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COLD PLASMA TREATMENT FOR FRESH-CUT MELON STABILIZATION

Silvia Tappi[a],* Giorgia Gozzi[a], Lucia Vannini[a,b], Annachiara Berardinelli[a], Santina Romani[a,b], Luigi Ragni[a,b], Pietro Rocculi[a,b]

[a] Department of Agricultural and Food Sciences, *Alma Mater Studiorum*, University of Bologna, P.zza Goidanich 60, 47521 Cesena (FC), Italy

[b] Inter-Departmental Centre for Agri-Food Industrial Research, *Alma Mater Studiorum*, University of Bologna, P.zza Goidanich 60, 47521 Cesena (FC), Italy

* Corresponding author. Tel.: +39 0547 338120; Fax: +39 0547 382348; E-mail address: silvia.tappi2@unibo.it (S. Tappi).

Abstract

The aim of this study was to evaluate the effect of gas plasma treatment on fresh-cut melon stability during controlled storage. Plasma treatments of 15+15 and 30+30 min were conducted on fresh cut melon using a Dielectric Barrier Discharge (DBD) generator. Samples were packed and stored for 4 days at 10°C and evaluated for qualitative, metabolic and microbiological indexes. Qualitative parameters of fresh-cut melon (titratable acidity, soluble solid content, dry matter, colour, texture) were only weakly affected by plasma treatment. Peroxidase (POD) and pectinmethylesterase (PME) activity were slightly inhibited by the treatment up to respectively about 17 and 7%. Tissue metabolic heat production decreased proportionally to the treatment duration, while a partial conversion to anaerobic metabolism was observed. Microbial results showed that a significant increase in microbial shelf-life was achieved following the 15+15 min plasma treatment due to a delayed growth of spoilage mesophilic and psychrotrophic microflora.

Industrial relevance

The demand for fresh-cut products characterized by high qualitative and nutritional values and an acceptable shelf-life has promoted the research for non-thermal treatments.

Fresh-cut melon is considered to be highly perishable and potentially hazardous foods because it can support the growth of spoilage microflora and several pathogens.

Cold plasma has shown its potentiality as an antimicrobial treatment and has been tested on different food products, but the impact on product quality and metabolism is still scarcely known.

The results obtained in this study contributed to deepen the knowledge on the effect of plasma treatment on microbial, qualitative and metabolic aspects of fresh-cut melon.

Keywords: DBD cold plasma; fresh-cut melon; quality; tissue metabolic activity; microbial shelf-life; spoilage

1. Introduction

Fresh-cut fruit and vegetables are products subjected to minimally processing operations, maintaining the fresh-like quality, with a high convenience value (Ragaert, Devlieghere, & Debevere, 2007). Minimal processing operations such as slicing, peeling and/or other mechanical injuries cause physical damages to the product, resulting in a number of physiological disorders called 'wounding response' (e.g. increased rate of respiration and ethylene production, enzymatic activity, quality degradation and dehydration), which favour also the growth of the spoilage microflora leading to a very limited shelf-life (Soliva-Fortuny & Martín-Belloso, 2003).

Furthermore, fresh-cut products such as melon, characterised by quite high pH (5.2-6.7) and water activity (0.97-0.99) values, are considered to be highly perishable and potentially hazardous foods because they can support the growth of spoilage microflora and several pathogens, including *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, particularly if they are not subjected to adequate preservative treatments and to cold storage (Harris et al., 2003; Lamikanra, Chen, Banks, & Hunter, 2000), or if their surface has been damaged prior to consumption (EFSA, 2014). Melon surface, characterized by a complex netting skin (peel), provide an environment on which bacteria can strongly attach (Ukuku, Olanya, Geveke, & Sommers, 2012; Vadlamudi, Taylor, Blankenburg, & Castillo, 2012) and from which they can be easily transferred onto melon flesh during cutting operations.

The U.S. Centres for Disease and Control and Prevention (CDC) identified 34 foodborne disease outbreaks related to the consumption of melons in the U.S. between 1973 and 2011 (CDC, 2011). Moreover, among the 16 outbreaks reported in 2012 due to contaminated fruits, which caused a total of 858 illnesses, four were associated to melons (CDC, 2012). The majority of these outbreaks were caused by *Salmonella*. Nevertheless, one of the most widespread outbreak of *Listeria monocytogenes* food poisoning in US, which caused 146 illness in 28 states and led to 32 deaths, resulted from contaminated cantaloupes (CDC, 2012).

Studies on innovative physico-chemical processes to improve the shelf-life of whole and fresh-cut melons mainly refer to the use of irradiation (Palekar, Taylor, Maxim, & Castillo, 2015) X-ray (Mahmoud, 2012), UV-C light (Manzocco, Rumignani, & Lagazio, 2013), gaseous ozone (Selma, Ibáñez, Cantwell, & Suslow, 2008), edible coatings (Martiñon, Moreira, Castell-Perez, & Gomes,

2014) or modified atmosphere packaging (MAP) (Zhang, Samapundo, Pothakos, Sürengil, & Devlieghere, 2013). On the other hand, most of the literature data are focused on sanitizers alternative to chlorine washing (Silveira, Conesa, Aguayo, & Artés, 2008; Ukuku, Huang, & Sommers, 2015), which is the procedure currently used at industrial level despite the widely diffused concerns of potential formation of harmful by-products.

The growing demand for fresh-cut products has pushed the researchers to develop new non-thermal treatments able to keep a desirable shelf-life, preserving the original fresh-like attributes of the raw material.

Recently, cold plasma, which in the past has been mainly used in the medical field, particularly for microbial decontamination of surfaces and living tissues (Emmert et al., 2013; Juwarkar, 2013; Weltmann et al., 2012), has drawn considerable attention as a novel non-thermal treatment for food products decontamination (Niemira, 2012). Plasma is considered the "fourth state of matter" and it is generated by applying energy to a gas mixture, causing the ionisation of the gas and the formation of active components, such as radicals, charged particles and UV radiations.

Non thermal plasma can be generated by microwaves, radio frequency, direct or alternating current; various set-ups such as dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ) and corona discharges (CD) (Ehlbeck et al., 2011; Laroussi, 2002; Ragni et al., 2010); different gas mixtures, including atmospheric gas (oxygen, nitrogen and carbon dioxide) as well as noble gases (e.g. helium and argon) can be used. The plasma composition greatly depends on the kind of gases in the mixture, the selected generator set-up, the operating conditions (flow, gas pressure, power of plasma excitation) and the exposure mode (direct or remote) (Misra, Tiwari, Raghavarao, & Cullen, 2011), and it includes reactive species such as oxygen and nitrogen species (ROS and RNS), atoms, free radicals and UV radiations.

Among all the different plasma constituents, the most important role in microbial inactivation and protein denaturation seems to be played by reactive species, such as free radicals (Laroussi, 2002; Li et al., 2011; Takai, Kitano, Kuwabara, & Shiraki, 2012).

The oxidative damage to the microbial cell surface can lead to the loss of functionality of the cell membrane and the exposure of the genetic material. Various authors have assessed the microbial decontamination of cold plasma on foodstuffs, such as the outer surface of various fruit and vegetables (Critzer, Kelly-Wintenberg, South, & Golden, 2007; Niemira & Sites, 2008; Misra et al., 2011; Baier et al., 2014) and on apple juice (Montenegro, Ruan, Ma, & Chen, 2002; Björn Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014). The effect was found to be highly dependent on the operative conditions (type of plasma generator, flow rate, treatment time, gas mixture), type of microorganism and matrix exposed to gas plasma.

Moreover, cold plasma treatments have recently drawn attention as possible treatments for fresh-cut vegetable products with the aim of inactivate endogenous enzymatic activity. In a previous study, Tappi et al. (2014) observed a significant reduction of PPO activity in fresh cut Pink lady apples (up to 45% compared to the control) and of browning reaction during storage. The observed reduction was probably due to reactions between the enzymes and the radicals produced during treatment. Protein structural modifications upon plasma treatment were observed in different studies, in which modifications in the aminoacidic side chain and the decrease in the amount of α-helix structures in various enzymes was detected by means of techniques, as circular dichroism spectroscopy and tryptophan emission fluorescence, and related to the loss of enzymatic activity (Pankaj, Misra, & Cullen, 2013; Bjoern Surowsky, Fischer, Schlueter, & Knorr, 2013; Takai et al., 2012).

Furthermore, few researches have evaluated the effect of plasma on bioactive compounds and antioxidant activity in lamb's lettuce (Grzegorzewski, Ehlbeck, Schlüter, Kroh, & Rohn, 2011) and fresh-cut kiwifruit (Ramazzina et al., 2015).

However, the differences among the type of plasma and the operating conditions used in these studies make difficult the comparison of the obtained results.

In this contest, the aim of this research was to evaluate the effects of cold plasma, generated by a DBD device, on fresh-cut melon quality and safety. In particular, the effect of different treatment times has been evaluated on qualitative, metabolic and microbiological aspects of fresh-cut melon during controlled storage.

2. Materials and Methods

2.1. Raw material, handling and storage

Melons (Cucumis *melo L.* var. *Reticolatus* cv. 'Raptor') grown in the Emilia-Romagna region of Italy were harvested in July 2013. The fruits were stored in plastic bins at 2 ± 0.5°C and approximately 100% RH in air for 2 weeks. After this period, 20 kg of fruits free from defects were selected, transported to our laboratory, placed in a dark refrigerated chamber at 4°C and saturated atmosphere for one week. At the beginning of experiments melons had a dry matter content of 15.73 g (± 0.29) 100 g fw^{-1}, a soluble solid content of 14.27 (± 0.35) % and a titratable acidity of 0.39 mg (± 0.03) of malic acid g fw^{-1} measured as an average of 10 fruit.

Before cutting operations, whole melons were washed and scrubbed with a sponge to eliminate dirt from the surface and then immersed for 2 min in a 200 ppm sodium hypochlorite solution, in order to sanitize the peel and avoid cross contamination during processing. Work surface and cutting tools were also sanitized with the same solution prior to use.

Melons were then halved and blossom and stem ends were eliminated. Skin and seeds were also removed. From the central part of each half, 10 mm slices were cut using a sharp knife and each slice was divided in 4 trapezoidal pieces (about 10 g each).

2.2. Gas plasma generator

A Dielectric Barrier Discharge (DBD) generator composed by three parallel pair electrodes made of brass and a 5 mm thick glass covering one electrode of each couple, was used for the treatment as described in previous works (Ragni et al., 2010; Berardinelli et al., 2012; Ramazzina et al., 2015). The electrodes were confined in a cabinet (about 3×10^{-2} m^3 of air volume) and were DC powered by three independent power supplies at 19 V and about 3 A. The high voltage (about 15 kV peak to peak and a dominant frequency of 12.5 kHz) was generated by switching transistors and transformers.

Air gas was used to generate the discharge (at 22°C and 60% of R.H.) and, according to previous studies (Ragni et al., 2010), it led to the formation of OH and N radicals and ions. Samples made of 15 melon pieces were placed at about 70 mm from the electrodes (Fig. 1). The plasma species were directed to the samples surface by three fans mounted over the electrodes (flow rate of about 7×10^{-3} m^3 s^{-1}).

2.3. Gas plasma treatments and samples storage

Preliminary tests were run in order to select treatment time and to avoid visible damages on the melon fresh-cut surface. On the basis of the obtained results, treatment durations of 30 min (15 min for each side) and 60 min (30 min for each side) were selected. Each treatment was repeated in triplicate. For each replication, samples of 30 pieces, obtained from 5 different melons, were randomly divided in two sub-samples of 15 pieces each, one used for plasma treatment and one used as control in order to minimize differences due to natural variability.

Control samples were stored at the same temperature and RH conditions for the duration of each treatment. Gas plasma treated samples and control ones were packed in propylene trays, sealed with a micro perforated polypropylene film in order to maintain atmospheric air composition avoiding dehydration, and stored for 4 days at 10°C and 90% RH. During storage, three packages for each sample were selected after 0, 1, 2 and 4 days for analytical determinations. Microbiological analyses were performed after 0, 1, 2, 3 and 4 days of storage.

2.4. Qualitative determinations

2.4.1. Chemical parameters

Dry matter content was determined gravimetrically by difference in weight before and after drying at 70°C, until a constant weight was achieved (AOAC International, 2002).

Soluble solids content (SSC) was determined at 20°C by measuring the refractive index of melon juice with a digital refractometer mod. PR1 (Atago Co. Ltd, Tokyo, Japan), calibrated with distilled water.

Titratable acidity (TA) was determined by titration with NaOH 0.1 N until pH 8.1 was reached (AOAC Official Method 942.15, 2000).

For each sample, SSC and TA were determined in triplicate on the juice obtained by crushing 10 melon pieces, after filtering through Whatman #1 filter paper.

2.4.2. Texture

Penetration tests were performed using a Texture Analyser mod. TA-HDi500 (Stable Micro Systems, Surrey, UK) equipped with a 50 N load cell and a 6 mm diameter stainless steel cylinder. Compression test speed was 0.5 mm s^{-1}, the test ended when a maximum deformation of 90% was reached. Tests were performed on 10 melon pieces for each sample.

Firmness F (N), as the first peak force value representing the limit of the flesh elasticity, and the gradient G between 0 and F were considered.

2.4.3. Colour

A spectrophotocolorimeter (Colorflex, Hunterlab) was used to measure surface colour of melon pieces (D_{65} illuminant and 10° standard observer). For each piece, measurements were performed on each side. The L*, a* and b* parameters of the CIELAB scale were measured, Hue angle (h° = arctan[$^{b*}/_{a*}$]) and chroma (Chroma= $\sqrt{a^{*2}+b^{*2}}$) values were also calculated (CIE, 1987). Results were expressed as average of 10 measurements for sample.

2.5. Metabolic determinations

2.5.1. Peroxidase (POD) and Pectinmetilesterase (PME) activities

POD activity was assayed using slight modifications of the spectrophotometric method of Morales-Blancas, Chandia, & Cisneros-Zevallos (2002). 25 g of sample were homogenized with a hand blender with 50 mL of cold (0-2°C) potassium phosphate buffer 0.1 M (pH 6.5) for 2 min. The

homogenized solution was filtered through filter paper and centrifuged for 30 min at 4 °C and 10000 × *g*. The supernatant (enzymatic extract) was collected.

POD substrate solution was prepared by mixing 0.1 mL of 99.5% guaiacol, 0.1 mL of 30% hydrogen peroxide, 99.8 mL of 0.1 M potassium phosphate buffer (pH 6.5). POD activity was tested by adding 150 μL of enzyme extract to 3.35 mL of substrate solution in 10-mm – pathlength glass cuvettes. The solution was mixed with a vortex for 3 s and by overturning the cuvette for 3 times. POD activity was measured at 25 °C by monitoring the increase in absorbance at 470 nm.

PME activity was assayed using slight modifications of the spectrophotometric method described by Hagerman & Austin (1986). 50 g of sample were homogenised with a hand blender with 50 mL of cold (0-2°C) NaCl 8.8% (w/v) for 2 min. The homogenized solution was stirred for 15 min, filtered through filter paper, and centrifuged for 30 min at 4 °C and 10000 × *g*. The pH of each enzymatic extract was adjusted to pH 7.5 by adding a few drops of 0.1 NaOH.

PME substrate was prepared by mixing 0.5 g of pectin from citrus peel in 100 mL of distilled water. The pH of the substrate was adjusted up to pH 7.5 with NaOH.

PME activity was assayed by adding 2000 μL of substrate, 100 μL of bromothymol blue solution, 740 μL of 3-mM potassium phosphate buffer (pH 7.5), 160 μL of enzyme extract, directly in 10-mm pathlength glass cuvettes. The solution was mixed with a vortex for 3 s and by overturning the cuvette for 3 times.

PME activity was measured at 25 °C by monitoring for 5 min the decrease in absorbance at 620 nm. Reaction rates were calculated from the slope ($\Delta A\ min^{-1}$) of the linear portion of the plot absorbance compared with time. Blank was prepared with water.

Residual enzymatic activity (%) was expressed as ratio of treated sample versus its control, according to Pizzocaro, Torreggiani, & Gilardi (1993), and measured just after gas-plasma treatment on three independent extracts.

2.5.3. Respiration rate

Respiration rate was evaluated using a static method as previously described (Tappi et al., 2014). Four cylinders (4 × 10 mm) were sampled from each melon piece, half were subjected to plasma treatments and half were used as control. Samples were then placed in sealed 20 ml glass ampoules and stored at 10°C for 24 h. O_2 and CO_2 percentage was measured in the ampoule headspace by a gas analyzer "check point O_2/CO_2" mod. MFA III S/L (Witt-Gasetechnik, Witten, Germany) after 1, 3, 5, 22 and 24 h. Respiration rate was calculated as mg of consumed O_2 (RRO_2) or produced CO_2 ($RRCO_2$) h^{-1} kg fw^{-1} according to the following equations:

$$RRO_2 = \frac{mm_{O_2} \cdot V_{head} \cdot \frac{(20.8 - \%O_{2,head})}{100} \cdot 101.325}{t \cdot m \cdot R \cdot 283}$$

$$RRCO_2 = \frac{mm_{CO_2} \cdot V_{head} \cdot \frac{\%CO_{2,head}}{100} \cdot 101.325}{t \cdot m \cdot R \cdot 283}$$

where mm_{O2} and mm_{CO2} refer to gases molar mass (g mol^{-1}), V_{head} represents the ampoule headspace volume (dm^3), % $O_{2,head}$ and % $CO_{2,head}$ refer to gases percentages in the ampoule headspace at time t (h); m is the sample mass (kg); R is the gas constant (8.314472 dm^3 kPa K^{-1} mol^{-1}). For each sample, the average of three replicates was considered.

2.5.4. Metabolic heat by isothermal calorimetry

Isothermal calorimetry allows the evaluation of the metabolic response to stress in fresh cut tissue through the determination of metabolic heat production as reported by Rocculi et al. (2012) and Tappi et al. (2014).

Four cylinders were sampled from each melon piece using a core borer and subjected to the different plasma treatments, then placed in 20 ml glass ampoule (about 2.5 g). A TAM-Air isothermal calorimeter (TA Instrument, New Castle, USA) previously described by Wadsö & Gómez Galindo (2009) was used to measure the rate of metabolic heat production.

Isothermal measurements were performed at 10°C for 24 h. For each sample, the average of four replicates was considered. Before and after the measurements, baseline was recorded for 30 min.

2.6. Microbiological analyses

Samples of untreated and plasma-treated melon (10 g) were suspended into sterile 0.1% (w/v) buffered peptone-water solution and homogenized with a Stomacher Lab Blender (Seward, PBI International, Whitstable, Kent, UK) for 2 min at room temperature.

Mesophilic lactobacilli and lactococci were determined on MRS agar (Oxoid Ltd, Basingstoke, Hampshire, UK) containing 0.1% of cycloheximide (Sigma Cemical Co.) and M17 agar (Oxoid Ltd), respectively at 30 °C for 48-72 h under anaerobic conditions. Yeasts were enumerated on Yeast Extract Peptone-Dextrose agar (YPD, Oxoid Ltd), added of 150 ppm chloramphenicol, at 30 °C for 72 h. Viable counts of total aerobic mesophilic and psychrotrophic bacteria were determined on Plate Count Agar (Oxoid Ltd) at 30 °C for 48 h and 4°C for 10 days, respectively.

Microbial analyses were carried out for melon samples exposed to three independent plasma treatments for each process time, which were analysed in duplicate (i.e. total six analyses for each process time).

2.7. Data Analysis

Significant differences in qualitative and metabolic parameters and in microbial loads, at the same sampling time, were assessed using the t-test, and significance of differences was defined at $p \leq 0.05$. Moreover, microbiological data of mesophilic and psychrotrophic bacteria collected over storage were modelled using the Gompertz equation as modified by Zwietering, Jongenburger, Rombouts, & Van't Riet (1990) in order to obtain the microbial growth parameters, *i.e*. maximum growth rate (μmax), lag phase length (λ) and maximum cell increase attained at the stationary phase (A). The growth parameters derived by the Gompertz equation in relation to plasma treatment times were then used to estimate the product shelf-life, which was calculated as the time necessary to attain a threshold level of 6 Log CFU g^{-1} as a critical cell load for the spoilage-associated microflora.

3. Results and discussion

3.1. Qualitative assessment of fresh-cut melon

As a consequence of minimal processing operations, wounding response promotes an increase in the vegetable products maturation processes due to higher respiration and conversion rate of starch (Beirão-da-Costa, Steiner, Correia, Empis, & Moldão-Martins, 2006).

As reported in Table 1, soluble solid content (SSC), titratable acidity (TA) and dry matter (DM) showed few significant differences among control and plasma treated melon samples. After 2 and 4 days of storage, 30+30 plasma treated samples showed higher DM and lower SSC contents than control ones. When significantly different, TA was lower in treated samples (after 0 and 2 days for 15+15 treatment and after 2 days for 30+30 treatment).

Generally, results seem to point out a higher water loss during storage, which was more evident when the longest plasma treatment was used. Similar results were obtained in previous experiments on fresh-cut kiwifruit (Ramazzina et al., 2015) and various fresh-cut fruit and vegetable (Wang et al., 2012); this behaviour was attributed to the moderate effect of the fan during the treatment.

Various authors (Aguayo, Escalona, & Artés, 2004; Fundo et al., 2014) have reported softening during storage of fresh-cut melon as a consequence of the degradation of the internal structures due to tissue ripening, and the solubilisation of the protopectinic fraction of the cell wall components and to the loss of cell adhesion (Varoquaux, Lecendre, Varoquaux, & Souty, 1990).

In this case, treated samples did not exhibit significantly different textural parameter values (Table 2) compared to control ones, with the exception of samples treated for 15+15 min that showed lower values for both firmness and gradient just after the treatment, and samples treated for 30+30 minutes that were characterised by lower values of gradient compared to the control ones after 2 and 4 days of storage.

Tappi et al. (2014) found a slight decrease in the crunchiness of fresh-cut apples subjected to plasma generated by the same DBD generator that was attributed to the destruction of the superficial layer of cells, but no significant differences in textural characteristics of kiwifruit slices were observed (Ramazzina et al., 2015). Other authors (Schnabel, Niquet, Schlüter, Gniffke, & Ehlbeck, 2014) did not detect any difference in apple flesh using a different device for plasma generation.

The colour changes occurring during storage in fresh cut melon can be attributed to the variation of pigments concentration and to induction of a translucent water-soaked tissue (glossy) caused by the loss of cellular compartmentation and water redistribution within the tissues (often tissue softening) since melon is not affected by enzymatic browning (Agar, Massantini, Hess-Pierce, & Kader, 1999). The development of translucency has been found to be the principal visual change of deterioration in fresh-cut melon stored under MAP (Aguayo et al., 2004; Bai, Saftner, Watada, & Lee, 2001; Oms-Oliu, Raybaudi-Massilia Martínez, Soliva-Fortuny, & Martín-Belloso, 2008).

Table 3 shows mean values and standard deviations of colorimetric parameters obtained for plasma treated samples and related controls. During storage, although significant differences were found only after 2 and 4 days between the 15+15 min treated and control samples, it can be trendily observed a reduction of all the colour parameters in the plasma treated samples. At the end of storage, treated melon samples appeared darker and more translucent compared to control ones.

The increased translucency may be due to a variation on the structure of the tissue that however did not affect textural parameters.

3.2 Metabolic evaluation

POD is an ubiquitous enzyme in vegetable cells and it can promote several reactions that adversely influence product quality, such as lipid and phenolic oxidations with consequent deterioration of

flavour, colour and nutritional quality (Morales-Blancas et al., 2002). It is characterized by an high stability to thermal (Anthon, Sekine, Watanabe, & Barrett, 2002; Lemmens et al., 2009) and pressure treatments, and by a substantial number of different isoenzymes.

As shown in Fig. 2a, POD activity underwent a significant reduction in plasma treated melon samples proportional to the treatment time, as the residual activity was found 91% (15+15) and 82% (30+30) compared to the control sample.

PME is a cell wall bound enzyme which is able to de-esterify pectins producing methanol and pectins with a lower degree of esterification (Alandes, Pérez-Munuera, Llorca, Quiles, & Hernando, 2009), that are further degraded by other pectolitic enzymes, causing tissue softening.

PME activity (Fig. 2b) was not affected by the 15+15 min treatment but after the 30+30 min the residual activity was found to be 94%.

The obtained reduction in the enzymatic activity is lower compared to the results obtained in our previous study on polyphenoloxidase (PPO) in Pink Lady apples (up to 54% after 15+15 min treatment) (Tappi et al., 2014). It can hence be assumed that different enzymes present a different resistance to denaturation by plasma agents, possibly due to their different structure and to the presence of isoenzymes. The type of fruit tissue, the specific microstructure, and porosity (e.g. 13.3 ± 0.6 % for melon and 27.3 ± 1.1 % for apple, as found by Muujica-Paz, Valdez-Fragoso, Loopez-Malo, Palou, & Welti-Chanes, 2003) can also play a role in the different treatment response.

Furthermore, the reduction observed in the enzymatic activity did not seem to have any relationship with colour and textural results.

In Table 4 data on respiration rate of fresh-cut plasma treated and control melon samples measured during 24 h at 10°C are reported. In terms of percentages, at the end of the experiment, the oxygen level within the ampoules was in the range of 14.8 and 17.3 % in the control and in the range of 16.1 and 17.8 % in the treated samples. Moreover, for either control or treated samples, CO_2 level never exceeded 5% that, according to Iversen, Wilhelmsen, & Criddle (1989) is the threshold for triggering anaerobic metabolism in vegetable tissues. Hence, it is possible to assume that the ratio between product and headspace amount was appropriate to maintain aerobic metabolism during the 24 h considered. However, the treatment effect has changed the normal respiration pathway of the product in aerobic conditions.

Actually, plasma treatment seemed to promote an increase in CO_2 production if compared to control sample, that was statistically significant after 22 h in the 15 + 15 sample and after 1, 3, 5 and 24 h in the 30 + 30 one. O_2 consumption, when significantly different (after 3 h in the 15 + 15 treated sample and after 5 h in the 30 + 30 treated sample), was lower in the treated samples than in the control ones. Generally, it seems that the plasma treatment has caused an higher CO_2 production

and a lower O_2 consumption, as can happen as a consequence of a partial conversion of the tissue respiratory metabolism from aerobic to anaerobic. These results confirmed what we found in a previous study on apple tissue subjected to the same kind of treatment (Tappi et al., 2014), hence contributing to highlight the complexity of the tissue response to plasma treatments.

Specific thermal power profiles of melon tissue cylinders during 24 h of analysis at 10°C are reported in Fig. 3.

For limitations of the instrument sample holders (22 mL vials), it was not possible to evaluate metabolic heat production on samples of the same size and surface-volume ratio as the one used for qualitative evaluation. Hence, considering that plasma treatment is considered to be mainly a surface treatment, the response of the tissue in larger pieces could be different. Nevertheless, the results can give useful information about the comparison of the effect of different treatment times on the melon tissue metabolism, particularly in terms of metabolic activity of regular cylindrical samples.

The heat production of the treated samples was lower compared to the controls for all the 24 h of analysis, proportionally to the treatment time. Metabolic profiles obtained by isothermal calorimetry have been integrated in order to calculate the total metabolic heat produced by fruit tissues during the first 12 h and during 24 h at 10°C. Differences among samples were more pronounced after 24 h of analysis than after 12 h, as it can be observed in Fig. 4. The variation in the respiratory pathway observed by the respiration rate results could be the cause of the decrease of the heat production detected. Plasma treatment could also affect cell vitality by decreasing it, as it has been observed for different minimal processing operations such as blanching for fresh carrot (Gómez, Toledo, Wadsö, Gekas, & Sjöholm, 2004) and osmo-dehydration for kiwifruit (Panarese, Tylewicz, Santagapita, Rocculi, & Dalla Rosa, 2012).

3.3 Microbiological evaluation

In order to evaluate the effects of gas plasma treatments on the microbial traits of melon samples, cell viability immediately after treatments and over refrigerated storage was measured for the spoilage-related microflora, i.e. total mesophilic and psychrotrophic bacteria, lactococci, lactobacilli and yeasts (Table 5 and Fig. 5).

Initial populations of total aerobic mesophilic and psychrotrophic microorganisms were about 3.4 and 2.5 Log CFU g^{-1} respectively, while lactic acid bacteria ranged between 2 and 3 Log CFU g^{-1}. On the other hand yeasts were roughly above the detection limit. Such values are in agreement with results reported by other authors for fresh-cut “Piel de Sapo” (Fernández, Picouet, & Lloret, 2010)

and cantaloupe (Fang, Liu, & Huang, 2013) melons, except for yeasts that were found at lower levels than literature data.

Overall, gas plasma treatments resulted in marked immediate reductions in cell viability of the indigenous bacteria by increasing the treatment time. The highest inactivation levels were observed for the mesophilic and lactic acid bacteria whose cell loads were under the detection limit in the 30+30 min treated samples which correspond to 3.4 and 2 log reductions, respectively. On the other hand cell load reductions not exceeding 1 Log CFU g^{-1} were recorded for the psychrotrophs.

The microbial inactivation observed after plasma exposure has been attributed to the effect of the various reactive species generated during the discharge. As reported in previous studies (Ragni et al., 2010; Ramazzina et al., 2015), the discharge generated by the DBD device showed the presence of nitrogen (N2+, NO•) and oxygen (OH•) reactive species. The inactivation mechanism has been related to plasma membranes damage due to oxidation of membrane lipids, and leakage of the intracellular components. Also, the oxidation of amino-acids and nucleic acids can contribute to cell injury or death (Critzer, Kelly-Wintenberg, South, & Golden, 2007).

The fate of the surviving cells over the refrigerated storage was quite different among the various microbial groups also in relation to plasma processing time. Yeasts showed the worst growth ability as the maximum cell loads attained in the gas plasma treated samples did not exceed 3.5 Log CFU g^{-1} regardless the treatment time, which was significantly ($p<0.05$) lower compared to the control fruit (7.5 Log CFU g^{-1}; Fig. 5A). Lactobacilli remained under the detection limit up to 2 days in the plasma treated samples, while they displayed a cell growth over 6 log units in untreated samples after 3 days of storage (Fig. 5B). Nevertheless final counts of 5.4 and 7 Log CFU g^{-1} were attained in 15+15 min and 30+30 min treated samples, respectively. Similar final cell loads were achieved also by lactococci although they showed slower growth dynamics compared to lactobacilli (Fig. 5C). Total mesophilic and psychrotrophic bacteria presented the highest growth ability reaching levels of 7-7.8, 5.4-5.9 and 7.3-7.6 Log CFU g^{-1} in the control, in the 15+15 min and 30+30 min samples, respectively. To better evaluate their recovery dynamics over storage and the effect on the products shelf-life, their cell count data were modelled with the Gompertz equation as modified by Zwietering et al. (1990). Table 5 reports the Gompertz parameters for mesophilic and psycotrophic bacteria in relation to gas plasma treatments as well as the time necessary to reach the value of 6.0 Log CFU g^{-1}, which was chosen as a spoilage threshold according to literature data (Patrignani, Vannini, Kamdem, Lanciotti, & Guerzoni, 2010). As expected, *K* values, corresponding to the initial levels of bacteria surviving the gas plasma processes, decreased by increasing the plasma exposure time. Also λ values were negatively affected by plasma treatments being significantly higher than in the control samples in the case of psychrotrophic bacteria. These data clearly indicate

that plasma treatments resulted in an inactivation and/or severe damages which however were repaired by microbial cells during storage. On the contrary, an opposite effect was detected for *μmax* values. In fact, significantly higher growth rates were found for treated melon samples compared to the control ones, and particularly for those corresponding to the longest process time.

Compared to the rinsing with traditional or emerging sanitizers (Silveira, Aguayo, & Artés, 2010), both the plasma treatments provided higher reductions of the initial contaminating microflora. In addition, the processing conditions used in this study negatively affected the fate of lactic acid bacteria and yeasts which are usually reported to be the main spoilage species for fruits including fresh-cut melon (Zhang et al., 2013). Likewise the growth of the mesophilic and psychrotrophic bacteria was markedly limited following the 15+15 min treatment thus leading to a significant increase in the melon samples shelf-life. However, unexpectedly the increase in processing time, although it allowed an immediate higher microbial inactivation, did not offer any additional advantage in terms of microbial shelf-life, despite no appreciable differences were observed for the qualitative indices. Current literature on cold plasma treatments on several raw fruit and vegetables reports that the inactivation level is generally time-dependent although non-linear inactivation curves are reported (Baier et al., 2014; Lee, Kim, Chung, & Min, 2015), but the evaluation is often limited to the immediate effect after the exposition and ignores the behaviour of the surviving microbial cells during further storage.

The overall effect observed in this study after the longest plasma treatment on microbial shelf-life was negligible if compared to that of untreated samples and comparable shelf-life values were obtained. Conversely, the 15+15 min exposure to gas plasma gave rise to a significant extension of the microbial shelf-life. In fact, the critical spoilage threshold was reached beyond four days in the treated samples, while the untreated melons were spoiled after 2.5-3 days.

This phenomenon could be related to tissue damages (e.g. cell wall weakening) caused by prolonged treatments resulting in the higher water loss recorded for the 30 + 30 min treated melons which probably made fruit more susceptible to microbial spoilage.

These results highlight the necessity of modulating treatment time not only by its immediate effects but also on the further shelf-life evaluation.

5. Concluding remarks and future perspectives

Among fresh-cut products, melon is considered highly perishable and potentially hazardous foods, as demonstrated by the number of foodborne diseases outbreaks registered in the recent years in developed countries.

The overall results obtained in this study indicate that the tested cold plasma treatment is very promising in order to stabilize fresh-cut melon, allowing efficient decontamination. Moreover, by modulating the treatment time a significant enhancement of microbial shelf-life was achieved due to a delayed growth over storage of the surviving spoilage microflora.

As far as quality characteristics are concerned, the treatment effect was very limited and mainly related to a slight increase of both dry matter content and translucent appearance during storage.

In addition, a slight reduction of enzymatic activity was observed but this effect was dependent on the type of enzyme considered, and did not seem to have any positive reflection on related qualitative parameters. A reduction of metabolic heat was obtained along with an alteration of the respiratory pathway, indicating a stress response of the tissue to the treatment that should be further clarified.

The potential application on in-packed cold plasma technology showed by Pankaj et al., (2014) makes this technique very encouraging for fresh-cut commodities stabilization, aiming to replace traditional chemical sanitizers such as chlorine and hydrogen peroxide.

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Figure 1. Schematic representation of the electrodes configuration.

Figure 2. (a) Peroxidase (POD) and (b) Pectinmethylesterase (PME) residual activity of melon samples treated with plasma for 15+15 and 30+30 min. * indicates samples that were significantly different from control sample at a p-level<0.05.

Fig 3. Specific thermal power profiles of melon tissue cylinders in relation to gas plasma treatments during 24 h of analysis at 10°C.

Fig 4 Normalized heat produced by melon samples during 12 and 24 h of analysis at 10°C. Different letters indicates samples that were significantly different at p-level<0.05.

Figure 5. Cell numbers of yeasts (A), lactobacilli (B) and lactococci (C) during storage at 10°C of melon samples.

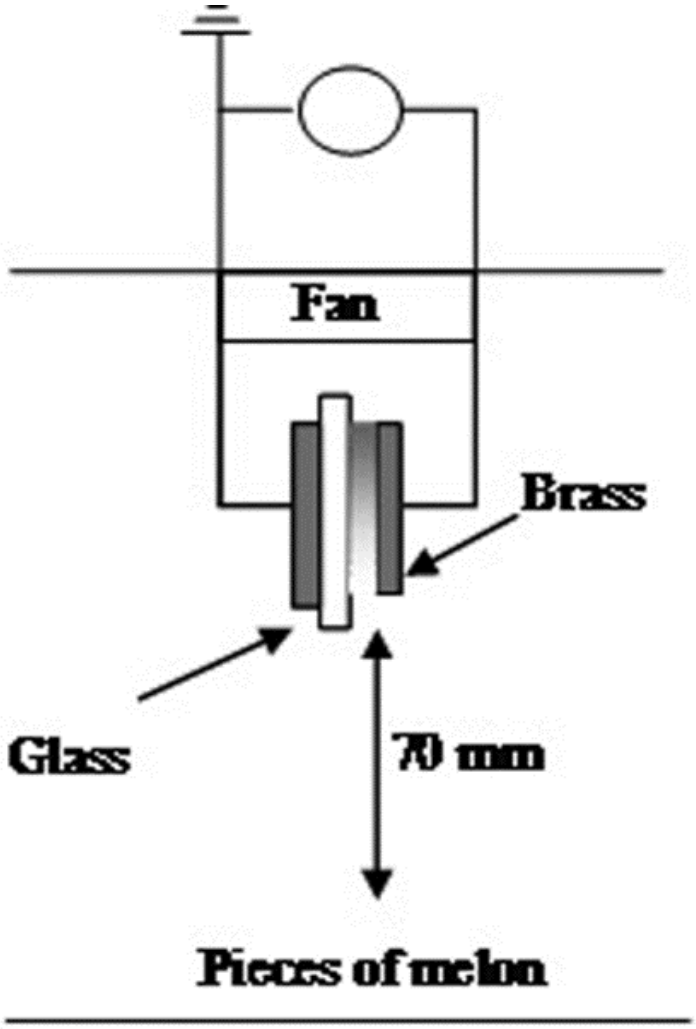

Figure 1

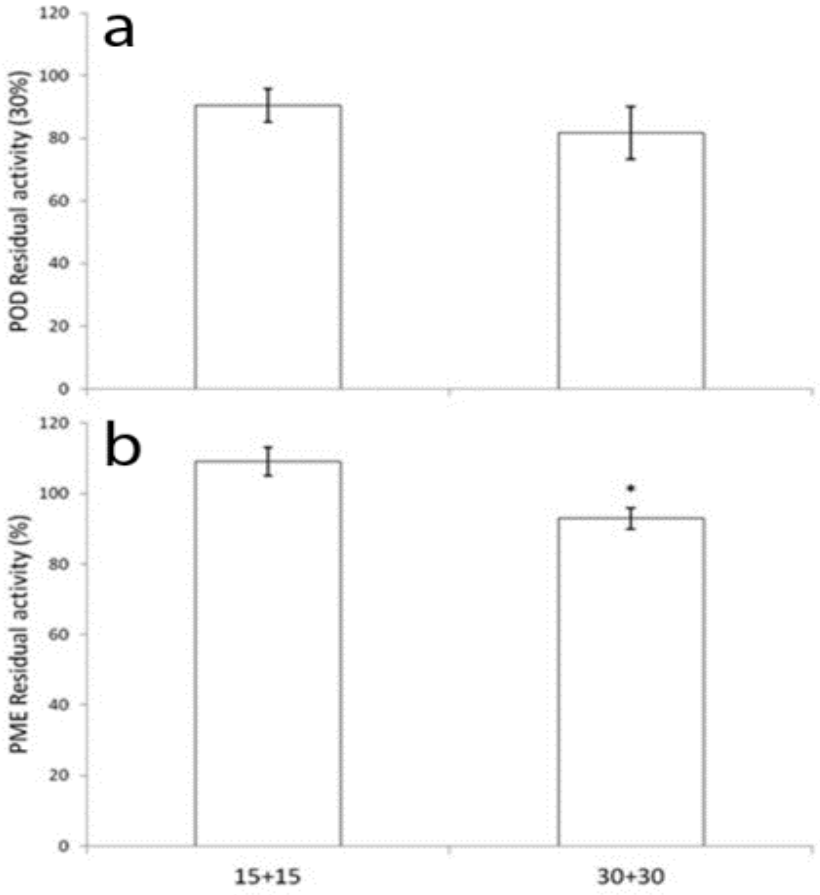

Figure 2

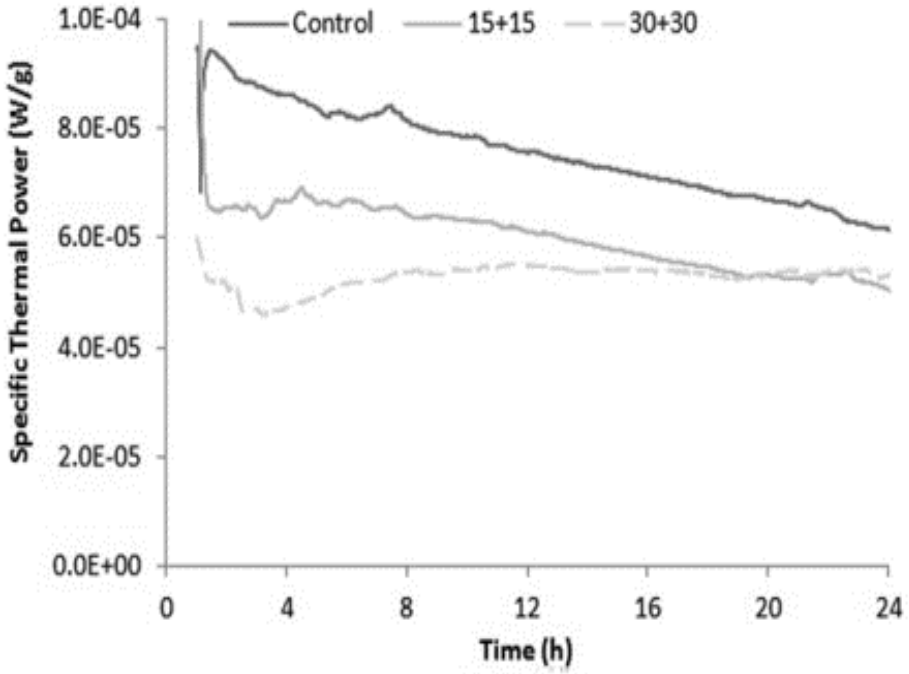

Figure 3

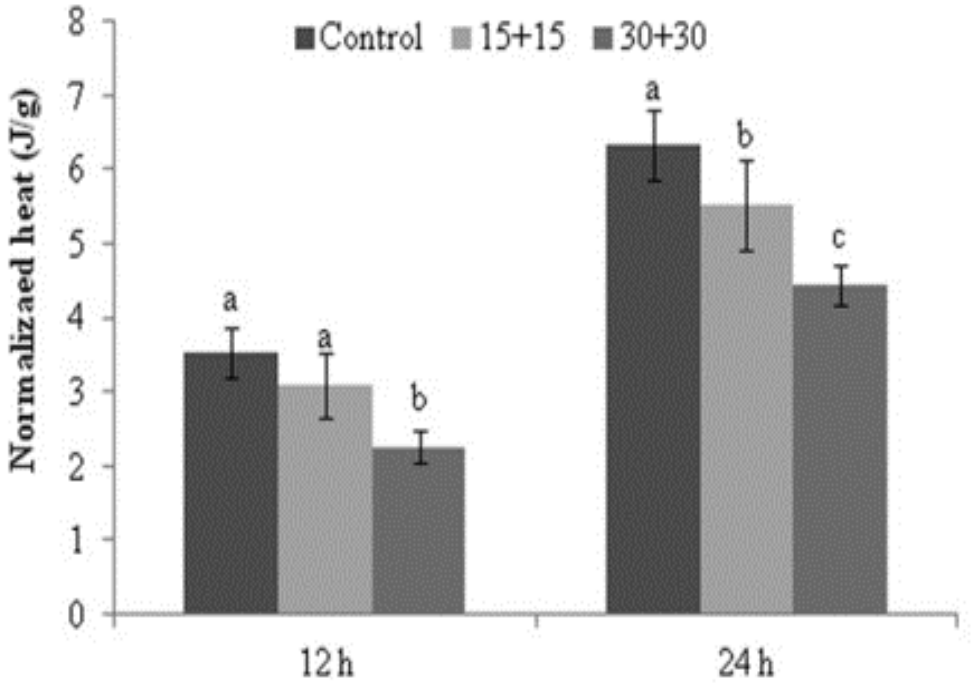

Figure 4

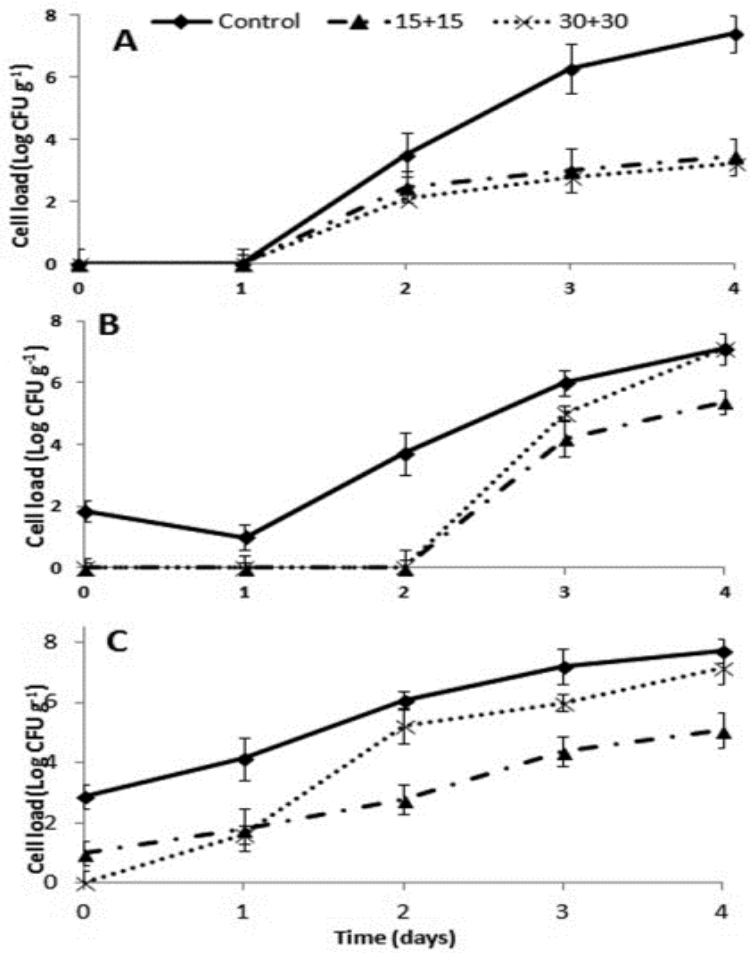

Figure 5

Table 1. Changes of soluble solid content (SSC), titratable acidity (TA) and dry matter (DM) in control (C) and plasma treated (T) melon samples during storage

Treatment	Storage (days)	SSC (%)		TA (mg malic acid 100 g fw^{-1})		DM (%)	
		C	T	C	T	C	T
15+15 min	0	1.41 ± 0.01[a]	1.41± 0.03[a]	0.0081 ± 0.0002[a]	0.0062 ± 0.0004[b]	9.8 ± 0.1[a]	8.9 ± 0.3[a]
	1	1.44 ± 0.05[a]	1.45± 0.18[a]	0.0058 ± 0.0007[a]	0.0058 ± 0.0002[a]	9.8 ± 0.4[a]	9.1 ± 0.2[a]
	2	1.51 ± 0.02[a]	1.48± 0.04[a]	0.0075 ± 0.0007[a]	0.0050 ± 0.0003[b]	8.8 ± 0.2[a]	9.3 ± 0.3[b]
	4	1.45 ± 0.07[a]	1.40± 0.06[a]	0.0102 ± 0.0002[a]	0.0093 ± 0.0003[a]	9.0 ± 0.5[a]	8.9 ± 0.3[a]
30+30 min	0	1.50 ± 0.07[a]	1.64± 0.08[a]	0.0080 ± 0.0003[a]	0.0081 ± 0.0003[a]	9.1 ± 0.6[a]	8.9 ± 0.6[a]
	1	1.41 ± 0.06[a]	1.47± 0.07[a]	0.0059 ± 0.0002[a]	0.0055 ± 0.0003[a]	9.2 ± 0.4[a]	9.1 ± 0.3[a]
	2	1.54 ± 0.03[a]	1.35± 0.02[b]	0.0099 ± 0.0004[a]	0.0065 ± 0.0002[b]	8.4 ± 0.2[a]	10.2 ± 0.3[b]
	4	1.46 ± 0.03[a]	1.36± 0.02[b]	0.0109 ± 0.0001[a]	0.0110 ± 0.0002[a]	8.5 ± 0.3[a]	9.2 ± 0.2[b]

Different letters indicate significant differences between control (C) and plasma treated sample (T) for each storage time ($p \leq 0.05$).

Table 2. Textural parameters of control (C) and plasma treated (T) melon samples during storage

Treatment	Storage (days)	Firmness (N)		Gradient ($N \cdot s^{-1}$)	
		C	T	C	T
15+15 min	0	13.70 ± 4.47[a]	9.28 ± 1.63[b]	3.88 ± 0.82[a]	3.16 ± 0.87[b]
	1	9.84 ± 2.31[a]	8.30 ± 2.33[a]	2.17 ± 0.64[a]	1.86 ± 0.43[a]
	2	9.64 ± 2.01[a]	9.91 ± 1.25[a]	2.32 ± 0.40[a]	2.53 ± 0.49[a]
	4	9.31 ± 2.80[a]	8.23 ± 2.49[a]	2.34 ± 0.72[a]	2.08 ± 0.51[a]
30+30 min	0	8.42 ±1.84[a]	9.68 ±2.61[a]	2.51 ± 0.54[a]	2.60 ± 0.53[a]
	1	9.31 ± 1.47[a]	9.44 ± 3.27[a]	2.05 ± 0.32[a]	2.00 ± 0.59[a]
	2	10.18 ±2.34[a]	9.33 ± 2.94[a]	2.63 ± 0.51[a]	2.15 ± 0.42[b]
	4	9.96 ± 3.10[a]	9.62 ± 2.95[a]	2.30 ± 0.64[a]	1.82 ± 0.50[b]

Different letters indicate significant differences between control (C) and plasma treated sample (T) for each storage time ($p \leq 0.05$).

Table 3. Colorimetric parameters of control (C) and plasma treated (T) melon samples during storage

Treatment	Storage (days)	L*		a*		b*		Hue angle		Chroma	
		C	T	C	T	C	T	C	T	C	T
15+15 min	0	58.7 ± 3.3^{a}	57.4 ± 2.4^{a}	18.0 ± 1.8^{a}	17.0 ± 1.0^{a}	37.3 ± 2.8^{a}	36.9 ± 2.0^{a}	244.3 ± 1.3^{a}	245.2 ± 0.9^{b}	41.4 ± 3.2^{a}	40.6 ± 2.2^{a}
	1	57.4 ± 5.6^{a}	57.0 ± 5.0^{a}	17.4 ± 2.2^{a}	16.7 ± 2.3^{a}	36.1 ± 3.4^{a}	34.2 ± 2.7^{a}	244.2 ± 1.4^{a}	244.0 ± 1.8^{a}	40.1 ± 3.9^{a}	38.1 ± 3.3^{a}
	2	58.6 ± 4.1^{a}	58.2 ±5.5^{a}	18.1 ± 2.5^{a}	16.1 ± 1.5^{b}	38.5 ± 3.5^{a}	36.1 ± 3.1^{a}	244.8 ± 1.3^{a}	245.9 ± 0.7^{b}	42.5 ± 4.2^{a}	39.5 ± 3.4^{b}
	4	57.9 ± 4.7^{a}	54.4 ± 3.5^{b}	20.8 ± 1.7^{a}	17.5 ± 2.7^{b}	46.6 ± 3.1^{a}	34.0 ± 3.7^{b}	245.9 ± 1.1^{a}	242.7 ± 0.8^{b}	51.0 ± 3.4^{a}	38.2 ± 4.5^{b}
30+30 min	0	59.4 ± 2.6^{a}	59.5 ± 2.9^{a}	17.2 ± 1.1^{a}	18.0 ± 1.8^{a}	37.2 ± 1.8^{a}	37.7 ± 2.7^{a}	245.1 ± 0.9^{a}	244.4 ± 1.0^{a}	41.0 ± 2.1^{a}	41.8 ± 3.1^{a}
	1	56.6 ± 3.4^{a}	57.3 ± 3.9^{a}	17.6 ± 1.8^{a}	17.0 ± 2.1^{a}	37.6 ± 3.2^{a}	35.8 ± 3.3^{a}	244.9 ± 0.7^{a}	244.6 ± 1.1^{a}	41.5 ± 3.7^{a}	39.6 ± 3.8^{a}
	2	57.7 ± 4.6^{a}	55.6 ± 3.1^{a}	17.5 ± 1.2^{a}	17.0 ± 2.3^{a}	38.2 ± 2.4^{a}	36.6 ± 3.7^{a}	245.3 ± 0.6^{a}	245.2 ± 1.1^{a}	42.0 ± 2.6^{a}	40.4 ± 4.3^{a}
	4	55.6 ± 6.6^{a}	52.8 ± 8.0^{a}	18.6 ± 2.9^{a}	17.2 ± 3.3^{a}	35.8 ± 5.0^{a}	33.6 ± 5.3^{a}	242.6 ± 1.0^{a}	242.9 ± 1.1^{a}	40.4 ± 5.7^{a}	37.8 ± 6.2^{a}

Different letters indicate significant differences between control (C) and plasma treated sample (T) for each storage time ($p \leq 0.05$).

Table 4. Respiration rate expressed as oxygen consumed and carbon dioxide produced during 24 hours of storage at 10°C.

Treatment	Storage time	$RRCO_2$(mg h^{-1} kg fw^{-1})		RRO_2 (mg h^{-1} kg fw^{-1})	
		C	T	C	T
15+15 min.	1	59.5 ± 11.19[a]	49.1 ± 6.91[a]	59.7 ± 0.21[a]	48.5 ± 7.44[a]
	3	38.2 ± 12.99[a]	38.3 ± 1.23[a]	34.5 ± 2.22[a]	23.6 ± 1.59[b]
	5	35.4 ± 3.659[a]	39.2 ± 4.58[a]	32.0 ± 0.54[a]	27.4 ± 4.44[a]
	22	25.5 ± 0.85[a]	31.9 ± 2.10[b]	22.1 ± 0.69[a]	18.6 ± 0.24[b]
	24	25.3 ± 4.37[a]	29.4 ± 0.19[a]	21.2 ± 1.82[a]	19.2 ± 1.42[a]
30+30 min.	1	41.0 ± 15.51[a]	66.8 ± 6.43[b]	27.8 ± 4.93[a]	35.5 ± 2.03[a]
	3	29.5 ± 3.54[a]	57.2 ± 2.12[b]	25.9 ± 1.75[a]	26.4 ± 1.16[a]
	5	30.6 ± 2.68[a]	44.1 ± 4.72[b]	24.7 ± 1.77[a]	21.2 ± 0.29[b]
	22	19.5 ± 3.26[a]	32.5 ± 10.59[a]	16.9 ± 2.44[a]	18.4 ± 0.29[a]
	24	20.8 ± 2.20[a]	36.9 ± 5.52[b]	17.5 ± 1.61[a]	17.1 ± 0.29[a]

Different letters indicate significant differences between control (C) and plasma treated sample (T) for each storage time ($p \leq 0.05$).

Table 5 . Gompertz parameters of mesophilic and psychrophilic bacteria recovery dynamics in melon samples during storage at 10°C in relation to the gas plasma treatment.

Microbial group	Gas plasma treatment time (min)	K	A	μmax	λ	R	Shelf-life (days) **
Mesophilic	**Control**	3.36	4.44	1.24	0.14	0.973	2.3
bacteria	**15+15 min**	1.48	3.92	1.55	0.20	0.998	>4§
	30+30 min	n.d. *	7.61	2.39	0.03	0.991	2.9
Psycotrophic	**Control**	2.48	4.34	1.45	0.07	0.987	2.9
bacteria	**15+15 min**	2.08	3.82	2.59	1.83	0.985	>4§
	30+30 min	1.48	5.77	3.69	1.25	0.999	2.6

Data are the mean of three different samples. The variability coefficients ranged between 5% and 7%.
*n.d. Under the detection limit
K= initial cell level (Log CFU g^{-1}) after gas plasma treatments
A= maximum cell increase attained at the stationary phase with respect to the initial cell load (Log CFU g^{-1})
μmax= maximum growth rate (Δ Log [CFU g^{-1}] per day)
λ= lag phase length (days)
R = correlation coefficient
**Time necessary to attain a cell count of 6 Log CFU g^{-1}, calculated using the predicted Gompertz parameters.
§ Since the threshold level of 6 Log CFU g^{-1} was not attained within 4 days, shelf-life could not be predicted by using mathematical models.

Industrial relevance

The demand for fresh-cut products characterized by high qualitative and nutritional values and an acceptable shelf-life has promoted the research for non-thermal treatments.

Fresh-cut melon is considered to be highly perishable and a potentially hazardous food because it can support the growth of spoilage microflora and several pathogens,

Cold plasma has shown its potentiality as an antimicrobial treatment and has been tested on different food products, but the impact on product quality and metabolism is still scarcely known.

The results obtained in this study contributed to deepen the knowledge on the effect of plasma treatment on microbial, qualitative and metabolic aspects of fresh-cut melon.

Highlights

- Cold plasma treatment is very promising in order to stabilize fresh-cut melon, allowing efficient decontamination
- By modulating the treatment time a significant enhancement of microbial shelf-life was achieved
- Quality characteristics were only slightly affected by the treatment
- The effect on enzymatic activity was limited and dependent on the type of enzyme considered
- A reduction of metabolic heat was observed along with an alteration of the respiratory pathway indicating a stress response of the tissue