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Department of Food Science & Technology

Increasing Pyranoanthocyanin Formation with Use of Caffeic Acid and Heat for Production of Naturally Derived Food Colorants

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ABSTRACT

There is consumer and regulatory push to replace artificial colorants with naturally derived ones for health and environmental reasons. Anthocyanins (ACN), plant pigments, are good antioxidants but are unstable in foods. In aged red wines, ACN slowly condensate with fermentation products to form the more stable pyranoanthocyanins (PACN). This study attempted to produce PACNS efficiently using high cofactor concentration and heat.

Semi-purified Elderberry anthocyanins were mixed with caffeic acid (CA), the cofactor, dissolved in 15% ethanol, and diluted with buffer to achieve ACN:CA molar ratios of 1:50 - 1:200 and incubated at 65°C for 5 days. PACN formation was monitored over time by uHPLC with PDA-MS/MS. The effect of temperature was tested using ACN samples incubated with or without CA at 25°C, 50°C, and 75°C for 7 days.

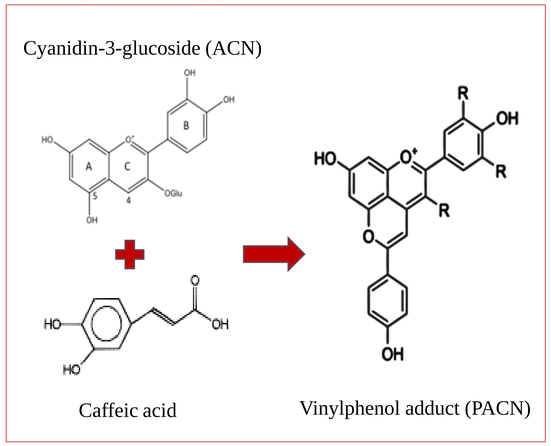
ACN:CA 1:150 ratio showed the highest tinctorial strength after 48 hr. PACN formation was fastest between 24-48 hr for all ACN:CA ratios. PACN formed faster at higher T°, reaching ~90% PACN in 24hr and ~100% PACN in 48hr at 75°C. However, incubation at 75°C also induced ACN degradation.

These results suggest PACN could be produced efficiently from elderberry ACN and CA if heated to produce more stable pigments with potential as food dyes.

INTRODUCTION

Food colorant toxicity has long been a matter of concern for consumers and regulatory bodies. In a double-blinded, randomized trial, children consuming foods with artificial colors had increased hyperactivity[1]. This increased need for naturally derived alternatives, like anthocyanins (ACN). These are plant pigments that are powerful antioxidants and provide health benefits but are unstable. Pyranoanthocyanins (PACN), first found in red wine, are derived from ACN. They naturally form in the aging process and provide the long-lasting color. PACN are more stable but are formed inefficiently.

Figure 1. Cyanidin-3-glucoside reacts with caffeic acid to produce vinylphenol adduct, a PACN



AIM

Produce PACN using caffeic acid (CA) as cofactor and temperature as catalyzer to increase formation efficiency for use in food industry

MATERIALS AND METHODS

Plant Material: *Sambucus nigra*

To obtain ACN
- Elderberry (*Sambucus nigra*) powder
- Dissolved in water
- Solution was semi-purified with C18 resin
- ACN concentrated with Rotavapor

CA was dissolved in 15% ethanol and pH 3.1 buffer[2,3]

The effect of temperature was tested in samples with or without CA incubated for 7 days at
- 25 °C
- 50 °C
- 75 °C

The effect of CA concentration was tested at 65°C for 8 days with ACN:CA molar ratios
- 1:0
- 1:50
- 1:100
- 1:150
- 1:200

PACN formation was monitored on a uHPLC-PDA-MS/MS
- Reverse phase chromatography
- C18 column
- Photo diode array detector
- Electrospray ionization
- Tandem mass spectrometry

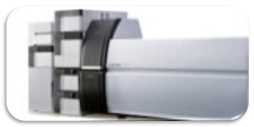
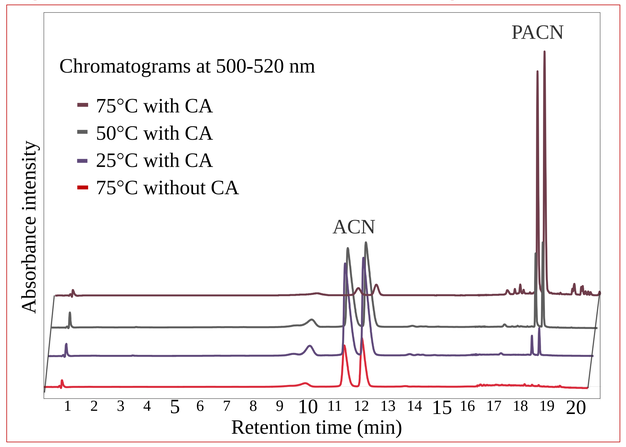


Figure 2. PACN formation and ACN remaining at 24 hr



RESULTS

- Higher temperatures led to lower ACN and higher PACN levels after 24hr (Fig. 2).
- PACN formed faster at higher temperatures (Fig. 3). Within 2 days, 100% of ACN converted to PACN.
- PACN levels reached at 75°C in 24 hr took 42 days to form at 25°C[3].
- Total pigment content decreased more rapidly at higher temperatures (Fig. 4).
- Higher ACN:CA ratios produced PACN more rapidly (Fig. 5)
- ACN:CA molar ratio 1:150 had greatest PACN formation, reaching 212% of the initial pigment absorbance (Fig. 6).

Figure 3. PACN formation

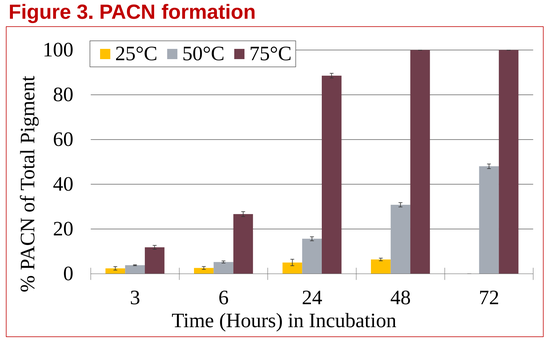


Figure 4. Pigment Degradation

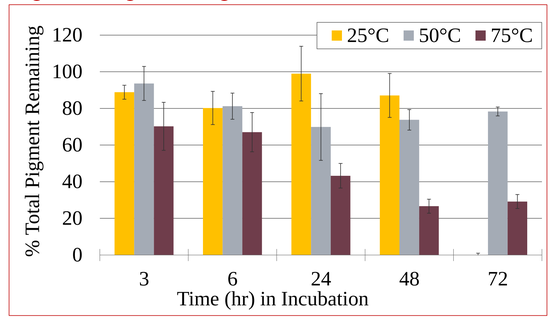


Figure 5. Percent PACN at different times

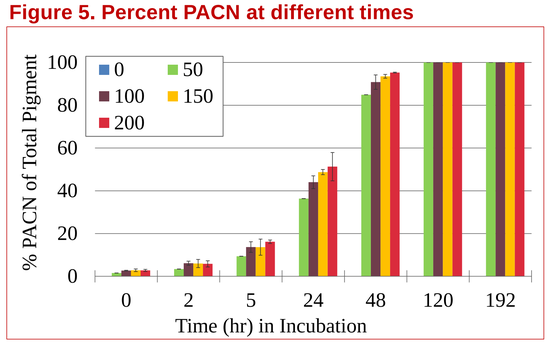
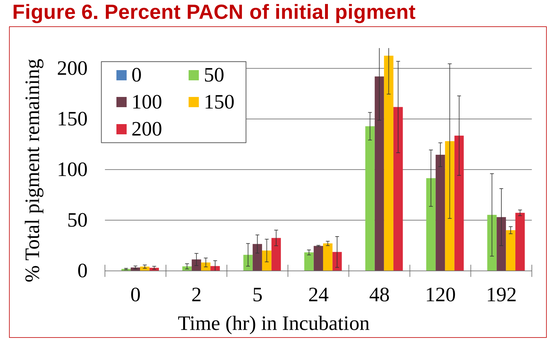


Figure 6. Percent PACN of initial pigment



CONCLUSIONS AND DISCUSSIONS

Elderberry ACN can be combined with CA and high heat to more efficiently produce the more stable PACN with potential application as naturally derived food dyes that can replace artificial dyes. Previous studies have reported increased PACN formation with increased temperature; however, none have reported formation at a rate as significant as reported here. Replacing artificial ingredients is a priority for many food consumers and producers. These results can lead to the development of a PACN colorant that can be applied to a variety of products throughout the food industry to replace artificial colorants with a naturally derived one that also provides antioxidative effects.

REFERENCES

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ACKNOWLEDGEMENTS

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