The Ohio State University/Department of Food Science and Technology

The interaction between thermally-induced whey protein (WP) and anthocyanin (ACN) by fluorescence quenching spectroscopy

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ABSTRACT

Our goal was to investigate anthocyanin binding to native and preheated whey protein using fluorescence quenching spectroscopy. Whey protein were preheated from 40-80℃ for 30 minutes, then mixed with various concentrations of anthocyanins (0~100 μM). Fluorescence spectra was recorded from 360-450nm at 25, 35, 45℃ with excitation at 280nm.

All anthocyanins strongly quenched whey protein's fluorescence. The fluorescence intensity of whey protein decreased 68%-73% and its λ_{max} increased (372-378nm) as anthocyanin concentration increased. The anthocyanin-whey protein quenching was determined to be static with only one binding side on the whey protein interacting with anthocyanins. Thermodynamic analysis showed that the binding between anthocyanin and whey protein was mainly through hydrophobic interaction. The binding affinity was higher for preheated whey protein and decreased gradually with increasing temperature due to whey protein aggregation.

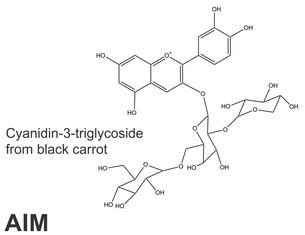
These results help better understand the protection mechanism of native or preheated whey protein on anthocyanin color stability, widening the application of anthocyanins as food colorants in food processing and storage.

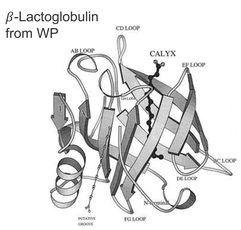
INTRODUCTION

Over the last decade, ACNs have been the most widely studied natural colorants to provide or improve color in food products because of their bright and attractive colors from red to purple, non-toxicity and water solubility (Montilla et al., 2011).

However, ACNs have limited chemical stability due to it is sensitive to environmental factors, such as pH, temperature, light, oxygen, metal ions and the presence of sulfur dioxide, ascorbic acid and enzymes (Torskangerpoll and Andersen, 2005). It is important and challenging to find an effective way to reduce the ACNs loss during food processing and storage.

At present, the complexation between WP and ACNs has received increasing attention. β-lactoglobulin (β-LG) in WP displays a strong binding affinity for various ligands, because it possesses a hydrophobic pocket located in the interior of the calyx structure (Liang and Subirade, 2012).



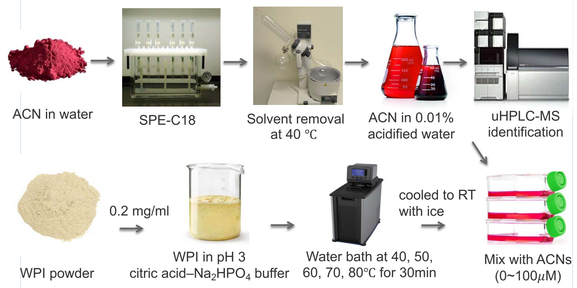


AIM

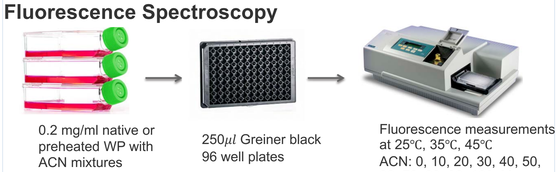
The purpose of this study was to investigate the interactions between preheated whey proteins and different anthocyanins (grape juice, black carrot and purple corn) by fluorescence quenching spectroscopy.

METHODS

Sample Preparation



Fluorescence Spectroscopy



Fluorescence quenching analysis

The fluorescence mechanism is described by the Stern-Volmer equation:

$$F_0/F = 1 + K_{SV}[Q] = 1 + K_q\tau_0[Q]$$

F_0 : fluorescence intensities of proteins alone;
F : fluorescence of proteins + ACN (quencher);
Q : concentration of free ACN;
K_{sv} : Stern-Volmer constant;
K_q : bimolecular quenching constant;
τ_0 : lifetime of the fluorophore being quenched, equals 10^{-8}s.

For static quenching, the binding constant (K_s) and number of binding sites (n) were calculated using nonlinear least squares fitting of the experimental data(Li et al., 2009):

$$\log[(F_0 - F)/F] = \log Ks + n \log[Q]$$

Thermodynamic Analysis

The energy equilibrium of protein-ligand are explained by the Gibbs free energy (ΔG_o), the binding enthalpy (ΔH_o) and entropy (ΔS_o). ΔG_o was calculated by:

$$\Delta G_o = -RT\ln K_s$$

The ΔH_o and ΔS_o were obtained from the linear fitting of the Van't Hoff equations (Mehranfar, Bordbar & Parastar, 2013):

$$\ln K_s = (-\Delta H_o/R)(1/T) + (\Delta S_o/R)$$
$$\Delta G_o = \Delta H_o - T\Delta S_o$$

T : absolute temperature (K);
K_s : binding constant associated with temperatures;
R : gas constant (8.314 J mol^{-1} K^{-1}).

Types of Binding Forces

The calculations above allowed to determine the type of non-covalent binding involved in the interaction between proteins and anthocyanins (Ross and Subramanian, 1981):

- Electrostatic and hydrophobic interactions: $\Delta H_o > 0$, $\Delta S_o < 0$;
- Hydrophobic interaction: $\Delta H_o > 0$, $\Delta S_o > 0$;
- Van der Waals forces or hydrogen bonding: $\Delta H_o < 0$, $\Delta S_o < 0$;
- Electrostatic interaction: $\Delta H_o < 0$, $\Delta S_o > 0$.

RESULTS

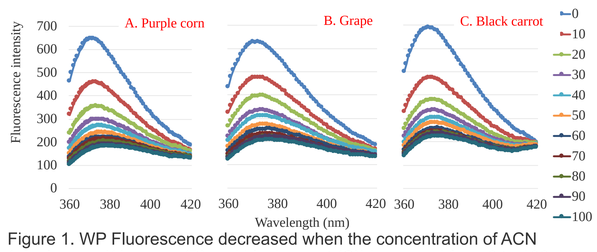


Figure 1. WP Fluorescence decreased when the concentration of ACN (purple corn (A), grape (B) and black carrot (C)) increased from 0 to 100μM at excitation wavelength of 280 nm at 25℃ and pH 3.

Table 1. The Stern-Volmer constants, quenching constants, binding constants and binding sites for purple corn, grape and black carrot ACNs binding to native WP at 25℃, 35℃ and 45℃ and preheated WP at 25 ℃ (pH 3) at excitation wavelength of 280 nm.

	Parameter	Temperature (℃)			Preheat Temperature (℃)					
		25	35	45	N/A	40	50	60	70	80
Purple corn	K_q ($\times10^{12}$ M^{-1})	3.02± 0.07	2.97± 0.13	3.10± 0.05	1.67± 0.09	1.69± 0.02	1.71± 0.09	1.82± 0.04	1.86± 0.07	1.98± 0.06
	K_s ($\times10^3$ M^{-1})	4.82± 0.15	4.94± 0.28	5.70± 0.19	3.33± 0.93	4.78± 0.95	4.75± 0.33	4.65± 0.71	4.08± 0.40	3.77± 0.75
	n	0.81± 0.04	0.81± 0.04	0.82± 0.04	0.59± 0.03	0.62± 0.04	0.62± 0.07	0.62± 0.01	0.60± 0.03	0.57± 0.03
Grape	K_q ($\times10^{12}$ M^{-1})	2.31± 0.07	2.33± 0.04	2.35± 0.07	1.44± 0.17	1.28± 0.09	1.29± 0.06	1.31± 0.07	1.60± 0.06	1.61± 0.03
	K_s ($\times10^3$ M^{-1})	4.09± 0.78	4.89± 0.97	5.99± 1.23	0.89± 0.26	1.32± 0.10	1.31± 0.03	1.25± 0.07	1.08± 0.53	0.97± 0.17
	n	0.82± 0.02	0.83± 0.02	0.85± 0.02	0.47± 0.04	0.52± 0.00	0.52± 0.00	0.51± 0.01	0.47± 0.06	0.46± 0.01
Black carrot	K_q ($\times10^{12}$ M^{-1})	2.44± 0.11	2.46± 0.04	2.52± 0.03	0.67± 0.05	0.67± 0.06	0.67± 0.08	0.68± 0.05	0.78± 0.10	0.88± 0.07
	K_s ($\times10^3$ M^{-1})	1.00± 0.21	1.03± 0.12	1.14± 0.18	1.07± 0.49	2.66± 0.23	2.54± 0.84	2.00± 0.15	1.42± 0.72	1.25± 0.77
	n	0.66± 0.02	0.66± 0.01	0.67± 0.02	0.67± 0.05	0.66± 0.05	0.65± 0.02	0.63± 0.01	0.58± 0.10	0.55± 0.07

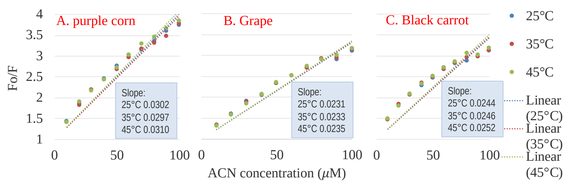


Figure 2. The Stern-Volmer plots for the quenching of whey protein by purple corn (A), grape (B) and black carrot (C) ACNs at 25℃, 35℃ and 45℃ (used for K_{sv} and K_q calculation). This showed that the quenching between anthocyanins and whey protein was static, regardless of the source.

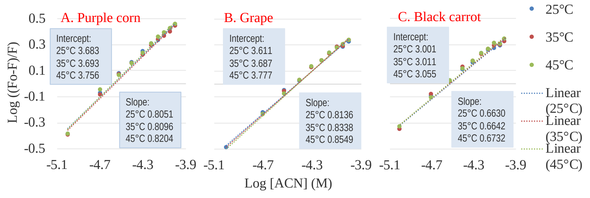


Figure 3. The double logarithm regression plots of log [(F_0-F)/F] versus log [ACN] (purple corn (A), grape (B) and black carrot (C)) of whey protein at 25℃, 35℃ and 45℃ (used for K_s and n calculation). This showed that the binding affinity increased at higher temperatures.

Table 2. Thermodynamic parameters for purple corn, grape and black carrot ACNs binding to whey protein at 25℃, 35℃ and 45℃ (pH 3).

ACN source	Parameters	Temperature		
		25°C	35°C	45°C
Purple corn	ΔH^o ($\times10^3$ J mol^{-1})		6.52	
	ΔG^o ($\times10^4$ J mol^{-1})	-2.10	-2.18	-2.29
	ΔS^o (J mol^{-1} K^{-1})	92.40	70.71	71.89
Grape	ΔH^o ($\times10^3$ J mol^{-1})		-6.49	
	ΔG^o ($\times10^4$ J mol^{-1})	-2.06	-2.10	-2.15
	ΔS^o (J mol^{-1} K^{-1})	47.36	68.29	67.77
Black carrot	ΔH^o ($\times10^3$ J mol^{-1})		4.90	
	ΔG^o ($\times10^4$ J mol^{-1})	-1.71	-1.78	-1.86
	ΔS^o (J mol^{-1} K^{-1})	73.89	57.64	58.49

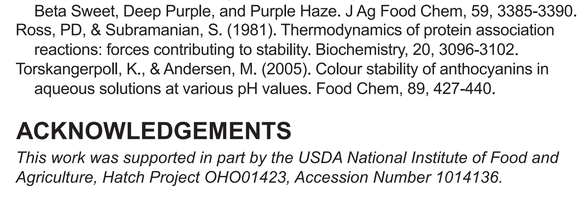


Figure 4. Fluorescence intensity of whey protein at 25°C increased with preheating temperatures (40-80℃) in the presence of 50μM purple corn (A), grape (B) and black carrot (C) ACNs at excitation wavelength of 280 nm at pH 3.

CONCLUSIONS

- ACNs quenched WP's fluorescence strongly (Fig. 1).
- The interaction between ACNs and WP decreased the fluorescence intensity of WP and increased its λ_{max} (Fig. 1), showing that the polarity microenvironment inside of WP increased with the addition of ACNs.
- WP-ACN interaction was mainly through static quenching (K_q value is higher than the limiting diffusion rate constant of the biomolecules 2×10^{10} $M^{-1}s^{-1}$ (Li et al., 2009)) and there was only one binding site in WP for ACN (Fig.1-2).
- Both native and preheated WP bound ACNs mainly via hydrophobic forces ($\Delta H_o > 0$, $\Delta S_o > 0$) (Table 2) .
- ACN binding affinity was higher for preheated WP than native WP (Fig.4). Affinity was higher when WP was preheated to 40°C and decreased gradually with increasing preheating temperature, likely due to protein aggregation (Table 1).

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