Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Colloidal gas aphrons based separation process for the purification and fractionation of natural phenolic extracts

G. Spigno[a,*], D. Amendola[a], F. Dahmoune[b], P. Jauregi[c]

[a] Institute of Oenology and Agro-Food Engineering, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy

[b] Laboratory of Biomathematics, Biochemistry, Biophysics and Scientometrics, Abderrahmane Mira University of Bejaia, Bejaia 06000, Algeria

[c] Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK

ABSTRACT

Following previous studies, the aim of this work is to further investigate the application of colloidal gas aphrons (CGA) to the recovery of polyphenols from a grape marc ethanolic extract with particular focus on exploring the use of a non-ionic food grade surfactant (Tween 20) as an alternative to the more toxic cationic surfactant CTAB. Different batch separation trials in a flotation column were carried out to evaluate the influence of surfactant type and concentration and processing parameters (such as pH, drainage time, CGA/extract volumetric and molar ratio) on the recovery of total and specific phenolic compounds. The possibility of achieving selective separation and concentration of different classes of phenolic compounds and non-phenolic compounds was also assessed, together with the influence of the process on the antioxidant capacity of the recovered compounds. The process led to good recovery, limited loss of antioxidant capacity, but low selectivity under the tested conditions. Results showed the possibility of using Tween 20 with a separation mechanism mainly driven by hydrophobic interactions. Volumetric ratio rather than the molar ratio was the key operating parameter in the recovery of polyphenols by CGA.

Keywords: Antioxidants; Colloidal gas aphrons; Grape marc; Phenolics; Surfactants

1. Introduction

Natural phenolic compounds have been investigated for their antioxidant and antimicrobial activity to quite some extent and this subject still raises a large interest due to the complex nature of the substances. Polyphenols are the biggest group of phytochemicals, with more than 8000 phenolic structures currently known, and among them over 4000 flavonoids identified (Löf et al., 2011). The polyphenols name includes different phenolic classes with even largelydifferent molecular

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; AOC, antioxidant capacity expressed as absorbance percent inhibition in the ABTS assay; CAE, caffeic acid equivalents; C_{APy}/C_{LPy}, concentration of compound y in the collapsed aphron phase/liquid phase at the end of separation trials; CGA, colloidal gas aphrons; CTAB, cetyltrimethylammonium-bromide; GAE_{TPI}/GAE_{FI}, gallic acid equivalents based on total phenol index/Folin Index; ME, malvidin-glucoside equivalents; $M_{y/feed}/M_{y/liq}$, total amount of compound y added in the feed/measured in the liquid phase in separation trials; QE, quercetin equivalents; RE_y, recovery of compound y in the aphron phase in separation trials; SF, separation factor; $V_{AP}/V_{LP}/V_{CGA}/V_{liquid\ drained}/V_{sample}$, volume of: aphron phase (measured after complete collapse)/liquid phase/CGA/liquid drained from complete collapse of CGA in the CGA characterisation/standard gallic acid solution or extract fed into the column in separation trials; ε/ε', gas hold-up from CGA characterisation/effective gas hold-up from separation trials.

* *Corresponding author*. Tel.: +39 0523 599181; fax: +39 0523 599232.
E-mail address: giorgia.spigno@unicatt.it (G. Spigno).

Available online 23 June 2014
http://dx.doi.org/10.1016/j.fbp.2014.06.002

structures, such as non-flavonoid phenolic acids, non-flavonoid stilbenes, anthocyanins, flavonols, flavanols, tannins, flavones and flavanones. The majority of studies available in literature deal with the nutritional, biochemical, or chemical structure aspects of natural phenolic compounds while a few studies address the physico-chemical and colloidal properties (Tsao, 2010) which are, however, of high importance in the understanding of the behaviour and functionality of the substances. One of the main factors preventing efficient and widespread applications of natural phenolic compounds is their limited solubility in aqueous or lipid media (depending on the phenolic class and molecular hydrophobicity), with things even more complicated when dealing with natural phenolic extracts which contain mixtures of different phenolic compounds (Amendola et al., 2010; Menese et al., 2013; Spigno et al., 2013). Production of standard phenolic compounds from natural sources, including agro-food by-products which represent a unique low-cost resource, requires also important purification and fractionation steps with quite expensive technologies (strategies including sequential extraction, liquid–liquid partitioning and other complex and expensive processes based on membrane or chromatographic technologies have been proposed). As it concerns the polluting character of natural phenolic compounds, besides the intrinsic antimicrobial properties, limited solubility can also prevent their efficient bioremediation. Limited stability due to easy thermal and oxidative degradation of natural phenolic compounds during storage and processing is another heavy limit to their application. Finally, binding of some natural phenolic compounds to macromolecules, primarily proteins/enzymes and polysaccharides, but also minerals (such as iron and copper), may have important anti nutritional effects or limit their bioavailability after ingestion, compromising their so commonly claimed health benefits, or compromising food quality (see precipitation of proteins/polyphenols complexes in wine and fruit juices).

Besides the intensive use of emulsifiers in the food industry (Hasenhuettl and Hartel, 2010) some works have been reported about the interactions between surfactants and phenolic compounds. For example, a few works have shown the potential of specific surfactants in micellar form to: solubilise or precipitate specific phenolic compounds (Löf et al., 2011); alter their partitioning in oil-in-water emulsions (Richards et al., 2002; SØrensen et al., 2008); enable phenolic compounds analytical determination exploiting different affinities (Wang et al., 2007); protect phenolic compounds from oxidation (Lin et al., 2007); solubilise aromatic compounds (Yoshida and Moroi, 2000; Wei et al., 2012); improve phenolic compounds efficiency in topical formulations (Scognamiglio et al., 2013; Yutani et al., 2012). Other works have investigated the use of surfactants for the recovery of natural phenolic compounds from wastewaters (Gortzi et al., 2008; Katsoyannos et al., 2012).

Surfactants and colloidal gas aphrons (CGA), which are surfactant-stabilized microbubbles (10–100 μm) generated by intense stirring of a surfactant solution at high speeds (>8000 rpm) (Jauregi and Dermiki, 2010), have been used for many separation processes such as protein and enzyme recovery (Fuda and Jauregi, 2006; Zidehsaraei et al., 2009; Cheng and Stuckey, 2012), carotenoids recovery (Dermiki et al., 2008; Alves et al., 2006); recovery of toxic wastes from soil, removal of dyes from wastewaters, stripping of dissolved gases and removal of dispersed oil droplets from water, bubble-entrained floc flotation. Depending on the type of surfactant used for the generation of CGA, e.g. cationic, anionic or non-ionic, the outer surface of the microbubble may be positively charged, negatively charged or neutral, to which oppositely charged or non-charged molecules will adsorb, resulting in their effective separation from the bulk liquid, and consequently the selectivity of adsorption can be controlled. CGA possess unique properties: (i) high interfacial area due to their small size; (ii) high stability compared to conventional foams; (iii) sufficient stability to allow them to be pumped from the generation point to the point of use without loss of their original structure; (iv) CGA can be easily separated from the bulk liquid without mechanical aid, as opposed to conventional liquid–liquid extraction methods and the aqueous two phase separations that need centrifugation for phase separation. If it is possible to use biodegradable and non-toxic surfactants, this could result in an environmentally friendly processes while the final product could also be safe for human consumption.

Previous research carried out by the authors (Spigno and Jauregi, 2005; Spigno et al., 2010;) demonstrated that the phenolic acid gallic acid in its anionic form (at neutral–basic pH conditions) can be recovered from aqueous solutions using CGA generated from the cationic surfactant cetyltrimethylammonium-bromide (CTAB). Gallic acid recovery was mainly affected by pH, ionic strength, surfactant/gallic acid molar ratio, mixing conditions and contact time. In further work it was demonstrated that total phenolic compounds could be recovered in high yield from a grape marc (the wine-making waste consisting in seeds and skins, which is typically removed after fermentation for red wines, and after pressing for white wines) ethanolic extract (Spigno et al., 2010). Here we take this work further onto investigating the application of CGA to the recovery of polyphenols from a grape marc ethanolic extract with particular focus on exploring the use of a non-ionic food grade surfactant Tween 20 as an alternative to a more toxic cationic surfactant. This could offer the additional advantage of extracting the polyphenols in a surfactant rich solution which could confer the product improved solubility properties. In particular this paper aims at getting a better insight into the following aspects of the application of CGA to the recovery of natural phenolic compounds from a crude ethanolic extract:

- Influence of surfactant type and concentration and processing parameters (such as pH, drainage time, CGA/extract volumetric and molar ratio) on the recovery of total and specific phenolic compounds.
- Selective separation and concentration of different classes of phenolic compounds and non-phenolic compounds.
- Influence of the process on the functional properties (antioxidant activity) of the recovered compounds.

2. Materials and methods

2.1. *Materials*

Gallic acid, caffeic acid, quercetin, CTAB (cetyltrimethylammonium bromide) and Tween 20 were supplied by Fluka (Milan, Italy); ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and malvidin-glucoside by Sigma (Milan, Italy); potassium persulphate by Carlo Erba (Milan, Italy); all other chemicals were of analytical grade.

Grape marc samples from two different red grape varieties (Barbera and off-skins fermented Pinot noir) were kindly provided by wineries in the Northern Italy. The samples were oven