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Synergistic effect of supercritical carbon dioxide and peracetic acid on microbial inactivation in shredded Mozzarella-type cheese and its storage stability at ambient temperature

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ABSTRACT

Supercritical carbon dioxide ($SCCO_2$) in combination with peracetic acid (PAA) could represent an effective decontamination technique to control microorganisms in shredded cheese. Preservation of shredded Mozzarella-type cheese (SMC) was assessed at 25 °C over 21 d, using $SCCO_2$ (9.9 MPa, 35 °C, 30 min) individually and combined with PAA at concentrations of 50 ($SCCO_2$/PAA50), and 100 ($SCCO_2$/PAA100) ppm with optional 30-min pre-conditioning time ($SCCO_2$/PAA100PC). Process efficacy was assessed based on achievable inactivation and treatment synergism reflected in counts of inoculated *Escherichia coli*, *Listeria innocua*, *Geobacillus stearothermophilus* spores and indigenous micro-flora such as total bacteria (TBC) and total yeasts and molds (TYMC). Complete inactivation of *E. coli* cells ($\geq$7.0 $\log_{10}$) in SMC was achieved with any of the combined treatments, whereas initial reduction of *L. innocua* was lower when $SCCO_2$/PAA50 and $SCCO_2$/PAA100 combinations were applied (2.9 and 4.6 $\log_{10}$, respectively). *G. stearothermophilus* spores exhibited the highest resistance, allowing for a reduction of up to 3.8 $\log_{10}$ ($SCCO_2$/PAA100) and indicating no advantage of pre-conditioning. However, PAA concentration significantly affected microbial inactivation, comparing $SCCO_2$/PAA100 to $SCCO_2$/PAA50 in TBC (minimum of 6.6 vs. 4.2 $\log_{10}$, respectively) ($P < 0.05$) and TYMC (minimum of 7.7 vs. 1.1 $\log_{10}$, respectively) ($P < 0.05$). The TBC and TYMC were not significantly decreased by stand-alone decontamination techniques ($P \geq 0.05$); however, synergistic treatment effects ($P < 0.05$) occurred for all microorganisms except for *E. coli*. Overall, the findings demonstrate great potential for a $SCCO_2$/PAA hurdle technology as an alternative to post-processing decontamination strategies for production of shelf-stable SMC at ambient temperatures.

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1. Introduction

Over the last decades, production of Mozzarella and Mozzarella-type cheeses has steadily increased, reflecting their growing popularity among consumers. In 2013 Mozzarella ranked the first, with a 33.4 percent share of 11.1 billion pounds of cheese produced in the United States (USDA, 2013). Its commercial relevance is apparent from preferences shown by mainstream consumers, food service or fast food chains, which use them, for instance, as topping in baked preparations such as pizza and gratin dishes or as an ingredient in other foods (Mastromatteo, Conte, Faccia, Del Nobile, & Zambrini, 2014). However, food safety concerns regarding microbial contamination of dairy products post processing exist, especially, with a scale-up of production and if the effectiveness and dimension of intervention methods remain at the same level as prior to the scale-up (Griffiths & Walkling-Ribeiro, 2012). This is crucial for shredded products like cheese as shredding greatly increase surface exposure for even airborne microbial contamination (Eliot, Vuillemard, & Emond, 1998). As a consequence of growing consumer demand for reduced- or low-sodium processed cheese a higher water activity is prevalent in these products, rendering the latter more sensitive to microbial spoilage and potentially affecting the storage stability (Taylor, 2013). While product spoilage associated with native aerobic bacteria and yeasts and molds could cause economic loss (Corbo, Lanciotti, Albenzio, & Sinigaglia, 2001; Eliot

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et al., 1998), a far more serious health risk comes from contamination with major pathogens such as *Escherichia coli* (Spano et al., 2003), *Listeria monocytogenes* (Stecchini, Aquili, & Sarais, 1995), and *Bacillus cereus* (Bonerba et al., 2010). These pathogens could be introduced in the absence of an effective system to ensure safety during cheese processing (i.e. good manufacturing practice (GMP), hazard analysis and critical control point (HACCP)) or incidentally, beyond the control and detection of a food safety system. In order to address these food microbiological challenges an innovative hurdle strategy, combining supercritical carbon dioxide ($SCCO_2$) and peracetic acid (PAA), could allow for safe preservation of shredded Mozzarella-like cheese (SMC) stored at ambient temperatures, thereby, also meeting the consumer trend for a convenient (i.e. long, non-refrigerated shelf stability) and environmentally sustainable (i.e. not requiring additional energy for cold storage) food supply chain (Schmidt Rivera, Espinoza Orias, & Azapagic, 2014). To date, few approaches have been proposed to inactivate the pathogenic and spoilage microorganisms in Mozzarella-type cheeses such as high pressure processing (Sheehan et al., 2005), ozone (Segat et al., 2014) and irradiation (Huo, Bai, Guo, & Zhao, 2013). The chemical nature of $SCCO_2$ fluids due to its molecular behavior in water (i.e. low intracellular pH due to formation of carbonic acid) brings about inhibitory action on microbial cells, making the treatment not entirely dependent on pressure (Sikin, Zoellner, & Rizvi, 2013). Physical properties such as adjustable densities, low viscosities, high diffusivities and low interfacial surface tension facilitate its penetration into various matrices (Sikin & Rizvi, 2011). Practically, it does not affect the stability of most food matrices and it can also be easily handled at industrial scales. It is also noteworthy, that $SCCO_2$ is nontoxic, nonflammable, chemically inert and a benign solvent with generally recognized as safe (GRAS) status, leaving no residue in the treated food products upon depressurization (Clifford & Williams, 2000).

$SCCO_2$ at 7.3 MPa and 31 °C or higher has unique properties which render it effective as a non-thermal decontamination technique for foods. Unlike liquid foods, application of $SCCO_2$ for microbial inactivation in solid foods suffers few limitations such as limited diffusion of CO_2 into solid matrices and bacterial cells, since the sample cannot be agitated, and much reduced levels of free water at the surface, which may limit the solubility of CO_2 into the food (Balaban & Duong, 2014; Ferrentino & Spilimbergo, 2011). In order to overcome this limitation, $SCCO_2$ is often combined with a co-solvent or an antimicrobial agent as a hurdle technology to achieve better microbial inactivation under milder treatment conditions ($\leq$10 MPa, $\leq$ 40 °C) and in shorter times ($\leq$60 min). Peracetic acid (PAA) is approved for use as a sanitizer in the United States on food contact surfaces (Code of Federal Regulation 21 CFR Part 178.1010) and for direct food contact with fruits and vegetables (Code of Federal Regulations 21 CFR Part 173.315) and meat, poultry and seafood (Code of Federal Regulations 21CFR Part 173.370) at a maximum concentration of 80, 85 and 110 ppm, respectively. For combined treatments, the role of highly diffusive $SCCO_2$ fluid is to act as vector, so that PAA can easily penetrate into the microbial cells and inactivate them. Thus, a rapid penetration of PAA into microbial cells and the release of oxygen and free radicals, critical for the oxidation and destruction of cellular enzymes, are likely associated with its efficacy (Pruss et al., 2001).

Hence, the aim of this work was to investigate the use of $SCCO_2$ and PAA, alone and in combination, to reduce the microbial load in SMC and to monitor its storage stability at 25 °C over a period of 21 d. In addition to the analysis of spoilage by native bacteria, yeasts and molds, non-pathogenic surrogates, such as *E. coli*, *Listeria innocua*, and *Geobacillus stearothermophilus* spores were used for inoculation in a selective approach to study their resistance and survival following single or combined $SCCO_2$ treatments.

2. Materials and methods

2.1. Inoculation and sample preparation

Shredded low-moisture part-skim Mozzarella cheese (C&S Wholesale Grocers Inc., Keene, NH, USA) was purchased from a local supermarket and stored under refrigeration (4 ± 1 °C) before treatments. *E. coli* American Type Culture Collection # 25922 (*E. coli* ATCC25922) and *L. innocua* Food Safety Laboratory #C2-008 (*Listeria innocua* FSL C2-008) were obtained from the −80 °C stock culture collection of the Food Microbiology and Safety Laboratory at Cornell University. Non-pathogenic *E. coli* and *L. innocua* strains were selected as surrogates, commonly used for challenge studies in the food industry, to simulate cheese contamination with respective gram-negative and -positive pathogens of great relevance (pathogenic *E. coli* and *L. monocytogenes*, respectively) to food product safety. A commercially available *Geobacillus stearothermophilus* (ATCC 9372, NAMSA Products, Northwood, OH, USA) endospore suspension of 10^6–10^7 colony forming units per ten mililiter (CFU/10 mL) was used as biological sterilization indicator and as a possible non-pathogenic surrogate for *Bacillus anthracis* (Guan, Chan, Brooks, & Rohonczy, 2013), for which, in turn, *Bacillus cereus* has also been regarded as a fitting pathogenic surrogate due to its genotypical and phenotypical resemblance (Greenberg, Busch, Keim, & Wagner, 2010). Based on the above established link between the three species it is suggested, that *G. stearothermophilus* may also function as an adequate surrogate for *B. cereus*. Prior to experiments, the culture was streaked onto trypticase soy agar (TSA; 236920 Difco™, BD, Sparks, MD, USA) and incubated for 24 ± 2 h at 37 ± 2 °C. For each of the vegetative challenge bacteria, a single isolated colony was transferred into trypticase soy broth (TSB; 296264 BBL™, BD, Sparks, MD, USA) and incubated for 24 ± 2 h at 37 ± 2 °C, under agitation at 225 rpm. A subsequent loop transfer into fresh TSB and incubation for 24 ± 2 h at 37 ± 2 °C, shaken at 225 rpm, was performed to produce an initial inoculum of about 10^9–10^{10} CFU/mL. A 10-mL spore suspension was diluted with 90 mL distilled water (1:10 (v/v) dilution) to prepare a final concentration of 10^8–10^9 CFU/mL for both *E. coli* ATCC 25922 and *L. innocua* FSL C2-008, and 10^5–10^6 CFU/mL of *G. stearothermophilus* ATCC 9372 spores.

For the inoculation with selective microorganisms (*E. coli* ATCC 25922, *L. innocua* FSL C2-008 and *G. stearothermophilus* spores), an aliquot of 80 g of cheese was submerged into 100 mL inoculum, that was previously 1:10 diluted with sterile, quarter-strength Ringer's solution (BR0052G, Oxoid Ltd., Basingstoke, UK) (v/v) for 15 min. For contamination with native microorganisms (total aerobic bacterial counts and total yeast and mold counts), cheese was spoiled at 25 °C for 72 h (ensuring high microbial growth, as determined in pretests, to allow for optimal evaluation of hurdle technologies) and, subsequently, immersed in a 100 mL suspension, made up of sterile TSB that was 1:10 diluted with sterile Ringer's solution (v/v). In the same manner negative control samples were drenched in sterile, uninoculated TSB diluted with Ringer's solution in order to assess microbial contamination of the commercial product prior to inoculation or spoilage. After a 15 min dwell period, 2 g of soaked cheese sample were weighed and dried for 15 min in weighing dishes (08-732-112 Fisherbrand, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) before aseptic transfer to gas-permeable 279.5 × 179.7 × 0.3 mm (length × depth × height) Tyvek bags (NovaSterilis, NY). Each bag was segmented into 8 pouches of equal size using a vacuum sealer (AGW Multivac Vakuum-Verpackungsmaschine, Sepp Haggenmüller KG, Wolfertsschwenden, Germany), allowing for 2 g of cheese sample to be vacuum-sealed in each pouch. Based on common practice in high pressure processing, bags were double-sealed (Ahmadi, Anany,

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