STUDY OF THE EFFECT OF NON-THERMAL TREATMENTS ON THE SAFETY OF SOME FRUITS AND VEGETABLES

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ABSTRACT

Thermal treatments, when conveniently applied, are efficient in reducing microbial load of fruits and vegetables. However, the negative impact of heat, especially at food texture level, makes non-thermal treatments promising technologies as minimal food processes.
The objective of this work was to study the effect of ultrasonication, and its combination with a milder heat treatment (thermosonication), UV-C radiation, and ozonation on the safety of strawberries (*Fragaria anannassa*), watercress (*Nasturtium officinale*) and red bell peppers (*Capsicum annuum,* L.). Results were compared to water-washings, performed as control.
For strawberries and watercress, safety was assessed in terms of total mesophiles and coliforms, respectively (endogenous flora). In red bell peppers, *Listeria innocua* was enumerated (artificial inoculation).
Results showed that UV-C radiation was less efficient than water-washings at 15 °C, for all microorganisms/products tested.
Ozone allowed, in average, 1 log-cycle reduction for mesophiles and coliforms, and 2 log-cycles reduction for *Listeria innocua* (higher than the values obtained by water-washings at 15 °C).
Ultrasonication treatments were equivalent to water-washings at the same temperatures. The temperature had the major effect. For 50 °C, 2 and 3 log-reductions were observed, respectively, for *Listeria innocua*/red bell pepper and coliforms/watercress. For 65 °C, reductions achieved 6 to 7 log-cycles. Mesophiles/strawberries were more heat resistant (2 log-reductions were obtained at 65 °C).

1. INTRODUCTION

The most common thermal technologies used to reduce the microbial load in food products are pasteurization and sterilization treatments. However, temperature has a negative impact at food textural level, and some nutritional contents may also suffer degradation.
Traditionally, fresh fruits and vegetables are washed with some chemical agents, such as, chorine, hydrogen peroxide and acetic acid solutions. The efficacy of those treatments rounds 2-3 log reductions of microbial content [1, 2, 3], which may not be efficient in cases of high initial contaminations.
New non-thermal treatments have been developed aiming at reducing pernicious effects of thermal processes, by preserving quality and nutritional attributes and decreasing the microbial load [1]. Ultrasonication and its combination with temperature (i.e. thermosonication), ultraviolet radiation and ozonation are examples of innovative technologies applied to fruits and vegetables [1, 4]. The treatment efficiency in reducing

microbial contamination depends on the microorganism sensitivity to the treatment and, consequently, variable results are commonly reported.

Ultrasound is defined as sound waves with a frequency over 20 kHz, which is about the upper limit of human hearing. Ultrasounds have an antimicrobial effect since they can disrupt biological structures when applied with convenient intensity. This effect can be increased when combined with temperature [5, 6]. Some recent works refer a microbial reduction between 5 and 6 log-cycles for liquid foods treated with power ultrasounds combined with mild thermal treatments [7, 8].

Ultraviolet radiation at 200-280 nm (UV-C) has a germicide action. This technology has been used to control post-harvest contaminations in fruits and vegetables, aiming at extending products' shelf-life. This effect was studied in several fruits, such as peaches [9], strawberries, cherries [10] and pomegranates [11]. In relation to vegetables, UV-C light was applied to zucchini [12] and lettuce [13].

Application of ozone in food industries is an emerging and challenging technology that may improve the value of fresh-like processed foods. Several studies demonstrated that ozone treatments increase shelf life of fruits and vegetables [14], and is often referred as an appropriate method to guarantee food quality and safety [15, 16, 17]. Ozone acts at cell level, oxidizing sulfhydryl groups and amino acids of enzymes, peptides and proteins to shorter peptides. Another proposed mechanism involves oxidation of polyunsaturated fatty acids to peroxides [18].

The objective of this study was to analyse the effect of ultrasonication and its combination with a milder heat treatment (thermosonication), UV-C irradiation and ozone treatments on strawberries, watercress and red bell peppers safety.

2. MATERIAL AND METHODS

2.1. Samples

Strawberries (*Fragaria anannassa*), watercress (*Nasturtium officinale*) and red bell pepper (*Capsicum annuum L.*) were acquired in a local market. For strawberries and watercress, safety was assessed in terms of total mesophiles and coliforms, respectively (endogenous flora). In red bell peppers, *Listeria innocua* was enumerated (artificial inoculation). Each red bell pepper sample was artificially inoculated at the surface, with 250 μL of the second *Listeria* subculture (see section 2.1.1. *Listeria innocua* culture). Contact time was 15 minutes. Analyses were carried out in fresh untreated samples and after treatments.

For ultrasonication and ozone treatments, all samples were cut in small portions (approximately 20g). For UV-C treatments, external surface of strawberries were cut and only watercress leaves were used in the experiments.

2.1.1. *Listeria innocua* culture

Listeria innocua NCTC 10528 was acquired commercially from Leatherhead Food Research Association (Leatherhead, UK). The first subculture was made in TSB, Tryptic Soy Broth (Lab M, Lancashire, UK), containing 0.6% yeast extract (Lab M, Lancashire, UK) – TSBYE - at 30ºC during 24h. The cultures were maintained at 7ºC on Tryptic Soy Agar, TSA (Lab M, Lancashire, UK), supplemented with 0.6% yeast extract - TSAYE.

The second subculture of *Listeria innocua* was prepared like the first one at 30ºC for ± 24h to yield stationary phase cultures.

2.2. Treatments

Ultrasonication (US) and thermosonication treatments were performed in ultrasound equipment (Bandelin Sonorex RK 100H) at 32 kHz. Samples were treated for 2 minutes at 15 °C (ultrasonication) and at 50-65 °C (thermosonication; US 50°C - US 65 °C). Control water washings, at the same temperatures, were also performed.

Ozone treatments were carried out in a pilot plant. An ozone generator (SPO3, model OZ5 from Sociedade Portuguesa de Ozono, Lda.) was interconnected to a container filled with tap water (volume of 30 L), forming a closed circuit ring apparatus. Ozone was continuously incorporated by bubbling in the water, and the ozone content was measured by potential difference (Redox probe; SZ 275 from B and C Electronics). The ozone generator operated at the highest production capacity, resulting in 700 mV of the ozonated water, which corresponded approximately to 0,25 p.p.m. of dissolved O_3. Samples were immersed in ozonated and non-ozonated (used as control) water baths and removed after 2 minutes.

Ultraviolet radiation experiments were performed for 2 minutes in an UV-C chamber (conceived by University of Algarve, Portugal) with 4 germicide lamps (average intensity of 12.36 Wm^{-2}; TUV G30T9, 16 W, Philips). The intensity of flux and dose of exposure (time x intensity) were continuously measured by an UV digital photometer (DO 9721 Delta Ohm).

Four true replicates of all these treatments were performed.

2.3. Microbiological analysis

After each treatment, samples were aseptically cut in small pieces and homogenised in a stomacher using 80 mL of Buffered Peptone Water, BPW (Lab M, Lancashire, UK), for 5 minutes. Decimal dilutions were carried out in BPW.

Total mesophiles enumeration was assessed, in duplicate, using Plate Count Agar, PCA (Lab M, Lancashire, UK). Samples were incubated at 30 °C during 3 days, for posterior counts.

Total coliforms enumeration was assessed, in duplicate, using Violet Red Bile Agar, VRBA (Lab M, Lancashire, UK). Samples were incubated at 30°C during 1 day, for posterior counts.

Listeria enumeration was assessed, in duplicate, using Palcam Agar containing selective supplement (Merck, Darmstadt, Germany). Samples were incubated at 30 °C during 3 days, for posterior counts.

2.4. Data analysis

The treatment effects were assessed by calculation of log-reduction of microbial content, in relation to fresh untreated samples (i.e. $\text{log-reduction} = -\log\left(\frac{\text{microbial load of treated samples}}{\text{microbial load of fresh untreated samples}}\right)$).

Results were compared by analyses of variance (one-way ANOVA, significance level of 5%), using SPSS® 14.0 FOR Windows® (2006 SPSS Inc., Chicago, USA). Duncan's test, for means comparison, was also performed.

3. RESULTS AND DISCUSSION

3.1. Total mesophiles in strawberries

Treatment effects (i.e. water-washings; ultrasonication – US; thermosonication – US 50 °C and US 65 °C; UV-C radiation and ozonation) in total mesophiles reduction of strawberries can be seen in Figure 1.

Ultrasonication at 15 °C (US) and thermosonication at 50 °C were equivalent to a water-washing at 15°C, reducing in average 0.5 log-cycles. Thermosonication at 65 °C reduced approximately 2.5 log-cycles. The treatment was more efficient than a water-washing at the same temperature.
For water-washings at 50 and 65 °C, reductions in total mesophiles counts were higher at the lowest temperature. This can be explained by different thermal resistances of the microorganisms included in mesophiles enumeration.
Ozone treatment provided the highest microbial reductions at 15 °C (~1 log-cycle).
Ultraviolet-C radiation did not inactivate mesophiles in strawberries. Further investigation is required for validation of these results.

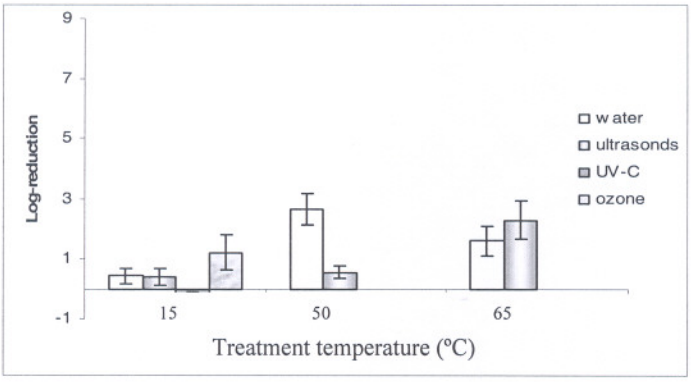

Figure 1. Treatment effects (water-washing, ultrasonication, thermosonication, UV-C radiation and ozonation) in total mesophiles reduction of strawberries. *The bars indicate standard deviation of values.*

3.2. Total coliforms in watercress

The treatments' impact in total coliforms reduction of watercress can be seen in Figure 2.
Ultrasonication treatments (at 15 °C), UV-C and ozonation were equivalent to a water-washing, reducing in average 1 log-cycle. No significant differences were detected between water-washings and thermosonication at the same temperatures (50, 55 and 65 °C).
The most efficient processes were water-washing and thermosonication at 65 °C, reducing approximately 6 log-cycles.

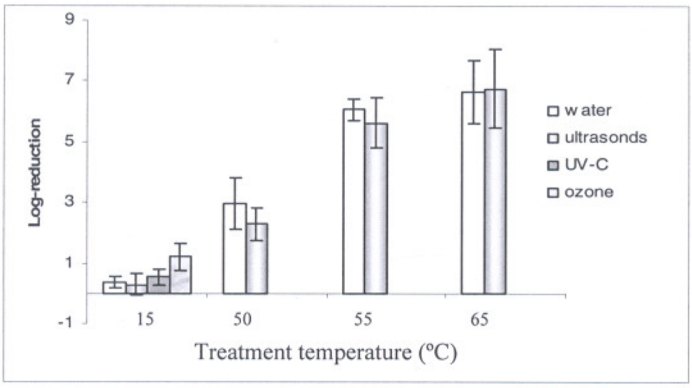

Figure 2. Treatment effects (water-washing, ultrasonication, thermosonication, UV-C radiation and ozonation) in total coliforms reduction of watercress. *The bars indicate standard deviation of values.*

3.3. *Listeria innocua* in red bell pepper

Results of *Listeria innocua* log-reductions in red bell peppers are presented in Figure 3. No significant differences were detected between the different technologies at the same temperatures. The mean of bacteria log-reductions fell within 1-2 log-cycles, at 15ºC.

The most efficient processes were water-washing and thermosonication at 65 ºC, reducing approximately 6 log-cycles.

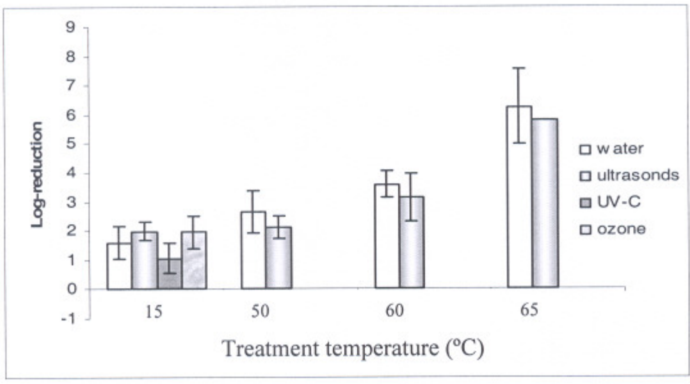

Figure 3. Treatment effects (water-washing, ultrasonication, thermosonication, UV-C radiation and ozonation) in *Listeria innocua* reduction of red bell pepper. *The bars indicate standard deviation of values.*

4. CONCLUSIONS

From the results it is possible to conclude that UV-C radiation was less efficient than water-washings at 15 ºC, for all microorganisms/products tested.

Ozone allowed, in average, 1 log-cycle reduction for mesophiles and coliforms, and 2 log-cycles reduction for *Listeria innocua*.

Ultrasonication treatments were equivalent to water-washings at the same temperatures. The temperature has the major effect. For 50 ºC, 2 and 3 log-reductions were observed, respectively, for *Listeria innocua*/red bell pepper and coliforms/watercress. For 65 ºC, reductions achieved 6 to 7 log-cycles. Mesophiles/strawberries were more heat resistant (2 log-reductions were obtained at 65 ºC).

5. ACKNOWLEDGEMENTS

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