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Assessment of the stability of catechin-enriched extracts obtained from *Arbutus unedo* L. fruits: Kinetic mathematical modeling of pH and temperature properties on powder and solution systems

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ABSTRACT

Arbutus unedo L. (strawberry-tree) fruits could be considered an alternative source of flavan-3-ols, in particular catechin and its derivatives. These compounds are known for a variety of applications in food (especially as food additives), pharmaceutical and cosmetic industry. Therefore, catechin-enriched extracts (60% flavan-3-ols and 22% of catechin) were obtained from *A. unedo* fruits and further submitted to physical and chemical stability studies, considering the main affecting variables (time, temperature and pH): i) a stability study of the extracts during the obtaining and storage procedures (powder system); and ii) a stability study of the extracts in simulated food environment (aqueous solution system). The measured responses were the flavan-3-ols and catechin contents, determined by High performance liquid chromatography couple to a diode array detector (HPLC-DAD), and the antioxidant activity of the extracts evaluated by hydrophilic assays. Mechanistic and phenomenological equations were used to describe the responses, and the optimal conditions for flavan-3-ols (including catechin) stability as powder extract during a month were $\text{pH} = 5.4$ and $T = -20\,^{\circ}\text{C}$; while its stability in aqueous solution remained during the 24 h of application at $\text{pH} < 4$ and $T < 30\,^{\circ}\text{C}$. These results provide useful information for: i) potential industrial use of *A. unedo* fruits as alternative sources of flavan-3-ols; and ii) shelf-life calculations and catechin loss predictions at specific conditions of temperature and pH. Finally, the results obtained showed a certain agreement with previous reports of catechin stability studies in powder and solution systems, but providing a new alternative source: *A. unedo* fruits.

1. Introduction

Arbutus unedo L. (common name strawberry-tree, Ericaceae family) is an ornamental species native from the Mediterranean region that produces an edible reddish sweet berry that is a good source of health promoting compounds such as tocopherols, dietary fiber, carbohydrates, proteins, sugars and flavonoids. It is known in the pharmacology, food and feed sectors for its antiseptic, diuretic, laxative and cardiovascular positive effects (Ziyyat et al., 2002). However, apart from some traditionally uses as preservatives in compotes, wines and liqueurs, the fruits are consider as flavorless having a reduce number of applications due to its decomposition rates after ripening (Tardío et al., 2006). Most of the fruit production is discarded, because it only reaches a pleasant flavor during a short period of time, besides the lack of commercial plantations (Celikel et al., 2008; Males et al., 2006; Ruiz-Rodríguez et al., 2011). Therefore, valorizing and producing added-value functional compounds from this underused fruit could be modern innovation approaches, highly desirable by the industrial sector and research community (Alarcão-E-Silva et al., 2001; Seker and Toplu, 2010).

Most of the beneficial health effects and bioactive properties of the strawberry-tree fruit are linked to its high content in

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flavonoids (Dimitrios, 2006; Heim et al., 2002; Pietta, 2000). This fruit is an excellent source of flavan-3-ols, such as catechin and procyanidins (Barros et al., 2010; Guimarães et al., 2014). Flavan-3-ols, and among them the catechin compound, have been used worldwide namely by the nutraceutical sector to enrich pharmaceutical/cosmetic products, dietary supplements and functional foods (Frauen et al., 2002; Nagao et al., 2009; Shakar et al., 2008; Wang et al., 2000; Zaveri, 2006). They can be added as plant extracts, taking advantage of the synergistic effects between compounds, or as individual molecules, after purification (Gadkari and Balaraman, 2015). The most popular source of catechin is green tea presenting ~1.5 mg/g dw (Ananingsih et al., 2013; Zaveri, 2006). Cocoa is the fruit known with the highest content of catechin (~3 mg/g dw), followed by black grape (~0.2 mg/g dw), apricot (~0.15 mg/g dw), white grape (~0.09 mg/g dw), and being also present in a lower content in other fruits such as apple, pear, strawberry, peach, among others (Gadkari and Balaraman, 2015; Tsanova-Savova et al., 2005; Vinson et al., 1999; Wang et al., 2011; Wei et al., 2011). In this scenario, strawberry-tree fruits could be considered as alternative sources of mainly catechin and other flavan-3-ol compounds with reported values of ~1.4 mg/g of fruit dry weight (Albuquerque et al., n.d.).

Extraction is a crucial step before the isolation of compounds from plant materials (Ghasemzadeh et al., 2014; Heleno et al., 2016; Lee et al., 2013; Pinela et al., 2016). However, flavan-3-ols and catechin are susceptible to degradation by many environmental factors and the stability of these compounds during storage and application processes is an essential preliminary criterion that needs to be examined cautiously, being as important as the classic extraction (technique and conditions) optimization approaches. Understanding flavan-3-ols and catechin stability is essential to ensure the quality and delivery of these bioactive components. Despite the existent studies about the stability of flavan-3-ols and catechin during storage as powder or in aqueous systems (Komatsu et al., 2014; Li et al., 2012), the results cannot be directly extrapolated, being highly dependent on the plant matrix source. Thus, the stability patterns of flavan-3-ols and catechin from strawberry-tree fruits need to be assessed. The stability reaction of both systems share the central affecting variables, which have been identified as time (t), temperature (T) and pH (Komatsu et al., 2014; Zhu et al., 2002). Nonetheless, other factors such as concentration, moisture content, oxygen concentration, etc., have also been pointed out as relatively important (Li et al., 2011).

The key issue lies down behind the fact that flavan-3-ols and catechin undergo kinetic degradation and epimerization during thermal processing and that the pH of the surrounding environment accelerates/decelerates the kinetic degradations rates (Komatsu et al., 2014; Ruiz-Rodríguez et al., 2011). The analytical solution of such a system through mathematical models is important and necessary, but not exempt of complexity due to the heterogeneous responses of the variables involved. Achieving a successful mathematical model solution would allow to control most factors that affect the system, helping to standardize the key variables for producing stable plant-based extracts and therefore, to optimize the complete extraction process.

Therefore, the aims of this study were to: 1) valorize the underused strawberry-tree fruits as alternative sources of functional compounds, by obtaining catechin-enriched extracts (CEE); 2) study the stability of the extracts as powder and in aqueous solution (indicative of its potential performance in food matrices) systems, according with the main affecting variables (t, T and pH); 3) develop kinetic mathematical models for the simultaneous description of flavan-3-ols degradation under different ranges of T and pH; and 4) achieve the best operating conditions to increase the physical and chemical stability of the CEE.

2. Material and methods

2.1. Overview of the methodology

Fig. 1 shows a diagram for the whole process, including: 1) obtaining the CEE from *A. unedo* fruits; 2) stability study of the CEE as powder systems along storage; 3) stability study of the CEE in aqueous solution systems; and 4) analyses performed in each stage considering the main affecting conditions (t, T and pH).

2.2. Standards and reagents

HPLC-grade acetonitrile was from Fisher Scientific (Lisbon, Portugal). Catechin was from Extrasynthese (Genay, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and formic acid were from Sigma (St. Louis, MO, USA). All the other chemicals were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Obtaining the catechin-enriched extracts

2.3.1. Source material

The fruits of *Arbutus unedo* L. (strawberry-tree berries) from Ericaceae were gathered in the Natural Park of Montesinho territory, Trás-os-Montes, Northeastern Portugal, in November 2008. The botanical identification was confirmed by the biologist, Dr. Ana Maria Carvalho, of Polytechnic Institute of Bragança (Trás-os-Montes, Portugal) according with a previous report of the authors (Guimarães et al., 2013). The fruits were lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) and stored in the deep-freezer at −20 °C for subsequent analyses.

2.3.2. Extraction procedure and purification

Each sample (1 g) was extracted by maceration with 30 mL of ethanol:water 20:80 (*v*/*v*) at 80 °C at 150 rpm during 90 min (Albuquerque et al., n.d.). The extract was filtered through Whatman no. 4 paper. The obtained extracts were evaporated at 40 °C (rotary evaporator Büchi R-210) to remove ethanol. For purification, the aqueous phase was deposited onto a C-18 solid phase column (Chromabond sorbent C_{18} ec, Macherey-Nagel, Duren, Germany), previously activated with ethanol followed by water; sugars and more polar substances were removed by passing through 50–80 mL of water and the purified extract was further eluted with 20–40 mL of ethanol. The purified extract was evaporated at 40 °C to remove ethanol.

2.4. Stability studies of the catechin-enriched extracts

2.4.1. Stability of the extracts as powder systems

The purified extract (5 mg) was dissolved in 5 mL of distilled water (control; pH = 3) and different pH values were adjusted to 2, 4, 5, 6, 7, 8 and 9 with solutions of hydrochloric acid or sodium hydroxide. The extracts with different pH, including the control, were lyophilized, and stored in Eppendorfs (5 mg of each lyophilized extract) at temperatures of −20, 5, 25 and 45 °C for a period of 0, 10, 20 and 30 days. In the end of each storage period, the samples were kept at −80 °C for subsequent analysis. A total of 112 individual experimental points (4t × 4 T × 7 pH) were used to understand the patterns behind the compound stability of the CEE powder. As responses, at each period the CEE powder was analyzed in terms of its content in flavan-3-ols (including catechin) and the remaining antioxidant activity (hydrophilic assays of DPPH scavenging

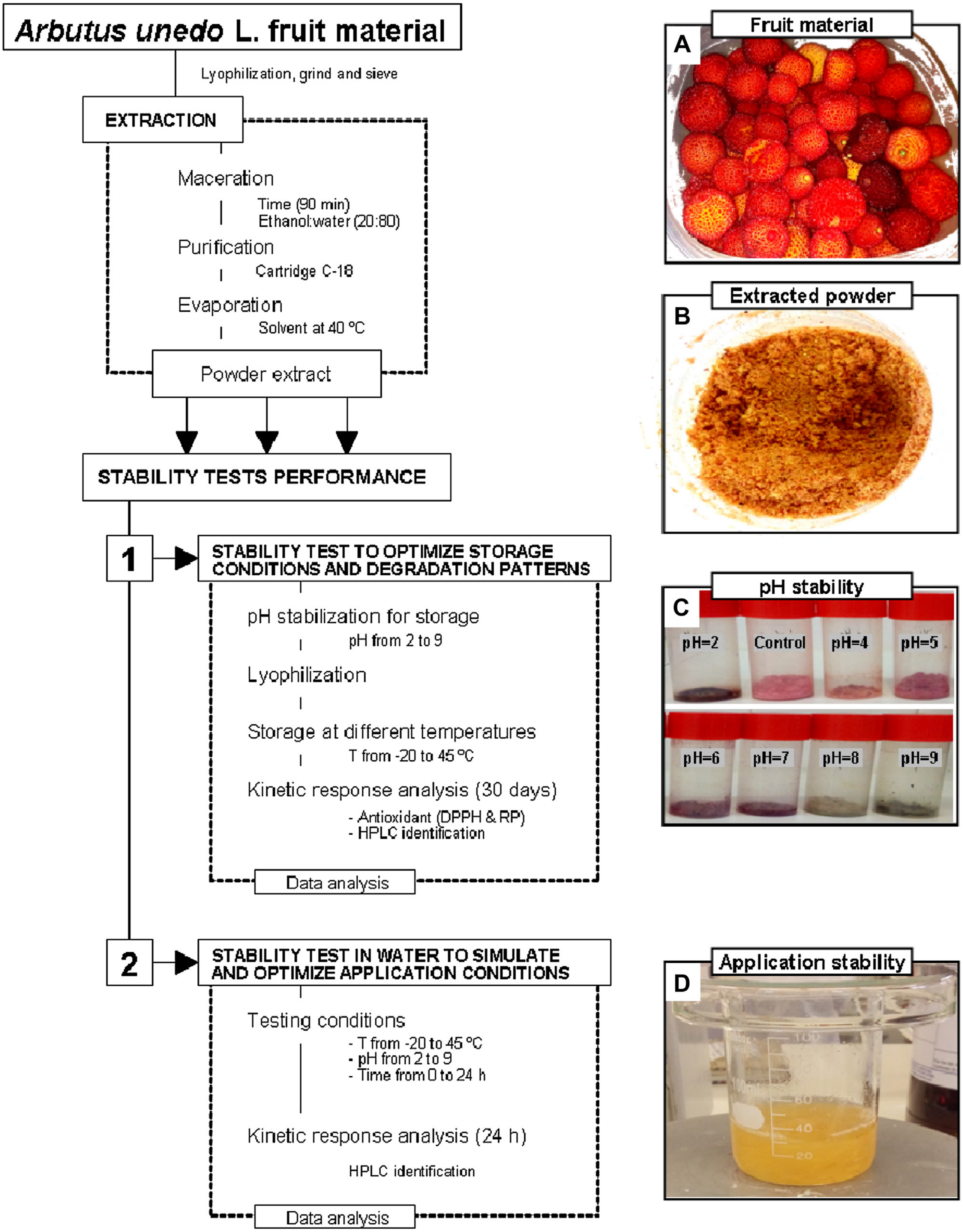

Fig. 1. Diagram describing the process extraction of the enriched extracts and the two stability tests performed: (1) stability of the extracts as powder systems according with the main affecting conditions involved in its obtaining and storage procedures; and (2) stability of the extracts in aqueous solution systems considering its potential application in food matrices.

activity and iron reducing power) of the extract. All independent measures were obtained in triplicate (n = 3).

2.4.2. Stability of the extracts in aqueous solution systems simulating food matrices

The purified extract (1 g) was dissolved in distilled water (30 mL). For the assays, 1.5 mL of this solution was poured in amber vials and the pH was adjusted with the addition of 0.5 mL of Britton-Robinson buffer solution pH 3, 5, 7 and 9. The samples were stored in a bath at 25, 40, 55, 70 and 80 °C for 1, 3, 5, 12 and 24 h. In the end of each storage period, the samples were kept at −80 °C for subsequent analysis. A total of 120 individual experimental points (6t × 5T × 4 pH) were used to understand the patterns behind the compound stability of the CEE in aqueous solution. As responses, at each period the content in flavan-3-ols and catechin were analyzed. All independent measures were obtained in triplicate (n = 3).

2.5. HPLC-DAD monitoring of flavan-3-ols, including catechin, content within the stability studies

The samples obtained in the stability studies (powder and solution systems) were analyzed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan) with a quaternary pump and a diode array detector (DAD) coupled to a LC solution software data-processing station. Separation was achieved using a Waters Spherisorb S3 ODS-2C_{18}, (3 μm, 4.6 mm × 150 mm) column thermostatted at 35 °C and formic acid in water 0.1% (A), acetonitrile (B) were used as solvents. The elution gradient established was 15% B for 5 min, 15% B to 20% B over 5 min, 20–25% B over 10 min, 25–35% B over 10 min, 35–50% B for 10 min, and re-equilibration of the column (15 min), using a flow rate of 0.5 mL/min. Detection was carried out in the DAD using 280 nm as preferred wavelength. Catechin was identified by comparing its UV spectra and retention times with commercial standard, and catechin derivatives were identified using previously published data on *A. arbutus* fruit (Guimarães et al., 2013). The quantitative analysis was performed using a calibration curve of catechin (y = 66243x–343411; R^2 = 0.999). The kinetic results were expressed in mg of catechin per g of extract and mg of flavan-3-ols (catechin and derivatives- procyanidins) per g of extract.

2.6. Antioxidant activity of the extracts within the stability studies evaluated by hydrophilic assays

2.6.1. DPPH free-radical scavenging activity

Different solution concentrations were prepared and were (30 μL) mixed with a methanolic solution (270 μL) containing DPPH free-radicals (6×10^{-5} mol/L) in a 96-well plate. The reaction mixture was left to stand for 60 min in the dark. After that, the reduction of DPPH free-radicals was determined by measuring the absorbance at 515 nm using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc, Winooski, VT, USA) (Pinela et al., 2012). The nonlinear dose-response of the asymptotic end-point values of the solutions was calculated by the Weibull model as previously described (Prieto et al., 2015a,b), using the following equation:

$$DPPH^{\bullet}(A) = N \exp\left[-(\ln 2)^{1-\alpha}\left(\frac{2v_m A}{N\alpha}\right)^{\alpha}\right] \quad (1)$$

in which A is the dose of antioxidant. The parameter *N* is the starting value of DPPH free-radicals (30 nM). The α shape parameter is related to the maximum slope of the response. The parameter v_m corresponds to the average number of DPPH molecules reduced per gram of CEE (nM DPPH/g), which is a value of maximal predictability and, therefore, was used as response.

2.6.2. Iron reducing power

The iron reducing power assay evaluates the capacity of the samples to convert potassium ferricyanide (Fe^{3+}) into potassium ferrocyanide (Fe^{2+}), which reacts with ferric chloride to form a ferric-ferrous complex that is monitored spectrophotometrically. The different solution concentrations (0.5 mL) were mixed with sodium phosphate buffer (200 mM, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and then trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in 48-well plates, with deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm using the microplate reader described above (Pinela et al., 2012). The dose-response results showed a linear relation and the following linear equation without intercept was used to compute the average number of reduced molecules:

$$RP(A) = mA \quad (2)$$

in which A is the dose of antioxidant. The slope parameter *m* corresponds to the average number of molecules that are reduced per gram of CEE (nM Fe^{2+}/g) and is used to compute the potential antioxidant activity of the extracts.

2.7. Mathematical models for the analysis of the response stability

Both stability tests were studied as function of functional equations typically applied in similar processes.

2.7.1. Individual model for the analysis of the stability variable effects

2.7.1.1. Effect of the time on the stability response. For the t effect, a typical exponential function was applied:

$$e(t) = k\exp(-rt) \quad (3)$$

where *k* represents the starting point and *r* is the decay degradation rate of the reaction.

2.7.1.2. Effect of the temperature on the stability response. The Arrhenius equation establishes that the rate constant of a chemical reaction is a function of the absolute *T* according to the following relation:

$$e(T) = A\exp\left(-\frac{Ea}{RT}\right) \quad (4)$$

Where the pre-exponential factor *A* represents the frequency of collisions among reacting molecules, *Ea* is the activation energy (kJ) and *R* the constant of gases (8.31 kJ/mol.K). In context, *A* and *Ea* can be considered as fitting parameters.

2.7.1.3. Effect of the pH on the stability response. In contrast with the effects of the previous variables, the pH has many possible behaviors and there is not an established single equation that could describe its effects. Therefore, authors are force to test several phenomenological models to be able to reproduce its conduct. Among those typical responses are the bell and the exponential profile solution:

1) The characteristic bell profile solution for the pH effect can be described by many bell functions (Di Marco and Bombi, 2001; Prieto and Murado, 2014). After an extensive evaluation, in which several equations were tested, the generalized exponential function without intercept (also called the modified Weibull distribution

function) was found to be the most satisfactory, with least number of parameters and highest accuracy:

$$e(pH) = k_m \left\{ \frac{i}{d} \left[1 - \left(\frac{pH}{pH_{opt}} \right)^{d} + \ln \left(\frac{pH}{pH_{opt}} \right) d \right] \right\} \tag{5}$$

in which the parameter d is related to the distance between the tails of the function, i a value related to the asymmetry of the bell profile, k_m the maximum response effect and pH_{opt} the pH at which k_m takes place. This model explicitly provides the characterizing parameters (k_m and pH_{opt}) of the response, and therefore their statistical significance can be tested through the determination of its confidence intervals.

2) Another solution for the description of the pH effect is the exponential function, similar to the one used for time effect, that is usually found in many biological system responses (Komatsu et al., 2014; Prieto et al., 2012b) and can be expressed as follows:

$$e(pH) = s\exp(-bpH) \tag{6}$$

where s represents the starting point and b is the degradation rate of the reaction.

2.7.2. Multivariable analysis

The development of a theoretical model is greatly facilitated by the possibility of combining all experimental data into a single master curve that is able to account for the important variables simultaneously (Prieto et al., 2012a,b). Such a solution allows to control most factors that affect the system, helping to standardize the key variables for producing reproducible protocols and therefore, to obtain reproducible results. Despite the existence of very rigorous results regarding the kinetics and the factors affecting the kinetic curve of specific molecules as function of variables such as T and pH (Prieto and Vázquez, 2014; Prieto et al., 2015a; Rosso et al., 1995; Tijskens et al., 1997), simultaneous solutions are not performed always, and by applying them, we can provide a more extensive knowledge to understand partially the governing mechanisms.

To be able to develop a multivariable analysis of these three variables, the logical approach is to insert the equations that take control of pH and T into Eq. (3) that governs the time variable by modifying its parameters, the starting value (k) and the degradation rate (r). Even if the three variables are fully independent, any event that may occur in the surrounding environment of a reaction always must be referred to the time variable. Therefore, a global possible description of the stability at the molecular level could be described by the following approach:

$$e(t, pH, T) = k^{\bullet}\exp(-r^{\bullet}t) \quad \text{where} \quad \begin{array}{l} k^{\bullet}(pH, T) = k \times e(T) \times e(pH) \\ r^{\bullet}(pH, T) = r \times e(T) \times e(pH) \end{array} \tag{7}$$

where $k^{\bullet}$ represents the starting point and $r^{\bullet}$ is the degradation rate of the reaction caused by the effect of time as described Eq. (3) but modified by the governing equations of the effect of T (Eq. (4)) and pH (Eqs. (5) or (6)).

2.8. Numerical methods and statistical analysis

All fitting procedures, coefficient estimates and statistical calculations were performed on a Microsoft Excel spreadsheet. Fitting and statistical analysis of the experimental results to the proposed equations were carried out in four phases:

- *Coefficients determination.* Parametric estimates were obtained by minimization of the sum of quadratic differences between observed and model-predicted values, using the nonlinear least-square (quasi-Newton) method provided by the macro Solver in Microsoft Excel 2003 (Kemmer and Keller, 2010), which allows quick testing of a hypotheses and its consequences.
- *Coefficients significance*. The determination of the parametric confidence intervals was calculated using the '*SolverAid*' (Prikler, 2009). The model was simplified by dropping terms, which were not statistically significant p-value (p) >0.05.
- *Model consistency*. The Fisher F test ($\alpha = 0.05$) test was used to determine whether the constructed models were adequate to describe the observed data (Shi and Tsai, 2002).
- *Other statistical assessment criteria.* To re-check the uniformity of the model the following criteria were applied: a) The 'SolverStat' macro (Comuzzi et al., 2003), which is used for the assessment of parameter and model prediction uncertainties; b) R^2 is interpreted as the proportion of the variability of the dependent variable explained by the model; c) Adjusted coefficients of multiple determination (R^2_{adj}), which is a correction to R^2 taking into account the number of variables used in the model.

3. Results and discussion

3.1. Production of a catechin-enriched extract from strawberry-tree fruits

The catechin-enriched extracts (CEE) obtained from *A. unedo* fruits presented 60% of flavan-3-ols and 22% of catechin (~425 and ~150 mg per 100 g dw, respectively) (Albuquerque et al., n.d.). Compounds were identified by comparing its UV spectra and retention time with a commercial standard and its derivatives were identified using a previous report of the authors (Guimarães et al., 2013). The flavan-3-ols values are over twenty times higher than those found in other typical plants from the Northeastern Portugal such as dog rose (*Rosa canina* L., 3.5 mg/100 g dw) and wild rose (*R. micrantha* Borrer ex Sm., 2.9 mg/100 g dw) (Guimarães et al., 2013), but much lower than those found in cacao (3–5 × 10^3 mg/100 g dw), which is considered the major source (Gu et al., 2006; Vinson et al., 1999). Strawberry-tree berries (*A. unedo*) presented similar contents of catechin to those found in green tea (~150 mg/100 g dw) and lower than the values found in cacao (~300 mg/100 g dw) (Zaveri, 2006), which are the main industrial sources of catechin. The good extraction efficiencies of catechin and derivatives reached in the fruit material of *A. unedo* in comparison with other sources emphasizes the need to perform more detailed evaluations of the stability of the compounds obtained.

Non-controllable variables such as soil cultivation, sun exposition, harvest time, etc., and controllable variables such as the extraction conditions (time, solvent, temperature, etc.) and techniques (ultrasound, maceration, microwave, etc.), may affect compounds concentration and could increase/decrease the yield efficiencies. The latter parameters have been previously optimized by the authors (Albuquerque et al., n.d.) and the best extraction conditions (maceration for 90 min, 80 °C, 20% of ethanol and 30 g/L) were used in order to obtain the CEE for the stability studies.

As reported previously, the stability of flavan-3-ols may be influenced by factors including t (Komatsu et al., 2014), pH (Su et al., 2003), T (Demeule et al., 2002), oxygen level (Labbé et al., 2008) and concentration of other compounds such as antioxidants level, metal ions and other compositional ingredients (Zhu et al., 1997). Although, being all of these parameters relevant, most of the authors agree that the essential ones are t, pH and T (Komatsu et al., 2014; Li et al., 2012, 2011), therefore, in the following sections the stability of flavan-3-ols will be monitored in function of t, pH and T within powder storage and application in a solution system.

Table 1

Results of the parametric and confidence intervals of the flavan-3-ols, catechin and antioxidant properties fitted to the time dependent model presented in Eq. (3) at different *pH* and *T* conditions when evaluating the stability during the process for obtaining the enriched extract.

pH	*T*	FLAVAN-3-OLS			Catechin			DPPH SCAV. Activity			Iron Reducing Power		
		k (t) (mg/g CEE)	*r (t) (day^{-1})*	R^2	*k (t) (mg/g CEE)*	*r (t) (day^{-1})*	R^2	*k (t) (nM DPPH/g CEE)*	*r (t) (nM DPPH/g CEE/day)*	R^2	*k (t) (nM Fe^{2+}/g CEE)*	*r (t) (nM Fe^{2+}/g CEE/day)*	R^2
2.0	***−20.0***	25.43 ± 2.37	0.0236 ± 0.002	0.9311	9.58 ± 0.76	0.0198 ± 0.002	0.8897	87.8 ± 8.8	0.019 ± 0.002	0.9994	4.12 ± 0.41	0.047 ± 0.005	0.9944
2.0	***5.0***	25.12 ± 2.46	0.0354 ± 0.003	0.9790	9.58 ± 0.81	0.0293 ± 0.002	0.8485	90.0 ± 8.0	0.038 ± 0.003	0.8895	4.09 ± 0.40	0.050 ± 0.005	0.9662
2.0	***25.0***	25.31 ± 2.48	0.0464 ± 0.005	0.9794	9.58 ± 0.78	0.0315 ± 0.003	0.8097	87.4 ± 8.4	0.044 ± 0.004	0.9667	4.08 ± 0.40	0.055 ± 0.005	0.9796
2.0	***45.0***	23.50 ± 2.16	0.0510 ± 0.005	0.9177	9.58 ± 0.83	0.0331 ± 0.003	0.8630	87.8 ± 8.1	0.063 ± 0.006	0.9228	4.04 ± 0.40	0.069 ± 0.007	0.9836
4.0	***−20.0***	41.34 ± 4.12	0.0265 ± 0.003	0.9962	19.32 ± 1.06	0.0083 ± 0.000	0.8475	110.9 ± 11.0	0.030 ± 0.003	0.9933	4.82 ± 0.48	0.044 ± 0.004	0.9997
4.0	***5.0***	41.61 ± 4.15	0.0304 ± 0.003	0.9980	19.32 ± 0.55	0.0074 ± 0.000	0.8828	111.2 ± 11.1	0.040 ± 0.004	0.9940	4.81 ± 0.48	0.050 ± 0.005	0.9999
4.0	***25.0***	42.67 ± 4.16	0.0325 ± 0.003	0.9743	19.32 ± 1.47	0.0177 ± 0.001	0.8613	109.7 ± 10.7	0.035 ± 0.003	0.9789	4.74 ± 0.47	0.059 ± 0.006	0.9901
4.0	***45.0***	42.37 ± 4.17	0.0354 ± 0.003	0.9852	19.32 ± 1.50	0.0132 ± 0.001	0.8744	111.3 ± 10.0	0.057 ± 0.005	0.9001	4.80 ± 0.48	0.085 ± 0.008	0.9997
5.0	***−20.0***	58.51 ± 5.79	0.0316 ± 0.003	0.9896	30.25 ± 2.56	0.0223 ± 0.002	0.8464	122.5 ± 12.0	0.044 ± 0.004	0.9786	4.92 ± 0.49	0.042 ± 0.004	0.9942
5.0	***5.0***	59.29 ± 5.92	0.0350 ± 0.003	0.9991	30.25 ± 2.79	0.0227 ± 0.002	0.9236	123.5 ± 11.9	0.031 ± 0.003	0.9656	4.74 ± 0.47	0.041 ± 0.004	0.9986
5.0	***25.0***	58.23 ± 5.72	0.0391 ± 0.004	0.9820	30.25 ± 2.46	0.0228 ± 0.002	0.8947	119.2 ± 11.9	0.054 ± 0.005	0.9981	5.10 ± 0.51	0.058 ± 0.006	0.9996
5.0	***45.0***	58.97 ± 5.88	0.0414 ± 0.004	0.9966	30.25 ± 2.53	0.0319 ± 0.003	0.9362	118.8 ± 11.8	0.094 ± 0.009	0.9938	4.69 ± 0.47	0.072 ± 0.007	0.9982
6.0	***−20.0***	80.48 ± 8.02	0.0377 ± 0.004	0.9966	23.88 ± 1.40	0.0151 ± 0.001	0.8871	134.8 ± 12.9	0.036 ± 0.003	0.9554	5.54 ± 0.55	0.040 ± 0.004	0.9851
6.0	***5.0***	80.70 ± 8.06	0.0409 ± 0.004	0.9990	23.88 ± 2.28	0.0146 ± 0.001	0.9562	139.7 ± 13.9	0.046 ± 0.005	0.9945	5.55 ± 0.55	0.047 ± 0.005	0.9922
6.0	***25.0***	80.66 ± 8.06	0.0442 ± 0.004	0.9989	23.88 ± 2.07	0.0193 ± 0.002	0.8649	137.9 ± 13.8	0.041 ± 0.004	0.9992	5.56 ± 0.55	0.054 ± 0.005	0.9933
6.0	***45.0***	78.59 ± 7.57	0.0509 ± 0.005	0.9634	23.88 ± 2.21	0.0217 ± 0.002	0.9267	138.1 ± 13.8	0.051 ± 0.005	0.9986	5.55 ± 0.55	0.061 ± 0.006	0.9914
7.0	***−20.0***	78.79 ± 7.83	0.0363 ± 0.004	0.9939	24.82 ± 1.81	0.0207 ± 0.002	0.9275	189.1 ± 18.4	0.042 ± 0.004	0.9753	5.55 ± 0.55	0.034 ± 0.003	0.9958
7.0	***5.0***	79.16 ± 7.91	0.0373 ± 0.004	0.9996	24.82 ± 1.74	0.0218 ± 0.002	0.9015	191.6 ± 17.9	0.038 ± 0.004	0.9351	5.58 ± 0.55	0.034 ± 0.003	0.9871
7.0	***25.0***	79.26 ± 7.92	0.0408 ± 0.004	0.9998	24.82 ± 1.80	0.0252 ± 0.002	0.9250	191.6 ± 18.8	0.047 ± 0.005	0.9817	5.72 ± 0.54	0.040 ± 0.004	0.9475
7.0	***45.0***	77.22 ± 7.56	0.0456 ± 0.004	0.9785	24.82 ± 2.21	0.0305 ± 0.003	0.8921	194.4 ± 18.7	0.068 ± 0.007	0.9611	5.62 ± 0.55	0.059 ± 0.006	0.9720
8.0	***−20.0***	55.29 ± 5.51	0.0282 ± 0.003	0.9971	17.58 ± 1.50	0.0149 ± 0.001	0.8549	130.5 ± 12.8	0.039 ± 0.004	0.9803	4.62 ± 0.46	0.042 ± 0.004	0.9931
8.0	***5.0***	54.21 ± 5.39	0.0328 ± 0.003	0.9938	17.58 ± 1.68	0.0174 ± 0.002	0.9539	130.8 ± 12.9	0.041 ± 0.004	0.9845	4.74 ± 0.45	0.038 ± 0.004	0.9524
8.0	***25.0***	55.81 ± 5.52	0.0347 ± 0.003	0.9898	17.58 ± 1.50	0.0148 ± 0.001	0.8531	132.1 ± 12.8	0.045 ± 0.004	0.9682	4.47 ± 0.43	0.050 ± 0.005	0.9718
8.0	***45.0***	55.87 ± 5.53	0.0372 ± 0.004	0.9890	17.58 ± 1.51	0.0164 ± 0.001	0.8566	133.2 ± 12.6	0.049 ± 0.005	0.9473	4.67 ± 0.46	0.052 ± 0.005	0.9788
9.0	***−20.0***	27.60 ± 2.72	0.0275 ± 0.003	0.9844	15.78 ± 1.42	0.0149 ± 0.001	0.8986	99.7 ± 10.0	0.039 ± 0.004	1.0000	3.56 ± 0.34	0.046 ± 0.004	0.9682
9.0	***5.0***	27.59 ± 2.73	0.0311 ± 0.003	0.9882	15.78 ± 1.41	0.0190 ± 0.002	0.8947	100.4 ± 10.0	0.039 ± 0.004	0.9988	3.57 ± 0.35	0.043 ± 0.004	0.9931
9.0	***25.0***	27.61 ± 2.73	0.0351 ± 0.003	0.9893	15.78 ± 0.96	0.0152 ± 0.001	0.9113	100.1 ± 10.0	0.044 ± 0.004	0.9994	3.57 ± 0.36	0.046 ± 0.005	0.9975
9.0	***45.0***	27.64 ± 2.74	0.0381 ± 0.004	0.9907	15.78 ± 1.38	0.0204 ± 0.002	0.8739	100.2 ± 9.8	0.040 ± 0.004	0.9793	3.53 ± 0.35	0.051 ± 0.005	0.9773

3.2. Stability of the extracts as powder systems

The CEE powder previously obtained was used to investigate the effects of t (0–30 days), pH (2–9) and T (−20 to 45 °C) on the stability of the major compounds (flavan-3-ols and catechin) and on the antioxidant activity (measured by DPPH scavenging activity (DPPH) and iron reducing power (RP) assays). For all these responses, the effect of t was described individually by Eq. (3); parametric responses and correlation coefficients are presented in Table 1. Then, the multivariable analyses were tested as described in Eq. (7), by inserting Eqs. (4) and (5) into Eq. (3), substituting both time dependent parameters of Eq. (3) (*k* and *r*). After testing the multivariable approach for all responses (flavan-3-ols, catechin, DPPH and RP), in all cases the pH effect had an influence on the asymptotic starting *k* parameter and the T effect had an exclusively effect on the *r* parameter. Therefore, a global multivariable model that predicts the effect of t, pH and T on the stability of compounds content of the CEE on powder system was described as follows:

$$e(t, pH, T) = k_m \left\{ \frac{i}{d} \left[1 - \left(\frac{pH}{pH_{opt}} \right)^{d} + \ln \left(\frac{pH}{pH_{opt}} \right) d \right] \right\} \exp \left(-A \exp \left(-\frac{Ea}{RT} \right) t \right) \tag{8}$$

All parametric notations were defined in the Material and methods section. The parametric solutions and correlation coefficients of the multivariable effects described by Eq. (8) for the stability analysis of compounds content (flavan-3-ols and catechin) and the antioxidant activity (DPPH and RP assays) are presented in Table 2 and Fig. 2, Fig. A1 (supplemental material), Fig. 3 and Fig. A2 (Supplemental material). Subsequent, in a more detail manner the results of these analyses were described for all the responses.

3.2.1. Monitoring the stability of flavan-3-ols and catechin compounds by HPLC-DAD

The graphical representation of the CEE powder stability results of the flavan-3-ols and catechin content as function of the t, T and pH are presented in Fig. 2 and Figure A1. Each of these figures is divided into two sections (A and B).

Section A shows the individual time-dependent analysis (during 30 days) of the degradation effects of the flavan-3-ols and catechin compounds for each of the pHs tested (2, 4, 5, 6, 7, 8 and 9). Points are the experimental data (●25 °C, ○5 °C, ♦25 °C, ◊45 °C) and lines (—) the modeled results using Eq. (3). The effects produced by the t and pH variables had a greater effect on the stability of flavan-3-ols and catechin compounds than the T variable. The tested range of T was large enough but was highly influenced by the pH. Only between pHs 5 and 7 the degradation rates caused by the T effect (r parameter, Table 1) showed a well differentiated response. Although the pHs between 5 and 7 showed the higher degradation rates at any T; the extraction performance and stability showed higher compounds content than any other pH as it can be evaluated by the parameter value k (Error! Reference source not found.) of Eq. (3). The maximum starting content obtained was 80.70 and 30.25 mg/g CEE for flavan-3-ols and catechin, respectively. The statistical information of the fittings performed is illustrated using two basic criteria, the basic R^2 coefficient and the parametric confidence intervals. In all cases, parameters were highly significant ($\alpha = 0.05$) and R^2 values were predominantly higher than 0.95 indicating the reliability of the analysis performed with Eq. (3). Therefore, to find a compromise range of values that will allow us to determine the exact variable range that would stabilize the CEE powder, the multivariable analysis model of Eq. (8) was applied and presented in section B of Fig. 2 and Fig. A1.

Section B shows, for both figures (flavan-3-ols and catechin content), the global multivariable fitting procedure (applying Eq. (8) with parametric results in Table 2) divided in three main subsections:

B1: Shows the patterns of the kinetic parameters *k* and *r* of Eq. (3) (points are individual results and lines multivariable response) as a function of their respective affecting variables (pH affecting the parameter *K* and the T affecting the parameter *r*). In both cases, the pH response shows a bell profile pattern with a clear peak at 6.7 for the flavan-3-ols content and 5.4 for the catechin content. In the case of the T variable, both cases showed an increasing linear profile with an expected identical *Ea* values (2.87 kJ) but dissimilar collision frequency (0.13 and 0.08 for flavan-3-ols and catechin content, respectively).

B2: Shows an illustration of the 3D response of the combined effect of the time with the other two variables (pH and T) at the best experimental conditions found for stabilizing the content of flavan-3-ols (T = −20 °C and pH = 6.7) and catechin (T = −20 °C and pH = 5.4) of the CEE in the powder system.

B3: Shows the correlation between the experimental values and the predicted ones obtained with the multivariable model presented in Eq. (8) for the prediction of the content stability in CEE powder of flavan-3-ols ($R^2 = 0.9741$) and catechin ($R^2 = 0.8741$).

These values are in agreement with those reported previously. As an example, Zhu et al. (2002) showed that the best shelf life conditions obtained for all flavan-3-ols from cocoa fruit was at a pH values between 2 and 4 independently of any storage temperature between 4 and 45 °C, and concluded that the stability is fundamentally pH-dependent.

3.2.2. Monitoring the remaining antioxidant activity of CEE compounds by hydrophilic assays

The graphical representation of the powder stability results of the DPPH scavenging activity and iron reducing power as function of the t, T and pH are shown in Fig. A2 and Fig. 3. Each of these figures is divided into the same two sections (A and B) and analyzed as those described in the previous heading. The individual analysis of the time remaining activity measured in both methods (Section A of Fig. A2 and Fig. 3) shows a stronger effect of the pH and t variable than the T, but with a T effect more notable than the one found in the analysis of the stability of the main compounds of the CEE. Parameter values of Eq. (3) of all antioxidant analyses are displayed in Table 1. The statistical information of the fittings showed $R^2 > 0.95$ in almost all cases and parametric confidence intervals highly significant ($\alpha = 0.05$). The multivariable analysis by Eq. (8) is graphically illustrated in section B of Fig. A2 and Fig. 3, and the parametric results are presented in Table 2. The patterns of the kinetic parameter *k* show a bell profile as a function of pH with a clear peak at 7.1 and 6.7 for DPPH and RP methods. In the case of the temperature-dependent parameter *r*, the DPPH scavenging activity and iron reducing power showed an increasing linear profile with similar *Ea* values (4.78 and 4.42 kJ, respectively) and collision frequency values (0.32 and 0.31, respectively). Therefore, at lower temperatures and pHs ranging between 6 and 7, the time degradation effects are reduced. Such a relation is illustrated by the 3D response of the combined effect of the time with the other two variables (pH and T) at the best stabilizing experimental conditions found for the antioxidant compounds content in CEE powder. Finally, subsection B3 shows the high correlation values between the experimental values and the predicted ones by Eq. (8), indicating once again the agreement of the developed solution.

3.3. Stability of the extracts in aqueous solution systems simulating food matrices

The information provided by the antioxidant activity analysis in the powder system cannot be replicated in the aqueous system because of the two time frames taking place in the response. In the

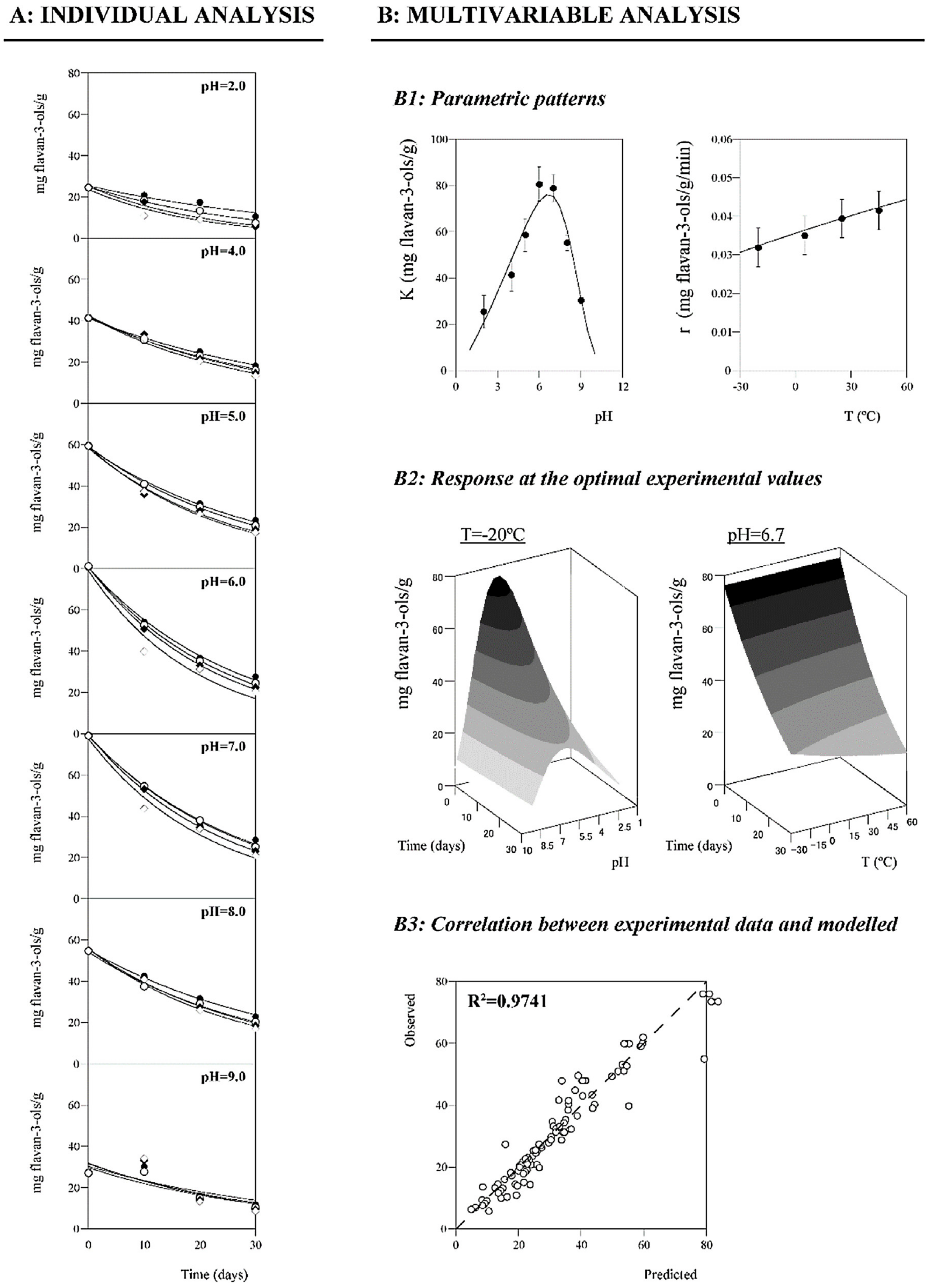

Fig. 2. Powder storage stability of the flavan-3-ols as function of the t, T and pH. Section **A** shows the individual kinetic analysis of the degradation effects of the flavan-3-ols. Points are the experimental data (●–25 °C, ○ 5 °C, ♦ 25 °C, ◊ 45 °C) and lines (—) the modeled results using Eq. (3) (parametric responses and correlation coefficients in Table 1). Section **B** shows the global multivariable (trivariate) fitting procedure: **B1** shows the parametric patterns of the kinetic parameters *k* and *r* of Eq [3] (points are univariate results and lines multivariable response) as a function of their respective affecting variables (pH affecting the parameter *K* and the T affecting the *r*); **B2** shows an illustration of the 3D response of the combined effect of the three variables at the best experimental conditions found (T = −20 °C and pH = 6.7); and **B3** shows the correlation between the experimental values and the predicted ones obtained with the multivariable model presented in Eq. (7).

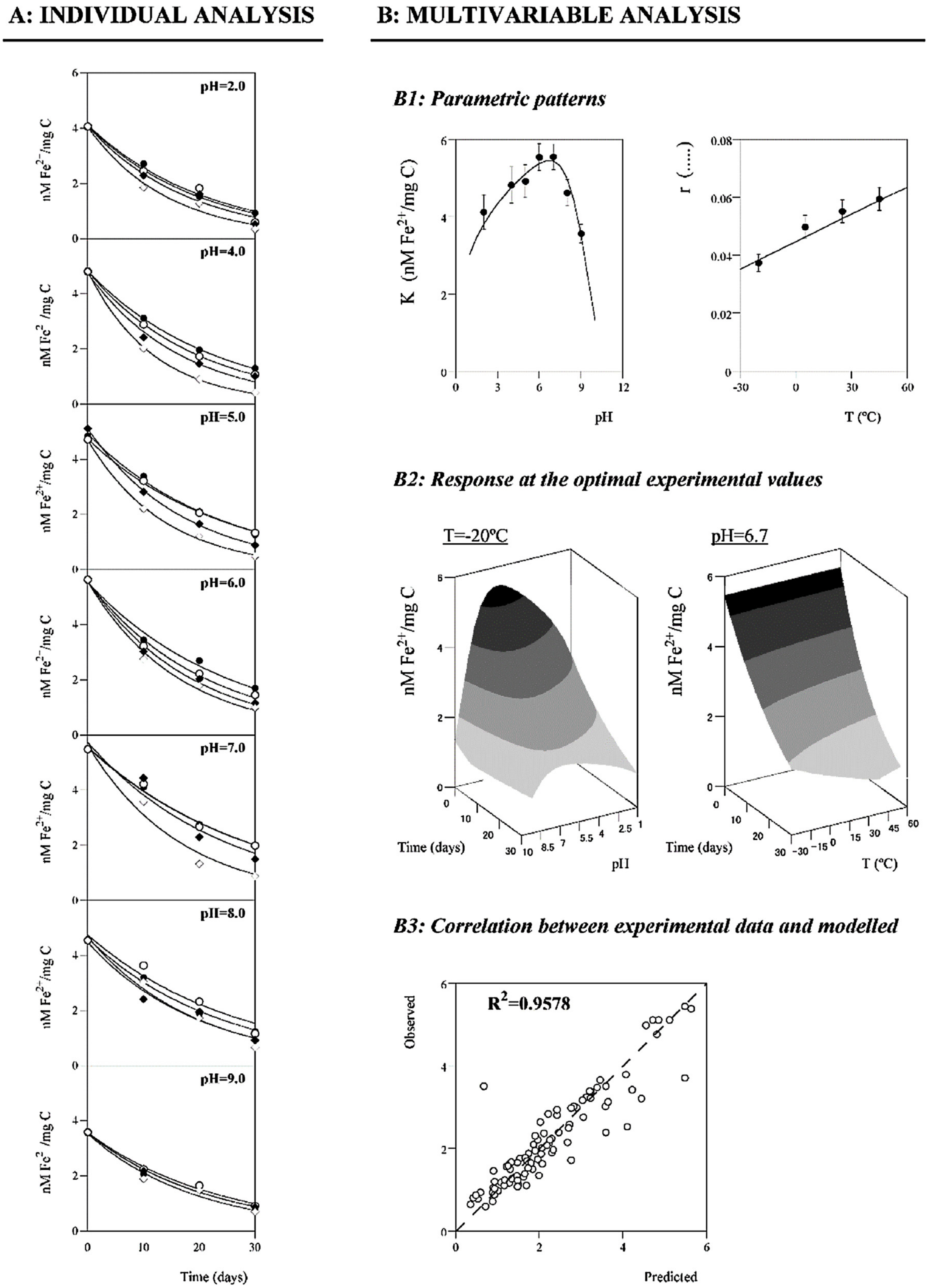

Fig. 3. Powder storage stability of the iron reducing power (RP) as function of the t, T and pH. Section **A** shows the individual kinetic analysis of the degradation effects of the antioxidant compounds. Points are the experimental data (●−25 °C, ○ 5 °C, ♦ 25 °C, ◊ 45 °C) and lines (—) the modeled results using Eq. (3) (parametric responses and correlation coefficients in Table 1). Section **B** shows the global multivariable (trivariate) fitting procedure: **B1** shows the parametric patterns of the kinetic parameters *k* and *r* of Eq [3] (points are univariate results and lines multivariable response) as a function of their respective affecting variables (pH affecting the parameter *K* and the T affecting the *r*); **B2** shows an illustration of the 3D response of the combined effect of the three variables at the best experimental conditions found (T = −20 °C and pH = 6.7); and **B3** shows the correlation between the experimental values and the predicted ones obtained with the multivariable model presented in Eq. (7).

Table 2
Results of the parametric and confidence intervals of the flavan-3-ols, catechin and antioxidant properties fitted to the multivariable (t, *T* and *pH*) model presented in Eq. (7) when evaluating the stability during the process for obtaining the enriched extract.

		FLAVAN-3-OLS	Catechin	DPPH SCAV. Activity	Iron Reducing Power
Parameters controlling the effect of the pH of the kinetic parameter k(t)					
R_{max} (pH)	(response units)	76.73 ± 2.18	30.21 ± 1.51	154.84 ± 5.17	5.46 ± 0.08
i (pH)		7.76 ± 1.17	1.49 ± 0.98	11.11 ± 3.76	9.70 ± 1.67
pH_{opt} (pH)	(pH units)	6.70 ± 0.14	5.43 ± 0.26	7.16 ± 0.31	6.73 ± 0.19
d (pH)		2.21 ± 0.15	3.87 ± 1.66	1.55 ± 0.09	1.33 ± 0.04
Parameters controlling the effects of the T of the kinetic parameter r(t)					
A(T)	(collision freq)	0.13 ± 0.02	0.08 ± 0.01	0.32 ± 0.04	0.31 ± 0.08
Ea (T)	(kJ)	2.87 ± 0.32	2.87 ± 0.80	4.78 ± 0.72	4.42 ± 0.62
Statistical information of the multivariable analysis (trivariate)					
Obs		120	120	112	112
df		113	113	105	105
R^2		0.9741	0.8792	0.8835	0.9578
R^2_{adj}		0.9318	0.8533	0.8648	0.9324

powder system, the two time frames were widely separated. We were measuring compounds decay over 30 days period and the 2 h period of processing the antioxidant response was not an influence on the output of the results. In contrast, the 24 h period analyzed in the aqueous system, not only showed extremely high degradation rates compared with the powder system, but also the two time frames overlap one to each other making the analysis fruitless. Although authors expected to be able to combine both time frames in order to overcome analytically this situation, the results obtained showed a complexity higher than expected and were discarded for further analysis. Therefore, the CEE powder obtained was used to investigate the effects of t, pH and T on the stability of compounds content (flavan-3-ols and catechin) in aqueous solution system simulating a food environment. For all these responses, the stability of the compounds was monitored by HPLC-DAD at different time sequences (1, 3, 5, 12 and 24 h) and the antioxidant activity was discarded.

The individual time-dependent graphical analysis of the aqueous solution stability results of the flavan-3-ols and catechin content as function of the T and pH are presented in Section A of Figure A3 (supplemental material) and Fig. 4, respectively. Each graph shows the time degradation effects (0–24 h) of each T tested (25, 40, 55, 70 and 80 °C). Points are the experimental data of the different pHs tested (● 3, ○ 5, ◆ 7 and ◇ 9) and lines (—) the results predicted by Eq. (3). The parametric results and correlation coefficients are presented in Table 3. Differently than the previous stability study, the compounds content (flavan-3-ols and catechin) is identical in all cases, thus the parameter *k* of Eq. (3) would not change as function of any variable and will remain constant (300 μg flavan-3-ols/mL CEE and 150 μg catechin/mL CEE). To be able to describe those effects in a multivariate form, the degradation rate parameter (*r*) takes in all perturbation effects caused by the T and pH variables. The increase in T and pH units causes an exponential increase on the degradation rate of CEE compounds content. The T effect behaves following the Arrhenius concept definition presented in Eq. (4). However, the pH effects cannot be described as previously with a bell profile function, because as stated before, the governing mechanisms of the effect of pH on basic chemical reactions or complex living organisms are dissimilar and heterogeneous. For the particular case of the stability of CEE compounds in aqueous system, the pH effect follows an exponential relation as described by Eq. (6). Therefore, a global multivariable model that controls the effect of t, pH and T on the stability in aqueous system can be developed by substituting the *r* parameter of Eq. (3) with the equations governing the T (Eq. (4)) and the pH effect (Eq. (6)), as follows:

$$e(t, pH, T) = k \exp\left(-p \exp\left(-\frac{Ea}{RT} + bpH\right) t\right) \qquad (9)$$

When substituting the *r* parameter by the multiplicative result of Eqs. (4) and (6), the new resulting expression share a pre-exponential factor (*A* and *s*) and their use in conjunction will be redundant therefore, a new factor its described and noted as *p*. All other parametric notations as defined in the Material and methods section. Section B of Figure A3 and Fig. 4. show the global multivariable fitting results of applying Eq. (9) to describe the full multivariable data. The parametric results were for flavan-3-ols ($k = 300.0 \pm 6.1$; $p = 40.21 \times 10^{-6}$; $Ea = 19.37$; $b = 67.31$) and for catechin ($k = 150.0 \pm 8.5$; $p = 40.21 \times 10^{-6}$; $Ea = 21.93$; $b = 64.09$). In all cases the statistical description was significant and the prediction of the CEE compounds content stability in solution by Eq. (9) showed highly consistent R^2 values for of flavan-3-ols and ($R^2 = 0.9629$) and catechin ($R^2 = 0.9585$). Subsection B1 shows the parametric net surface pattern of the kinetic *r* as a function of their respective affecting variables (pH and T). The conclusions are in concordance with previous results in powder systems and data previously reported (Komatsu et al., 2014; Li et al., 2012, 2011), finding that at T < 20 °C and pH < 4.5 the CEE is more stable lowering as much as possible the degradation rate of flavan-3-ols and catechin.

As in the powder system, flavan-3-ols and catechin of the strawberry tree in aqueous solution system proved to be fundamentally pH-dependent. At pH values higher than 7 the flavan-3-ols and catechin compounds decay completely in a few minutes independent of T. This phenomenon was also detected by others authors that studied the stability of catechin and derivatives from others matrices such as green tea and cacao (Komatsu et al., 2014; Li et al., 2012; Zhu et al., 2002, 1997). In similar terms with other authors (Li et al., 2012), results indicated that catechin enriched extracts in aqueous solution remained stable in pH values lower than 4 and temperature below 30 °C for a period of 24 h. Even at high thermal process conditions (85 °C) at pHs lower than 4 the flavan-3-ols and catechin are detected for 1 h period without great losses. These results may limit the CEE direct application, favoring acid foods as some chesses, fruit juice, vegetable/fruit products, mayonnaises and yogurts (Food Ingredients Brasil, 2008).

4. Conclusions

The analysis of the flavon-3-ols and catechin compounds stability from the strawberry tree fruits is crucial for predicting the shelf life behavior of the compounds in various processing situations. In fact, food processing or other processes are factors that affect directly on the integrity of the molecules. Controlling the conditions of t, pH and T among others are essential aspects for keeping the process efficiency and for obtaining high quality products. The flavan-3-ols and catechin degradation in powder systems is different from the degradation in aqueous solutions, and the results showed that powder systems are more stable than solu-

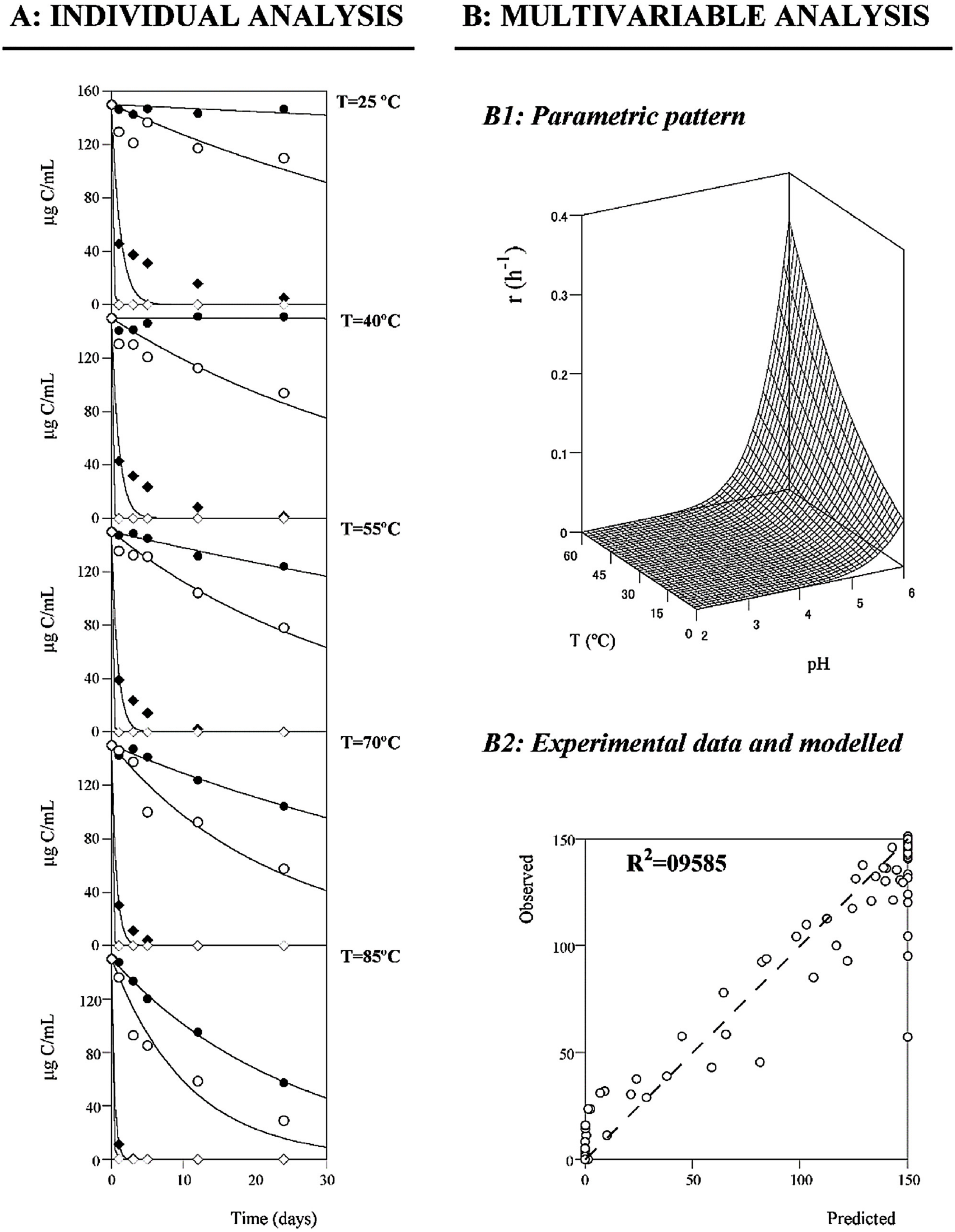

Fig. 4. Catechin stability in aqueous solution systems simulating food matrices. Section **A** shows the individual time-dependent graphical analysis of the stability results based on the catechin content as function of the T and pH. Each graph shows the time degradation effects (0–24 h) of each T tested (25, 40, 55, 70 and 80 °C). Points are the experimental data of the different pHs tested (● 3, ○ 5, ♦ 7 and ◊ 9) and lines (—) the results predicted by Eq. (3). The parametric results and correlation coefficients are presented in Table 3. Section **B** shows the global multivariable fitting results of applying Eq. (9) to describe the full multivariable data: **B1** shows the parametric net surface pattern of the kinetic *r* as a function of their respective affecting variables (pH and T); and **B2** shows the correlation between the experimental values and the predicted ones obtained with the multivariable model presented in Eq. (9).

Table 3
Results of the parametric and confidence intervals of the flavan-3-ols and catechin responses fitted to the time dependent model presented in Eq. (3) at different *pH* and *T* conditions when evaluating the application stability of the enriched extract.

T	*pH*	FLAVAN-3-OLS			Catechin		
		k (t) (μg/mL)	*r (t) (h^{-1})*	R^2	*k (t) (μg/mL)*	*r (t) (h^{-1})*	R^2
25.0	**3.0**	300.0 ± 29.06	0.011 ± 0.003	0.9687	150.0 ± 14.95	0.040 ± 0.006	0.9965
25.0	**5.0**	300.0 ± 29.78	0.081 ± 0.024	0.9926	150.0 ± 14.30	0.094 ± 0.013	0.9536
25.0	**7.0**	300.0 ± 30.00	3.138 ± 0.941	0.9888	150.0 ± 15.00	2.598 ± 0.390	0.9989
25.0	**9.0**	300.0 ± 30.00	7.952 ± 2.386	0.9981	150.0 ± 15.00	6.094 ± 0.914	0.9990
40.0	**3.0**	300.0 ± 26.60	0.002 ± 0.001	0.8866	150.0 ± 14.51	0.015 ± 0.002	0.9671
40.0	**5.0**	300.0 ± 29.35	0.058 ± 0.017	0.9783	150.0 ± 13.92	0.043 ± 0.006	0.9280
40.0	**7.0**	300.0 ± 30.00	2.543 ± 0.763	0.9961	150.0 ± 14.93	1.555 ± 0.232	0.9955
40.0	**9.0**	300.0 ± 30.00	6.156 ± 1.847	0.9977	150.0 ± 15.00	7.828 ± 1.174	0.9997
55.0	**3.0**	300.0 ± 30.00	0.001 ± 0.000	0.9988	150.0 ± 14.31	0.008 ± 0.001	0.9541
55.0	**5.0**	300.0 ± 29.55	0.045 ± 0.013	0.9849	150.0 ± 14.65	0.029 ± 0.004	0.9767
55.0	**7.0**	300.0 ± 30.00	1.820 ± 0.546	0.9979	150.0 ± 14.64	1.211 ± 0.177	0.9758
55.0	**9.0**	300.0 ± 30.00	5.156 ± 1.547	0.9989	150.0 ± 15.00	7.828 ± 1.174	0.9998
70.0	**3.0**	300.0 ± 12.98	0.002 ± 0.000	0.4328	150.0 ± 8.09	0.001 ± 0.001	0.9397
70.0	**5.0**	300.0 ± 28.98	0.038 ± 0.011	0.9659	150.0 ± 13.37	0.023 ± 0.003	0.8916
70.0	**7.0**	300.0 ± 30.00	1.374 ± 0.412	0.9999	150.0 ± 14.24	1.004 ± 0.143	0.9492
70.0	**9.0**	300.0 ± 30.00	4.156 ± 1.247	0.9989	150.0 ± 15.00	7.828 ± 1.174	0.9991
85.0	**3.0**	300.0 ± 6.42	0.000 ± 0.000	0.2140	150.0 ± 0.45	0.002 ± 0.001	0.9301
85.0	**5.0**	300.0 ± 28.70	0.033 ± 0.009	0.9566	150.0 ± 10.71	0.016 ± 0.002	0.9141
85.0	**7.0**	300.0 ± 29.99	1.085 ± 0.326	0.9997	150.0 ± 13.78	0.840 ± 0.116	0.9190
85.0	**9.0**	300.0 ± 30.00	3.545 ± 1.064	0.9991	150.0 ± 15.00	6.131 ± 0.920	0.9991

tions. Mathematical models were developed and multiple graphical plots were conducted to establish and illustrate the optimum values of the independent variables studied. Thus, the kinetic models could be used for calculating shelf-life and predicting compounds stability at given T and pH conditions for powder and aqueous systems. The optimal stability conditions for flavan-3-ols (including catechin) in powder systems was higher at $pH = 5.4$ and $T = -20\,^{\circ}C$ during a month, while in aqueous solution remained intact at $pH < 4$ and $T < 30\,^{\circ}C$ for a period of at least 24 h. High correlations and significant parameter confidence intervals were always obtained. Therefore, it is proposed that these semi-empirical kinetic models could be applied to predict the effects of T and pH on the shelf life of compounds in powder and aqueous system. Moreover, using the optimal processing conditions, it is possible to produce functional extracts with high potential as nutraceuticals or as active ingredients in the design of functional foods, which can be also extended to other industrial fields such as pharmaceutical and cosmeceutical industries.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.02.002.

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