

Article

# Water–Organic Solvent Extraction of Phenolic Antioxidants from Brewers’ Spent Grain

Antonio Zuorro , Annalaura Iannone and Roberto Lavecchia \*

Department of Chemical Engineering, Materials and Environment, Sapienza University, 00185 Rome, Italy; antonio.zuorro@uniroma1.it (A.Z.); annalaura.iannone@uniroma1.it (A.I.)

\* Correspondence: roberto.lavecchia@uniroma1.it

Received: 7 January 2019; Accepted: 18 February 2019; Published: 1 March 2019



**Abstract:** Brewers’ spent grain (BSG) is the most abundant by-product of the brewing process. BSG is currently disposed of or used as a supplement for animal feed, although it contains significant amounts of bioactive compounds of great interest to the pharmaceutical, cosmetic and food sectors. In this study we investigate the feasibility of using a simple solvent extraction procedure to recover phenolic antioxidants from BSG. Acetone–water and ethanol–water mixtures were used as extraction solvents. Phenolic extracts obtained by treatment of BSG with the two solvent systems were characterized in terms of total phenolics and antioxidant activity. For both systems, the extraction yield was maximum at 60% (v/v) organic solvent concentration. At all solvent compositions, mixtures containing acetone provided higher extraction yields. As suggested by the strong correlation between the antioxidant activity of BSG extracts and their phenolic content, the antioxidant capacity of the extracts can be mainly attributed to polyphenols. Overall, the obtained results strongly support the exploitation of BSG as a source of phenolic antioxidants and the possibility of recovering them by a mild and green extraction process.

**Keywords:** brewers’ spent grain; phenolic compounds; solvent extraction; waste valorization

## 1. Introduction

Beer is the oldest and most consumed alcoholic beverage in the world. The annual world production of beer is estimated to around 1.8 billion hectoliters, with an average per capita consumption of about 27 L/year [1]. The beverage is usually obtained by fermentation of malted barley, although other types of cereals, such as wheat, rice, corn, spelt and oats, are suitable for its production [2].

From the brewing process, large amounts of solid by-products, including surplus yeast, spent hops and brewers’ spent grain (BSG), are generated. BSG is the most abundant by-product, representing about 85% of the total solid waste [3].

The world production of BSG has been estimated to be on the order of 40 million tons per year [4]. This waste consists primarily of barley grain husks, with minor fractions of pericarp and seed coat layer fragments. Its specific chemical composition depends on several factors, including barley variety, harvest time, malting conditions and the type of adjuncts used in the brewing process [5]. Basically, BSG is a lignocellulosic material, with fiber (cellulose and hemicellulose), protein and lignin being the major constituents. On a dry basis, fiber amounts to about 50% of total weight, while proteins can reach up to 25% [6]. Essential amino acids represent approximately 30% of the total protein content, with lysine being the most abundant (14.3%) [4]. The presence of large amounts of lysine is a peculiarity of barley grain, as most cereals are deficient in this amino acid. For this reason, this waste material is mainly used as protein supplements for animal feed. However, BSG is also a rich source of important bioactive compounds [7]. In particular, phenolic compounds can be present in an amount of up to 8 mg/g [3]. The high levels of phenolic compounds in BSG can be explained by the fact that,

during the development of barley seeds, these compounds are specifically accumulated in the grain husk. Ferulic and *p*-coumaric acids are the main phenolics, followed by caffeic, sinapic and syringic acids [8]. In addition to being powerful antioxidants, these compounds exhibit anti-inflammatory and anti-carcinogenic activities [9], thus supporting the use of BSG as a raw material for the recovery of valuable bioactive phenolics.

In most cases, solid–liquid extraction can be advantageously used for the recovery of natural compounds from untreated or pretreated biomass materials [10–13]. A large number of studies indicate that the extraction yields and the properties of the recovered compounds are significantly dependent on the structural and compositional features of the plant matrix, the type of compounds and the strength with which they are bound to the matrix [14–16]. As a result, a deep knowledge of the characteristics of the system under investigation is required. Furthermore, the process conditions should be carefully controlled in order to maximize the recovery of bioactives while preserving their functional properties. However, a review of the literature shows that, contrary to other polyphenol-containing materials, scarce attention has so far been paid to the recovery of phenolic compounds from BSG [17,18].

In this study we investigate the recovery of phenolic compounds from BSG using two water–organic solvent mixtures: acetone–water and ethanol–water. The main purpose of the study was to compare the extraction yields obtained with the two solvent systems and evaluate the antioxidant activity of the resulting extracts. In addition, we wanted to provide a physical interpretation of the results in terms of solute–solvent affinity. To this end, we used the Hansen solubility parameter (HSP) theory, which is briefly outlined in Appendix A. According to this theory, the affinity between a solute and a solvent can be described by considering three main contributions to the total cohesive energy: the dispersion, the polarity and the hydrogen bonding energies. HSP theory was initially developed in the field of paints and coatings to provide a rigorous tool for the selection of paint components [19]. Subsequently, it was used to describe many other phenomena, such as partial miscibility of liquids, pigment deposition on solid surfaces and the interaction of a drug with excipients or carriers in solid dispersions [20].

## 2. Materials and Methods

### 2.1. Chemicals and Waste Material

Acetone, ethanol, methanol, sodium carbonate and hydrochloric acid (37%) were purchased from Carlo Erba (Milan, Italy). Gallic acid (3,4,5-trihydroxybenzoic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and the Folin–Ciocalteu reagent were obtained from Sigma-Aldrich (Milan, Italy). All chemicals were used as received.

BSG was provided by a brewery in the Province of Rome (Italy). As soon as received, the material was dried to a final moisture content <3% in a forced-air dehydrator (Stöckli, Switzerland) at 50 °C, ground in an electric mill (Moulinex, Ecully, France) to a particle size less than 0.5 mm and stored in the dark at room temperature until use.

### 2.2. Analytical Methods

Moisture measurements were made by oven drying at 105 °C. The initial phenolic content of BSG was determined by solid-liquid extraction under agitation, as described in [21]. This method allows for achieving a nearly complete removal of phenolic compounds from the plant material through four extraction stages. In each stage the temperature was kept to 60 °C, the extraction time was 1 h and aqueous acetone (60% v/v) was used as the solvent. The liquid-to-solid ratio was equal to 20, 10, 5 or 5 mL g<sup>-1</sup>.

Total phenolics were determined by the Folin–Ciocalteu method following the procedure reported elsewhere [22]. Briefly, 200 µL of the sample, 150 µL of Folin–Ciocalteu's reagent and 5 mL of 0.1 M HCl were put into a glass tube. Then, a 20% w/v Na<sub>2</sub>CO<sub>3</sub> solution was added to a final volume of 10 mL. The tube was kept in the dark at room temperature for 1 h. Finally, the absorbance at 525 nm was measured. The results were expressed as gallic acid equivalents (GAE) using a calibration curve obtained with gallic acid standards between 0.02 and 0.3 g L<sup>-1</sup> (Figure S1).

Antioxidant activity was determined by the DPPH method according to Maietta et al. [23]. A work solution of DPPH in methanol at a concentration of  $0.05 \text{ g L}^{-1}$  ( $1.2 \cdot 10^{-4} \text{ M}$ ) was initially prepared. Then, a suitable amount of the sample to be assayed (100–300  $\mu\text{L}$ ) was poured into an optical glass cuvette and the DPPH solution was added to obtain a final volume of 4 mL. The cuvette was incubated in the dark at room temperature for 30 min. Finally, the absorbance of the solution at 517 nm was measured against methanol. The antioxidant activity of the sample was evaluated from the values of the initial and final absorbance as  $100 \cdot (A_0 - A_f) / A_f$ . The results were expressed as Trolox equivalents (TE) using a calibration curve obtained with Trolox standards between 2 and  $10 \text{ mg L}^{-1}$  (Figure S2).

Optical measurements were made on a double-beam UV-Vis spectrophotometer (UV-2700, Shimadzu, Kyoto, Japan).

### 2.3. Extraction Procedure

Batch extraction experiments were carried out in screw-capped sealed glass flasks equipped with a magnetic bar. The flasks were placed in a water bath at  $60 \pm 0.1 \text{ }^\circ\text{C}$ . Then, 20 mL of water–organic solvent mixture of the desired composition (0, 20, 40, 60, 80, 100% v/v) and 1 g of BSG were put into the flasks and stirred at 400 rpm for 60 min. After this time, the flasks were rapidly quenched under tap water and an aliquot of the suspension was centrifuged at  $7000 \times g$  for 5 min. Finally, the supernatant liquid was removed and assayed for total phenolics and antioxidant activity.

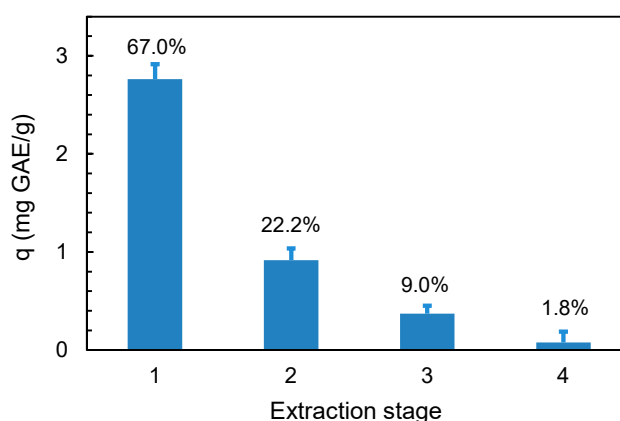
### 2.4. Statistical Analysis

Extraction experiments were carried out in duplicate, while analytical measurements were repeated at least three times. All results were expressed as mean  $\pm$  standard deviations. The values of the coefficient of variation (standard deviation over mean) were always  $<4\%$ . Statistical analysis was performed using Microsoft Excel 2010.

## 3. Results

### 3.1. Extraction of Phenolic Compounds

The initial moisture content of BSG was  $79.8 \pm 0.4 \text{ wt } \%$  and was reduced to  $2.8 \pm 0.1 \text{ wt } \%$  after drying. The overall phenolic content, as determined by the four-stage extraction procedure, was  $4.126 \text{ mg GAE g}^{-1}$ . About 90% of the total amount of phenolic compounds was recovered in the first two stages and less than 2% in the fourth stage (Figure 1).



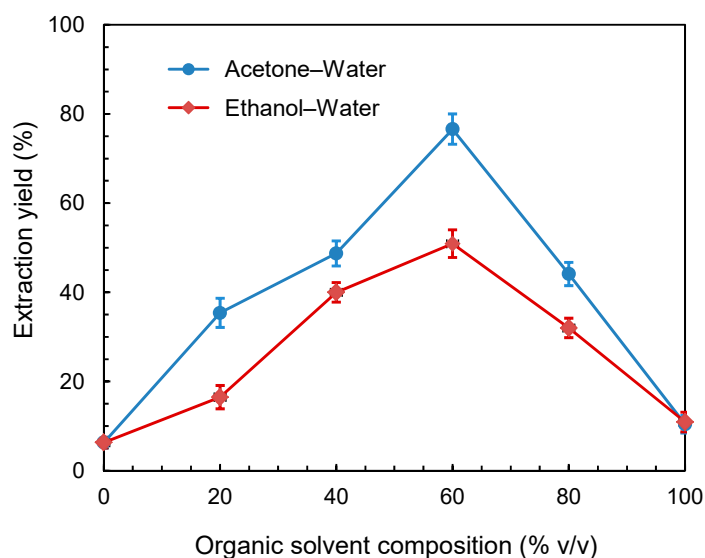
**Figure 1.** Amount of recovered phenolic compounds per unit weight of dry solid ( $q$ ) in each extraction stage, with the associated recovery percentages.

Experiments aimed at assessing the effects of solvent type and composition on the amount of recovered phenolics gave the results listed in Table 1. In the same table, the antioxidant activity of the extracts determined by the DPPH assay are reported. In both solvent systems, the two quantities

exhibited a non-monotonic trend with solvent composition, with a maximum at 60% (v/v) acetone or ethanol. From the phenolic content of BSG, the percent extraction yields, defined as the ratio between the amount of recovered phenolic compounds and the total amount of the same compounds, was obtained (Figure 2).

**Table 1.** Concentration of phenolic compounds (c) and antioxidant activity (AA) of water–organic solvent extracts from brewers’ spent grain (BSG). Data are expressed as mean  $\pm$  standard deviation ( $n = 3$ ).

Solvent	v/v (%)	c (mg GAE/L)	AA (mg TE/L)
Water	100	0.013 $\pm$ 0.002	0.011 $\pm$ 0.001
Acetone	20	0.073 $\pm$ 0.003	0.034 $\pm$ 0.003
	40	0.101 $\pm$ 0.006	0.051 $\pm$ 0.003
	60	0.160 $\pm$ 0.005	0.064 $\pm$ 0.005
	80	0.091 $\pm$ 0.004	0.048 $\pm$ 0.004
	100	0.022 $\pm$ 0.001	0.017 $\pm$ 0.002
Ethanol	20	0.034 $\pm$ 0.002	0.019 $\pm$ 0.003
	40	0.083 $\pm$ 0.004	0.029 $\pm$ 0.003
	60	0.106 $\pm$ 0.005	0.043 $\pm$ 0.005
	80	0.067 $\pm$ 0.004	0.033 $\pm$ 0.004
	100	0.023 $\pm$ 0.002	0.019 $\pm$ 0.003



**Figure 2.** Percent phenolic extraction yields in water–organic solvent mixtures.

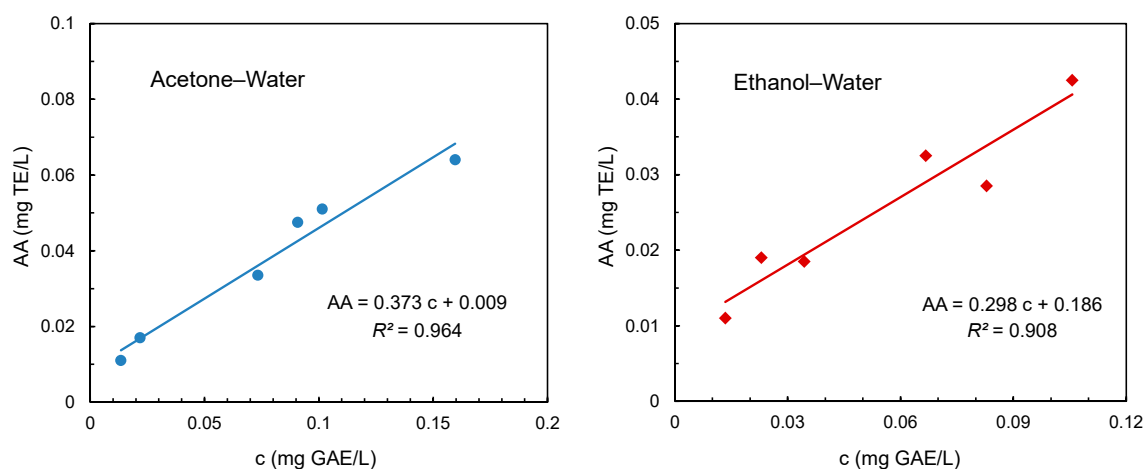
At every solvent composition, systems containing acetone resulted in higher yields compared to ethanol–water. At the optimum composition (60% v/v), the percent extraction yields in aqueous acetone and ethanol were equal to 70.6% and 50.9%, respectively. The corresponding antioxidant activities of the extracts were 1.28 and 0.85 mg TE g<sup>-1</sup>.

For both solvent systems, a sharp linear dependence ( $R^2 > 0.9$ ) was observed when the antioxidant activity of the extracts was plotted against the concentration of phenolic compounds (Figure 3).

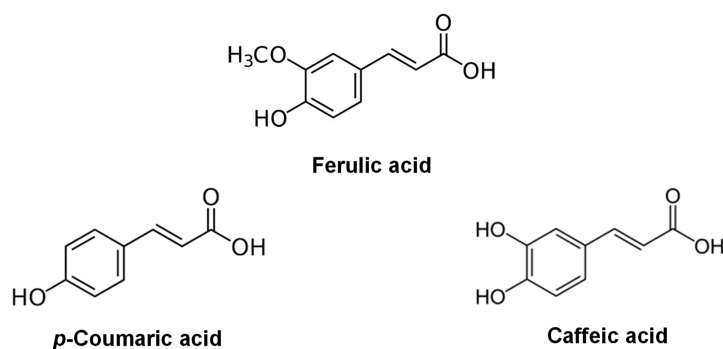
### 3.2. Analysis of HSPs

HSPs were used to estimate the affinity of phenolic compounds for the investigated solvents. Three of the major phenolics present in BSG, that is, ferulic, caffeic and *p*-coumaric acids, were selected as representatives of the whole class of phenolic compounds in this material (Figure 4). Their partial solubility parameters ( $\delta_D$ ,  $\delta_P$ ,  $\delta_H$ ) were calculated from Equations (A5)–(A7) using the group contribution values reported in [24]. Then, the total solubility parameter ( $\delta_T$ ) was determined

from Equation (2). The molar volumes were estimated by the group contribution method of Fedors [25]. The results are presented in Table 2.



**Figure 3.** Antioxidant activity (AA) of BSG extracts against total phenolic compounds concentration (c) in water–organic solvent systems.



**Figure 4.** Molecular structures of the three major phenolic compounds in BSG.

**Table 2.** Molecular properties and Hansen solubility parameters of solvent components (MW: molecular weight; MV: molar volume;  $\delta_i$ : Hansen solubility parameters).

Compound	MW (Da)	MV (cm <sup>3</sup> /mol)	$\delta_D$ (MPa <sup>0.5</sup> )	$\delta_P$ (MPa <sup>0.5</sup> )	$\delta_H$ (MPa <sup>0.5</sup> )	$\delta_T$ (MPa <sup>0.5</sup> )
Water	18.01	18.02	15.5	16	42.3	47.8
Acetone	58.08	73.40	15.5	10.4	7.0	19.9
Ethanol	46.07	58.68	15.8	8.8	19.4	26.5
Ferulic acid	194.19	136.2	22.7	5.7	15.6	28.1
Caffeic acid	180.16	108.9	25.5	10.0	21.4	34.8
<i>p</i> -Coumaric acid	164.16	117.9	20.4	5.6	16.0	26.5

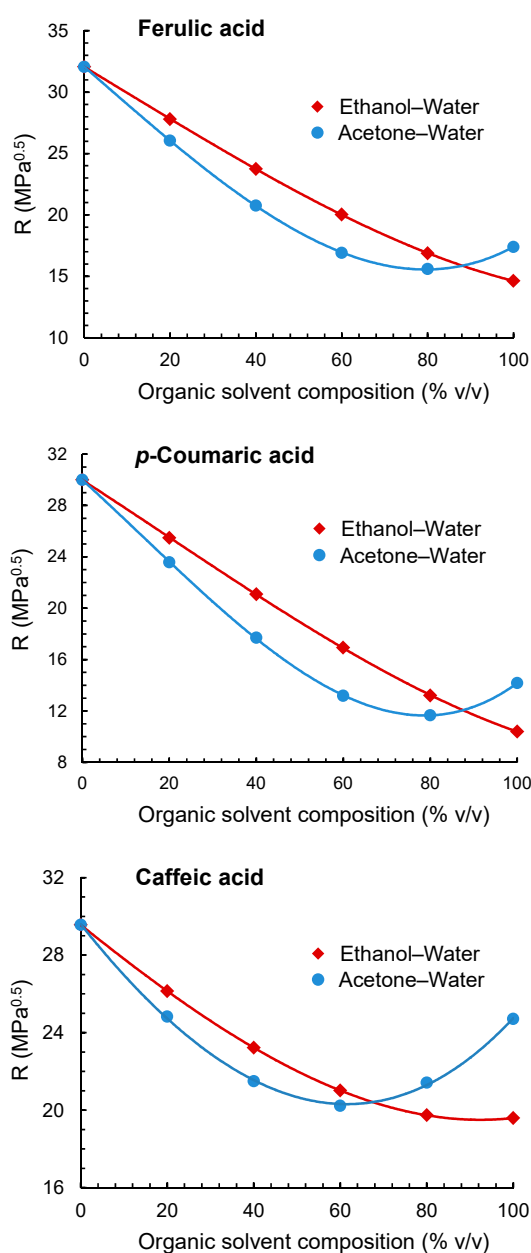
Hansen solubility parameters and molar volumes of solvent components (water, acetone and ethanol) were taken from the literature [26]. HSPs of mixed solvents ( $\delta_{mix}$ ) were determined from Equation (A8).

Finally, the solubility parameter distance (R) for the three phenolic compounds in the acetone–water and ethanol–water mixtures was calculated using Equation (4), leading to the results displayed in Figure 5.

#### 4. Discussion

The results presented here indicate that BSG is a good source of phenolic compounds, their total amount being on the order of 4 mg GAE per g of dry waste. For comparison, a value of 4.61 mg

$\text{g}^{-1}$  was obtained by Szwajgier et al. [27] using ferulic acid esterase from *Lactobacillus acidophilus* to favor the release of phenolic compounds from BSG. Meneses et al. [17] evaluated the effect of different solvents on the extraction of phenolics from BSG and recovered  $3.59 \text{ mg g}^{-1}$  (pure water) to  $9.9 \text{ mg g}^{-1}$  (aqueous acetone) of total phenolic compounds. In another study by Socaci et al. [18], the amount of recovered phenolic compounds ranged from  $0.41 \text{ mg g}^{-1}$  (pure ethanol) to  $1.14 \text{ mg g}^{-1}$  (aqueous acetone). Although these values are, on average, lower than those found in other fruit or vegetable wastes, such as apple and berry fruit peels [28,29] or spent coffee grounds [30], the large availability throughout the year of BSG at low or no cost makes it a promising raw material for the recovery of phenolic compounds. It should also be considered that the levels of these compounds are highly dependent on barley variety and growth conditions and could be increased through appropriate cultivation practices. Interestingly, as attested by the strong correlation between phenolic content and antioxidant activity, this class of compounds seems to be the main one responsible for the antioxidant properties of BSG extracts.



**Figure 5.** Hansen solubility parameter distance (R) of ferulic, *p*-coumaric and caffeic acids in water–organic solvent mixtures.

Regarding the influence of solvent type and composition on the extraction yields, from Figure 2 it can be seen that aqueous mixtures containing 60% *v/v* organic solvent were the most effective. Of the two organic solvents, acetone provided a higher recovery of phenolic compounds over the entire solvent composition range. Similar results were obtained in other studies on BSG [17,18] or different plant materials, such as spent coffee grounds [22], artichoke waste [31], mango by-products [32] and beetroot [33].

It is generally agreed that the existence of an optimal solvent composition in aqueous solvents is due to the fact that phenolic compounds exhibit a wide range of solubilities in single mixture components and are often more soluble in solvents less polar than water [22,34]. Thus, the observed differences in the extraction efficiencies by the two solvent systems can be primarily ascribed to differences in their affinity for the phenolic compounds present in BSG. An estimation of the contribution of affinity to the overall extraction yield can be made by comparing the Hansen solubility parameters of the solutes with those of the solvent. To this end, ferulic acid, *p*-coumaric acid and caffeic acid were selected as representatives of the whole class of BSG phenolics [27]. During plant development, these and other phenolic compounds accumulate in the husk of barley grain, where ferulic acid, accounting for approximately 68% of total phenolics, reaches concentrations from about 150  $\mu\text{g g}^{-1}$  to over 400  $\mu\text{g g}^{-1}$  [35].

As can be seen in Figure 5, the solubility parameter distance for the three phenolic compounds in aqueous acetone was generally lower than that in aqueous ethanol, suggesting that they have a higher affinity for acetone than for ethanol. Furthermore, in acetone–water mixtures, *R* exhibited a non-monotonic trend with the organic solvent concentration and a minimum located approximately between 60% and 80% (*v/v*) appeared. This behavior indicates that the solubility parameters of solute and solvent exhibit a different dependence on the composition of the mixture and that at a certain composition they become very close to each other, leading to the observed minimum. However, an examination of the results displayed in Figures 2 and 5 reveals that the affinity of phenolic compounds for the solvent cannot completely explain the observed dependence of extraction yields on mixture type and composition. This is particularly true for the ethanol–water system, as the solubility parameter distance of the three phenolic compounds decreases monotonically with the concentration of ethanol in the mixture. On the other hand, it should be considered that the extraction of a natural substance from a plant material is a very complex heterogeneous process involving different steps, such as the penetration of solvent molecules in the plant matrix, the breakage of solute–matrix interactions and the solubilization of the solute. Therefore, in addition to affinity, factors related to the other process steps should be considered.

In cereal grains, phenolic compounds are mainly located in the pericarp, which constitutes the outermost part of the grain [36]. As a result, the solvent molecules must penetrate the cell walls of the plant tissue before reaching the solutes. Plant cell walls are composite three-dimensional structures containing cellulose, hemicellulose and pectin as major polysaccharide components [37]. Small amounts of proteins with a structural or functional role are also present. Cellulose is organized in microfibrils with a diameter of 2–10 nm composed of well-packed hydrogen-bonded stretches of crystalline cellulose and less ordered amorphous regions. These cellulose microfibrils are assembled into fibers of larger diameter cross-linked by hemicelluloses and embedded in a gel-like matrix of pectic polysaccharides [38].

Plant tissue is poorly permeable to the solvent, but its compactness can be reduced by swelling. This phenomenon originates from the adsorption of polar solvent molecules on the hydroxyl and carboxyl groups of cellulose fibers [22]. Following adsorption, the distances between the fibers increase, causing expansion of the material and thus increasing the penetration of the solvent into the plant tissue [22]. Swelling by protic solvents, such as water and ethanol, occurs mainly within the amorphous region of cellulose [39], leading to an appreciable disruption of the strong hydrogen bonded network. In contrast, swelling by aprotic solvents, such as acetone, depends more on solvent–cellulose dipolar interactions [40]. Due to its small molar volume and high hydrogen bonding capability, water is a



powerful swelling agent, which supports its key role in the recovery of phenolic compounds from BSG and other plant materials. It can therefore be hypothesized that the observed optimum in the extraction yields at 60% (v/v) solvent composition is the result of two main factors: affinity of phenolic compounds for solvent components and solvent effects related to the swelling of the plant tissue. As for the second factor, water can be expected to play the major role due to its high swelling capacity.

However, other solvent-related factors may also contribute to the observed behavior. For example, it is known that phenolic compounds in cereals can be either in free or bound forms [41]. Bound phenolic compounds are ester- or ether-linked to cell wall polysaccharides [42]. Solvent components can promote the breakage of these bonds, favoring the release of phenolic compounds. Finally, organic solvents like acetone and ethanol may also cause protein denaturation [43]. The underlying mechanisms are complex and only partially elucidated, as an organic solvent may affect different types of the interactions responsible for protein stability, such as the electrostatic, hydrogen bonding and hydrophobic ones. The resulting effects are solvent- and protein-specific, and usually increase with the organic solvent concentration. Since BSG has a high protein content, up to 25% on a dry weight basis [6], denaturation of structural proteins by solvent components can be expected to contribute to weakening and loosening the cell-wall structure, improving the penetration of solvent molecules and the recovery of phenolic compounds.

## 5. Conclusions

The results of this study indicate that BSG is a potentially valuable source of phenolic antioxidants that could be easily recovered by extraction with aqueous organic solvents. We have also shown that an optimal solvent composition exists that maximizes the extraction yields and is likely the result of different solvent-related effects.

Like other agro-industrial waste, the characteristics of BSG can vary both with the type of raw materials used and the process conditions. Therefore, further research should be devoted to investigating other types of BSG and characterizing the phenolic extracts obtained. A deeper insight into the role of solvent components in the recovery of phenolic components should also be gained in future studies.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2227-9717/7/3/126/s1>, Figure S1: Calibration curves for evaluation of total phenolics, Figure S2: Calibration curves for evaluation of DPPH antioxidant activity.

**Author Contributions:** A.Z. designed the experiments, analyzed the data and wrote the paper; A.I. performed the experiments; R.L. contributed to the design of experiments, interpreted the data and wrote the paper.

**Funding:** This research was partially supported by grants from Sapienza University of Rome (Italy).

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A. HSP Theory

The classic solubility theory developed by Hildebrand and Scott [44] states that the solubility parameter of a substance ( $\delta$ ) can be expressed as the square root of the cohesive energy density:

$$\delta = \sqrt{\frac{\Delta E}{v_m}}, \quad (\text{A1})$$

where  $\Delta E$  is the cohesive energy and  $v_m$  is the molar volume.

Hansen proposed to evaluate the total cohesive energy as the sum of three contributions: the dispersion energy (related to the Van der Waals forces), the polarity energy (related to permanent dipoles) and the hydrogen bonding energy [26]. Accordingly, the total solubility parameter ( $\delta_T$ ) can be determined from the three individual parameters as follows:

$$\delta_T^2 = \delta_D^2 + \delta_P^2 + \delta_H^2, \quad (\text{A2})$$



where  $\delta_D$ ,  $\delta_P$  and  $\delta_H$  represent the dispersion, the polar and the hydrogen-bonding solubility parameters.

The affinity of a solute for a solvent can therefore be evaluated by comparing the values of the corresponding total solubility parameters. In particular, the closer the two parameters, the higher the solubility of the solute in that solvent. In Hansen space, the distance ( $d_{AB}$ ) between the points representing the solute ( $\delta_{D,A}$ ,  $\delta_{P,A}$ ,  $\delta_{H,A}$ ) and the solvent ( $\delta_{D,B}$ ,  $\delta_{P,B}$ ,  $\delta_{H,B}$ ), calculated as:

$$d_{AB} = \sqrt{(\delta_{D,A} - \delta_{D,B})^2 + (\delta_{P,A} - \delta_{P,B})^2 + (\delta_{H,A} - \delta_{H,B})^2}, \quad (\text{A3})$$

which provides a measure of the solute–solvent affinity.

Some authors suggested that good miscibility is achieved if  $d \leq 5 \text{ MPa}^{0.5}$  [45]. However, Hansen and Skaarup [46] proposed to modify the above equation by defining the solubility parameter distance ( $R$ ) between the two components as:

$$R = \sqrt{4(\delta_{D,A} - \delta_{D,B})^2 + (\delta_{P,A} - \delta_{P,B})^2 + (\delta_{H,A} - \delta_{H,B})^2}, \quad (\text{A4})$$

Small  $R$ -values indicate a high solubility of  $A$  in  $B$ , as the interaction forces acting between the molecules of the two components are similar.

Hoftyzer and van Krevelen developed a group contribution method to estimate the partial solubility parameters of polymers and pure organic compounds. They provided the following equations [24]:

$$\delta_D = \frac{\sum F_{D,i}}{v_m} \quad (\text{A5})$$

$$\delta_P = \frac{\sqrt{\sum F_{P,i}^2}}{v_m} \quad (\text{A6})$$

$$\delta_H = \frac{\sqrt{\sum E_{H,i}}}{v_m}, \quad (\text{A7})$$

where  $F_{D,i}$ ,  $F_{P,i}$  and  $F_{H,i}$  are the group contributions to individual solubility parameters and  $v_m$  is the molar volume of the substance.

Finally, in the case of multicomponent solvents, the HSPs of the solvent ( $\delta_{mix}$ ) can be determined as follows:

$$\delta_{mix} = \sum_i \varphi_i \delta_i, \quad (\text{A8})$$

where  $\varphi_i$  is the volume fraction of the  $i$ -th component in the solvent mixture and  $\delta_i$  is the partial or total HSP of that component.

## References

1. FAOSTAT. Food and Agricultural Commodities Production. Final 2013 Data. Available online: <http://faostat3.fao.org/> (accessed on 17 December 2018).
2. Eaton, B. An Overview of Brewing. In *Handbook of Brewing*, 3rd ed.; Stewart, G.G., Russell, I., Anstruther, A., Eds.; CRC Press: Boca Raton, FL, USA, 2017; pp. 53–66.
3. Xiros, C.; Christakopoulos, P. Biotechnological potential of brewers spent grain and its recent applications. *Waste Biomass Valorization* **2012**, *3*, 213–232. [CrossRef]
4. Mussatto, S.I. Brewer's spent grain: A valuable feedstock for industrial applications. *J. Sci. Food Agric.* **2014**, *94*, 1264–1275. [CrossRef] [PubMed]
5. Mussatto, S.I.; Dragone, G.; Roberto, I.C. Brewers' spent grain: Generation, characteristics and potential applications. *J. Cereal Sci.* **2006**, *43*, 1–14. [CrossRef]
6. Lynch, K.M.; Steffen, E.J.; Arendt, E.K. Brewers' spent grain: A review with an emphasis on food and health. *J. Inst. Brew.* **2016**, *122*, 553–568. [CrossRef]

7. Wannemacher, J.; Gastl, M.; Becker, T. Phenolic substances in beer: Structural diversity, reactive potential and relevance for brewing process and beer quality. *Compr. Rev. Food. Sci. Food Saf.* **2018**, *17*, 953–988. [[CrossRef](#)]
8. McCarthy, A.L.; O’Callaghan, Y.C.; Piggott, C.O.; FitzGerald, R.J.; O’Brien, N.M. Brewers’ spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: A review. *Proc. Nutr. Soc.* **2013**, *72*, 117–125. [[CrossRef](#)] [[PubMed](#)]
9. Fărcaș, A.C.; Socaci, S.A.; Dulf, F.V.; Tofană, M.; Mudura, E.; Diaconeasa, Z. Volatile profile, fatty acids composition and total phenolics content of brewers’ spent grain by-product with potential use in the development of new functional foods. *J. Cereal Sci.* **2015**, *64*, 34–42. [[CrossRef](#)]
10. Ameer, K.; Shahbaz, H.M.; Kwon, J.-H. Green extraction methods for polyphenols from plant matrices and their byproducts: A review. *Compr. Rev. Food. Sci. Food Saf.* **2017**, *16*, 295–315. [[CrossRef](#)]
11. Chaturvedi, A.K. Extraction of nutraceuticals from plants by microwave assisted extraction. *Syst. Rev. Pharm.* **2018**, *9*, 31–35. [[CrossRef](#)]
12. Zuorro, A.; Lavecchia, R.; Medici, F.; Piga, L. Use of cell wall degrading enzymes for the production of high-quality functional products from tomato processing waste. *Chem. Eng. Trans.* **2014**, *38*, 355–360.
13. Zuorro, A.; Maffei, G.; Lavecchia, R. Optimization of enzyme-assisted lipid extraction from *Nannochloropsis* microalgae. *J. Taiwan Inst. Chem. Eng.* **2016**, *67*, 106–114. [[CrossRef](#)]
14. Kammerer, D.R.; Kammerer, J.; Valet, R.; Carle, R. Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients. *Food Res. Int.* **2014**, *65*, 2–12. [[CrossRef](#)]
15. Zuorro, A. Optimization of polyphenol recovery from espresso coffee residues using factorial design and response surface methodology. *Sep. Purif. Technol.* **2015**, *152*, 64–69. [[CrossRef](#)]
16. Xu, C.C.; Wang, B.; Pu, Y.Q.; Tao, J.S.; Zhang, T. Advances in extraction and analysis of phenolic compounds from plant materials. *Chin. J. Nat. Med.* **2017**, *15*, 721–731. [[CrossRef](#)]
17. Meneses, N.G.T.; Martins, S.; Teixeira, J.A.; Mussatto, S.L. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer’s spent grains. *Sep. Purif. Technol.* **2013**, *108*, 152–158. [[CrossRef](#)]
18. Socaci, S.A.; Fărcaș, A.C.; Diaconeasa, Z.M.; Vodnar, D.C.; Rusu, B.; Tofană, M. Influence of the extraction solvent on phenolic content, antioxidant, antimicrobial and antimutagenic activities of brewers’ spent grain. *J. Cereal Sci.* **2018**, *80*, 180–187. [[CrossRef](#)]
19. Hansen, C.M. The three dimensional solubility parameter—Key to paint component affinities. I. Solvents, plasticizers polymers, and resins. *J. Paint Technol.* **1967**, *39*, 104–117.
20. Mohammad, M.A.; Alhalaweh, A.; Velaga, S.P. Hansen solubility parameter as a tool to predict cocrystal formation. *Int. J. Pharm.* **2011**, *407*, 63–71. [[CrossRef](#)] [[PubMed](#)]
21. Lavecchia, R.; Zuorro, A. Evaluation of olive pomace as a source of phenolic antioxidants for the production of functional cosmetics. *Int. J. Appl. Eng. Res.* **2015**, *14*, 34405–34409.
22. Zuorro, A.; Lavecchia, R. Influence of extraction conditions on the recovery of phenolic antioxidants from spent coffee grounds. *Am. J. Appl. Sci.* **2013**, *10*, 478–486. [[CrossRef](#)]
23. Maietta, M.; Colombo, R.; Lavecchia, R.; Sorrenti, M.; Zuorro, A.; Papetti, A. Artichoke (*Cynara cardunculus* L. var. *scolymus*) waste as a natural source of carbonyl trapping and antiglycative agents. *Food Res. Int.* **2017**, *100*, 180–190.
24. Hoftyzer, P.J.; Van Krevelen, D.W. *Properties of Polymers*, 2nd ed.; Elsevier: Amsterdam, The Netherlands, 1976; pp. 152–155.
25. Fedors, R. A method for estimating both the solubility parameters and molar volumes of liquids. *Polym. Eng. Sci.* **1974**, *14*, 147–154. [[CrossRef](#)]
26. Hansen, C.M. *Hansen Solubility Parameters: A User’s Handbook*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2007; pp. 27–43.
27. Szwajgier, D.; Waško, A.; Targoński, Z.; Niedźwiadek, M.; Bancarzewska, M. The use of a novel ferulic acid esterase from *Lactobacillus acidophilus* K1 for the release of phenolic acids from brewer’s spent grain. *J. Inst. Brew.* **2010**, *116*, 293–303. [[CrossRef](#)]
28. Kowalska, H.; Czajkowska, K.; Cichowska, J.; Lenart, A. What’s new in biopotential of fruit and vegetable by-products applied in the food processing industry. *Trends Food Sci. Technol.* **2017**, *67*, 150–159. [[CrossRef](#)]
29. Kumar, K.; Yadav, A.N.; Kumar, V.; Vyas, P.; Dhaliwal, H.S. Food waste: A potential bioresource for extraction of nutraceuticals and bioactive compounds. *Bioresour. Bioprocess.* **2017**, *4*, 18. [[CrossRef](#)]

30. Panusa, A.; Zuorro, A.; Lavecchia, R.; Marrosu, G.; Petrucci, R. Recovery of natural antioxidants from spent coffee grounds. *J. Agric. Food Chem.* **2015**, *61*, 4162–4168. [[CrossRef](#)] [[PubMed](#)]
31. Zuorro, A.; Maffei, G.; Lavecchia, R. Effect of solvent type and extraction conditions on the recovery of phenolic compounds from artichoke waste. *Chem. Eng. Trans.* **2014**, *39*, 463–468.
32. Dorta, E.; Lobo, M.G.; Gonzalez, M. Reutilization of mango byproducts: Study of the effect of extraction solvent and temperature on their antioxidant properties. *J. Food Sci.* **2012**, *77*, C80–C88. [[CrossRef](#)] [[PubMed](#)]
33. Guiné, R.P.F.; Gonçalves, F.; Lerat, C.; El Idrissi, T.; Rodrigo, E.; Correia, P.M.R.; Gonçalves, J.C. Extraction of phenolic compounds with antioxidant activity from beetroot (*Beta vulgaris* L.). *Curr. Nutr. Food Sci.* **2018**, *14*, 350–357. [[CrossRef](#)]
34. Liu, F.F.; Ang, C.Y.W.; Springer, D. Optimization of extraction conditions for active components in *Hypericum perforatum* using response surface methodology. *J. Agric. Food Chem.* **2000**, *48*, 3364–3371. [[CrossRef](#)] [[PubMed](#)]
35. Hernanz, D.; Nuñez, V.; Sancho, A.I.; Faulds, C.B.; Williamson, G.; Bartolomé, B.; Gómez-Cordovés, C. Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley. *J. Agric. Food Chem.* **2001**, *49*, 4884–4888. [[CrossRef](#)] [[PubMed](#)]
36. Dykes, L.; Rooney, L.W. Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World* **2007**, *52*, 105–111. [[CrossRef](#)]
37. Albersheim, P.; Darvill, A.; Roberts, K.; Sederoff, R.; Staehelin, A. *Plant Cell Walls: From Chemistry to Biology*; Garland Science: New York, NY, USA, 2010.
38. Lavecchia, R.; Zuorro, A. Cellulase Applications in Pigment and Bioactive Compound Extraction. In *New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Cellulase System Properties and Applications*; Gupta, V.K., Ed.; Elsevier: Amsterdam, The Netherlands, 2016; pp. 209–222.
39. El Seoud, O.A. Understanding solvation. *Pure Appl. Chem.* **2009**, *81*, 697–707. [[CrossRef](#)]
40. Fidale, L.C.; Ruiz, N.; Heinze, T.; El Seoud, O.A. Cellulose swelling by aprotic and protic solvents: What are the similarities and differences? *Macromol. Chem. Phys.* **2008**, *209*, 1240–1254. [[CrossRef](#)]
41. Idehen, E.; Tang, Y.; Sang, S. Bioactive phytochemicals in barley. *J. Food Drug Anal.* **2017**, *25*, 148–161. [[CrossRef](#)] [[PubMed](#)]
42. Sun, R.C.; Sun, X.F.; Wang, S.Q.; Zhu, W.; Wang, X.Y. Ester and ether linkages between hydroxycinnamic acids and lignin from wheat, rice rye, and barley straws, maize stems, and fast-growing poplar wood. *Ind. Crop Prod.* **2002**, *15*, 179–188. [[CrossRef](#)]
43. Damoderan, S. Protein: Denaturation. In *Handbook of Food Science, Technology and Engineering*; Hui, Y.K., Ed.; CRC Press: Boca Raton, FL, USA, 2005; pp. 6-1–6-14.
44. Hildebrand, J.H.; Scott, R.L. Solutions of nonelectrolytes. *Annu. Rev. Phys. Chem.* **1950**, *1*, 75–92. [[CrossRef](#)]
45. Güner, A. The algorithmic calculations of solubility parameter for the determination of interactions in dextran/certain polar solvent systems. *Eur. Polym. J.* **2004**, *40*, 1587–1594. [[CrossRef](#)]
46. Hansen, C.M.; Skaarup, K. The three dimensional solubility parameter—Key to paint component affinities III. *J. Paint Technol.* **1967**, *39*, 511–514.

