Food Chemistry 173 (2015) 305–312

Contents lists available at ScienceDirect

Food Chemistry

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

Thermal degradation of green tea flavan-3-ols and formation of hetero- and homocatechin dimers in model dairy beverages

Brian J. Song [a,1], Chris Manganais [a,1], Mario G. Ferruzzi [a,b,*]

[a] Department of Food Science, Purdue University, 745 Agriculture Mall Dr., West Lafayette, IN 47906, USA
[b] Department of Nutrition Science, Purdue University, 700 W. State St., West Lafayette, IN 47907, USA

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 12 August 2014
Accepted 4 October 2014
Available online 15 October 2014

Keywords:
Flavan-3-ols
Thermal stability
Oxidation
Theasinesin
P-2 dimer
Protein–polyphenol interaction

ABSTRACT

Interactions between polyphenols and macromolecules may impact polyphenol stability and bioavailability from foods. The impact of milk on tea flavan-3-ol stability to thermal treatment was investigated. Single strength (36.2 protein per L), quarter strength (9.0 g protein per L) milk, and control model beverages were incubated with epigallocatechin gallate and green tea extract at 62 or 37 °C for 180 min. Intact flavan-3-ols and select auto-oxidation products [theasinesins (THSNs) and P-2 dimers] were quantified by LC–MS. Generally, greater polyphenol to protein ratios increased first order degradation rates, consequently decreasing formation of oxidation products. The presence of galloyl and hydroxy moieties was associated with higher stability of monomeric flavan-3-ols with increasing protein concentrations suggesting potential for protein affinity to stabilise flavan-3-ols to thermal treatment. Absence of these moieties led to no observable improvements in stability. These results suggest that protein interactions may be useful in stabilising flavan-3-ols through thermal processing.

1. Introduction

Teas are one of the most commonly consumed beverages worldwide. Green tea contains high concentrations of polyphenols, predominantly composed of flavan-3-ol monomers including epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Flavan-3-ols have a generic backbone while the presence of a galloyl moiety or an additional hydroxy substitution on the B-ring determines the exact compound (Fig. 1). Diets rich in flavan-3-ols have been associated with reduced risk of chronic diseases including cardiovascular diseases, certain cancers, diabetes, and obesity (Arts & Hollman, 2005; Crespy & Williamson, 2004; Hughes et al., 2008; Theodoratou et al., 2007). Therefore, the bioavailability, chemistry, and physiological responses of dietary flavan-3-ols have been the focus of recent studies. However, they are vulnerable to several degradative reactions accelerated by heat, food ingredients, elevated pH (>5) conditions, and presence of dissolved oxygen or other reactive oxygen species (Chen, Zhu, Tsang, & Huang, 2001; Zhu, Zhang, Tsang, Huang, & Chen, 1997; Zimeri & Tong, 1999). Although there are many mechanisms of degradation, the formation of simple auto-oxidation products including theasinesin (THSN) and P-2 dimers has been the focus of recent studies (Fig. 1) (Haslam, 2003). In general, the auto-oxidation of flavan-3-ols occurs by the formation of a radical semiquinone structure and is stabilised via multiple resonance structures available to the flavan-3-ol (Castañeda-Ovando et al., 2009). Further oxidation will result in the formation of a stabilised quinone structure (Dangles, Fargeix, & Dufour, 1999; Graham, 1992). Once flavan-3-ol quinones are formed, they may react with other flavan-3-ols or quinones to produce a THSN dimer or its quinone analogue, respectively (Sang, Yang, Buckley, Ho, & Yang, 2007). Additional oxidation of the THSN dimer will produce its quinone analogue as well, which can then be further oxidised to form the P-2 dimer (Sang et al., 2007). The dimers have been shown to be naturally present in oolong and black teas and also form during storage and digestion of green tea (Neilson et al., 2007). Furthermore, studies have investigated the bioavailability of the dimers in both rodent and cell culture models demonstrating their potential biological relevance (Neilson, Song, Sapper, Bomser, & Ferruzzi, 2010; Qiu et al., 2012). However, the preservation of monomeric forms is believed to be more critical to delivery of health benefits.

* Corresponding author at: Department of Food Science, Purdue University, 745 Agriculture Mall Dr., West Lafayette, IN 47906, USA. Tel.: +1 (765) 494 0625; fax: +1 (765) 494 7953.
E-mail address: mferruzz@purdue.edu (M.G. Ferruzzi).
[1] Present address: PepsiCo Global R&D, 617 West Main Street, Barrington, IL 60010, USA.

http://dx.doi.org/10.1016/j.foodchem.2014.10.026
0308-8146/

One factor believed to alter stability and bioavailability of flavan-3-ols is the presence of milk protein. Polyphenols have previously been reported to interact with macromolecules in the food matrix or in vivo. For instance, Bennick et al. described interactions between polyphenols and salivary proteins as a main contributor to an astringent sensation (Bennick, 2002). A molecular model for astringency based on protein–polyphenol interactions has been described (Jobstl, O'Connell, Fairclough, & Williamson, 2004). These interactions are not isolated to the consumption of polyphenol rich beverages as they can also occur during storage and production. Siebert et al. investigated protein–polyphenol interaction and the relation to haze formation in beer, wine, and apple juice (Siebert, Carrasco, & Lynn, 1996). Hydrophobic and hydrogen bonding forces between polyphenols and proline rich segments have been reported to be important driving factors for these interactions (Hasni et al., 2011). Thus, proline rich proteins including β-casein and salivary proteins have been targeted by recent studies. However, studies have not investigated the impact of these interactions on the stability of flavan-3-ols and their potential involvement in modulation of auto-oxidation reactions in food systems.

Recently, our research group investigated the presence of monomeric flavan-3-ols in human milk from 17 mothers during 3 lactation stages (Song, Jouni, & Ferruzzi, 2013). EC, ECG, and EGCG were detected in milk samples at 63.7–828.5, 55.7–645.6, and 215.1–2364.7 nmol/L, respectively. In contrast, EGC was not detected in any of the milk samples and its absence is caused, in part, by dietary habits as well as the chemistry of EGC. For instance, EGC is more susceptible to degradative reactions than EC, ECG, and EGCG (Zhu et al., 1997). Furthermore, EGC has a lower binding affinity to milk and human serum proteins than ECG and EGCG (Minoda et al., 2010; Xiao et al., 2011). The potential for flavan-3-ol binding may limit their availability to oxidative reactions and thus alter the stability of monomeric flavan-3-ol. Therefore, EGC may be more likely to degrade below the analytical limit of detection before ECG or EGCG in a protein rich matrix. However, the degradation of monomeric flavan-3-ols in milk protein rich model beverages has not been fully investigated. The current study investigates the degradation of monomeric flavan-3-ols and the formation of dimers at multiple polyphenol to protein ratios in both a model dairy system at human body (37 °C) and batch pasteurisation (62 °C) temperatures.

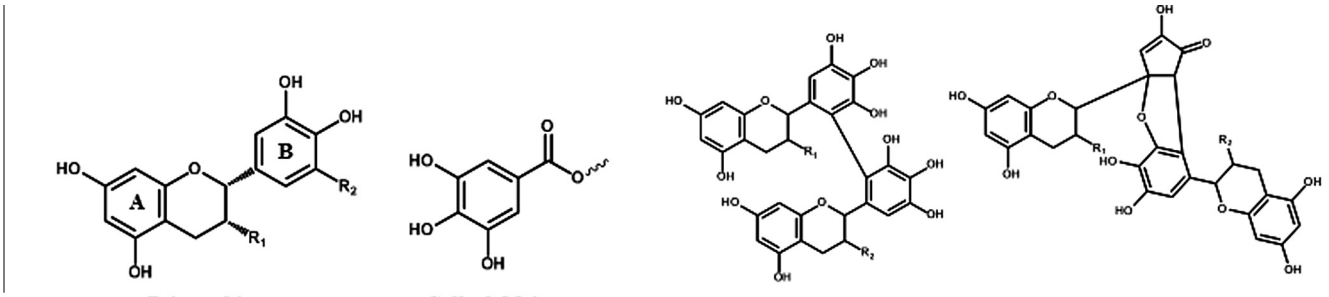
2. Materials and methods

2.1. Materials

L-Ascorbic acid, Na_2-ethylenediaminetetraacetic acid (EDTA), pepsin (# P7000), citric acid, sodium phosphate dibasic, formic acid, EC, EGC, ECG, and EGCG were purchased from Sigma Aldrich (St. Louis, MO, USA). Solvents including mass spectroscopy grade water, methanol, isopropanol, and HCl were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Green tea extract (GTE) and Teavigo®, >94% EGCG concentrate, were donated to our research group from Nestlé PTC Marysville and DSM, respectively. Saco® (Middleton, WI, USA) non-fat dry milk (NFDM) was purchased from a local market.

2.2. Model dairy beverages

A range of polyphenols and model dairy beverages were selected in order to investigate multiple polyphenol to protein ratios. 10% and 2.5% w/w NFDM in double distilled water were used as our model dairy beverages corresponding to single strength milk (SSM) and quarter strength milk (QSM), respectively. These preparations delivered approximately 36.2 and 9.0 g protein per L finished beverage, respectively. NFDM concentrations were selected to approximate the protein content of dairy beverages (U.S. Department of Agriculture & A. R. S, 2012). A 0% w/w NFDM buffered to pH 6.30 ± 0.02 using citric acid and sodium phosphate dibasic was used as a zero protein control. Model dairy beverages were impregnated with GTE at 500, 100, 0.5, and 0.1 mg/L and Teavigo® at 100, 0, 1, and 0.1 μM. EC, EGC, ECG, and EGCG were present in the GTE at 37.4, 40.4, 196.9, and 240.1 μmol/g. Thus, 500 mg/L of GTE was equivalent to 18.7, 20.2, 48.5, and 120.1 μM of EC, EGC, ECG, and EGCG, respectively. Similarly, 100, 0.5 and 0.1 mg/L GTE solution contained the same ratio of flavan-3-ols at their equivalent dilutions. No detectable quantities of THSN or P-2 dimers were present in stock GTE when immediately stabilised in 2% acetic acid in water. These results confirm that the GTE extract used in this study is a lightly processed and minimally oxidised green tea. The wide range in of GTE and Teavigo concentrations were used to approximate EGCG concentrations found in both teas and ready to drink tea beverages (2.62–283 μM) and human milk (0.215–2.36 μM) (Chen et al., 2001; Song et al., 2013).



Compound	m/z	Abbreviation	R1	R2
Epicatechin	289	EC	OH	H
Epigallocatechin	305	EGC	OH	OH
Epicatechin gallate	441	ECG	Galloyl	H
Epigallocatechin gallate	457	EGCG	Galloyl	OH

Compound	Monomer Composition	m/z	R1	R2
THSN A/D	2 EGCG	913	Galloyl	Galloyl
THSN C/E	2EGC	609	OH	OH
THSN B	EGCG + EGC	761	Galloyl	OH
P-2	2 EGCG	883	Galloyl	Galloyl
P-2 analog	2 EGC	579	OH	OH
P-2 analog	EGCG + EGC	731	Galloyl/OH	OH/Galloyl

Fig. 1. Structures of monomeric flavan-3-ols and autooxidation derived THSN and P-2 dimers.