Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Exploring different strategies of separation of antioxidant compounds from winery by-products via surfactant-assisted processes for process intensification and integration

D. Carullo[a], M. Bosi[b], M. Dermiki[c], A. Bassani[a], P. Jauregi[d,e], G. Spigno[a,*]

[a] Università Cattolica del Sacro Cuore, Department for Sustainable Food Process (DiSTAS), Via Emilia Parmense 84, 29122 Piacenza, Italy
[b] Humana Italia s.p.a., Viale Liguria 22, 20143 Milan, Italy
[c] School of Science, Institute of Technology Sligo, Sligo, Ireland
[d] Department of Food and Nutritional Sciences, School of Chemistry, Food and Pharmacy, University of Reading, Reading RG6 6AP, United Kingdom
[e] AZTI, Food Research, Basque Research and Technology Alliance (BRTA), Parque Tecnológico de Bizkaia, Astondo Bidea, Edificio 609, Derio, Bizkaia 48160, Spain

ARTICLE INFO

Article history:
Received 3 June 2021
Received in revised form 16 November 2021
Accepted 23 November 2021
Available online 29 November 2021

Keywords:
Antioxidants
Colloidal gas aphrons (CGA)
Food by-products valorization
Grape pomace
Polyphenols
Surfactants

ABSTRACT

The impact of Tween20 surfactant, applied in native form or as colloidal gas aphrons (CGAs), on the extractability/purification enhancement of antioxidant compounds from grape skins (GS), compared to conventional solid–liquid extraction (SLE), was evaluated.

Three strategies were developed (S1: partial ethanol replacement during SLE with aqueous Tween20 solutions; S2: CGAs application to GS-extract suspension from SLE; S3: Tween20 addition to extracts before spray-drying). The effect of surfactant/solvent concentration (S1), extract/CGAs volumetric ratio (S2), and surfactant addition (S3) on total phenolic compounds (TPC), total anthocyanin compounds (TAC), and antioxidant power of recovered phases, was investigated.

In S1, using surfactants could not significantly reduce ethanol percentage in solvent to achieve a given TPC yield, but similar TAC yields than those from SLE were obtained when partially replacing ethanol with Tween20. In S2, CGAs allowed an averaged 40% recovery of TPC, and TAC contained in crude extract, with only a slight detected additional TAC recovery from exhausted GS. Adding surfactants within spray-drying processes (S3) enhanced the water solubility of powdered extracts, despite reducing the powder/phenolics yield due to increased sample stickiness.

Results demonstrated the potential of employing food-grade surfactants at different stages of grape pomace valorization process for the recovery of antioxidant extracts.

1. Introduction

The re-utilization of food industry-derived by-products, being capable to retain large amounts of valuable compounds, represents an interesting approach to meet the bioeconomy challenges, due to their economically and environmentally sustainable valorization (Galanakis, 2012, 2015; Rocha et al., 2018).

An interesting case study is given by the industrial processing of grapes (Vitis Vinifera L.) for winemaking, whose world production, considering either red or white cultivars, was estimated to slightly exceed 63 million tonnes in 2018 (FAOSTAT, 2018). During harvesting and industrial processing phases, about 20 % by weight of the total raw material is

* Corresponding author.
E-mail address: giorgia.spigno@unicatt.it (G. Spigno).
https://doi.org/10.1016/j.fbp.2021.11.007

discarded as waste (Andrade et al., 2019). The majority of this biomass is constituted by highly perishable vine shoots, stems, grape pomace (skins, and seeds), and wine lees/sediments, which might lead to detrimental effects on the environment, together with dramatic economical losses, if unutilized and not properly disposed (Antoniolli et al., 2015; Bustamante et al., 2008; Negro et al., 2003; Prasad et al., 2015).

In the last decades, there has been an increasing attention towards the valorization of by-products from the industrial vinification process, among which grape pomace, being a cheap source of high-added value molecules such as phenolic compounds, of interest for food, feed, pharmaceutical, and cosmetic sectors, as well as for agricultural and oenological purposes (Nayak et al., 2018; Nunes et al., 2017; Sette et al., 2019; Sirohi et al., 2020). Several literature works highlighted the capability of phenolic compounds to exert many protective benefits on human health, since they act as antioxidant, antimutagen, anticarcinogen, and antimicrobial agents (Frontuto et al., 2019; Makris, 2018).

The exploitation of such antioxidant compounds may be achieved by their proper solubilization from vacuoles of food by-products cells where they are closely embedded, being surrounded by a rigid cell/wall membrane system. This operation generally occurs via a conventional solid/liquid extraction (SLE) step, involving an intimate contact between the solid matrix and organic solvent or solvent mixture with a high affinity for the target compounds. However, in order to intensify SLE process efficiency in terms of extraction yields, long maceration times, high temperatures, and large amounts of organic and polluting solvents are typically required, thus eventually causing the loss of functionality of target compounds, as well as leading to co-extraction of undesired molecules (Angiolillo et al., 2015; Barba et al., 2016).

Hence, these underlined drawbacks have triggered research to explore safer, environmental-friendly, and more sustainable methods for the extraction/purification of food by-products-derived phenolic compounds. For instance, it was demonstrated that pre-treating grape pomace with mild cell wall/membrane demolishing processes, such as those based on the use of enzymes or pulsed electric fields, could dramatically enhance the extraction yield of phenolic compounds with respect to a simple hydroalcoholic SLE (Binaschi et al., 2018; Brianceau et al., 2015).

More recently, the utilization of approaches exploiting the interaction of phenolic compounds with surface-active molecules (surfactants), applied either in their native state or in the colloidal gas aphrons (CGAs) form, has been proposed and extensively investigated for extraction/purification purposes (Dahmoune et al., 2013; Löf et al., 2011; MohdMaidin et al., 2018, 2019; Spigno and Jauregi, 2005; Spigno et al., 2010, 2015).

Specifically, CGAs are stable microbubbles (10–100 μm) created by intense stirring (5000–10000 rpm) of a surfactant solution above its critical micellar concentration (CMC) (Jarudilokkul et al., 2004; Prasad et al., 2015), composed of a gaseous inner core (≈ 65% by volume) surrounded by a double layer of surfactant molecules with thin surfactant film (Corpuz et al., 2019; Yan et al., 2020).

The characteristic structure of CGAs confers them several important features, namely high foam stability, easy phase separation, and capability to absorb particles/molecules to the encapsulating shell, which could be properly tuned by varying the type of surfactant implied for their production (Fuda and Jauregi, 2006; Jauregi and Varley, 1998; Jauregi and Dermiki, 2010; Spigno et al., 2015). Within this frame, different scientists have previously reported the successful application of CGAs for the efficient recovery of proteins from whey (Amiri and Valsaraj, 2004; Jarudilokkul et al., 2004), cellulose from paper mill wastewaters (Hashim and Sen Gupta, 1997), oil from water (Corpuz et al., 2019), and antioxidants from artichoke bracts/stems (Noriega et al., 2018).

In our recent studies (Dahmoune et al., 2013; MohdMaidin et al., 2018; Spigno et al., 2015), we demonstrated that high extraction yields of polyphenols, and in particular anthocyanins, could be obtained when applying CGAs, generated either by the cationic surfactant CTAB or the non-ionic surfactant Tween20, to hydroalcoholic extracts of grape pomace. More in detail, the highest values of recovery and separation ratio of the investigated classes of compounds were detected at conditions maximizing both the electrostatic and hydrophobic interactions (use of CTAB at pH > 2). However, differently from what was reported in the case of CTAB-generated CGAs, no substantial losses of antioxidant capacity were observed in recovered fractions when Tween20 was utilized (Spigno et al., 2015). These results suggested the possibility to preserve intrinsic properties of extracted compounds, thus enabling CGAs utilization in replacement to conventional separation techniques (e.g., SLE).

To our knowledge, no work in the current literature explored the integration of surfactants at different stages of the grape pomace valorization process (e.g. SLE, separation/purification) to develop a low-impact method for polyphenols exploitation. Therefore, this work aimed to assess the potential of surfactants, applied either in their native form or as CGAs, to intensify the extraction/selective recovery of polyphenols from grape pomace, as well as to increase the stability of powders obtained after spray-drying of crude extracts. Specifically, the following strategies were investigated:

- S1: integration of native surfactants within SLE step;
- S2: integration of surfactants in the form of CGAs after SLE step;
- S3: integration of native surfactants within the spray-drying process of extracts,

with the products obtained characterized in terms of total or specific (e.g., anthocyanins) phenolic compounds recovery, and antioxidant capacity.

2. Materials and methods

2.1. Raw materials and chemicals

Wine processing by-products, mainly composed of skins, were gently provided by a winery located in Northern Italy. For this work, grape pomace was obtained upon industrial pressing of grapes belonging to the “Barbera” variety, which were field-grown in the Piedmont region in 2015. In particular, pomace was collected into plastic containers (20 kg) and transported within 1 day to the research laboratories. Upon its arrival, the biomass was dried at 55 ± 2 °C until reaching a residual moisture content of <7% dry weight (DW) and subsequently skins were manually separated from seeds. Afterward, skins were milled up to a final particle size of less than 2 mm. Dried milled grape skins (DMGS), used for carrying out all the experiments, were packed in airtight bags and kept in a dark place until their use.

Gallic acid, sodium carbonate, and Tween20 were purchased from Fluka (Milan, Italy); potassium chloride, sodium acetate, and hydrochloric acid were obtained from Carlo Erba (Milan, Italy); ethanol and Folin-Ciocalteau reagent were supplied by VWR Chemicals (Milan, Italy); ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) was purchased from Sigma-Aldrich (Milan, Italy); maltodextrins (Glucidex 120 IT 12 DE (dextrose equivalent)) were obtained from Roquette Italia s.p.a. (Alessandria, Italy), while potassium persulfate was provided by Merck (Darmstadt, Germany).

2.2. Conventional solid–liquid extraction (SLE) step

Conventional SLE step was performed adopting the method previously optimized by Amendola et al. (2010), using aqueous ethanol as extraction solvent (60%, v/v). Accordingly, many studies have highlighted the greater efficiency of an ethanol/water mixture in extracting phenolic constituents from winery by-products with respect to the corresponding mono-component solvent systems (Alonso et al., 1991; Pinelo et al., 2005; Yilmaz and Toledo, 2006).

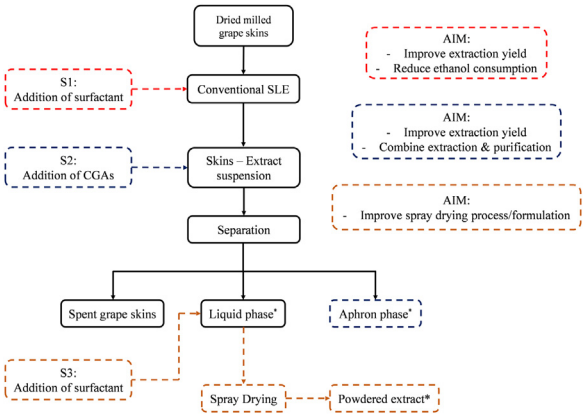

Fig. 1 – Flow diagram of the followed experimental plan, with common steps for investigated strategies (S1, S2, and S3) being enclosed within black-edged boxes. Legend: * (product subjected to analysis).

Specifically, 125 g of DMGS were mixed with 1 L of solvent, thus applying a solid-to-liquid ratio (SLR) of 1:8 (g/mL), and the mixture was kept under stirring at 3500 rpm (mixer Silverson L5M) for 1 h at 60 °C (temperature maintained by means of an electric heating plate). At the end of the SLE process, the solid/liquid separation was achieved by centrifugation at 5000 rpm for 10 min (Centrifuge ALC 4237R), with the supernatant (extract) collected in glass flasks and stored under refrigerated conditions (T = 4 °C) for further analysis (by 24 h).

2.3. *Intensification of SLE process*

In order to gain insight into the effect of the implementation of surfactants, used either in their native form or as CGAs, to recover antioxidant compounds (e.g. phenolic compounds, anthocyanins) from grape skins and their ethanolic extracts, as well as to assist the spray-drying step of the latter products, three different strategies were proposed and alternatively applied (S1, S2, and S3), with their schematization briefly illustrated in the flow diagram of Fig. 1.

2.3.1. *S1: surfactant-assisted SLE step*

S1 consisted of the conventional SLE with the partial or complete replacement of the reference extraction solvent (60% ethanol, v/v) with aqueous or hydroalcoholic solutions of the Tween20 surfactant (10–20 mM, 30–60% ethanol v/v). Extraction and further solid–liquid separation steps were carried out according to the procedure illustrated in the previous paragraph (2.2) and Fig. 1.

2.3.2. *S2: CGAs-assisted separation step*

S2 involved the direct application of the CGAs into the ethanol suspension (DMGS + extract) immediately after the conventional SLE. The ethanol suspension is composed of a liquid part, where the phenols are dissolved, and a solid part that still contains phenolic compounds. Thus, this operation was carried out to possibly improve the extractability of phenolic compounds from partially spent DMGS by exploiting their affinity with CGAs, as well as to provide an integrated process for a single stage of extraction/purification.

Firstly, CGAs were generated from a 1 L Tween20 solution (20 mM) by intense stirring at 8000 rpm with the Silverson mixer for 5 min. Afterward, CGAs were pumped to a flotation glass column (Dahmoune et al., 2013; Spigno et al., 2015) employing a peristaltic pump (Watson Marlow 505 U) from the bottom, while the DMGS/extract suspension (feed), obtained employing the protocol illustrated in Section 2.2, was manually transferred into the column from the top. Experimental trials were performed at different sample/CGAs volumetric ratios (1/9, 1/12, and 1/24 $V_{Extract}/V_{CGAs}$). Once the column was filled, the mixture was left standing for 5 min before pumping out the separated bottom liquid phase (LP) and, after complete collapse, also the upper aphron phase (AP). The latter was then subjected to filtration in order to separate the exhausted skins from the clear extract. The volumes of the separated liquid phase and collapsed aphron phase were measured and used to calculate the amount (in mg) of target compounds recovered in both streams. The percentage recovery of target compounds in the aphron phase was calculated according to (Eq. 1):

$$AP\ recovery\ (\%) = \frac{mg_{i,\,AP}}{mg_{i,\,Feed}} \quad (1)$$

where i represents the investigated species to be recovered (phenolic compounds, anthocyanin compounds).

2.3.3. *S3: surfactant-assisted spray-drying process*

This step aimed to assess the capacity of Tween20 surfactant to partially replace maltodextrins as encapsulation material during a spray-drying process of DMGS hydroalcoholic extracts.

Specifically, the extracts collected from the application of conventional SLE step were concentrated three times under vacuum by using an R-114 Rotavapor (BÜCHI Labortechnik AG, Flawil, Switzerland) set at 30 °C, and then diluted to their initial volume via water or aqueous solutions of Tween20 surfactant of constant concentration (20 mM). Since a partial precipitation phenomenon occurred after the dilution with Tween20 solution, the extract was left in darkness for one night and then transferred to another flask to remove the precipitate.

Experimental trials for the encapsulation of extracts, added or not with Tween20, were executed employing a laboratory scale spray-dryer (Büchi Mini Spray Dryer B-290, Switzerland), equipped with an atomization cylinder (0.48 m in height, 0.16 m in diameter). The liquid feed, composed of extract and maltodextrins previously mixed at variable DE/GAE (dextrose equivalent/gallic acid equivalent) molar ratios (0.64, 1.28, 2.44, and 3.85 mol_{DE}/mol_{GAE}), was sent to the drying system via a peristaltic pump working at a constant flow rate of 4 mL/min. Compressed air at 38.5 m3/h was used to co-currently disperse the liquid in fine droplets through a 0.7 mm nozzle to be subsequently dried in the atomization cylinder at constant inlet temperature (T = 150 °C). For each test, the aspiration rate was set at 100%, with the obtained powder being collected into a cyclone separator and stored for further analyses. The total wet weight sample recovery, and the total phenols and anthocyanins recovery in the powder was calculated by comparing their content in the spray-dried product with that of the initial extract (Eqs. 2 – 5).

$$Wet\ powder\ recovery\ (Re_{WP}) = \frac{m_{WP}}{m_{extract}} \quad (2)$$

$$TPI_{280}\ recovery\ (RE_{TPI280}) = \frac{m_{WP,\,TPI_{280}}}{m_{extract,\,TPI_{280}}} \quad (3)$$

$$TPC_{Folin}\ recovery\ (RE_{TPCFolin}) = \frac{m_{WP,\,TPC_{Folin}}}{m_{extract,\,TPC_{Folin}}} \quad (4)$$

$$TAC\ recovery\ (RE_{TAC}) = \frac{m_{WP,\,TAC}}{m_{extract,\,TAC}} \quad (5)$$

where m_{WP} is the mass of recovered wet powder after spray-drying, $m_{extract}$ is the amount of solids present in the liquid extract, $m_{WP,TPI280}$ represents the amount of phenolic compounds detected in the collected powder by TPI method, $m_{extract,TPI280}$ is the mass of phenolic compounds present in the liquid extract by TPI method, $m_{WP,TPCFolin}$ is the amount of phenolic compounds detected in the collected powder by Folin-Ciocalteau method, $m_{extract,TPCFolin}$ is the mass of phenolic compounds present in the liquid extract by Folin-Ciocalteau method, $m_{WP,TAC}$ is the amount of anthocyanin compounds detected in the collected powder, and $m_{extract,TAC}$ stands for the mass of anthocyanin compounds present in the liquid extract.

2.4. Analyses

2.4.1. Flow measurement tests

Steady-state flow tests of surfactant solutions utilized throughout the application of S1, with or without the addition of ethanol (10–20 mM, 30–60% ethanol v/v), were carried out using a controlled stress and strain rheometer (MCR 302, Anton Paar, Gratz, Austria), fitted with a cup and bob geometry. The system was thermally regulated by a Peltier plate and a circulating water bath (FP 50, Julabo, Milan, Italy). For the analysis, 20 mL of sample were transferred into the cup and kept at a fixed temperature (25 °C) for 2 min to allow stress relaxation and thermal equilibration. The apparent viscosity (η, mPa s) of the samples was determined in the range of shear rates (γ, s^{-1}) between 50 and 200 s^{-1}. A new sample was used for each determination.

2.4.2. Characterization of CGAs

CGAs obtained from solutions of Tween20 surfactant at 1 mM, 10 mM, and 20 mM (200 mL), were characterized for stability in terms of gas hold up (ε) and half-life ($t^{1/2}$) in the generation vessel (Jauregi and Dermiki, 2010). Higher surfactant concentrations were not tested since, as similarly reported by Dermiki et al. (2010), no additional effects on CGAs structure were observed. For the experiments, freshly produced CGAs were transferred into a 500 mL volumetric cylinder and the volume of drained liquid was registered at regular time intervals within the range 0–25 min, until complete foam collapse. ε was calculated according to (Eq. 6):

$$\varepsilon = \frac{V_{CGAs,\,t=0} - V_{DL,\,collapse}}{V_{CGAs,\,t=0}} \quad (6)$$

where $V_{CGAs,t=0}$ is the initial volume of produced CGAs, and $V_{DL,collapse}$ is the volume of drained liquid after complete foam collapse (200 mL). Instead, the $t^{1/2}$ was calculated as the time required for the first 100 mL to drain, corresponding to half of the initial aqueous Tween20 solution volume.

2.4.3. Total phenolic compounds (TPC)

All the extracts and powders obtained from the application of S1, S2, and S3, were subjected to TPC measurements adopting two different methods, as previously reported by Amendola et al. (2010):

- Total phenolic index (TPI_{280}): when required, samples were diluted with distilled water, and their absorbance was measured at 280 nm using a UV-1601 spectrophotometer (Shimazu, Milan, Italy). Gallic acid, previously dissolved in aqueous ethanol or Tween20/aqueous ethanol mixture, was used to generate a five-point standard calibration curve and the results were expressed as mg of gallic acid equivalent (GAE) per L of sample (mg_{GAE}/L) or per g of dry weight grape skins (mg_{GAE}/g_{DW}).
- Folin-Ciocalteau method (TPC_{Folin}): for the analysis, 25 mL of water, 0.5 mL of extract or diluted powder sample, 2.5 mL of undiluted Folin-Ciocalteau reagent, and 5 mL of sodium carbonate (20%, v/v in water) were mixed, brought to 50 mL, and allowed to stand for 30 min at 40 °C. A mixture of water and reagents was used as a blank. The absorbance of the reacting mixture was then spectrophotometrically measured at 750 nm. Gallic acid was used as the standard for the calibration curve and the TPC_{Folin} values were expressed as for those of TPI_{280}.

2.4.4. Total anthocyanins content (TAC)

Total anthocyanins of liquid extract samples were evaluated by diluting them in acid-ethanol (ethanol:water:HCl, 70:30:1, v:v:v) and reading their absorbance at 538 nm against the same acid-ethanol. In this case, a calibration curve was not performed but the value was multiplied by a dilution factor and by 26.6, being a literature conversion coefficient used for a mixture of the five main grape anthocyanins (Di Stefano and Cravero, 2001). TAC values were expressed as mg of wine anthocyanins equivalents (WAE) per L of sample (mg_{WAE}/L) or per g of dry weight grape skins (mg_{WAE}/g_{DW}).

Instead, as far as powdered extracts are concerned, the pH differential method optimized by Lee et al. (2005) was applied, since preliminary studies revealed the interference of maltodextrins upon the usage of acid-ethanol mixtures (data not shown). Specifically, samples were diluted by two different buffer solutions (sodium chloride pH 1.0, and sodium acetate pH 4.5) and their absorbances were spectrophotometrically measured at specific wavelengths (520 nm, and 700 nm) against water (blank sample). The concentration of anthocyanins was expressed as mg of cyanidin-3-glucoside equivalents (C3GE) per L of diluted powder (mg_{C3GE}/L) or per g of dry powdered extract (mg_{C3GE}/g_{DW}).

2.4.5. Antioxidant capacity

The antioxidant power of all investigated samples was assessed by the ABTS assay (Re et al., 1999). In brief, a radical solution was prepared with 7 mM ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and 2.45 mM potassium persulfate and subsequently kept in the dark at room temperature for 16 h before its use. Such a solution was then diluted with absolute ethanol up to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. For the analysis, 2 mL of the diluted radical solution were mixed with 20 μL of the sample, opportunely diluted with water, and the mixture absorbance was read after 6 min at 734 nm against ethanol 50%. A blank sample (2 mL of diluted ABTS mixed with 20 μL of water) and a control sample (2 mL of diluted ABTS) were also included and utilized to calculate the antioxidant capacity in terms of percentage inhibition (AOC%), as previously reported by Amendola et al. (2010).

2.4.6. Water solubility index (WSI)

The WSI of the powders obtained from spray-drying was determined using the method described by Kha et al. (2010). In brief, 2.5 g of powder was added to 30 mL of distilled water in a 50 mL centrifuge tube. The obtained mixture was then vig-

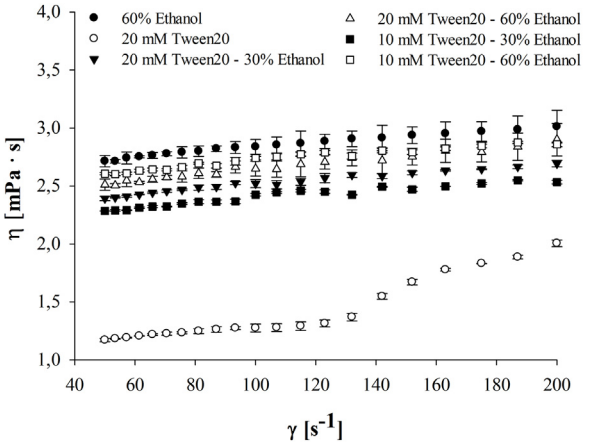

Fig. 2 – Curves of apparent viscosity (η, mPa s) against the shear rate (γ, s^{-1}), for the hydroalcoholic Tween20 solutions. Standard deviations were used as error bars ($p \leq 0.01$).

orously shaken on a vortex, and then incubated at 37 °C in a water bath for 30 min. Afterward, the suspension was centrifuged for 20 min at 10,000 rpm, with the supernatant being collected in a pre-weighed crucible and dried in an oven at 105 °C until achieving constant weight. The WSI (%) was calculated as the percentage of solubilized powder (the residual solids of supernatant dried at 105 °C) with respect to the initial powder mass.

2.5. *Statistical analysis*

All experiments and analyses were executed in triplicate, with all the obtained results reported as mean ± standard deviation (SD). ANOVA test was carried out in order to evaluate the influence of specific process variables on measured parameters. In case of significant influence, assessed at a 99 % confidence level, variance homogeneity was checked and Tukey's post-hoc test was applied for means discrimination ($p \leq 0.01$). The statistical analysis was performed using the IBM SPSS Statistics 19 software (SPSS Inc, Chicago, USA).

3. Results and discussion

3.1. *S1: surfactant-assisted SLE step*

The results of apparent viscosity measurements, carried out on aqueous/ethanolic solutions of Tween20 surfactant, are depicted in Fig. 2.

The hydroalcoholic solution (60%, v/v) was characterized by the highest values of η (2.84 mPa s, on average), exhibiting a pure Newtonian fluid behavior due to the absence of shear effects on flow resistance, in line with the findings of Kadlec et al. (2010). Instead, the aqueous solution of Tween20 (20 mM), having significantly ($p \leq 0.01$) lower η values than those observed for 60% ethanol, showed a typical shear-thickening behavior, with an increasing trend as a function of the shear rate. Interestingly, regardless of either the surfactant or ethanol concentration in the extraction system, all the flow curves associated with hydroalcoholic Tween20 solutions, reporting a similar behavior to that detected for 60% (v/v) ethanol, never exceeded that of the reference solvent.

The results shown so far seem to suggest the possibility to use surfactant-based hydroalcoholic solutions, in place of only hydroalcoholic solutions (60% ethanol, v/v), to potentially enhance the extractability of target intracellular compounds from DMGS (S1). In particular, the lower the solvent viscosity, the higher its diffusivity coefficient which enables a better penetration within the pores of the given plant materials and, hence, greater recovery of their bioactive constituents (Wijekoon et al., 2011). Within this frame, Table 1 reports the extraction yields of total polyphenols and anthocyanins obtained after either a conventional or surfactant-assisted SLE step.

The hydroalcoholic solution allowed to efficiently recover antioxidant intracellulars from DMGS, showing significantly ($p \leq 0.01$) higher TPC extraction yields than those observed in the presence of Tween20, with or without ethanol. Moreover, no statistical differences ($p > 0.01$) in terms of phenolic yields could be detected when increasing the surfactant concentration in the extraction medium. This suggests a lower capability of Tween20 to efficiently interact with the solid matrix, in comparison with the ethanol solution implied for SLE. However, it should be also noted that similar TAC values than those observed when employing 60% ethanol as extraction medium were detected ($p > 0.01$) when partially replacing the organic solvent with surfactant (10–20 mM, 30% ethanol v/v), thus indicating a particular affinity of Tween20 towards anthocyanin compounds, in accordance with the results reported in our previous study (Spigno et al., 2015). Such a statement is also reinforced by the lower TPC/TAC ratios detected in the presence of surfactant, rather than for conventional SLE.

Additional information on the quality of achieved extracts, related to the occurrence of polyphenols oxidation phenomena, is given by the ratio between the Folin index (TPC_{Folin}) and total polyphenols index (TPI_{280}). For instance, a higher ratio indicates a reduced oxidation extent of the recovered compounds (the Folin index is more influenced by the oxidative status of the molecules than the total polyphenol index), as well as a different composition of the extract (Spigno et al., 2015). In this context, the results of Table 1 reflected the combined potential of Tween20 and ethanol in protecting the recovered compounds from oxidation reactions, with respect

Table 1 – Extraction yields of total phenolic compounds (TPC) and total anthocyanin compounds (TAC) in extracts from dried Barbera skins, as a function of the concentration of Tween20 (mM) and ethanol (% v/v) in the extraction system. The results are expressed as mean ± SD. Values with different superscript letters within the same column are significantly different ($p \leq 0.01$).

Tween20 [mM]	Ethanol [% v/v]	TPI_{280} [mg_{GAE}/g_{DW}]	TPC_{Folin} [mg_{GAE}/g_{DW}]	TAC [mg_{WAE}/g_{DW}]	TPC_{Folin}/TAC	TPC_{Folin}/TPI_{280}
0	60	24.6 ± 1.2^{d}	24.3 ± 2.8^{c}	6.4 ± 0.9^{b}	3.79	0.99
20	0	4.3 ± 0.5^{a}	4.1 ± 0.3^{a}	2.6 ± 0.1^{a}	1.56	0.94
20	30	8.9 ± 0.1^{b}	11.8 ± 0.8^{b}	5.8 ± 0.4^{b}	2.04	1.33
	60	12.7 ± 0.1^{c}	20.5 ± 0.2^{c}	7.0 ± 0.0^{b}	2.94	1.62
10	30	8.9 ± 0.7^{b}	13.6 ± 1.2^{b}	5.5 ± 1.0^{b}	2.12	1.53
	60	13.1 ± 0.1^{c}	20.1 ± 0.6^{c}	7.2 ± 0.1^{b}	2.80	1.53

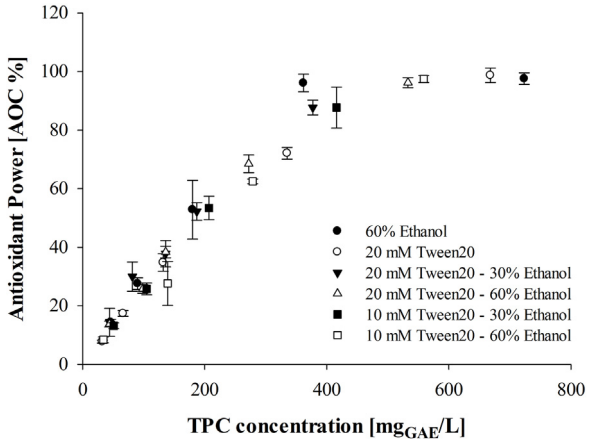

Fig. 3 – Antioxidant capacity (AOC% based on ABTS test) of the extracts obtained from dried Barbera skins, as a function of the TPC, for different concentrations of Tween20 (mM) and ethanol (% v/v) in the extraction system. Standard deviations were used as error bars ($p \leq 0.01$).

to the application of single-component systems for extraction purposes.

Remarkable outcomes were achieved in the research study of Papaioannou and Karabelas (2012), who investigated the influence of an enzymatic pre-treatment, applied alone or in presence of different non-ionic surfactant agents, on the extractability of lycopene from tomato peels. Specifically, the authors found that the utilization of Span20 surfactant dramatically enhanced lycopene recovery, whose extent reached 4-fold and 10-fold increases as compared to only enzymatically pre-treated and untreated samples, respectively. Therefore, following a similar "green" approach, in our work the direct application of Tween20 solutions during the organic SLE step of DMGS could represent a low-cost method for the intensification of bioactive compounds recovery. For instance, according to the results reported in Table 1, it might be hypothesized to perform a first extraction stage with Tween20 10 mM/ethanol 30% to recover the majority of anthocyanins together with half of the other phenolic compounds, followed by a minor SLE step with ethanol 60% for the solubilization of the remaining polyphenols fraction.

The analysis of the antioxidant capacity of all the obtained extracts via ABTS assay (Fig. 3) furtherly confirmed the mildness of surfactant-assisted extraction protocol, since no significant reduction in the antiradical activity of the phenolic compounds could be detected when Tween20 was integrated into the extraction solvent. This eventually endorses the possibility of using Tween20 to reduce ethanol consumption during the SLE step without affecting the functionality of recovered compounds. However, deeper studies on surfactant-assisted extraction are strictly necessary in order to better elucidate the interaction occurring between surfactant and solvent during SLE, aiming at maximizing the extraction yields of specific classes of phenolic compounds by possibly reducing either the solvent volume or the extraction time.

3.2. *S2: CGAs-assisted separation step*

In this work, S2 was implemented for potentially achieving a double aim, that is to integrate the extract-solids separation step with a purification/fractionation step, and to furtherly increase the recovery of phenolic compounds from exhausted DMGS.

Table 2 – Evaluation of CGAs stability, in terms of gas hold up (ε) and half-life ($t^{1/2}$), as a function of Tween20 concentration in the starting aqueous solutions. Results are expressed as mean ± SD. Values with different superscript letters within the same column are significantly different ($p \leq 0.01$).

Tween20 [mM]	ε [-]	$t^{1/2}$ [s]
1	0.49 ± 0.00^{a}	173 ± 1^{a}
10	0.65 ± 0.01^{b}	523 ± 1^{b}
20	0.71 ± 0.01^{c}	701 ± 4^{c}

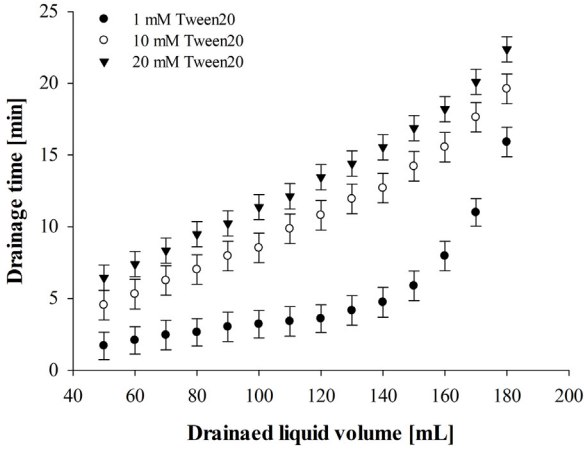

Fig. 4 – Evolution of the drainage time (min) against the drained liquid volume (mL) for the CGAs generated from aqueous Tween20 solutions. Standard deviations were used as error bars ($p \leq 0.01$).

The stability of CGAs, generated by aqueous solutions of surfactant (1–20 mM Tween20) was assessed by measuring the gas hold-up (ε) and half-life ($t^{1/2}$, in s), with the results schematized in Table 2.

The increase in surfactant concentration in aqueous solutions contributed to improving the stability of achieved structures, as testified by the significant ($p \leq 0.01$) increase in both observed parameters along the investigated domain. As expected, the lowest stability was observed for a 1 mM aqueous solution of Tween20, being the considered concentration very close to the reported value of CMC in water (0.08 mM) by Kim and Hsieh (2001).

The greater stability of CGAs generated from a 20 mM Tween20 solution is also corroborated by the results depicted in Fig. 4, showing the dependence of drainage time from the produced liquid volume during sampling. In particular, the system containing the highest amount of surfactant agent, showing the greatest $t^{1/2}$ value (Table 2), underwent a complete collapse approximately 3.5 min later than the foam obtained from the solution at the intermediate concentration of Tween20 (10 mM).

In light of these results, a 20 mM concentration of Tween20 was selected and utilized to perform the experiments on CGAs-assisted purification of bioactive molecules from DMGS extracts.

Table 3 schematizes the yields/amounts of total phenolic compounds and anthocyanins calculated on the basis of the collected volume of the separated aphron phase, for different extract/CGAs volumetric ratios. As it clearly emerges from the results, maximum values of TPC and TAC extraction yields were obtained at 1:24 $V_{Extract}/V_{CGAs}$, being significantly higher ($p \leq 0.01$) than those observed within the range 1:9 –

Table 3 – Extraction yields and quantity of collected mass in the Aphron phase (AP) of total phenolic compounds (TPC) and total anthocyanin compounds (TAC), as a function of the ethanol suspension/CGAs volumetric ratio. The results are expressed as mean ± SD. Values with different superscript letters within the same column are significantly different ($p \leq 0.01$).

$V_{Extract}/V_{CGAs}$ [–]	TPI_{280}		TPC_{Folin}		TAC	
	Yield [mg_{GAE}/g_{DW}]	mg_{GAE} (AP)	Yield [mg_{GAE}/g_{DW}]	mg_{GAE} (AP)	Yield [mg_{WAE}/g_{DW}]	mg_{WAE} (AP)
1/9	3.6 ± 0.1^a	33.8 ± 1.1^b	6.0 ± 0.2^a	56.2 ± 2.2^b	1.5 ± 0.0^a	13.9 ± 0.1^b
1/12	6.4 ± 0.5^b	44.4 ± 3.2^c	10.1 ± 0.9^b	70.2 ± 6.0^c	1.9 ± 0.1^b	16.5 ± 0.1^c
1/24	7.4 ± 0.3^c	25.8 ± 1.0^a	11.0 ± 0.9^b	38.4 ± 3.1^a	2.6 ± 0.1^c	9.1 ± 0.5^a

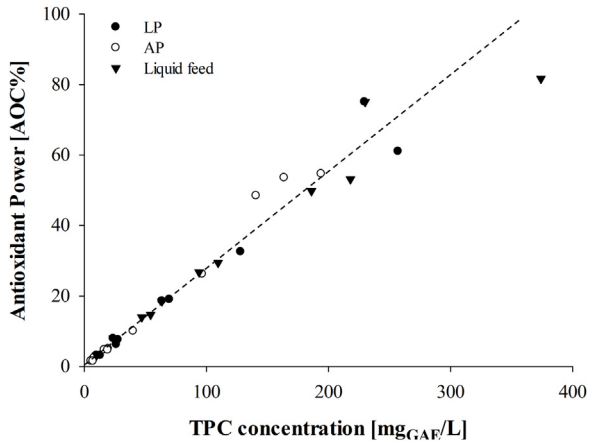

Fig. 5 – Antioxidant capacity (AOC% based on ABTS test) as a function of the TPC for the liquid feed, aphron phase (AP), and liquid phase (LP) collected upon the application of S2.

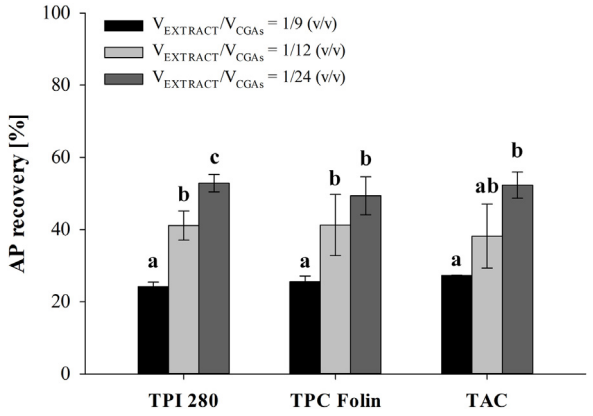

Fig. 6 – Recovery (%) of total phenolic compounds (TPC) and total anthocyanin compounds (TAC) in the aphron phase, calculated according to Eq. (1), as a function of the ethanol suspension/CGAs volumetric ratio. For each investigated class of compounds, different letters above the bars indicate significant differences among the mean values ($p \leq 0.01$).

1:12 $V_{Extract}/V_{CGAs}$. Conversely, if we consider the total amount of antioxidant compounds retained in the aphron phase for the different trials, the highest values ($p \leq 0.01$) were achieved in correspondence of the intermediate volumetric ratio, likely due to a maximization of hydrophobic forces participating in the separation of extracted compounds from the liquid feed. Additionally, no significant losses in antioxidant activity of compounds recovered in the aphron phase were detected over those of the crude extract or liquid phase (Fig. 5).

However, in order to enable the comparison of our data with those reported in previous research works, the percentage recovery of interest compounds in the aphron phase was calculated according to Eq. (1), with the results reported in the histograms of Fig. 6.

It should be noted that the percentage recovery of TPC and TAC (even at 1:24 $V_{Extract}/V_{CGAs}$) were significantly lower than those achieved by Spigno et al. (2015) when applying CGAs generated from a 10 mM Tween20 solution at similar volumetric ratios (1:22 $V_{Extract}/V_{CGAs}$) to a DMGS liquid extract (74 % and 80% for TPC and TAC, respectively). This discrepancy could be due to the higher surfactant concentration used in the current work (20 mM), previously selected for imparting the greater foam stability (Table 2), which might have prevented a good interaction among micelles and phenolic compounds. As similarly reported by Das et al. (2008), it could be likely that the presence of more surfactant agent (higher molarity) at the CGAs/extract interface has caused an increase in mass transfer resistance with a reduced intake of phenolic compounds to the internal CGAs core. Another possible explanation for the observed trend could arise from the presence of solid particles contained in the ethanolic DMGS extracts, which might have consistently decreased the contact surface between interest phases. However, this aspect is worth investigating in future works, due to the lack of comparative studies reported in the current literature on the CGAs-assisted purification of solid–liquid extracts.

Nevertheless, the trends of TPC and TAC observed in Fig. 6 are in good agreement with the findings of Noriega et al. (2018), who detected a gradual enrichment of the aphron phase with phenolics extracted from artichoke wastes when increasing the volumetric ratio, independently of the surfactant agent utilized for CGAs generation (Tween20, CTAB).

As per the literature survey, several previous authors have assessed the efficiency of various methods for the purification/fractionation of phenolic compounds, in particular anthocyanins, obtained from different food by-products. For instance, Negro et al. (2003) performed a purification step of "Negro Amaro" grape skins, preliminarily homogenised in 80% ethanol (v/v), by solid-phase extraction using a C18 column, which yielded a 35% recovery of the total anthocyanins contained in the processed biomass. Better results were reported in the recent work of Pazir et al. (2021) on the effect of either temperature, pressure, or time applied during a supercritical CO_2 (SC-CO_2) extraction from grape pomace on the achieved anthocyanins yield. Specifically, the above-mentioned authors showed that increasing the contact time between SC-CO_2 and the investigated biomass positively affected the total monomeric anthocyanins content (TMAC) of collected extracts, which rose up to a saturation extraction efficiency value of 63% after 180 min of processing. Instead, in the work of Jampani et al. (2014) the adsorption and desorption capacity of seven different resins towards water-extracted anthocyanins from Jamun fruit seeds was tested. The authors detected the highest anthocyanins desorption ratio (87%) using the Amberlite XAD7HP resin, despite the elution was assisted by organic solvents (e.g., ethanol). Although

Table 4 – Values of TPC and TAC recovered in both the aphron (AP) and liquid (LP) phases, in comparison with those contained in the ethanol suspension feeding the flotation column (Feed), as a function of the ethanol suspension/CGAs volumetric ratio. The results are expressed as mean ± SD. When reported, the symbol * indicates a significant difference ($p \leq 0.01$) between the class of compounds (TPC, TAC) in the considered samples (AP + LP, and Feed), at a fixed volumetric ratio.

$V_{Extract}/V_{CGAs}$ [–]	TPI_{280}		TPC_{Folin}		TAC	
	AP + LP [mg_{GAE}]	Feed [mg_{GAE}]	AP + LP [mg_{GAE}]	Feed [mg_{GAE}]	AP + LP [mg_{GAE}]	Feed [mg_{GAE}]
1/9	137.9 ± 1.1	138.3 ± 2.5	224.8 ± 3.1	222.6 ± 9.6	55.9 ± 0.6*	51.9 ± 1.3*
1/12	102.7 ± 7.2	108.6 ± 12.6	161.1 ± 8.2	155.1 ± 1.4	38.6 ± 0.6	40.5 ± 4.1
1/24	49.8 ± 1.6	48.9 ± 0.8	71.1 ± 6.4	78.1 ± 1.1	18.5 ± 1.3	17.3 ± 0.2

any comparison with data from the aforementioned literature works is very difficult due to the different separation methods and raw materials utilized, in our investigation we found that the application of CGAs induced approximately a 50 % recovery of the total anthocyanins dissolved in the crude DMGS extract without involving the additional usage of organic solvents, thus standing as a potential low-cost alternative purification method.

The results of Table 4 showed the poor capability of CGAs to additionally extract bioactive compounds from exhausted DMGS. Peculiarly, regardless of the considered extract/CGAs volumetric ratio, statistically similar ($p > 0.01$) values were detected when comparing TPC content of the output processing streams (AP + LP) with that initially contained in the liquid feed. However, only at 1:9 $V_{Extract}/V_{CGAs}$ of volumetric ratio, a slight but significant ($p \leq 0.01$) increase in the TAC recovery over the crude extract was observed, thus indicating a further extraction from the solid matrix. Therefore, based on this result, it could be speculated that the utilization of lower volumetric ratios than the minimum one adopted in this work (1:9 $V_{Extract}/V_{CGAs}$) leads to even higher extraction yields of valuable compounds from grape skins.

Evaluation of the scale-up feasibility and techno-economic analysis of the proposed strategy were not carried out during this work but previous research (Dermiki et al., 2010) tested both batch and continuous operation modes of CGA-based separation process, showing potential scalability of the process. In our case, the presence of particles would surely complicate the process and additional trials would be required for testing continuous operation mode in terms of separation efficiency and economic viability.

Overall, collected results corroborated the greater affinity of Tween20 towards anthocyanin compounds, in total agreement with the results of Table 1, as well as with previous literature findings (MohdMaidin et al., 2018; Spigno et al., 2015).

3.3. *S3: surfactant-assisted spray-drying process*

Due to the formation of a sticky dark red precipitate after the Tween20 addition to the concentrated extract (data not shown), samples to be spray-dried were again analyzed in terms of TPI_{280}, TPC_{Folin}, and TAC, with the results schematized in Table 5.

Precipitation led to a partial mass loss of around 34–39 % for total phenolic compounds (TPC_{Folin} and TPI_{280}, respectively), while the TAC concentration was not significantly ($p > 0.01$) varied after the surfactant addition. The observed result may be explained by considering that the applied pH differential method is capable of determining only the monomeric anthocyanin pigment, while polymerized anthocyanin compounds are not detected. Thus, it could be likely that the addition of Tween20 to concentrated DMGS extract has caused the separation of polymerized anthocyanins from the system via precipitation, due to their greater affinity with the surfactant agents (Spigno et al., 2015). On the same line, Dahmoune et al. (2013) observed that pumping CGAs in a flotation column, previously filled with extracts from the pomace of "Pinot noir" grape variety, implied the formation of insoluble dark red agglomerates in the aphron phase which could not be recovered, thus remaining unquantified. Nevertheless, in order to confirm our hypothesis and to better characterize the achieved products after Tween20 usage, further analyses on the precipitated fraction need to be performed.

Table 6 reports the principal characteristics of powders obtained from spray-dried DMGS extracts added with Tween20, as a function of the DE/GAE ratio, in terms of mass, TPC, and TAC recovery (%), as well as of WSI (%). Moreover, for two specific values of DE/GAE ratio (0.64, and 2.44 mol_{DE}/mol_{GAE}), experimental trials were also performed on DMGS extracts without the addition of the surfactant agent.

In the absence of Tween20, results clearly show that, as long as the DE/GAE ratio was raised, a higher mass yield and a slightly higher protective effect against degradation of phenolic compounds were observed. The latter can be inferred from the comparison of the phenolics and anthocyanins recovery at the two investigated DE/GAE ratios, and also from the comparison of the phenolics and mass yield. In fact, similar values of yields indicate that no degradation phenomena occurred during the spray-drying process.

Conversely, when Tween20 was added to the feeding extract, dramatically reduced mass yields were recorded as compared to those from powders obtained without surfactant addition, at a constant DE/GAE ratio. As far as the degradation of phenolic compounds is concerned, this is evident from the recovery values of TPC_{Folin} and TAC, being significantly lower than the corresponding mass powder recovery. Despite this, the yield of TPI_{280} remained almost constant. This is, anyway, related to the fact that such parameter is not affected by the oxidative status of the phenolic compounds (Spigno et al., 2015).

However, apart from the abovementioned degradative effect induced towards phenolic compounds, the evident reduction in RE_{TPI280}, $RE_{TPCFolin}$, and RE_{TAC} when diminishing the amount of coating agent utilized during spray-drying could be likely attributed to the increased hydrophilicity of the DMGS extracts upon the addition of surfactant, thus imparting a sticky behavior to the product being processed and, hence, complicating the separation of water from solids by evaporation. Similar results were achieved in the work of Adhikari et al. (2009), who assessed the influence of low-molecular-weight surfactants, such as Tween80, on the surface stickiness of a rich-sugar product utilized for carrying out spray-drying tests. Specifically, the authors underlined the difficulty in

Table 5 – Concentrations of phenolic and anthocyanin compounds in extracts to be spray–dried, before (pre) and after (post) the addition of Tween20 solution. The results are expressed as mean ± SD. Values with different superscript letters within the same column are significantly different ($p \leq 0.01$).

DMGS extract	TPI_{280} [mg_{GAE}/g_{DW}]	TPC_{Folin} [mg_{GAE}/g_{DW}]	TAC [mg_{WAE}/g_{DW}]
pre	14.04 ± 0.06^{b}	26.48 ± 0.01^{b}	1.93 ± 0.05^{a}
post	8.62 ± 0.03^{a}	17.55 ± 1.85^{a}	2.09 ± 0.20^{a}

Table 6 – Recovery (%) of total wet powder (WP) mass, total phenolic/anthocyanin compounds, and WSI of powders obtained from spray-drying of DMGS extracts, with or without Tween20, as a function of the DE/GAE ratio. The results are expressed as mean ± SD. Different lowercase and uppercase letters within the same column express significant differences ($p \leq 0.01$) among powder characteristic parameters due to the effect of DE/GAE ratio, in the presence and absence of Tween20, respectively. When reported, the symbol * indicates significant differences ($p \leq 0.01$) between powder characteristic parameters, at constant DE/GAE ratio, only due to the effect of surfactant agent.

DE/GAE [–]	RE_{WP} [%]	RE_{TPI280} [%]	$RE_{TPCFolin}$ [%]	RE_{TAC} [%]	WSI [%]
3.85 (+Tween20)	71.0 ± 1.3^{b}	90.0 ± 0.1^{c}	$*57.0 \pm 1.9^{c}$	55.0 ± 5.6^{b}	93.0 ± 0.1^{c}
2.44 (+Tween20)	$*73.0 \pm 0.2^{b}$	$*75.0 \pm 0.2^{b}$	49.0 ± 0.9^{b}	$*47.0 \pm 1.7^{b}$	$*90.0 \pm 0.1^{b}$
1.28 (+Tween20)	26.0 ± 3.3^{a}	26.0 ± 2.7^{a}	18.0 ± 2.3^{a}	12.0 ± 2.4^{a}	89.0 ± 0.6^{a}
0.64 (+Tween20)	$*25.0 \pm 2.5^{a}$	$*24.0 \pm 2.0^{a}$	$*19.0 \pm 1.9^{a}$	$*15.0 \pm 3.8^{a}$	$*88.0 \pm 0.6^{a}$
2.44	$*91.0 \pm 1.0^{B}$	$*88.0 \pm 0.1^{A}$	$*80.0 \pm 3.2^{A}$	$*93.0 \pm 5.5^{A}$	$*86.0 \pm 0.2^{B}$
0.64	$*80.0 \pm 1.7^{A}$	$*86.0 \pm 2.1^{A}$	$*81.0 \pm 0.9^{A}$	$*85.0 \pm 2.1^{A}$	$*74.0 \pm 0.9^{A}$

obtaining high powder recoveries after processing, reaching an almost null value when Tween80 was integrated into the starting solutions. Such outcome was ascribed to the so-called "orogenic displacement" phenomenon, which consisted in the dislodgement of the proteins added to the starting formulation from the surface of the produced droplets, as caused by the non-ionic surfactant agent, thus remaining unprotected and, hence, depositing on the spray-dryer walls.

Looking at the water solubility of the obtained powders (Table 6), as expected, an increase in the maltodextrin amount led to an increase in WSI, such as the addition of Tween20, which would furtherly confirm the hypothesized greater hydrophilic behavior of DMGS extracts in the presence of surfactant. In conclusion, the application of surfactants for spray-drying of bioactive compounds remains worth investigating due to their capability to mainly modify/improve the solubility of powders and the further release of stored compounds. However, additional efforts are required to optimize their dosage within liquid formulations to avoid the observed losses in both the mass and antioxidant power of final powders.

4. Conclusions

This work assessed the feasibility to integrate food-grade surfactant agents at different stages of the grape skins valorization process for intensifying the extraction/purification of their main bioactive constituents (polyphenols, anthocyanins). As regarding the surfactant-assisted SLE (S1), a sustainable and low solvent consuming two-steps process could be proposed (1^{ST} extraction: 10 mM Tween20/30 % ethanol, 2^{ND} extraction: 60 % ethanol) in order to selectively and efficiently recover anthocyanins and other phenolic compounds from dried milled grape skins, thus potentially leading to a more economical and environmentally friendly process due to a reduced consumption of organic solvents. Instead, when Tween20 was used in the form of CGAs (S2), maximum recoveries of phenolics (49.3 %) and anthocyanins (52.3 %) were obtained at the highest investigated extract/CGAs volumetric ratio, without leading to substantial losses of antioxidant capacity of extracted compounds. The addition of surfactant to the liquid extracts before spray-drying (S3) caused an increase in the water solubility of the resulting powders, despite the percentage recovery of mass, TPC, and TAC were significantly lower, as compared to those obtained from crude extracts in the absence of surfactant.

Additional studies need to be addressed to better clarify the behavior of surfactant agents in the presence of solid particles like grape skins during the CGAs-assisted extraction step, as well as the generated impact on the achieved degree of extract purification and on the process energy requirements. Viability and techno-economic analysis of a scaled-up version of the proposed technology will have to be established.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The research activities carried out under this work received financial support from: Agrisystem - Doctoral School for the Agro-Food system, Università Cattolica del Sacro Cuore, 3 years Ph.D. studentship for the XXVII Cycle; Progetto Ager "Valorvitis - Valorisation of wine-making industry wastes for the recovery of high added-value compounds", Grant n. 2010-2222; 2013 Seligman APV Fellowship, by SCI.

References

Adhikari, B., Howes, T., Wood, B.J., Bhandari, B.R., 2009. The effect of low molecular weight surfactants and proteins on surface stickiness of sucrose during powder formation through spray drying. J. Food Eng. 94, 135–143, http://dx.doi.org/10.1016/j.jfoodeng.2009.01.022.

Alonso, E., Bourzeix, M., Revilla, E., 1991. Suitability of water/ethanol mixtures for the extraction of catechins and proanthocyanidins from Vitis vinifera seeds contained in a winery by-product. Seed Sci. Technol. 19, 542–552.

Amendola, D., De Faveri, D.M., Spigno, G., 2010. Grape marc phenolics: extraction kinetics, quality and stability of

extracts. J. Food Eng. 97, 384–392, http://dx.doi.org/10.1016/j.jfoodeng.2009.10.033.
Amiri, M.C., Valsaraj, K.T., 2004. Effect of gas transfer on separation of whey protein with aphron flotation. Sep. Purif. Technol. 35, 161–167, http://dx.doi.org/10.1016/j.seppur.2003.08.007.
Andrade, M.A., Lima, V., Sanchez-Silva, A., Almeida Vilarinho, F.S., Conceição Castilho, M., Khwaldia, K., Ramos, F., 2019. Pomegranate and grape by-products and their active compounds: are they a valuable source for food applications? Trends Food Sci. Technol. 86, 68–84, http://dx.doi.org/10.1016/j.tifs.2019.02.010.
Angiolillo, L., Del Nobile, M.A., Conte, A., 2015. The extraction of bioactive compounds from food residues using microwaves. Curr. Opin. Food Sci. 5, 93–98, http://dx.doi.org/10.1016/j.cofs.2015.10.001.
Antoniolli, A., Fontana, A.R., Piccoli, P., Bottini, R., 2015. Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. Food Chem. 178, 172–178, http://dx.doi.org/10.1016/j.foodchem.2015.01.082.
Barba, F.J., Zhu, Z., Koubaa, M., Sant'Ana, A.S., Orlien, V., 2016. Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: a review. Trends Food Sci. Technol. 49, 96–109, http://dx.doi.org/10.1016/j.tifs.2016.01.006.
Binaschi, M., Duserm Garrido, G., Cirelli, C., Spigno, G., 2018. Biotechnological strategies to valorise grape pomace for food applications. Chem. Eng. Trans. 64, 367–372, http://dx.doi.org/10.3303/CET1864062.
Brianceau, S., Turk, M., Vitrac, X., Vorobiev, E., 2015. Combined densification and pulsed electric field treatment for selective polyphenols recovery from fermented grape pomace. Innov. Food Sci. Emerg. Technol. 29, 2–8, http://dx.doi.org/10.1016/j.ifset.2014.07.010.
Bustamante, M.A., Moral, R., Paredes, C., Pérez-Espinosa, A., Moreno-Caselles, J., Pérez-Murcia, M.D., 2008. Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. Waste Manag. 28, 372–380, http://dx.doi.org/10.1016/j.wasman.2007.01.013.
Corpuz, A.G., Pal, P., Banat, F., 2019. Effect of temperature and use of regenerated surfactants on the removal of oil from water using colloidal gas aphrons. Sep. Purif. Technol. 227, 115678, http://dx.doi.org/10.1016/j.seppur.2019.115678.
Dahmoune, F., Madani, K., Jauregi, P., De Faveri, D.M., Spigno, G., 2013. Fractionation of a red grape marc extract by colloidal gas aphrons. Chem. Eng. Trans. 32, 1903–1908, http://dx.doi.org/10.3303/CET1332318.
Das, C., Rungta, M., Arya, G., DasGupta, S., De, S., 2008. Removal of dyes and their mixtures from aqueous solution using liquid emulsion membrane. J. Hazard. Mater. 159 (2), 365–371, http://dx.doi.org/10.1016/j.jhazmat.2008.02.027.
Dermiki, M., Bourquin, A.L., Jauregi, P., 2010. Separation of astaxanthin from cells of phaffia rhodozyma using colloidal gas aphrons in a flotation column. Biotechnol. Prog. 26, 477–487, http://dx.doi.org/10.1002/btpr.340.
Di Stefano, R., Cravero, M.C., 2001. Metodi per lo studio dei polifenoli dell'uva. Riv. Viticoltura Enologia 2, 37–45.
FAOSTAT (Food and Agriculture Organization of the United Nations), (2018). http://www.fao.org/faostat/en/#data/QC. (November 2020).
Frontuto, D., Carullo, D., Harrison, S.M., Brunton, N.P., Ferrari, G., Lyng, J.G., Pataro, G., 2019. Optimization of pulsed electric fields-assisted extraction of polyphenols from potato peels using response surface methodology. Food Bioproc. Technol. 12, 1708–1720, http://dx.doi.org/10.1007/s11947-019-02320-z.
Fuda, E., Jauregi, P., 2006. An insight into the mechanism of protein separation by colloidal gas aphrons (CGA) generated from ionic surfactants. J. Chromatogr. B 843, 317–326, http://dx.doi.org/10.1016/j.jchromb.2006.06.032.
Galanakis, C.M., 2012. Recovery of high added-value components from food wastes: conventional, emerging technologies and commercialized applications. Trends Food Sci. Technol. 26, 68–87, http://dx.doi.org/10.1016/j.tifs.2012.03.003.
Galanakis, C.M., 2015. In: Galanakis, C.M. (Ed.), Food Waste Recovery: Processing Technologies and Industrial Techniques. , ISBN: 978-0-12-800351-0.
Hashim, M.A., Sen Gupta, B., 1997. The application of colloidal gas aphrons in the recovery of fine cellulose fibres from paper mill wastewater. Bioresour. Technol. 64, 199–204, http://dx.doi.org/10.1016/S0960-8524(97)00169-7.
Jampani, C., Naik, A., Raghavarao, K.S.M.S., 2014. Purification of anthocyanins from jamun (*Syzygium cumini* L.) employing adsorption. Sep. Purif. Technol. 125, 170–178, http://dx.doi.org/10.1016/j.seppur.2014.01.047.
Jarudilokkul, S., Rungphetcharat, K., Boonamnuayvitaya, V., 2004. Protein separation by colloidal gas aphrons using nonionic surfactant. Sep. Purif. Technol. 35, 23–29, http://dx.doi.org/10.1016/S1383-5866(03)00111-4.
Jauregi, P., Dermiki, M., 2010. Separation of value added bioproducts by colloidal gas aphrons (CGA) flotation and applications in the recovery of value-added food products. In: Rizvi, S.S.H. (Ed.), Separation, Extraction and Concentration Processes in Food, Beverage and Nutraceutical Industries. Woodhead Publishing Limited, Cambridge, pp. 284–313.
Jauregi, P., Varley, J., 1998. Colloidal gas aphrons: a novel approach to protein recovery. Biotechnol. Bioeng. 59, 471–481, http://dx.doi.org/10.1002/(SICI)1097-0290(19980820)59:4<471::AID-BIT10>3.0.CO;2-D.
Kadlec, P., Henke, S., Bubník, Z., 2010. Properties of ethanol and ethanol-water solutions — tables and equations. Sugar Industry 135, 607–613, http://dx.doi.org/10.36961/si10420.
Kha, T.C., Nguyen, M.H., Roach, P.D., 2010. Effect of spray-drying conditions on the physicochemical and antioxidant properties of the Gac (*Momordica cochinchinensis*) fruit aril powder. J. Food Eng. 98, 385–392, http://dx.doi.org/10.1016/j.jfoodeng.2010.01.016.
Kim, C., Hsieh, Y.L., 2001. Wetting and absorbency of nonionic surfactant solutions on cotton fabrics. Colloid Surf. A 187–188, 385–397, http://dx.doi.org/10.1016/S0927-7757(01)00653-7.
Lee, J., Durst, R.W., Wrolstad, R.E., 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. J. AOAC Int. 88, 1269–1278, http://dx.doi.org/10.1093/jaoac/88.5.1269.
Löf, D., Schillén, K., Nilsson, L., 2011. Flavonoids: precipitation kinetics and interaction with surfactant micelles. J. Food Sci. 76, N35–N39, http://dx.doi.org/10.1111/j.1750-3841.2011.02103.x.
Makris, D.P., 2018. Green extraction processes for the efficient recovery of bioactive polyphenols from wine industry solid wastes — recent progress. Curr. Opin. Green Sustain. Chem. 13, 50–55, http://dx.doi.org/10.1016/j.cogsc.2018.03.013.
MohdMaidin, N.M., Michael, N., Oruna-Concha, M.J., Jauregi, P., 2018. Polyphenols extracted from red grape pomace by a surfactant based method show enhanced collagenase and elastase inhibitory activity. J. Chem. Technol. Biotechnol. 93, 1916–1924, http://dx.doi.org/10.1002/jctb.5459.
MohdMaidin, N., Oruna-Concha, M.J., Jauregi, P., 2019. Surfactant TWEEN20 provides stabilisation effect on anthocyanins extracted from red grape pomace. Food Chem. 271, 224–231, http://dx.doi.org/10.1016/j.foodchem.2018.07.083.
Nayak, A., Bhushan, B., Rosales, A., Rodriguez Turienzo, L., Cortina, J.L., 2018. Valorisation potential of Cabernet grape pomace for the recovery of polyphenols: process intensification, optimisation and study of kinetics. Food Bioprod. Process. 109, 74–85, http://dx.doi.org/10.1016/j.fbp.2018.03.004.
Negro, C., Tommasi, L., Miceli, A., 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol. 87 (1), 41–44, http://dx.doi.org/10.1016/S0960-8524(02)00202-X.
Noriega, D., Zuniga, M.E., Soto, C., MohdMaidin, N., Michael, N., Jauregi, P., 2018. Colloidal Gas Aphrons separation to obtain polyphenol rich fractions from artichoke agro-industrial discards. Food Bioprod. Process. 110, 50–59, http://dx.doi.org/10.1016/j.fbp.2018.04.007.

Nunes, M.A., Rodrigues, F., Oliveira, M.B.P.P., 2017. Grape processing by-products as active ingredients for cosmetic proposes. In: Galanakis, C.M. (Ed.), Handbook of Grape Processing By-Products. Academic Press, pp. 267–292.

Papaioannou, E.H., Karabelas, A.J., 2012. Lycopene recovery from tomato peel under mild conditions assisted by enzymatic pre-treatment and non-ionic surfactants. Acta Biochim. Pol. 59, 71–74.

Pazir, F., Koçak, E., Turan, F., Ova, G., 2021. Extraction of anthocyanins from grape pomace by using supercritical carbon dioxide. J. Food Process. Preserv. 45, e14950, http://dx.doi.org/10.1111/jfpp.14950.

Pinelo, M., Del Fabbro, P., Marzocco, L., Nunez, M.J., Vicoli, M.C., 2005. Optimization of continuous phenol extraction from Vitis vinifera byproducts. Food Chem. 92, 109–117, http://dx.doi.org/10.1016/j.foodchem.2004.07.015.

Prasad, K.N., Spigno, G., Jauregi, P., Misra, N.N., Cullen, P.J., 2015. Emerging macro- and micromolecules separation. In: Galanakis, C.M. (Ed.), Food Waste Recovery: Processing Technologies and Industrial Techniques. Academic Press, pp. 227–248.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved abts radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237, http://dx.doi.org/10.1016/S0891-5849(98)00315-3.

Rocha, C.M.R., Genisheva, Z., Ferreira-Santos, P., Rodrigues, R., Vicente, A.A., Teixeira, J.A., Pereira, R.N., 2018. Electric field-based technologies for valorization of bioresources. Bioresour. Technol. 254, 325–339, http://dx.doi.org/10.1016/j.biortech.2018.01.068.

Sette, P., Fernandez, A., Soria, J., Rodriguez, R., Salvatori, D., Mazza, G., 2019. Integral valorization of fruit waste from wine and cider industries. J. Clean. Prod. 242, 118486, http://dx.doi.org/10.1016/j.jclepro.2019.118486.

Sirohi, R., Tarafdar, A., Singh, S., Negi, T., Kumar Gaur, V., Gnansounou, E., Bharathiraja, B., 2020. Green processing and biotechnological potential of grape pomace: current trends and opportunities for sustainable biorefinery. Bioresour. Technol. 314, 123771, http://dx.doi.org/10.1016/j.biortech.2020.123771.

Spigno, G., Jauregi, P., 2005. Recovery of gallic acid with colloidal gas aphrons (CGA). Int. J. Food Eng. 1, 1–10, http://dx.doi.org/10.2202/1556-3758.1038.

Spigno, G., Dermiki, M., Pastori, C., Casanova, F., Jauregi, P., 2010. Recovery of gallic acid with colloidal gas aphrons generated from a cationic surfactant. Sep. Purif. Technol. 71, 56–62, http://dx.doi.org/10.1016/j.seppur.2009.11.002.

Spigno, G., Amendola, D., Dahmoune, F., Jauregi, P., 2015. Colloidal gas aphrons based separation process for the purification and fractionation of natural phenolic extracts. Food Bioprod. Process. 94, 434–442, http://dx.doi.org/10.1016/j.fbp.2014.06.002.

Wijekoon, M.M.J.O., Bhat, R., Karim, A.A., 2011. Effect of extraction solvents on the phenolic compounds and antioxidant activities of bunga kantan (Etlingera elatior Jack.) inflorescence. J. Food Compos. Anal. 24, 615–619, http://dx.doi.org/10.1016/j.jfca.2010.09.018.

Yan, Y.L., Zhang, Y., Una, C.-C., 2020. On the nature of colloidal aphrons. Colloid Interface Sci. Commun. 34, 100232, http://dx.doi.org/10.1016/j.colcom.2019.100232.

Yilmaz, Y., Toledo, R.T., 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. J. Food Compos. Anal. 19, 41–44, http://dx.doi.org/10.1016/j.jfca.2004.10.009.