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SHORT COMMUNICATION

Kinetic study of sulforaphane stability in blanched and un-blanched broccoli (*Brassica oleracea* var. *italica*) florets during storage at low temperatures

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Abstract Sulforaphane is a health-promoting compound found in broccoli. Given its high thermo-lability, its preservation through high-temperature processes seems inconvenient. Accordingly, storage at low temperature is an alternative. There are no studies about the evolution of sulforaphane content during storage at low temperatures. The change of sulforaphane content in blanched and un-blanched broccoli florets during storage at 10, − 1, − 21 and − 45 °C for 83 days was studied. In blanched broccoli, sulforaphane content followed a first-order degradation kinetics ($R^2 \geq 0.95$). A two-consecutive irreversible reactions model described adequately the evolution of sulforaphane content in un-blanched broccoli ($R^2 \geq 0.94$). Activation energies from Arrhenius equation resulted in 19.4 kJ/mol for blanched and 30 kJ/mol (formation) and 58 kJ/mol (degradation) for un-blanched broccoli. Storage of un-blanched broccoli at − 45 °C for 40 days maximized sulforaphane content. These results could be useful to propose broccoli storage conditions that preserve or maximize sulforaphane content.

Keywords Sulforaphane · Broccoli · Low temperature · Storage · Kinetics

List of symbols

C_G	Glucoraphanin concentration (μmol g^{-1} DM)
C_{G0}	Initial glucoraphanin concentration (μmol g^{-1} DM)
C_S	Sulforaphane concentration (μmol g^{-1} DM)
C_{S0}	Initial sulforaphane concentration (μmol g^{-1} DM)
C_T	Thiourea concentration (μmol g^{-1} DM)
DM	Dry matter
Ea	Activation energy (kJ/mol)
ESP	Epithiospecifier protein
GFN	Glucoraphanin
GSL	Glucosinolate
k	Degradation rate constant for sulforaphane in blanched broccoli (d^{-1})
k_0	Frequency factor in the Arrhenius equation (d^{-1})
k_1	Formation rate constant for sulforaphane in un-blanched broccoli (d^{-1})
k_2	Degradation rate constant for sulforaphane in un-blanched broccoli (d^{-1})
r	Correlation coefficient
R	Universal gas constant (kJ mol^{-1} K^{-1})
R^2	Determination coefficient
SFN	Sulforaphane
t	Time (d)
T	Temperature (°C)

Introduction

Sulforaphane (SFN) is a health-promoting compound naturally occurring in food (Ganai 2016). The precursor glucosinolate (GSL) of sulforaphane is glucoraphanin (GFN), which mainly occurs in broccoli (*Brassica oleracea* var. *italica*). The hydrolysis of GFN to yield sulforaphane proceeds through the action of myrosinase (β-Thioglucosidase Glucohydrolase, EC 3.2.1.147), which is physically segregated from GFN in the plant. Accordingly, sulforaphane formation occurs after disruption of the vegetable tissue. Competing reactions yield mainly nitriles, epithionitriles and thiocyanates, depending on the chemical

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conditions and on the presence of cofactors and other enzymes, such as epithiospecifier protein (ESP) (Gu et al. 2012). High-pressure processing (Westphal et al. 2017), blanching (Pérez et al. 2014), incubation (Mahn and Pérez 2016) and drying (Mahn et al. 2016) were proposed to maximize SFN formation in broccoli. However, SFN degrades above 40 °C, thus hindering its preservation through drying. An alternative preservation method is storage at low temperature; however, the information about the changes in sulforaphane content during the storage is scarce.

The degradation of sulforaphane and other antioxidant compounds during thermal treatments follows first order kinetics (Wu et al. 2014). Olivero et al. (2011) investigated the effect of moisture content (13–82%) and temperature (60–100 °C) on glucosinolates degradation kinetics in broccoli, and found that degradation of GSL follows a first order kinetics. Mahn et al. (2016) studied SFN evolution during tray drying, reporting formation and degradation of SFN depending on GFN availability and temperature. The authors showed that a two-consecutive irreversible reactions kinetic model described adequately the process, agreeing with Tanongkankit et al. (2011).

Knowledge about the stability of sulforaphane in broccoli during storage at low temperatures will contribute in the design of new preservation strategies in order to exploit its potential as functional food. The aim of this work was to investigate the evolution of sulforaphane content in blanched and un-blanched broccoli florets during storage at low temperatures.

Materials and methods

Chemicals

All chemicals were HPLC grade. Methylene chloride was purchased from J.T. Baker (Center Valley, PA, USA), anhydrous sodium sulfate and HPLC standards were purchased for Sigma–Aldrich (Schnelldorf, Germany). Organic solvents were purchased from Merck (Darmstadt, Germany).

Plant material

Broccoli (*Brassica oleracea var. italica*) cultivar Avenger heads were purchased at the local market (Santiago, Chile) to a single supplier. Broccoli heads were washed immediately after purchasing, cut into 5-cm × 0.7 cm width pieces, and divided in two groups: one group was blanched at 57 °C for 13 min in a thermostatic water bath (Stuart, United Kingdom, Great Britain), in order to maximize the sulforaphane content (Pérez et al. 2014). Blanched and un-blanched broccoli florets were put in an ice-water bath for 5 min; then put in sealed plastic bags and stored at different temperatures as stipulated by the experimental design. Temperature was monitored continuously.

Experimental design

Blanched and un-blanched broccoli florets were stored at 10 °C (refrigerator), − 1 °C (upper compartment of a refrigerator), − 21 °C (household freezer) and − 45 °C (industrial freezer), for up to 83 days, depending on the preservation status of the vegetable. In each storage condition 39 bags with blanched broccoli and 39 bags with un-blanched broccoli were used, each one contained 50-g broccoli. In each sampling, three bags containing blanched broccoli and three bags containing un-blanched broccoli were withdrawn. For storage at 10 and − 1 °C, sampling was conducted every two days until day 15, and every three days until day 41, when the vegetable was decomposed. For storage at − 21 and − 45 °C, samples were taken every 5 days until day 15 and once a week until day 83.

Sulforaphane content

Sulforaphane (SFN) was quantified by reverse phase HPLC (Pérez et al. 2014). 1 g of pulverized broccoli was extracted twice with 10-mL methylene-chloride combined with 0.5 g anhydrous sodium sulfate. The equipment was a HPLC–DAD (Shimadzu, Kyoto, Japan) equipped with a C18 column (5 μm particle size, 250 × 4.6 mm) (Agilent Technologies, Santa Clara, CA, USA). The solvent consisted of 20% acetonitrile in water; changing linearly over 10 min to 60% acetonitrile and maintained at 100% acetonitrile for 5 min. The temperature was set at 30 °C, the flow rate was 1 mL-min^{-1}, and injection volume was 20 mL. Absorbance at 254 nm was recorded. All measurements were made in triplicate.

Kinetic models

Blanching maximizes the conversion of glucoraphanin into sulforaphane (Pérez et al. 2014), then no SFN formation should occur during storage, but only its degradation. An irreversible first-order degradation kinetic model describes this behavior. The model considers that the main non-volatile product is N,N-di-(methylsulfinyl)butyl thiourea [Eq. (1)] (Jin et al. 1999). Equation (2), as suggested for glucosinolates and other bioactive compounds (Olivero et al. 2011; Gonçalves et al. 2011) described sulforaphane degradation in blanched broccoli. Here, k is the rate constant at temperature T, t is the storage time, C_S and C_{S0} are the sulforaphane content at t = t and t = 0, respectively.

$$Sulforaphane \xrightarrow{k} N,N-di-(methylsulfinyl)butylthiourea \quad (1)$$

$$\frac{C_S}{C_{S0}} = e^{-k_{(T)}\cdot t} \quad (2)$$

The temperature dependence of the rate constant follows the Arrhenius equation [Eq. (3)], where k_{ref} is the frequency factor at the reference temperature.

$$k = k_{ref}\cdot e^{-\frac{E_a}{R}\cdot\frac{1}{T}} \quad (3)$$

Combining Eqs. (2) and (3) gives a global model that describes the decrease of sulforaphane content in time as a function of temperature [Eq. (4)]. Here, C_S and C_{S0} are the sulforaphane content at t = t and t = 0, respectively. The model solution considered that the initial sulforaphane content was the maximum (at t = 0) (Table 1).

$$C_S = C_{S0}\cdot e^{\left(-k_{ref}\cdot e^{\left(-\frac{E_a}{R}\frac{1}{T}\right)}\right)\cdot t} \quad (4)$$

In un-blanched broccoli, myrosinase is active, the initial GFN content is high and therefore SFN formation occurs during storage until GFN depletion. Given the unstable nature of sulforaphane, its content should decrease after GFN depletion. Accordingly, sulforaphane evolution in unblanched broccoli was described by two-irreversible-consecutive reactions (Mahn et al. 2016), as shown in Eq. (5), where k_1 and k_2 are the rate constants.

$$GFN + H_2O \xrightarrow{k_1} SFN + D-glucose \xrightarrow{k_2} N,N-di-(methylsulfinyl)butylthiourea \quad (5)$$

Equations (6) and (7) represent the kinetic model, where C_S, C_G and C_T are the concentrations of sulforaphane, glucoraphanin and thiourea, respectively.

$$-\frac{dC_G}{dt} = k_1\cdot C_G \quad (6)$$

$$\frac{dC_S}{dt} = k_1\cdot C_G - k_2\cdot C_S \quad (7)$$

$$\frac{dC_T}{dt} = k_2\cdot C_S \quad (8)$$

Table 1 Kinetic parameters estimates (R^2 and MSE) of sulforaphane degradation in blanched broccoli stored at different temperatures

Temperature (°C)	K (d^{-1})	R^2	MSE	C_{s0} (μmol g^{-1} DM)
10	0.122	0.989	0.002	2.84 ± 0.04
− 1	0.052	0.961	0.007	2.83 ± 0.04
− 21	0.028	0.979	0.002	2.97 ± 0.24
− 45	0.014	0.950	0.004	2.98 ± 0.20

K is the rate constant, R^2 is the determination coefficient, MSE is the mean squared error and C_{S0} is the initial sulforaphane concentration

The analytical solution of the system composed by Eqs. (6) (7) and (8) is given in Eq. (9), where C_{G0} and C_{S0} are the initial (at t = 0) concentration of glucoraphanin and sulforaphane, respectively (Table 2). The initial concentration of thiourea was zero. The model was solved by estimating k_1, k_2 from the experimental data.

$$\frac{C_S}{C_{S0}} = e^{-k_2 t} + \frac{C_{G0}}{C_{S0}}\frac{k_1}{k_2-k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \quad (9)$$

Statistical analyses

The model adjustment to the experimental data resulted from minimization of the sum of squares. The fit quality was assessed by the determination coefficient and squared mean error (R^2 and SME, respectively).

Results and discussion

Blanched broccoli

Blanching maximized the conversion of glucoraphanin into sulforaphane (Pérez et al. 2014), then initial GFN content was low (0.17 ± 0.05 μmol g^{-1} DM). Figure 1 shows that at 10 and − 1 °C the normalized sulforaphane concentration decreased homogeneously following a first order kinetics. At − 21 °C and − 45 °C, the slope changed after 30 and 37 days of storage, respectively. This biphasic behavior may obey to higher release of sulforaphane and leakage from the vegetable cells due to tissue damage produced by the formation of ice crystals (at − 1 °C) or enzymatic degradation of cell wall (10 °C).

Despite the slope change observed at the lowest temperatures, the evolution of sulforaphane content was well described by a first order kinetic model (Fig. 1), with $R^2 \geq 0.95$ and MSE lower than 0.007 (Table 1). Higher temperatures produced faster degradation of the compound, as confirmed by the rate constants.

The rate constants fluctuated between 0.014 and 0.122 d^{-1}. Wu et al. (2014) investigated sulforaphane degradation in broccoli extract at temperatures between 60 and 90 °C, and found degradation rate constants of 0.24 and 11.3 d^{-1}, respectively. These values are higher than ours, probably because of the higher temperature used by the authors. Torres-Contreras et al. (2017) studied handling of fresh broccoli in order to enhance sulforaphane formation, concluding that sulforaphane content increased in 65% (final content of 2.4 μmol/g-DW) in intact broccoli florets stored for 24 h at 20 °C. The authors reported only two points: 0 and 24 h of storage, and reported no kinetics data. Gonçalves et al. (2011) studied the loss of ascorbic acid in

Table 2 Kinetic parameters estimates (R^2 and MSE) of sulforaphane degradation in un-blanched broccoli stored at different temperatures

Temperature (°C)	K_1 (d^{-1})	K_2 (d^{-1})	C_{G0} (μmol g^{-1} DM)	(μmol g^{-1} DM)	R^2	MSE
10	0.780	1×10^{-2}	1.31 ± 0.01	0.60 ± 0.19	0.936	0.060
− 1	0.635	4×10^{-3}	2.25 ± 0.03	0.79 ± 0.35	0.969	0.027
− 21	0.270	1 x 10^{-4}	2.01 ± 0.01	0.61 ± 0.20	0.954	0.093
− 45	0.038	3 x 10^{-5}	4.01 ± 0.03	0.60 ± 0.19	0.935	0.433

K_1 is the formation rate constant, K_2 is the degradation rate constant, C_{G0} is the initial glucoraphanin concentration, C_{S0} is the initial sulforaphane concentration, R^2 is the determination coefficient and MSE is the mean squared error

broccoli stored at − 15 °C, and found rate constants between 0.007 and 0.008 d^{-1}, one order of magnitude lower than our values. The slower degradation of ascorbic acid obeys to its higher stability in comparison with sulforaphane. Jaiswal et al. (2012) studied the degradation of antioxidants in cabbage during blanching at 80 to 100 °C. They reported kinetic constants between 150 and 292 d^{-1}, being four orders of magnitude higher than our constants. This difference may relate to the different compounds studied by the authors and the different food matrixes. Olivero et al. (2012) described the degradation of glucosinolates in broccoli at 60–120 °C by means of a first-order kinetic model, finding rate constants between 13 and 114 d^{-1} at 100 °C. Glucosinolates are more stable than sulforaphane, and then a lower degradation rate was expected. However, this is not true in this case, probably because of the high temperature used by the authors that makes it difficult to compare with our results.

The activation energy (Ea) from the Arrhenius equation was 19.4 kJ/mol, being in the same order of magnitude of Ea reported for thermal degradation of sulforaphane at pH 6.0 (70 kJ/mol) in a broccoli extract (Wu et al. 2014). The higher Ea found by the authors relates to the diffusional hurdles that occur in the vegetable matrix, which are absent in an extract.

The Ea found in our work is comparable with those reported for other antioxidant compounds in studies that consider first order degradation kinetics. Gonçalves et al. (2011) found an Ea of 60.2 kJ/mol for ascorbic acid. Olivero et al. (2012) reported an Ea of 96 kJ/mol for glucoraphanin (the precursor of sulforaphane) in broccoli during hydrothermal treatment. Jaiswal et al. (2012) found an Ea of 11.5 kJ/mol for phenolic compounds in cabbage during blanching.

Un-blanched broccoli

Un-blanched broccoli had higher glucoraphanin content, and then sulforaphane formation occurred during storage. After GFN depletion, sulforaphane degradation dominated until the end of the storage periods (Fig. 1). Despite the formation rate was lower at low temperatures; the lowest storage temperature gave the maximum relative concentration of sulforaphane, probably because of the formation of ice crystals that facilitate hydrolysis and extraction. The highest C/C_0 was obtained after 40 days of storage at − 45 °C, corresponding to a sevenfold increase of SFN content in comparison with the fresh vegetable. This value is similar to that reported by Mahn and Pérez (2016), who maximized the SFN content in broccoli florets by means of a three-step process: blanching, crushing and incubation. The authors reported a 5.8-fold increase in SFN content with respect to fresh broccoli. Then, our results suggest that it is possible to maximize sulforaphane formation by means of storage at − 45 °C for 40 days.

The lowest C/C_0 was attained in storage at 10 °C, unlike in storage below 0 °C, where tissue damage occurred due to formation of ice crystals. Then, myrosinase and glucoraphanin interact easily resulting in higher SFN formation. Additionally, during storage there is SFN formation and degradation simultaneously. As glucoraphanin is available for reaction, sulforaphane formation is the dominant phenomenon. Approaching glucoraphanin depletion, sulforaphane degradation dominates. The degradation rate was higher at higher temperatures. At − 45 °C no sulforaphane degradation was observed, probably because of the strong temperature dependence of the degradation reaction. Besides, since un-blanched broccoli suffered no thermal treatment, the action of other enzymes such as ESP would result in compounds different from sulforaphane. Consequently, the maximum relative sulforaphane content was lower at higher temperatures.

Sulforaphane formation and degradation were faster at higher temperatures, agreeing with the rate constants shown in Table 2. The two-consecutive irreversible reactions model adjusted adequately the experimental data, showing $R^2 \geq 0.94$. The lowest MSE came from storage at 10, − 1 and − 21 °C (MSE < 0.1). The fit at − 45 °C was more modest (MSE = 0.4). This relates with the low sulforaphane degradation rate observed in this condition, which the model could not describe satisfactorily. The

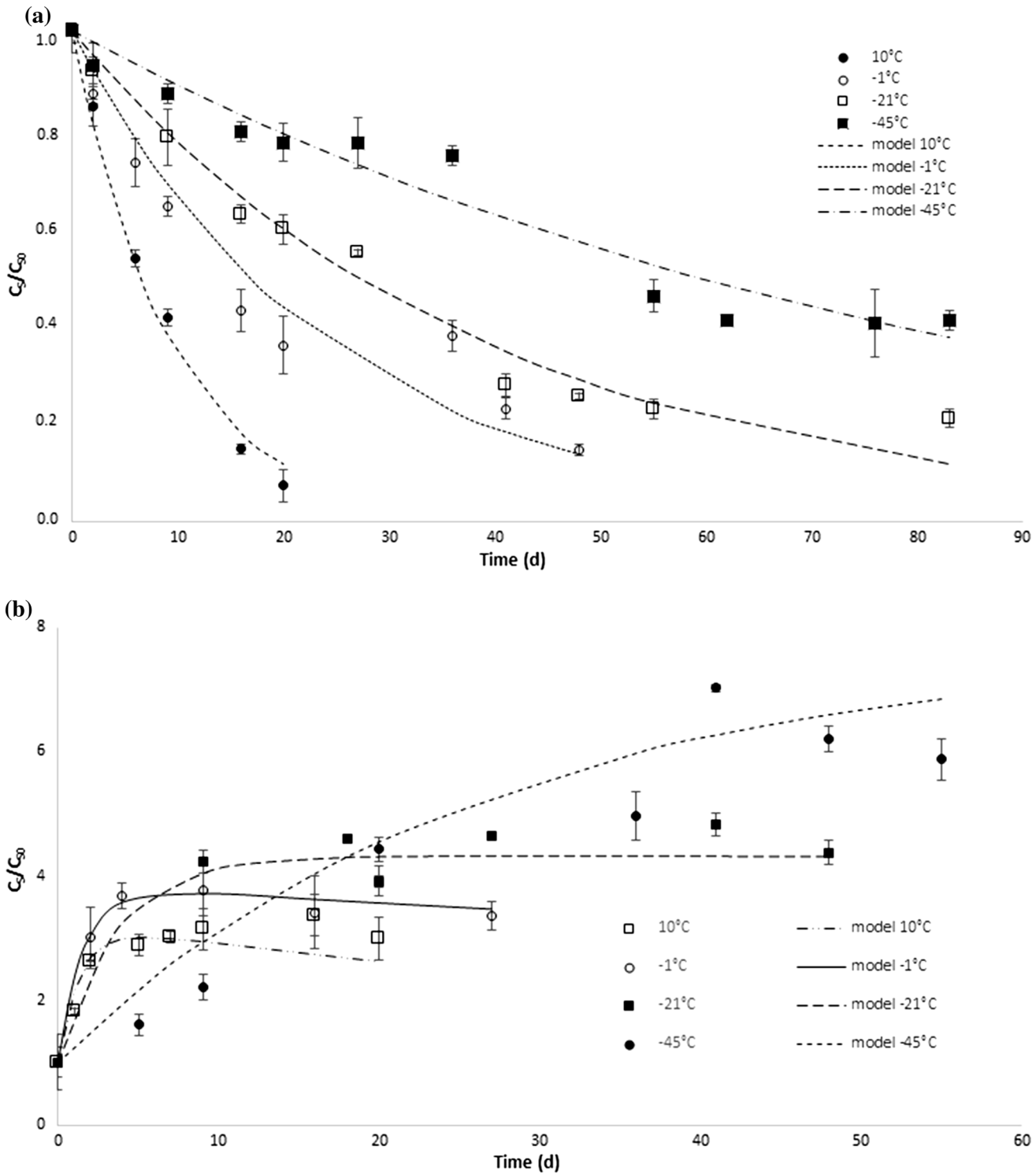

Fig. 1 Evolution of sulforaphane content in **a** blanched broccoli and **b** un-blanched broccoli, and model adjustment. Individual dots correspond to the experimental data, and the lines correspond to the kinetic model. Bars correspond to standard deviation at 95% confidence

model gave good fit to sulforaphane formation in this condition (Fig. 1).

The rate constants for sulforaphane formation (k_1) fluctuated between 0.038 and 0.780 d^{-1}, and the degradation rate constants (k_2) varied between $3{\cdot}10^{-5}$ and $1{\cdot}10^{-2}$ d^{-1}. Our results agree with values reported for phenolics in mandarin slices stored at 4 °C considering

two-consecutive reactions kinetics (Amodio et al. 2014) (k_1 and k_2 equal to 0.24 and 0.04 d^{-1}, respectively).

Ea for sulforaphane formation in un-blanched broccoli was 30 kJ/mol and Ea for degradation was 58 kJ/mol. No literature about the evolution of sulforaphane content in un-blanched broccoli during storage at low temperature was available. However, similar kinetic models described sulforaphane evolution during drying processes. Tanongkankit et al. (2011) studied the evolution of sulforaphane content in outer cabbage leaves during hot air drying. They considered two irreversible consecutive reactions and proposed a semi-empirical heat transfer and kinetic model. The authors reported activation energy for sulforaphane formation in the order of 10^3 [kJ/mol], two orders of magnitude higher than the values found in our work. This difference relates with the different food matrixes used by the authors. Mahn et al. (2016) studied the evolution of sulforaphane content in broccoli florets during drying at 60–80 °C, using a similar kinetic model. The authors reported activation energies for sulforaphane formation (k_1) between 48 and 112 kJ/mol and for sulforaphane degradation (k_2) between 58 and 70 kJ/mol, showing the same order of magnitude than Ea values obtained in this work. These similar outcomes obey probably to the use of the same food matrix, and agree with the behavior expected for the same reactions occurring under different temperatures.

Ea obtained for sulforaphane degradation in un-blanched broccoli was threefold the Ea estimated for blanched broccoli. Although these values fall within the same order of magnitude, we expected both values to be closer, because they correspond to the same reaction. This may owe to the blanching step, since this process alters the vegetable structure leading to an easier release of molecules from the cells. Accordingly, the degradation reaction in un-blanched broccoli apparently occurred at a slower rate because of diffusional hurdles. This explains the higher Ea found in this case. Another fact to consider is the presence of different enzymes that remain active in the un-blanched broccoli while totally (the case of ESP) or partially inactivated in blanched broccoli.

Conclusion

A first-order kinetic model described the evolution of sulforaphane content in blanched broccoli, while the two-consecutive irreversible reactions model represented it appropriately in un-blanched broccoli. The rate constants were proportional to temperature and the activation energies were in the same order of magnitude than those reported for other bioactive compounds. Ea obtained for sulforaphane degradation in un-blanched broccoli was threefold higher than that estimated for blanched broccoli, probably because of diffusional hurdles. Storage of un-blanched broccoli at − 45 °C for 40 days maximized sulforaphane content. However, the quality parameters were seriously impaired making the consumption of stored broccoli is inviable. The energy demand in storage at − 45 °C should be analyzed. Storage at − 45 °C can be proposed to obtain a bioactive extract from sulforaphane-rich broccoli intended to be a functional food or nutraceutical.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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