 ± 0.1	3.7 ± 0.1
	60	3581 ± 197	0.93 ± 0.09	72.3 ± 4.3	13.7 ± 1.8	8.2 ± 1.7	4.2 ± 0.1	3.7 ± 0.2
	120	3168 ± 240	0.83 ± 0.02	67.4 ± 1.9	13.4 ± 2.8	11.4 ± 0.9	3.9 ± 0.2	4.0 ± 0.1
	300	2509 ± 98	0.65 ± 0.08	66.7 ± 9.3	12.8 ± 2.9	23.7 ± 1.5	3.6 ± 0.6	5.0 ± 0.9
k^0_{rfla}*			-0.0012±0.0001					
3/7	0	3683 ± 305	1.00	62.5 ± 0.13	26.5 ± 0.2	3.1 ± 0.1	4.4 ± 0.1	3.5 ± 0.1
	15	3460 ± 284	0.94 ± 0.02	66.0 ± 1.68	21.5 ± 2.2	4.6 ± 0.6	4.1 ± 0.1	3.8 ± 0.1
	30	3469 ± 250	0.94 ± 0.05	68.5 ± 1.25	17.6 ± 1.2	6.0 ± 0.7	4.0 ± 0.2	3.9 ± 0.3
	60	3273 ± 245	0.89 ± 0.07	69.8 ± 2.54	14.5 ± 3.1	7.7 ± 1.0	4.0 ± 0.3	3.9 ± 0.3
	120	2968 ± 563	0.81 ± 0.14	68.9 ± 2.55	11.3 ± 2.9	13.8 ± 2.0	3.5 ± 0.1	4.0 ± 0.5
	300	2042 ± 147	0.55 ± 0.07	53.8 ± 0.99	14.5 ± 3.1	22.0 ± 5.1	2.3 ± 0.2	5.6 ± 0.8

k^0_{rfla}*			-0.0014±0.0001					
1/1	0	3545 ± 280	1.00	63.1 ± 0.49	27.0 ± 0.4	3.0 ± 0.3	4.4 ± 0.2	3.7 ± 0.3
	15	3224 ± 146	0.91 ± 0.05	67.7 ± 1.18	21.6 ± 0.9	5.1 ± 0.8	4.0 ± 0.4	4.3 ± 0.6
	30	3453 ± 245	0.97 ± 0.05	72.2 ± 3.19	15.8 ± 1.3	5.9 ± 0.2	3.7 ± 0.2	3.9 ± 0.3
	60	2694 ± 338	0.76 ± 0.06	67.3 ± 3.54	19.0 ± 3.8	6.4 ± 1.6	3.4 ± 0.1	3.8 ± 0.3
	120	2327 ± 452	0.66 ± 0.08	65.3 ± 4.83	17.4 ± 3.5	11.4 ± 3.0	2.1 ± 0.1	4.9 ±1.0
	300	1607 ± 210	0.45 ± 0.08	52.5 ± 1.30	19.8 ± 3.8	20.7 ± 5.0	1.8 ± 0.1	5.4 ± 1.3
k^0_{rfla}*			-0.0018±0.0002					

*FLA; total flavonoids; RFLA: total flavonoids, relative to Time zero; Q 4 GLC: quercetin 4-glucoside; Q 3,4′ DGLC: quercetin 3,4′-diglucoside; Q: quercetin aglycone; I 4 GLC: isorhamnetin 4-glucoside; others: other FLA components.

**Broccoli/onion ratio: 1/9, 3/7 and 1/1.

***k^0_{rfla}: zero order rate kinetic constant for flavonoids.

Table 2S

Calculated parameters for the overall relation between total GL and glucoraphanin contents, relative to Time zero, time of heating and onion relative content of the model systems**.**

parameters*	**total GLs**	**glucoraphanin**
RCi	1.01±0.02	1.00 ± 0.02
Ik^2	0.040 ± 0.03	0.040 ± 0.03
Dk	-0.027 ± 0.04	-0.027 ± 0.05
overall r^2	0.93 **	0.91 **