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Short communication

Inactivation of *Salmonella* Typhimurium in fresh vegetables using water-assisted microwave heating

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ABSTRACT

A water-assisted microwave treatment was studied against the pathogenic bacterium *Salmonella* Typhimurium in fresh jalapeño peppers and coriander foliage. Vegetables were immersed in water and treated in a microwave oven at 950 W to reach up 63 °C: jalapeño pepper for 25 s and coriander foliage for 10 s. After the microwave heating, samples were cooled in water at 4 °C. Samples were observed with confocal microscope before and after treatment. The proposed protocols resulted in a reduction of 4–5 log cycles on the *Salmonella* population, which is the main issue from the microbiological viewpoint. Even color of the vegetables was affected by the treatments ($p < 0.05$) mainly by darkening in both vegetables and loss of greenness in jalapeño pepper, no changes in firmness were observed. Sensory acceptance of a salsa formulated with the treated vegetables had high scores (7.21 in a 9-points hedonic scale).

1. Introduction

Jalapeño pepper (*Capsicum annuum* L.) is a spicy green vegetable used widely in Mexican food. Coriander leaves or *cilantro* (*Coriandrum sativum* L.) is other green vegetable widely used in Mexican cuisine, also in some Italian and Oriental recipes. On June 2008, the FDA (2008) reported a *Salmonella* outbreak in the U.S. caused by a Mexican salsa containing fresh tomato, coriander leaves and jalapeño pepper. Enteric bacteria may contaminate vegetables, depending on water quality for irrigation, post-harvest handling and conditions during food preparation (Abougrain, Nahaisi, Madi, Saied, & Ghenghesh, 2010), and coriander leaves may have substantial amounts of microorganisms, such as thermophiles, enterobacteriaceae, *Pseudomonas* spp. and molds (Mitkowska, Hickey, Alonso-Gomez, & Wilkinson, 2011). *Salmonella* is a Gram-negative and facultative anaerobic bacterium, which contaminates many foods.

In the industry, *Salmonella* is inactivated by applying thermal treatments during food processing. For raw products, some disinfectant treatments in domestic or industrial kitchens are carried out, including application of ozone, chlorine or iodine solutions (Mossel & Garcia, 2003). However, and due to the reported outbreaks, it is possible that current disinfecting procedures are not being properly used or are not enough to inhibit the bacteria. Other proposed treatment for disinfesting coriander leaves was reported by Kamat, Pingulkar, Bhushan, Gholap, and Thomas (2003), throughout application of low dose gamma irradiation from 1 to 3 kGy; the treatment was effective against pathogenic bacteria (*Listeria* spp. and *Yersinia* spp.). In the case of jalapeño peppers, immersion in solutions such as 200 ppm of sodium hypochlorite, acidified sodium chlorite, or peroxy acetic acid for 10 min, could reduce the population of *Salmonella* Saintpaul in 1.5–1.7 log units for stem/calyx by and 2.1 to 2.4 log units for the flesh of the peppers (Liao, Cooke, & Niemira, 2010).

An easy thermal treatment could be applied to these vegetables in many facilities using a domestic microwave oven, since this kind of ovens are very popular. Microwaves have been used as a blanching method in herbs like marjoram and rosemary resulting in higher quality herbs compared with to water and steam blanching methods (Singh, Raghavan, & Abraham, 1996). Microwaves have not been used for disinfecting purposes in coriander foliage, just for drying of the herb (Sarimeseli, 2011; Shaw, Meda, Tabil, & Opoku, 2007), neither applied to jalapeño peppers. Thus, the objective of this study was to develop microwave treatments in order to have safe products without affecting their physical and sensory characteristics.

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2. Materials and methods

2.1. Vegetables

Medium-size jalapeño peppers (*C. annuum* L.) between 19 and 25 g and coriander foliage (*C. sativum* L.) were acquired in a local market (Puebla, Mexico). Similar and desirable color, texture and overall appearance features were found in both raw products. Jalapeño pepper had initial color 30.4 ± 0.6, −8.8 ± 0.2, 9.1 ± 0.5 in L^*, a^*, b^* scale, and firmness of 10.9 ± 1.3 N; coriander foliage had initial color 25.9 ± 0.1, −5.6 ± 0.1, 7.1 ± 0.2 in L^*, a^*, b^* scale, and firmness of 6.1 ± 1.5 N.

2.2. Salmonella inoculum preparation

Inoculum preparation was made by triplicate, prior to the microwave treatment. For that, one loopful of *Salmonella* Typhimurium ATCC 14028 strain was inoculated in 100 mL of triptycase soy broth, TSB (Becton Dickinson de México, Cuatitlán Izcalli, Estado de México) for 24 h at 35 ± 2 °C. A medium size jalapeño pepper or 9 g of coriander foliage were immersed in a liter of distilled water with 1 mL of the TSB inoculated with *Salmonella* and it was let stand for 15 min to reach a high population of the bacteria. The vegetables were drained under sterile conditions. In order to verify the initial inoculum in the vegetables, as well as to count the viable cells of *Salmonella* after treatments, 10 g of vegetable was homogenized with 90 mL of peptone water (1% p/v); serial dilutions were made and plated on *Salmonella–Shigella* agar (Becton Dickinson de México, Cuatitlán Izcalli, Estado de México). Plates were incubated (24 h at 35 °C) and counted. Sampling, plating and counting was made by duplicate.

2.3. Confocal image analysis

Cuts of about 20 μm of the peduncle and the surface of the jalapeño pepper and coriander leaves were obtained. 500 μL of a 1:5000 solution of propidium iodide was employed as a dye. After 1 min, the samples were washed 3 times with distilled water and transferred to a slide. A drop of mounting medium for fluorescence (Vectashield, H-1000, Vector Laboratories, USA) was added to the samples; then, they were covered with a slide and observed with a confocal microscope (LSM5 Pascal, Axioscope 2MOT, Zeiss, Germany) at wavelengths of 532 and 543 nm. Images were built in two dimensions.

2.4. Microwave treatments

Output power of the microwave oven used for the study (1200 W, 2450 MHz, Panasonic, China) was determined following the method 60705 IEC (Martin, 2008). Temperatures were monitored with optical fiber sensors and FISO Commander software (FISO Technologies, Quebec, Canada). The temperature was measured by introducing the sensor just under the skin, parallel to the surface of the jalapeño body or the coriander stem. One jalapeño pepper (19–25 g) was immersed in distilled water in a 1:1 pepper:water proportion; for cilantro, 9 g were immersed in 1:1.5 coriander: water proportion. A Pyrex glass cylindrical container was used for the treatments, which was previously sterilized. Temperature was monitored till reach up 63 °C in the samples. This value has been reported as the target temperature for *Salmonella* inactivation (FDA, 2008). In order to have a short treatment time, the maximum power was applied. After heat treatment, samples were cooled in cold water at 4 °C for 10 min. Protocols, which include microwave heat treatment to reach 63 °C and cooling for every studied vegetable, were made by triplicate.

2.5. Physical-chemical and sensory analyses

Color for both jalapeño pepper and coriander foliage was measured by triplicate with a colorimeter using the CIELab scale (Sarimeseli, 2011). Our colorimeter (BYK Gardner, System/05, Reston VA, USA) was previously calibrated with the black and white tiles, and expressed through the L^* (lightness), a^* (green-red contribution) and b^* (blue-yellow contribution) parameters. For jalapeño pepper firmness, an aluminum 60° cone moving at 1 mm/s was used to penetrate 5 mm the skin in a whole piece. For coriander, foliage (leaves and stem) were stacked and rolled to form a 2.5 cm thick roll. Samples were cut 50% of their thickness (Bourne, 1982) using a stainless steel cutting wire moving at 2 mm/s. A Texture Analyzer TA.XT2 (Stable Micro Systems, Haslemere, England) was used to measure the maximum force (N) for both vegetables. Texture is a complicated measurement, due to the high variation between samples, even coming from the same batch. To avoid high deviations in firmness determination in vegetables, some authors recommend to analyze around 20 samples (Horak et al., 2006). 16 samples of each vegetable (jalapeño pepper or coriander) were used in our study.

Samples of Mexican salsa were prepared with 60% tomato, 20% white onion, 10% jalapeño pepper and 10% coriander leaves. One sample contained both jalapeño pepper and coriander treated with microwaves (non-inoculated with *Salmonella*) and other, a control, was formulated with non-treated vegetables. An affective test with hedonic scale (Horak et al., 2006; Larmond, 1976; Tochampa, Jittrepotch, Kongbangkerd, Kraboun, & Rojsuntornkitti, 2011) was applied to 20 non-trained judges, tasting the two salsas, to evaluate their general acceptance.

2.6. Data analysis

Results were analyzed through an analysis of variance (ANOVA) using a statistical software Minitab Release 14.0 software (Minitab Inc., State College, PA, USA); differences were established at the significant level of $P = 0.05$.

3. Results and discussion

After inoculation, average *Salmonella* population was 3×10^8 CFU/g in both vegetables, reaching up a level to simulate a severe microbial contamination.

Fig. 1 shows the temperature profiles in the sample, for jalapeño pepper and coriander, while they were treated at 950 W (the

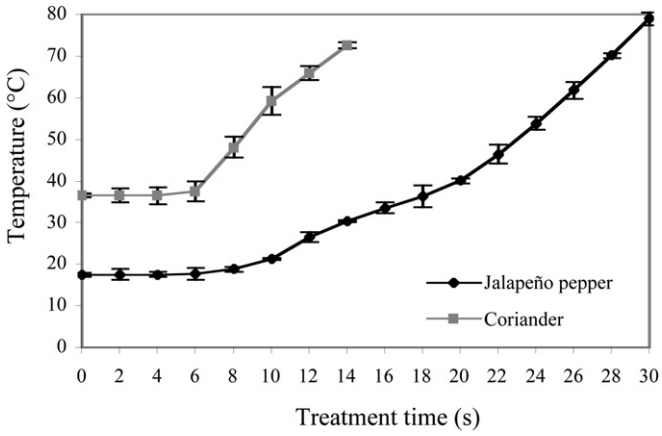

Fig. 1. Average temperature profiles during the water-assisted microwave treatment for jalapeño pepper and coriander. Temperatures were acquired by triplicate.