Sustainable production of low molecular weight phenolic compounds from Belgian Brewers' spent grain

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Abstract:

Brewers' spent grain (BSG) is a brewery co-product rich in phenolic compounds which only within Europe, 3-4Mt is generated annually. This study aims to sustainably isolate low-molecular-weight phenolic compounds from Belgian BSG using a microwave-assisted method (MW). Two types of BSG (from light and red malted barley grains) were subjected to MW at 600 and 800W for 5, 15 and 30min. MW showed a significant and positive effect on the release of 4-vinylguaiacol, a bioactive compound derived from the thermal decarboxylation of ferulic acid. The amounts of 4-vinylguaiacol in the extracts increased by about 80% after 30min of MW despite the power applied. In the same time interval, total phenolic contents (TPC) increased of about 70% for light and 60% for red

BSG. The highest TPC (13.23mg/gDM) was obtained at 30min and 600W. This sustainable method could help add value to BSG by improving the extractability of bioactive phenolics.

Keywords: phenolic; BSG; antioxidant; microwave; 4-vinylguaiacol.

1. Introduction

Phenolic compounds are secondary metabolites that play an important role in protecting plants against environmental stresses. They have highly variable structures ranging from a simple substituted aromatic ring, such as phenolic acids, to complex high molecular weight polymers such as lignin or condensed tannins (Alves-Santos *et al*., 2020).

Low molecular weight phenolic compounds (LMWPC) are highly reactive with antioxidants (Shahidi & Ambigaipalan, 2015), antimicrobial (Suriyarak et al., 2014) and anticarcinogenic (Raphaelli et al., 2021) properties.

Barley residues correspond to 50 Mt/year of the total cereal crops production, constituting 15 % of the cereal production in Europe (Camia A. et al., 2018). Brewers' spent grain (BSG), which mainly consists of the malted grain husks of barley, is obtained as solid residue after wort production (Lynch et al., 2016). BSG is a rich source of phenolic compounds sold as animal feed and for a meagre profit margin. The difference between light and dark beers is the type and treatment of the grain used in the brewing process. Barley roasting temperatures can raise from 80 to about 200°C (McCarthy et al., 2013).

The phenolic profile of BSG can vary according to barley variety, germination conditions, harvest time and malt pressing and roasting processes (Carvalho et al., 2015; Munekata

et al., 2016). LMWPC can be generated from the hydrolysis of lignin during natural plant processes or during mechanical or heating treatments of the biomass (Azadfar et al., 2015). The primary phenolic acids in European barley and BSG are ferulic (4-hydroxy-3-methoxycinnamic acid) and p-coumaric [(E)-3-(4-hydroxyphenyl)prop-2-enoic acid] (70% of the total phenolic content) followed by sinapic, and caffeic acids. Ferulic acid (FA) is usually found cross-linked with proteins, lignin, hemicellulose and/or polysaccharides through ether or ester bonds in the cell wall (Azadfar et al., 2015; Malunga & Beta, 2016;).

During roasting step, FA and p-coumaric acid (CA) can be decarboxylated via Maillard reactions into their corresponding vinylphenols: vinyl-guaiacol (2-Methoxy-4-vinylphenol) and p-vinylphenol (4-hydroxystyrene), respectively.

4-vinylguaiacol (4VG) is a flavour molecule with a particularly high value in the food industry (Baqueiro-Pena et al., 2010). Vinylic derivatives have proven to present higher bioactivity than their correspondent hydroxycinnamic acid. The presence of a methoxyl group associated with the single phenolic hydroxyl and the terminal double bound in resonance with the aromatic ring in the chemical structure of 4-vinylguaiacol was responsible for a small but significant higher scavenging capacity in comparison with its corresponding hydroxycinnamic acid (Tańska et al., 2018).

The low phenolic contents in the non-pre-treated BSG may be due to the resistance of the plant cell walls to which some of the phenolic compounds tend to remain bounded (**Table 2**). Studies showed that thermal pre-treatments using microwaves modifies the cell wall by increasing its porosity and facilitating the extraction of phenolic compounds (Azadmard-Damirchi et al., 2010). The phenomenon of microwave selective heating on the extraction of phenolic compounds was described by (Hayat et al., 2019). Phenolic

compounds are thermal unstable, so microwave selective heating could weaken physical binding forces between phenolics and the plant with low degrading effects. The molecules strongly bound to the matrix can then be released by thermal hydrolysis (Chipurura et al., 2010; Dai & Mumper, 2010).

In this study, a sustainable MW was used to extract LMWPC from BSG in order to reduce extraction time, use of solvents, and to improve extraction efficiency (Patrignani et al., 2021). The specific objectives of this study were: i) Selection of two different samples of brewing by-products from the production of light (LB) and red (RB) ales of Belgian beer; ii) Study and selection of a sustainable method for the extraction of the major LMWPC from Belgian BSG; iii) Analysis and quantification of the total phenolic contents (TPC) on the selected BSG and characterization of the individual phenolic profile of the samples; iv) Promotion of the formation of the bioactive 4-VG in the selected BSG, particularly by evaluating the influence of microwave pre-treatments of BSG as a sustainable process potentially applicable later on at industrial scale (Li et al., 2016); v) Evaluation of the antioxidant activity of the obtained extracts and analysis of the correlation between the radical inhibition capacity and the phenolic profiles of BSG extracts.

The study and selection of a sustainable process for the obtention of extractible high active molecules from BSG could promote adding value to this co-product.

2. Material and Methods

2.1. Material

The BSG from the production of light and red beers were kindly provided by En Stoemelings, a local microbrewery in the region of Brussels, Belgium. The following chemicals were purchased from Merck Life Science – Sigma-Aldrich (Overijse,

Belgium): Folin Ciocalteau reagent (2N), ferulic acid (4-hydroxy-3-methoxycinnamic acid) (≥ 98.0%), p-coumaric acid [(E)-3-(4-hydroxyphenyl) prop-2-enoic acid] (≥ 98.0%), triethylamine (N,N-diethylethanamine) (synthesis grade), DPPH (2,2-Diphenyl-1-picrylhydrazyl), (silica gel (60 Å, 230-400 mesh particle size, 40-63 μm particle size). TLC plates (silica gel F254, 0.25 mm thick, Merck, Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from ChemLabAnalytical (Zedelgem, Belgium). All solvents used were of HPLC (> 99%) or analytical grades and were supplied by Chem Lab Analytical (Redu, Belgium). These solvents were used without prior distillation.

2.2. Characterization of BSG: water and lipid contents

For the water content measurement, samples were kept in an oven at 105°C for 96 h. The percentage of water in the samples calculated by the difference between the mass of the sample before and after drying.

Prior to extracting the phenolic compounds, BSG was oven-dried at 60°C for 24 h (Carciochi et al., 2018) and subsequently ground in a coffee bean mill (PRINCESS brand, Model: 221040 Multi Chopper and Grinder). For this, 10.0 g of sample is ground for 5 min. The grinded BSG was 0.75-0.075 mm particle size.

The lipid was extracted by a Soxhlet type extractor with n-hexane at 40-45°C for eight hours. The thimble containing the defatted BSG is then air-dried and weighted. The lipid content of each sample is calculated from the difference between the mass of the non-delipidated and the delipidated BSG. The values are expressed as a percentage of lipids per gram of dry BSG.

2.3. Preliminary extraction studies

Preliminary studies were performed to evaluate the LMWPC extraction capacity of ultrasound-assisted extraction (UAE) and thermal extraction (TE) methods. Three different solvent mixtures of ethanol:water (70:30, v/v), acetone:water (60:40, v/v) and acetone:water (40:60, v/v) were tested using both UAE and TE. The solvent mixtures selection was based on previous studies (Carciochi et al., 2018; Meneses et al., 2013; Socaci et al., 2018).

2.3.1. Ultrasound-Assisted Extraction (UAE)

For each UAE extraction, one gram of the dried and delipidated BSG was added to a centrifuge tube and ultrasonicated (Sonics Vibra-cell VCX 130 PB, 130 W, 20 Hz) with 10 mL of the solvent mixture for one minute at 50 % amplitude (Carciochi et al., 2018). The supernatant was recovered, and the BSG submitted to the same extraction procedure for two more times. The three crude phenolic extract portions were combined and centrifuged at 6000 rpm (4024 g) for 10 min. The supernatant containing the phenolic compounds was recovered and stored at –4°C for further analysis. The extractions were performed in triplicate.

2.3.2. Thermal Extraction (TE)

The TE was based on the method described by (Carciochi et al., 2018) with some modifications. In a closed cap glass tube, 0.5 g of light beer BSG was weighed, and 15 mL of each solvent mixtures are added. The phenolic compounds were extracted for 60 min under stirring (300 rpm) at 80°C. After cooling, the samples were centrifuged at 6000 rpm (4024 g) for 10 min, the supernatant containing the phenolic compounds was

collected and stored at −4 °C for further analysis. The extractions were performed in triplicate.

2.4. The microwave pre-treatment and the extraction of low-molecular weight phenolic compounds

The microwave pre-treatments were carried out using a SAMSUNG domestic microwave device (Model MS23F301EAK, 2450 MHz). In a MARSXpress™ capped reaction tube of 90 mL (suitable for microwave use), 0.5 g of dried and delipidated BSG was added. Three different heating time intervals of 5, 15 and 30 min, and two different microwave powers of 600 and 800 W were tested on the final contents of LMWPC from BSG. The experimental conditions are described in **Table *3***.

At the end of the treatments, the temperature was immediately measured using an infrared thermometer (BOSCH, Universal Temp, Reference 603683100). After cooling down to room temperature, the pre-treated BSG were added to screw-top glass test tubes for the extraction of LMWPC. All the experiments were done in triplicates.

The extraction of the LMWPC was carried out following the method described by (Carciochi et al., 2018) with some modifications. The solvent to sample ratio was 30/1 (v/w). Typically, 0.5 g of the delipidated sample was weighed into a screw-top test tube and 15 mL of the solvent mixture ethanol:water (70:30, v/v) was added. The closed tubes were kept under magnetic stirring in an oil bath at 80°C for 60 min. The tubes were then cooled to room temperature, the supernatant was collected, and the extraction process was repeated for a second time. The two extracted portions were combined and centrifuged for 10 min at 6000 rpm (4024 g). The solvent was evaporated under reduced pressure at 40°C using a Buchi Rotavapor R-100 rotary evaporator (Flawil, Switzerland) and

protected from the light. The dry extracts were stored at −20 °C until further use and resolubilized in ethanol:water (70:30, v/v) for further analysis.

2.5. Synthesis of 4-vinylguaiacol

FA can be converted into a monosubstituted styrene derivative, i.e. 4-hydroxy-3-methoxystyrene, known as 4-vinylguaiacol (4VG) via decarboxylation. The methodology used is based on (Takeshima et al., 2017). This method describes the preparation of vinylphenols via decarboxylation according to a mechanism described by Nomura et al., 2005; and Bernini et al., 2007).

In a 50 mL flask, commercial FA (1 g, 5 mmol) was dissolved in DMF (5 mL). Triethylamine (1.5 mL, 10 mmol) was added to this medium, and the mixture was maintained under magnetic stirring (250 rpm) in an oil bath at 100°C. At the end of the reaction, the medium was cooled to room temperature. The decarboxylation of FA was followed by thin-layer chromatography (TLC) and eluted with the n-hexane/ethyl acetate/formic acid mixture (70/30/0.1, v/v/v). The spots were identified using a 254 nm UV lamp for thin layer chromatography (Merck, Darmstadt, Germany). The retention factors (R_f) were 0.12 for FA and 0.60 for 4-VG. The reaction medium was diluted with diethyl ether and washed three times with water and one time with brine. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated off under reduced pressure. Further purification was conducted by column chromatography on silica gel. The concentrated reaction medium was dissolved in ethyl acetate and eluted with n-hexane:ethyl acetate, 70:30 (v/v). The purified fraction yielded almost pure 4-VG (0.70 g, 93 % yield) as light-yellow oil. The chemical structure of the synthesized 4VG was

196 confirmed by nuclear magnetic resonance (NMR), high resolution mass (HR-MS), and
197 infrared (IR) spectroscopy.
198
199 **2.6. Analytical methods**
200 **2.6.1. Total phenolic contents (TPC)**
201 The content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteu
202 method adapted from (Carciochi et al., 2018). The dry extracts were resolubilized in the
203 ethanol:water (70:30, v/v) solution to a final volume of 15 mL. In a 5 mL plastic
204 Eppendorf, 0.1 mL of extract was mixed with 0.3 mL of aqueous sodium carbonate
205 solution (20%, w/v). Then, 0.1 mL of a Folin-Ciocalteu (2 N) solution was added, and the
206 medium was completed to a final volume of 2 mL with milli-Q water. After 120 min of
207 incubation, the samples were placed in a glass cuvette, and their absorbance read at 765
208 nm in a Thermo Fisher, GENESYS™ 10S UV-Vis spectrophotometer at room
209 temperature. The total concentration of phenolic compounds was calculated from a
210 calibration curve established by taking FA as standard phenolic compound at
211 concentrations between 0.001 to 0.5 mg/mL of FA diluted in the ethanol:water (70:30,
212 v/v) solution (R^2 = 0.991). The results are expressed in ferulic acid equivalents (FAE) in
213 milligrams per gram of dry matter (mg/g DM).
214
215 **2.6.2. Determination of the antioxidant capacity of BSG phenolic extracts**
216 Antioxidant capacity was determined by measuring the ability of the extracts to scavenge
217 the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). 50 µL of each extract was added
218 to 1950 µL of fresh prepared DPPH solution (60 µM in methanol) and allowed to stand
219 for 30 min in darkness at room temperature before measuring the absorbance at 517 nm.

Results were expressed as a percentage of radical scavenging (DPPH inhibition, %), as previously described by (Carciochi et al., 2018; Meneses et al., 2013).

2.6.3. Liquid Chromatography (LC) analysis of individual phenolics in BSG extracts

The individual content of phenolic compounds of interest (FA, CA and 4VG) in each sample was quantified by HPLC using an Agilent 1100 model HPLC System equipped with diode array (DAD) & fluorescence (FLD) Detectors (Santa Clara, CA, USA). The separation was carried out with a reversed phase silica column Symmetry C18 (5 µm, 250 mm x 4.6 mm, Waters Corporation, Milford, MA, USA). For this, the dry extracts obtained are resolubilized in 3 mL of ethanol:water (70:30, v/v), then filtered (Millex 0.45 µm, Millipore, Bedford, MA, USA) and analysed by HPLC. The analytical method involves a gradient elution using two mobile phases: water with 0.2% (v/v) acetic acid (A) and methanol with 0.2% (v/v) acetic acid (B). The elution gradient applied is as follows: 20 - 40% B (0-10 min), 40 - 50% B (10 - 30 min), 50 - 100% B (30 - 35 min), 100 - 20% B (35 - 40 min), 20% B (40 - 45 min). The detection wavelengths were 325 nm for FA, 312 nm for CA and 264 nm for 4-vinylguaiacol. The injection volume was 10.0 µL, the flow rate 1 mL/min and the analysis done at room temperature. The retention times are 14.7 min for CA, 15.3 min for FA and 28.4 min for 4VG (Supplementary Information). Data were acquired and analysed using Agilent ChemStation B.04.03(16) software (Santa Clara, CA, USA). External calibration curves were obtained from methanolic solutions of FA, CA and 4VG. The concentrations of these solutions ranged from 0.1 to 10 µg/mL with $R^2 = 0.9973$ for FA, $R^2 = 0.9999$ for CA and $R^2 = 0.9990$ for 4VG (Supplementary Information).

2.6.4. Nuclear magnetic resonance analysis (NMR)

The structure of the synthesized 4-vinylguaiacol (section 2.5) was confirmed by 1H NMR spectroscopy (Bruker Avance 300 spectrometer operating at 7.0 T (300 MHz for ^{1}H), Bremen, Germany) and ^{13}C NMR spectroscopy (JEOL JNM-ECZ400R/S3 spectrometer operating at 9.4 T (101 MHz for ^{13}C), Tokyo, Japan). The compound was dissolved in DMSO-*d6* or $CDCl_3$. For $CDCl_3$, the spectra were referenced to the internal residual solvent signals of $CHCl_3$ at 7.26 ppm and 77.0 ppm for ^{1}H and ^{13}C respectively. In the case the compound was dissolved in DMSO-*d6*, the chemical shifts were referenced to the internal residual solvent signals of DMSO at 2.5 ppm and 39.5 ppm for ^{1}H and ^{13}C, respectively. Data was processed and analysed using MestReNova 14.1.2–25024 software (Mestrelab Research, Santiago de Compostela, Spain). Acquisition and processing parameters were T = 25°C, pulse width = 30°, relaxation delay = 2 s, FID apodization (single exponential) = 0.5 Hz, zero filling = 256 K.

2.6.5. High resolution mass spectroscopy analysis (HR-MS)

HR-MS analyses was performed using methanol and 0.2% formic acid as solvent on an ESI-MS apparatus (Q-TOF 6520 Agilent Technology) equipped with a TOF detector.

2.6.6. Infrared spectroscopy (IR)

IR spectrum was recorded on a Bruker Tensor 27 FTIR spectrometer equipped with a Specac Golden Gate™ single reflection ATR (Attenuated total reflection) unit (diamond). All the system was purged with dry air and sample dried with gaseous N_2. 128 scans were acquired with a resolution of 2 cm^{-1}.

2.6.7. Statistical Analysis

3. Results were reported as mean values ± of the standard deviation (SD) of triplicate analysis. The differences between mean values for the beer type, microwave pre-treatment time intervals and power (**Table 3**) were determined using a factorial design consisting of of three independent factors, including beer type (LB and RB), microwave power (0, 600W, and 800W), and time (0, 5, 15, and 30 minutes). Data points were checked initially for their normality and logarithmic and square root transformations were conducted accordingly to obtained normalized data (Pallant, 2011). Data analysis was carried out using the general linear multiple regression model using the two-way analysis of variance (ANOVA). Multiple mean comparisons were performed using Tukey's test at the level of significance of 0.05 ($p < 0.05$) (Pallant, 2011). All the data analysis tests were assessed by SPSS statistical software version 22 (IBM, New York, USA).

4. Results and Discussion

4.1. Characterization of BSG: Water and lipid contents

Lipids constitute a significant part of BSG and have been found to comprise from 4 to 10 % (w/w) of the dried matter. BSG lipids are predominantly triglycerides (67 %, w/w) and free fatty acids (18 % w/w), with lower amounts of monoglycerides (1.6 %, w/w) and diglycerides (7.7 %, w/w) (del Río et al., 2013). The lipid content of BSG from light beer was 4.2 ± 0.2 % (w/w) and for red beer was 10.5 ± 0.7 % (w/w) (**Figure 1**A).

The differences in the lipid contents for the different BSG can be explained by the different process conditions used during brewing, the cereals used and their proportions (Bonifácio-Lopes et al., 2020) (**Table 1**).

Once obtained from the lautering step, BSG can rapidly spoil, providing high microbial activity. Therefore, it is important to dry and keep this material to prevent microbial degradations. The samples used in this work presented very similar water contents indicating consistency in the Brewery's mashing process: 75.8 ± 0.8 and 73.4 ± 0.5 % (w/w) of water for light and red beer BSG, respectively (**Figure 1**B). The final moisture detected for the dried samples was 6 % (w/w).

4.2.Synthesis of 4-vinylguaiacol

4-vinylguaiacol (2-methoxy-4-vinylphenol) can be obtained from the decarboxylation of FA which is the predominant hydroxycinnamic acid on BSG. However, the isolation and purification of this compound from BSG its difficult and tedious and provide low concentrations of 4-VG, especially in laboratory scale. Consequently, the 4-VG used as a standard for the characterization of the BSG extracts was synthesized according to a protocol described in the literature (Takeshima et al., 2017). The protocol from the literature is based in the presence of a molar excess of the base to induce the formation of a double negatively charged aromatic structure derived from the hydroxycinnamic acid. Cinnamic acids having a hydroxyl group at the para position such as FA and CA can be deprotonated by strong bases such as DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) and triethylamine to get the intermediates as the quinone methide which can undergo a rapid decarboxylation and easily rearomatize to yield the corresponding 4-vinilphenols (Bernini et al., 2007; Nomura et al., 2005).

The conversion of FA measured by ^{1}H NMR was nearly quantitative (93 % yield, 4.7 mmols) and the purity of the product 4-VG (>97 %) analysed by LC. These values are in accordance with those obtained by (Takeshima et al., 2017): 100 % yield and a 4-VG purity of >99 % (Supplementary Information). Assignment by comparison with the literature (Darapureddi & Nayak, 2016):

^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.09 (s, 1H, OH), 7.07 (d, J = 2.0 Hz, 1H, H2), 6.88 (dt, J = 8.1, 1.2 Hz, 1H, H5), 6.76 (dd, J = 8.1, 0.8 Hz, 1H, H6), 6.63 (dd, J = 17.6, 10.9 Hz, 1H, H7), 5.65 (dt, J = 17.6, 1.0 Hz, 1H, H8), 5.08 (dt, J = 10.8, 0.9 Hz, 1H, H8), 3.81 (d, J = 0.8 Hz, 3H, Me) ppm.

^{13}C NMR (101 MHz, Chloroform-*d*):
δ = 56.0 (Me), 109.4 (C2), 112.3 (C8), 114.1 (C5), 119.5 (C5), 131.9 (C1), 136.5 (C7), 148.0 (C4), 149.7 (C3) ppm.

The HR-MS calc. for $C_9H_{10}O_2$ $[M+H]^+$: 150.0681, found: 150.0674. The main peaks characteristics of the 4VG structure found by the ATR-FTIR analysis were as follows: ν between 2850-3363 cm^{-1} were attributed to the aromatic alkenes phenol. The aromatic C-H, and the methyl vibration peaks appeared between 1423-1586 cm^{-1}. Guaiacyl units were described to present a characteristic band at 1260 cm^{-1} (Monteil-Rivera et al., 2013). Peaks from 1034 to 1364 cm^{-1} were attributed to the ether bond C-O-C, and the peaks between 1562-1653 cm^{-1} were attributed to the vinyl C=C stretching vibrations (Supplementary Information).

4.3. The microwave pre-treatments

4.3.1. Ultrasound-Assisted Extraction (UAE) and Thermal Extraction (TE)

The use of mixtures of organic solvents with water can provide green and safe extracts. The efficiency of the extraction of phenolic compounds from plant matrices depends on the accessibility of the solvent to the molecules and of the solvent polarity (Ameer et al., 2017; Rodríguez-Rojo et al., 2012). Ultrasound-Assisted Extraction (UAE) and Thermal Extraction (TE) were studied. Extraction by ultrasound occurs by simple immersion or by percolation. The effectiveness of this technique is due to so-called "acoustic pressure," which generates several important phenomena such as cavitation, surface friction of molecules, and increasing diffusion speed (Chiremba et al., 2012; Hayat et al., 2019). Ultrasounds have been applied to extract numerous plant compounds by considerably reducing the extraction time and increasing the maximum yield of extraction (Carciochi et al., 2018). Increases in the extraction temperature can improve the solubility of the compounds to be extracted. In addition, at higher temperatures, the solvent's viscosity and surface tension are reduced, favouring its accessibility within plant matrices. However, the readily oxidizable phenolic compounds, long extraction periods and/or exposure to high temperatures promote their oxidation. Therefore, it is important to obtain the best solvent/temperature/extraction time and solid to liquid ratio to maximize the yield of the process and the stability of the target compounds.

When UAE was applied, there were no significant differences between the TPC values regardless of the solvent mixtures used. However, for TE, the best solvent to extract LMWPC was ethanol:water (70:30 %, v/v) (TPC = 2,6 FAE/mg DM) in comparison to acetone:water in either proportions of 60:40 (v/v) or 40:60 (v/v) showed significantly lower TPC (**Figure 2**B).

362 However, significant differences between the efficiency of both methods (UAE or TE)
363 were observed independently of the solvent mixture used (**Figure 2**). The thermal
364 extraction provided 40-60 % (w/w) higher amounts of TPC than the UAE. This is
365 probably due to the effect of temperature on improving the mass transfer of the extracting
366 medium.
367 Alonso-Riano et al., (2020) compared the phenolic profile of ultrasonic extracts with
368 acid/basic and enzymatic hydrolysis and found that ultrasound was less effective than
369 basic hydrolysis in releasing phenolic acids that esterified to the plant cell wall. Some
370 studies showed that UAE improves the release of saccharides and proteins from BSG
371 (Reis et al., 2015). Birsan et al., (2019) compared conventional maceration, microwave
372 and ultrasound-assisted extraction using BSG from light and dark beer and acetone:water
373 (60:40, v/v) as the solvent mixture.
374 The presence of water in organic solvents can improve the polarity of the medium and
375 may help the extraction of high polar compounds (Socaci et al., 2018). Moreover, the
376 efficiency of pure water as an extraction solvent is reduced since LMWPC are generally
377 more soluble in organic solvents with lower polarity than water (Herrera-Pool et al.,
378 2021).
379 Meneses et al., (2013) studied the thermal (60-80°C, 30 min) extraction of phenolic
380 compounds from BSG using different solvents: methanol, ethanol, acetone, water, and
381 the mixtures of methanol, ethanol, and acetone with water in ratios of 80-20%, v/v. The
382 highest TPC (9.9 ± 0.4 mg gallic acid equivalents GAE/g BSG) and the best antioxidant
383 activity (18.5 ± 0.9 % of DPPH inhibition) were found for the acetone:water (60:40, v/v)
384 phenolic extract. Socaci et al., (2018) tested 12 solvent mixtures based on methanol,
385 ethanol, acetone, and their mixture with water in ratios of 60:40 or 40:60 (v/v) solvent to

386 water, for the extraction of phenolic from a dark lager beer BSG. The highest
387 concentrations in phenolics (0.9 – 1.1 mg GAE/g DM) were observed for the methanolic
388 extracts and the solvent mixtures of ethanol/water (60:40 %, v/v) and acetone/water
389 (40:60 %, v/v) (**Table 2**).
390 The TPC and FA levels of both untreated BSG were comparable with those found in the
391 literature. However, these levels increased significantly when a microwave step pre-
392 treatment of 600W and 30 min was applied. Under these conditions, the present study
393 provided extracts with high TPC (12.5 ± 0.4 and 13.23 ± 0.02 mg/g DM for light and red
394 beer BSG, respectively) and FA (34 ± 4 and 15.00 ± 0.02 µg/g DM for light and red beer
395 BSG, respectively). The efficacy of MW was demonstrated by the increase of the TPC
396 and the amounts of individual LMWPC in the extracts obtained from the MW pre-treated
397 BSG.
398
399 **4.3.2. Total phenolic contents (TPC) and Individual phenolic contents (FA,**
400 **AC, and 4VG)**
401 The total phenolic contents may vary according to the variety of the plant, growing
402 conditions, industrial treatments of the grains and extraction conditions. As shown in
403 **Table 2**, BSG TPC can vary from 0.14 to 13.23 mg/g DM depending on the barley type,
404 pre-treatment and extraction methods. Only a small fraction of phenolic acids exists in
405 free form. Most are found either as ester, ether, or acetal or bound to structural
406 components of the plant (sugars, kaempferols), proteins or hydrates of carbon (Azadfar
407 et al., 2015; Malunga & Beta, 2016). Microwave energy was described to improve
408 releasing of plant-bonded compounds (Rodríguez-Rojo et al., 2012). In our study, we

409 applied microwave to dried and delipidated BSG of light and red beer to improve the
410 extraction of LMWPC.
411 As shown in **Figure 3**, microwave power did not significantly affect the TPC on both
412 light and red beers BSG extracts. However, the heating time showed a positive influence
413 in the release of LMWPC confirmed by the increase in the TPC for both light and red
414 beers BSG. In light beer BSG (**Figure 3**A), TPC increases linearly with the time of pre-
415 treatment independently on the power applied (600 or 800W). For both types of BSG,
416 heating the BSG for only 5 min, independently of the power applied, was insufficient to
417 induce higher energy in the system to allow the release and/or production of free
418 LMWPC. Accordingly, no significant increases in the individual phenolic contents (FA,
419 CA, and 4VG) were observed at 5 min MW pre-treatment. However, the higher level of
420 pre-treatment time intervals positively affected the release of FA, CA, and 4-VG from
421 BSG cell walls as it improved the TPC. For LB, 30 min pre-heating increased the FA
422 concentration by about 80 % regardless of the power used (**Table 3**). In the case of RB,
423 the effect of time was more pronounced at 800 W. While at 600 W about 66 % FA
424 contents increased at 30 min, at 800 W more than 80 % of FA was observed at the same
425 time. The highest amounts of FA (33.30 ± 10.30 µg/g DM) and 4VG (4.0 ± 0.7 µg/g DM)
426 were found in light beer BSG extracts and at the higher levels of time and power (30 min,
427 800 W). In **Table 3**, it is possible to observe that 4VG concentration rises 65 % while FA
428 concentration was reduced by 44 % for LB-12. For LB the highest FA concentration (LB-
429 16 = 33.30 ± 10.30 µg/g DM) was obtained using 800 W for 30 min (**Table 3**, **Figure 3**A
430 & B). Stefanello et al., (2018) studied the MW extraction of phenolic compounds from
431 Brazilian BSG using a mixture of 50:50 (v/v) acetone/water and a BSG to solvent ratio
432 of 1:20 (w/v). The system's temperature was kept at 100°C and the extraction under

magnetic stirring (200 rpm) for 15 min. They found a concentration of CA = 8.4 ± 2.1 µg/g of dried BSG and of FA = 5.6 ± 1.1 µg/g of dried BSG. In the case of this study, pre-heating the BSG for 30 min increased CA amounts of about 65 % for 600 W and 78 % for 800 W for LB (**Figure 3A**). For RB, the trend was different, while CA contents increased with the time level for 600 W pre-treatments, for 800 W, heating the BSG for 30 min was deleterious. The concentration on CA was about 20 % lower than when heating the sample for 15 min. The same behaviour was observed for FA (**Figure *3*B**). Patrignani et al., (2021) had similar results when they found that CA (38 µg/ gDM) and FA (86 µg/ gDM) concentrations increased with microwave pre-treatments under moderate conditions (1200W, 6 min, 120°C). The explanation of the observed effect is due to the thermal degradation of hydroxycinnamic acid ester bonds on the plant cell walls. As observed for red beer BSG, when the power intensity of microwave pre-treatments increased, increase in time reduced the concentration of these phenolic compounds significantly (**Figure 3**B), probably because of their involvement in Maillard reactions. Lower microwave power, such as 600W consists of the emission of microwaves in cycles varying by time intervals. Lower power intensity might prevent phenolics from thermal degradation avoiding the constant emission of microwaves and maintaining high temperatures, which could justify the absence of significant increments in the TPC as observed. The power increase did not improve the release of bond FA and CA. However, the production of 4VG was significantly and negatively affected by applying high power levels (800W). Again, this could be related to the thermal sensitivity, high volatility and reactivity of this molecule. No significant differences were observed between both beer colours.

For comparison, the microwave pre-treatments produced less individual phenolic contents for the BSG from red beer than the pre-treatment for BSG from light beer. It would be expected that RB-10 would present higher levels of FA, CA, and 4-VG than LB-10 since in the first case, the barley has been submitted to roasting processes which tend to improve the release of these compounds by thermal hydrolysis and/or decarboxylation in the of 4VG. However, RB-10 presented lower amounts of both FA and 4VG and not significant difference for CA compared to LB-10 (**Table 3**). This phenomenon could be explained by the thermal degradation of the free phenolic compounds during the roasting step of barley (before fermentation), light exposition and/or oxidation processes. As observed in **Figure 3**, from LB-10 to LB-12, the amounts of 4VG increase while FA decrease. Decreases in the amounts of FA after microwave pre-treatments could be justified by the conversion of this FA into 4VG by decarboxylation). However, the same behaviour is not observed for RB. Despite that, an increase on the TPC was observed. The gap between the amounts of FA, CA, and 4VG and the TPC might be due to the release of minor phenolic compounds present in the BSG such as catechin, protocatechuic acid, syringic acid, p-hydroxybenzoic, protocatechuic acid, chlororogenic acid etc. The microwaves frequency increases the energy on the system and leads to the cleavage of the ester bonds of the hydroxycinnamic acids present in the lignin. The higher the exposition to the microwaves, the higher the release of free LMWPC. This increase in the temperature as a result of MW caused the decarboxylation of FA at higher temperatures as demonstrated by the increase in the concentrations of 4-VG from control (LB-10 and RB-10) (0.9 ± 0.1 μg/ gDM for light beer, 0.35 ± 0.04 μg/g DM for red beer) to pre-treated BSG at both 600 W (LB-16, 4.0 ± 0.7 μg/g DM) and 800 W (RB-16, 2.2 ± 0.2 μg/ gDM) (**Figure 3**).

The composition of BSG depends on the barley variety, cultivation and harvest conditions, the malting and mashing processes, and the type and quality of secondary raw materials added during the brewing process (Bonifácio-Lopes et al., 2020; Petrón et al., 2021). Indeed, depending on the heat treatment undergone during the roasting of malt, the phenolic composition of the BSG can vary. Increases in the temperature of malt roasting, increase the thermal energy in the grain. Consequently, the esterified bonds of the hydroxycinnamic acids to the plant cell walls tend to be cleaved and LMWPC are released. Patrignani et al., (2021) confirmed that microwave treatments of BSG resulted in the formation of Maillard reaction products. These products were found to be derivatives of hydroxycinnamic acids, which possessed at the same time a high antioxidant activity. Besides that, free hydroxycinnamic acids can also undergo decarboxylation by increasing the medium thermal energy (Bernini et al., 2007), which can enhance TPC.

Some studies found that microwave treatment of BSG increased bioaccessible antioxidants as products of the Maillard reaction of hydroxycinnamic acids. For Patriagnani et al. (2021), higher amounts of FA (86 ± 3 µg/g DM) were obtained at 120°C and 6 min, while CA was obtained at a concentration of 112 ± 4 µg/g DM at 150°C and 2 min. For Hayat et al., (2019), the amounts of FA in the unheated fennel seed were 0.85 mg/g DM and increased to 1.16 and 1.74 mg/g DM when heated for 5 min at 300 W and 500 W, respectively. Chiremba et al., (2012) studied the influence of microwave-assisted extraction on the profile of the bound phenolic acids in sorghum and maize. They found that microwave influenced the phenolic acid composition of both plant matrices. Ferulic acid ester and/or ether bonds to plant matrix are responsible for inhibiting the biodegradability of plant cell wall polysaccharides and lignin (Du & Yu, 2010). Despite

504 their similar chemical structures, the analogue hydroxycinnamic acid, CA, did not present
505 the same inhibitory effect from its covalent bonds to the plant cell wall as FA. Du & Yu,
506 (2010) also related genotypic variations of barley seeds such as growth year to the
507 hydroxycinnamic acid profiles in BSG. Moreira et al., (2012) noted that the type of malt
508 used may have a crucial impact on the phenolic composition and antioxidant properties
509 of BSG.
510 For the statistical analysis of TPC of the main effects, all main effects were significant
511 (Supplementary Information).
512 In the case of individual phenolic compounds, except for beer type * time interaction, all
513 the other two-way interactions were significantly different for FA (0.364), CA (0.136),
514 and 4VG (0.184) (Supplementary Information).
515
516 **4.3.3. The antioxidant capacity of BSG extracts**
517 The ability to inhibit free radicals in organic medium of the obtained phenolic extracts as
518 well as the major individual phenolics in BSG was evaluated. For this, DPPH was chosen
519 as the free radical and the antioxidant capacity of samples was measured as by their ability
520 to reduce DPPH• in methanol. In the case of the individual phenolic compounds studied
521 in this work (FA, CA, and 4VG), their antioxidant capacity was compared at different
522 concentrations ranging from 0.01 to 1.00 mg/mL.
523 For the BSG phenolic extracts, except for BSG type * time (0.640) and power * time
524 (0.204) interactions, all other main effects had significant effect on DPPH
525 (Supplementary Information).
526 As shown in **Figure 3 C** & **D**, time had a positive influence on the extracts' antioxidant
527 activity. Increases in MW pre-treatment time increased the scavenging capacity of the

528 phenolic extracts from light beer BSG probably as a consequence of the production of
529 higher amounts of 4VG during MW heating. The power does not seem to affect the TPC
530 in LB BSG.
531 The time of microwave pre-treatment had a positive influence on the antioxidant activity
532 of the extracts. For both 600 and 800 W, the percentage of inhibition of DPPH increases
533 from 7.4 ± 0.8 to 54.1 ± 2.9 from 5 to 30 min of pre-treatment. MW pre-heating at 15 and
534 30 min improved the antioxidant capacity of the phenolic extracts of about 30 % and
535 50 %, respectively, regardless the power applied. In the 5 min pre-treatment, power
536 positively affected the DPPH inhibition (7 % for 600 W and 16 % for 800 W). In the
537 present work, 4-vinylguaiacol, the product of the decarboxylation of FA, showed the
538 highest percentage of inhibition of DPPH among the three studied phenolics (**Figure 3 C**
539 **& D**).
540 The radical scavenging capacity of the extracts proved to be linearly (Pearson correlation
541 coefficient r = 0.9907) dependent on their concentration on phenolic compounds (**Figure**
542 **3 C & D**), as expected. The observed antioxidant capacity of the obtained phenolic
543 extracts follows a proportional correlation to the TPC in the extracts (**Figure 3 C & D**).
544 Moreira et al., (2012) noted that the type of malt used might have a crucial impact on the
545 phenolic composition and antioxidant properties of BSG.
546 Time had a positive influence on the antioxidant activity of the extracts for both beer
547 colours independently of the power applied (**Table 3**) (**Figure 3 C & D**). This trend
548 corresponds to the previous TPC results: when the pre-treatment is longer, the percentage
549 of inhibition of DPPH increases. The treatment at 800W for 30 min leads to 52.2 ± 7.0 %
550 of DPPH inhibition (RB-16) sorely superior to the non-treated BSG (RB-10) (9.0 ±
551 3.3 %). Pre-treating the BSG from light beer at 800 W for 30 min leads to the highest

552 percentage of inhibition of DPPH (56,9 ± 4,9 % DPPH inhibition) which corresponds to
553 an augmentation of 51,1 ± 2,6 % DPPH inhibition. These results demonstrated the ability
554 of microwaves to release antioxidant phenolics bounded to the cell wall. Rahman et al.
555 (2021) obtained the highest DPPH radical scavenging capacity when BSG was oven pre-
556 treated at 160°C, generating the largest amount of TPC (from non-treated 22.7 ± 6.9 to
557 oven heated 46.3 ± 2.2 μmol/g of the defatted meal). Otherwise, the DPPH radical
558 scavenging capacity of his BSG extracts was enhanced according to the heat treatment
559 temperature. For Carciochi et al. (2018) phenolic extracts from BSG presented the highest
560 DPPH inhibition of 10.99 and 11.93 % when using ethanol/water as the solvent mixture
561 at 60:40 and 80:20 (v/v) ratio, respectively. The respective TPC on these samples were
562 3.6 and 3.2 μg/g DM. Socaci et al. (2018) studied the influence of the extraction solvent
563 on phenolic content and antioxidant activities of BSG from barley mated dark lager beer
564 (**Table 2**). Thus, the highest DPPH radical inhibition (41.14 %) was attributed to the
565 phenolic extract obtained by a mixture of acetone/water (60:40, v/v) (TPC = 1.14 mg
566 GAE/g DM) followed by the solvent mixture ethanol/water (60:40, v/v) (DPPH inhibition
567 = 29.23 %) and ethanol/water (40:60, v/v) (DPPH inhibition = 28.17 %).
568 The FA derivative, 4VG, had the highest antioxidant activity among the tested phenolic
569 compounds. Besides Trolox, 4-vinylguaiacol showed the highest ability to inhibit the free
570 radical DPPH in a methanolic medium, as observed in **Figure 4**. The production of higher
571 amounts of 4VG in the extracting medium can justify the increase in the radical
572 scavenging power as observed in **Table 3**. The percentage of inhibition of DPPH of 0.5
573 mg/mL of 4VG is 96 % (**Figure 4**), whereas FA equals 75 % at the same concentrations.
574 It was proposed that a methylated phenolic hydroxyl group in FA structure may confer
575 better resonance stability to the phenolic structure while for CA, the lower stability may

576 be attributed to fewer substituents in the aromatic ring (Chiremba et al., 2012) which
577 could justify the differences in their capacity to inhibit the DPPH radical. Their structural
578 differences could explain the difference in reactivity between CA and FA. The presence
579 of a methoxy group, in the ortho position, on the aromatic ring of FA tends to enhance the
580 electronegativity of the hydroxyl group, making its proton more labile. This promotes the
581 formation of the quinone methide intermediate which can justify the highest ability of FA
582 to inhibit the free radical DPPH than CA. It is important to highlight that, electronic
583 effects and the affinity of the antioxidant molecule to the medium can play an important
584 role in the antioxidant capacity (Tańska et al., 2018). An electron-donating substituent
585 such as a methoxy coupled to a phenol tends to increase the molecule's antioxidant
586 activity while electron withdrawing groups as carboxyl may decrease the antioxidant
587 activity (Wright et al., 2001). In the case of hydroxycinnamic acids, an electro-donating
588 double bond coupled to the aromatic ring improves the stability of the intermediate
589 quinone methide by resonance, avoiding the harmful effect of a carboxy group in the
590 molecule chemical structure. This effect is even more evident when the hydroxycinnamic
591 acid is decarboxylated. The release of the carboxyl group and the presence of the terminal
592 double bond linked to the phenol may be responsible for the observed higher antioxidant
593 activity of 4-vinylguaiacol in comparison to its correspondent hydroxycinnamic acid
594 (FA).
595

596 **5. Conclusions**

597 It was possible to obtain extracts from Belgian BSG rich in bioactive LMWPC and by
598 using a sustainable extraction method. MW pre-treatments of BSG from light and red
599 beer permitted a significant (< 0.05) increase in the TPC, the antioxidant capacity and the

600 individual phenolic contents (FA, CA, and 4VG) when compared to non-treated BSG.
601 Time was the most important factor. Power changes did not appear to significantly
602 interfere. The effect of MW pre-treatments leads to the decarboxylation of FA and the
603 production of 4VG, which presented a higher capacity of inhibition of the DPPH radical
604 in organic medium.
605

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Acknowledgements

E.Z would like to acknowledge the postdoctoral fellowship provided by the European Program IF@ULB - MARIE SKŁODOWSKA-CURIE Cofund Action (European Horizon 2020). En Stoemelings is particularly acknowledged for the supply of the Brewers' spent grains. The content is solely the responsibility of the authors and does not necessarily represent the official views of the above-mentioned funding agencies.

Figure captions:

Figure 1. Contents in (**A**) lipid and (**B**) water of the ■ light and ■ red beers BSG samples.

Figure 2. Total phenolic contents (in equivalents of FA per grams of dried matter, FAE mg/g DM) of the extracts obtained by (**A**) ultrasonication and (**B**) thermal extraction. The extracting solvent mixtures tested were: ■ acetone:water (60:40, v/v), ■ acetone:water (40:60, v/v), and ■ ethanol:water (70:30, v/v).

Figure 3. Total phenolic contents (TPC) □ and individual phenolic contents (■ FA, ■ CA, and ■ 4VG) of the extracts obtained by different microwave pre-treatments of BSG from (**A**) light and (**B**) red beer production. Correlation between □ TPC (FAE mg/g DM) and antioxidant activity (percentage of inhibition of DPPH) for the phenolic extracts obtained from different microwave pre-treatments of (**C**) ▬ light beer BSG and (**D**) ▬ red beer BSG.

Figure 4. Capacity of inhibition of the DPPH radical (%) of ■ Trolox (used as a positive control), ■ FA, ■ CA, and ■ 4VG in concentrations from 0.01 to 1.0 mg/mL.

Tables:

Table 1. Lipid and water contents in different types of BSG.

BSG type	Lipid content (%, w/w)	Water content (%, w/w)	Authors
Unroasted BSG (aqueous extract)	nd	nd	(Connolly et al., 2021)
Malted barley (ALE beer)	9.2 ± 0.2	nd	(del Río et al., 2013)
Dark lager beer 100% malted grain	nd	75 - 80	(Socaci et al., 2018)
Irish red ale	1.1 ± 0.2	nd	(Lordan et al., 2019)
Not cited	9.4 ± 0.1	nd	(Dajun Yu et al., 2020)
Not cited	8.20	81.03	(Stefanello et al., 2018)
Not cited	5.90 ± 0.01	80	(Alonso-Riano et al., 2020)
Light beer (malted barley)	4.2 ± 0.2	75.8 ± 0.8	This work
Red beer (triple ALE)	10.5 ± 0.7	73.4 ± 0.5	This work

nd: not determined.

Table 2. Recent described methods for the extraction of phenolic compounds from different types of BSG and their contents in (FA, CA) and the total phenolic contents (TPC) in mg/g DM.

Authors	Type of BSG	Type of pre-treatment	Extraction method	TPC (mg/g DM)	FA (mg/g DM)	CA (mg/g DM)
(Meneses et al., 2013)	BSG	-	Acetone:water (60:40, v/v) 60-80°C, 30 min, BSG/solvent (1:20, w/v)	9.9 ± 0.4	-	-
(Socaci et al., 2018)	Dark lager beer BSG (100 % malted grain)	-	Acetone:water (60:40, v/v) 30 min, UAE BSG/solvent (1:10, w/v)	1.14	-	-
(Carciochi et al., 2018)	Pale barley malt (Pilsen type)	-	Ethanol:water (72:28, v/v) 80°C, 60 min, BSG/solvent (1:30, w/v)	3.57	-	-
(Rahman et al., 2021)	BSG	Control	70:30 (v/v) acetone:water 20 min UAE BSG/solvent (1:20, w/v)	1.0 ± 0.2	0.009 ± 0.001	0.003 ± 0.0002
	BSG	100°C, 30 min	70:30 (v/v) acetone:water 20 min UAE BSG/solvent (1:20, w/v)	-	0.01 ± 0.01	0.003 ± 0.0002
	BSG	140°C, 30 min	70:30 (v/v) acetone:water 20 min UAE BSG/solvent (1:20, w/v)	1.41 ± 0.06	0.016 ± 0.01	0.007 ± 0.0009
	BSG	160°C, 30 min	70:30 (v/v) acetone:water 20 min UAE BSG/solvent (1:20, w/v)	1.73 ± 0.07	0.010 ± 0.001	0.011 ± 0.0005
(Andres et al., 2020)	Pilsen barley malt/wheat malt (50:50)	-	100% Water, 30°C, 2 h, BSG/solvent (1:10, w/v)	5.42	0.0009 ± 0.0001	0.0003 ± 0.0001
(Kumari et al., 2019)	Light Beer	Control	100% distilled water BSG/Solvent (1:6, w/w)	0.83	-	-
	Light Beer	Pulsed electric field pre-treatment (2.8 kV/cm, 3000 pulses of 20 µs)	100% water BSG/Solvent (1:6, w/w)	1.4	-	-

	Dark Beer	Control	100% water BSG/Solvent (1:6, w/w)	4.88	-	-
	Dark Beer	Pulsed electric field pre-treatment (2.8 kV/cm, 3000 pulses of 20 µs)	100% water BSG/Solvent (1:6, w/w)	3.97	-	-
(Alonso-Riano et al., 2020)	BSG	Mechanical grinding	Ethanol:water (20:80, v/v) UAE (20 kHz, 100 % amplitude) 47°C, 30 min BSG/Solvent (1:21.7 w/v)	3.55 ± 0.07	0.0090 ± 0.000	-
(Shih et al., 2020)	India Pale Ale	Impingement drying	Acetone:water 60:40 (v/v) 30°C, 20 min, UAE BSG/Solvent (1:10 w/v)	2.21	-	-
(Zhang et al., 2021)	BSG	-	Acetone:water 60:40 (v/v) 30°C, 20 min, UAE BSG/Solvent (1:10 w/v);	0.66	-	-
This work	Light Beer	-	Ethanol:water (70:30, v/v) 80°C, 60 min BSG/solvent (1:30, w/v)	3.5 ± 0.1	0.006 ± 0.003	0.0030 ± 0.000
This work	Light Beer	MW: 600 W, 30 min	Ethanol:water (70:30, v/v) 80°C, 60 min BSG/solvent (1:30, w/v)	12.5 ± 0.4	0.034 ± 0.004	0.007 ± 0.003
This work	Red Beer	-	Ethanol:water (70:30, v/v) 80°C, 60 min BSG/solvent (1:30, w/v)	4.5 ± 0.8	0.00500 ± 0.000	0.0030 ± 0.000
This work	Red Beer	MW: 600 W, 30 min	Ethanol:water (70:30, v/v) 80°C, 60 min BSG/solvent (1:30, w/v)	13.23 ± 0.02	0.01500 ± 0.000	0.009 ± 0.002

TPC: total phenolic contents (mg/g DM)
FA: ferulic acid (mg/g DM)
CA: p-coumaric acid (mg/g DM)
RT: room temperature

Table 3. Concentration of FA, CA, and 4VG in the extracts obtained by microwave pre-treatment of BSG from light (LB) and red beer (RB).

Samples	t (min)	P (W)	T (°C)	Ferulic acid	p-coumaric acid	4-vinylguaiacol	TPC (FAE mg/g DM)	DPPH (% of Inhibition)
LB-10	-	-	-	6.5 ± 2.73	2.6 ± 0.8	0.9 ± 0.4	3.46 ± 0.09	5.8 ± 1.6
LB-11	5	600	43. ± 8	7.0 ± 0.2	2.27 ± 0.03	1 ± 1	3.1 ± 0.2	7.4 ± 0.8
LB-12	15	600	77. ± 15	3.63 ± 0.01	3 ± 1	2.7 ± 0.2	8.2 ± 1.5	36.3 ± 7.9
LB-13	30	600	89. ± 7	34 ± 4	7 ± 3	2.0 ± 0.5	12.5 ± 0.4	54.1 ± 2.9
LB-14	5	800	62 ± 5	10 ± 2	2.62 ± 0.22	0.6 ± 0.2	6.0 ± 1.5	15.9 ± 6.1
LB-15	15	800	99 ± 16	25 ± 3	4.86 ± 1.30	2.5 ± 1.9	8.9 ± 1.0	36.6 ± 11.4
LB-16	30	800	124 ± 15	33 ± 10	11.9 ± 4.0	4.0 ± 0.7	12.20 ± 0.33	56.9 ± 4.9
RB-10	-	-	-	5.04 ± 0.02	3.3 ± 0.6	0.35 ± 0.04	4.5 ± 0.8	10.2 ± 1.4
RB-11	5	600	49 ± 2	3.5 ± 0.6	2.8 ± 0.9	0.45 ± 0.04	4.1 ± 0.2	9.0 ± 3.3
RB-12	15	600	97 ± 27	4.3 ± 1.2	4.8 ± 1.6	0.48 ± 0.04	5.0 ± 0.4	21.1 ± 7.81
RB-13	30	600	103 ± 12	14.83 ± 0.02	9.1 ± 2.3	0.7 ± 0.3	13.23 ± 0.02	47.3 ± 7.8
RB-14	5	800	63 ± 1	3.3 ± 0.4	3.0 ± 0.2	1.0 ± 0.1	4.3 ± 0.8	13.3 ± 3.8
RB-15	15	800	116. ± 15	29.6 ± 1.2	13.4 ± 0.7	2.3 ± 0.3	12.1 ± 0.8	48.9 ± 9.6
RB-16	30	800	146 ± 24	20.96 ± 5.03	11.2 ± 1.5	2.2 ± 0.2	12.2 ± 0.6	52.2 ± 7.0

LB: light beer; RB: red beer.
LB-10 and RB-10 are the control samples, no microwave pre-treatment was applied to these samples before the phenolic extraction.

Figures:

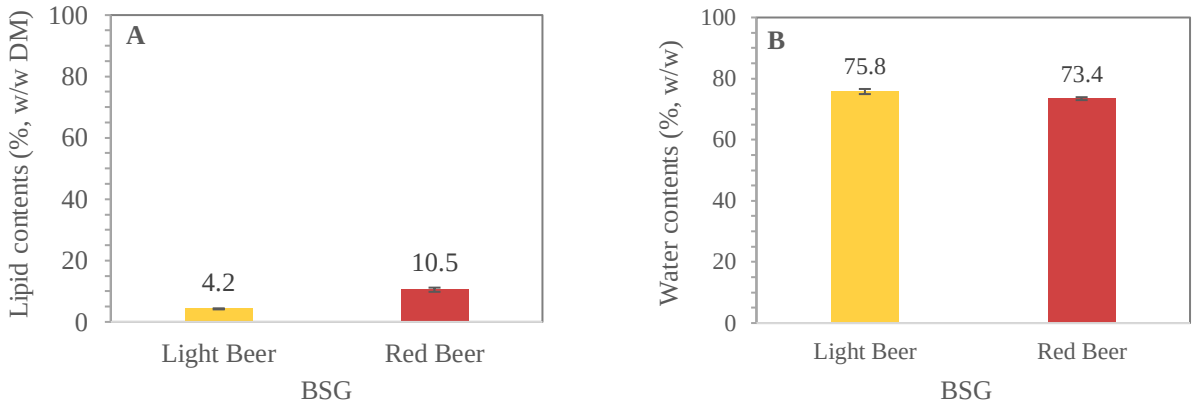

Figure 1. Contents in (**A**) lipid and (**B**) water of the ■ light and ■ red beers BSG samples.

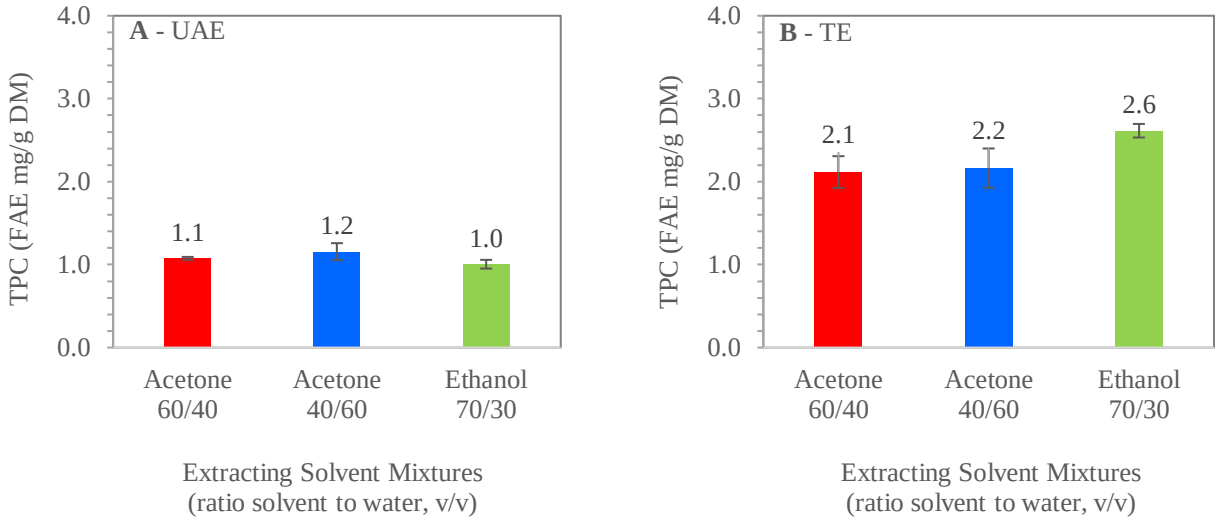

Figure 2. Total phenolic contents (in equivalents of FA per grams of dried matter, FAE mg/g DM) of the extracts obtained by (**A**) ultrasonication and (**B**) thermal extraction. The extracting solvent mixtures tested were: ■ acetone:water (60:40, v/v), ■ acetone:water (40:60, v/v), and ■ ethanol:water (70:30, v/v).

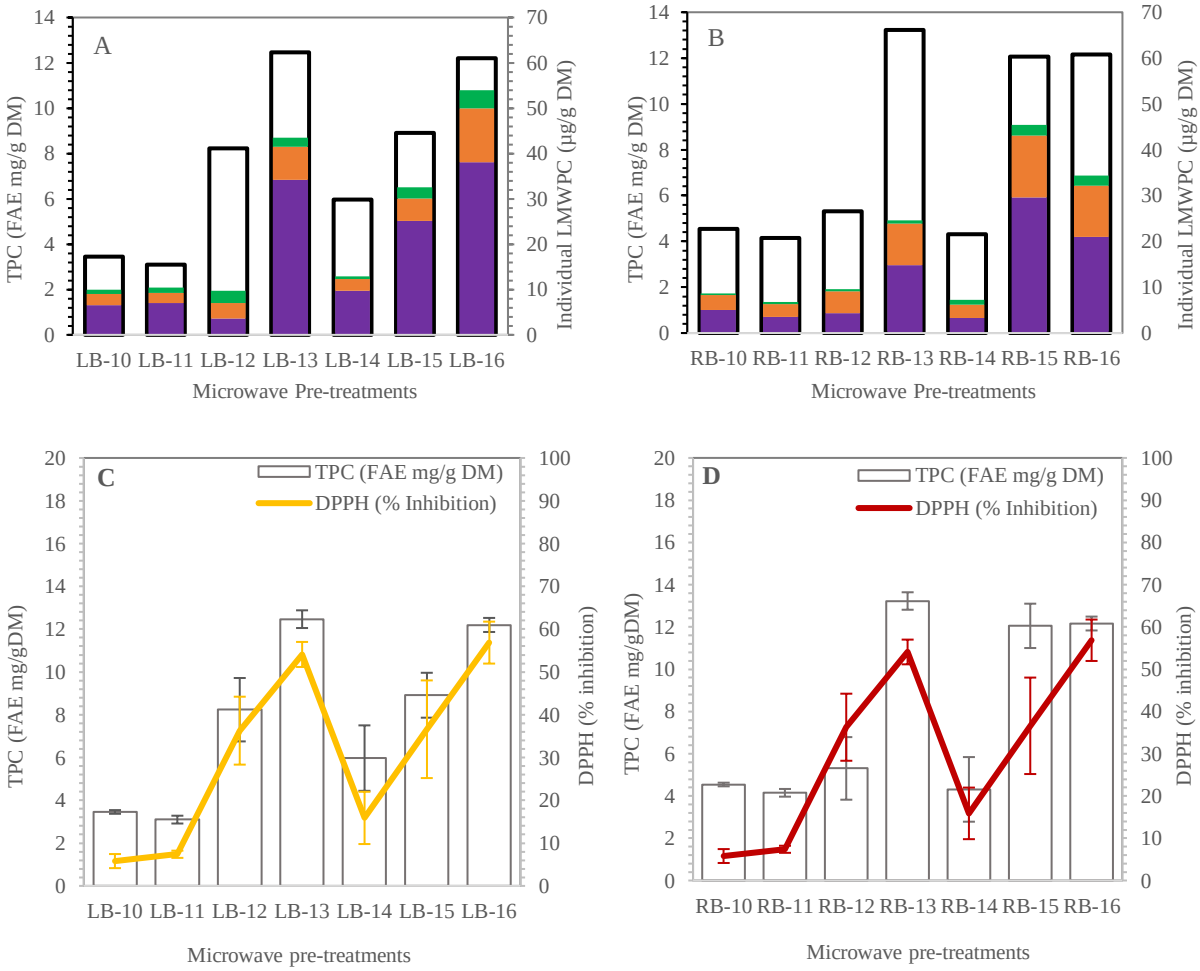

Figure 3. Total phenolic contents (TPC) □ and individual phenolic contents (■ FA, ■ CA, and ■ 4VG) of the extracts obtained by different microwave pre-treatments of BSG from (**A**) light and (**B**) red beer production. Correlation between □ TPC (FAE mg/g DM) and antioxidant activity (percentage of inhibition of DPPH) for the phenolic extracts obtained from different microwave pre-treatments of (**C**) ▬ light beer BSG and (**D**) ▬ red beer BSG.

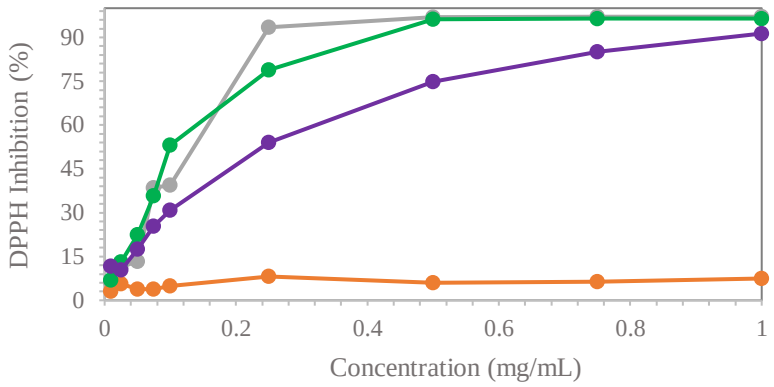

Figure 4. Capacity of inhibition of the DPPH radical (%) of ■ Trolox (used as a positive control), ■ FA, ■ CA, and ■ 4VG in concentrations from 0.01 to 1.0 mg/mL.