Food Research International 51 (2013) 274–282

Contents lists available at SciVerse ScienceDirect

Food Research International

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

Application of ferric anthocyanin chelates as natural blue food colorants in polysaccharide and gelatin based gels

M. Buchweitz, J. Brauch, R. Carle, D.R. Kammerer *

Hohenheim University, Institute of Food Science and Biotechnology, Chair Plant Foodstuff Technology, Garbenstrasse 25, D-70599 Stuttgart, Germany

ARTICLE INFO

Article history:
Received 5 July 2012
Accepted 28 November 2012

Keywords:
Blue food colorant
Pectin
Agar–agar
Elderberry
Red cabbage
Purple carrot

Dedicated to Prof. Dr. Dr. h.c. Rainer Wild, Heidelberg, on the occasion of his 70th birthday.

ABSTRACT

Ferric anthocyanin chelates based on elderberry (EB-E) and purple carrot (PC-E) extracts as well as red cabbage juice (RC-J) were applied to different gel matrices to confirm their potential as natural blue food colorants. Blue color evolution and stability during storage at 4 and 20 °C in the dark and at 25 °C under illumination was monitored at two colorant concentrations by determination of lightness (L*), red (a*) and blue (b*) hue values as derived from tristimulus reflectance measurements. Intense blue hues were observed for gels based on gelatin (G) and a blend of agar–agar with amidated pectin (AA/AP). Under each of the storage conditions color stabilities of the gels with PC-E were excellent, being superior to those with added EB-E and RC-J. While room temperature (20 °C) and especially VIS light significantly affected blue color stability in gels dyed with EB-E and RC-J, color of the gels prepared with PC-E was almost fully retained independent of temperature and light exposure. Generally, enhanced stabilities were observed at higher colorant dosages. Gelatin improved color stability significantly compared to AA/AP gels, except for the PC-E colorant, where the difference between both matrices was negligible. Blue color decay in gels, as monitored by increasing b* values, partially deviated from first-order kinetics depending on colorant and storage conditions. Hence, kinetic calculations by exclusive consideration of the b* values were unsuitable for describing and predicting blue color loss. The formation of dairy based gums by substitution of water with yoghurt, buttermilk and milk was only supported by the aid of a gelatin matrix, resulting in pink, violet and slightly blue hues, respectively. In summary, the successful application of ferric anthocyanin chelates to food matrices was demonstrated, confirming their potential as promising natural blue food colorants.

1. Introduction

Due to increasing health awareness, substitution of artificial colorants by their natural counterparts is a major goal of the food, pharmaceutical and cosmetics industry (Stintzing & Carle, 2004). In particular, since the study of McCann et al. (2007), widely known as "Southampton study", has provided evidence that some azo dyes may be detrimental to children's health, the European Commission imposed labeling of these additives (Directive 1333/2008 (EC)). For replacing red, orange and yellow hues, natural alternatives such as hydrophilic anthocyanins (E 163), betalains (E 162) and lipophilic carotenoids (carotenes, E 160 a–e; xanthophylls, E 161a–j) are available to dye foods (Giusti & Wrolstad, 2003; Henry, 1996; Malien-Aubert, Dangles, & Amiot, 2001; Wissgott & Bortlik, 1996). To avoid E numbers or chemical terms in the list of ingredients, coloring foodstuffs such as elderberry, red beet or carrot juice concentrates are preferred for dying uncolored products such as candies, confectionary, soft drinks and drugs (Downham & Collins, 2000). In contrast, to achieve blue tones, only a few natural colorants are commercially available. Usually, extracts of *Spirulina* spp. are applied; however, mainly for coatings, and data regarding color evolution and stability are scarce. Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, and Galán-Vidal (2009) suggested intense blue anthocyanin–metal chelates to be an interesting option for natural blue colorants. Surprisingly, no further attempt was undertaken to realize this concept. Blue anthocyanin based colors have been investigated in various flowers. These exceptionally intense and stable hues are due to several stabilizing mechanisms which have been summarized by Yoshida, Mori, and Kondo (2009). In many cases, anthocyanin–metal chelates and additional co-pigmentation with colorless polyphenols in a non-stoichiometric ratio are responsible for the blue hues; however, such fuzzy metal chelates are only stable in their vacuolar matrix, fading away during complex isolation and crystallization. In previous studies we observed a stabilizing effect of pectic structures on the latter preventing complex precipitation in aqueous solutions (Buchweitz, Nagel, Carle, & Kammerer, 2012a). Furthermore, the hydroxylation pattern of the anthocyanin B ring, the presence of uncolored phenolics and

Abbreviations: ACN, anthocyanin; AA, agar–agar; AP, amidated citrus pectin; DE, degree of esterification (%); DA, degree of amidation (%); GalA, galacturonic acid; ss, soluble solids; a_w, water activity; G, gelatin; M, gelatin gel with milk; BM, gelatin gel with buttermilk; Y, gelatin gel with yoghurt; EB, elderberry; RC, red cabbage; PC, purple carrot; J, juice; E, phenolic extract; %, w/w, mass concentration; D, destruction value; L*, lightness; a*, red-green hue; b*, yellow-blue hue; ΔE, color difference.

* Corresponding author. Tel.: +49 711 459 22995; fax: +49 711 459 24110.
E-mail address: Dietmar.Kammerer@uni-hohenheim.de (D.R. Kammerer).

0963-9969/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2012.11.030

further matrix compounds as well as structural prerequisites of pectic compounds to achieve blue color evolution and enhanced stability were thoroughly investigated in model solutions (Buchweitz, Carle, & Kammerer, 2012b; Buchweitz et al., 2012a). In small scale model experiments promising blue hues, storage and heat stabilities were demonstrated for ferric anthocyanin chelates (Buchweitz, Brauch, Carle & Kammerer, in press).

Therefore, the objective of the present study was to transfer these results to real foods such as edible gels, demonstrating the applicability of natural anthocyanin-based blue pigments in food. Colorants based on extracts of elderberry (*Sambucus nigra* L., EB-E) and purple carrot (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef., PC-E) as well as red cabbage (*Brassica oleracea* L. ssp. *capitata* f. *rubra*) juice (RC-J), the latter even enabling its labeling as coloring foodstuff, should be developed and added to protein and polysaccharide based gels under conditions commonly applied in industrial practice. Color evolution and impact of storage conditions (temperature and light) on color stability should be thoroughly evaluated.

2. Materials and methods

2.1. Anthocyanin solutions

For preparing the phenolic extracts (E), concentrates of elderberry (EB; Exberry GNT Europe, Aachen, Germany) and purple carrot (PC; Wild, Heidelberg, Germany) were diluted with water and applied to solid phase extraction on a polystyrene adsorber resin (Amberlite FPX66, Rohm & Haas, Philadelphia, PA, USA) following the procedure described previously (Buchweitz et al., in press). Red cabbage juice (RC-J) was prepared by dilution of a concentrate (Exberry GNT Europe, Aachen, Germany) with water. All stock solutions were adjusted to total anthocyanin concentrations of $2.01 \pm 0.02 \cdot 10^{-3}$ mol/L and stored at −18 °C until further use. Demineralized water was used throughout.

A comprehensive chemical characterization of these anthocyanin containing stock solutions, including sugars and acids as well as HPLC chromatograms and polyphenol assignment is provided as supplementary material (Data S1).

2.2. Hydrocolloids

Low methoxylated amidated citrus pectin (AP; degree of esterification (DE) 27.4%, degree of amidation (DA) 22.8%, content of galacturonic acid (GalA) 93.5%) and a commercial pectin formulation (Pektin Amid CS 025-B) based on amidated pectin (DE 25.8%, DA 20.1%), recommended for gum and jelly products, were donated by Herbstreith & Fox (Neuenbürg, Germany). Commercially available agar–agar (AA; 20% in maltodextrin) and gelatin (G) were from Ruf Lebensmittelwerk (Quakenbrück, Germany).

2.3. Miscellaneous

Potassium sorbate p.a. was purchased from Merck (Darmstadt, Germany) and glucose syrup from Schließmann (Schwäbisch Hall, Germany). Crystalline sucrose was from Südzucker (Mannheim, Germany). Cow's milk (M; 1.5% fat), buttermilk (BM, <1% fat) and yoghurt (Y; 0.1% fat) were from Milbona (Ravensburg, Germany) and purchased on local markets.

2.4. Preparation of the colorant

Amidated pectin (AP) was suspended in water (1%, w/w) and shaken over night. 224 mL of this solution was mixed with 32 mL of the respective anthocyanin stock solution ($2 \cdot 10^{-3}$ mol/L). 48 mL of aqueous ferric stock solution ($4 \cdot 10^{-3}$ mol/L $FeCl_3 \cdot 6H_2O$, Fluka, Buchs, Switzerland) was added slowly. The pH value of both stock solutions was adjusted to pH 3.3 immediately before mixing. The final concentrations of AP, anthocyanins and ferric ions amounted to 0.74% (w/w), 2.11 and $6.32 \cdot 10^{-4}$ mol/L, respectively, and a molar anthocyanin to ferric ion ratio (ACN:Fe^{3+}) of 1:3 was obtained. After adjusting the pH value to 5.0 and continuous stirring over night, aliquots of the colorant were filled in polyethylene bottles and stored at −18 °C until further use. Each colorant was prepared in duplicate.

2.5. Model gels

2.5.1. Gels prepared from a commercial pectin formulation (matrix CS 025-B)

25 g of pectin formulation (Pektin Amid CS 025-B) was blended with 100 g sucrose and 1.8 g potassium sorbate. This mixture was stirred into 220 g water and boiled while stirring until the pectin was completely dissolved. Glucose syrup (475 g) and sucrose (260 g) were added, and the solution was cooked to a final soluble solid content (ss) of 78.0 ± 0.1% (measured by refractometer RX-5000, Atago, Japan) as recommended for jelly production by the supplier of the pectin formulation. The final concentration of pectin formulation amounted to 2.5%, and the pH value was 4.5. Subsequently, to each portion of 200 g, 60 mL of colorant together with 20 mL water and 80 mL of colorant for colorant dosages 1 and 2, respectively, were added at 80 °C.

2.5.2. Gels prepared from amidated pectin (matrix AP)

The procedure was analogous to Section 2.5.1 using amidated pectin AP instead of the pectin formulation (Pektin Amid CS 025-B).

2.5.3. Gels prepared from agar–agar and amidated pectin (matrix AA/AP)

Potassium sorbate (1.2 g) was dissolved in 400 mL water, and 20 g agar–agar (AA, 20%, w/w) was added under stirring. The solution was boiled for 4 min, and 300 g sucrose blended with 2 g amidated pectin (AP) was added. The mixture was cooked to a final ss content of 49%. The concentrations of AA and AP were 0.6 and 0.3%, respectively, and a pH value of 5.5 was determined. Subsequently, to each portion of 200 g, 60 mL colorant and 20 mL water (colorant dosage 1) and 80 mL (colorant dosage 2) were added at 80 °C. The ss content and pH value of the blue gels were 35.7 ± 0.1% and 5.3 ± 0.0, respectively.

2.5.4. Gels prepared from gelatin (matrix G)

Potassium sorbate (1.25 g) was dissolved in 138 mL water, and 575 g sucrose was added with subsequent heating of the mixture to 100 °C. Gelatin (25 g) was soaked for 5 min in cold water, drained and dissolved in the hot sucrose solution without boiling. The mixture was cooked to a final ss content of 70% (3.4% gelatin, w/w) at a pH value of 5.7. Subsequently, to each portion of 200 g, 40 mL colorant with 20 mL water and 60 mL colorant for colorant dosages 1 and 2, respectively, were added at 80 °C. The ss content and pH value of the blue gels were 58.5 ± 0.1% and 5.7 ± 0.1, respectively.

2.5.5. Gelatin based gels prepared with dairy products (matrices M, BM, Y)

The basic gelatin gel (2.5.4.) was modified by replacing water with the respective amount of milk (M, pH 6.6), buttermilk (BM, pH 4.6) and yoghurt (Y, pH 4.4). To each portion of 200 g, 60 mL of the colorant based on red cabbage juice (RC-J) and 20 mL water were added (colorant dosage 1) and pH values of 6.1, 5.1 and 4.9 were determined, respectively. Additionally, milk-containing gels (M) with the addition of 80 mL RC-J colorant per 200 g (colorant dosage 2) were prepared.

All gels were prepared in duplicate (A and B samples). Aliquots of ~12 g were filled in Petri dishes (35/10 mm, Greiner bio-one, Frickenhausen, Germany), cooled to room temperature, closed and sealed with Parafilm®. Uncolored gels (blanks) were prepared similarly by adding water instead of colorant. All samples were stored for 48 h at 20 ± 0.2 °C in the dark to establish equilibrium (t = 0).