sustainability

MDPI

Article

Optimization of Phenolic Compound Extraction from Brewers' Spent Grain Using Ultrasound Technologies Coupled with Response Surface Methodology

Rosamaria Iadecola [1], Roberto Ciccoritti [2,*], Brunella Ceccantoni [1], Andrea Bellincontro [1] and Tiziana Amoriello [3,*]

[1] Department for Innovation in Biological, Agro-Food and Forest Systems, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy; r.iadecola@gmail.com (R.I.); b.ceccantoni@unitus.it (B.C.); bellin@unitus.it (A.B.)
[2] CREA Research Centre for Olive, Citrus and Tree Fruit, Via di Fioranello 52, 00134 Rome, Italy
[3] CREA Research Centre for Food and Nutrition, Via Ardeatina 546, 00178 Rome, Italy
* Correspondence: roberto.ciccoritti@crea.gov.it (R.C.); tiziana.amoriello@crea.gov.it (T.A.)

Abstract: Brewers' spent grain (BSG) is the main solid by-product from the brewery industry, rich in valuable nutrients and bioactive compounds. The aim of this study was to valorize this by-product, recovering phenolic compounds from BSG using ultrasound-assisted extraction (UAE) and chemometric techniques, such as the response surface methodology (RSM). Therefore, UAE process parameters (temperature and time) and solvent composition (ethanol aqueous mixtures) were optimized using a three-level Box–Behnken design, in order to carry out the maximum yield in phenols. Then, the extract obtained under optimal conditions was characterized for the total phenolic content and antioxidant capacity (2,20-azino-bis(3-ethylbenothiazoline-6-sulphonic acid, ABTS, and 2,2-diphenyl-1-picrylhydrazyl, DPPH), and individual phenolic compounds were identified using HPLC-DAD. The results show the highest level of total soluble phenolic content (4.1 ± 0.1 mg GAE/g d.w.) at 80 °C, 50 min and 65:35% ethanol:water, with a high goodness of fit between experimental and predicted values (R^2 = 0.987), and a high antioxidant potential (DPPH: 0.42 ± 0.01 mg TE eq/g d.w.; ABTS: 5.82 ± 0.04 mg TE eq/g d.w.). A comparison between the classic extraction techniques and the UAE with the same solvent showed an increase of 156% in the phenol yield. The characterization of phenolic profile revealed that ferulic acid (1.5 ± 0.2 mg/L), vanillic acid (0.78 ± 0.18 mg/L) and p-coumaric acid (0.12 ± 0.03 mg/L) were the prevalent ones. UAE coupled with RSM was a useful tool to inexpensively and quickly recover bioactive phenolic compounds from BSG, which can be used in the food, pharmaceutical or cosmetic industries.

Keywords: brewers' spent grain; drying process; extraction condition optimization; antioxidant activity; phenolic profile

Citation: Iadecola, R.; Ciccoritti, R.; Ceccantoni, B.; Bellincontro, A.; Amoriello, T. Optimization of Phenolic Compound Extraction from Brewers' Spent Grain Using Ultrasound Technologies Coupled with Response Surface Methodology. *Sustainability* **2022**, *14*, 3309. https://doi.org/10.3390/su14063309

Academic Editor: Attila Gere

Received: 7 February 2022
Accepted: 8 March 2022
Published: 11 March 2022

1. Introduction

The European green deal strategies aim to boost the efficient use of resources by moving to a clean, circular economy, stop environmental degradation and climate change, revert biodiversity loss and cut pollution [1]. The coronavirus pandemic has only highlighted the need to accelerate the transition processes to make the economy competitive and inclusive, implementing resource efficiency pathways and providing a high standard of living with much lower environmental impacts. To address these global challenges, it is necessary to rethink and reshape the entire global economy, building an economic system based on circular economy models, with measures covering the full life cycle of products, from production and consumption to waste management and the market for secondary raw materials [2]. In this context, industries must develop innovative processes, valorizing residual flows, obtaining new high added-value products and minimizing the disposal of their residuals in the environment [3].

The brewing industry generates large amounts of by-products and waste: wastewater, brewers' spent grain (BSG), spent yeast, spent hops, hot trub and diatomaceous earth [3–5]. Among these, BSG is the main solid waste (around 20 kg hL^{-1} beer), accounting for around 85% of the total by-products produced [6]. BSG basically consists of residues from barley grain and other cereals (rice, wheat, maize, sorghum, oat, etc.) after the mashing and filtering processes [4]. In particular, BSG is mainly composed of the grain husk, and pericarp, seed coat and fragments of endosperm to a lesser extent [4,7].

BSG is mainly composed of lignocellulosic material rich in fibers (20–70%), proteins (19–30%), lipids (about 10%), minerals (2–5%), and vitamins and polyphenols (0.7–2.0%) [3]. However, these constituents can vary due to many factors, such as cereal species and varieties used in the brewing process, pedoclimatic condition, soil management [8] and technological processes such as the malting and mashing processes, etc. [9]. Owing to its significant chemical composition, BSG can be used for different purposes. In general, BSG is sold as animal feed, for energy production and agricultural applications, but it can be very attractive for application in the food, pharmaceutical and cosmetic industries [10–26].

In the last few years, great attention has been paid to the phenolic compounds due to their antioxidant, antiallergenic, anti-inflammatory and antimicrobial properties [3,27]. Phenolic compounds comprise flavonoids, phenolic acids, and tannins. In cereals, they can be found as insoluble bound forms, soluble conjugate, and free forms (the latter soluble in extraction solvents) [28]. Among them, the ferulic acid and p-coumaric acid are the most abundant in BSG [21,29,30]. However, BSG recovery using green chemistry techniques and chemometric tools is still under exploited. Different techniques of extraction, namely, supercritical fluid extraction, microwave-assisted extraction, saponification, enzymatic hydrolysis, pressurized liquid extraction, and liquid-solid extraction, were tested [23,31–37]. Recently, the ultrasound-assisted extraction (UAE) technique was considered as a green approach for an efficient, environmentally friendly, sustainable and cost-effective recovery of high added-value bioactive components, such as phenolic compounds, from agri-food by-products [38]. Furthermore, this technique is up-and-coming for the extraction of thermolabile phenolic compounds also due to the reduced solvent use and energy consumption in comparison with conventional extraction methods. The UAE technique consists in using mechanic vibrations caused by sound waves with frequencies higher than 20 kHz [35]. The vibrations generate local hotspots on the macroscopic scale with high shear stress and temperature by producing cavitational bubbles throughout the solvent (in contact with a sample) that collapse, causing pressure and temperature changes and, therefore, enhancing the rate of the mass transfer of analytes to the solvent. In fact, when the cavitation bubbles burst at the surface of the sample matrix, shockwave-induced damage to the cell wall enhances the mass transfer of phenolic compounds across cellular membranes into solution [39]. Furthermore, the choice of the solvent used in the extraction procedure must be taken into account. Methanol, acetone and ethanol are the most used solvents in different extraction techniques. However, among these, ethanol, and its mixture with water, can be considered as a green solvent at the same time.

The efficiency of a UAE extraction is related to the optimization of different operative parameters, such as time, temperature, and solvent choice. To determine the optimal conditions, a Response Surface Methodology approach was considered. It allows one to achieve the desired maximum phenolic content values, to save chemicals, raw material and consumables, to reduce laboratory time and costs, and to save energy. To the best of our knowledge, two studies were carried out to determine optimal conditions for the extraction of phenols from BSG [37,40], but neither considered UAE and ethanol as a solvent.

In light of these considerations, the aim of this study was to investigate the potentiality of UAE as a green extraction technique for the recovery of phenolic compounds from BSG. To do this, process factors such as temperature, time and solvent composition (water and ethanol) were optimized to lead to a maximum yield in phenols. The optimal phenolic content was also compared with that obtained by extraction with traditional solvent, i.e., acidified methanol or with a mixture of ethanol and water, in order to determine the

efficiency of the proposed technique. At last, the antioxidant activity and the composition of phenols from BSG extracts were determined.

2. Materials and Methods

2.1. Raw Materials

Fresh brewers' spent grains were kindly supplied by an industrial brewery (Birra Peroni s.r.l., Rome, Italy). BSG was obtained from a single lot after the filtration stage during the beer production, and immediately oven-dried until the moisture content was less than 10%. Then, the dried BSG was ground by a Bühler MLI 203 sifter (Milan, Italy) and sieved to obtain a fine flour with particles size from 400 to 500 µm. BSG was derived from a blend of barley malt and corn, also provided by the brewery.

2.2. BSG Drying Process

Since wet BSG contains more than 80% water mixed with fermentable sugars, in order to make it conservable and avoid microbiological spoilage, the water content was reduced from about 83% by weight (value of the sample just filtered) to values under 10%, below which microbial growth is strongly inhibited [4]. To determine the optimal BSG drying conditions, three tests were carried out by varying the temperature (50, 60, 70 °C) and time. The time value for each of the three tests was identified as a function of the achievement of the constant weight of the dried samples. Therefore, 500 g of BSG inside aluminum trays was placed simultaneously in three pre-set ventilated stoves of the Intercontinental mod. DAS 46010 (Anzio, Italy). Samples were replicated three times. The optimal drying was identified in terms of preservation of phenolic compounds and energy saving.

2.3. Proximate Composition

Moisture, proteins, lipids and ashes were determined by the ICC standard methods 110/1, 105/2, 136, and 104/1, respectively [41]. Protein content was estimated using the conversion factor 5.83 for barley malt and 6.25 for BSG and corn flours. Total dietary fiber (TDF) content was measured according to Lee et al. [42], using a reagent kit (K-TDFR, Megazyme Int., Wicklow, Ireland). All determinations were made in triplicate and result expressed on dry weight (d.w.).

2.4. Color Measurement

Color measurements were taken on raw materials using a Chroma Meter CR-200 (Konica Minolta, Tokyo, Japan) and (CIE) L*a*b* scale. The results (L*, a*, b*) are the average of measurements of five different points per sample.

2.5. FTIR Analysis

FTIR-ATR spectrometer (Nicolet iS 10 FT-IR Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a diamond crystal cell (ATR) was used for MIR spectra acquisition. The spectra were acquired as described by Amoriello et al. [43] and then processed with the OMNIC™ software (Thermo Fisher Scientific Inc., USA).

2.6. Extraction of Phenolic Compounds from BSG by Ultrasound-Assisted Extraction

Phenolic compounds in BSG were extracted through ultrasound-assisted extraction (UAE) by an ultrasonic bath (ElmasonicS30H, Elma Ultrasonic Technology, Singen, Germany). Briefly, for each extraction batch, 2 g of dried and milled BSG was weighted in a tube and diluted with 40 mL of different solvent mixed and sonicated at 37 kHz for different time. The solvent used for the extraction was a mixture of ethanol and water (with different ratio). On the contrary, acidified methanol:water (80:20, 0.1% HCl) was used to distinguish TCP from UAE and TPC from a classic extraction technique.

Extraction time, solvent composition, and input power are the three major factors affecting UAE extraction efficiency. Optimization of the conditions can be performed using a response surface methodology (RSM) approach. Therefore, experiments were

established based on a Box–Behnken design (BBD) with three independent factors (solvent composition, extraction time, and extraction temperature), known to affect extraction yield and phytochemical contents [44]. BBD is a spherical, rotatable design, which is viewed on a cube, and consists of a central point and middle points of the edges [45]. BBD considers a specific subset of the factorial combinations from the 3^k factorial design. The design included 15 runs (three at the central point); each run was replicated three times. Each factor was coded at three levels, −1, 0, and +1 (Table 1). Solvent composition (X_1) is a mixture of water and ethanol; ethanol percentage ranged between 50 % and 80 %. Extraction time (X_2) varied between 50 and 70 min, whereas extraction temperature (X_3) ranged from 70 to 80 °C. These values were assessed by the literature [35–37,40] and resulted from a pilot study (data not shown). The maximum value for extraction temperature was set at 80 °C, because this value corresponded to the maximum settable temperature of the ultrasound equipment. Regarding the range for extraction time, the pilot study showed higher TPC values around 60 °C. Therefore, we chose the range between 50 and 70 °C.

Table 1. Box–Behnken design with coded and uncoded parameters of ultrasound-assisted extraction (UAE), and experimental and predicted response values (Total Phenolic Content, TPC) in the extracts using response surface methodology (RSM).

Run	X_1	X_2	X_3	X_1 (%)	X_2 (min)	X_3 (°C)	TPC_{exp} (mgGAE/g d.w.)	TPC_{RSM} (mgGAE/g d.w.)
1	−1	−1	0	50	50	75	3.10 ± 0.02	3.13
2	1	−1	0	80	50	75	3.05 ± 0.01	3.01
3	−1	1	0	50	70	75	2.79 ± 0.03	2.87
4	1	1	0	80	70	75	2.87 ± 0.02	2.87
5	−1	0	−1	50	60	70	2.01 ± 0.01	1.94
6	1	0	−1	80	60	70	2.33 ± 0.02	2.35
7	−1	0	1	50	60	80	3.53 ± 0.02	3.55
8	1	0	1	80	60	80	2.92 ± 0.05	3.03
9	0	−1	−1	65	50	70	2.81 ± 0.06	2.89
10	0	1	−1	65	70	70	2.73 ± 0.03	2.76
11	0	−1	1	65	50	80	4.10 ± 0.08	4.11
12	0	1	1	65	70	80	3.89 ± 0.03	3.84
13	0	0	0	65	60	75	3.77 ± 0.07	3.75
14	0	0	0	65	60	75	3.77 ± 0.07	3.75
15	0	0	0	65	60	75	3.77 ± 0.07	3.75

Legend: X_1 = solvent composition (%); X_2 = extraction time (min); X_3 = extraction temperature (°C).

Powdered BSG was solved in the extraction solvent and the mixture was heated and sonicated for a time as in Table 1. Sample-to-solvent ratio 20:80 (v/v) was fixed in this study.

At the end of sonication, extracted product was left to cool at room temperature, then centrifuged at 3000 rpm for 20 min at 4 °C. At last, each sample was filtered with nylon syringe filter and the supernatants were immediately analyzed.

2.7. The RSM Model and the Optimization Procedure

The predicted response of TPC was obtained using a second-order polynomial equation, as follows:

$$Y_i = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^2 + \sum_{ij,\, i<j} \beta_{ij} X_i X_j + e_i \quad (1)$$

where Y = total phenolic content (TPC); X_1 = solvent composition (%); X_2 = extraction time (min); X_3 = extraction temperature (°C); β_0 = intercept; β_i, β_{ii}, β_{ij} = linear, quadratic and interactive coefficients, respectively; e_i = error term.

The statistical significance of the main effects, the interactions and the quadratic terms, regression coefficients and model fitting were found by analysis of variance (ANOVA). Goodness of fit of the second order equation was checked by the coefficient of determination

R^2, the adjusted coefficient of determination $R_{adj}{}^2$, the lack of fit value, and the absolute average deviation (AAD) value. ADD was defined as follows [46]:

$$ADD = 100 \times \left\{ \left[\sum_{i=1}^{n} \left(\left| Y_{i,exp} - Y_{i,pred} \right| \right) / Y_{i,pred} \right] / n \right\} \quad (2)$$

where $Y_{i,exp}$ and $Y_{i,pred}$ are the experimental and calculated responses, respectively, and n is the number of experimental run. In order to obtain a better accuracy, R^2 and $R_{adj}{}^2$ must be close to 1.0 and the AAD between the predicted and experimental data has to be as small as possible, better if less than 5 [46].

Three-dimensional plots were outlined to understand the relationships between the response and experimental levels of each factor.

A desirability-based method for yielding compromise solutions with desired response properties was used to assess optimal variable settings of each factor [47]. The overall desirability (D) is defined as the geometric mean of the individual desirability of each response. It ranges between 0 and 1, where 0 is completely undesirable and 1 is most favorable response. The optimal combinations of factors will be those that maximize the overall desirability.

2.8. Determination of Total Phenolic Content

The total phenolic content (TPC) of extracts was determined using the Folin–Ciocalteu (F–C) method as reported by Ciccoritti et al., [48]. TPC was calculated from a calibration curve, using Gallic acids as a standard. Results are expressed as micrograms of Gallic acid equivalents (GAE) per g of whole milled spent grain (d.w.).

2.9. Identification and Quantification of Phenols from BSG Extracts by HPLC-DAD

A liquid chromatography apparatus, Dionex (Dionex Corporation Sunnyvale, Sunnyvale, CA, USA), controlled by Chromeleon software (version 6.50) and equipped with P680 quaternary pump, manual injector (Rheodyne) with 20 µL loop, TCC-100 thermostatic oven, and PDA 100 detector (Photodiode Array Detector) was used.

The separation was carried out with a Dionex Acclaim® 120 C18, 5 µm, 4.6 × 250 mm column thermostated at 30 °C. The mobile phase consists of a ternary gradient consisting of: solvent A = 50 mM ammonium dihydrogen phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B = 20% solvent A and 80% acetonitrile; solvent C = 0.2 M orthophosphoric acid adjusted to pH 1.5 with NaOH according to the method developed by Ritchey and Waterhouse [49].

Phenolic compounds were identified on the basis of their retention time and the characteristics of their Uv-Vis spectra at wavelengths of 280 nm for hydroxybenzoic acids and 316 nm for hydroxycinnamic acids. The HPLC analysis was replicated three times for samples and calibration points (n = 3). The HPLC method was validated by assessing precision, linearity, limit of detection (LOD) and limit of quantification (LOQ). Precision was evaluated by intra-day and inter-day repeatability, as relative standard deviation of both retention time and ratio of the analyte response to that of the standard for all phenolic acids (PA) considered, which were analyzed in triplicate during the same day and over three consecutive days. Linearity was evaluated by analyzing mixtures of phenolic acid standard solutions at five equally spaced concentrations within appropriate ranges, employing linear least-squares regression analysis to calculate slope, intercept, and correlation coefficient of the calibration graphs constructed as reported above. The limit of detection (LOD) and the limit of quantification (LOQ) were estimated as the concentrations of phenolic acids producing chromatographic peaks with a height at least three times and ten times as high as the baseline, respectively.

2.10. Determination of Antioxidant Activity

The DPPH radical dot quenching capacity of BSG extracts was determined spectrophotometrically. Briefly, 500 µL of sample extracts was mixed with 500 µL of pure methanol and 250 µL the DPPH radical solution diluted in absolute. The analysis was performed in triplicate and the total antioxidant capacity was expressed as mg of Trolox equivalent per gram of sample on a dry weight basis (mg TE g d.w.) by mean of a Trolox dose–response curve.

The ABTS values were determined according to the method described by Ciccorittiet al. [48] with minor modifications. Briefly, 20 µL of sample extracts were mixed to 980 µL of the ABTS radical solution diluted in absolute ethanol to reach an absorbance of 0.7 OD at 734 nm. The antioxidant capacity was expressed as mg of Trolox equivalent per g (mg TE g d.w.) using a Trolox dose–response curve. Samples with values over the range of calibration were opportunely diluted with ethanol before the analysis.

2.11. Statistical Analysis

All tests were replicated three times, and mean values and standard deviations were calculated. A one-way analysis of variance (ANOVA) employing the Kruskal–Wallis non-parametric test at a significance level of 5% was carried out to determine significant differences in all measured properties. Data were processed using SPSS statistical software (version 22, SPSS, Chicago, IL, USA).

The experimental design, RSM analysis and optimization procedure were carried out using Statistica statistical package software (Stat Soft Inc., Tulsa, OK, USA).

3. Results and Discussion

3.1. Optimal Drying Process

The BSG optimal drying process was determined by considering at the same time the higher total polyphenol content as a function of the set temperatures and the time obtained to reach a constant moisture content of the samples. Table 2 did not show statistical differences between polyphenol content in the extract from samples treated at 50 and 60 °C (2.3 ± 0.2 and 2.1 ± 0.2 mg GAE/g d.w., respectively), whereas, in the extract from samples treated at 70 °C, TPC was lower.

Table 2. Total polyphenol content of BSG samples extracted with acidified 80:20 methanol.

Temperature (°C)	Moisture (g/100 g)	Time (h)	TPC (mg GAE/g d.w.)
50	7.7 ± 0.2	48	2.3 ± 0.2 a
60	3.7 ± 0.2	24	2.1 ± 0.2 a
70	1.9 ± 0.1	18	1.6 ± 0.1 b

Legend: TPC = total soluble phenolic content; h = hours; d.w. = dry weight. Different letters indicate that averages are significantly different from each other ($p < 0.05$).

Summing up, the extractability of phenolic compounds decreased after hot air-treatment at 70 °C, in agreement with a previous study on the effects of thermal treatment on cereals or BSG [9,48,50]. Taking into account that twice the drying time was required at 50 °C than at 60 °C, and therefore with an increase in energy cost, the BSG samples were dried at 60 °C.

3.2. Raw Material Characterization

3.2.1. Proximate Composition

Chemical and physical composition (moisture, ash, protein, lipids, fiber components and color coordinates) of samples of corn, barley malt and BSG is shown in Table 3.

As expected, the highest moisture content (82.9 g/100 g of fresh weight) was observed in BSG before the oven drying process, in accordance with Mathias et al. [50] and Santos et al. [9], deriving BSG from the enzymatic conversion phase (mashing), during which barley malt and corn were mixed with water. The moisture values of barley malt

and corn were much lower than fresh BSG. After the oven drying process, BSG moisture level decreased at 5.2 g/100 g of fresh weight.

Table 3. Biochemical composition of raw materials.

	Corn	Barley Malt	BSG
Moisture (g/100 g)	13.1 ± 0.2	6.3 ± 0.1	82.9 ± 0.9
Protein (g/100 g d.w.)	6.7 ± 0.1	10.4 ± 0.5	26.9 ± 0.5
Ash (g/100 g d.w.)	0.40 ± 0.01	2.17 ± 0.01	3.63 ± 0.02
Lipids (g/100 g d.w.)	1.7 ± 0.1	3.4 ± 0.1	10.7 ± 0.1
TDF (g/100 g d.w.)	3.3 ± 0.4	16.8 ± 0.1	50.8 ± 0.9
TPC (mg GAE/g d.w.)	1.55 ± 0.16	6.54 ± 0.17	3.16 ± 0.03
L*	89.62 ± 0.09	82.70 ± 0.29	54.11 ± 0.65
a*	−0.49 ± 0.07	1.50 ± 0.04	6.34 ± 0.05
b*	37.89 ± 0.69	14.34 ± 0.35	21.36 ± 0.35

Legend: TDF = total dietary fiber; TPC = total soluble phenolic content; L* = luminosity; a* = redness; b* = yellowness; d.w. = dry weight.

The BSG total protein content was 26.9 g/100 g d.w., which was more than double that of barley malt and four times that of corn, similar to levels observed by Waters et al. [51] and Celus et al. [52]. In general, the protein content of BSG can vary quite considerably but typically is present at levels of about 20% per dry weight basis [53]. The ash content of BSG was 3.63 g/100 g d.w., which was in excess of the levels in barley malt or corn. The fat content value of BSG (10.7 g/100 g d.w.) was in keeping with results from Kanauachi et al. [14], but in excess of those reported in previous studies [9,20,54]. However, as reported by Lynch et al. [53], lipids in BSG can vary between 3 and 13 g/100 g d.w. Regarding the total dietary fiber, corn showed the lowest value (3.0 g/100 g d.w.), followed by barley malt (16.8 g/100 g d.w.). BSG is confirmed as a ligno-cellulosic material rich in fiber, which can reach up to 70% of its composition. In our sample, TDF was equal to 50.8 g/100 g d.w., in the range of the results of other authors [36,51]. At last, the total soluble phenolic content showed the highest level for the barley malt sample (6.54 mg GAE/g d.w.). As regards BSG, its value is influenced by the presence of corn, which strongly reduced the phenol content. In addition, the differences in TPC observed between malt and BSG could be due to a free phenol from solubilization in water during the water and malt mashing process.

The differences in values of all parameters recorded by different authors might be due to the different brewery process conditions (efficiency of malting and mashing), to the cereal used and their proportions, growing conditions and time of harvesting for cereals, etc. [36,53]. Moreover, the different localization of the nutrients and bioactive compounds throughout the kernel could affect BSG biochemical composition. In particular, all investigated parameters except for proteins, which are found also in kernel endosperm, are characteristic of the layers of seed coat, pericarp and husk that covered the original barley grain. As is well known, the malt carbohydrates, which mainly make up the endosperm of the seed, are solubilized during the mashing section of the brewing process, causing the BSG to be mainly characterized by the outermost layers of the caryopsis. Furthermore, in relation to the mashing efficiency, a more or less starchy endosperm content and variable empty aleurone cell walls may remain [53], causing a significant variation in carbohydrate with respect to the raw materials.

Color parameters of raw materials are reported in Table 3. BSG showed a higher darkness (L* = 54.11) in comparison with barley malt (L* = 82.70) and corn (L* = 89.62). As for the redness variable, higher a* values indicate a redshift, whereas lower a* a greenness. At the same time, the b* coordinate describes the yellowness, from blue (−b *) to yellow (+ b *). In our samples, the lowest a* and highest b* values were observed for corn, and the highest a* and lowest b* for BSG.

3.2.2. Infrared Spectra of Raw Materials

FTIR-ATR spectra of BSG, corn and barley malt were collected in the range 4000–650 cm^{-1} to characterize the chemical structure of raw materials by identifying their functional groups, including carboxyl, hydroxyl, amino and amide groups of hydrocarbons, and proteins [55]. In particular, a variable number of characteristic absorption peaks revealed at different wavenumbers in relation to the different raw materials (from 14 to 15) was clearly noticeable (Figure 1, Table 4) and revealed a different chemical composition of samples.

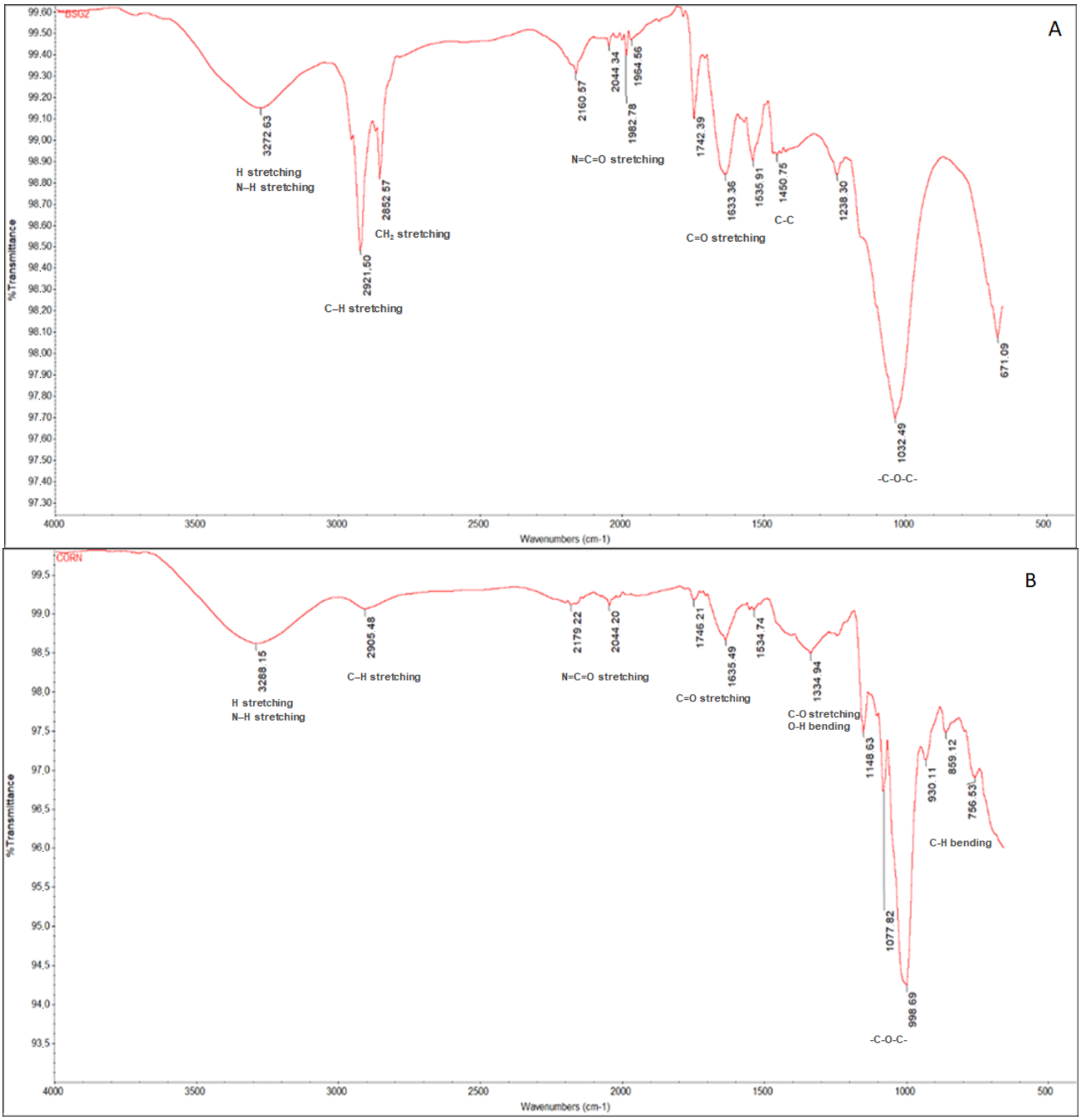

Figure 1. *Cont.*

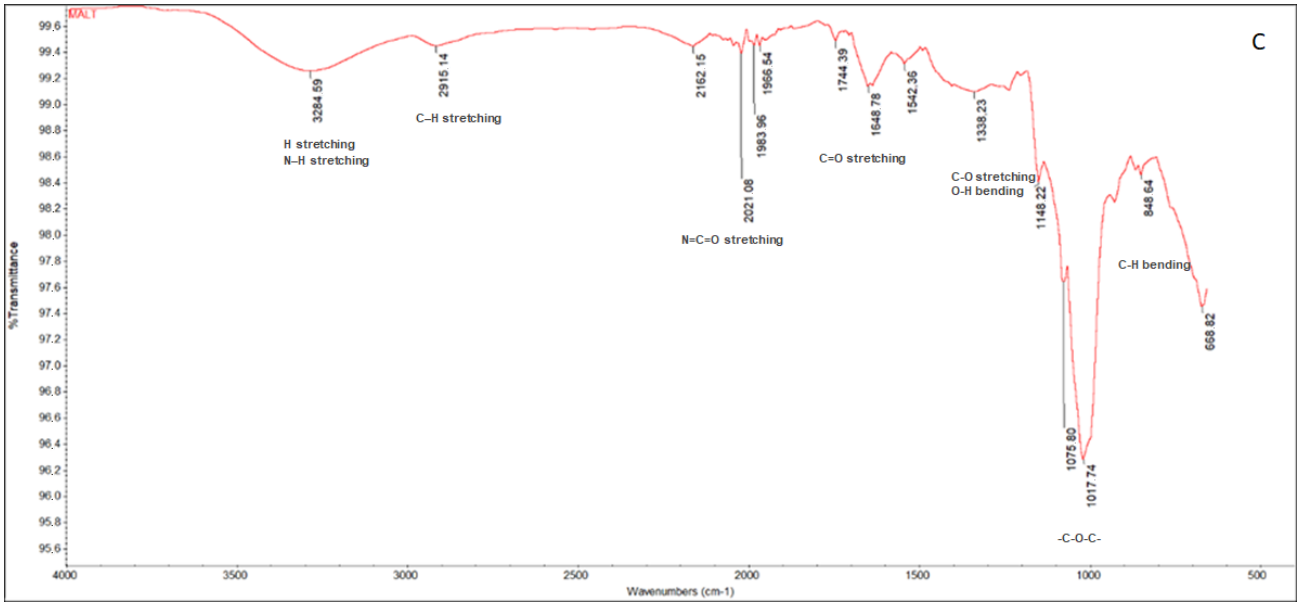

Figure 1. Typical average FTIR spectra and characteristic peaks of measured raw material samples ((**A**) BSG, (**B**) corn, and (**C**) barley malt).

Table 4. Spectral features of raw materials.

Main Peak (cm^{-1})	Wave Number Range (cm^{-1})	Raw Material	Typical Band
3272 3288 3284	3290–3250	BSG Corn Malt	cellulose, lignin or hemicellulose H stretching vibration of OH groups primary amines N–H stretching
2921 2905 2915	2930–2900	BSG Corn Malt	cellulose, lignin or hemicellulose C–H stretching vibrations in aliphatic chains
2852	2880–2840	BSG	Lipid –carbohydrate (CH_2) and (CH_2) stretching
1964, 1983, 2044 and 2160 2179, 2044 1953, 1962, 2044 and 2162	2200–1940	BSG Corn Malt	Isocyanate asym. stretch N=C=O
1742 and 1633 1746 and 1635 1744 and 1648	1750–1620	BSG Corn Malt	Carbonyl group C=O stretching
1536 1534 1542	1550–1530	BSG Corn Malt	Lignin aromatic ring C-C bonds
1334 1338	1340–1320	Corn Malt	C-O Stretching, O-H bending vibration presence of alcohol
1238	1240–1200	BSG	aryl-alkyl ether bonds (CAOAC)
1032 1148,1077, 998 1148, 1075 1017	1120–980	BSG Corn Malt	Carbohydrate (C-O-C) of polysaccharides
930, 859 848	979–800	Corn Malt	Out of plane C-H bending of polysaccharides

In detail, the first peak (around 3288–3272 cm^{-1}) detected in all samples could be assigned to the H stretching vibration of cellulose, lignin or hemicellulose hydroxyl groups and to the N–H stretching in primary amines [19,56]. Peaks at 2920 and 2850 cm^{-1} correspond to C–H stretching vibrations in aliphatic chains that may belong to cellulose, lignin or hemicellulose [55]. These last two peaks showed a greater amplitude of signal in the BSG spectra. In fact, BSG is characterized by a large amount of fiber and low content of starch with respect to corn and barley malt. In addition, Patrignani et al. [57] highlighted that the signals at 2852 cm^{-1} indicated structural modification in BSG when the Maillard reaction took place during beer productions. The spectral region between 2162 and 1950 cm^{-1} was characterized by 4 main peaks (at 1953, 1962, 2044 and 2162 cm^{-1}) in malt and BSG, while only two peaks (2044 and 2162 cm^{-1}) were observed for corn. This region was usually assigned by isocyanate asymmetric stretch N=C=O.

In general, the spectral regions between 1750 and 1620 cm^{-1} are characteristic of carbonyl group C=O stretching vibration in ketones, ethers, aldehydes and carboxylic acids for the first one, and assigned to the carbonyl group in aromatic rings found in lignin for the second. The peak at 1742 cm^{-1} can be attributed either to the acetyl and uronic ester groups of the hemicelluloses or to the ester linkage of the carboxylic group of the ferulic and p-coumeric acids of lignin and/or hemicelluloses [58].

The peak at 1526 cm^{-1} represented C-C bonds in the aromatic ring of lignin, whereas the peak at 1247 cm^{-1} corresponded to aryl-alkyl ether bonds (CAOAC). The peaks around 1053 to 895 cm^{-1} were directly related to the CAO stretching and CAH vibrations, distinctive of cellulose content in BSG [59].

3.3. Box–Behnken Design and Model Adequacy

A Box–Behnken design (BBD) for three factors (solvent composition, extraction time, extraction temperature) and three levels was employed to gain a second-degree model, which allows us to determine the optimal conditions of UAE extraction. A series of experiments, as described in Table 1, was carried out leading up to a region that is believed to contain the location of the optimum response. Table 1 also showed the design settings and the corresponding observed and predicted response values.

The experimental total phenolic content (TPC) found in the different BSG extracts varied from 2.01 to 4.10 mg GAE/100 g d.w. (runs 5 and 11, respectively; Table 1). The lowest TPC value was found at a solvent composition equal to 50%, extraction time 60 min, and extraction temperature 70 °C. On the contrary, the highest TPC was achieved at 65 % solvent composition, 50 min extraction time, and 80 °C extraction temperature.

The multiple regression analysis on the experimental data returned a model for the predicted response TPC (TPC_{RSM}). The relationship between dependent and independent variables can be expressed by the following quadratic polynomial equation:

$$TPC_{RSM} = -105.02807 + 0.64121\,X_1 - 0.00325\,X_1^2 + 0.08445\,X_2 - 0.00049\,X_2^2 + 2.17278\,X_3 - 0.01211\,X_3^2 + 0.00021\,X_1X_2 - 0.00311\,X_1X_3 - 0.00066\,X_2X_3$$

where X_1, X_2, and X_3 are the coded variables for solvent composition, extraction time, and extraction temperature, respectively. The magnitude and sign of the coefficients for intercept, linear, quadratic and interaction effects pointed out the influence of each factor.

The adequacy of the model was performed by the analysis of variance (ANOVA), as shown in Table 5. The linear, quadratic and two-factor interaction effect coefficients, the statistical parameter F-values, the coefficient of determination R^2, the adjusted coefficient of determination R_{adj}^2, the lack of fit value, and the absolute average deviation (AAD) value are summarized in Table 5. The high F value for all responses indicated that the model obtained was statistically significant. The second-order polynomial model was highly significant ($p < 0.0001$), describing a high degree correlation between the experimental and predicted values, as shown by its coefficient of determination R^2 (0.987) and adjusted R_{adj}^2 (0.984). Although the *p*-value of lack of fit was slightly significant ($p = 0.0321$), the sum of square of lack of fit was less than the sum of square of pure error. Therefore, this model can

be accepted. As an additional verification of the model adequacy, the AAD test was used. ADD resulted equal to 0.370, far below the threshold of 5, indicating that the model fitted the experimental data accurately.

Table 5. Analysis of variance for the second-order polynomial equation for ultrasound-assisted extraction of total phenolic compounds (TPC).

	Sum of Square	Degree of Freedom	Mean Square	F-Value	*p*-Value	Significance
X_1	0.02519	1	0.02519	4.423	0.0427	*
X_2	0.23029	1	0.23029	40.438	<0.0001	***
X_3	7.79274	1	7.79274	1368.403	<0.0001	***
X_{12}	5.91159	1	5.91159	1038.072	<0.0001	***
X_{22}	0.02622	1	0.02622	4.605	0.0389	*
X_{32}	1.01581	1	1.01581	178.375	<0.0001	***
X_1 X_2	0.01141	1	0.01141	2.004	0.1657	ns
X_1 X_3	0.65492	1	0.65492	115.004	<0.0001	***
X_2 X_3	0.01292	1	0.01292	2.269	0.1410	ns
Residual	0.19932	35	0.00570			
Lack of fit	0.09316	3	0.03105	5.361	0.0321	
Pure error	0.10615	32	0.00332			
Total SS	15.52326	44				
R^2	0.987					
R_{adj}^2	0.984					
AAD	0.370					

Legend: X_1 = solvent composition, X_2 = extraction time, X_3 = extraction temperature. * and *** indicate significance at $p < 0.05$ and 0.001, respectively; ns indicates not significant.

Solvent composition (X_1) showed a slightly significant ($p = 0.0427$) and negative linear effect on TPC, and a highly significant ($p < 0.0001$) positive quadratic effect. The extraction time (X_2) displayed a highly significant ($p < 0.0001$) and positive linear effect on TPC, and a slightly significant ($p = 0.0389$) and negative quadratic effect. The linear and quadratic terms of the extraction temperature (X_3) were both highly significant ($p < 0.0001$). However, the linear term was positively correlated, whereas the quadratic term was negatively correlated. As regards the interaction terms, only X_1X_3 resulted highly significant ($p < 0.0001$).

3.4. Analysis of Response Surface, Optimization of Extracting Parameters, and Validation of the Model

The effects on the overall response desirability of different combinations of levels of each pair of independent variables are evaluated using 3D-response surface plots (Figure 2). The desirability function showed the desirability of TPC (which can range from 0.0 for undesirable up to 1.0 for very desirable) across the observed range of each class.

When the extraction temperature was fixed at 0 level, the solvent composition (X_1) demonstrated quadratic effects on the extraction yields. In fact, the shape of the response surface was parabolic and the quadratic term of X_1 resulted highly significant (Table 5). Yields initially increased with increasing ethanol concentration until reaching maximum levels and then started to decrease above this proportion, as found by other authors [60,61]. The response surface showed that a desirable proportion for the solvent composition was between 55% and 70%. At the same time, the extraction time (X_2) did not strongly affect the extraction yields, as reported by previous studies [37,62], although values below 60 min are desirable.

When the extraction time was fixed at 0 level, the interaction between the solvent composition (X_1) and the extraction temperature (X_3) was highly significant, as shown by the elliptical profile of the contour plot. The three-dimensional plot revealed optimal yields for extraction time values (X_2) lower than 60 min, as reported by Andres et al. [37], and an extraction temperature (X_3) between 76 and 80 °C. In general, temperature can play an important role in TPC extraction. As the temperature increases, the solubility of the

compounds and the diffusion coefficients increase, while the viscosity of the solvent and the surface tension decrease [37]. At the same time, the phenolic-protein and phenolic-polysaccharide bonds weaken [37], favoring the migration of phenolic compounds into the extraction solvent [35,63]. However, too high temperature may cause degradation of phenolics and a decrease in the extraction yield due to the occurrence of degradative mechanisms, such as oxidative phenomena and the degradation of thermolabile compounds [61].

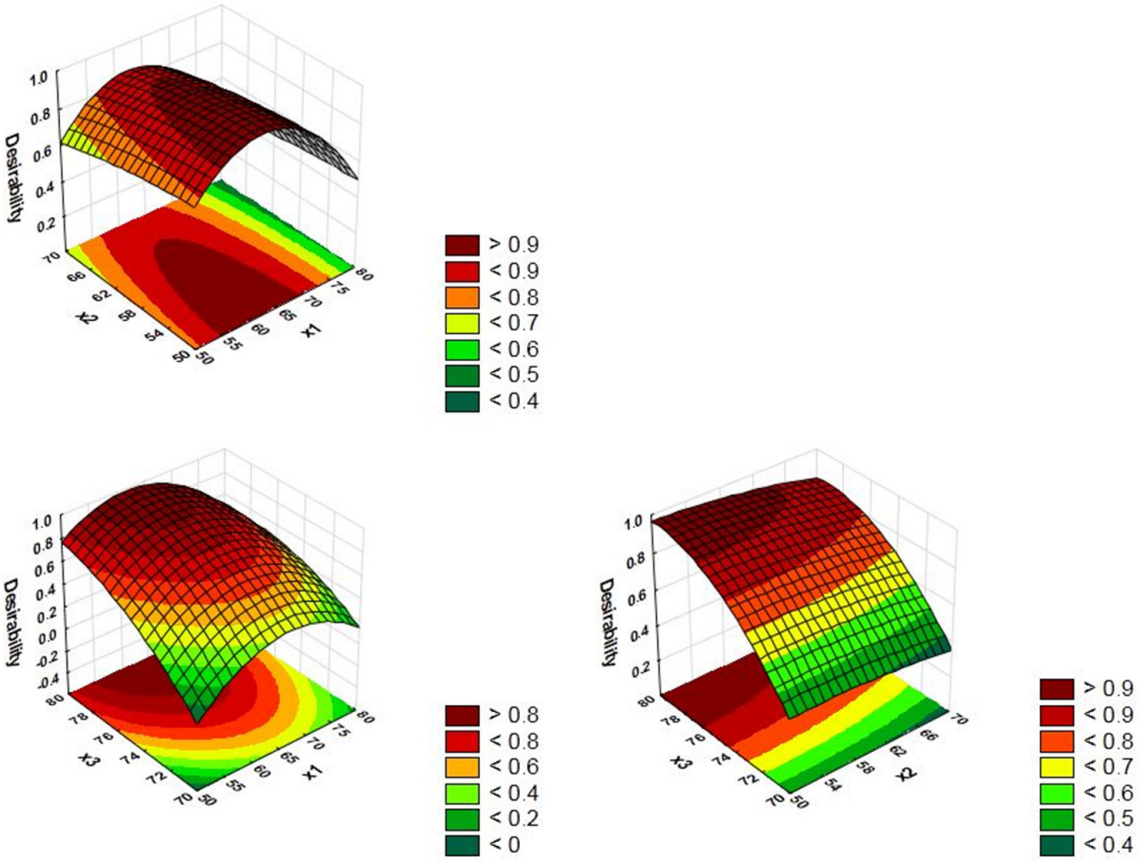

Figure 2. Response surface and contour plots of BSG extract.

At last, when the solvent composition was fixed at 0 level, optimal yields were carried out when extraction time values (X_2) were lower than 60 min and the extraction temperature (X_3) was between 76 and 80 °C.

The model optimization was carried out by maximizing the desirability of the response TPC. From a theoretical point of view, the maximal desirability should be at the maximum concentration of TPC. The desirability profile indicated that the maximum desirability level can be achieved with solvent composition X_1 = 65%, extraction time X_2 = 50 min, and extraction temperature X_3 = 80 °C.

Under these optimal conditions, the extraction yield of TPC was 4.1 ± 0.1 mg GAE/g d.w., and the predicted yield was 4.11 mg GAE/g d.w. Therefore, there was a perfect agreement ($p < 0.001$) between experimental and predicted data.

3.5. Comparison of Polyphenol Content among Different Extraction Techniques

The UAE has proved to be an excellent technique for increasing the extraction yield of phenolic compounds from complex matrices, such as BSG, if compared to other techniques. In fact, the extraction with traditional solvent, i.e., acidified methanol water solution (methanol:water 80:20 *v*:*v*) or with a mixture of ethanol and water (ethanol:water 80:20 *v*:*v*) without UAE carried out lower yields than those with UAE. In particular, the application of ultrasound resulted in an increase of 156% compared to simple extraction with ethanolic solvent (4.1 ± 0.1 mg GAE/g d.w. vs. 1.6 ± 0.1 mg GAE/g d.w., respectively)

and 28% compared to extraction with methanolic solution (4.1 ± 0.1 mg GAE/g d.w. vs. 3.2 ± 0.1 mg GAE/g d.w., respectively). These results were in keeping with those from Guido and Moreira [35]. At the same time, these authors also observed a reduction in extraction times. As is well known, the UAE technique allows for the manipulation of the solvent physical properties to reduce its superficial tension, to increase the solute solubility, and to improve the mass transfer rate; in some cases, these manipulations can also induce changes in the solvent polarity [64,65]. Ultrasound has been shown to enhance the recovery of bioactive compounds, such as polyphenols, from different plant by-products [64,66,67]. Some aspects related to the stability of the compounds extracted have not been fully addressed; however, recent studies revealed that the UAE of phenolic compounds was less degraded than others [68].

3.6. UAE Bioactive Compounds and Antioxidant Activity Characterization of Optimized Extraction

3.6.1. Bioactive Compounds and Antioxidant Activity Evaluation

The antioxidant capacity of the BSG extracts was investigated using two assays, the DPPH and ABTS radical scavenging method, widely used for plant extracts. Table 6 showed the antioxidant capacity of the BSG extract under optimal UAE conditions. DPPH was found to be 0.42 ± 0.01 mg TE eq/g d.w., and ABTS was 5.82 ± 0.04 mg TE eq/g d.w.

Table 6. Total phenolic content (TPC) and antioxidant capacity of the BSG extracts (mean ± standard deviation).

	TPC (mg GAE/g d.w.)	**DPPH (mg TE eq/g d.w.)**	**ABTS (mg TE eq/g d.w.)**
BSG	4.1 ± 0.1	0.42 ± 0.01	5.82 ± 0.04

Legend: ABTS = antiradical capacity vs. ABTS+•; DPPH = antiradical capacity vs. DPPH•.

Comparing DPPH and ABTS assays, the antioxidant potential was consistently lower (about 10 times) when DPPH assay is used. This discrepancy is closely related the different affinity of the two molecules with respect to hydrophilic (ABTS) and lipophilic (DPPH) compounds [69]. In fact, ABTS can be solubilized in aqueous and in organic solvents, in which the antioxidant activity can be measured due to the hydrophilic and lipophilic nature of the compounds [37,69]. In contrast, DPPH can only be dissolved in organic solvents (especially in alcoholic solvents), and it can represent a limitation for hydrophilic antioxidants [37,69]. Consequently, the BSG extract could be mainly characterized by water-soluble phenolic compounds, such as ferulic acid and phenolic acids in general, compared to lipophytic compounds. According to Zhu et al. [70], a high concentration of phenolic acids in solution can contribute to increasing the antioxidant potential when the ABTS assay is used. However, not only phenolic compounds can contribute to the total antioxidant capacity of the extract. Indeed, previous studies highlighted a potential antioxidant activity of the melanoidins generated during kilning by the Maillard reaction, whose content increased with temperature. These compounds are widely reported to have antioxidant properties [71], although other studies reported that melanoidins can trap polyphenols within their structure lowering the content of free phenolic compounds [72].

3.6.2. HPLC Phenolic Characterization

Since most of the phenolic compounds of the barley grain are contained in the husk and hydroxycinnamic acids accumulate in the cell walls, BSG is a potentially valuable source of phenolic acids [27]. Indeed, the characterization of the UAE extract of BSG by HPLC-DAD has revealed that this matrix was mainly composed of phenolic acids.

The identification of individual phenolic acid (PA) of BSG extract was performed by the developed high-performance liquid chromatography (HPLC) method on the basis of their retention times and UV spectra. In detail, the most abundant PA was ferulic acid (about 1.5 ± 0.2 mg/La), followed by vanillic acid (0.8 ± 0.2 mg/L) and by p-coumaric acid (0.12 ± 0.03 mg/L). Similar concentrations for ferulic acid and p-coumaric in BSG were

also reported by Lynch et al. [53], who also found small quantities of sinapic, caffeic and syringic acids. On the contrary, p-hydroxybenzoic, caffeic, syringic and sinapic acids in the optimized UAE extract were not detected, because of the low quantities of these compounds. A hydrolysis treatment would have made it possible to extract even conjugated or bound phenols more efficiently [73]. Shakeel et al. [74] highlighted a poor solubility of ferulic acid in aqueous solutions differently from hydroalcoholic ones used for our extraction. Differences among quantified phenolic compounds reported by various authors can be ascribed to many factors, such as the cereals used for beer production, the malting and mashing conditions, the oven drying process of BSG, the technique and solvents used for the extraction. As regards the HPLC method, validation reveals that the intra-day and inter-day repeatability of peak area ratio (analyte/standard) resulted to be better than 8.4% and 9.9%, respectively, whereas that of retention times was better than 0.9% and 1.5%, respectively. Linear calibration graphs with correlation coefficients better than 0.990 were obtained for all PA standards. The LOD values for ferulic acid, vanillic acid and p-coumaric acid were 0.05, 0.21 and 0.05 mg/L, respectively, whereas the LOQ values were 0.17 for ferulic acids, 0.70 for vanillic acid and 0.16 for p-coumaric acid.

4. Conclusions

The new challenge in the agri-food sector concerns reducing waste production or reuse through applying green technologies. In light of these considerations, the aim of this study was optimizing the UAE parameters, such as temperature, time and solvent composition (water and ethanol), using the RSM technique to lead to a maximum yield in phenols. The optimum conditions using the RSM model were achieved at 50 min of extraction time, 80 °C of extraction temperature and a mixture of 65% ethanol and 35% water. The RSM model showed a high value of R^2 and low values of statistical metrics (lack of fit and absolute average deviation), which indicated high accuracy and predictability of the RSM model. The UAE extraction under optimal conditions carried out a better phenol yield (+156%) in comparison with the classic extraction techniques. Thus, the UAE at RSM-optimized conditions could be a desirable method for extracting phenols from BSG. Moreover, the results of this work show an interesting bioactive compound content of BSG characterized by a large amount of phenolic compound that strongly influenced the antioxidant capacity. The characterization of phenolic profile revealed that ferulic acid, vanillic acid and p-coumaric acid were the prevalent ones. However, the extraction process strongly affected the phytochemical recovery both from a qualitative and a quantitative point of view. The parameter optimization is crucial. Our findings indicate that the modulation of the physical parameters of the UAE process promoted the recovery of TPC most involved in the biological activities. BSG extract obtained through this optimized process is rich in phenols. Therefore, it can be used as an additive or ingredient to obtain healthy natural products requested by consumers and the food, pharmaceutical and cosmetic industries. Nevertheless, further investigation is needed for the evaluation of eco-friendly extract applications.

Author Contributions: Conceptualization, T.A. and R.C.; methodology, T.A. and R.C.; software, T.A.; validation, T.A., R.C. and A.B.; formal analysis, R.I. and B.C.; investigation, T.A., R.C., R.I., B.C. and A.B.; data curation, R.I., B.C., T.A. and R.C.; writing—original draft preparation, T.A. and R.C.; writing—review and editing, T.A., R.C., R.I., B.C. and A.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Grateful acknowledgements are given to Francesco Mellara, Luigi Bartoli and Paolo Gabrielli for technical assistance.

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

References

1. European Commission. Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions- The European Green Deal. *European Commission.* 2019. Available online: https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_1&format=PDF (accessed on 1 January 2022).
2. European Commission. Directorate-General for Communication, Circular economy action plan: For a cleaner and more competitive Europe, Publications Office. 2020. Available online: https://data.europa.eu/doi/10.2779/717149 (accessed on 1 January 2022).
3. Amoriello, T.; Ciccoritti, R. Sustainability: Recovery and Reuse of Brewing-Derived By-Products. *Sustainability* **2021**, *13*, 2355. [CrossRef]
4. Mussatto, S.I.; Dragone, G.; Roberto, I.C. Brewer's spent grain: Generation, characteristics and potential applications. *J. Cer. Sci.* **2006**, *43*, 1–14. [CrossRef]
5. Tišma, M.; Juric, A.; Bucic-Kojic, A.; Panjicko, M.; Planinic, M. Biovalorization of Brewers' Spent Grain for the Production of Laccase and Polyphenols. *J. Inst. Brew.* **2018**, *124*, 182–186. [CrossRef]
6. Mussatto, S.I.; Roberto, I.C. Chemical charaterization and liberation of pentose sugars from brewer's spent grain. *J. Chem. Technol. Biotechnol.* **2006**, *81*, 268–276. [CrossRef]
7. Parek, I.; Khanvillar, A.; Naik, A. Barley-Wheat brewers' spent grain: A potential source of antioxidant rich lipids. *J. Food Process. Preserv.* **2017**, *41*, 13244. [CrossRef]
8. Nocente, F.; De Stefanis, E.; Ciccoritti, R.; Pucciarmati, S.; Taddei, F.; Campiglia, E.; Radicetti, E.; Mancinelli, R. How do conventional and organic management affect the healthy potential of durum wheat grain and semolina pasta traits? *Food Chem.* **2019**, *297*, 124884. [CrossRef] [PubMed]
9. Santos, M.; Jimenez, J.J.; Bartolome, B.; Gomez-Cordoves, C.; Nozal, M.J. Variability of brewer's spent grain within a brewery. *Food Chem.* **2003**, *80*, 17–21. [CrossRef]
10. Farcas, A.C.; Socaci, S.A.; Mudura, E.; Dulf, F.V.; Vodnar, D.C.; Tofana, M.; Salant, L.C. Exploitation of Brewing Industry Wastes to Produce Functional Ingredients. In *Brewing Technology*; Kanauchi, M., Ed.; IntechOpen: London, UK, 2017.
11. Nocente, F.; Taddei, F.; Galassi, E.; Gazza, L. Upcycling of brewers' spent grain by production of dry pasta with higher nutritional potential. *LWT-Food Sci. Technol.* **2019**, *114*, 108421. [CrossRef]
12. Barbosa-Pereira, L.; Bilbao, A.; Vilches, P.; Angulo, I.; Luis, J.; Fité, B.; Paseiro-Losada, P.; Cruz, J.M. Brewery waste as a potential source of phenolic compounds: Optimization of the extraction process and evaluation of antioxidant and antimicrobial activities. *Food Chem.* **2014**, *145*, 191–197. [CrossRef] [PubMed]
13. Nadolny, B.; Heineck, R.G.; Bazani, H.A.G.; Hemmer, J.V.; Biavatti, M.L.; Radetski, C.M.; Almerindo, G.I. Use of brewing industry waste to produce carbon-based adsorbent: Paracetamol adsorption study. *J. Environ. Sci. Health A* **2020**, *55*, 947–956. [CrossRef]
14. Kanauchi, O.; Mitsuyama, K.; Araki, Y. Development of a functional germinated barley foodstuff from brewers' spent grain for the treatment of ulcerative colitis. *J. Am. Soc. Brew. Chem.* **2001**, *59*, 59–62.
15. Assandri, D.; Pampuro, N.; Zara, G.; Cavallo, E.; Budroni, M. Suitability of Composting Process for the Disposal and Valorization of Brewer's Spent Grain. *Agriculture* **2021**, *11*, 2. [CrossRef]
16. Ferreira, S.; Monteiro, E.; Brito, P.; Castro, C.; Calado, L.; Vilarinho, C. Experimental Analysis of Brewers' Spent Grains Steam Gasification in an Allothermal Batch Reactor. *Energies* **2019**, *12*, 912. [CrossRef]
17. Jackowski, M.; Semba, D.; Trusek, A.; Wnukowski, M.; Niedzwiecki, L.; Baranowski, M.; Krochmalny, K.; Pawlak-Kruczek, H. Hydrothermal Carbonization of Brewery's Spent Grains for the Production of Solid Biofuels. *Beverages* **2019**, *5*, 12. [CrossRef]
18. Hejna, A.; Barczewski, M.; Skórczewska, K.; Szulc, J.; Chmielnicki, B.; Korol, J.; Formela, K. Sustainable upcycling of brewers' spent grain by thermo-mechanical treatment in twin-screw extruder. *J. Clean. Prod.* **2021**, *285*, 124839. [CrossRef]
19. Amoriello, T.; Fiorentino, S.; Vecchiarelli, V.; Pagano, M. Evaluation of spent grain biochar impact on hop (*Humulus lupulus* L.) growth by multivariate image analysis. *Appl. Sci.* **2020**, *10*, 533. [CrossRef]
20. Amoriello, T.; Mellara, F.; Galli, V.; Amoriello, M.; Ciccoritti, R. Technological Properties and Consumer Acceptability of Bakery Products Enriched with Brewers' Spent Grains. *Foods.* **2020**, *9*, 1492. [CrossRef] [PubMed]
21. Estevez, A.; Padrell, L.; Inarra, B.; Orive, M.; San Martin, D. Brewery by-products (yeast and spent grain) as protein sources in gilthead seabream (*Sparus aurata*) feeds. *Aquaculture* **2021**, *543*, 736921. [CrossRef]
22. Jackowski, M.; Niedźwiecki, Ł.; Mościcki, K.; Arora, A.; Saeed, M.A.; Krochmalny, K.; Pawliczek, J.; Trusek, A.; Lech, M.; Skřínský, J.; et al. Synergetic Co-Production of Beer Colouring Agent and Solid Fuel from Brewers' Spent Grain in the Circular Economy Perspective. *Sustainability* **2021**, *13*, 10480. [CrossRef]
23. Fărcas, A.C.; Ancu, S.; Chi, M.S.; Pop, O.L.; Fogarasi, M.; Păucean, A.; Igual, M.; Michiu, D. Reintegration of Brewers Spent Grains in the Food Chain: Nutritional, Functional and Sensorial Aspects. *Plants* **2021**, *10*, 2504. [CrossRef]

24. Arranz, J.I.; Sepúlveda, F.J.; Montero, I.; Romero, P.; Miranda, M.T. Feasibility Analysis of Brewers' Spent Grain for Energy Use: Waste and Experimental Pellets. *Appl. Sci.* **2021**, *11*, 2740. [CrossRef]
25. Bedo, S.; Rozbach, M.; Nagy, L.; Fehér, A.; Fehér, C. Optimised Fractionation of Brewer's Spent Grain for a Biorefinery Producing Sugars, Oligosaccharides, and Bioethanol. *Processes* **2021**, *9*, 366. [CrossRef]
26. Naibaho, J.; Korzeniowska, M.; Wojdyło, A.; Figiel, A.; Yang, B.; Laaksonen, O.; Foste, M.; Vilu, R.; Viiard, E. The Potential of Spent Barley as a Functional Food Ingredient: Study on the Comparison of Dietary Fiber and Bioactivity. *Proceedings* **2021**, *70*, 86.
27. Mussatto, S.I. Brewer's spent grain: A valuable feedstock for industrial applications. *J. Sci. Food Agric.* **2014**, *94*, 1264–1275. [CrossRef] [PubMed]
28. Ikram, S.; Zhang, H.; Ming, H.; Wang, J. Recovery of major phenolic acids and antioxidant activity of highland barley brewer's spent extracts. *J. Food Process Preserv.* **2020**, *44*, 14308. [CrossRef]
29. Crowley, D.; O'Callaghan, Y.; McCarthy, A.L.; Connolly, A.; Fitzgerald, R.J.; O'Brien, N.M. Aqueous and enzyme-extracted phenolic compounds from brewers' spent grain (BSG): Assessment of their antioxidant potential. *J. Food Biochem.* **2017**, *41*, 12370. [CrossRef]
30. Teixeira, J.; Gaspar, A.; Garrido, E.M.; Garrido, J.; Borges, F. Hydroxycinnamic acid antioxidants: An electrochemical overview. *BioMed Res. Int.* **2013**, *2013*, 251754. [CrossRef]
31. Mussatto, S.I.; Dragone, G.; Roberto, I.C. Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. *Ind. Crop Prod.* **2007**, *25*, 231–237. [CrossRef]
32. Bartolome, B.; Faulds, C.; Williamson, G. Enzymic release of ferulic acid from barley spent grain. *J. Cereal Sci.* **1997**, *25*, 285–288. [CrossRef]
33. Moreira, M.M.; Morais, S.; Carvalho, D.O.; Barros, A.A.; Delerue-Matos, C.; Guido, L.F. Brewer's spent grain from different types of malt: Evaluation of the antioxidant activity and identification of the major phenolic compounds. *Food Res. Int.* **2013**, *54*, 382–388. [CrossRef]
34. Meneses, N.G.T.; Martins, S.; Teixeira, J.A.; Mussatto, S.I. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Sep. Purif. Technol.* **2013**, *108*, 152–158. [CrossRef]
35. Guido, L.F.; Moreira, M.M. Techniques for extraction of brewer's spent grain polyphenols: A review. *Food Bioprocess. Technol.* **2017**, *10*, 1192–1209. [CrossRef]
36. Bonifácio-Lopes, T.; Boas, A.A.; Coscueta, E.R.; Costa, E.M.; Silva, S.; Campos, D.; Teixeira, J.A.; Pintado, M. Bioactive extracts from brewer's spent grain. *Food Funct.* **2020**, *11*, 8963–8977. [CrossRef] [PubMed]
37. Andres, A.I.; Petron, M.J.; Lopez, A.M.; Timon, M.L. Optimization of Extraction Conditions to Improve Phenolic Content and In Vitro Antioxidant Activity in Craft Brewers' Spent Grain Using Response Surface Methodology (RSM). *Foods* **2020**, *9*, 1398. [CrossRef] [PubMed]
38. 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]
39. Al Jitan, S.; Alkhoori, S.A.; Yousef, L.F. Phenolic Acids from Plants: Extraction and Application to Human Health. In *Studies in Natural Products Chemistry*; Elsevier: Amsterdam, The Netherlands, 2018; Volume 58, pp. 389–417.
40. Alonso-Riaño, P.; Sanz Diez, M.T.; Blanco, B.; Beltrán, S.; Trigueros, E.; Benito-Román, O. Water Ultrasound-Assisted Extraction of Polyphenol Compounds from Brewer's Spent Grain: Kinetic Study, Extract Characterization, and Concentration. *Antioxidants* **2020**, *9*, 265. [CrossRef]
41. International Association for Cereal Science and Technology. *ICC Standard Methods (Methods No. 104/1, 105/2, 110/1, 136)*; ICC: Vienna, Austria, 2003.
42. Lee, S.C.; Prosky, L.; DeVries, J.W. Determination of total, soluble, and insoluble, dietary fibre in foods-enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. *J. Assoc. Off. Anal. Chem.* **1992**, *75*, 395–416.
43. Amoriello, T.; Mellara, F.; Amoriello, M.; Ceccarelli, D.; Ciccoritti, R. Powdered seaweeds as a valuable ingredient for functional breads. *Eur. Food Res. Technol.* **2021**, *247*, 2431–2443. [CrossRef]
44. Dai, J.; Mumper, R.J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* **2010**, *15*, 7313–7352. [CrossRef]
45. Ferreira, S.L.C.; Silva Junior, M.M.; Felix, C.S.A.; Da Silva, D.L.F.; Santos, A.S.; Santos Neto, J.H.; De Souza, C.T.; Cruz Junior, R.A.; Souza, A.S. Multivariate optimization techniques in food analysis—A review. *Food Chem.* **2019**, *273*, 3–8. [CrossRef]
46. Bas, D.; Boyac, I.H. Modelling and optimization I: Usability of response surface methodology. *J. Food Eng.* **2007**, *78*, 836–845. [CrossRef]
47. Derringer, G.; Suich, R. Simultaneous optimization of several response variables. *J. Qual. Technol.* **1980**, *12*, 214–219. [CrossRef]
48. Ciccoritti, R.; Taddei, F.; Gazza, L.; Nocente, F. Influence of kernel thermal pre-treatments on 5-n-alkylresorcinols, polyphenols and antioxidant activity of durum and einkorn wheat. *Eur. Food Res. Technol.* **2021**, *247*, 353–362. [CrossRef]
49. Ritchey, J.G.; Waterhouse, A. A Standard Red Wine: Monomeric Phenolic Analysis of Commercial Cabernet Sauvignon Wines. *Am. J. Enol. Vitic.* **1999**, *50*, 91–99.
50. Mathias, T.R.S.; Alexandre, V.M.; Cammarota, M.C.; De Mello, P.P.; Sérvulo, E.F. Characterization and determination of brewer's solid wastes composition. *J. Inst. Brew.* **2015**, *121*, 400–404. [CrossRef]
51. Waters, D.M.; Jacob, F.; Titze, J.; Arendt, E.K.; Zannini, E. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. *Eur. Food Res. Technol.* **2012**, *235*, 767–778. [CrossRef]

52. Celus, I.; Brijs, K.; Delcour, J.A. The effects of malting and mashing on barley protein extractability. *J. Cereal Sci.* **2006**, *44*, 203–211. [CrossRef]
53. 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]
54. Ktenioudaki, A.; Chaurin, V.; Reis, S.; Gallagher, E. Brewer's spent grain as a functional ingredient for breadsticks. *Int. J. Food Sci. Technol.* **2012**, *47*, 1765–1771. [CrossRef]
55. Ferraz, A.I.; Amorim, C.; Tavares, T.; Teixeira, J.A. Chromium (III) biosorption onto spent grains residual from brewing industry: Equilibrium, kinetics and column studies. *IJEST* **2015**, *12*, 1591–1602. [CrossRef]
56. Tarley, C.R.T.; Arruda, M.A.Z. Biosorption of heavy metals using rice milling by-products. Characterisation and application for removal of metals from aqueous effluents. *Chemosphere* **2004**, *54*, 987–995. [CrossRef]
57. Patrignani, M.; Del Sol Gonzalez-Forte, L. Characterisation of melanoidins derived from Brewers' spent grain: New insights into their structure and antioxidant activity. *Int. J. Food Sci. Technol.* **2020**, *56*, 384–391. [CrossRef]
58. Sun, X.F.; Xu, F.; Sun, R.C.; Fowler, P.; Baird, M.S. Characteristics of degraded cellulose obtained from steam-exploded wheat straw. *Carbohydr. Res.* **2005**, *340*, 97–106. [CrossRef] [PubMed]
59. Ravindran, R.; Jaiswal, S.; Abu-Ghannam, N.; Jaiswal, A.K. A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. *Bioresour. Technol.* **2018**, *248*, 272–279. [CrossRef] [PubMed]
60. Silva, E.; Rogez, H.; Larondelle, Y. Optimization of extraction of phenolics from Inga edulis leaves using response surface methodology. *Sep. Purif. Technol.* **2007**, *55*, 381–387. [CrossRef]
61. Yilmaz, M.; Karaaslan, M.; Vardin, H. Optimization of extraction parameters on the isolation of phenolic compounds from sour cherry (*Prunus cerasus* L.) pomace. *J. Food Sci. Technol.* **2015**, *52*, 2851–2859. [CrossRef] [PubMed]
62. Liyana-Pathirana, C.M.; Shahidi, F. Antioxidant and free radical scavenging activities of whole wheat and milling fractions. *Food Chem.* **2007**, *101*, 1151–1157. [CrossRef]
63. Viacava, G.E.; Roura, S.I.; Agüero, M.V. Optimization of critical parameters during antioxidants extraction from butterhead lettuce to simultaneously enhance polyphenols and antioxidant activity. *Chemometr. Intell. Lab.* **2015**, *146*, 47–54. [CrossRef]
64. Azmir, J.; Zaidul, I.S.M.; Rahman, M.M.; Sharif, K.M.; Mohamed, A.; Sahena, F.; Jahurul, M.H.A.; Ghafoor, K.; Norulaini, N.A.N.; Omar, A.K.M. Techniques for extraction of bioactive compounds from plant materials: A review. *J. Food Eng.* **2013**, *117*, 426–436. [CrossRef]
65. Esclapez, M.D.; Garcia-Perez, J.V.; Mulet, A.; Carcel, J.A. Ultrasound-assisted extraction of natural products. *Food Eng. Rev.* **2011**, *3*, 108–120. [CrossRef]
66. Vilkhu, K.; Mawson, R.; Simons, L.; Bates, D. Applications and opportunities for ultrasound assisted extraction in the food industry. *Innov. Food Sci. Emerg.* **2008**, *9*, 161–169. [CrossRef]
67. Virot, M.; Tomao, V.; Le Bourvellec, C.; Renard, C.M.C.G.; Chemat, F. Towards the industrial production of antioxidants from food processing by-products with ultrasound-assisted extraction. *Ultrason. Sonochem.* **2010**, *17*, 1066–1074. [CrossRef] [PubMed]
68. Pingret, D.; Fabiano-Tixier, A.S.; Le Bourvellec, C.; Renard, C.M.; Chemat, F. Lab and pilot-scale ultrasound-assisted water extraction of polyphenols from apple pomace. *J. Food Eng.* **2012**, *111*, 73–81. [CrossRef]
69. Arnao, M.B. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. *Trends Food Sci. Technol.* **2000**, *11*, 419–421. [CrossRef]
70. Zhu, M.; Huang, Y.; Wang, Y.; Shi, T.; Zhang, L.; Chen, Y.; Xie, M. Comparison of (poly) phenolic compounds and antioxidant properties of pomace extracts from kiwi and grape juice. *Food Chem.* **2019**, *271*, 425–432. [CrossRef] [PubMed]
71. Inns, E.L.; Buggey, L.A.; Booer, C.; Nursten, H.E.; Ames, J.M. Effect of modification of the kilning regimen on levels of free ferulic acid and antioxidant activity in malt. *J. Agric. Food Chem.* **2011**, *59*, 9335–9343. [CrossRef]
72. Maillard, M.N.; Berset, C. Evolution of antioxidant activity during kilning: Role of insoluble bound phenolic acids of barley and malt. *J. Agric. Food Chem.* **1995**, *43*, 1789–1793. [CrossRef]
73. Martini, D.; Ciccoritti, R.; Nicoletti, I.; Nocente, F.; Corradini, D.; D'Egidio, M.G.; Taddei, F. From seed to cooked pasta: Influence of traditional and non-conventional transformation processes on total antioxidant capacity and phenolic acid content. *Int. J. Food Sci. Nutr.* **2018**, *69*, 24–32. [CrossRef] [PubMed]
74. Shakeel, F.; Salem-Bekhit, M.M.; Haq, N.; Siddiqui, N.A. Solubility and thermodynamics of ferulic acid in different neat solvents: Measurement, correlation and molecular interactions. *J. Mol. Liq.* **2017**, *236*, 144–150. [CrossRef]