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Stability and colour characteristics of PEF-treated cyanidin-3-glucoside during storage

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Abstract

The stability and colour characteristics of PEF (pulsed electric field)-treated cyanidin-3-glucoside (Cy-3-glu) was investigated during storage at 4, 24 and 37 °C. The degradation of Cy-3-glu was analyzed using a first reaction kinetics while its colour characteristics was evaluated using colour indices such as colour density (*CD*) and *CIE* $L^*a^*b^*$ parameters. PEF had no post-effect on the stability and colour characteristics of Cy-3-glu during storage while the storage temperature had a significant effect ($p < 0.05$). The degradation of PEF-treated Cy-3-glu during storage conformed to the first-order reaction kinetics with regression coefficients R^2 greater than 0.9300. Corresponding to 4, 24 and 37 °C, the degradation rate constant k in Cy-3-glu significantly increased in the exponential order level of 10^{-4}, 10^{-3} and 10^{-2}, while the $t_{1/2}$ (the time that 50% Cy-3-glu degradation would take) and D-value (the time that 90% Cy-3-glu degradation would take) of Cy-3-glu during storage decreased in the exponential order level of 10^3, 10^2 and 10. The reduction of *CD* was closely related to the Cy-3-glu content in this study, the Cy-3-glu content was perfectly predicted using *CD* with a higher coefficient $R^2 > 0.9999$. A significant decrease in b^* and H^0 value was obtained at all storage temperature ($p < 0.05$). A positive correlation was found between the Cy-3-glu content and b^* value or H^0 value with a coefficients $R^2 > 0.8400$ during storage. ΔE increased significantly, ΔE was less than 2 at 4 °C while it was greater than 2 at 24 and 37 °C.

Keywords: Pulsed electric field (PEF); Cyanidin-3-glucoside (Cy-3-glu); Stability; Colour characteristics

1. Introduction

Anthocyanins are widely present in plant materials (Markakis, 1982) and are noted for their attractive colour and potential as substitute for synthetic colorants. In addition to their colour properties, interest in anthocyanins has intensified because of their possible role in reducing the risk of coronary heart disease, cancer and stroke (Wrolstad, 2004). It is well known that anthocyanins are not stable and tend to decolorize or degrade during storage of food (Seeram, Bourquin, & Nair, 2001). Temperature, oxygen, water activity and time during storage are considered to influence the stability of anthocyanins (Tsai, Delva, Yum, Huang, & Dufossé, 2005). As described by Rodríguez-Saona, Giusti, and Wrolstad (1999), extraction method also has an effect ($p < 0.01$) on the degradation of monomeric anthocyanins in potato and radish at 25 °C during storage. Cabrita, Fossen, and Andersen (2000) investigated the absorbance change of Cy-3-glu during storage at 10 °C and 23 °C for 60 days. Torskangerpoll and Andersen (2005) investigated changes in colour characteristics of Cy-3-glu solution during storage at 10 °C. The results indicated that temperature was an important factor influencing the degradation of anthocyanin during storage. The degradation of anthocyanins influenced by temperature of storage was well fitted to a first-order rate law (Cemeroğlu, Velioglu, & Isik, 1994; Gradinaru, Biliaderis, Kallithraka, Kefalas, & Garcia-Viguera, 2003; Iversen, 1999; Kirca & Cemeroğlu, 2003; Ochoa, Kesseler, Michelis, Mugridge, & Chaves, 2001; Ochoa, Kesseler, Vullioud, & Lozano,

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1999). Iversen (1999) reported that the degradation reaction rate constant of anthocyanin in black currant nectar was 4.2×10^{-3} and 3.2×10^{-3} in light and in darkness during storage at 20 °C, respectively. The reaction rate constants were statistically different. As described by Eder (1996), the degradation rate constants of delphinidins and cyanidins were between 6.3×10^{-3} and 7.5×10^{-3} (days) during storage at 17 °C.

In recent years, there has been an increasing interest in possible use of pulsed electric field (PEF) in research areas, including the extraction, and dehydration, among others (Bazhal & Vorobiev, 2000; Fincan, Devito, & Dejmek, 2004; Guderjan, Töpfl, Angersbach, & Knorr, 2005; Rastogi, Eshtiaghi, & Knorr, 1999; Yin, Han, & Han, 2006). The PEF-assisted extraction of pigment, polysaccharides, sugar, protein and plant oil has been previously reported (Chalermchat, Fincan, & Dejmek, 2004; Fincan et al., 2004; Guderjan et al., 2005; Yin et al., 2006; Zhang et al., 2006). The effect of PEF-assisted extraction on the yields of anthocyanins in red raspberry fruits was investigated earlier (Zhang et al., 2006). Moreover, we discussed the effect of PEF on anthocyanins and reported the kinetics of Cy-3-glu degradation and its colour change by pulsed electric field (PEF) at mild electric intensity (Zhang et al., 2007). It was found that the degradation rate constant of Cy-3-glu exposed to PEF was 10^4 times that of thermal treatment. However, no more information is available on the stability and colour characterization of PEF-treated anthocyanins during storage. Although Min, Jin, and Zhang (2003) have reported that the content of lycopene was not significantly different in thermally processed and PEF processed tomato juice during storage at 4 °C for 112 days. Due to the complexity of chemical reactions taking place in natural systems, e.g. juice, concentrates, jams and jellies, it has been difficult to explain changes of pigments in a single factor. Therefore, the evaluation of a particular parameter requires model systems in order to closely control the composition and factors influencing pigments stability (Garzón & Wrolstad, 2001).

The degradation of anthocyanin during storage of foods could have a dramatic impact on colour quality and may also affect nutritional properties. Modern colour instrumentation have made measurement of *CIE* $L^*a^*b^*$ indices practical and easy. Indices of lightness (L^*), chroma (C^*), and hue angle (H^0) are particularly useful for tracking colour changes (Wrolstad, Durst, & Lee, 2005).

The objective of this study was to investigate the degradation and colour characteristics of PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C.

2. Materials and methods

2.1. Materials

The berry fruits of red raspberry (*Rubus idaeus* L., heritage variety) were used in the experiment for isolating Cy-3-glu. Fruits were picked at commercial maturity during the 2003 harvest season at the Agriculture Experiment Base of Chinese Academy of Forestry in Miyun county (Beijing, China). The berry fruits were kept at −28 °C until used.

All chemicals in the investigation were of analytical grade, which were purchased from Beijing Chemical Reagent Co. (Beijing, China), except methanol (MeOH) that was of chromatographic grade (Fisher Chemicals, Waltham, MA, USA) used in HPLC/ESI-MS and HPLC.

2.2. Purification of Cy-3-glu

The preparation of Cy-3-glu was performed according to the method described in a previous paper (Zhang et al., 2007). Briefly, frozen berry fruits were extracted with 0.5% trifluoroacetic acid (TFA, Beijing Chemical Reagent Co., Beijing, China) in 2 L methanol. After standing for 24 h at 0 °C, the mixture was centrifuged at 2700*g* for 15 min by a centrifugation (TDL-5-A, Anqing Lab Instrument Co., Shanghai, China) at 28 °C. The methanolic pigments were collected and concentrated at 40 °C in a rotary evaporator (SENCQ R-501, Shenshun Biotechnology Co., Shanghai, China). Then, the concentrated extracts were purified by partition against ethyl acetate (Beijing Chemical Reagent Co., Beijing, China) three times before application on an Amberlite XAD-7 column (40 × 1.6 cm, Sigma, St. Louis, MO, USA). After washing the column with 0.5% TFA aqueous solution, the isolated anthocyanins were eluted with methanol containing 0.5% TFA (Fossen, Cabrita, & Andersen, 1998; Fossen, Slimestad, & Andersen, 2003). Then the concentrated methanolic pigments were fractioned using Sephadex LH-20 chromatography (70 × 2.6 cm, Sigma, St. Louis, MO, USA) using 0.5% TFA aqueous solution–MeOH (7:3, v/v) as eluent (Fossen et al., 1998, 2003). The flow rate was 0.3 mL min^{-1}. The purified Cy-3-glu was checked for homogeneity by analytical HPLC and was identified by HPLC/ESI-MS (Agilent 1100 MSD series, Agilent Technologies Co. Ltd., Palo Alto, CA, USA).

The fraction of Cy-3-glu was collected and stored at −18 °C before use. The pH of the resulting solution was about 1.6.

2.3. Sample treatment

PEF treatment was performed using a laboratory scale pulse generator system, which was used in previous investigations (Zhang et al., 2007). This apparatus consisting of a high voltage pulse generator, a high voltage pulse treatment chamber, a peristaltic pump, and the parallel-plate electrodes was used in the investigation. Equipment parameters included exponentially decaying wave, 300 μs pulse duration (pulse width), 1 Hz pulse frequency, 0.5 μF capacitor and 60 mL treatment chamber. The electrodes were stainless steel and their configuration was parallel-plate type. The gap between the high voltage electrode and the ground electrode was 4.5 cm. The high voltage was monitored by a Trek oscilloscope (Tektronix TDS 210, Tek-

tronix, OR, USA). A thermocouple was attached to the exit of the chamber to monitor the post-treatment temperature.

Sample was pumped to the PEF chamber from one sample bottle by peristaltic pump (BT02, Xinyu Technology Co., Beijing, China) until the chamber was full, then was treated by PEF at the desired electric field intensity of 1.2, 2.2 and 3.0 kV/cm, respectively. The number of pulses was 300. PEF-treated samples to be measured were pumped into new bottles. The temperature of samples was less than 47 °C by thermocouple determined.

The PEF-treated samples in same volume were stored at 4, 24 and 37 °C, respectively. All experiments were performed in triplicates.

2.4. Cy-3-glu analysis

The quantification of Cy-3-glu was evaluated using HPLC analysis. The HPLC system consisted of a Shimadzu (Kyoto, Japan) liquid chromatography system equipped with LC-10AT pump, a model SPD-M10A photodiode array detector and a model SCL-10A communications bus module. Samples (1 mL) were filtered through a 0.22 μm filter and analyzed on an Cosmosil $5C_{18}$-RA-II column (Cosmosil, Kyoto, Japan) 250 × 4.6 mm I.D. 5 μm at a column temperature of 35 °C. The mobile phase HCO_2H–H_2O (3:97, v/v) (A) and HCO_2H–H_2O–MeOH (3:47:50, v/v/v) (B) were used. The elution profile for analytical HPLC consisted of isocratic elution (60% A, 40% B) in 2 min, a linear gradient from 40% B to 100% B over next 60 min. The flow rate was 0.8 mL min^{-1}. The Cy-3-glu was detected at 280 and 520 nm. The sample injection volume was 20 μL.

2.5. Kinetic analysis

The degradation data of Cy-3-glu were subjected to the regression analysis using the following first-order model:

$$\ln(C_t/C_0) = kt \quad (1)$$

where C_t and C_0 are the Cy-3-glu content at time t and t_0, respectively, k was the reaction rate constant (day^{-1}), and t is the storage time (days). Furthermore, half-life value $t_{1/2}$ of Cy-3-glu was calculated as $t_{1/2} = \ln 2/k$, D-value was also calculated as a kinetics parameter of Cy-3-glu degradation. D-value meant the time that the degradation of 90% Cy-3-glu would take and was calculated as $D = 1/k$.

2.6. Colour measurement

The methods described by Somers and Evans (1977) were used to determine the colour density (CD) of PEF-treated Cy-3-glu during storage and the CD was the sum of the absorbance at 420 nm and 520 nm of Cy-3-glu.

CIE $L^*a^*b^*$ colour parameters were recorded as L^* (lightness), a^* (redness), and b^* (yellowness) with a colour difference meter (SC-80C, Kangguang Instrument Co., Beijing, China) using the transmission mode. Samples were filled in 5 × 3 × 1 cm glass cell and measured. Colour results were expressed as follows: L^*, a^* and b^* indicated lightness, redness and yellowness, respectively. H^0 ($H^0 = \tan^{-1} b^*/a^*$) indicated sample hue angle (0° or 360° = red; 90° = yellow; 180° = green; 270° = blue). ΔE indicated the total colour difference between two samples, calculated as $[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ (Torskangerpoll & Andersen, 2005).

2.7. Data analysis

Results were presented as means ± standard deviation. Statistical analysis of experimental results was based on analysis of variance. Significant difference was statistically considered at the level of $p < 0.05$. The difference of degradation rate constant between PEF-treated and control Cy-3-glu was analyzed by t-test, significant difference was statistically considered at the level of $p < 0.01$. The analyses were performed with SAS, version 9.0 (SAS Institute Inc., Cary, NC, USA). All experiments were performed in triplicates.

3. Results and discussion

3.1. Degradation of PEF-treated Cy-3-glu during storage

The content of Cy-3-glu was significantly decreased when exposed to PEF at 1.2, 2.2 and 3.0 kV/cm in an earlier study (Zhang et al., 2007). However, the stability of PEF-treated Cy-3-glu during storage was not available in the earlier study, thus it was worth investigating whether PEF had a post-effect on the stability of Cy-3-glu or not. Fig. 1 illustrates the reduction of PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C for 90 days. The profile of Cy-3-glu degradation curves was very similar during storage for all cases, a significant decrease was observed for Cy-3-glu after 90-day storage ($p < 0.05$). A retention of PEF-treated Cy-3-glu during storage at 4 °C for 90 days was 95.08%, 94.72% and 93.31% in Fig. 1a, corresponding to 1.2, 2.2 and 3.3 kV/cm, respectively. As the storage temperature increased, a significant decrease of Cy-3-glu content was observed for all samples ($p < 0.05$), indicating that the storage temperature had a significant effect on the stability of Cy-3-glu. The result was also similar to previous findings, which documented that the degradation of anthocyanins was affected by storage temperature. Martí, Pérez-Vicente, and García-Viguera (2001) found that anthocyanin losses in pomegranate juice stored for two months were about 60% at 5 °C and 85% at 25 °C. Plocharski and Zbroszcyzk (1992) described that the retention of anthocyanin in black chokeberry juice were only 53% at 4 °C and 11% at 20 °C after a year of storage.

Based on the above observations, the degradation of PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C was analyzed using Eq. (1). The kinetic parameters are

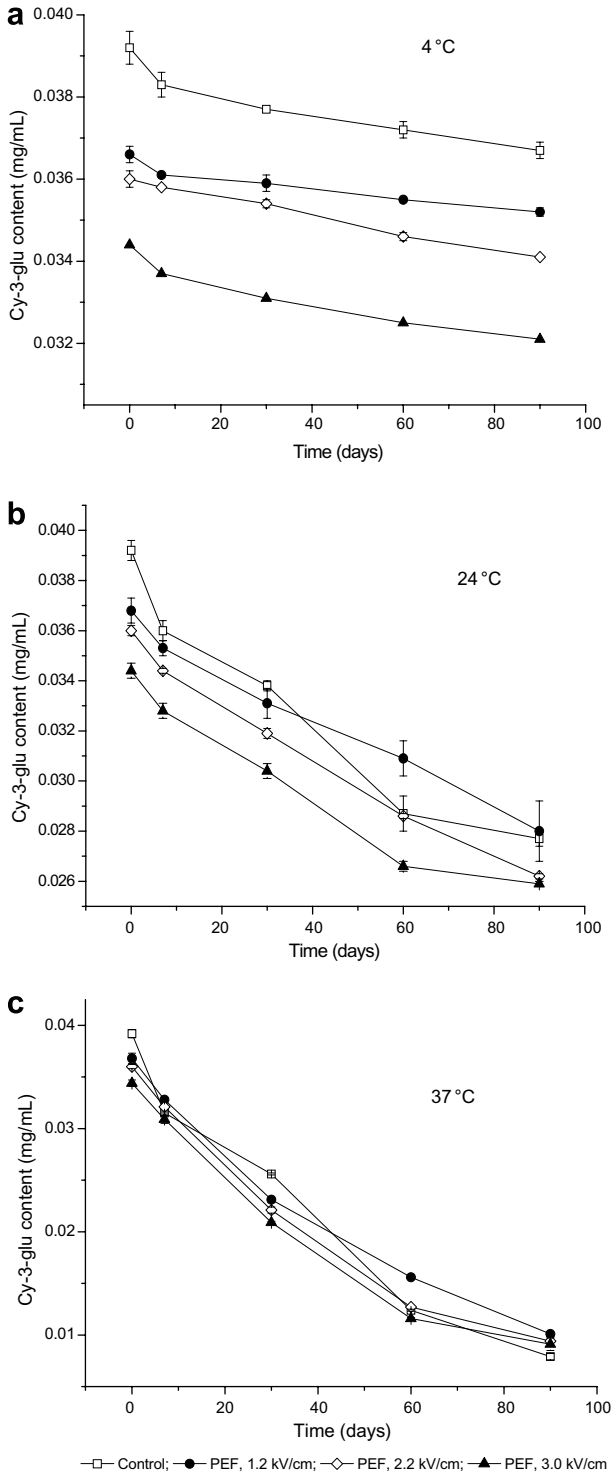

Fig. 1. Change of Cy-3-glu content as a function of time during storage at 4, 24 and 37 °C.

Table 1
Degradation kinetics parameters of PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C

Parameters	Control	1.2 kV/cm	2.2 kV/cm	3.0 kV/cm
$k \times 10^{-4}$	5.0401	5.4202	6.0566	5.8406
R^2	0.9897	0.9973	0.9911	0.9828
$t_{1/2} \times 10^3$ (days)	1.6911	1.2788	1.1444	1.1868
D-value $\times 10^3$ (days)	1.9841	1.8450	1.6511	1.7122
$k \times 10^{-3}$	3.4000	2.7400	3.3200	3.0000
R^2	0.9395	0.9947	0.9432	0.9423
$t_{1/2} \times 10^2$ (days)	2.0387	2.5298	2.0878	2.3104
D-value $\times 10^2$ (days)	2.9410	3.6500	3.0120	3.3330
$k \times 10^{-2}$	1.8200	1.4100	1.5100	1.5200
R^2	0.9434	0.9993	0.9853	0.9721
$t_{1/2} \times 10$ (days)	3.8085	4.9159	4.5904	4.5602
D-value $\times 10$ (days)	5.4900	7.0900	6.6200	6.5800

shown in Table 1. All regression coefficients (R^2) were greater than 0.9300, indicating that the degradation of PEF-treated Cy-3-glu during storage conformed to the first-order reaction kinetics at all temperatures examined. The observations were in accordance with previous findings. Kirca, Özkan, and Cemeroğlu (2006) described that anthocyanin degradation of black carrot anthocyanins in fruit juices and nectars during storage at 4–37 °C followed a first-order reaction kinetics. As shown in Table 1, the degradation rate constant k of PEF-treated Cy-3-glu during storage at 4 °C varied from 5.4202×10^{-4} to 6.0566×10^{-4}, the degradation rate constant k of control Cy-3-glu during storage was 5.0401×10^{-4}, the data indicated that no significant difference ($p > 0.01$) in degradation rate constant k was presented among PEF-treated Cy-3-glu and control Cy-3-glu. It seems that the degradation rate constant k of PEF-treated Cy-3-glu during storage is not related to the electric intensity of PEF. Similar observations were found during storage at 24 and 37 °C. Thus, PEF also had no post-effect on the degradation rate constant k during storage ($p > 0.01$). Moreover, at 4, 24 and 37 °C, the corresponding degradation rate constant k in PEF-treated Cy-3-glu and control Cy-3-glu, significantly increased in the exponential order level of 10^{-4}, 10^{-3} and 10^{-2}, this result shows that the degradation rate constant k of all the samples in the investigation is closely related to the storage temperature. The temperature dependence of degradation rate constant k during storage agreed with earlier investigations. Cemeroğlu et al. (1994) described that the degradation rate constant of anthocyanin in sour cherry concentrate at 45° Brix was 1.000×10^{-4}, 5.290×10^{-3} and 1.844×10^{-2} at 5, 20 and 37 °C, respectively. Kirca et al. (2006) also showed that the black carrot anthocyanin degradation rate in grape juice was 6.9×10^{-4}, 8.5×10^{-3} and 5.48×10^{-2} at 4, 20 and 37 °C, respectively.

Moreover, $t_{1/2}$ and D-value of all the Cy-3-glu samples during storage was also calculated. As shown in Table 1, the $t_{1/2}$ and D-value of PEF-treated Cy-3-glu and control Cy-3-glu during storage decreased in the exponential order level of 10^3, 10^2 and 10, as increasing the storage temperature. No significant difference for the $t_{1/2}$ and D-value was noted at the same storage temperature among all the Cy-3-glu samples ($p > 0.01$). The result was in agreement with previous reports (Cemeroğlu et al., 1994; Kirca et al., 2006).

Table 2
The colour characteristics of PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C

Treatment	Storage time (days)	4 °C				24 °C				37 °C			
		CD	b^*	H^0	ΔE	*CD*	b^*	H^0	ΔE	*CD*	b^*	H^0	ΔE
Control	0	1.473 ± 0.011	−44.83 ± 0.06	347.0 ± 0.0	–	1.473 ± 0.011	−45.20 ± 0.06	346.9 ± 0.0	–	1.473 ± 0.011	−45.20 ± 0.06	346.9 ± 0.0	–
	7	1.447 ± 0.002	−45.21 ± 0.08	346.9 ± 0.0	1.19 ± 0.05	1.350 ± 0.008	−47.16 ± 0.05	346.4 ± 0.0	2.53 ± 0.13	1.160 ± 0.008	−50.66 ± 0.35	345.4 ± 0.1	5.72 ± 0.25
	30	1.435 ± 0.006	−45.82 ± 0.21	346.8 ± 0.0	1.42 ± 0.08	1.248 ± 0.048	−49.77 ± 0.90	345.7 ± 0.3	6.01 ± 0.09	0.673 ± 0.001	−52.62 ± 0.06	344.8 ± 0.0	16.6 ± 0.18
	60	1.423 ± 0.004	−46.04 ± 0.05	346.7 ± 0.0	1.34 ± 0.05	1.065 ± 0.005	−53.52 ± 0.06	344.6 ± 0.0	8.84 ± 0.18	0.422 ± 0.043	−65.99 ± 0.95	341.2 ± 0.3	22.31 ± 0.51
	90	1.393 ± 0.006	−46.16 ± 0.09	346.6 ± 0.0	1.26 ± 0.08	0.961 ± 0.006	−55.68 ± 0.19	344.0 ± 0.0	10.93 ± 0.33	0.235 ± 0.031	−69.38 ± 1.07	340.1 ± 0.3	25.09 ± 1.02
1.2 kV/cm	0	1.400 ± 0.003	−46.73 ± 0.12	346.4 ± 0.0	–	1.400 ± 0.003	−46.73 ± 0.12	346.4 ± 0.0	–	1.400 ± 0.003	−46.73 ± 0.12	346.4 ± 0.0	–
	7	1.366 ± 0.002	−47.24 ± 0.20	346.4 ± 0.1	1.88 ± 0.03	1.298 ± 0.010	−47.94 ± 0.31	346.2 ± 0.1	3.50 ± 0.16	1.213 ± 0.001	−49.41 ± 0.30	345.7 ± 0.1	3.74 ± 0.25
	30	1.346 ± 0.012	−47.44 ± 0.33	346.2 ± 0.1	1.18 ± 0.01	1.252 ± 0.017	−49.92 ± 0.69	345.6 ± 0.2	5.06 ± 0.42	0.849 ± 0.015	−57.27 ± 0.36	343.5 ± 0.1	11.88 ± 0.35
	60	1.315 ± 0.019	−47.73 ± 0.08	346.2 ± 0.0	1.53 ± 0.03	1.164 ± 0.027	−50.29 ± 0.78	345.5 ± 0.2	5.46 ± 0.31	0.606 ± 0.011	−61.25 ± 0.64	342.4 ± 0.2	15.16 ± 0.55
	90	1.293 ± 0.001	−47.97 ± 0.37	346.1 ± 0.1	1.62 ± 0.02	1.032 ± 0.013	−54.15 ± 0.43	344.4 ± 0.1	8.50 ± 0.37	0.404 ± 0.026	−64.80 ± 0.71	341.3 ± 0.2	18.48 ± 0.59
2.2 kV/cm	0	1.371 ± 0.005	−47.27 ± 0.07	346.2 ± 0.0	–	1.371 ± 0.005	−47.30 ± 0.07	346.2 ± 0.0	–	1.371 ± 0.005	−47.30 ± 0.07	346.2 ± 0.0	–
	7	1.353 ± 0.003	−47.69 ± 0.08	346.2 ± 0.0	1.58 ± 0.02	1.286 ± 0.002	−48.58 ± 0.39	346.0 ± 0.1	3.05 ± 0.22	1.194 ± 0.004	−49.58 ± 0.58	345.7 ± 0.2	2.32 ± 0.11
	30	1.333 ± 0.004	−47.72 ± 0.16	346.1 ± 0.0	0.63 ± 0.00	1.224 ± 0.008	−51.00 ± 0.34	345.3 ± 0.1	4.75 ± 0.28	0.818 ± 0.020	−57.64 ± 0.55	343.3 ± 0.1	10.94 ± 0.42
	60	1.310 ± 0.008	−47.91 ± 0.06	346.1 ± 0.0	1.34 ± 0.02	1.087 ± 0.008	−52.95 ± 0.23	344.7 ± 0.1	6.14 ± 0.24	0.486 ± 0.018	−65.18 ± 0.93	341.4 ± 0.3	19.71 ± 0.06
	90	1.274 ± 0.001	−48.17 ± 0.26	346.1 ± 0.1	1.71 ± 0.01	0.934 ± 0.004	−55.17 ± 1.29	344.1 ± 0.4	9.54 ± 0.13	0.275 ± 0.046	−67.85 ± 0.21	340.5 ± 0.1	21.27 ± 0.21
3.0 kV/cm	0	1.326 ± 0.003	−48.07 ± 0.08	346.0 ± 0.0	–	1.326 ± 0.003	−48.34 ± 0.08	345.9 ± 0.0	–	1.326 ± 0.003	−48.34 ± 0.08	345.9 ± 0.0	–
	7	1.308 ± 0.007	−48.60 ± 0.15	346.0 ± 0.0	1.68 ± 0.04	1.239 ± 0.005	−49.60 ± 0.06	345.7 ± 0.0	2.78 ± 0.13	1.147 ± 0.006	−51.00 ± 0.38	345.2 ± 0.1	3.36 ± 0.16
	30	1.281 ± 0.004	−48.85 ± 0.09	345.9 ± 0.0	0.49 ± 0.02	1.162 ± 0.007	−52.05 ± 0.18	345.0 ± 0.0	4.57 ± 0.20	0.750 ± 0.005	−59.29 ± 0.05	342.9 ± 0.0	11.31 ± 0.15
	60	1.266 ± 0.003	−48.94 ± 0.06	345.8 ± 0.0	1.25 ± 0.01	1.012 ± 0.023	−54.64 ± 0.43	344.3 ± 0.1	6.85 ± 0.38	0.437 ± 0.021	−65.90 ± 0.35	341.1 ± 0.1	18.46 ± 0.43
	90	1.223 ± 0.003	−49.10 ± 0.40	345.8 ± 0.1	1.46 ± 0.02	0.944 ± 0.002	−56.25 ± 0.05	343.8 ± 0.0	8.40 ± 0.13	0.221 ± 0.002	−70.48 ± 0.40	339.8 ± 0.1	23.11 ± 0.49

3.2. Colour characteristics of PEF-treated Cy-3-glu during storage

The anthocyanin degradation resulted in a colour change and the colour change could be characterized by colour density (*CD*) and *CIE* $L^*a^*b^*$ system. The *CD* reduction in PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C is shown in Table 2. The higher the temperature, the greater the *CD* decrease, the greatest decrease of *CD* was observed stored at 37 °C. The *CD* decrease resulted from both reductions in absorbance at 420 nm and 520 nm. The reduction in absorbance at 420 nm during the storage in all samples in the study was different from the earlier observations in complex matrices. As previously investigated, the absorbance at 420 nm increased due the transformation of monomeric anthocyanin into polymeric anthocyanin which had generally a brownish shade (Garzón & Wrolstad, 2002; Reyes & Cisneros-Zevallos, 2007). It was also shown that thermal degradation of anthocyanins in food led to increasing browning material (Reyes & Cisneros-Zevallos, 2007). Thus, the polymeric colour in our study was detected using the method described by Somers (1971). However, the polymeric colour could not be detected in our study, implying that the transformation of monomeric anthocyanin into polymeric anthocyanin did not occur in the investigation, this observation disagrees with the findings by Garzón and Wrolstad (2001), who reported that the polymeric colour increased with the decrease of monomeric anthocyanin in a simple system (Garzón & Wrolstad, 2001) similar to our study system. In Vis–UV spectrum of Cy-3-glu, there were two identified absorbance peak at 280 nm and 520 nm bands and a shoulder at 420 nm bands (Markakis, 1982; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999). Therefore, the reduction in absorbance at 520 nm and at 420 nm was only due to the degradation of Cy-3-glu during storage, it was concluded that the change of *CD* was closely related to the Cy-3-glu content in our model system. The correlation between the Cy-3-glu content and *CD* is illustrated in Fig. 2. A positive linear relationship with a higher correlation coefficient $R^2 > 0.8400$ (in Table 3) was displayed between the Cy-3-glu content and *CD* during storage at 4, 24 and 37 °C, indicating that the Cy-3-glu content could be characterized by the *CD* in our system. As shown in Fig. 3, the Cy-3-glu content was predicted using *CD*, a perfect correlation with $R^2 > 0.9999$ between the predicted data and the observed data.

Other colour indices of PEF-treated Cy-3-glu during storage at 4, 24 and 37 °C were evaluated by *CIE* $L^*a^*b^*$ system. The change in b^*, H^0 and ΔE is shown in Table 2. During storage, b^* value of PEF-treated Cy-3-glu moved significantly towards the negative direction ($p < 0.05$). H^0 value significantly decreased from 346° to 339° ($p < 0.05$), indicating the colour of Cy-3-glu changed from red to lilac colour. The control Cy-3-glu presented a similar decrease behavior. A positive correlation was found between the Cy-3-glu content and b^* value or H^0 value with a higher coefficients $R^2 > 0.8400$ (in Table 3) during storage at 4, 24 and 37 °C. ΔE increased significantly in all cases, induced mainly by the decrease of b^* value. However, ΔE was less than 2 at 4 °C, while it was greater than 2 at 24 and 37 °C.

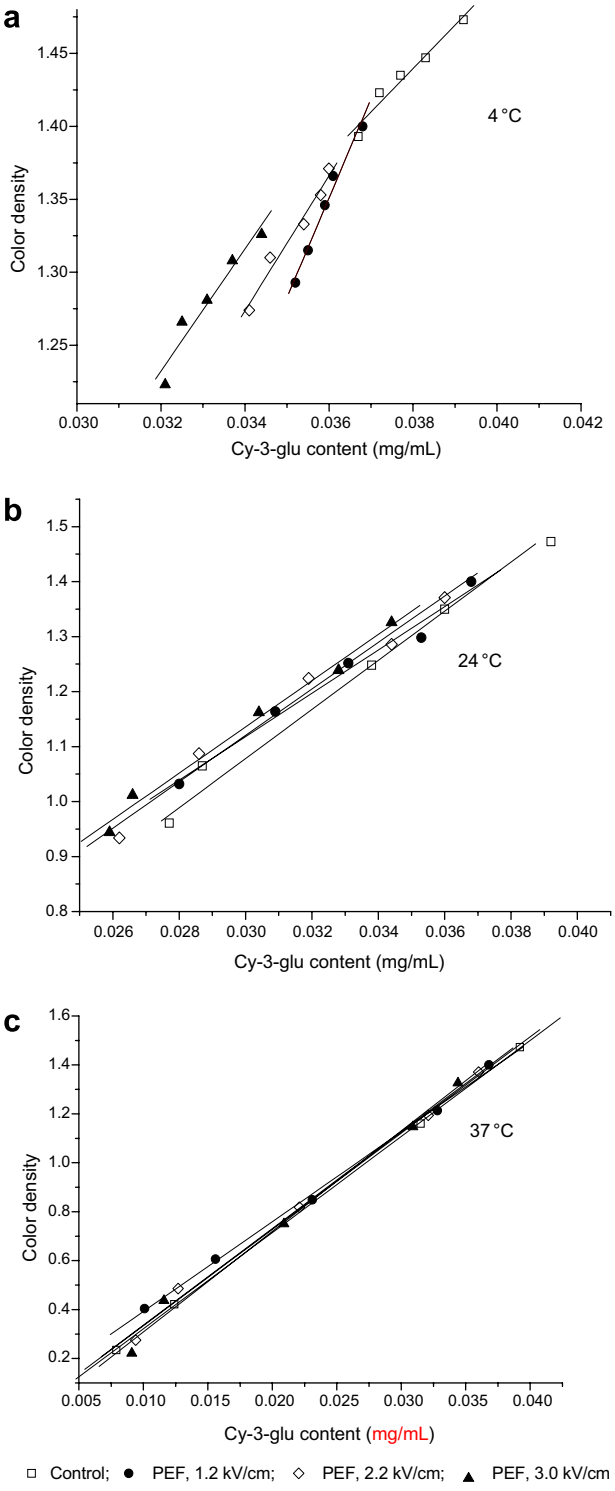

Fig. 2. Linear correlation between Cy-3-glu content and colour density in PEF-treated samples during storage at 4, 24 and 37 °C.

Table 3
Correlation coefficients between the Cy-3-glu content and colour indices during storage at 4, 24 and 37 °C

Colour indices	Temperature (°C)	Correlation coefficients (R^2)			
		Control	1.2 kV/cm	2.2 kV/cm	3.0 kV/cm
CD	4	0.9596	0.9835	0.9721	0.9340
	24	0.9893	0.9828	0.9772	0.9885
	37	0.9999	0.9977	0.9958	0.9917
b^*	4	0.9618	0.9988	0.8495	0.9284
	24	0.9837	0.9538	0.9943	0.9873
	37	0.9835	0.9948	0.9990	0.9911
H^0	4	0.9326	0.8924	0.9160	0.9617
	24	0.9788	0.9451	0.9917	0.9800
	37	0.9846	0.9841	0.9979	0.9893

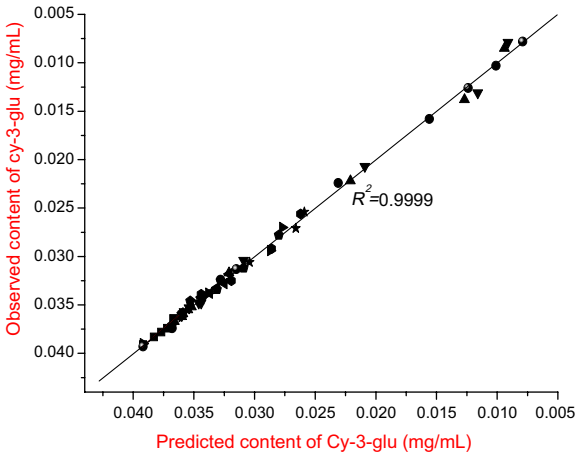

Fig. 3. Correlation between predicted and observed data for Cy-3-glu content.

4. Conclusions

PEF had no post-effect on the stability and colour characteristics of Cy-3-glu during storage while the storage temperature had a significant effect ($p < 0.05$). The degradation of PEF-treated Cy-3-glu during storage conformed to the first-order reaction kinetics with regression coefficients R^2 greater than 0.9300. Corresponding to 4, 24 and 37 °C, the degradation rate constant k in Cy-3-glu significantly increased in the exponential order level of 10^{-4}, 10^{-3} and 10^{-2}, while the $t_{1/2}$ (the time that 50% Cy-3-glu degradation would take) and D-value (the time that 90% Cy-3-glu degradation would take) of Cy-3-glu during storage decreased in the exponential order level of 10^3, 10^2 and 10. The *CD* was reduced in Cy-3-glu during storage The reduction of *CD* was closely related to the Cy-3-glu content in our model system, the Cy-3-glu content was perfectly predicted using *CD* with a coefficient $R^2 > 0.9999$. As the storage time increased, a significant decrease in b^* and H^0 value was obtained at all storage temperature ($p < 0.05$). A positive correlation existed between the Cy-3-glu content and b^* value or H^0 value with a higher coefficients $R^2 > 0.8400$ during storage. ΔE increased significantly, ΔE was less than 2 at 4 °C while it was greater than 2 at 24 and 37 °C.

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