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Original article

The effect of UV-C in combination with H_2O_2 treatments on microbial response and quality parameters of fresh cut pear discs

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Summary The response of *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090 and *Zygosaccharomyces bailii* NRRL 7256 in fresh-cut pear to simultaneous and serial combined treatments involving H_2O_2 immersion (3% w/v; pH 3.0; 25 °C; 2.0 or 5.0 min) and UV-C exposure (7.5 min; 3.7 kJ m^{-2}) was investigated. For selected treatments, native flora, sensory and colour changes were also evaluated. *E. coli* and *L. innocua* were more sensitive than *Z. bailii*. Serial H_2O_2/UV-C treatments were more effective than the simultaneous arrangement. The single effect of UV-C was enhanced by the combination with 5 min H_2O_2, hence avoiding the recovery of the surviving population. The combined treatment kept optimal microbial stability and exhibited more luminosity than the single UV-C treatment. Texture profile analysis conducted using a trained panel showed that H_2O_2/UV-C processed pear discs were perceived with significantly less hardness and fracturability but as juicy as untreated discs. Consumers found them pleasant.

Keywords Colour, combined treatment, cut pear, hydrogen peroxide, sensory attributes, ultraviolet light.

Introduction

Pears (*Pyrus communis*) are versatile fruits, popular around the world mainly as fresh produce; however, they are also consumed in processed forms such as canned pears and pear juice. The composition of the fruit tissue, the pH value and the type of acid itself play a major role in the nature and extension of the microbial populations that grow on the product. Moreover, pear fruit is a suitable substrate for various micro-organisms including *Listeria* species, *Salmonella* spp, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Escherichia coli*, moulds and yeasts (Martinez *et al*., 2000).

Short-wave ultraviolet light (UV-C) is a radiation in the range 200–280 nm in the UV spectrum, which mainly breaks down DNA molecules resulting in germicidal effect on bacteria, virus, protozoa, fungi and algae (Unluturk *et al*., 2008). UV-C does not produce byproducts or generate chemical residues that could change the sensory characteristics of the final product. Several researchers have demonstrated that UV-C light can be used for the inactivation of pathogens without adversely modifying the overall quality of food (Fonseca & Rushing, 2006). However, UV-C radiation has a limited penetration depth (Bintsis *et al*., 2000).

The use of hydrogen peroxide (H_2O_2) has been proposed as an alternative for decontaminating fruits and vegetables. H_2O_2 is a strong oxidising agent that has been shown to damage bacterial proteins, DNA and cellular membranes (Juven & Pierson, 1996). Its toxicity is due to its capacity as an intermediary in oxygen reduction to generate more reactive oxygen species such as the hydroxyl radical. H_2O_2 rapidly degrades into oxygen and water after contacting organic material, thus having no long-term residual activity. H_2O_2 is classified as Generally Regarded as Safe (GRAS) for use in food products as a bleaching agent, oxidising and reducing agent and antimicrobial agent (Sapers & Miller, 1998).

Hydrogen peroxide (H_2O_2) and ultraviolet-C (UV-C) irradiation are being extensively used alone in industrial sterilization or disinfection processes and in food preservation. Both processes can act synergistically to kill bacteria, both vegetative cells and spores. Waites *et al*. (1988) suggested that the mechanism for UV-C-H_2O_2 synergy is related to enhanced production of hydroxyl radicals from H_2O_2 due to irradiation rather

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than to any direct interactions of UV light with DNA. Many questions about the nature of killing by the combined agents still remain unanswered.

During industrial processing, fruits suffer mechanical injury that results in cellular delocalization of enzymes and their substrates, leading to biochemical deteriorations such as enzymatic browning, off-flavours and texture breakdown. In particular, colour and texture are directly related to consumer perception of appearance (Gómez *et al*., 2010). There are a lot of works about microbiological aspects, but only a few studies which examine the effects of UV-C light on shelf life, flavour, colour or nutrient content of fresh fruit have recently been published (Gómez *et al*., 2011; Manzocco *et al*., 2011).

This research was aimed to investigate the effect of combined UV-C light and H_2O_2 treatment on some micro-organisms inoculated on fresh-cut pear discs. Additionally, native flora, surface colour and some sensory texture attributes of fresh-cut pear discs were examined after being processed and/or during refrigerated storage.

Materials and methods

Inocula

Experiments were performed using *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090 and *Zygosaccharomyces bailii* NRRL 7256. Bacteria strains were weekly subcultured in Trypticase Soy Broth (Difco Laboratories, Detroit MI USA) supplemented with 0.1% w/w Yeast Extract (TSBYE) and Trypticase Soy plus Yeast Extract Agar (TSAYE) at 37 ± 0.2 °C and stored at 4 ± 1 °C. The bacteria cultures were prepared by transferring a loopful (10 μL) of a stock culture maintained on agar slants to 20 mL of TSBYE contained in 50 mL-Erlenmeyer flasks. The micro-organisms were incubated at 37 ± 0.2 °C until they reached the stationary phase (≈ 18 h). The initial inoculum of yeast was prepared by transferring a loopful of a stock culture maintained on Potato Dextrose Agar slants to flasks with 20 mL of Potato Dextrose Broth, both from Biokar Diagnostics, Beauvais, France. The organism was grown at 27 ± 0.2 °C until it reached the stationary phase (≈ 24 h).

Preparation of produce samples

Ripe pears (*William cv,* pH ∼ 4.2) were purchased in a local market. For inactivation assay, before being cut, they were rinsed with 0.02% sodium hypochlorite and sterile water to eliminate the surface microbial load and finally they were gently dried with a sterile cloth. A household machine disinfected with 1% hypochlorite solution and equipped with backed stainless steel razor blades was used for slicing. Pears were peeled off and sliced into 3 mm thick pieces. A stainless steel sharp cutting disc was used for obtaining a pear disc (3 cm in diameter) from the centre of each pear slice. Finally, discs (∼2.5 g in weight) were transferred into a sterile Petri dish. The overall preparation of pear samples was made inside a Class II Security Cabinet (Nuaire, Plymouth, MN, USA). For native flora assay, discs were not obtained as aseptically as described above.

Pear disc inoculation

Inocula (0.1 mL) were widespread onto the surface of each pear disc at initial levels of approximately 5×10^5 –10^6 CFU g^{-1} of pear. Before being treated, the inoculated pear discs were kept inside the safety cabinet for 30 min, for the micro-organisms to adhere to the surface of the discs, avoiding any detachment into the peroxide solution. Inoculated and untreated pear discs were used as controls.

Treatments

Ultraviolet chamber and UV-C treatment

The UV-C irradiation device consisted of one bank of two reflectors with unfiltered germicidal emitting lamps (TUV-15W G13 T8 55 V germicidal lamp, Philips, Amsterdam, the Netherlands) that mainly emit 253.7-nm UV light, located 10 cm above the tray. The UV-C lamps and treatment area were enclosed in a wooden box (covered with aluminium foil with a cover protection for the operator). A ventilation device was installed in a corner of the box to avoid temperature increase because of UV-C radiation (Fig. S1). The average temperature during the treatments was 27 ± 1 °C. The UV-C dose emitted from the lamps was determined by using the iodide/iodate chemical actinometer according to the technique proposed by Rahn (1997). Previous studies had shown that for irradiation times larger than 7.5 min (3.7 kJ m^{-2}) the inactivation rate of micro-organisms inoculated in cut pear discs significantly fell down (Schenk *et al*., 2008). Because of this, the UV-C radiation applied in this work was 3.7 kJ m^{-2} (7.5 min). For the UV-C treatments, a Petri dish with three inoculated pear discs was placed over the treatment tray, within a uniform area of the radiation field determined by actinometry (Schenk *et al*., 2008). This area was delimited by a zone below the line between both lamps, equidistant of the ends of the lamps (Fig. S1).

Hydrogen peroxide treatment

Experiments were carried out at 25 °C in a Petri dish with three inoculated discs submerged in 15 mL sterile citric acid-Na_2HPO_4 buffer solution (pH 3.0) containing 3.0% w/v H_2O_2, during 2 or 5 min. These experiment

conditions were based on results obtained from previous studies where lower concentrations of H_2O_2 (1% and 2%) scarcely inhibited micro-organisms and treatment times above 10 min provoked detrimental changes on pear tissue (data not shown). The reaction was stopped by neutralisation. Neutralising solutions were obtained from 4% w/v $Na_2S_2O_3.5H_2O$ diluted in 0.25 M KH_2PO_4 buffer solution adjusted at pH 7.0 with 1N NaOH (Raffellini *et al.*, 2008). All reagents were purchased from Anedra S.A. (Bs. As.; San Fernando, Argentina).

Combined UV-C and hydrogen peroxide treatment

The combined treatments were performed using two different arrangements to detect interaction effects, if any. Consecutive treatments were designed involving first, UV-C treatment (7.5 min) followed by H_2O_2 treatment (2 or 5 min) or *vice versa*. The simultaneous application of both agents (UV-C + H_2O_2) during 2 and 5 min was also studied. The inoculated pear discs were put into a sterile Petri dish with 15 mL of a 3.0% w/v H_2O_2 aqueous solution (pH 3.0) and immediately treated in the UV-C chamber. In both configurations, after the application of H_2O_2, discs were submerged in the neutralising solutions to stop the inactivation reaction. Then, samples were placed into sterile bags (Whirl-Pak; Nasco, Fort Atkinson, WI, USA) and stored in the dark at 5 ± 1 °C for 6 days. During the storage, samples were taken at preset time intervals for the analysis of survivors. For each condition, three replicate samples were used and two independent experiments were carried out.

Microbial enumeration

For microbial enumeration, 20 mL of sterile peptone water (0.1% w/v) were incorporated into each sterile bag containing the pear disc. Subsequently, the bag was pummelled in a laboratory blender (AES Laboratoires, Bruz, France) at high speed for 3 min. Ten-fold dilutions of homogenate samples were made in peptone water and 0.1 mL sample suspension was surface plated using Tryptone Soy Agar (bacteria) or Dextrose Potato Agar (yeast). Two plates were used for each dilution. Plates were incubated at 37 ± 0.2 °C for 48 h in the case of bacteria and at 27 ± 0.2 °C during 72 h in the case of yeasts. A colony counter (Comecta S.A., Barcelona, Spain) was used for colonies enumeration.

Native flora

Control and treated (3% w/v H_2O_2 5 min immersion followed by 7.5 min UV-C irradiation) pear discs were later stored individually in sterile bags in the dark at 5 ± 1 °C for 8 days. Samples were processed as described above and analysed for total mesophilic aerobic populations on Plate Count Agar incubating for 72 h at 37 ± 0.2 °C, and yeasts and moulds populations on Chloramphenicol Glucose Agar incubating at 25 ± 0.2 °C for 5 days before colonies were counted.

Colour measurement

Samples were measured with a handheld tristimulus reflectance spectrocolorimeter Model CM-508-d (Minolta Co., Tokyo, Japan) using a 1.4 cm measuring aperture and a white background. Values were obtained for C illuminant and 2º observer. The instrument was calibrated with a standard white provided by the manufacturer.

The CIE colour coordinates (X, Y, Z) and the L^*, a^*, b^* components of the CIELAB space were recorded. These numerical values were converted into ''browning index'' (BI) colour function using the following equations:

$$\mathrm{BI} = [100(x - 0.31)]/0.172 \quad \text{where } x = X/(X + Y + Z)$$

Colour was measured at 0, 2, 4 and 8 days of storage at 5 °C in the following samples: (i) pear discs without any treatment (control), (ii) pear discs irradiated with UV-C during 7.5 min (3.7 kJ m^{-2}), and (iii) pear discs processed by the combined treatment including immersion in H_2O_2 solution (3.0% w/v, pH 3.0, 25 °C, 5 min) followed by 7.5 min UV-C irradiation.

Ten independent samples were used for each condition with three readings taken at different positions on the surface of each sample. The mean ± standard deviation of the mean (mean ± SD) of colour parameters was reported.

Sensory analysis

Sensory analysis was carried out by trained and untrained panellists. The trained panel was composed of individuals who were previously trained to identify texture attributes of the product. An untrained consumer panel was used to measure the overall acceptability of the treated pear discs. The tested samples were (i) pear discs treated with UV-C irradiation (3.7 kJ m^{-2}), and (ii) pear discs treated with hydrogen peroxide (3% w/v, pH 3.0, for 5 min) followed by UV-C irradiation (3.7 kJ m^{-2}). The samples were presented to the panellists in red plastic glasses identified by numbers of three digits randomly chosen. The evaluations were carried out in individual booths under white light.

Consumer panel

The acceptability panel consisted of 51 randomly untrained volunteers, from Buenos Aires University staff who were invited to taste treated pear disc samples.

Participants evaluated the overall acceptability of the treated samples by using a 9-point hedonic scale in which 1 represented ''highly unacceptable'' and 9 represented ''highly acceptable''. Data obtained from panellists were analysed by converting assigned positions to numbers.

Trained panel

The sensory panel was composed of nine panellists (a group of three men and six women), all between the ages of 21–38. For the selection of panellists twenty people were recruited from the staff of Buenos Aires University. They were trained with the texture profile method following the procedures described by Civille & Szczesniak (1973) during 35–40 h (2 h per week) for them to be able to recognise the most relevant attributes in this type of samples, that is to say hardness, fracturability and juiciness. The definitions for sensory texture parameters used to train the panel were the following: *hardness* was the force required to compress a substance between molar teeth (in the case of solids) or between tongue and palate (in the case of semi-solids); *fracturability* was the force with which a sample crumbles cracks or shatters and *juiciness* was the a mount of juice released on the first three chews (Szczesniak, 1963). For the training and use of texture scales, sensory data corresponding to the standard food references were obtained from García Loredo & Guerrero (2011) for hardness and fracturability scales and from Szczesniak & Ilker (1988) for juiciness scale. Judges used the reference samples prepared according to the descriptions by these authors regarding type, size, temperature, brand and manufacturer of the food products.

The textural attributes of the pear disc samples were analysed by texture profile method (IRAM 20013, 2001). Two well-known food references and an evaluation form were also provided with the sample. This form included instructions and the line scale corresponding to the texture attribute with the positions of the references indicated on it (García Loredo & Guerrero, 2011).

Statistical analysis

UV-C and hydrogen peroxide data were analysed by one-way analysis of variance (ANOVA) to establish the presence or absence of significant differences between treatments. Statistical analyses for colour measurements of pear samples were carried out using InfoStat Versión 2009 (InfoStat Group, FCA-UNC, Córdoba, Argentina). Colour data were analysed by two-way multivariate analysis of variance (MANOVA) to establish the presence or absence of significant differences in colour parameter values according to the factors ''treatment'', ''time'' and the interaction ''treatment*time''. Significance level was set at $P < 0.05$. In case of finding significant differences, post-hoc multiple comparisons among multivariate means of factors were performed by Hotelling tests based on Bonferroni correction. Principal components analysis (PCA) was applied as an extension of multivariate analysis to illustrate the relationship between colour parameters and samples. Multivariate outliers were detected by Mahalanobis distance and removed from data set.

Trained panel data were analysed by two-way analysis of variance (ANOVA) to establish the presence or absence of significant differences among samples or panellists. Overall acceptability individual values were processed by one- way analysis of variance (ANOVA) in order to establish significant differences among samples.

For all statistical analysis, significance level was set at $P < 0.05$. In case of finding significant differences, Tukey's test was performed.

Results and discussion

Effects of combined treatments on inoculated micro-organisms

The inactivation effects of consecutive treatments UV-C/H_2O_2 or H_2O_2/UV-C and simultaneous treatment H_2O_2-UV-C on *E. coli* inoculated in pear discs for 2 and 5 min of immersion in H_2O_2 solution are presented in Table 1.

The observed inactivation with the simultaneous treatment was limited to only 1.3–1.5 log cycles reduction, being this inactivation lower than those corresponding to the single treatments. Rosenfeldt *et al.* (2006) reported that hydroxyl radical formation from H_2O_2 in the presence of oxidisation agents, such as UV-C light at 254 nm, is enhanced at temperatures above 50 °C. The temperature of the simultaneous experiment H_2O_2-UV-C presented in this work was near 25 °C making the formation of radicals less probable. Hence, the presence of H_2O_2 could stand as a physical barrier, limiting the UV-C penetration in the pear discs and reducing its effectiveness (Unluturk *et al.*, 2008).

When the treatment was applied using consecutive arrangements 7.5 min UV-C irradiation/2 min H_2O_2 immersion or *vice versa* at 25 °C, *E. coli* inactivation did not significantly improve compared with single treatments. However, when immersion of pear discs in H_2O_2 solution was extended to 5 min, greater inactivation was observed in both consecutive treatments, being the reduction $\sim$ 3.6 log cycles. The combined inactivation effect observed in both 5 min. H_2O_2 consecutive treatments was lower than additive. The order of the sequence in the consecutive arrangement did not have a significant impact on the inactivation observed (Table 1).

Because hydrogen peroxide could promote the formation of hydroxyl radicals, the consecutive arrange-

Table 1 Log reductions of different micro-organisms in pear discs subjected to single UV-C and combined H_2O_2/UV-C treatments

Treatment (min)	Micro-organism		
	Escherichia coli	*Listeria innocua*	*Zygosaccharomyces bailii*
UV-C (7.5)	2.51 ± 0.05[b]	2.24 ± 0.02[b]	2.29 ± 0.03[b]
H_2O_2 (2)	2.53 ± 0.08[b]	–	–
H_2O_2 (5)	2.45 ± 0.15[b]	1.39 ± 0.08[c]	0.25 ± 0.01[c]
UV-C (7.5)-H_2O_2 (2) CT	2.11 ± 0.16[b]	–	–
H_2O_2 (2)-UV-C (7.5) CT	2.43 ± 0.41[b]	–	–
H_2O_2/UV-C (2) ST	1.33 ± 0.02[c]	–	–
UV-C (7.5)-H_2O_2 (5) CT	3.74 ± 0.12[a]	–	–
H_2O_2 (5)-UV-C (7.5) CT	3.63 ± 0.15[a]	2.44 ± 0.05[a]	2.73 ± 0.10[a]
H_2O_2/UV-C (5) ST	1.47 ± 0.11[c]	–	–

CT, consecutive treatment; ST, simultaneous treatment; –, not tested.
In each column, means with the same letter are not significantly different ($P \leq 0.05$).

ment 5 min H_2O_2/7.5 min UV-C was selected to evaluate *L. innocua and Z. bailii* inactivation in pear discs (Table 1). In general, lower inactivation effect was observed with both micro-organisms, exhibiting similar response to the treatment corresponding to 7.5 min UV-C irradiation (~2.3 log reduction) but being *L. innocua* more sensitive to 5 min H_2O_2 decontamination (~1.4 log reduction) than the yeast (~0.3 log reduction).

In experiments where *L. innocua* was used, the application of the combined treatment did not markedly improve the log reduction observed when UV-C was applied as a single treatment (~2.4 log cycles). When *Z. bailii* was used, it was observed that the total inactivation increased significantly, achieving a reduction of 2.7 log cycles. These results are consistent with those reported by Block (2001), who states that 3% H_2O_2 solution is more effective against Gram-negative bacteria (D: 0.5–0.7 min) than against Gram-positive bacteria (D: 1.50–2.35 min) or yeasts (D: 4–18 min) in cell suspensions. It has been reported by Crowe *et al.* (2007) that using a combination of UV-C and H_2O_2 did not result in greater inactivation of the microflora associated with blueberries compared with that when H_2O_2 was applied alone. In both cases, it is possible that the lack of a visible synergistic effect was a consequence of applying H_2O_2 at ambient temperature, in which the generation of free radicals would be lower.

Storage studies were carried out to analyse the possibility of micro-organism recovery in pear discs processed by the combined consecutive treatment immersion in (pH 3.0; 3% w/v) H_2O_2 solution for 5 min, and then exposure to 3.7 kJ m^{-2} UV-C light. Figure 1 shows the evolution of microbial counts during 6-day storage of pear discs at 5 °C. It was observed that *E. coli* population slightly decreased in the first day of storage (~1 log cycle), remaining almost constant and without recovery during the storage. *L. innocua* population exhibited an oscillating behaviour of survival

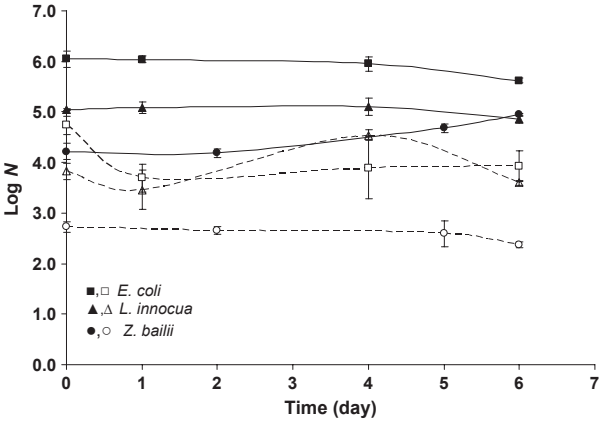

Figure 1 Survival curves of different micro-organisms in pear discs after the combined treatment H_2O_2 (5 min)/UV-C (7.5 min) during storage (5 °C). Solid symbol: control sample; Open symbol: treated sample.

during 6-day storage of pear discs at 5 °C. *L. innocua* counts scarcely decreased within the first day of storage. However, an increase was observed until the fourth day and then it started decreasing towards the end of storage. The surviving population of *Z. bailii* remained roughly constant throughout the storage while in the control system it slightly increased during the storage as it was expected.

The applied combined treatment H_2O_2/UV-C reduced the initial microbial population in pear discs which could not repair induced damage and recover during storage, remaining almost constant. This could be due to the fact that hydrogen peroxide generates free radicals that cause sublethal damage on the enzymes of micro-organisms altering membrane permeability, favouring the action of UV-radiation, and what is more destabilizing the membrane and damaging DNA

molecule and proteins producing a range of significant physiological alterations (Crowe *et al.*, 2007). In the case of bacteria, the absence of recovery could be attributed to the low storage temperature which was not conductive to the growth of *E. coli* or *L. innocua* as it can be seen in the survival curves corresponding to control systems (Fig. 1). This emphasises the fact that the application of the combined treatment reduces the initial load. In the case of yeast, the application of the combined treatment was more useful, avoiding yeast proliferation.

Native flora

The evolution of native flora (mesophilic aerobic microorganisms and yeasts and moulds) present in pear discs without any treatment (control) and after the single 7.5 min UV-C and the combined 5 min H_2O_2/7.5 min UV-C treatments during 8 day-storage (5 °C) are shown in Fig. 2. Though growth was observed in untreated and UV-C treated samples, native microflora counts were higher in control samples than in UV-C treated samples (Fig. 2a). Similar results were obtained by Fonseca & Rushing (2006), who applied UV-C light (4.1 kJ m^{-2}) in watermelon cubes with subsequent storage for 7 days at 3 °C, observing an increase of about ~ 2.5 log cycles in samples treated with UV-C after 7 days of storage. Different results were obtained by Erkan *et al.* (2001), who found that after 5 days of storage of squash slices, the mesophilic bacterial populations were higher in non-irradiated samples and in those scarcely irradiated (1 min- UV-C; dose 0.49 kJ m^{-2}) than in samples irradiated for 10 or 20 min (4.93 or 9.8 kJ m^{-2}) at 10 °C. At the sixth day of storage, they observed that the mesophilic bacterial populations remained relatively constant in all treatments until the end of storage (14 days).

For yeasts and moulds enumeration in control and single UV-C treated samples, the development of some filamentous fungi was observed but with majority of yeast population. At the onset of storage, counts of yeasts and moulds in untreated pear discs were low (2.4×10^2 CFU g^{-1}). However, the counts increased quickly from the third to the eighth day of storage, reaching 3.6×10^4 CFU g^{-1} of pear. The UV-C treatment inhibited the development of these micro-organisms within the first day of storage, after which, the counts increased to 1.1×10^4 CFU g^{-1} on the eighth day of storage (Fig. 2b). Similar results were found by Allende *et al.* (2006) when lettuce was irradiated with UV-C (doses: 1.18, 2.37 and 7.11 kJ m^{-2}), and yeast growth decayed within the first day, but then increased the counts towards the end of storage.

This did not occur with the pear samples treated with the combined H_2O_2/UV-C treatment, where native population reduction at the onset of storage was observed without further recovery (Fig. 2a and b). The pear discs processed with the combined treatment H_2O_2/UV-C retained optimal microbiological stability throughout the refrigerated storage.

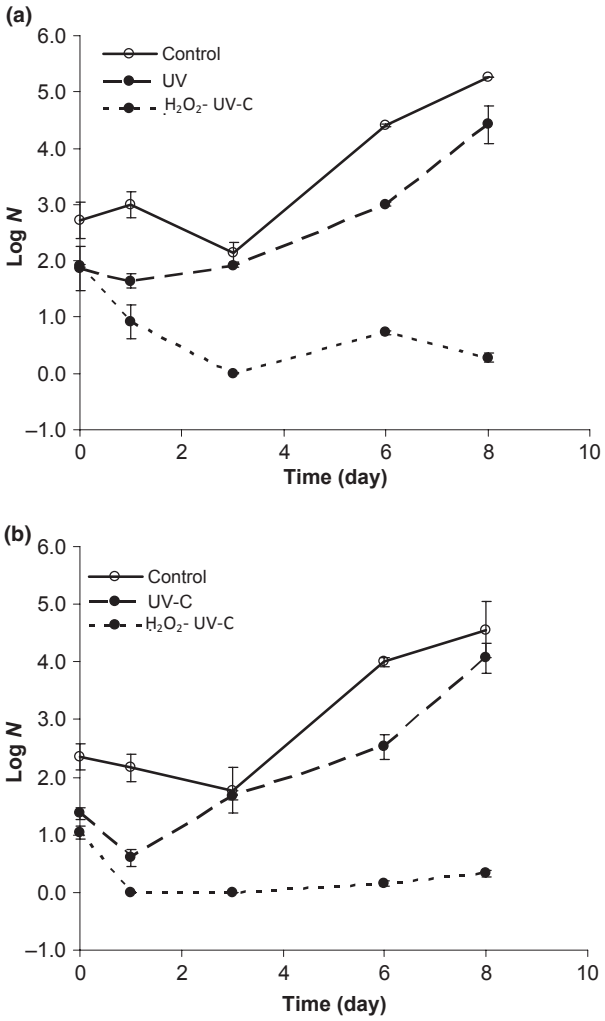

Figure 2 Evolution of native flora (log N) in processed pear discs stored at 5 °C. (a) Mesophilic aerobic counts, (b) yeast and mould counts, N: (CFU g^{-1}).

Colour study

The evolution of average L^*, a^* and b^* values as well as BI function of processed pear discs during refrigerated storage are presented in Table 2. The two-way MANOVA did not find significant differences for the interactions ''Time*Treatment'' of colour measurements ($F_{24, 432} = 1.3$; $P = 0.154$) indicating that the evolution of pear disc colour through time did not depend on the applied treatment. However, there were significant differences for the single factor ''time'' ($F_{12, 321} = 3.52$; $P =$

Table 2 Mean values and standard deviations (SD) of L^*, a^* and b^* colour parameters and BI function corresponding to different samples along storage[1]

Treatment	Time (day)	L^* ± SD	a^* ± SD	b^* ± SD	BI ± SD
Control	0	60.8 ± 4.9^{Aa}	-0.7 ± 1.0^{Aa}	17.9 ± 2.0^{Aa}	26.8 ± 4.6^{Aa}
	2	60.2 ± 5.6^{Ab}	0.2 ± 1.5^{Ab}	18.8 ± 1.8^{Ab}	29.7 ± 6.4^{Ab}
	4	60.8 ± 6.1^{Ab}	0.4 ± 1.6^{Ab}	18.8 ± 1.8^{Ab}	29.7 ± 6.3^{Ab}
	8	60.9 ± 6.2^{Ab}	0.6 ± 1.8^{Ab}	19.2 ± 2.1^{Ab}	30.5 ± 7.5^{Ab}
UV-C	0	59.7 ± 2.3^{Ba}	-0.7 ± 1.1^{Ba}	18.1 ± 2.1^{Ba}	27.3 ± 3.4^{Ba}
	2	54.5 ± 3.0^{Bb}	1.3 ± 1.2^{Bb}	19.0 ± 1.5^{Bb}	33.5 ± 3.9^{Bb}
	4	53.3 ± 2.6^{Bb}	1.6 ± 1.2^{Bb}	17.9 ± 1.6^{Bb}	32.5 ± 3.9^{Bb}
	8	51.6 ± 2.0^{Bb}	1.8 ± 1.2^{Bb}	17.6 ± 1.6^{Bb}	33.1 ± 3.8^{Bb}
H_2O_2/UV-C	0	$65.2 \pm 3.7A^{a}$	$-0.4 \pm 1.6A^{a}$	$19.8 \pm 4.2A^{a}$	28.5 ± 9.0^{Aa}
	2	58.6 ± 6.6^{Ab}	1.0 ± 1.8^{Ab}	18.8 ± 3.4^{Ab}	31.5 ± 9.3^{Ab}
	4	57.1 ± 7.4^{Ab}	1.3 ± 2.0^{Ab}	18.2 ± 3.7^{Ab}	31.6 ± 10.1^{Ab}
	8	54.8 ± 7.6^{Ab}	1.4 ± 1.7^{Ab}	17.3 ± 2.4^{Ab}	31.1 ± 8.1^{Ab}

[1]In each column, different upper-case superscripts indicate significant differences among treatments at the same storage time; different lower-case superscripts indicate significant differences among samples processed by the same treatment at different storage times.

0.0001) between the beginning of the storage (time 0) and the rest of storage time.

L^*, a^*, b^* and BI values of untreated pear samples did not show any significant variation throughout 8 days of storage. Nevertheless, pear discs subjected to both emerging treatments (single UV-C or combined H_2O_2/UV-C) exhibited comparable initial colour parameter values but along the storage, L^* and b^*values decreased and a^* and BI values increased.

The decrease of L^* and b^* and the increase of a^* and BI mean values indicated that treated pear disc surface became darker and more brownish. There were significant differences between treatments ($F_{8,\ 212} = 5.71$; $P = 0.0001$) (Table 2). At the end of storage, treated samples turned darker than control samples and this effect was more pronounced in pear discs treated with single UV-C treatment.

Erkan *et al.* (2001) measured the browning presented by the surface of the zucchini exposed to UV-C (doses between 5 and 10 kJ m^{-2}). They attributed the observed browning to an accumulation of phenolic compounds induced by the UV-C irradiation. Fonseca & Rushing (2006) reported that the application of 13.7 kJ m^{-2} UV-C light on minimally processed watermelon produced an adverse effect on the colour pulp inducing tissue discoloration.

Gómez *et al.* (2010) commented that the modifications in colour of UV-C irradiated apple discs (doses between 4.5 and 11 kJ m^{-2}) during refrigerated storage could be, at least partially, ascribed to the breakage of cellular membranes, which would cause a loss of funcional cell compartmentalisation. This would increase enzyme-substrate contact with the consequent increase in tissue browning.

Principal component analysis (PCA) showed the spatial relationships of the four parameters for each sample. A two-dimensional representation of principal component 1 and 2 is presented in Fig. 3. The first two principal components (PC 1 y PC 2) explained 73.7% and 24.5% of the variance, respectively. The PC 1 contrasted L^* values positively and a^* and BI values negatively. The second axis (PC 2) was defined positively by b^* parameter. Untreated and treated pear discs corresponding to the beginning of storage (day 0) were placed to the right on the graph, indicating that these samples have more luminosity than the rest. In particular, the H_2O_2/UV-C treated pear discs showed an increase in L^* respect to the single UV-C treated pear discs, indicating the bleaching effect of hydrogen peroxide. Untreated pear discs (C2, C4 and C8) increased b^* values during the storage (arrow direction in Fig. 3).

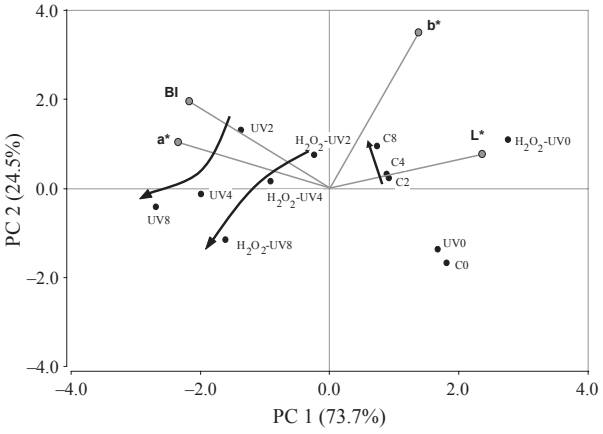

Figure 3 Principal component analysis (PCA) bi-plot of colour parameters of pear discs. C, control; UV, UV-C treatment; H_2O_2-UV$_i$, combined treatment; i, 0, 2, 4 or 8 days of storage.

All treated samples corresponding to different storage times (2, 4 and 8 days) were placed to the left on the

graph. A clear trend of colour changes depending on storage time was observed in these samples. They showed an increase in a^* and BI values and a decrease in L^* due to storage time compared with the same samples at the beginning of the storage (day 0), evidencing the detrimental effects of H_2O_2 and UV-C treatments on the colour of pear tissue. The displacement of combined treated samples to the right along the first axis proves that the hydrogen peroxide improved the colour of samples with respect to UV treated samples, increasing their luminosity. In fact, at first sight, combined H_2O_2/UV-C treated pear discs presented colour parameters which were more similar to the ones presented by the untreated fruit. All results were consistent with the MANOVA analysis previously presented.

Sensory characteristics

Texture

A trained panel tested some texture attributes of untreated, single UV-C or combined H_2O_2/UV-C treated pear discs to determine the influence of the different preservation treatments on the texture perception of the samples. Food references selected to show the most representative texture parameters in pear discs: hardness, fracturability, and juiciness scales were well identified by the panellists during the training process and were well assigned into the corresponding scale during the successive sessions. These results indicate that the panel acquired a good degree of training and was able to quantitatively evaluate the individual mechanical parameters of texture (García Loredo & Guerrero, 2011). Two-way analysis of variance (ANOVA) of data showed that there were no significant differences among panellists (Tukey's test $P < 0.05$), demonstrating consistency in results (data are not shown). Table 3 shows the texture attribute means and the standard deviations of the sensory ratings assigned by the panellists to the pear discs samples.

Hardness of pear discs decreased as more treatments were applied to the samples, showing significant differences between pear samples without any treatment and those processed with the combined H_2O_2/UV-C treatment ($F_{2,35} = 3.89$, $P = 0.0394$). Gómez *et al.* (2011) analysed the effect of UV-C radiation (dose 11.2 kJ m^{-2}), with or without an anti-browning pretreatment on the instrumental texture, sensory texture and ultrastructure of cut apple. As far as hardness is concerned, significant differences between untreated and UV-C treated cut apples were found.

Table 3 Mean values and standard deviations corresponding to the sensory parameters hardness, fracturability and juiciness of pear discs subjected to different treatments

Treatment	Hardness	Fracturability	Juiciness
C	4.83 ± 0.72[a]	4.41 ± 2.01[a]	4.92 ± 1.01[a]
UV-C	4.31 ± 0.65[a,b]	3.91 ± 2.19[a,b]	5.33 ± 1.40[a]
H_2O_2/UV-C	4.18 ± 0.65[b]	3.70 ± 2.28[b]	5.23 ± 0.61[a]

Different superscripts in the same column indicate significant differences between the mean values. C, control, untreated samples; UV-C, single 7.5 min UV-C treatment; H_2O_2/UV-C, combined 5 min 3% H_2O_2/7.5 min UV-C treatment.

As regards fracturability, there was more discrepancy in the judgment due to the difficulty in the measurement of this attribute and to the biological variability of the pear samples. At any rate, it was concluded that the control samples were more breakable than the combined H_2O_2/UV-C treated samples ($F_{10,35} = 4.06$; $P = 0.0048$).

Gómez *et al.* (2011) found that during refrigerated storage, the ultrastructural differences in the surface of cut apples subjected to UV-C light included rupture of membranes, swelling of cells and alteration of cell walls. As a consequence of vacuole rupture induced by UV-C, the interactions between cell walls and cellular contents (organic acids, hydrolytic enzymes, etc.) would be facilitated and could allow cell wall degradation during storage. Accordingly, they found significant differences among untreated and UV-C treated and stored cut apples regarding hardness and fracturability. In this work, these changes on fruit texture parameters observed during the storage of the UV-C treated samples by Gómez *et al.* (2011) could be equivalent to changes in the combined H_2O_2/UV-C treated pear discs without storage, with the action of hydrogen peroxide accelerating the detrimental process.

There were no significant differences in the perception of juiciness of pear disc samples with/without any treatment ($F_{2,35} = 0.47$, $P = 0.6325$). This implies that the applied treatments did not modify the multidimensional perception of juiciness. Gómez *et al.* (2011) also reported significant differences among all samples regarding juiciness. They explained that this unexpected response could be related to fractures causing alteration in the other texture parameters, more related to cell to cell debonding than to the breaking of cell walls. Thus, individual cells would not break, open and release their contents and the juiciness of the treated samples could be perceived similar to the untreated ones.

Overall acceptability

The single UV-C and combined H_2O_2/UV-C treatments were evaluated by consumers in order to assay the overall impression of pear slices processed by a novel technology. The average overall acceptability of samples treated with single 7.5 min UV-C was 6.9 ± 1.1, corresponding to the category "like moderately" while the mean value of samples subjected to the combined treatment 5 min

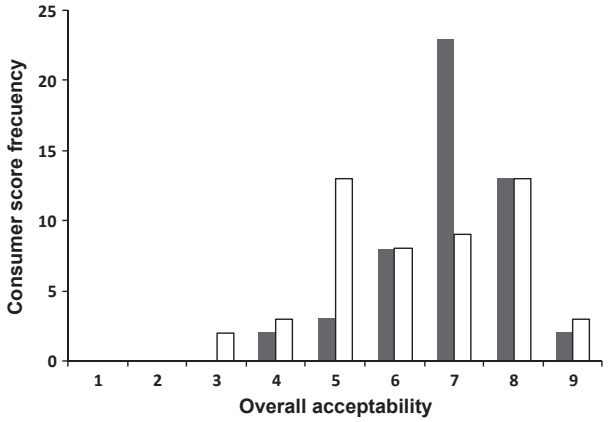

Figure 4 Frequency distribution of scores given by consumers for the evaluation of overall acceptability of pear discs treated with: (■) UV-C and (□) H_2O_2-UV-C.

H_2O_2/7.5 min UV-C was 6.4 ± 1.4, corresponding to the category ''like slightly''. Analysis of variance (ANOVA) showed significant differences ($F_{1,50}$ = 4.67; $F_{critical}$ = 4.03) in overall acceptability of samples processed with both treatments. Figure 4 shows the frequency distributions of the scores assigned by the panellists to both types of processed samples (single 7.5 min UV-C and combined 5 min H_2O_2/7.5 min UV-C treatments). Frequency distribution of scores assigned to samples processed by the single UV-C treatment was described a Gaussian shape with most of consumers choosing category 7 ''like moderately'' but Fig. 4 put on evidence discrepancies between consumers evaluating samples processed with the combined treatment. There were two main groups of frequencies, one group chose the ''Neither like nor dislike'' category (point 5 in the 9-point scale), but other group chose the ''like very much'' category (point 8 in the 9-point scale), suggesting that the combined treatment introduced small sensory changes to pear discs which were, in general, well accepted by consumers. Comments made by panellists through open-ended questions remarked that the pear samples processed with combined treatment were ''unfamiliar to pear consumers and somewhat less sweet but pleasant and with good texture''.

Conclusions

This study has contributed to give alternatives to the use of novel technologies when applied to real food systems and to show how they influence over some food quality parameters. In particular, this study provided useful quantitative information about the inactivation of some target micro-organisms and the native flora, and overall quality of fresh-cut pear discs irradiated by UV-C light (7.5 min) or combined treated by immersion in hydrogen peroxide (5 min) followed by UV-C irradiation (7.5 min). On the one hand, it was noted that, though the combined treatment reduced micro-organism levels on produce, there were always residual survivors. However, these survivors could not repair damage induced by the treatment since they did not proliferate during post-treatment storage of pear discs. When UV-C treated pear discs were stored at 5 °C, there was a relatively rapid growth of the residual native flora but this did not occur when the combined H_2O_2/UV-C treatment was applied since these populations significantly decreased along refrigerated storage. The combination of UV-C irradiation with hydrogen peroxide treatment turned out to be a promising approach since browning effect observed along storage was less pronounced. Though some texture parameters of pear discs changed due to the applied single or combined treatments, they were well accepted by consumers. This investigation also demonstrated that the way combined treatments are performed, may change their efficiency. The knowledge of the interaction characteristics of the factors in combination as well as the effect of the preservation system on food quality could contribute to the rational design of minimal processes for food preservation using emerging agents in combination.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Schematic diagram of the UV-C irradiation device.

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