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Effect of pulsed electric field (PEF)-treated kombucha analogues from *Quercus obtusata* infusions on bioactives and microorganisms

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ABSTRACT

Pulsed electric field (PEF) is a promising non-thermal food preservation technology. The objective was to study inactivation of yeasts in PEF-treated kombucha analogues prepared from *Quercus obtusata* infusions. Fermentation conditions of infusions from *Q. obtusata* were time (7 days), sugar (10%), starting culture (10%), and inoculum (2.5%, at 25 °C). The PEF treatment considered using square waves, an electric field strength (37.3–53.4 kV/cm), PEF processing time (445.3–1979.2 μs), an output temperature (18.31 ± 0.98 °C), an input energy (21.2 - 136.5 KJ/L), and two feed flow rates (51.42 and 102.85 L/h). pH, °Brix, color determinations, microbiological testing, total phenolic, flavonoid content, DPPH test, and UPLC/ESI/MS/MS analysis were done. No changes at different PEF conditions were observed for pH and °Brix. Higher color changes were observed at higher specific energies. Acid-acetic bacteria were more sensitive to PEF than yeasts. Lower specific energies render products with higher polyphenolic content and antioxidant capacity.

Industrial relevance: Pulse electric field is an interesting alternative to preserve kombucha analogues from oak leave infusions with minimal changes in physicochemical characteristics, antioxidant activity and bioactive compounds. The present work describes the effect of feed flow and specific energy on the several characteristics of fermented beverages, determining conditions for best processing.

1. Introduction

Thermal treatments are effective on enzyme inactivation and microbiological control. However, they imply an important loss of nutrients and phytochemicals such as phenolic compounds (Manzocco, Mastrocola, & Nicoli, 1998), although several reports have claimed opposite results (Manzocco, Anese, & Nicoli, 1998; Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997).

The conventional way to determine the efficiency of a thermal process is based on the assumption that survival curves of microbial cells and bacterial spores are governed by a first-order kinetic law (Mafart, Couvert, Gaillard, & Leguerinel, 2002). As a complement to or replacement of traditional thermal pasteurization, pulsed electric field (PEF) is a promising non-thermal food preservation technology (Toepfl, Heinz, & Knorr, 2007). Several investigations have been performed in various fruit juices to evaluate microbial resistance to PEF treatments (Raso, Calderón, Góngora, Barbosa-Cánovas, & Swanson, 1998a, 1998b; Timmermans et al., 2013). Wouters, Alvarez, & Raso (2001) indicate that the main process parameters that affect microbial inactivation by PEF are electric field strength, pulse length, pulse shape, number of pulses, and start temperature (MacGregor, Farish, Fouracre, Rowan, & Anderson, 2000). Also, type of microorganism, species, strains, size and shape are related with the efficiency of PEF to inactivate microorganisms (Wouters et al., 2001).

Electric field strength and treatment time are the most studied parameters related with microbial inactivation by PEF. The main process parameters that affect microbial inactivation by PEF are electric field strength, pulse length, pulse shape, number of pulses, and starting temperature (Barbosa-Canovas, Pothakamury, Gongora-Nieto, & Swanson, 1999; Saldaña, Álvarez, Condón, & Raso, 2014; Siemer, Toepfl, & Heinz, 2014). In general, increasing the intensity of these factors enhances microbial inactivation; however, their relationship with the survival fraction is unclear (Wouters et al., 2001). Not only process parameters are important, but also product parameters are significant too.

PEF treatment has been applied to a range of different products as fruit juices, milk, liquid eggs, and dry herbs (Barba et al., 2015). Also the influence of pH and conductivity has been studied by several groups. Wouters et al. (2001) found that a change in the medium conductivity affected the pulse energy; Vega-Mercado, Pothakamury, Chang, Barbosa-Cánovas, & Swanson (1996), concluded that it is better to

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process products with low conductivity in order to obtain a higher inactivation. Microbial characteristics are important because microbial inactivation by PEF is dependent on the type of microorganisms, species and strains (Mosqueda-Melgar, Elez-Martínez, Raybaudi-Massilia, & Martín-Belloso, 2008). It has been proposed that cell size or shape may influence the inactivation kinetics (Wouters et al., 2001). On the other hand, emulsions and fermented beverages had received little or no attention at all.

Mathematical relationships between these parameters and inactivation rates have been reported by several research groups (Morales de La Peña, Elez-Martínez, & Martín-Belloso, 2011). However, Huang, Yu, Wang, Gai, & Wang (2014) claimed that these parameters are strongly related and could be affected by other parameters. Other research groups claim that the evaluation of energy necessary to inactivate microorganisms is a better form to evaluate PEF. On this approach, energy input is considered a better control parameter as it involves different parameters such as conductivity, pulse width, volume, and electrical resistance of the treatment chamber, as well as the initial temperature of the sample (Saldaña, Puértolas, Monfort, Raso, & Alvarez, 2011).

Information on pasteurization of fermented beverages by pulsed electric fields is scarce; few reports are available about pasteurized beer (Walkling-Ribeiro, Rodríguez-González, Jayaram, & Griffiths, 2011), and fermented beverages (Zhang, Gao, Zhang, Shi, & Xu, 2010). However, in our best knowledge, pasteurization by pulsed electric fields of fermented beverages such as kombucha analogues has not been reported yet.

Additionally, the influence of PEF on bioactive compounds into fermented beverages is limited. However, several works about fruit juices have been done (Barba et al., 2012), as well as for fruit juice–soymilk beverages (Morales de la Peña et al., 2011). Total phenolic acids and total flavonoids seemed to be highly stable during storage (56 days at 4 °C), although the behavior of the individual phenolic compounds was not clear. The content of some of the phenolic compounds increased with time, while others decreased or remained with no significant changes in the initial values. Therefore our aim was to study the inactivation by pulsed electric field of yeasts present in kombucha analogues prepared from *Quercus obtusata* infusions and to evaluate the effect of PEF on their bioactive compounds (polyphenols).

2. Materials and methods

2.1. Setup experimental design

A graphical setup experimental design is shown in Fig. 1.

2.2. Sample material

Q. obtusata leaves were obtained from trees located 9.2–9.4 km from the Mezquital–Charcos Road in Southern Durango, Mexico. The leaves were air dried in the shade at 25 °C followed by milling and sieving to a particle size of 0.7 mm. Kombucha consortium was obtained from a Mexican trading house (Healthy, Natural Life, Tlaquepaque, Jal., Mexico).

2.3. Chemicals

Catechin, epicatechin, rutin, myricetin, quercetin, dimethyl sulfoxide (DMSO), 2,2-diphenyl-picrylhydrazyl (DPPH), Folin Ciocalteu reagent (2N), and sodium carbonate were from Sigma-Aldrich (St Louis, MO, USA), and methanol LC–MS grade is from J. Baker.

2.4. Preparation of herb infusions

Infusions were prepared as follows: 1 g of ground leaves (*Q. obtusata*) was added to 100 mL of water and heating the mixture for 10 min at 80 °C, followed by centrifugation at 4500 rpm for 10 min and filtered through a 0.5 mm pore size filter.

2.5. Started cultures and fermentation

The starting kombucha consortium was maintained in sweetened (sucrose 10%) black tea at 25 °C. Freshly cultured kombucha was used for further subcultures or fresher fermentation batches. Fermentation conditions were established according to Vázquez-Cabral et al. (2014). Briefly, fermentation variables were time (7 days), sugar concentration (10%), starting culture portion (10%), inoculum (2.5%) and temperature (25 °C). The total volume of fermented *Q. obtusata* leaf infusion was 60 L.

2.6. Physicochemical properties

The pH was determined by the use of pH-meter ATAGO (DPH-2, Tokyo, Japan) following the method NMX-F-317-NORMEX-2013. For °Brix determination, a hand refractometer (ATAGO) was used, following the method NMX-F-103NORMEX-2009. Conductivity was measured using an electrical conductivity meter ATAGO (DEC-2, Tokyo, Japan).

The color parameters are Hunter L*, a*, and b*, where L* = 100 is white, and 0 is black. −a is green, +a is red, −b is blue and +b is yellow and the total color difference is defined by the expression: $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$. Depending on the value of ΔE, the color difference between the treated and untreated samples can be estimated

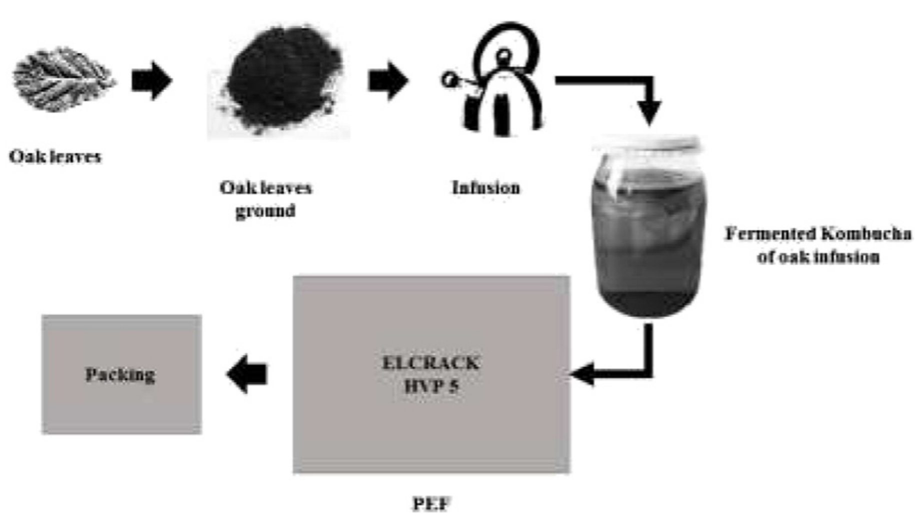

Fig. 1. Graphical setup experimental design.