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The use of optical coherence tomography for the evaluation of textural changes of grapes exposed to pulsed electric field

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ABSTRACT

Application of pulsed electric fields (PEF) to food is a nonthermal technology of food processing. The short pulses of high intensity electric field can modify the internal structures of fruits and vegetables by affecting the cell permeability. In the presented study three popular grape wine cultivars – *Johanniter*, *Hibernal* and *Marechal Foch* were exposed to PEF at electric field strengths of 3.3 kV/cm and 5 kV/cm. The significant textural changes of near peel grape layers influenced by electric field were observed in optical coherence tomography (OCT) images, using infrared light of 1300 nm. These changes were expressed by the variation of entropy, standard deviation or lacunarity features and evaluated in a dedicated software tool developed by the authors in Matlab environment. The OCT is a non-destructive technique in which no sample preparation is needed and grapes still remain intact (undamaged) during imaging. The OCT cross-sections revealed the progressive process of expanding zone with strong echo in sub-peel layers what may indicate cell permeabilization or even loosing parenchyma cells integrity. Also grape surface deformations under PEF were quantified. It has been shown that the values of considered textural features in near peel grape tissue were related to the intensity of electric field. *Marechal Foch* cultivar appeared to be more resistant to PEF than two other grape varieties.

1. Introduction

One of the nonthermal, fast methods of food treatment is subjecting it to the pulsed electric field (PEF). This method is used most often to increase the efficiency of the process for obtaining juices from vegetables and fruits, as well as to improve the efficiency of food drying and preservation (Puertolas et al., 2010; Walkling-Ribeiro et al., 2011a,b; Monfort et al., 2011, 2012; Oziembłowski et al., 2014). The PEF-treatment allows the preservation of the natural quality, pigment, flavouring agents and vitamin constituents of food products.

In wine industry the application of the PEF treatment leads to improved wine quality parameters such as phenolic content, total polyphenols index and visual characteristic such as colour intensity and clarity (López et al., 2008b, 2009; López-Alfaro et al., 2013; Delsart et al., 2014; Donsì et al., 2010; López-Giral et al., 2015). The application of PEF treatment also reduces the maceration time during vinification (López et al., 2008a,b, 2009; Delsart et al., 2014). The process of compounds releasing, mostly from grape skin, is strictly dependent on grape variety (Garde-Cerdán et al., 2013), due to differences in the grape skin layer thickness and the construction of its cell wall (Ortega-Regules et al., 2008).

It has been found that several other techniques such as high fermentation temperature, extending maceration time, freezing as well as the use of maceration enzymes can also enhance the extraction of phenolic compounds through the degradation or permeabilization of the grape skin cells (Sacchi et al., 2005). However, these methods can lead to a poorer quality of the product or prolonged production time. When compared with novel technologies such as high electrostatic pressure or ultrasonics, PEF tends to predominate, particularly in the increasing the antioxidant activity of the extracts (Corrales et al., 2008). The PEF procedure also has the advantage that it provides lower energy costs and greater product quality than other processing methods (Lebovka et al., 2016).

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The problem of determining the relevant PEF influence on the tissue and its scale evaluation is inherently multidisciplinary and integrates different biological, electro-physical and chemical processes (Vorobiev and Lebovka, 2008). To extract the contents from plant tissue, the cell membrane must be ruptured or permeabilized through a phenomenon called electroporation (Ade-Omowaye et al., 2001). Electroporation can be either reversible or irreversible, depending on parameters of the electric pulses. For reversibility the cell membrane potential has to be kept below the critical value which is between 200 mV and 1 V (Zimmermann, 1982; Tsong, 1991; Kandušer and Miklavčič, 2008). When this value is exceeded, irreversible electroporation results in cell membrane disintegration, large pores formation, and loss of cell viability (Angersbach et al., 2000).

The tissue damage degree can be defined as the ratio of the damaged cells to the total number of cells. The damage degree can be estimated through microscopic observation of the PEF-treated tissue, using TEM, SEM or cell viability staining techniques (Fincan and Dejmek, 2002; Cholet et al., 2014; Delsart et al., 2014; Janositz and Knorr, 2010; Ersus and Barrett, 2010). These techniques provide good quality images to confirm changes in grape cells under PEF but require specific preparation of samples. The cell disintegration index was also proposed for the description of tissue deformation (Angersbach et al., 1999). This index comprises the extent of the cell membrane damage relative to the initially intact cells and it is based on conductivity of cell materials before and after treatment. Evaluation of cell disintegration index requires determination of electrical conductivity from supplementary measurements for maximally damaged material after freeze-thawing or strong PEF-treatment with high strength electric field and long duration of PEF-treatment (Lebovka et al., 2007a). Another method is based on electrical conductivity measurements at low (≈1 kHz) and high (3–50 MHz) frequencies (Angersbach et al., 2002). Generally, the disintegration index shows the average cell disintegration in the whole tissue cross-section and describes the transition of a cell from intact to ruptured state.

It is possible to estimate the damage degree from diffusion coefficient measurements in the PEF-treated biological materials (Lebovka et al., 2007b). The apparent diffusion coefficient can be determined from solute extraction or convective drying experiments. Unfortunately, diffusion techniques are indirect and invasive for plant material, and they may impact the tissue arrangement.

The disintegration of grape tissue seems to be the most interesting just under the fruit cuticle, as this area accumulates the most of aromatic and phenolic compounds (Borbalán et al., 2003; Pinelo et al., 2006; Delsart et al., 2014; López-Giral et al., 2015). To study the grape flesh in this regard, one should apply fully non-invasive visual inspection. Therefore the authors propose imaging with optical coherence tomography (OCT) and then the determination of the textural features of peel layers and parenchymatous grape tissue in acquired images. The texture analysis of plant tissue has been applied widely in the food industry for quality evaluation and inspection (Kondo et al., 2000; Thybo et al., 2004; Zheng et al., 2006; Valous et al., 2010). In the present case, the texture change in near skin area is used as an indicator of the range of cells degradation as well as confirmation of the PEF method effectiveness.

Optical coherence tomography (OCT) is a novel optical imaging method, which allows the inspection of cross-sections in a non-destructive and contactless manner. The advantage of this method for plant and animal tissues is complete safety of radiation emitted by the interferometer.

OCT systems in most applications use light with wavelength between 800 nm and 1550 nm (Drexler and Fujimoto, 2008). The wavelength appropriate in specific cases depends on the ability of scattering of photons in the deep layers of the material.

Most biological objects including food and agricultural products are specific and difficult study materials for methods related to the near infrared radiation, because of their optical opacity. It has been shown that with high resolution OCT scanning small structures in the upper part of fruits and leaves can be successfully distinguish. The observable objects are e.g. parenchyma cell vacuoles, outer integument of kiwifruit seeds, juice vacuoles, membranes of juice sac and oil glands in orange flavedo (Loeb and Barton, 2003). They are also the intercellular spaces filled by protective materials in plant roots (Sekulska-Nalewajko et al., 2014) as well the outer and inner structure of the apple skin (Leitner et al., 2011; Verboven et al., 2013).

In the presented study OCT scanning can only register the refraction changes of infrared radiation penetrating tissue material at the constant optical magnification usually of 5–10 times. It is impossible to get an accurate, complete image of the cells, or cell walls. The information of tissue changes is contained in texture modifications. To quantify the sub-skin grape texture variations the authors proposed the methods of measuring entropy, standard deviation (Haralick et al., 1973) and lacunarity analysis. Lacunarity analysis was originally developed to describe fractal properties now and it is used for texture appearance characterization in muskmelon epidermis, sliced pork-ham, soil material or forest images (Valous et al., 2009; Pendleton et al., 2005; Plotnick et al., 1993; Li et al., 2012). This analysis dedicated to binary images was preceded by the creation of local binary patterns at various scales (Ojala et al., 2002). Such an approach has been recently proposed by Quan et al. (2014). The signal standard deviation and entropy features used in the paper represent the most general characteristics of any texture, which can also be applied for near peel grape tissue images.

The purpose of the present study is to demonstrate that the structural changes of near peel grape tissue can be observed and also quantitatively assessed by the fast and non-invasive OCT imaging technique. The proper selection and then OCT measurement of grape textural features varying under the influence of electric field will allow for strict control of the PEF process in the future.

2. Materials and methods

2.1. Plant material

The tests have been subjected to three kinds of grapes: red *Marechal Foch* and two white *Johanniter* and *Hibernal* which are widespread and highly rated in the wine industry.

2.2. PEF process

Before the experiment, the grapes were cleaned and stalks peeled. Then they were portioned in 200 g parts and were subjected to the high voltage operation in a Teflon container.

As the result of carrying out the number of samples, where the number of pulses, the voltage value, the interval between pulses and the temperature of the sample were changed, the following electroporation process parameters were selected: for the voltage value 20 kV, the number of pulses = 200, time interval between two pulses = 10 s, energy equals 3 W h/kg. For the voltage of 30 kV the number of pulses = 200, time interval between two pulses = 15 s, and the energy was 3 W h/kg. The PEF generator