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ORIGINAL PAPER

Comparison of the degradation kinetics of A-type and B-type proanthocyanidins dimers as a function of pH and temperature

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Abstract The degradation behaviors of four different proanthocyanidins dimers including B-type (e)picatechin (E)C dimer and A-type (epi)catechin (E)C dimer, A-type (epi)catechin-3-*O*-gallate (E)CG dimer and A-type (epi)gallocatechin-3-*O*-gallate (E)GCG dimer as affected by pH and temperature were compared in this study. The results showed that the stability of proanthocyanidins dimers was not only temperature and pH dependent, but also structure dependent. Proanthocyanidins dimers were found to be unstable at pH as low as 1.5 and physiological pH conditions, and they were rather unstable at alkaline conditions and temperature above 25 °C. Among the four dimers tested, B-type (E)C dimer was the least stable, while A-type (E)C dimer was the most stable. In general, B-type dimers showed less stability than A-type ones. The higher the degree of galloylation and the more the hydroxyl groups in the molecular, the less stable the proanthocyanidins dimers.

Keywords A-type dimers · B-type dimers · Stability · pH · Temperature · Degradation kinetics

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Introduction

Proanthocyanidins (PAs), which are a group of compounds widely presented in fruits, vegetables, and other food products, are well known for their strong antioxidant and anticarcinogenic properties as well as their protective effects against chronic diseases [1–5]. According to the linkage type of the interflavan bonds, PAs can be divided into B-type proanthocyanidins, which are linked by C4–C8 or C4–C6 bond and A-type proanthocyanidins, which are doubly linked by a C4–C8/C6 bond and an additional C2–O–C7 or C2–O–C5 ether bond.

It is known that the structural features of PAs including the monomer compositions, the degree of galloylation and the linkage type of the interflavan bonds have great influence on their bioactivities. However, knowledge of the influence of the structural features on the stability of PAs is lacking. In order to fully understand the biological effects and bioavailability of proanthocyanidins, it is important to study their stability. It was reported that tea catechins such as epigallocatechin gallate (EGCG) have poor stability during processing and storage. Studies concerning the stability of the monomeric flavan-3-ols such as epicatechin (EC), catechin (C), EGCG, epigallocatechin (EGC) and epicatechin gallate (ECG) are available. For example, Wang et al. [6] investigated the reaction kinetics of degradation and epimerization of EGCG in aqueous system over a wide temperature range. Komatsu et al. [7] reported the effects of temperatures and pH on the reaction kinetics of tea catechins. Li et al. [8] studied the effects of temperature and relative humidity on the degradation kinetics of catechins in green tea powder. Friedman et al. [9] revealed that tea catechins, especially ECGC

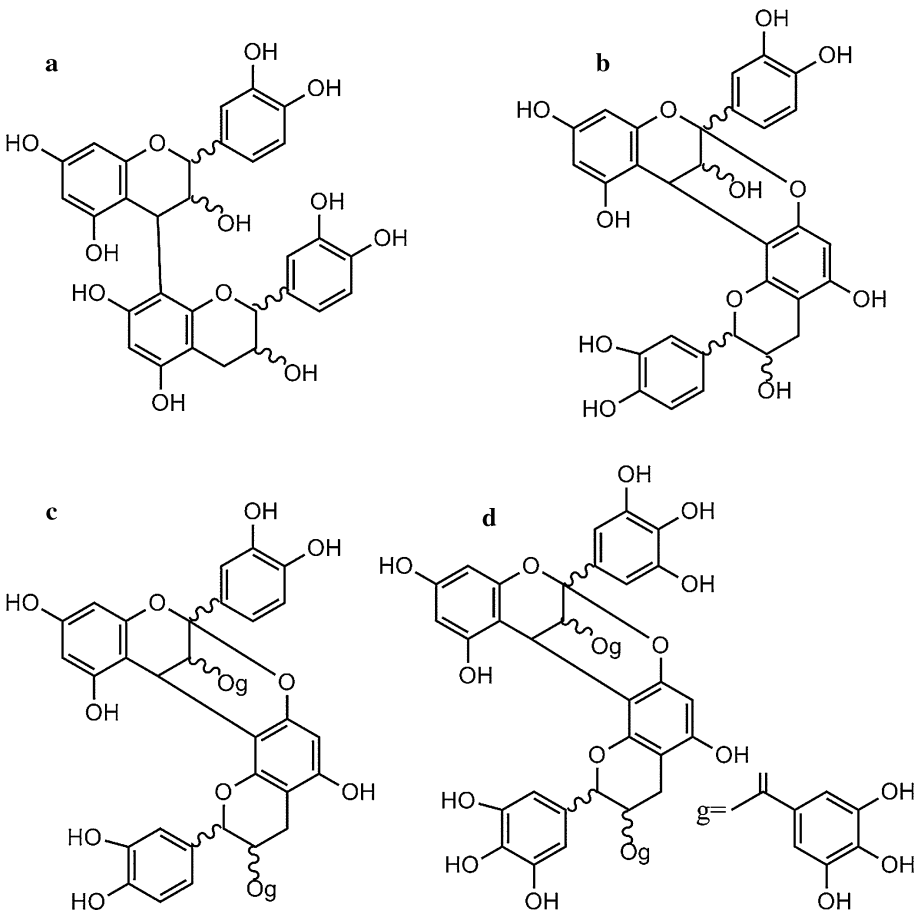

Fig. 1 Structures of the four PA dimers. **a** The structure of B-type (E)C dimer from Granny Smith apple; **b** The structure of A-type (E)C dimer extracted from peanut red skins; **c** The structure of A-type (E)CG dimer presents from persimmon; and **d** The structure of A-type (E)GCG dimer also comes from persimmon

and ECG, were unstable during storage even in the dry state. Unfortunately, due to the structure difference, the stability behaviors and the reaction kinetics of monomeric flavan-3-ols under different conditions may differ significantly from that of the proanthocyanidins oligomers. Zhu et al. [10] reported that cocoa PA dimers B-2 and B-5 were unstable under physiological condition and degraded in a few hours. Lu et al. [11] indicated that A-type proanthocyanidin dimer A2 (PA2) from longan flowers was more stable in acidic condition than in weak alkaline condition, and it was also quite unstable in common cell culture medium. However, up to now, no studies concerning the effects of monomer compositions, the degree of galloylation and the linkage type of the interflavan bonds on the stability of proanthocyanidins oligomers are available. In our previous studies, we established simple methods for preparing large quantities of A-type proanthocyanidins dimers and B-type proanthocyanidins dimers from peanuts, apple and persimmon. Therefore, the aim of this study is to document the influence of the structural features on the stability of PA dimer as well as the degradation of the reaction kinetics of four different dimers.

Materials and methods

Chemicals

Proanthocyanidin A2 and B1 (purity >99 %) were purchased from Extrasynthèse (Genay Cedex, France). HPLC grade acetonitrile and methanol were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade. pH conditions were adjusted using 2 mol/L sodium hydroxide and 6 mol/L HCl solutions.

Sample preparation

B-type (E)C dimer (Fig. 1a) was isolated from Granny Smith apples (*Malus domestica*) pulp and characterized by HPLC–MS/MS as we previously reported [12]. Its purity was confirmed by HPLC to be 87.57 % using commercial proanthocyanidin B1 as the standard. A-type (E)C dimer (Fig. 1b) was prepared from peanut (*Arachis hypogaea*) skins, and A-type (E)CG dimer (Fig. 1c) and A-type (E)GCG dimer (Fig. 1d) were prepared from persimmon (*Diospyros kaki L.*) pulp with the method previously

reported [13]. Their purity was analyzed by HPLC and calculated to be 88.72, 82.56 and 93.28 %, respectively, using PA2 as the reference. The exact spatial structural assignments of the dimers cannot be made.

Stability of the four PA dimers at different temperatures

PAs dimers were dissolved in distilled water with the concentration of 0.67 mg/mL and were kept in a water bath (25, 40 °C) and a refrigerator (4 °C), respectively. All samples were prepared in triplicate. The PA dimers solution was then delivered into HPLC for quantitative analysis with an interval of 2.25 h.

The effects of pH on the four PA dimers

The stability of the four dimers was assessed at different pH values (1.5, 6.8, 7.4, 10.0). Dimers were prepared in distilled water (3 mg/mL). 200 μL of the dimer solution was mixed with 1.8 mL of KCl–HCl solution (pH 1.5), Tris–HCl buffer (pH 6.8, 7.4), glycine–sodium hydroxide buffer (pH 10.0), respectively, to obtain the aimed pH value. All samples were prepared in triplicate and were stored at 25 °C. The samples were then periodically analyzed by HPLC.

HPLC–ESI-MS/MS analysis

HPLC analysis was operated on a Shimadzu prominence LC-20A spectrometer (Shimadzu, Japan) equipped with a dual-channel UV detector and LC solution workstation. The column used was a Hypersil ODS C18 cartridge column (250 × 4.6 mm, i.d.) with a guard column of the same materials (Grace Davison, Deerfield IL USA). A gradient method was employed using mobile phase A consisting of distilled TFA: water (0.13/99.87, v/v) and phase B consisting of TFA-acetonitrile (0.1/99.9, v/v). The flow rate was 0.8 mL/min. The analysis was achieved in a 45-min program as follows: 0 min, 5 % B, 0–10 min, 5 % B, 10–15 min, increase to 20 % B, 15–20 min, 20 %B, 20–25 min, increase to 55 % B, 25–30 min, 55 % B, 30–40 min decrease to 5 % B, 40–45 min, re-equilibrate at 5 % B. The eluant was monitored at 280 nm. Quantitative determination of dimers was based on the external standard method. The peak area for the appropriate PA peak was integrated and compared with a standard curve, and the percentage of specific dimer remained in solution calculated as compared to its initial concentration.

Mass measurement was performed in the negative ion mode under the following condition: capillary temperature 350 °C, sprayer needle voltage 3.5 kV, gas flow rate 20 mL/min. The full scan mass spectra of the depolymerized mixture ranging from m/z 100 to 1,000 were recorded.

Reaction kinetics

To analyze pH and temperature effects on the kinetics of the PA dimers loss, first-order models were used to fit the data collected on the concentration of PA dimers that remained in the mixed solution over time. Moreover, the Arrhenius equation was applied to appraise the temperature dependence of the reaction rate constant k. The dimers contents can be formatted by the following equation: [14]

$$\ln (c_o - c) = kt \quad (1)$$

where k is the reaction rate constant (min^{-1}), and c_o is the initial concentration, c is the concentration of the four dimers at time t (min).

The Arrhenius equation was used to describe the temperature dependence of the rate constant k: [15]

$$k = A \cdot e^{-Ea/RT} \quad (2)$$

where Ea, A, k, T, R represent activation energy (KJ/mol), frequency factor of collision, rate constant (min^{-1}), temperature (k), gas constant (8.3145 J $(\text{mol K})^{-1}$), respectively.

Statistical analysis

All operations were performed at least in triplicate. SAS 8.0 (SAS Institute Inc., Cary, NC) and Prism Graph Pad (5.0) were conducted to analyze experiment statistics and linear regressions. P values <0.05 were regarded as significant.

Results and discussion

Stability of the four PA dimers at different temperatures

The degradation of the four PA dimers at different temperatures was shown in Fig. 2. It was obvious that all four dimers degraded over time, but the degradation of PA dimers was not only temperature dependent, but also structure dependent. Generally, the higher the temperature, the less stable the dimers. All dimers were more stable at temperature below 25 °C than at 40 °C, which is close to physiological temperature. Among the four dimers, A-type (E)C dimer was the most stable, while B-type (E)C dimer and A-type (E)GCG dimer were the least stable. The highest loss amounting to 15 % was observed in the case of A-type (E)GCG dimer and 13 % of B-type (E)C dimer at 40 °C for a 11.25 h of storage. In turn, A-type (E)C dimer only lost 8 % in the same condition.

When plotted the log of the ratio of the remaining dimers to the initial concentration for each temperature against the processing time (Fig. 3), we found the first-order kinetic model that fitted the experimental data well (R^2 = 0.8491–0.9734) (Table 1), indicating an apparent

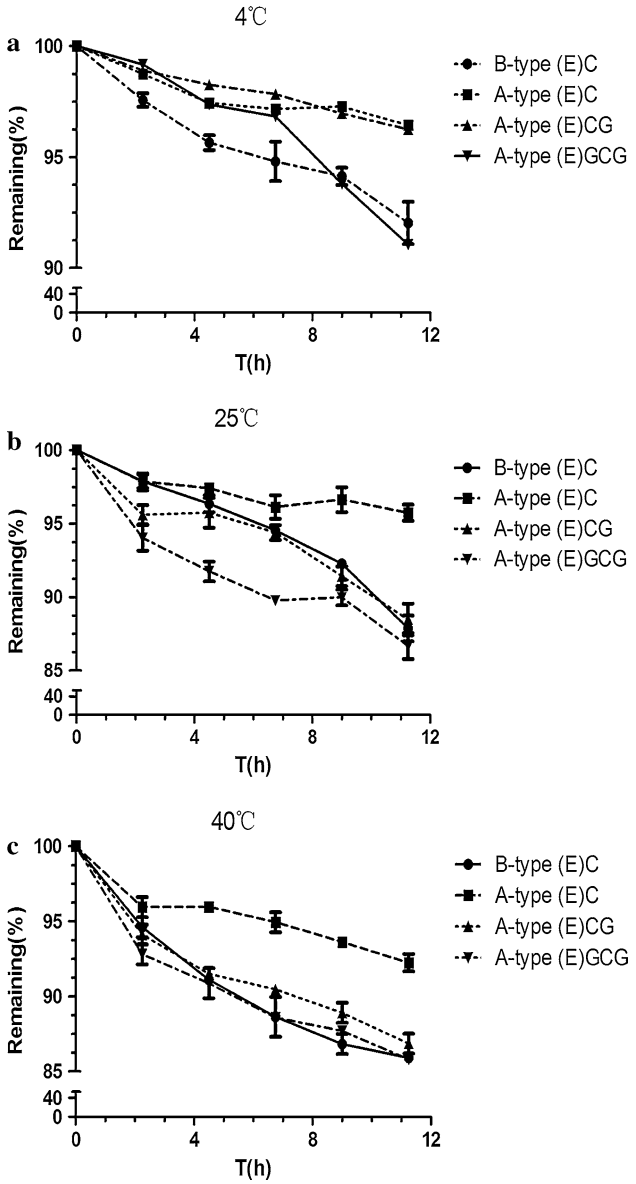

Fig. 2 Time-dependent changes of the four PA dimers in different temperature: 4 °C (**a**), 25 °C (**b**), 40 °C (**c**), respectively

first-order reaction kinetics of PA dimers in solution. The Arrhenius equation was applied to calculate the reaction rate constant k values and the half-life ($t_{1/2}$) values of each dimer at different temperatures. As indicated in Table 1, A-type (E)C dimer showed the lowest reaction rate and the highest half-life at all three temperatures, suggesting it was the most stable. B-type (E)C dimer and A-type (E)GCG dimer had similar reaction rate and half-life at three temperatures tested. A-type (E)CG dimer was more stable at temperature below 25 °C, but rather unstable when temperature increased to 40 °C. By applying the Arrhenius equation (Eq. 2), the activation energy (*Ea*) values of four dimers were calculated. And the parameters of

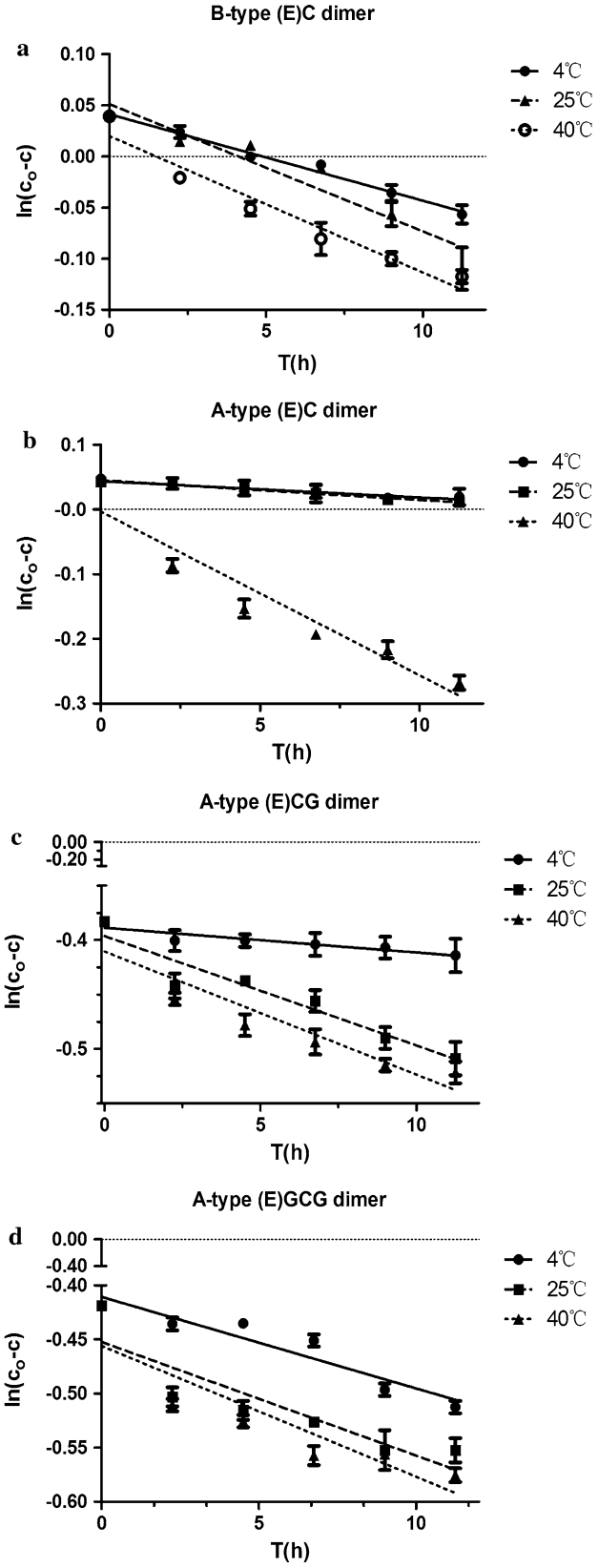

Fig. 3 First-order degradation regression lines of the four PA dimers at different temperatures from 4 to 40 °C: B-type (E)C dimer (**a**), A-type (E)C dimer (**b**), A-type (E)CG dimer (**c**) and A-type (E)GCG dimer (**d**)

Table 1 Temperature effects on rate constants and $t_{1/2}$ values in the dimers solution

Sample	Temp (°C)	k * 10^{-3} (h^{-1})	$t_{1/2}$ (h)	R^2	E_a (kJ/mol)
G1	4	8.352 ± 0.32[b]	82.964 ± 3.12[a]	0.9734	
	25	10.67 ± 0.40[a]	64.948 ± 2.50[b]	0.9448	8.281 ± 0.65
	40	12.57 ± 0.42[a]	55.131 ± 2.38[b]	0.9420	
P1	4	2.668 ± 0.12[b]	259.746 ± 8.33[a]	0.9560	
	25	3.001 ± 0.31[b]	230.923 ± 3.22[a]	0.9098	40.224 ± 2.52
	40	12.012 ± 0.47[a]	57.708 ± 2.17[b]	0.8592	
T1	4	2.375 ± 0.08[c]	291.790 ± 12.5[a]	0.9426	
	25	8.985 ± 0.28[b]	77.129 ± 3.57[b]	0.9156	33.112 ± 2.28
	40	11.86 ± 0.35[a]	58.432 ± 2.85[c]	0.9249	
T2	4	8.366 ± 0.72[b]	82.835 ± 1.38[a]	0.9350	
	25	10.38 ± 0.26[a]	66.763 ± 3.84[a]	0.8491	6.726 ± 0.59
	40	11.74 ± 0.68[a]	59.029 ± 1.46[b]	0.8625	

G1, P1, T1 and T2 represented B-type (E)C dimer, A-type (E)C dimer, A-type (E)CG dimer and A-type (E)GCG dimer, respectively. All dimers were incubated in buffer with pH 6.8. Each data point shows the mean ± SD ($n = 3$). Values with different letter are significantly different from each other ($p < 0.05$)

Table 2 The parameters of Arrhenius equation with the four PA dimers

	P1	G1	T1	T2
Arrhenius fittings				
Slope × 10^{-3}	−0.996	−5.211	−3.982	−0.809
Intercept	3.496	10.522	6.110	4.158
R^2	0.9993	0.9821	0.9504	0.9995

G1, P1, T1 and T2 represented B-type (E)C dimer, A-type (E)C dimer, A-type (E)CG dimer and A-type (E)GCG dimer, respectively

Arrhenius equation with the four PA dimers were shown in Table 2. As an indicator of temperature sensitivity, *Ea* value varied significantly in four dimers. The order of *Ea* value below 40 °C was A-type (E)C dimer > A-type (E)CG dimer > B-type (E)C dimer > A-type (E)GCG dimer, suggesting that A-type dimer was more stable than B-type ones. B-type (E)C dimer and A-type (E)C dimer have the same monomer composition, while the B-type (E)C dimer is less stable than A-type (E)C dimer, which may be due to the additional C2–O–C7 ether linkage in A-type (E)C dimer instead of a phenolic OH group as in B-type (E)C dimer [16, 17]. The subunits of A-type (E)C dimer are linked by two covalent bonds, making it more stable than B-type ones, as indicated by the higher *Ea* value than that of B-type (E)C dimer in the same experimental conditions (Table 1).

Beside the linkage type of the interflavan bonds, the monomer compositions and the degree of galloylation also affected the stability of PA dimers significantly. A-type (E)CG dimer and A-type (E)GCG dimer were less stable than A-type (E)C dimer, and A-type (E)GCG dimer was the least stable. It was reported that among the green tea catechins, EC was the most stable, while EGCG was more susceptible to degradation than ECG and EC [18]. The degradation of PA dimers in solution included multiple pathways such as oxidation, epimerization and degradation [19]. The greater number of hydroxyl-type substituent in the A-type (E)GCG dimer could explain its less stability than A-type (E)C dimer and A-type (E)CG dimer under the same conditions [20]. In addition, compared with A-type (E)CG and (E)C dimer, A-type (E)GCG dimer has three adjacent hydroxyl groups in its molecular, which is more reactive than the two adjacent hydroxyl groups in A-type (E)CG and (E)C dimer, making A-type (E)GCG dimer more instable [21]. The additional galloylation groups,

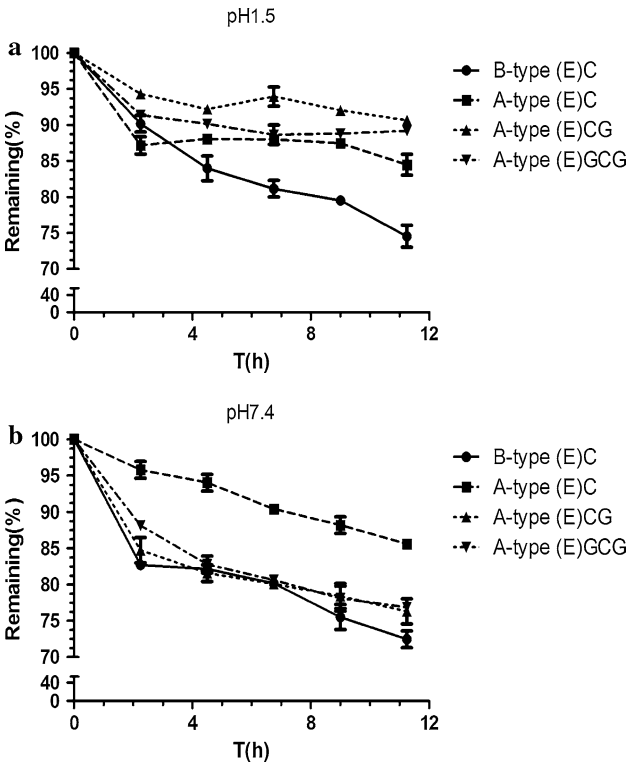

Fig. 4 Time-dependent changes of the four PA dimers at different pH, namely pH 1.5 (**a**) and pH 7.4 (**b**)

which owned a bigger steric hindrance were very susceptible to be hydrolyzed in the aqueous solution, gave another explanation for the more instability of A-type (E)CG dimer and A-type (E)GCG dimer. It is worth noting that A-type (E)CG dimer was stable at 4 °C, as indicative as low reactive rate constant and long half time (Table 1), but it was rather instable when the temperature increased, which might be due to hydrolysis of the galloylation group at higher temperature [22].

Impact of pH on the stability of the four PA dimers

Effect of pH on the stability of PA dimers was assessed at pH 1.5, 7.4 and 10.0, respectively. We choose these pH values because pH 1.5 and 7.4 are close to gastric and intestinal pH, separately, and pH 10.0 could represent an extreme pH value in food. All dimers degraded rapidly at pH 10.0. It was observed that the color of PA dimers in the pH 10.0 solution varied from light yellow to dark brown in a minute. After 2.25 h of incubation, A-type (E)GCG and (E)CG dimer almost degraded completely, and above 80 % of A-type (E)C and B-type of (E)C were lost (Data not shown), indicating PAs were extremely unstable at pH 10.0. Our results were in line with that of Zhu et al. [10], who reported that flavan-3-ols monomers such as epicatechin and catechin and related B-type dimeric proanthocyanidins were rapidly degraded at a pH greater than pH 9.0.

Our results suggested that dimeric proanthocyanidins, no matter it was linked by A-type or B-type interflavan bonds, were unstable at extremely alkaline conditions. The degradation of the PA dimers as a function of time at pH 1.5 and 7.4 was shown in Fig. 4. B-type (E)C dimer was the least stable with a loss of 27 and 28 % at pH 1.5 and 7.4, respectively. High degradation of A-type (E)CG dimer (26 %), A-type (E)GCG dimer (25 %) and A-type (E)C dimer (14 %) at pH 7.4 was also observed. Comparatively, A-type dimers were more stable at pH 1.5 than at pH 7.4, with about 10 % of loss during 11.25 h of storage. Similar results were also reported by Zhu et al. [10], who observed that procyanidins B2 and B5 were more stable at lower pH than at higher pH. And Lu et al. [11] reported that PA2 was rather stable under acidic condition, whereas it was unstable under neutral or alkaline conditions, which could be explained, at least in part, by the direct increase in oxidation rates with an increasing pH. Although it is generally regarded that polyphenols are stable in acidic pH conditions, they are unstable at extremely low pH conditions. In the present study, we noticed content loss in both A-type and B-type dimers at pH 1.5, which may be due to the degradation and isomerization reaction under acid conditions. Similar to the temperature dependence of the degradation of the dimers, an apparent first-order reaction kinetics of PA dimers in different pH values was also

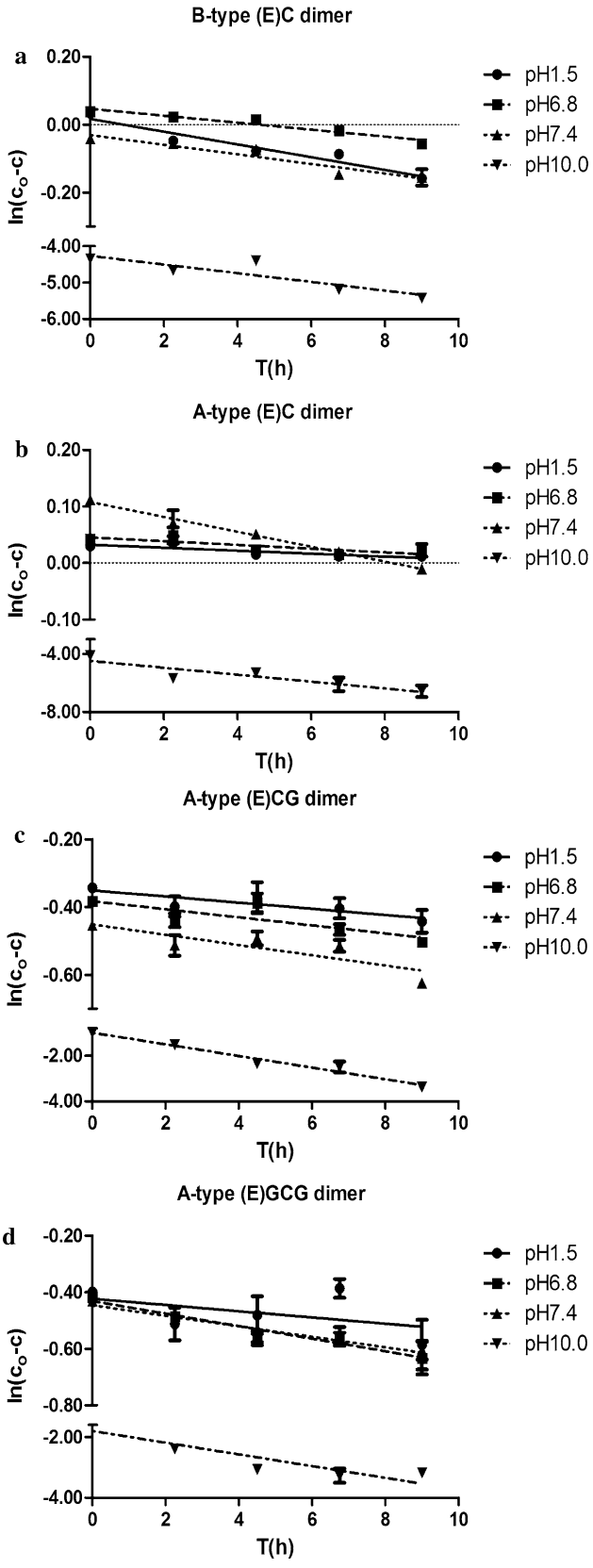

Fig. 5 First-order degradation regression lines of the B-type (E)C dimer (**a**), A-type (E)C dimer (**b**), A-type (E)CG dimer (**c**) and A-type (E)GCG dimer (**d**) at four different pH

observed (Fig. 5). As shown in Table 3, the degradation rate (k) raised evidently with increased pH, while half-life ($t_{1/2}$) of the four dimers reduced correspondingly. B-type (E)C dimer exhibited the highest reaction rate constant and the lowest half-life at both pH, indicating it was the most unstable. The half-life ($t_{1/2}$) of A-type (E)CG dimer, A-type (E)C dimer and A-type (E)GCG dimer at pH 1.5 was quiet longer than at pH 7.4. Likewise, *Ea* decreased as the pH was increased from 1.5 to 7.4, providing further support for those A-type dimers that were more stable at pH 1.5 than at pH 7.4. At pH values of both 1.5 and 7.4, the Ea for B-type (E)C loss was significantly lower than those of A-type dimers, suggesting that A-type dimer was more stable than B-type ones. It was seen that A-type (E)C dimer and A-type (E)CG dimer had the highest *Ea* value, while B-type (E)C owned the lowest value at pH 1.5 and 7.4, suggesting B-type (E)C dimer was the most unstable, while A-type (E)C dimer and A-type (E)CG dimer were more stable. Because the A-type linkage is not susceptible to degrade under acidic conditions, it is not surprising that B-type (E)C dimer was more unstable than A-type ones at pH 1.5 [23]. Malin-Aubert et al. [24] reported only 30 % of procyanidin B2 and B3 lost in pH 3, 50 °C oenin solutions after 50 h. However, Kosińska et al. [25] reported that Cocoa procyanidin B2 lost by 22 % in pH 7.4 HBSS buffers after 2 h, while only 40 % of dimer B5 remained after 2 h. The degradation of procyanidin A2 in DMEM was reported to be more rapid, with half-life of only about 15 min [11]. These results indicated that the degradation of procyanidin dimers is not only structure dependent, but also affected significantly by the storage conditions such as pH values and temperature.

We tried to detect some degraded products by thorough examination of the HPLC–MS/MS chromatograms from the original samples and samples after storage at different pH conditions. Under pH 1.5, we detected monomeric (E)C with a molecular ion [M–H]- at 289 as well as a new peak with m/z 425 (Data not shown), which resulted from the RDA cleavage of B-type (E)C dimer [26, 27]. A small amount of gallic acid with m/z 169 was detected both in A-type (E)GCG and A-type (E)CG dimers. And monomeric (E)GCG and (E)CG were also detected in A-type (E)GCG and A-type (E)CG dimers, separately. Although a new peak appeared after storage of A-type (E)C dimer at pH 1.5 (Data not shown), it showed the same molecular ion [M–H]- at 575 with that of the original sample, indicating epimerization happened. We failed to detect other new peak in A-type (E)C dimer, which might be due to that the minor decomposed products could not be detected under our current analytical conditions. These results suggested that all dimers decomposed in a similar pathway under pH 1.5. Our results agreed with that of Spencer et al. [28] well, who revealed that procyanidin oligomers are unstable under conditions of the acidic environment and decompose essentially to monomeric units.

It is well known that polyphenols are unstable under alkaline conditions. Autoxidation and epimerization were demonstrated to be two major reactions causing the instability of polyphenols under alkaline conditions. Lu et al. [11] found that proanthocyanidin A2 conversed to its isomers under physiological pH conditions. Although some polymerized oxidant products such as theasinensin (EGCG dimer) were identified from EGCG solutions [29] and a dimeric quercetin oxidation product [30] and some putative dimers derived from anthocyanidins [31, 32] have been reported, new oxidant products identified from proanthocyanidin dimers are scarce. Autoxidation can lead to either oxidative degradation or oxidative polymerization. Yoshino et al. [33] reported that EGCG could either degrade to gallic acid or polymerize to theasinensins (THSNs) A and D (m/z 913) in mouse plasma (pH 7.8) and authentic intestinal juice (pH 8.5). While Neilson et al. [34] reported that EGCG oxidative polymerization under simulated digestion not only resulted in the production of THSNs A and

Table 3 Impact of pH on rate constants and $t_{1/2}$ values in the dimers solution

Sample	pH	k * 10^{-3} (h^{-1})	$t_{1/2}$ (h)	R^2	E_a (kJ/mol)
G1	1.5	20.37 ± 1.28^a	34.021 ± 0.78^a	0.9421	5.354 ± 0.48^a
	7.4	14.42 ± 0.84^a	48.058 ± 1.19^a	0.9431	6.163 ± 0.62^a
P1	1.5	2.675 ± 0.35^b	259.06 ± 3.85^a	0.9636	41.743 ± 3.41^a
	7.4	13.48 ± 0.74^a	51.410 ± 1.35^b	0.9816	37.901 ± 3.32^b
T1	1.5	3.128 ± 0.33^b	221.55 ± 3.03^a	0.8796	35.727 ± 2.96^a
	7.4	10.99 ± 0.61^a	63.057 ± 1.64^b	0.9317	32.608 ± 2.77^b
T2	1.5	2.149 ± 0.27^b	319.50 ± 4.70^a	0.8118	19.856 ± 1.23^a
	7.4	14.87 ± 1.17^a	46.604 ± 0.85^b	0.9606	15.056 ± 1.65^b

G1, P1, T1 and T2 represented B-type (E)C dimer, A-type (E)C dimer, A-type (E)CG dimer and A-type (E)GCG dimer, respectively. All dimers were incubated in buffer with temperature 25 °C. Each data point shows the mean ± SD ($n = 3$). Values with different letter are significantly different from each other ($p < 0.05$)

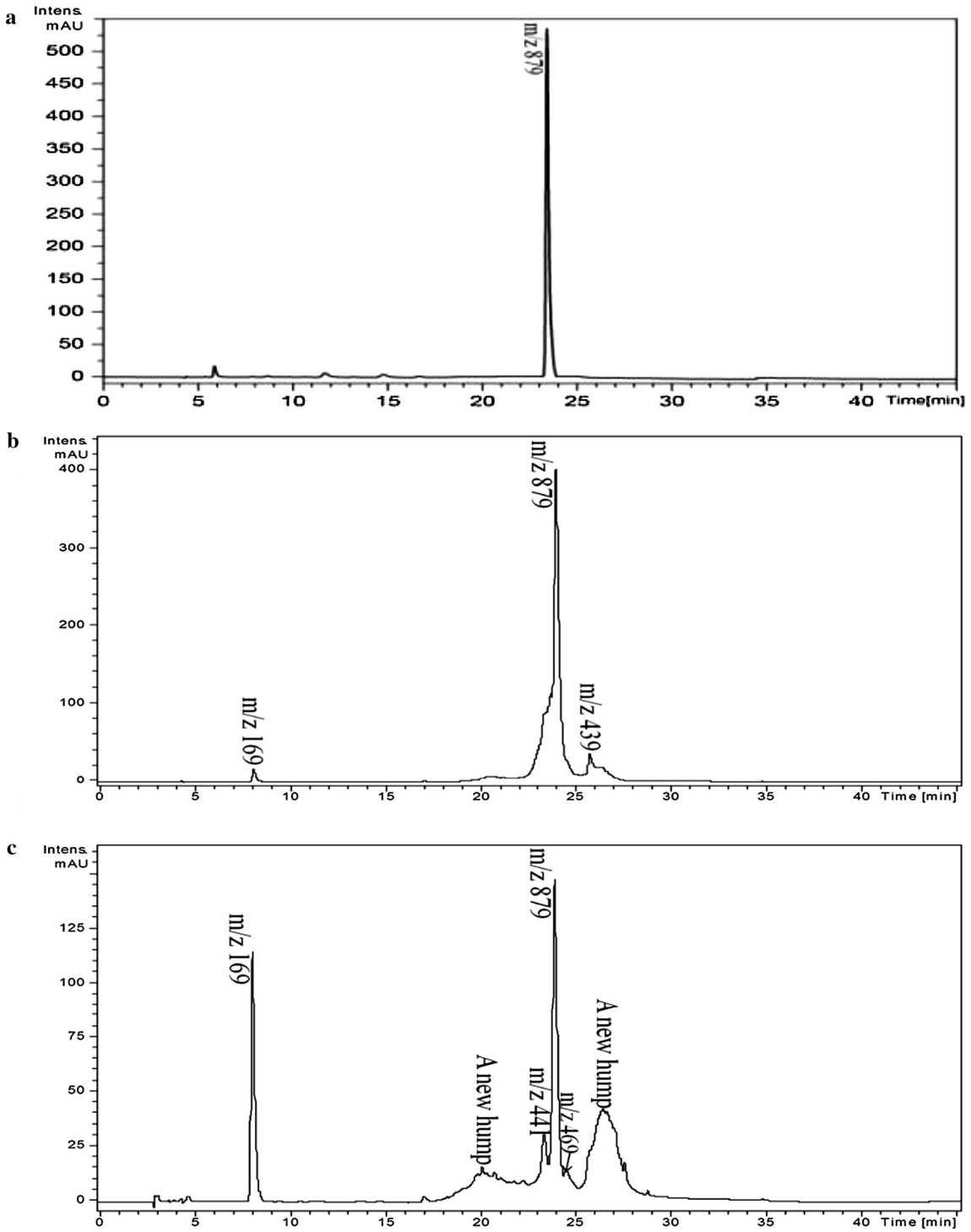

Fig. 6 Chromatograms of the A-type (E)CG dimer in distilled water (**a**), pH 1.5 (**b**) and pH 7.4 (**c**) buffer solution after they were water-bathed 9 h at 25 °C

D (m/z 913), but also P-2 (m/z 883), while ECG formed homodimers analogous to the THSNs (m/z 897). Under our current analytical conditions, we detected some oxidative degradation products in all dimers. HPLC–MS/MS analysis of B-type (E)C dimers showed the formation of three new peaks with m/z 289, 1,151 and 591, respectively (Data not shown), indicating both oxidative degradation and oxidative polymerization happened. Meanwhile, a new peak with a mass [M–H]- of 423 was detected in A-type (E)C dimers, which was a product resulted from the RAD cleavage of

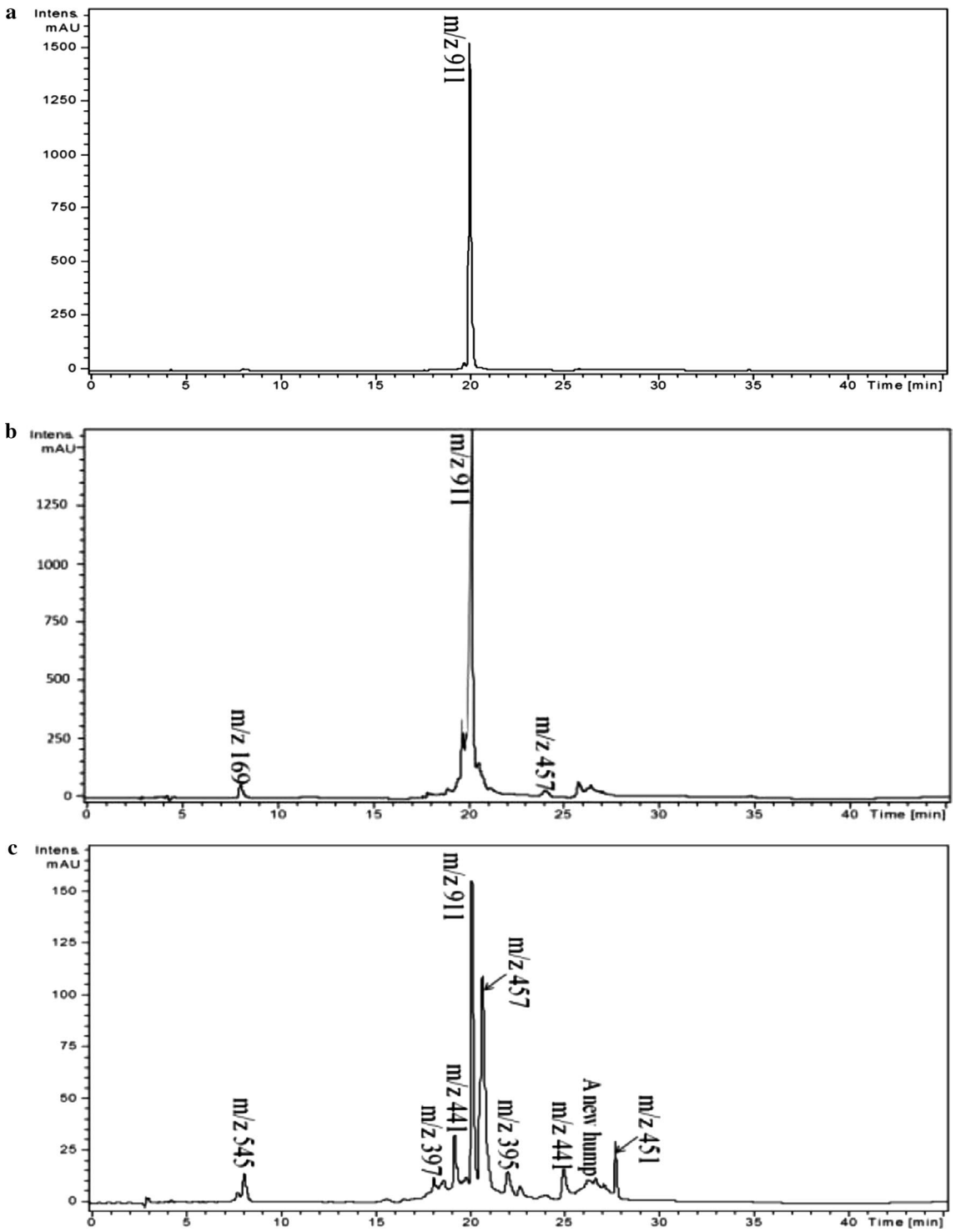

Fig. 7 Chromatograms of the A-type (E)GCG dimer in distilled water (**a**), pH 1.5 (**b**) and pH 7.4 (**c**) buffer solution after they were water-bathed 9 h at 25 °C

A-type (E)C dimer [35]. Similarly, the formation of a series of peaks with masses [M–H]- of 169, 404, 441 and 469, respectively (Fig. 6), was seen in A-type (E)CG dimer solution, and more peaks with masses [M–H]- of 545, 399, 606, 397, 441, 395, 469 and 451, respectively (Fig. 7), were observed in A-type (E)GCG dimer solution. Although the detailed structures of the newly formed peaks were unclear, these results strongly suggested that

oxidative degradation was one main degradation pathway for all dimers in alkaline condition. Compared with A-type (E)CG and (E)C dimer, A-type (E)GCG dimer has greater number of hydroxyl groups, especially three adjacent hydroxyl groups, which is more susceptible to undergo autoxidation reactions, making it more instable than A-type (E)CG and (E)C dimer [20], and more degradation products were formed. Although dissimilar to that in B-type (E)C dimer, no polymerized product was detected in all three A-type dimers under the current analytical conditions, the HPLC chromatograms in Fig. 7 clearly displayed a large hump eluting at the end between 25 and 30 min, such a hump in the chromatographic profile could be attributed to polymeric polyphenols, suggesting polymerization reaction happened under the alkaline condition.

In addition, oxygen may also play an important role in the autoxidation reactions of polyphenols in solutions. Talcott et al. [36] reported that reducing oxygen could result in a better color maintenance of polyphonic materials after processing and during storage, especially in low and medium acid food systems. However, Bermúdez-Soto et al. [37] found that the degraded rates of chokeberry polyphenols exposed to atmospheric O_2 and light or in the absence of light and O_2 in vitro gastric and pancreatic digestion conditions had no significant difference. Results of Spencer et al. [28] revealed that Cocoa procyanidin oligomers (trimer to hexamer) were hydrolyzed to mixtures of epicatechin monomer and dimer, thus improving the potential for their absorption in the small intestine, but they also ignore the possible influence of oxygen in their study. Oligomeric procyanidins were reported to be decomposed by intestinal microflora in anaerobic condition [38]; however, the presence of numerous intestinal microflora makes the conditions more complex and significantly different from the current condition. The influence of oxygen on the decomposition pathways of dimers may differ significantly in different systems. The degradation pathways for the dimers might be quite different in anoxic environments and in the presence of O_2, and this needs further study in the future work. The differences in the stability of different proanthocyanidin dimers as well as their distinct degradation products in stimulated gastric and intestinal conditions could explain, at least in part, their different bioactivities in vivo. And the detailed mechanisms also need to be explored in the future work.

Conclusion

In summary, the stability of proanthocyanidin dimers was not only temperature and pH dependent, but also structure dependent. Proanthocyanidin dimers were found to be unstable at pH as low as 1.5 and physiological pH conditions, and they were rather unstable at alkaline conditions and temperature above 25 °C. In general, B-type dimers showed less stability than A-type ones. The higher the degree of galloylation and the more the hydroxyl groups in the molecular, the less stable the proanthocyanidins dimers.

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