



The impact of pulsed electric fields and ultrasound on water distribution and loss in mushrooms stalks



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ABSTRACT

Pulsed electric fields (PEF) and ultrasound (US) are promising innovative technologies with the potential to increase mass transfer when combined with further processes which in turn can provide potential benefits in the recovery of valuable compounds from food by-products. To provide evidence of the mechanism of mass transfer enhancement, the present study assessed the impact of PEF and US treatments, applied individually and in combination, at low and high temperatures, on the tissue microstructure of mushroom stalks. Different indices such as quantitative water redistribution, water loss and qualitative release of compounds were evaluated. The combination of these physical methods demonstrated that PEF redistributed a greater proportion of intracellular water into extracellular spaces than US. However, the application of high temperature treatments alone showed an even greater proportion of intracellular water migration compared to PEF. When PEF was combined with US at low temperatures the difference was not significant.

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1. Introduction

Recent research on innovative technologies such as pulsed electric fields (PEF) and ultrasound (US) have shown great potential for applications in a number of areas within the food industry. As an example, they have been studied as an alternative to traditional thermal treatments for microbial inactivation (Raso & Barbosa-Cánovas, 2003) or for the enhancement of mass transfer in various processes (Donsì, Ferrari, & Pataro, 2010). Pulsed electric fields treatments create pores at the level of biological membranes which can be transient and reseal or pores can be permanent leading to irreversible cell disruption. The extent to which transient/permanent pores occur is a function of the treated matrix and the applied PEF protocol (Vorobiev & Lebovka, 2009). Overall these phenomena are generally recognized as electroporation. Recent research, used electroporation in its irreversible form for the valorisation of food by-products and waste with a view to reducing the waste disposal problems (Barba et al., 2015). Generally, electric field strengths between 0.5 and 3 kV and short pulses (pulse width of 1–100 µs) at the frequency of 1–100 Hz have been successful for irreversible electroporation of plant tissues (Vorobiev & Lebovka, 2009). Similarly, the application of ultrasound leads to an increase in mass

transfer phenomena by physically affecting the treated tissue. US induces the formation of gas bubbles which collapse generating high energy shock waves and intense shear forces, a phenomena known as the cavitation effect (Patist & Bates, 2008). As a consequence, extraction and purification processes of food by-products assisted by high intensity ultrasound, commonly 100–400 W applied at 20–100 kHz, resulted in high recoveries of valuable compounds from several plant materials (Roselló-Soto, Galanakis et al., 2015).

These innovative technologies were tested for the recovery of different components from mushroom by-products (Cheung, Siu, Liu, & Wu, 2012; Cheung, Siu, & Wu, 2013; Parniakov, Lebovka, Van Hecke, & Vorobiev, 2014; Xue & Farid, 2015), being rich in several high value compounds, namely proteins, chitins, glucans and other polysaccharides from cell wall, polyphenols and ergosterol (Roselló-Soto, Parniakov, et al., 2015). The potential simultaneous extraction of several compounds promotes the recovery of the by-products for different industrial sectors such as food, pharmaceuticals and cosmetic. For example, cell walls of *Agaricus bisporus* are an important source of chitinous biopolymers, which can be used as antimicrobial agent or coating material in the food sector. Its sustainable extraction from mushroom by-products can also reduce allergenic problems and inconveniences arisen from the use of harsh solvents that are needed for the extraction from the traditional sources like shrimp and crab shells (Wu, Zivanovic,

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Draughon, & Sams, 2004). Moreover, taking advantage of their non-thermal mechanisms of action, PEF and US can affect the quality of the extracted material, preventing the damage of some thermolabile components which usually occurs with traditional methods when heat is provided to ease the extraction. In previous studies (Luo, Han, Zeng, Yu, & Kennedy, 2010; Pingret, Fabiano-Tixier, & Chemat, 2013), various food materials subjected to heat, US and PEF treatments were examined to find possible degradations of different chemical compounds. Non-thermal technologies, i.e. PEF and US, resulted in lower degradation of thermolabile vitamins and proteins when compared to heat processes. Conversely, polysaccharides, including those that compose the mushroom cell wall, were influenced by PEF and US processes that produced changes of their morphology and molecular weight. The induced modifications might result in a different water uptake of these biopolymers and in a different viscosity of the extracts.

Rapid methods are very valuable in screening the impact of innovative process technologies applied across a range of conditions. Appropriate methods include acoustic, texture and colour measurements (Lespinard, Bon, Cárcel, Bénédict, & Mascheroni, 2015; Parniakov, Lebovka, Bals, & Vorobiev, 2015; Wiktor et al., 2016) but assessments can also be made using direct measures of cell metabolite release (Dellarosa, Tappi, et al., 2016; Luengo, Condón-Abanto, Condón, Álvarez, & Raso, 2014). In addition to the aforementioned methods, measuring the changes in electrical impedance, known as cell disintegration index, has been extensively investigated in recent years and it is the most commonly accepted quantitative index of the extent of electroporation (Angersbach, Heinz, & Knorr, 1999; Lebovka, Bazhal, & Vorobiev, 2002). Such indices provide an estimate of microstructural modifications in the inner compartments of plant tissues (Vorobiev & Lebovka, 2009) and have been recently adopted to compare the effect of different technologies on the food matrix (Barba, Brianceau, Turk, Boussetta, & Vorobiev, 2015; Barba, Galanakis, Esteve, Frigola, & Vorobiev, 2015). Another approach to assess microstructural effects of novel processes is based on the optical and electronic microscopy, but these results are qualitative (Faridnia, Burritt, Bremer, & Oey, 2015; Fincan & Dejmek, 2002; Nowacka, Tylewicz, Laghi, Dalla Rosa, & Witrowa-Rajchert, 2014) and can be vulnerable to the subjectivity of the analyst. Further quantitative methods focused on tissue microstructure and take into consideration water distribution through the food matrix. Indeed, water drip loss as a function of technological treatments also provides an index of the extent of cell damage which is generally accompanied with the release of intra-cellular water (Sun & Li, 2003). In addition, direct insights into water distribution and possible redistribution upon the application of different process technologies were recently obtained by means of time domain nuclear magnetic resonance (TD-NMR) (Dellarosa, Ragni, et al., 2016; McDonnell et al., 2013; Santagapita et al., 2013). The analysis of the transverse relaxation time (T_2) curves led to the non-destructive estimation of water distribution through the cell compartments, including the intra/extra-cellular water repartition.

The present study was focused on microstructural changes of mushroom stalks, an abundant by-product of the mushroom industry. The main goal was to gain insight into fine modifications which affect the mechanisms of mass transfer, aiming at improving the extraction of valuable compounds. Different innovative technological treatments were tested, i.e. pulsed electric fields (PEF) and ultrasound (US), and compared to traditional water extraction processes. A novel multianalytical approach which combined several physical techniques, such as cell disintegration index, drip loss and water distribution by TD-NMR, was employed. Moreover, various combinations of different technologies were experimented to highlight possible synergistic or antagonistic effects of the different mechanisms of action. Results of the study provide useful tools for

the evaluation of the feasible and efficient industrial application of the tested technologies.

2. Materials and methods

2.1. Mushroom stalks

Mushrooms (*Agaricus bisporus*) were purchased from a local market in Dublin (Ireland) and were analysed within 2 days of purchase. The stalks were manually separated from the caps and cut with a knife. In order to obtain a homogenous size distribution and standardize the surface-to-volume ratio, the cut stalks were sieved and pieces between 1.70 and 4.75 mm were selected for experimental trials.

2.2. Sample preparation

For all treatments 10 g of mushroom stalks were immersed in tap water (conductivity = 0.6 mS/cm) for 10 min at a standardized solid/liquid ratio of 1/10 and constant agitation by magnetic stirrer at 60 rpm. The temperature was controlled by an external water bath (DMS360, Fisher Scientific, UK) and monitored by a digital thermocouple (TC-08, Pico Technology, UK). Two different temperatures were used for the experiments to be representative of the room temperature (25 °C) and the ideal temperature for the extraction of valuable compounds in mushrooms (90 °C). The latter was also chosen because it is the lowest temperature value within the optimal range of temperatures reported in the literature (Xujie & Wei, 2008), so to minimize the cost of the process from an industrial angle. Each sample batch was weighed before and after treatments and water, possibly evaporated at 90 °C, was added again in order to restore the solid/liquid ratio. Control samples were obtained by only dipping mushroom stalks at both 25 °C (DIP) and 90 °C (H). Pulsed electric fields, as a pre-treatment, and/or ultrasound were tested, either alone or in combination, at both temperatures, resulting in the following sample groups: PEF, US, PEF + US, US + H, PEF + H, PEF + US + H.

2.2.1. Pulsed electric fields (PEF)

PEF was applied, as a pre-treatment either alone or in combination with other technologies, using a 5 kW PEF generator ELCRAK HVP 5 (DIL ELEA, Quakenbrück, Germany). The electric pulses of near-rectangular shape were delivered in a batch $4 \times 4 \times 4$ cm chamber with two 4×4 cm stainless steel parallel electrodes.

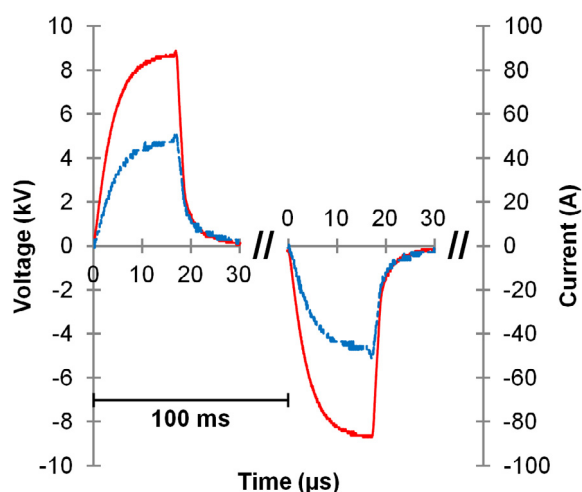


Fig. 1. An example of bipolar electric pulses used in the present investigation; solid and dashed lines are voltage and current, respectively.

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