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Effect of pulsed electric fields – assisted extraction on anti-inflammatory and cytotoxic activity of brown rice bioactive compounds

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ABSTRACT

The bioactive compounds of brown rice exhibit many beneficial health effects, ranging from antioxidant to cytotoxic activities. Pulsed Electric Field (PEF) pretreatment can significantly enhance their extraction, through the induction of the electro-permeabilization of the cell membranes.

This paper aims to demonstrate that PEF-assisted extraction of brown rice enables not only enhanced yields of antioxidant compounds, such as γ-oryzanol, polyphenols and phenolic acids, and of saturated and unsaturated fatty acids, but also increased cytotoxic effects on cancer cells.

Initially, the PEF-assisted extraction conditions have been defined by the assessment of the cell permeabilization index via impedance measurements and the DPPH antioxidant activity. Subsequently, the biological effects of PEF have been evaluated on the cytotoxicity and anti-inflammatory properties against human colon cancer cell line HT29. The results show that PEF-assisted extraction, enhancing the yield of bioactive compounds, with respect to untreated extracts, significantly promotes their antioxidant activity, which is correlated with an increased HT29 cells cytotoxicity. In addition, PEF extracts of brown rice substantially inhibit also gene expression and interleukin production in colon cancer cells, suggesting their exploitation as natural anti-inflammatory agents.

The integration of PEF pretreatment in the solvent extraction process of bioactives from brown rice appears, therefore, as a promising practice to significantly enhance their biological activity.

1. Introduction

Brown rice, owing to its content of vitamins, fibers, fatty acids and minerals, which helps to regulate digestion, reduce weight, as well as cholesterol and triglycerides (Anderson & Hanna, 1999; Panlasigui & Thompson, 2006), can be regarded within reason as a superfood. Its consumption has been reported to have also beneficial health effects on diabetes, immune system, cancer, and heart disease, owing to its high content also of bioactive compounds, such as tocopherols, tocotrienols, γ-oryzanols, phytic acid, and other phenolic compounds (Jun, Song, Yang, Youn, & Kim, 2012).

Recent studies have highlighted that the pool of soluble and insoluble antioxidant compounds (the most abundant being ferulic, *p*-coumaric, isoferulic, syringic, vanillic, sinapic and caffeic acids, (+)-catechin, *p*-hydroxybenzoic and protocatechuic acids) contained in rice bran synergistically contributes to the anti-inflammatory, hypocholesterolemic and hypotriglyceridemic activities upon its consumption (Shao & Bao, 2015). However, the consumption of whole rice as a regular meal might be problematic especially for those patients, which are affected by colon cancer and intestinal chronic inflammatory diseases, because of limited digestion capability, and may require different forms of administration, which for example include the extraction of the antioxidant compounds from whole rice or from rice bran.

Previous studies have reported the use of organic solvent extraction to recover bioactive compounds from different varieties of pigmented rice and brown rice bran, highlighting significant differences in antioxidant and chelating properties, with black and red rice exhibiting the highest activities (Jun et al., 2012). The observed differences in the extracts composition and activity are due not only to the biological diversity of the matrices but also to the specific mass transfer resistances to the transport of intracellular bioactive compounds through rice bran cell membranes.

Pulsed electric fields (PEF) treatment is one of the most promising novel physical methods to improve the efficiency of several processes of the food industry, based on mass transfer phenomena, such as extraction, juice expression, drying, and osmotic dehydration (Barba et al., 2015).

PEF treatment involves the application of short duration (from μs to ms) electric field pulses of moderate intensity (0.5–10 kV/cm) to plant tissues placed between two electrodes, causing the permeabilization not only of the cell membrane (Bouzrara & Vorobiev, 2003; Donsì,

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Ferrari, & Pataro, 2010a), but also of cell vacuoles (Fincan & Dejmek, 2002), where some metabolites are contained.

More specifically, the enhancement in the extraction yield of phenolic compounds, flavonoids, anthocyanins, proteins, and carbohydrates from agricultural and food processing by-products of grapes (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008), blueberries (Bobinaite et al., 2015), flaxseed hulls (Boussetta, Soichi, Lanoiselle, & Vorobiev, 2014), papaya peel and seeds (Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2014), and mango peels (Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2016) has been reported when PEF-assisted extraction with solvents has been used.

The main idea behind this paper is to demonstrate that PEF-assisted extraction of brown rice enables not only enhanced yields of antioxidant compounds and of nutritive compounds with relevant biological activity, such as unsaturated fatty acids, but also increased cytotoxic effects on cancer cells. In particular, despite PEF-assisted extraction has been tested on different plant matrices (Donsì et al., 2010a), no previous studies focused on brown rice. We aim to demonstrate that PEF treatment significantly enhances not only the extraction yield and the antioxidant activity of the extracts, but also their composition in terms of both polyphenols and fatty acids. Moreover, the biological relevance of antioxidant compounds for cancer cure can be correlated with their cytotoxicity against colon cancer cell line HT29 (Baricault et al., 1995). Therefore, we also aim to show that the integration of PEF pretreatment in the solvent extraction process of bioactives from brown rice positively affects their anti-cancer potential.

Brown rice (*Oryza sativa*), despite not being the rice variety with the highest content of antioxidant compounds, has been selected as the matrix of study because of its widespread distribution all over the world, as well as for the large amounts of bran available from its processing to white dehulled rice.

2. Experimental

2.1. Materials

Biological brown rice (*Oryza sativa*) from “Il Fior di loto S.r.l.” (Velezzo Lomellina, Italy) was used through the entire experimental campaign.

Extraction of phenolic compounds from brown rice was carried out using Ethyl Acetate (anhydrous, ≥99.8%), Methanol (anhydrous, ≥99.8%), Acetone (for HPLC, ≥99.8%), and Ethanol (anhydrous, ≥99.5%) from Sigma-Aldrich (Milan, Italy) and bidistilled water.

Standards of 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, and ascorbic acid and reagents for derivatization and GC–MS analysis, such as hydrochloric acid, dichloromethane, BSTA/TMS (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 10% w/w trimethylchlorosilane), and hexane were also obtained from Sigma-Aldrich (Milan, Italy).

The chemicals and reagents for cellular studies were: Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), and Penicillin-Streptomycin, obtained from Gibco, Life Technology (Italy), MTT-WST-8 from Dojindo Molecular Technologies Inc. (Rockville, MD, US) and lipopolysaccharides (LPS) obtained from Sigma-Aldrich (Milan, Italy). Reagents for gene expression studies, such as TRIzol reagent, primers, and Superscript First-Strand Synthesis System Kit were obtained from Invitrogen (Thermo Fisher Scientific, Milan, Italy).

All chemicals and reagents used in the study were of analytical grade.

2.2. Extraction procedure

All the organic solvents, except ethyl acetate, were mixed in different proportions with water to obtain solutions at different concentrations (0%, 20%, 40%, 60%, 80% and 100% v/v of solvent). Ethyl acetate was instead tested pure (100% v/v), owing to its lack of solubility in water. 4 g of brown rice were added to 20 mL of the extraction solutions in glass tubes, which were kept under agitation at 37 °C in a thermostated orbital shaker, with rotational speed set at 150 rpm. After 24 h, samples were centrifuged at 1000 $\times g$ for 15 min at 25 °C (ALC PK 130R, VWR International PBI Srl, Italy), and the extracted solution was filtered through filter paper (Whatman No. 4), as previously suggested (Jun et al., 2012). The liquid extracts were then analyzed for total phenolic compounds and antioxidant activity.

After the preliminary determination of optimal solvent, different pretreatment procedures of the brown rice were evaluated. In particular, rice was cooked in boiling water under agitation for 10, 20, 30 or 40 min. After the pretreatment, the rice was drained in a colander, and the weight increase due to water uptake was measured. After cooling to 20 °C, organic solvent and water were added in such a proportion that, together with the water uptaken by the rice during the pretreatment and the eventual water added during the PEF treatment, the desired composition of the extraction solvent was achieved, maintaining a ratio of 4 g of rice (dry basis) in 20 mL of solvent. Extraction was then carried out as described above.

The effect of PEF treatment was evaluated on pretreated rice and performed immediately before solvent extraction.

At the end of the extraction process, the solvent was removed from the extracts by evaporation at 30 °C and 50 mbar in a Rotavapor R210 (Buchi, Italia), followed by freeze-drying. Dried extracts were used for the identification of the main constituents of the extracts by GC–MS analysis and for cytotoxicity studies on colon cancer cells.

2.3. PEF treatment

PEF treatments of different intensities were carried out in a laboratory-scale batch system, previously described in details (Bobinaite et al., 2015). Briefly, the system consisted of a specifically designed treatment chamber made of a vertical spacer of polycarbonate material, closed on both ends by two cylindrical electrodes in stainless steel, with the upper one able to slide within the spacer, in order to apply a predetermined compression (0.3 bar) on the sample. The electrode area was 7.1 cm^2, and the distance between the two electrodes could be adjusted (up to 5 cm) depending on the volume of the treated sample. The two electrodes of the PEF chamber were connected to an electric pulses generator (Modulator PG, ScandiNova, Uppsala, Sweden) able to deliver monopolar square wave pulses of different intensities. Special probes connected to an oscilloscope were used to measure the actual voltage and current signals in the treatment chamber. The maximum electric field intensity (E, in kV/cm) and total specific energy input (W_T, in kJ/kg) were calculated as previously described (Bobinaite et al., 2015).

Rice samples of 2 g each (dry basis) after pretreatment (boiling for 10 or 20 min) were loaded in the treatment chamber. Water was added to fill the empty space between the grains of rice in order to improve the electrical contact between the electrodes and the sample. The applied electric field E was varied from 1 to 3 kV/cm, the number of pulses N_p from 10 to 1000 at a constant pulse duration τ_p (100 μs) and pulse repetition frequency (5 Hz). The total specific energy delivered to the brown rice grains was comprised between 0.16 and 144 kJ/kg. In all experiments, the initial temperature of the samples was 20 ± 1 °C and the final temperature never exceeded 23 ± 1 °C.

In order to quantify the degree of cellular permeabilization attained by each treatment, the cell membrane permeabilization index Z_p was evaluated on the basis of the measurement of the absolute values of the complex impedance of the intact and PEF treated tissues in the low (1 kHz) and high (10 MHz) frequency ranges, as previously described (Donsì et al., 2010a). The value of this index varies between 0, for the intact tissue, and 1, for the fully permeabilized tissue.

2.4. Total polyphenols and antioxidant activity of the extracts

The content of total polyphenols in the extracts was determined using the Folin-Ciocalteu colorimetric method (Slinkard & Singleton,