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Combination of pulsed electric fields, mild heat and essential oils as an alternative to the ultrapasteurization of liquid whole egg

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ABSTRACT

The production of microbiologically safe liquid whole egg (LWE) by industrial ultrapasteurization is restricted by the high thermal sensitivity of LWE components. This research proposes an alternative treatment based on the application of pulsed electric fields (PEF) and mild heat, in the presence of natural essential oils (EOs) or their individual components (ICs). The obtained results indicate that the successive application of PEF (25 kV/ and 100 kJ/kg) followed by heat (60 °C during 3.5') to LWE added with 200 μL/L of lemon EO would reach 4 $\log_{10}$ -cycles of inactivation of *Salmonella* Senftenberg 775 W and *Listeria monocytogenes*, when any of these barriers acting alone inactivated less than 1.5 $\log_{10}$ cycles of either bacteria. Therefore, the synergism between lemon EO and the successive application of PEF and heat would provide a safety level similar to that of ultrapasteurization treatment for *Salmonella* Senftenberg 775 W and *L. monocytogenes*, but at a lower temperature. To a lesser extent, synergism with the successive application of PEF and heat was also observed in the presence of 200 μL/L of carvacrol, citral, (+)-limonene, or mandarin EO, reaching about 3.5 $\log_{10}$ cycles of inactivation in *Salmonella* Senftenberg and 3.0 $\log_{10}$ cycles in *L. monocytogenes*, respectively. A sensory test on LWE containing 200 μL/L of each additive in the form of omelets and sponge cakes revealed that this concentration of mandarin EO, lemon EO, or (+)-limonene did not decrease the sensory acceptability of the LWE-containing products, and lemon EO and mandarin EO even increased the hedonic acceptability of sponge cakes. In conclusion, this process could be applied in the food industry to obtain microbiologically safe LWE, which could be used to produce egg-based products without decreasing (and even increasing) their sensory appeal.

1. Introduction

Eggs are recognized as highly nutritive complete products whose technological and flavoring properties endorse their use as a multifunctional ingredient in the food industry. Unfortunately, the role of eggs and egg products acting as vehicles for foodborne diseases is still a major public concern in the United States and Europe (EFSA and ECDC, 2012; Jackson et al., 2013). Food manufacturers chiefly substitute shell egg with liquid egg products such as liquid whole egg (LWE) due to its easier handling and lower microbiological risk (Nemeth et al., 2011). Current efforts are being made to produce LWE free of *Salmonella* spp. and *Listeria monocytogenes*, the pathogens of concern in this product (European Commission, 2007). Besides good agricultural and handling practices, pasteurization treatments are needed to achieve the required sanitary level for LWE. Thus far, the prevalent treatment applied in the egg industry is thermal pasteurization due to its high efficacy in inactivating pathogens. Although the minimal performance standards in the USA require pasteurization of LWE at 60 °C for 3.5 min (NACMCF, 2006), ultrapasteurization at 70 °C for 1.5 min was developed to provide a higher level of safety than traditional pasteurization (Ball et al., 1987). Unfortunately, LWE components are very sensitive to high temperatures, so ultrapasteurization leads to the coagulation of proteins and entails deleterious effects against egg quality (Dawson and Martinez-Dawson, 1998). Some of these effects, like the loss of foaming and emulsifying and gelling capacities, may limit LWE's utility as a food ingredient.

In the search of preservation procedures capable of ensuring microbial safety without detriment to the functional, nutritional and sensory properties of LWE, recent research has focused on treatments based on the use of non-thermal technologies, such as pulsed electric fields (PEF), high hydrostatic pressure, UV light, or ultrasound waves (Amiali et al., 2007; de Souza and Fernández, 2011; Huang et al., 2006; Monfort et al., 2010a, 2012a). However, these technologies have demonstrated limited efficacy in inactivating target pathogens (Monfort et al., 2010a, 2012b). Consequently, lethal efficacy similar to ultrapasteurization could only be achieved through intelligent combination of hurdles acting synergistically, as proposed by Leistner and Gorris (1995). In many

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occasions, this synergism has been ascribed to the effectiveness of a hurdle in preventing the recovery of damages inflicted by other barriers (Ait-Ouazzou et al., 2012; Arroyo et al., 2010).

Within this context, Monfort et al. (2012b) designed a combined process based on the successive application of PEF and mild heat in the presence of the additives EDTA or triethyl citrate (TC). This procedure allows obtaining LWE with a similar level of safety against the target heat-resistant pathogens *Salmonella* Senftenberg 775 W and *L. monocytogenes* as ultrapasteurized LWE, but with better quality in terms of color, viscosity, foaming, and emulsifying and gelling properties. However, the use of EDTA or TC is in conflict with the recent trend of green consumerism, which is prompting the food industry to search for natural alternatives to traditionally used preservatives, so that food products can be considered as "free of synthetic additives" and "environmentally friendly" (Burt, 2004). Among naturally occurring substances with antimicrobial properties, essential oils (EOs) and their individual components (ICs) are gaining special interest due to their GRAS (generally recognized as safe) status and their wide acceptance from consumers (Burt, 2004). Since incorporation of high doses of EOs or ICs may adversely affect the organoleptic properties of food (Gutierrez et al., 2009), minimization of the concentration is strongly recommended. The addition of a low concentration (200 μL/L at most) of a high variety of EOs and ICs has been demonstrated to enhance the preservation potential of other physical technologies in fruit juices, yogurt drinks and other liquid foods (Ait-Ouazzou et al., 2013; Amiali et al., 2007; Espina et al., 2012; Mosqueda-Melgar et al., 2008). In LWE, the ICs carvacrol and cinnamaldehyde have been tested in combination with physical treatments such as heat, ionizing radiation, or ultrasound (Alvarez et al., 2007; Valverde et al., 2011), and the IC allyl isothiocyanate has been proven effective in active packaging for LWE (Jin and Gurtler, 2011). Since scarce research has been conducted in this food product despite the observed synergism between physical technologies and EOs in other media, and also considering the high efficacy of the process developed by Monfort et al. (2012b), we decided to investigate the preservation potential of the combination of mild heat, PEF and EOs or ICs in LWE.

Amidst the totality of EOs and ICs whose bactericidal activities have been tested by our research group, we selected three ICs ((+)-limonene, citral, and carvacrol), and three EOs (lemon EO, mandarin EO, and rosemary EO) for their high synergy in combination with PEF and/or heat in the inactivation of foodborne pathogens in laboratory and food media (Ait-Ouazzou et al., 2012, 2013; Espina et al., 2012, 2014; Somolinos et al., 2010). Rosemary EO (whose major IC is 1,8-cineole) and lemon EO and mandarin EO (being mainly composed of (+)-limonene) were also selected for their common culinary use.

The objectives of this work were (i) to evaluate the lethal efficacy of the successive application of PEF followed by heat treatment on the population of *Salmonella* Senftenberg 775 W and *L. monocytogenes* in LWE and to establish the relationship between its efficacy with the occurrence of sublethal damage inflicted by PEF; (ii) to determine the lethal efficacy of the combined process based on successive application of PEF followed by heat in the presence of carvacrol, (+)-limonene, citral, lemon EO, mandarin EO or rosemary EO on the population of *Salmonella* Senftenberg 775 W and *L. monocytogenes* in LWE; and (iii) to evaluate the sensory acceptability of omelets and sponge cakes elaborated with LWE containing each EO or IC.

2. Materials and methods

2.1. Preparation of LWE

Extra-large, grade-A eggs were purchased from a local supermarket. The eggshells were thoroughly washed with 70% ethanol and then allowed to air dry. The sanitized eggs were aseptically broken, transferred to a sterile stomacher bag (Tekmar Co., Cincinnati, Ohio, USA), and homogenized for 2 min at 230 rpm in a stomacher laboratory blender (model 400, Tekmar Co., Cincinnati, Ohio, USA). The obtained LWE was centrifuged at 102×*g* for 2 min (Heraeus, model Megafuge 1.0 R, Newport Pagnell, UK) to eliminate air and maintained at 2–4 °C for a maximum of 4 h until ready for use. The pH of LWE was 7.5 ± 0.3, and its electric conductivity was 0.67 ± 0.03 S/m.

2.2. Preparation of LWE with additives

The additives used were carvacrol, citral, (+)-limonene, rosemary EO from *Rosmarinus officinalis* L., lemon EO from *Citrus lemon* L., and mandarin EO from *Citrus reticulata* L. lemon and mandarin EOs were obtained as described by Espina et al. (2011), and rosemary EO was obtained as described by Ait-Ouazzou et al. (2012). The composition of these EOs was analyzed and reported in the cited papers. Carvacrol, citral and (+)-limonene were purchased from Sigma Aldrich (Sigma–Aldrich Chemie, Steinheim, Germany).

Each additive was added directly into 20 mL of LWE at a final concentration of 200 μL/L. This concentration was chosen according to published results about the additives' antimicrobial activities when added to laboratory media and foods (Ait-Ouazzou et al., 2012, 2013; Espina et al., 2012, 2014; Somolinos et al., 2010). These compounds neither modified the pH nor the electrical conductivity of LWE.

2.3. Microorganisms

The strains of *Salmonella enterica* subsp. *enterica* serovar Senftenberg 775 W (CECT 4565) and *L. monocytogenes* (CECT 5672) used in this investigation were supplied by Colección Española de Cultivos Tipo (CECT). Broth subcultures of each serotype were prepared in flasks containing 50 mL of tryptic soy broth (Biolife, Milan, Italy) plus 0.6% (w/v) of yeast extract (Biolife) (TSBYE) inoculated to an initial concentration of approximately 10^6 CFU/mL. The cultures were incubated under agitation (135 rpm) (mod. Rotabit, Selecta, Spain) at 37 °C until the stationary growth phase was reached. Before treatment, bacterial suspensions were centrifuged at 6000×*g* for 5 min at 4 °C and re-suspended in 1 mL of LWE with the corresponding additive at a concentration of approximately 10^9 CFU/mL. LWE with additives, inoculated with the corresponding microbial population, was immediately subjected to PEF and/or thermal treatments. The additives in the concentrations did not affect the number of survivors during the storage time at 4 °C before the treatments (data not shown).

2.4. PEF, heat, and PEF followed by heat treatments

A volume of 0.3 mL of LWE with or without each additive and 10^9 CFU/mL of the bacterial suspension was placed in the PEF treatment chamber, as previously described (Monfort et al., 2010b). Microorganisms were treated in a tempered batch parallel-electrode treatment chamber (15.0 ± 0.1 °C) with a distance between electrodes of 0.4 cm and an area of 0.79 cm^2. 3 μs-square waveform pulses were applied at 0.5 Hz. The temperature of the LWE was always lower than 35 °C. The energy per pulse (W') was calculated using Eq. (1):

$$W' = \int_0^{\infty} k \cdot E(t)^2 dt \quad (1)$$

where k (S/m) is the electrical conductivity of LWE, E (V/m) is the electric field strength, and t (s) is the duration of the pulse. The total energy applied (kJ) was calculated by multiplying the energy per pulse (W') by the number of pulses. The total specific energy (W) applied (expressed in kJ/kg) was determined by dividing the total energy by the mass of treated LWE. Treatments were applied at an electric field strength of 25 kV/cm and a specific energy of 100 kJ/kg (8 pulses). This treatment intensity was chosen according to previously published work and established as energetically suitable, both in static and continuous conditions (Monfort et al., 2012b).