Food Chemistry 210 (2016) 221–227

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Preheated milk proteins improve the stability of grape skin anthocyanins extracts

Zhiyong He [a,*], Mingzhu Xu [a], Maomao Zeng [a], Fang Qin [a], Jie Chen [a,b,*]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China
[b] Synergetic Innovation Center of Food Safety and Nutrition, Jiangnan University, Wuxi, Jiangsu 214122, PR China

ARTICLE INFO

Article history:
Received 30 January 2016
Received in revised form 23 April 2016
Accepted 25 April 2016
Available online 26 April 2016

Keywords:
Preheat treatment
Whey proteins
Casein
Anthocyanins degradation
Stability
Grape skin anthocyanins extracts

ABSTRACT

The effects of casein and whey proteins, preheated at 40–100 °C and 45–60 °C for 15 min, respectively, on color loss and anthocyanins degradation in grape skin anthocyanins extracts (GSAE) at pH 3.2 and 6.3 were evaluated. Preheating milk proteins effectively improved their protective effects against color loss and anthocyanins degradation in GSAE solutions during thermal treatment (at 80 °C for 2 h), H_2O_2 oxidation (0.005% H_2O_2 for 1 h) and illumination (at 5000 lx for 5 d). Whey proteins and casein, preheated at 50 °C and 60 °C for 15 min, respectively, demonstrated the optimal protective effects. However, preheated whey proteins had a better protective effect on the thermal, oxidation and photo stability of GSAE, decreasing the thermal, oxidative and photo degradation of anthocyanins in GSAE 71.59%, 32.22% and 56.92% at pH 3.2 and 54.91%, 22.89% and 46.68% at pH 6.3, respectively.

1. Introduction

Anthocyanins are the most abundant water-soluble flavonoids occurring in the fruits, leaves and flowers of plants. They are pigments that may appear as red, blue or purple depending on the pH (Li et al., 2013; Strack & Wray, 1994). As the pH varies from acidic to neutral and alkaline, the color changes from red to purple, blue and green (Wu, Guan, & Zhong, 2015). Anthocyanins are used as natural plant pigments in the food industry due to their biological activities and health benefits, such as their antioxidative, neuroprotective and anticarcinogenic properties (Philpott, Gould, Lim, & Ferguson, 2004; Talavéra et al., 2006; Wu et al., 2015). Grape skin anthocyanins extracts (GSAE), mainly obtained from vinification or grape juice pressing, are often used as colorants in wines, beverages, jams and candy produced in China. GSAE is composed of many anthocyanidin-3-*O*-glucosides; the main anthocyanidins forming the glycosides include malvidin, petunidin, cyanidin, peonidin and delphinidin (Gordillo et al., 2015; Ortega-Regules, Romero-Cascales, López-Roca, Ros-García, & Gómez-Plaza, 2006).

Anthocyanins are susceptible to food processing (especially thermal processing) (Sadilova, Stintzing, & Carle, 2006), due to their poor stability in aqueous solutions and sensitivity to environmental conditions, such as pH, temperature, oxygen, metal ions, light, sulfur dioxide, enzymes and ascorbic acid (Torskangerpoll & Andersen, 2005; Xiong, Melton, Easteal, & Siew, 2006). The instability of anthocyanins limits their industrial application as natural pigments. Therefore, reducing the loss of anthocyanins during food processing and storage is a significant challenge. Although previous studies have demonstrated that copigmentation, structural modification, microencapsulation, and food additives improve the stability of anthocyanins (Bakowska-Barczak & Kolodziejczyk, 2011; Kopjar, Jakšić, & Piližota, 2012; Malaj, De Simone, Quartarolo, & Russo, 2013; Wiczkowski, Szawara-Nowak, & Topolska, 2013), studies on the protection of anthocyanins by natural macromolecules (e.g., food proteins) have been limited.

Milk is widely recognized as a unique source of nutrients and compounds that are beneficial to the growth and health of children and adults (Yazdi & Corredig, 2012). Casein and whey proteins are two major types of proteins in milk that are defined according to their solubility at pH 4.6 at 25 °C. Casein and whey proteins account for 80% and 20%, respectively, of the total protein content of milk. Casein and whey proteins are widely used in the food industry because of their nutritional and functional properties. Casein exists as micelles and is made of the polypeptides α-, β- and κ-casein at an approximate molar ratio of 5:4:1.3. These polypeptides have molecular weights of approximately 24 kDa and different hydrophilic and hydrophobic regions along their protein chains (Dalgleish, 2011; Yazdi & Corredig, 2012). Due to its low intrinsic hydrophobicity and high net charge, casein appears as a

* Corresponding authors at: State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China (J. Chen).
E-mail addresses: zyhe@jiangnan.edu.cn (Z. He), chenjie@jiangnan.edu.cn (J. Chen).

http://dx.doi.org/10.1016/j.foodchem.2016.04.116
0308-8146/

unique unfolded structure in its native form. Casein is thus less susceptible to heating than whey proteins (Yazdi & Corredig, 2012). β-Lactoglobulin, a globular protein, is the most abundant whey proteins (approximately 50–55%) and is folded into a calyx known as a "β-barrel". The β-barrel is formed by eight anti-parallel β-strands, a three-turn α-helix and a ninth β-strand located on its outer surface (He, Chen, & Moser, 2015; Kanakis et al., 2011; Liang & Subirade, 2012; Shpigelman, Israeli, & Livney, 2010, 2012). At neutral pH and room temperature, β-lactoglobulin exists mainly as a dimer with a molecular weight of 36 800 Da. However, it dissociates into monomers at pH < 3, undergoing a conformational change and subsequent aggregation as temperature increases (He et al., 2015; Liang & Subirade, 2012; Palazolo, Rodríguez, Farruggia, Picó, & Delorenzi, 2000).

It has been shown that α-casein, β-casein and β-lactoglobulin can be used as vehicles for delivering various hydrophobic and amphiphilic compounds, such as vitamin D, vitamin A, fatty acids, β-carotene, phospholipids, folic acid, quercetin, resveratrol, genistein, curcumin, norbixin, procyanidins, tannic acid and epigallocatechin gallate (EGCG), through hydrophilic and/or hydrophobic interactions (Acharya, Sanguansri, & Augustin, 2013; Bohin et al., 2014; Bourassa, Bariyanga, & Tajmir-Riahi, 2013; Bourassa & Tajmir-Riahi, 2012; Helal, Tagliazucchi, Conte, & Desobry, 2012; Liang & Subirade, 2012; Mehranfar, Bordbar, & Parastar, 2013; Perez, Andermatten, Rubiolo, & Santiago, 2014; Yazdi & Corredig, 2012; Zorilla, Liang, Remondetto, & Subirade, 2011). The interaction between milk proteins and phenols has been shown to form complexes and affect the stability of phenols. Jing and Giusti (2005) found that milk proteins protect anthocyanins from degradation. Zheng, Bucheli, and Jing (2009) showed the positive effects of whey proteins on the absorbance, antioxidant activity and anthocyanins content of bog bilberry anthocyanins extract. Our previous research also showed that casein prevents the color loss and decreases the thermal, oxidative and photo degradation of anthocyanins in GSAE solutions (He, Xu, Zeng, Qin, & Chen, 2016). Moreover, some studies have shown that preheated proteins demonstrate enhanced protective effects on phenols. Yazdi and Corredig (2012) reported that casein from thermally treated milk (at 80 °C for 10 min) has a stronger affinity for curcumin than that from unheated milk, due to heat-induced conformational changes that result in enhanced hydrophobic interactions. Li, Du, Jin, and Du (2012) and Shpigelman et al. (2012) also reported that thermally treated β-lactoglobulin (at 70–120 °C) at a neutral pH exhibits a greater affinity for EGCG than native or heat-denatured β-lactoglobulin at an acidic pH, effectively preserving the antioxidant activity of EGCG. However, little information is available on the influence of pre-heated milk proteins on the stability of anthocyanins.

Therefore, the aim of this study is to evaluate the effects of thermally treated milk proteins (specifically, casein and whey proteins) on the color stability and degradation of anthocyanins in GSAE solutions at acidic and neutral pH. The results of this study are expected to facilitate the industrial application of GSAE.

2. Materials and methods

2.1. Materials and chemicals

Casein with a protein content of 86% (w/w, dry basis) was purchased from the Kerry Group (Beloit, WI, USA). Whey proteins with a protein content of 90% (w/w, dry basis) was purchased from Davisco Foods International Inc. (Eden Prairie, MN, USA). GSAE with an anthocyanins content of 2.5%, was purchased from AAFUD Industry Co., Ltd. (Zhu Hai, China). Malvidin-3-*O*-glucoside (97% purity) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preheat treatment of milk proteins

The commercial casein (0.4 mg/ml) and whey proteins (0.3 mg/ml) solutions were prepared in PBS solutions of pH 3.2 and 6.3, respectively. The 0.4 mg/ml casein solutions were heated in a water bath at 40 °C, 60 °C, 80 °C and 100 °C for 15 min and were then quickly cooled to room temperature in an ice bath. Similarly, the 0.3 mg/ml whey proteins solutions were heated in a water bath at 45 °C, 50 °C, 55 °C and 60 °C for 15 min and were then quickly cooled to room temperature. The preheated casein and whey proteins stock solutions and their unheated counterparts were stored separately at 4 °C until used.

2.3. Preparation of the GSAE-milk protein mixtures

GSAE stock solutions were freshly prepared for each experiment by dissolving 1.6 mg of GSAE powder in 1 ml of PBS solution at pH 3.2 and 6.3. The GSAE and milk proteins mixtures were prepared by blending GSAE with unheated and preheated casein and whey proteins stock solutions at a volume ratio of 1:1 for further stability testing. The final GSAE concentration in the mixtures was 0.8 mg/ml, and the final casein and whey proteins concentrations were 0.2 mg/ml and 0.15 mg/ml, respectively.

2.4. Thermal, oxidation and photo stability tests

According to our previous research (He et al., 2016), the GSAE-milk protein mixtures and the GSAE-only samples (i.e., not mixed with milk protein) were all subjected to thermal, oxidation and photo stability tests. The thermal stability test was carried out by heating the samples in 15 ml glass tubes wrapped in tin foil in a water bath at 80 °C for 2 h and then rapidly cooling them down to room temperature in an ice bath for further color measurement and anthocyanins content analysis. The oxidation stability test was performed by adding H_2O_2 to the samples at a final concentration of 0.05 mg/ml and oxidizing them in the dark at room temperature for 1 h. The oxidized samples were then collected for further analysis. The photo stability test was carried out by illuminating the samples, which had been sealed in 15 ml transparent glass tubes for 5 days at ambient temperature, in an SHP-150 illumination incubator with fluorescent lamps (Samsung Laboratory Instrument Co., Shanghai, China) at an illumination intensity of 5000 ± 500 lx. The samples were randomly interchanged once every day to minimize the effects of unequal exposure. After illumination, the samples underwent stability analysis.

2.5. Color measurement

Color measurements of the heated, oxidized and illuminated samples and the untreated GSAE sample were performed following the method described by He et al. (2016) using an UltraScan PRO-1166 (Hunter Associates Laboratory Inc., Reston, VA, USA) with a quartz cuvette with a path length of 0.5 cm (2 ml of the sample). The results were expressed as L^* (lightness), a^* (redness-greenness) and b^* (yellowness-blueness) and were used to evaluate the color changes of the samples. Color change (ΔE) was calculated according to Eq. (1) (Mercali, Schwartz, Marczak, Tessaro, & Sastry, 2014). The treated and untreated GSAE samples not mixed with milk proteins acted as the treated and untreated controls, respectively.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

where L_0^*, a_0^* and b_0^* are the color values of the untreated GSAE solution (0.8 mg/ml).

2.6. HPLC-DAD analysis of anthocyanins

Similar to our previous study (He et al., 2016), 5 ml of the treated GSAE solutions with and without milk proteins and 5 ml of the untreated GSAE solution were mixed with 5 ml of 1% (v/v) formic acid in methanol. The mixtures were sonicated for 3 min and centrifuged at 10,000*g* for 10 min at 4 °C. The supernatant was collected and filtered through a 0.45 μm PTFE filter for the HPLC anthocyanins analysis. The analyses were performed with a Shimadzu Prominence HPLC system equipped with an LC-20AD high-pressure pump, an SIL-20AC auto sampler, a CTO-20AC column oven and an SPD-M20A diode array detector (DAD) (Shimadzu Co., Tokyo, Japan). Separation was achieved using a TSK-GEL ODS-100V C18 column (5 μm, 250 × 4.6 mm i.d.; Tosoh Co., Tokyo, Japan). The column temperature was 30 °C. Water/-formic acid (solvent A) (90:10, v/v) and acetonitrile (solvent B) were used as the eluents at a flow rate of 1 ml/min. The gradient conditions for the sample elution were isocratic 10% B, 0–10 min; linear gradient from 10% B to 60% B, 10–40 min; to 100% B, 40–45 min; isocratic 100% B, 45–50 min; linear gradient from 100% B to 10% B, 50–60 min; and isocratic 10% B, 60–65 min. The anthocyanins were detected at 528 nm. The chromatograms were integrated using LCsolution Software (Shimadzu Co.). Malvidin-3-*O*-glucoside was used as the reference standard. Individual anthocyanins were quantified by integrating the peak area and using the malvidin-3-*O*-glucoside calibration curve. The total anthocyanins contents of the GSAE samples were obtained by adding individual anthocyanins contents and expressing them as mg of malvidin-3-*O*-glucoside per liter of solution (mg/L).

2.7. Statistical analysis

All of the experiments were carried out three times. The data were expressed as the mean ± the standard deviation (SD). Statistical analyses were carried out using the general linear model procedure of the Statistix 9.0 software (Analytical Software, Tallahassee, FL, USA). Significant differences ($p < 0.05$) among the colorimetric parameters of GSAE solutions in the presence and absence of preheated milk proteins at different temperatures, as well as among their total anthocyanins contents and degradation rates, were identified by the least significant difference procedure of all pairwise multiple comparisons.

3. Results

3.1. The effects of preheated milk proteins on the thermal stability of GSAE

The effects of unheated and preheated milk proteins (casein and whey proteins) on the thermal stability of GSAE at pH 3.2 and 6.3 are shown in Tables 1 and 2, respectively. The L* and b* values of

Table 1
Effect of preheated milk proteins on colorimetric parameters, total anthocyanins contents and degradation rate of GSAE solutions after thermal test (at 80 °C for 2 h) at pH 3.2.[A]

	Preheat treatment temperature (°C)	L*	a*	b*	ΔE	Total anthocyanins (mg/L)	Anthocyanins degradation rate[B] (%)
No protein	Control	53.60 ± 0.38	27.28 ± 0.24	0.48 ± 0.01		22.17 ± 0.52	
		58.83 ± 0.24 a	16.37 ± 0.17 h	9.41 ± 0.03 a	15.04 ± 0.22 a	11.36 ± 0.01 i	48.78 ± 0.15 a
Casein (0.2 mg/ml)	Unheated	56.82 ± 0.12 cd	17.91 ± 0.10 f	8.26 ± 0.21 c	12.60 ± 0.22 c	15.51 ± 0.08 g	30.02 ± 0.12 c
	40	56.61 ± 0.19 d	18.83 ± 0.12 e	8.32 ± 0.15 c	11.91 ± 0.08 d	16.58 ± 0.02 e	25.22 ± 0.20 ef
	60	55.81 ± 0.07 e	21.83 ± 0.17 a	5.53 ± 0.26 g	7.75 ± 0.18 g	18.57 ± 0.03 b	16.26 ± 0.18 i
	80	55.82 ± 0.13 e	19.64 ± 0.36 d	8.70 ± 0.08 b	11.44 ± 0.20 e	16.97 ± 0.07 d	23.46 ± 0.04 g
	100	54.85 ± 0.47 g	17.66 ± 0.30 f	9.19 ± 0.05 a	13.03 ± 0.20 b	15.32 ± 0.00 h	30.90 ± 0.27 b
Whey proteins (0.15 mg/ml)	Unheated	57.23 ± 0.18 b	18.70 ± 0.10 e	6.74 ± 0.06 e	11.22 ± 0.14 e	15.71 ± 0.08 f	29.14 ± 0.11 d
	45	57.10 ± 0.07 bc	18.58 ± 0.02 e	6.61 ± 0.11 ef	11.20 ± 0.09 e	16.62 ± 0.10 e	25.02 ± 0.20 f
	50	55.89 ± 0.11 e	21.31 ± 0.22 b	4.88 ± 0.10 h	7.76 ± 0.13 g	19.10 ± 0.14 a	13.86 ± 0.35 j
	55	55.43 ± 0.13 f	20.43 ± 0.24 c	6.36 ± 0.29 f	9.21 ± 0.27 f	17.73 ± 0.14 c	20.03 ± 0.37 h
	60	55.34 ± 0.09 f	17.22 ± 0.13 g	7.14 ± 0.16 d	12.19 ± 0.16 d	16.51 ± 0.05 e	25.51 ± 0.05 e

[A] Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).
[B] Degradation rate (%) = 100% × (total anthocyanins in initial GSAE − total anthocyanins in tested GSAE)/total anthocyanins in initial GSAE.

Table 2
Effect of preheated milk proteins on colorimetric parameters, total anthocyanins contents and degradation rate of GSAE solutions after thermal test (at 80 °C for 2 h) at pH 6.3.[A]

	Preheat treatment temperature (°C)	L*	a*	b*	ΔE	Total anthocyanins (mg/L)	Anthocyanins degradation rate[B] (%)
No protein	Control	55.12 ± 0.22	23.62 ± 0.48	0.92 ± 0.04		20.42 ± 0.08	
		64.51 ± 0.27 a	1.26 ± 0.15 i	15.83 ± 0.29 a	28.47 ± 0.17 a	5.27 ± 0.05 h	74.19 ± 0.14 a
Casein (0.2 mg/ml)	Unheated	58.95 ± 0.34 c	4.80 ± 0.10 h	11.65 ± 0.52 b	22.00 ± 0.09 b	7.55 ± 0.08 g	63.03 ± 0.25 b
	40	57.77 ± 0.32 d	7.23 ± 0.07 f	6.86 ± 0.09 e	17.64 ± 0.01 d	9.36 ± 0.00 d	54.16 ± 0.18 e
	60	58.04 ± 0.04 d	10.82 ± 0.66 d	3.16 ± 0.09 i	13.32 ± 0.62 g	12.77 ± 0.01 b	37.46 ± 0.20 g
	80	57.99 ± 0.16 d	9.32 ± 0.08 e	5.22 ± 0.21 g	15.21 ± 0.08 f	10.43 ± 0.01 c	48.92 ± 0.15 f
	100	57.67 ± 0.19 d	6.44 ± 0.05 g	7.62 ± 0.13 d	18.61 ± 0.06 c	8.92 ± 0.00 e	56.32 ± 0.17 d
Whey proteins (0.15 mg/ml)	Unheated	60.55 ± 0.59 b	11.39 ± 0.26 c	10.63 ± 0.16 c	16.54 ± 0.38 e	8.02 ± 0.17 f	60.78 ± 0.68 c
	45	60.69 ± 0.14 b	12.48 ± 0.36 ab	5.97 ± 0.58 f	13.45 ± 0.43 g	12.76 ± 0.09 b	37.51 ± 0.20 g
	50	60.66 ± 0.11 b	12.82 ± 0.05 a	4.05 ± 0.44 h	12.53 ± 0.13 h	13.59 ± 0.16 a	33.45 ± 0.52 h
	55	60.73 ± 0.15 b	12.12 ± 0.07 b	5.16 ± 0.12 g	13.48 ± 0.06 g	12.82 ± 0.03 b	37.22 ± 0.10 g
	60	60.41 ± 0.08 b	10.48 ± 0.18 d	6.81 ± 0.15 e	15.34 ± 0.12 f	10.42 ± 0.05 c	48.97 ± 0.04 f

[A] Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).
[B] Degradation rate (%) = 100% × (total anthocyanins in initial GSAE − total anthocyanins in tested GSAE)/total anthocyanins in initial GSAE.

the heat-treated (80 °C for 2 h) GSAE samples (both with and without milk proteins) increased, whereas the a* values decreased, compared to the initial values (L_0^*, a_0^* and b_0^*) of untreated GSAE samples, indicating GSAE color loss during the thermal test. The notable decrease in total anthocyanins content showed that the anthocyanins of the GSAE samples had been significantly degraded. The ΔE values and anthocyanins degradation rates of the GSAE at pH 3.2 were far lower than those at pH 6.3 after thermal treatment.

As shown in Tables 1 and 2, after thermal treatment the L*, b* and ΔE values of the GSAE samples mixed with unheated and preheated milk proteins were significantly lower ($p < 0.05$), whereas their a* values and total anthocyanins content were significantly higher ($p < 0.05$) than those of the GSAE samples without milk proteins. Accordingly, the anthocyanins degradation rates of the GSAE samples mixed with casein and whey proteins were 16.26–30.90% and 13.86-29.14%, respectively, at pH 3.2 and 37.46–63.03% and 33.45–60.78%, respectively, at pH 6.3, much lower than the 48.18% and 74.19% degradation rates of the GSAE-only samples at pH 3.2 and 6.3, respectively. The ΔE values and anthocyanins degradation rates of the GSAE mixed with unheated casein were significantly higher ($p < 0.05$) than those of the GSAE mixed with unheated whey proteins, suggesting that native whey proteins had a stronger preventative effect against GSAE color loss and anthocyanins degradation than native casein. The native whey proteins and casein reduced the thermal anthocyanins degradation rates 40.26% and 38.46% at pH 3.2, respectively, and 18.08% and 15.04% at pH 6.3, respectively.

When casein and whey proteins were respectively preheated at 40–80 °C and 45–60 °C for 15 min at pH 3.2, the ΔE values and degradation rates of the GSAE samples mixed with preheated casein and whey proteins were significantly lower ($p < 0.05$) than those of the GSAE samples mixed with unheated casein and whey proteins (Table 1). The GSAE samples mixed with casein preheated at 100 °C for 15 min showed higher ΔE values and degradation rates than when mixed with native casein. However, at pH 6.3, all of the ΔE values and degradation rates of the GSAE samples mixed with casein and whey proteins that had been preheated at 40–100 °C and 45–60 °C, respectively, for 15 min, were significantly lower ($p < 0.05$) than those of the GSAE samples mixed with unheated casein and whey proteins (Table 2). Moreover, when casein and whey proteins were preheated at 60 °C and 50 °C, respectively, for 15 min, the corresponding ΔE values and anthocyanins degradation rates of the GSAE were the minimum among the respective preheated samples at different temperatures. This suggests that the GSAE samples mixed with casein and whey proteins preheated at 60 °C and 50 °C, respectively, for 15 min have relatively better thermal stabilities at pH 3.2 and 6.3 than the samples with milk proteins preheated at the other temperatures. As shown in Tables 1 and 2, whey proteins preheated at 50 °C for 15 min reduced the thermal degradation rates of anthocyanins in the GSAE 71.59% at pH 3.2 and 54.91% at pH 6.3, which is slightly higher than the 66.67% and 49.51% of casein preheated at 60 °C for 15 min, at pH 3.2 and 6.3, respectively.

3.2. The effects of preheated milk proteins on the oxidation stability of GSAE

The effects of unheated and differentially preheated milk proteins on the oxidation stability of the GSAE samples at pH 3.2 and 6.3 are shown in Tables 3 and 4. Similar to the thermal-induced change of GSAE, the a* values of the oxidation-treated (0.005% H_2O_2 for 1 h) GSAE samples decreased, whereas their L* and b* values increased, compared to the L_0^*, a_0^* and b_0^* values, indicating that the GSAE samples experienced color loss due to H_2O_2 oxidation. Moreover, the total anthocyanins content of the GSAE samples showed a sharp decrease after oxidation due to the heavy degradation of anthocyanins. The ΔE values and anthocyanins degradation rates of the GSAE after oxidation at pH 3.2 were significantly lower ($p < 0.05$) than those after oxidation at pH 6.3.

Similarly, the L* and ΔE values of the GSAE mixed with milk proteins after H_2O_2 oxidation were significantly lower ($p < 0.05$), whereas their a* values and total anthocyanins content were significantly higher ($p < 0.05$), than those of the GSAE samples without milk proteins. At pH 3.2 the anthocyanins degradation rates of the oxidation-treated GSAE mixed with casein and whey proteins were 61.02–79.87% and 61.17–78.80%, respectively. At pH 6.3 they were 74.49–88.58% and 74.49–83.06%, respectively. These rates were significantly lower ($p < 0.05$) than the 90.25% and 96.18% anthocyanins degradation rates of the GSAE-only samples at pH 3.2 and 6.3, respectively. As shown in Tables 3 and 4, the GSAE samples mixed with unheated casein demonstrated slightly higher ΔE values and anthocyanins degradation rates compared to the samples mixed with unheated whey proteins after oxidation. The unheated whey proteins and casein decreased the oxidative degradation of anthocyanins in the GSAE 12.69% and 11.50%, respectively, at pH 3.2 and 13.64% and 7.90%, respectively, at pH 6.3.

When casein and whey proteins were preheated at 40–100 °C and 45–60 °C, respectively, for 15 min, the ΔE values and degradation rates of the oxidation-treated GSAE mixed with preheated

Table 3
Effect of preheated milk proteins on colorimetric parameters, total anthocyanins contents and degradation rate of GSAE solutions after oxidation test (0.005% H_2O_2 for 1 h) at pH 3.2.[A]

	Preheat treatment temperature (°C)	L*	a*	b*	ΔE	Total anthocyanins (mg/L)	Anthocyanins degradation rate[B] (%)
No protein	Control	53.60 ± 0.38	27.28 ± 0.24	0.48 ± 0.01		22.17 ± 0.52	
		59.98 ± 0.12 a	19.53 ± 0.26 g	1.30 ± 0.07 bcd	10.07 ± 0.22 a	2.16 ± 0.03 g	90.25 ± 0.11 a
Casein (0.2 mg/ml)	Untreated	58.10 ± 0.16 bc	19.16 ± 0.18 g	1.41 ± 0.03 ab	9.33 ± 0.13 b	4.46 ± 0.14 f	79.87 ± 0.61 b
	40	57.63 ± 0.15 cd	20.68 ± 0.31 cd	1.21 ± 0.05 def	7.77 ± 0.28 d	6.26 ± 0.16 c	71.75 ± 0.67 e
	60	56.37 ± 0.12 e	22.46 ± 0.35 a	1.05 ± 0.04 gh	5.59 ± 0.20 g	8.64 ± 0.06 a	61.02 ± 0.14 g
	80	56.33 ± 0.35 e	21.41 ± 0.20 b	1.23 ± 0.05 cde	6.52 ± 0.29 f	6.75 ± 0.05 b	69.54 ± 0.13 f
	100	55.95 ± 0.92 ef	20.00 ± 0.53 f	1.50 ± 0.08 a	7.72 ± 0.42 d	5.73 ± 0.07 d	74.14 ± 0.24 d
Whey proteins (0.15 mg/ml)	Untreated	58.40 ± 0.04 b	20.13 ± 0.06 ef	1.33 ± 0.05 bc	8.65 ± 0.10 c	4.70 ± 0.11 e	78.80 ± 0.46 c
	45	57.51 ± 0.03 d	21.03 ± 0.05 bc	1.11 ± 0.03 fg	7.40 ± 0.13 de	6.68 ± 0.05 b	69.88 ± 0.13 f
	50	56.30 ± 0.05 e	22.42 ± 0.12 a	1.12 ± 0.12 efg	5.60 ± 0.11 g	8.61 ± 0.11 a	61.17 ± 0.39 g
	55	56.15 ± 0.38 ef	21.19 ± 0.42 b	0.99 ± 0.12 h	6.62 ± 0.24 f	8.57 ± 0.06 a	61.36 ± 0.14 g
	60	55.71 ± 0.16 f	20.52 ± 0.09 de	0.94 ± 0.04 h	7.10 ± 0.26 e	6.81 ± 0.04 b	69.30 ± 0.08 f

[A] Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).
[B] Degradation rate (%) = 100% × (total anthocyanins in initial GSAE − total anthocyanins in tested GSAE)/total anthocyanins in initial GSAE.

Table 4
Effect of preheated milk proteins on colorimetric parameters, total anthocyanins contents and degradation rate of GSAE solutions after oxidation test (0.005% H_2O_2 for 1 h) at pH 6.3.[A]

	Preheat treatment temperature (°C)	L*	a*	b*	ΔE	Total anthocyanins (mg/L)	Anthocyanins degradation rate[B] (%)
No protein	Control	55.12 ± 0.22	23.62 ± 0.48	0.92 ± 0.04		20.42 ± 0.08	
		65.77 ± 0.40 a	2.05 ± 0.04 h	3.63 ± 0.06 a	24.21 ± 0.14 a	0.78 ± 0.11 h	96.18 ± 0.52 a
Casein (0.2 mg/ml)	Untreated	58.25 ± 0.11 f	5.73 ± 0.22 g	1.90 ± 0.10 b	18.19 ± 0.34 b	2.33 ± 0.02 g	88.58 ± 0.21 b
	40	58.16 ± 0.12 f	7.93 ± 0.36 e	1.07 ± 0.03 d	15.99 ± 0.38 d	3.47 ± 0.04 e	83.01 ± 0.13 d
	60	58.21 ± 0.22 f	11.31 ± 0.59 a	0.25 ± 0.14 e	12.71 ± 0.58 h	5.21 ± 0.04 a	74.49 ± 0.10 h
	80	57.43 ± 0.25 g	8.56 ± 0.25 d	1.04 ± 0.10 d	15.24 ± 0.29 e	4.11 ± 0.00 d	79.87 ± 0.08 e
	100	57.27 ± 0.19 g	6.77 ± 0.20 f	1.27 ± 0.03 cd	16.99 ± 0.18 c	3.27 ± 0.05 f	83.99 ± 0.18 c
Whey proteins (0.15 mg/ml)	Untreated	60.97 ± 0.14 b	8.06 ± 0.05 e	0.43 ± 0.16 e	16.63 ± 0.02 c	3.46 ± 0.09 e	83.06 ± 0.37 d
	45	60.05 ± 0.03 c	8.93 ± 0.05 d	0.19 ± 0.09 e	15.52 ± 0.04 e	4.10 ± 0.08 d	79.92 ± 0.31 e
	50	59.23 ± 0.44 d	11.56 ± 0.05 a	0.42 ± 0.05 e	12.76 ± 0.18 h	5.21 ± 0.10 a	74.49 ± 0.39 h
	55	58.80 ± 0.06 e	10.76 ± 0.15 b	1.03 ± 0.21 d	13.37 ± 0.16 g	4.57 ± 0.01 b	77.62 ± 0.04 g
	60	58.48 ± 0.29 ef	9.37 ± 0.22 c	1.32 ± 0.33 c	14.65 ± 0.16 f	4.27 ± 0.05 c	79.09 ± 0.16 f

[A] Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).
[B] Degradation rate (%) = 100% × (total anthocyanins in initial GSAE − total anthocyanins in tested GSAE)/total anthocyanins in initial GSAE.

casein and whey proteins were significantly lower ($p < 0.05$) than those of the GSAE samples mixed with native casein and whey proteins. Additionally, the ΔE values and anthocyanins degradation rates of the oxidation-treated GSAE samples mixed with casein and whey proteins preheated at 60 °C and 50 °C, respectively, for 15 min were the lowest among the samples preheated at different temperatures. Casein preheated at 60 °C for 15 min and whey proteins preheated at 50 °C for 15 min reduced the oxidative degradation of anthocyanins in the GSAE 32.39% and 32.22%, respectively, at pH 3.2 and 22.89% at pH 6.3, demonstrating similar effects on the oxidative degradation of GSAE.

3.3. The effects of preheated milk proteins on the photo stability of GSAE

As shown in Tables 5 and 6, the L* and b* values of the illuminated (at 5000 lx for 5 d) GSAE samples increased, whereas their a* values decreased, compared to the L_0^*, a_0^* and b_0^* values of the non-illuminated GSAE samples. This revealed that GSAE color loss was induced by illumination, similarly to the color changes induced by heat and oxidation. The total anthocyanins content of the GSAE also exhibited significant reduction ($p < 0.05$) after illumination due to the photo degradation of anthocyanins. Similar to the heat- and oxidation-treated GSAE, the ΔE values and anthocyanins degradation rates of the illuminated GSAE samples at pH 6.3 were much higher than at pH 3.2.

After illumination, the GSAE samples mixed with milk proteins showed significant decreases in L*, b* and ΔE values, and increases in a* values and total anthocyanins content, compared to the GSAE-only samples ($p < 0.05$). The anthocyanins degradation rates of GSAE samples mixed with casein and whey proteins were 36.14–66.16% and 35.95–63.52%, respectively, at pH 3.2 and 54.75–82.36% and 49.17–74.38%, respectively, at pH 6.3. These results were significantly lower ($p < 0.05$) than the 83.45% and 92.21% degradation rates of the GSAE samples not mixed with milk proteins at pH 3.2 and 6.3, respectively. As shown in Tables 5 and 6, the ΔE values and anthocyanins degradation rates were significantly lower ($p < 0.05$) for the illuminated GSAE samples mixed with unheated whey proteins than for the GSAE samples mixed with unheated casein. The unheated whey proteins and casein lowered the photo degradation rate of anthocyanins in GSAE 23.88% and 20.72%, respectively, at pH 3.2 and 19.34% and 10.68%, respectively, at pH 6.3.

As casein and whey proteins were preheated at 40–100 °C and 45–60 °C, respectively, for 15 min, the illuminated GSAE samples mixed with preheated casein and whey proteins showed significant decreases ($p < 0.05$) in their ΔE values and degradation rates compared to the GSAE samples mixed with native milk proteins. Furthermore, as shown in Tables 5 and 6, among all of the

Table 5
Effect of preheated milk proteins on colorimetric parameters, total anthocyanins contents and degradation rate of GSAE solutions after illumination test (at 5000 lx for 5 d) at pH 3.2.[A]

	Preheat treatment temperature (°C)	L*	a*	b*	ΔE	Total anthocyanins (mg/L)	Anthocyanins degradation rate[B] (%)
No protein	Control	53.60 ± 0.38	27.28 ± 0.24	0.48 ± 0.01		22.17 ± 0.52	
		59.07 ± 0.31 a	13.55 ± 0.10 g	11.05 ± 0.04 a	18.17 ± 0.13 a	3.67 ± 0.01 g	83.45 ± 0.02 a
Casein (0.2 mg/ml)	Untreated	56.76 ± 0.17 b	16.03 ± 0.03 f	5.20 ± 0.48 c	12.60 ± 0.21 b	7.50 ± 0.01 f	66.16 ± 0.08 b
	40	57.17 ± 0.37 b	17.28 ± 0.05 e	4.70 ± 0.30 d	11.43 ± 0.16 d	9.82 ± 0.02 d	55.73 ± 0.08 d
	60	55.90 ± 0.07 cd	20.19 ± 0.09 a	4.07 ± 0.07 e	8.27 ± 0.14 f	14.16 ± 0.03 a	36.14 ± 0.10 h
	80	54.98 ± 0.11 ef	18.83 ± 0.13 c	4.66 ± 0.10 d	9.53 ± 0.20 e	11.28 ± 0.17 b	49.12 ± 0.63 f
	100	54.35 ± 0.33 f	17.05 ± 0.20 e	5.71 ± 0.29 b	11.51 ± 0.28 d	8.17 ± 0.06 e	63.17 ± 0.15 c
Whey proteins (0.15 mg/ml)	Untreated	56.93 ± 0.51 b	16.20 ± 0.02 f	4.21 ± 0.09 e	12.16 ± 0.14 c	8.09 ± 0.03 e	63.52 ± 0.00 c
	45	57.02 ± 0.33 b	17.37 ± 0.20 e	4.10 ± 0.54 e	11.09 ± 0.37 d	10.56 ± 0.01 c	52.35 ± 0.14 e
	50	56.06 ± 0.60 c	19.98 ± 0.62 ab	3.42 ± 0.04 g	8.25 ± 0.43 f	14.20 ± 0.04 a	35.95 ± 0.06 h
	55	55.47 ± 0.50 cde	19.69 ± 0.04 b	3.59 ± 0.05 fg	8.41 ± 0.18 f	14.20 ± 0.07 a	35.95 ± 0.09 h
	60	55.26 ± 0.61 de	18.29 ± 0.17 d	4.02 ± 0.15 ef	9.80 ± 0.36 e	11.38 ± 0.07 b	48.68 ± 0.14 g

[A] Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).
[B] Degradation rate (%) = 100% × (total anthocyanins in initial GSAE − total anthocyanins in tested GSAE)/total anthocyanins in initial GSAE.

Table 6
Effect of preheated milk proteins on colorimetric parameters, total anthocyanins contents and degradation rate of GSAE solutions after illumination test (at 5000 lx for 5 d) at pH 6.3.[A]

	Preheat treatment temperature (°C)	L*	a*	b*	ΔE	Total anthocyanins (mg/L)	Anthocyanins degradation rate[B] (%)
No protein	Control	55.12 ± 0.22	23.62 ± 0.48	0.92 ± 0.04		20.42 ± 0.08	
		64.44 ± 0.06 a	0.59 ± 0.05 h	18.84 ± 0.13 a	30.63 ± 0.05 a	1.59 ± 0.10 i	92.21 ± 0.46 a
Casein (0.2 mg/ml)	Untreated	58.95 ± 0.25 d	2.65 ± 0.28 g	14.66 ± 0.38 b	25.36 ± 0.27 b	3.60 ± 0.03 h	82.36 ± 0.08 b
	40	58.12 ± 0.33 f	7.46 ± 0.16 ef	7.85 ± 0.14 d	17.84 ± 0.16 d	5.76 ± 0.09 f	71.79 ± 0.33 d
	60	58.26 ± 0.16 ef	10.11 ± 0.02 b	4.42 ± 0.12 gh	14.31 ± 0.05 g	9.24 ± 0.02 c	54.75 ± 0.08 g
	80	57.93 ± 0.18 fg	8.68 ± 0.12 d	6.65 ± 0.11 e	16.25 ± 0.12 e	7.29 ± 0.01 e	64.30 ± 0.09 e
	100	57.50 ± 0.21 g	7.33 ± 0.21 f	7.75 ± 0.29 d	17.82 ± 0.07 d	5.79 ± 0.00 f	71.65 ± 0.11 d
Whey proteins (0.15 mg/ml)	Untreated	59.95 ± 0.44 b	7.70 ± 0.14 e	8.48 ± 0.40 c	18.28 ± 0.17 c	5.23 ± 0.02 g	74.38 ± 0.00 c
	45	59.47 ± 0.26 bc	8.74 ± 0.22 d	5.16 ± 0.06 f	16.07 ± 0.27 e	9.73 ± 0.09 b	52.35 ± 0.25 h
	50	59.12 ± 0.59 cd	11.68 ± 0.18 a	4.62 ± 0.24 g	13.14 ± 0.10 h	10.38 ± 0.19 a	49.17 ± 0.73 i
	55	58.79 ± 0.08 d	9.58 ± 0.06 c	4.14 ± 0.09 h	14.86 ± 0.03 f	9.76 ± 0.10 b	52.20 ± 0.30 h
	60	58.69 ± 0.07 de	8.51 ± 0.06 d	5.09 ± 0.06 f	16.08 ± 0.06 e	7.51 ± 0.06 d	63.22 ± 0.15 f

[A] Values are expressed as the mean ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).
[B] Degradation rate (%) = 100% × (total anthocyanins in initial GSAE − total anthocyanins in tested GSAE)/total anthocyanins in initial GSAE.

differentially preheated casein samples, the GSAE samples mixed with casein preheated at 60 °C for 15 min had the lowest Δ*E* value and anthocyanins degradation rate at pH 3.2 and 6.3 after illumination. However, for the preheated whey proteins, minimum values occurred for the GSAE samples mixed with whey proteins preheated at 50 °C and 55 °C for 15 min at pH 3.2, whereas samples with preheated whey proteins at 50 °C for 15 min yielded minimum values at pH 6.3. Additionally, casein preheated at 60 °C for 15 min decreased the photo degradation rate of anthocyanins 56.69% at pH 3.2 and 40.62% at pH 6.3. The decreasing percentages in anthocyanins degradation rates for whey proteins preheated at 50 °C for 15 min were 56.92% and 46.68% at pH 3.2 and 6.3, respectively.

4. Discussion

The effects of preheated milk proteins, casein and whey proteins, on the thermal, oxidation and photo stability of GSAE at pH 3.2 and 6.3 were investigated. Color and anthocyanins content are the two important features of naturally pigmented anthocyanins products. The stability of natural anthocyanins pigments can be evaluated by measuring the changes in color and anthocyanins content in the accelerated tests (He et al., 2016). As shown in Tables 1–6, the color of GSAE samples faded and their anthocyanins contents decreased significantly after heat treatment, oxidation and illumination. At pH 6.3, the color loss and anthocyanins degradation of GSAE were much more severe than at pH 3.2, which suggests that GSAE was more susceptible to thermal, oxidative and photo degradation at neutral rather than acidic conditions. Many studies have shown that anthocyanins is very sensitive to some factors, such as pH, heat, light and oxygen. Anthocyanin's pyrylium ring easily opens and forms a chalcone structure due to its susceptibility to hydration and oxidation at the C2 position, which results in anthocyanins degradation (He et al., 2016; Torskangerpoll & Andersen, 2005; Wu et al., 2015; Xiong et al., 2006).

As shown in Tables 1–6, when mixed with milk proteins, all of the GSAE samples demonstrated reduced color change and anthocyanins loss during the stability tests, indicating that milk proteins prevented color loss and anthocyanins degradation in GSAE samples subjected to thermal treatment, oxidation and illumination. Native whey proteins had slightly stronger preventative effects than native casein. Previous studies have shown that milk proteins, such as α-casein, β-casein and β-lactoglobulin, can form complexes with bioactive compounds, such as retinol, folic acid, resveratrol, EGCG and malvidin-3-*O*-glucoside, through hydrophilic and/or hydrophobic interactions. This is thought to contribute substantially to the protective effects of milk proteins on these bioactive ligands (He et al., 2016; Liang, Zhang, Zhou, & Subirade, 2013; Zorilla et al., 2011). Thus, the slightly stronger protective effect of native whey proteins on GSAE is likely due to the stronger molecular binding interaction of its globular lactoglobulins with the anthocyanins in GSAE than the natively unfolded casein proteins.

As shown in Tables 1–6, when mixed with casein and whey proteins preheated at 40–100 °C and 45–60 °C for 15 min, respectively, GSAE samples showed significantly less ($p < 0.05$) color change and anthocyanins degradation compared to the samples mixed with the native milk proteins during the thermal, oxidation and photo stability tests. The GSAE mixed with casein and whey proteins preheated at 60 °C and 50 °C for 15 min, respectively, demonstrated the weakest color changes and lowest anthocyanins degradation rates, respectively. These results indicate that preheating casein and whey proteins effectively improved their protective effects against the thermal-, oxidation- and photo-induced color loss and anthocyanins degradation of GSAE. Additionally, GSAE samples mixed with casein and whey proteins, respectively preheated at 60 °C and 50 °C for 15 min, demonstrated relatively good thermal, oxidation and photo stability. Previous studies have also demonstrated that heat-treated milk proteins (e.g., casein and β-lactoglobulin) preserve curcumin and EGCG more effectively than native milk proteins, thus attributing the enhanced protective effects of preheated proteins to their stronger binding affinity for these bioactive ligands than unheated proteins (Li et al., 2012; Shpigelman et al., 2012; Yazdi & Corredig, 2012). When subjected to heat treatment, milk proteins, such as β-casein and β-lactoglobulin, generally undergo structural and conformational changes, which vary according to pH and temperature. At acidic pH and low temperatures, their structures were stable and remained mostly intact. However, at neutral pH and/or increasing temperatures, β-casein and β-lactoglobulin exhibited more open conformations due to structural unfolding, thus exposing more interior residues and enhancing the binding interactions between the milk proteins and ligands (e.g., EGCG) via hydrophobic interactions and hydrogen bonding (He, Chen, Moser, Jones, & Ferruzzi, 2016; He et al., 2015). Furthermore, as shown in Tables 1–6, whey proteins preheated at 50 °C for 15 min had stronger protective effects than casein preheated at 60 °C for 15 min on the thermal stability of GSAE at pH 3.2 and 6.3 and photo stability at pH 6.3. However, they had similar effects as casein preheated at 60 °C for 15 min on the oxidation stability of GSAE at pH 3.2 and 6.3 and

photo stability at pH 3.2. In general, whey proteins preheated at 50 °C for 15 min exhibited comparatively better protective effects on GSAE stability than casein preheated at 60 °C for 15 min.

5. Conclusions

The addition of whey proteins and casein significantly prevented color loss and anthocyanins degradation ($p < 0.05$) in GSAE samples during heat treatment, oxidation and illumination at pH 3.2 and 6.3. Furthermore, thermal pretreatment of milk proteins effectively increased their protective effects on the thermal, oxidation and photo stability of GSAE. Whey proteins preheated at 50 °C for 15 min and casein preheated at 60 °C for 15 min produced optimal effects. Comparatively, whey proteins preheated at 50 °C for 15 min had a better protective effect on GSAE stability, which reduced the thermal, oxidative and photo degradation rates of anthocyanins in GSAE to a greater extent. These results should help facilitate the widespread application of GSAE as natural and functional colorants in the food industry.

Acknowledgments

This work was funded in part by the Innovation and Exploration Fund of State Key Laboratory of Food Science and Technology, Jiangnan University (No. SKLF-ZZA-201504), the Fundamental Research Funds for the Central Universities (No. JUSRP21108), the Science and Technology Infrastructure Program of Jiangsu (No. BM2014051) and the National High-Tech Research and Development Program of China (No. 2013AA102204).

References

Acharya, D. P., Sanguansri, L., & Augustin, M. A. (2013). Binding of resveratrol with sodium caseinate in aqueous solutions. *Food Chemistry, 141*, 1050–1054.

Bakowska-Barczak, A. M., & Kolodziejczyk, P. P. (2011). Black currant polyphenols: Their storage stability and microencapsulation. *Industrial Crops and Products, 34*, 1301–1309.

Bohin, M. C., Vincken, J., Westphal, A. H., Tripp, A. M., Dekker, P., van der Hijden, H. T., & Gruppen, H. (2014). Interaction of flavan-3-ol derivatives and different caseins is determined by more than proline content and number of proline repeats. *Food Chemistry, 158*, 408–416.

Bourassa, P., Bariyanga, J., & Tajmir-Riahi, H. A. (2013). Binding sites of resveratrol, genistein, and curcumin with milk α- and β-caseins. *The Journal of Physical Chemistry B, 117*, 1287–1295.

Bourassa, P., & Tajmir-Riahi, H. A. (2012). Locating the binding sites of folic acid with milk α- and β-caseins. *The Journal of Physical Chemistry B, 116*, 513–519.

Dalgleish, D. G. (2011). On the structural models of bovine casein micelles-review and possible improvements. *Soft Matter, 7*, 2265–2272.

Gordillo, B., Rodríguez-Pulido, F. J., González-Miret, M. L., Quijada-Morín, N., Rivas-Gonzalo, J. C., García-Estévez, I., Heredia, F. J., et al. (2015). Application of differential colorimetry to evaluate anthocyanin−flavonol−flavanol ternary copigmentation interactions in model solutions. *Journal of Agricultural and Food Chemistry, 63*, 7645–7653.

He, Z. Y., Chen, J., & Moser, S. E. (2015). Interaction of β-lactoglobulin with (−)-epigallocatechin-3-gallate under different processing conditions of pH and temperature by the fluorescence quenching method. *European Food Research and Technology, 241*, 357–366.

He, Z. Y., Chen, J., Moser, S. E., Jones, O. G., & Ferruzzi, M. G. (2016). Interaction of β-casein with (−)-epigallocatechin-3-gallate assayed by fluorescence quenching: effect of thermal processing temperature. *International Journal of Food Science & Technology, 51*, 342–348.

He, Z. Y., Xu, M. Z., Zeng, M. M., Qin, F., & Chen, J. (2016). Interactions of milk α-, β-casein with malvidin-3-*O*-glucoside and their effects on the stability of grape skin anthocyanin extracts. *Food Chemistry, 199*, 314–322.

Helal, A., Tagliazucchi, D., Conte, A., & Desobry, S. (2012). Antioxidant properties of polyphenols incorporated in casein/sodium caseinate films. *International Dairy Journal, 25*, 10–15.

Jing, P., & Giusti, M. M. (2005). Characterization of anthocyanin-rich waste from purple corncobs (*Zea may* L.) and its application to colour milk. *Journal of Agricultural and Food Chemistry, 53*, 8775–8781.

Kanakis, C. D., Hasni, I., Bourassa, P., Tarantilis, P. A., Polissiou, M. G., & Tajmir-Riahi, H. (2011). Milk β-lactoglobulin complexes with tea polyphenols. *Food Chemistry, 127*, 1046–1055.

Kopjar, M., Jakšić, K., & Piližota, V. (2012). Influence of sugars and chlorogenic acid addition on anthocyanin content, antioxidant activity and color of blackberry juice during storage. *Journal of Food Processing and Preservation, 36*, 545–552.

Li, B., Du, W. K., Jin, J. C., & Du, Q. Z. (2012). Preservation of (−)-epigallocatechin-3-gallate antioxidant properties loaded in heat treated β-lactoglobulin nanoparticles. *Journal of Agricultural and Food Chemistry, 60*, 3477–3484.

Li, J., Li, X., Zhang, Y., Zheng, Z., Qu, Z., Liu, M., Zhu, S., et al. (2013). Identification and thermal stability of purple-fleshed sweet potato anthocyanins in aqueous solutions with various pH values and fruit juices. *Food Chemistry, 136*, 1429–1434.

Liang, L., & Subirade, M. (2012). Study of the acid and thermal stability of β-lactoglobulin-ligand complexes using fluorescence quenching. *Food Chemistry, 132*, 2023–2029.

Liang, L., Zhang, J., Zhou, P., & Subirade, M. (2013). Protective effect of ligand-binding proteins against folic acid loss due to photodecomposition. *Food Chemistry, 141*, 754–761.

Malaj, N., De Simone, B. C., Quartarolo, A. D. N., & Russo, N. (2013). Spectrophotometric study of the copigmentation of malvidin 3-*O*-glucoside with *p*-coumaric, vanillic and syringic acids. *Food Chemistry, 141*, 3614–3620.

Mehranfar, F., Bordbar, A., & Parastar, H. (2013). A combined spectroscopic, molecular docking and molecular dynamic simulation study on the interaction of quercetin with β-casein nanoparticles. *Journal of Photochemistry and Photobiology B: Biology, 127*, 100–107.

Mercali, G. D., Schwartz, S., Marczak, L. D. F., Tessaro, I. C., & Sastry, S. (2014). Ascorbic acid degradation and color changes in acerola pulp during ohmic heating: Effect of electric field frequency. *Journal of Food Engineering, 123*, 1–7.

Ortega-Regules, A., Romero-Cascales, I., López-Roca, J. M., Ros-García, J. M., & Gómez-Plaza, E. (2006). Anthocyanin fingerprint of grapes: Environmental and genetic variations. *Journal of the Science of Food and Agriculture, 86*, 1460–1467.

Palazolo, G., Rodríguez, F., Farruggia, B., Picó, G., & Delorenzi, N. (2000). Heat treatment of β-lactoglobulin: Structural changes studied by partitioning and fluorescence. *Journal of Agricultural and Food Chemistry, 48*, 3817–3822.

Perez, A. A., Andermatten, R. B., Rubiolo, A. C., & Santiago, L. G. (2014). β-LG heat-induced aggregates as carriers of polyunsaturated fatty acids. *Food Chemistry, 158*, 66–72.

Philpott, M., Gould, K. S., Lim, C., & Ferguson, L. R. (2004). In situ and in vitro antioxidant activity of sweet potato anthocyanins. *Journal of Agricultural and Food Chemistry, 52*, 1511–1513.

Sadilova, E., Stintzing, F. C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science, 71*, C504–C512.

Shpigelman, A., Israeli, G., & Livney, Y. D. (2010). Thermally-induced protein–polyphenol co-assemblies: Beta lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG. *Food Hydrocolloid, 24*, 735–743.

Shpigelman, A., Israeli, G., & Livney, Y. D. (2012). Thermally-induced β-lactoglobulin EGCG nanovehicles: Loading, stability, sensory and digestive-release study. *Food Hydrocolloid, 29*, 57–67.

Strack, D., & Wray, V. (1994). The anthocyanins. In J. B. Harborne (Ed.), *The flavonoids: Advances in research since 1986* (pp. 1). London, UK: Chapman and Hall.

Talavéra, S., Felgines, C., Texier, O., Besson, C., Mazur, A., Lamaison, J., & Rémésy, C. (2006). Bioavailability of a bilberry anthocyanin extract and its impact on plasma antioxidant capacity in rats. *Journal of the Science of Food and Agriculture, 86*, 90–97.

Torskangerpoll, K., & Andersen, M. (2005). Colour stability of anthocyanins in aqueous solutions at various pH values. *Food Chemistry, 89*, 427–440.

Wiczkowski, W., Szawara-Nowak, D., & Topolska, J. (2013). Red cabbage anthocyanins: Profile, isolation, identification, and antioxidant activity. *Food Research International, 51*, 303–309.

Wu, J., Guan, Y., & Zhong, Q. (2015). Yeast mannoproteins improve thermal stability of anthocyanins at pH 7.0. *Food Chemistry, 172*, 121–128.

Xiong, S., Melton, L. D., Easteal, A. J., & Siew, D. (2006). Stability and antioxidant activity of black currant anthocyanins in solution and encapsulated in glucan gel. *Journal of Agricultural and Food Chemistry, 54*, 6201–6208.

Yazdi, S. R., & Corredig, M. (2012). Heating of milk alters the binding of curcumin to casein micelles. A fluorescence spectroscopy study. *Food Chemistry, 132*, 1143–1149.

Zheng, N., Bucheli, P., & Jing, H. (2009). Effects of casein- and whey protein-dextran conjugates on the stability of bog bilberry anthocyanin extract. *International Journal of Food Science and Technology, 44*, 1452–1458.

Zorilla, R., Liang, L., Remondetto, G., & Subirade, M. (2011). Interaction of epigallocatechin-3-gallate with β-lactoglobulin: Molecular characterization and biological implication. *Dairy Science and Technology, 91*, 629–644.