Food Chemistry 178 (2015) 38–44

Contents lists available at ScienceDirect

Food Chemistry

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

Grape seed and apple tannins: Emulsifying and antioxidant properties

Maria Cruz Figueroa-Espinoza [a], Andrea Zafimahova [a,b,c,d], Pedro G. Maldonado Alvarado [a,b,c,d], Eric Dubreucq [a], Céline Poncet-Legrand [b,c,d,*]

[a] Montpellier SupAgro, UMR1208 IATE, F-34060 Montpellier, France
[b] INRA, UMR1083 SPO, F-34060 Montpellier, France
[c] Montpellier SupAgro, UMR1083 SPO, F-34060 Montpellier, France
[d] Université Montpellier UMR1083 SPO, F-34060 Montpellier, France

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form 7 January 2015
Accepted 8 January 2015
Available online 14 January 2015

Dedicated to the memory of Gérard Mazerolles.

Keywords:
Antioxidant
Polyphenols
Tannins
Catechin
Emulsion
CAT assay
Poly(vinyl alcohol)

ABSTRACT

Tannins are natural antioxidants found in plant-based foods and beverages, whose amphiphilic nature could be useful to both stabilize emulsions and protect unsaturated lipids from oxidation. In this paper, the use of tannins as antioxidant emulsifiers was studied. The main parameters influencing the stability of emulsions (i.e. tannins structure and concentration, aqueous phase pH, and ionic strength) were identified and optimized. Oil in water emulsions stabilized with tannins were compared with those stabilized with two commercial emulsifying agents, poly(vinyl alcohol) (PVA) and polyoxyethylene hydrogenated castor oil. In optimized conditions, the condensed tannins allowed to obtain a stability equivalent to that of PVA. Tannins presented good antioxidant activity in oil in water emulsion, as measured by the conjugated autoxidizable triene (CAT) assay.

1. Introduction

Emulsions are formed when two immiscible liquids are mixed. One liquid (the dispersed phase) is dispersed as droplets (from 0.10 μm to a few μm of diameter) in the other (the continuous phase) (McClements, 2007). Forming emulsions requires energy to increase the interfacial area between continuous and dispersed phases. Emulsified systems are thermodynamically unstable because of the surface tension between oil and water, which opposes to the increase of interfacial area. Emulsions can be stabilized by amphiphilic molecules, which adsorb in the oil–water interface, decreasing the surface tension. They can also be stabilized by solid particles which adsorb onto the interface between the two phases and are called Pickering emulsions (Pickering, 1907). Besides, the stability of emulsions is conditioned by the competition between attractive (Van der Waals, hydrophobic interactions, electrostatic attractions, hydrogen bonds) and repulsive forces (electrostatic repulsion, steric repulsion) between the dispersed droplets (Guzey & McClements, 2007; Tcholakova, Denkov, Sidzhakova, Ivanov, & Campbell, 2005). Stabilizing agents increase repulsions between droplets and slow down coalescence and phase separation phenomena. The stabilization of emulsions also depends on constituents of the emulsion, namely the concentration in emulsifier or stabilizing agent, pH, viscosity and ionic strength of the aqueous phase, and the concentration of the organic phase (Chanamai & McClements, 2000; Tcholakova, Denkov, Sidzhakova, & Campbell, 2006). Emulsified systems raise the problem of oxidation: dispersal of lipids in emulsified systems increases the specific area in contact with oxygen and some pro-oxidizing species (Coupland & McClements, 1996), which are detrimental to lipids.

On another hand, polyphenols are known for their antioxidant properties. They are molecules found in large amounts in plant-based foods and beverages. They are constituted by one or several rather hydrophobic aromatic nuclei bearing polar hydroxyl groups (–OH) (Poncet-Legrand, Cartalade, Putaux, Cheynier, & Vernhet, 2003). Among them, a particular class of polyphenols called tannins is produced in large amounts by distilleries and wine industries. These compounds are polymers of flavan-3-ol units primarily linked by C4–C8 bonds, with C4–C6 bonds giving rise to some degree of polymer branching (Fig. 1). For example, the

* Corresponding author at: INRA, UMR1083 SPO, F-34060 Montpellier, France. *E-mail address:* poncetc@supagro.inra.fr (C. Poncet-Legrand).

http://dx.doi.org/10.1016/j.foodchem.2015.01.056
0308-8146/

constitutive units of grape seed tannins are (+)-catechin (C), (−)-epicatechin (Ec), and (−)-epicatechin gallate (EcG) (Prieur, Rigaud, Cheynier, & Moutounet, 1994). Conversely, apple condensed tannins present an homogeneous structure, with primarily (−)-epicatechin as the constitutive flavanol unit (Guyot, Doco, Souquet, Moutounet, & Drilleau, 1997). The antibacterial tannin activity is related to surface chemistry and to their ability to non-covalently associate with proteins and other macromolecular structures (Vidal et al., 2003).

Their structure (aromatic hydrophobic rings, hydroxyl hydrophilic groups) suggests that some tannins may have surface active properties, and might thus stabilize emulsions. Although there are already numerous natural surfactant agents, the interest to use tannins as stabilizers lies in the fact that they also have important antioxidant capacities. They are highly polymerized and possess many phenolic hydroxyl groups. In addition, the "B" ring in the flavanols is responsible for most of the antioxidant activity, as it contains the catechol or trihydroxy functionality (Hagerman et al., 1998; Pazos, Gallardo, Torres, & Medina, 2005; Torres et al., 2002; Van Acker et al., 1996). However, due to their high chemical reactivity, they undergo numerous reactions when they are in solution, leading to the formation of new compounds which possess chemical structures, conformation in solution, and water solubility different from those of native tannins.

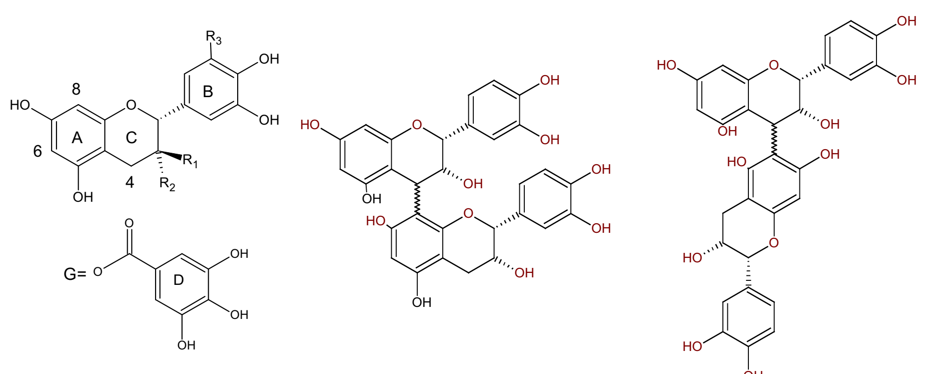
In this paper, the following properties of catechin, grape seed and apple tannins were studied: oil in water emulsion stabilization capacity and antioxidant activity in emulsified system. The effect of the tannin's structure (chemical composition and polymerization degree) and concentration, as well as of pH and ionic strength on the emulsion stability was also investigated.

2. Materials and methods

2.1. Materials

Methyl oleate (the oil phase) and Eumulgin ® HRE40, an ether of polyoxyethylene cetyl stearyl alcohol, were supplied by Cognis (Monheim, Germany; now BASF). Phosphate buffers (pH 3–9, ionic strengths 50–100 mM) were prepared in deionised water (MilliQ system, Millipore, USA) from reagents obtained from Sigma Aldrich (St. Louis, MO, USA). Poly(vinyl alcohol) (PVA) was purchased from Merck (Whitehouse Station, NJ, USA).

In this paper, tannins will be referred to as Gn or An (respectively for Grape and Apple), where n stands for the number average degree of polymerization, followed by the suffix "ox" when they were oxidized. The polyphenols used in this study were a commercial monomer (catechin, DP1) supplied by Sigma (St. Louis, MO), tannin fractions purified from Grape seed (*Vitis vinifera*, var. Shiraz) (G4ox and G15ox) obtained as referred in the literature (Poncet-Legrand et al., 2003), and Apple (*Malus sylvestris* var. Kermerrien) (A6 and A15) as in reference (Michodjehoun-Mestres et al., 2009). Their degree of polymerization was determined by depolymerization followed by HPLC analysis, as described by Preys et al. (2006) immediately after their purification. Depolymerization was also performed prior to emulsion preparation, and we observed a drop in depolymerization yield compared to the initial one with the grape seed fractions (but not the apple tannins) and this was attributed to oxidation. In this case, the characterization of fractions by standard methods becomes inaccurate (Mouls & Fulcrand, 2012; Poncet-Legrand et al., 2010; Vernhet et al., 2011): the effective degrees of polymerization of these oxidized tannins is larger. In a second set of experiments, emulsions were prepared with freshly purified apple tannins (A15b). Oxidized catechin (D1ox) was obtained by stocking a catechin (D1) solution (3 mg mL^{-1} in phosphate buffer pH 6.5, 50 mM) at 28 °C for 7 days. To obtain oxidized apple tannins (A15box), 33 mg of apple tannins (A15b) were solubilized in 11 mL of filtered (0.22 µm) phosphate buffer (pH 6.5, 50 mM), and left in contact with air during 15 days at 28 °C. The buffers were filtered in order to minimize the risks of bacterial growth. Oxidized tannins exhibit a different color from the non-oxidized ones: their color in solution is ranging from dark yellow to brown. Depending on the application (food or non-food application) these color changes may or may not be accepted from a consumer point of view.



(a) **(b)** **(c)**

Flavonol	R_1	R_2	R_3
Catechin	OH	H	H
Epicatechin	H	OH	H
Epicatechin gallate	H	G	H

Fig. 1. Chemical structure of the studied monomers (a) and condensed tannins linked by the C4–C8 (b) or C4–C6 (c) interflavanol bonds. Apple condensed tannins are made of epicatechin units linked by C4–C6 or C4–C8 interflavanol bonds, whereas grape seed tannins are made of catechin, epicatechin, and epicatechin gallate.