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Residual brewing yeast as a source of polyphenols: Extraction, identification and quantification by chromatographic and chemometric tools

María Eugenia León-González *, Esther Gómez-Mejía, Noelia Rosales-Conrado, Yolanda Madrid-Albarrán

Departamento Química Analítica, Facultad Ciencias Químicas, Universidad Complutense de Madrid, Madrid 28045, Spain

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ABSTRACT

A method combining aqueous extraction, reversed-phase high-performance capillary liquid chromatography with photodiode array detection (cLC-DAD) and chemometric tools, was developed to determine phenolic compounds in residual brewing yeast. The effect of temperature, nature of extraction solvent and method for separation of extract solution were studied to optimize the extraction conditions on the basis of total phenolic content (TPC), total flavonoids content (TFC) and antioxidant capacity. Polyphenols were determined by cLC-DAD. Flavonols as rutin and kaempferol, flavonoids as naringin, phenolic acids as gallic acid and antioxidants as *trans*-ferulic and *p*-coumaric acids were found and quantified in the brewing residue. Data were subjected to evaluation using multifactor ANOVA and principal component analysis (PCA), both showing that lyophilization pretreatment affects the content of individual polyphenols and that residual brewing yeast contains higher polyphenol amounts than the liquid beer waste. The obtained results suggest that residual brewing yeast could be a source of polyphenols.

1. Introduction

Adding value to agri-food residues is an emerging trend in food technology. The agro-industrial residues are the most abundant and renewable resources on earth and can be processed to yield valuable added products which can be suitable for food, pharmaceutical or cosmetic industry (Farcăs et al., 2013; Moreira, Morais, Barros, Delerue-Matos, & Guido, 2012; Podpora, Swiderski, Sadowska, Piotrowska, & Rakaeska, 2015; Shrikanta, Kumar, & Govindaswamy, 2015).

Polyphenols are a kind of chemicals with many beneficial effects on human health, due to their antioxidant properties (Vijayalaxmi, Jayalakshmi, & Sreeramulu, 2015), playing an important role in preventing and/or decreasing the incidence of degenerative diseases, such as cancers, and cardiovascular and neurodegenerative illnesses (Pandey & Rizvi, 2009). Therefore, the recovery of polyphenols from food processing residues for added value use is a topic of great interest in many research areas. There are several reports in the literature on the recovery of phenolic compounds from food residues and by-products. For instance, polyphenols have been extracted from orange peel (Mereyem, Chahrazed, & Loic, 2015), and bioactive proanthocyanidins have been isolated from different fruit peels (Li et al., 2015). In peanut skins, the major phenolic compounds detected were coumaric acid and quercetin (Ma et al., 2014), while naringin was the predominant flavonoid found in the peel of pummelo varieties (Xi, Fang, Zhao, Jiao, & Zhou, 2014). Gallic acid, ferulic acid, quercetin and kaempferol have been extracted from different agricultural residues (Vijayalaxmi et al., 2015) and gallic acid, coumaric acid and resveratrol from several underutilized fruits (Shrikanta et al., 2015). The phenolic profile of both chestnut leaves and beer residue extracts have been analyzed by Munekata et al. in order to evaluate their potential application as a functional food ingredient (Munekata, Franco, Trindade, & Lorenzo-Rodriguez, 2016). Polyphenols have been also obtained from essential oils (Sánchez-Vioque et al., 2013). Finally, the extraction of polyphenols retained in black tea wastes from the commercial production of tea beverages was also investigated by Naoki et al. (2012).

The residual brewing yeast is the second-largest by product from brewing industry and it contains a great amount of carbohydrates, vitamins, minerals and proteins, reason why this residue has been currently utilized as ingredient in animal feed and food supplements (Podpora et al., 2015). Additionally, some studies have reported the capability of yeast (such as *Saccharomyces cerevisiae*) either to absorb polyphenols during fermentation processes or to recover phenolic compounds from tea (Jilani, Cilla, Barberá, &

* Corresponding author at: Dpto. Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain.
E-mail address: leongon@ucm.es (M.E. León-González).

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Hamdi, 2015) and olive leaf infusions (Jilani, Cilla, Barberá, & Hamdi, 2016). In this line, Rizzo et al. determined rutin and gallic acid adsorbed on 23 types of *Saccharomyces cerevisiae* yeasts grown in media containing high levels of polyphenols from grape seeds and grape skin (Rizzo, Ventrice, Varone, Sidari, & Cadari, 2006). *S. cerevisiae* based biosorbents are generally considered safe and could be easily accepted by the public when applied for nutritional purposes. This fact suggests the potential interest of residual brewing yeast as a valuable source of bioactive polyphenols for added value used in cosmetic, food and pharmaceutical industry.

Therefore, the objective of this work was to determine and quantify phenolic compounds extracted from residual brewing yeast. For this purpose, an analytical methodology combining a cost efficient and green extraction procedure, spectrophotometric and chromatographic methods was developed. Moreover, multivariate ANOVA and principal component analysis (PCA) were employed as chemometric tools for optimizing the best conditions for extracting phenolic compounds from residual brewing yeast.

2. Materials and methods

2.1. Residual brewing yeast and chemicals

The residual brewing yeasts employed in this study were kindly provided by the Mahou-San Miguel brewery group (one of the biggest producer of beer in Spain) and by Cerveza Henares (a local beer producer). In both cases, the brewing yeast employed was a lager yeast (bottom-fermenting yeast).

The residual brewing yeast was provided as a yeast slurry and therefore it was subsequently submitted to a filtration and centrifugation process to separate the liquid phase (liquid beer waste). The resulting solid residue (residual brewing yeast) was dried by using two methodologies: air drying and lyophilization. Both solid and liquid residues were stored in the dark at 4 °C for further analysis. Because of the fact that brewers use their own yeast for brewing, differences in polyphenols content could be found between their corresponding residual brewing yeast. For this purpose, wastes obtained from the two providers mentioned above were characterized. In order to clarify the data presented in the current work, residual brewing yeast provides for the group Mahou–San Miguel will be named through the manuscript as Residual brewing yeast 1 (RBY1) and the corresponding Liquid residue as liquid beer waste 1 (LBW1). Similarly, residual brewing yeast provides for the Cerveza Henares will be named as (RBY2) and the corresponding Liquid residue as (LBW2).

All reagents and solvents were of analytical grade, and purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used in all procedures. Methanol (MeOH), acetonitrile (MeCN) and ethanol of gradient HPLC grade were supplied by Scharlab (Barcelona, Spain). Trifluoroacetic acid (TFA, 99%) and Folin-Ciocalteu's phenol reagent 2 N were provided by Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate anhydrous, sodium hydroxide pellets, sodium nitrite, tri-sodium phosphate 12-hydrate, aluminium chloride 6-hydrate, ammonium molybdate tetrahydrate and sulphuric acid (95–98%) were purchased from Panreac (Barcelona, Spain). 3,4,5-Trihydroxybenzoic acid monohydrate (gallic acid monohydrate, ≥98.0%), *trans*-4-hydroxycinnamic acid (*p*-coumaric acid, ≥98.0%), *trans*-4-hydroxy-3-methoxycinnamic acid (*trans*-ferulic acid, 98%), quercetin-3-rutinoside trihydrate (rutin trihydrate), 3,3′,4′,5,5′,7-hexahydroxyflavone (myricetin), 3,4′,5-trihydroxy-*trans*-stilbene (resveratrol, ≥99%), 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin, ≥95%), 3,4′,5,7-tetrahydroxyflavone (kaempferol, ≥97.0%), 4′,5,7-trihydroxyflavanone 7-rhamnoglucoside (naringin, ≥95%) analytical standards were obtained from Sigma-Aldrich.

The appropriate amount of each standard was dissolved into methanol to obtain stock solutions with a final concentration of 200 mg·L^{-1}. Quercetin stock solution was prepared using a mixture ethanol-water 80:20 (v/v) as solvent. Analyte stock solutions were stored in the dark at 4 °C for no longer than one month, except for *trans*-ferulic acid, which was stored at −80 °C to prevent fast analyte degradation. Fresh working standard solutions were daily prepared by suitable dilution of stock solutions as required.

Nylon membrane filters from Teknokroma (Barcelona, Spain), with 0.45 μm and 0.22 μm pore size, were used for mobile phase filtration before HPLC analysis. Ash-free filter paper supplied by Scharlab (Barcelona, Spain), PTFE membrane filters, polyester membrane filters and nitrocellulose membrane filters supplied by Teknokroma (Barcelona, Spain) were tested for sample filtration.

2.2. Apparatus and instrumentation

Chromatographic analysis by cLC was performed by an Agilent cLC instrument Mod. 1100 Series (Agilent Technologies, Madrid, Spain) which was equipped with a G1376A binary capillary pump, a G1379A degasser and a G1315B diode array detector (500 nL, 10 mm pathlength). Data acquisition and processing were made using the Agilent Chemstation software package for Microsoft Windows. An external stainless steel loop with a volume of 10 μL was placed into a Rheodyne® injection valve. Reversed-phase separation was made on a Synergi™ Fusion 4 μm C18 (150 mm × 0.3 mm I.D.) capillary analytical column supplied by Phenomenex (Torrance, CA, USA).

Absorbance measurements were carried out using a diode array HP8543 UV/Vis spectrophotometer (Agilent Technologies), by means of the HP Chemstation software.

A Unicen centrifuge model 21 provided by Ortoalresa (Madrid, Spain) was used for performing centrifugation of aqueous or ethanol-water residual brewing yeast extracts before chromatographic assays. For determination of residual brewing yeast moisture content, a P-Selecta oven with temperature control range from 10 to 200 °C, equipped with ceramic dishes from Panreac (Barcelona, Spain), was employed. The pH of residual brewing yeast extract was measured using a Teknokroma pH-meter model pH 555, equipped with a Crison 52–02 glass combination electrode (Barcelona, Spain). Lyophilization of beer residues was made on a Lyophilizer Sher Freeze-110 from AAPPTec LLC (Lousville, KY, USA).

2.3. Extraction of polyphenols from residual brewing yeast

An amount of 0.5 g of dried residual brewing yeast was added to 100 mL of Milli-Q water or a mixture of ethanol-water (20:80 v/v) at 25 °C or 95 °C for spectrophotometric analysis. In the case of chromatographic analysis, the same conditions were applied to an amount of 0.2 g of the sample and 10 mL of the extract. The mixture was extracted for 5 min with magnetic stirring, and then it was cooled down at 25 °C. Between 0.5 and 5 mL of obtained extracts were directly taken for the determination of total polyphenolic and flavonoid content, previous filtration or centrifugation. When performing chromatographic analysis, extraction times of 10 and 30 min were also evaluated. The resulting mixture was centrifuged at 2963*g* during 20 min at 25 °C, and different volumes depending of the type of residual brewing yeast were taken, varying between 100 and 200 μL. In the case of liquid beer waste, between 100 and 200 μL were taken for performing total polyphenolic and flavonoid content and chromatographic analysis. Samples were prepared in triplicate in all studied conditions.

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