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Effect of cold plasma treatment on jackfruit puree: decontamination of *Aspergillus niger* spores and quality attributes

Paloma Patricia Casas-Junco¹, Josué Raymundo Solís-Pacheco²,
Juan Arturo Ragazzo-Sánchez¹, Blanca Rosa Aguilar-Uscanga²
and Montserrat Calderón-Santoyo^{1*}

¹Laboratorio Integral de Investigación en Alimentos, Tecnológico Nacional de México/Instituto Tecnológico de Tepic, Av. Tecnológico # 2595, Col. Lagos de Country, Tepic, 63175, Nayarit, México.

²Laboratorio de Microbiología Industrial, Centro Universitario de Ciencias Exactas e Ingeniería, Universidad de Guadalajara, Guadalajara, Jalisco 44430, México. E-mail: *mcalderon@tepic.tecnm.mx

ABSTRACT

Jackfruit puree was artificially contaminated with *Aspergillus niger* spores and then treated with cold plasma (30 W, 1.5 L/min Helium, time 0 to 16 min). After treatment, samples were stored at 25 °C for 60 days, and microbiological and physicochemical parameters were evaluated. A reduction of 4 log (CFU /g) in *Aspergillus niger* spores was achieved after 8 min treatment at 30 W and 850 V by Helium gas. *A. niger*, coliforms and mesophilic aerobic bacteria were completely inhibited up to 8 min of treatment and there was no growth during storage. Treated samples did not show changes in total soluble solids, color or a_w during storage. Minimal changes were found in pH and total acidity. There were no significant differences in aromatic profiles for treated and untreated samples. Also, the cold plasma treatment did not alter the sensory properties of jackfruit puree. Cold atmospheric plasma is a promising non-thermal technology to be used in processed fruit products.

Keywords: cold atmospheric plasma, jackfruit puree, *Aspergillus niger*, quality attributes.

Efecto de tratamiento con plasma frío en puré de jaca: descontaminación de esporas de *Aspergillus niger* y atributos de calidad

RESUMEN

El puré de jaca se contaminó artificialmente con esporas de *Aspergillus niger* para tratarlo con plasma frío (30 W, 1.5 L / min de Helio, en un lapso de 0 a 16 min, posteriormente las muestras se almacenaron a 25 °C durante 60 días en los que no hubo crecimiento, ni cambios en los sólidos solubles totales, color o a_w , sin diferencias significativas en los perfiles aromáticos y tampoco en las muestras no tratadas, en el pH y la acidez total los cambios fueron mínimos. Se logró una reducción de 4 log (UFC / g) en las esporas de *Aspergillus niger*, coliformes y bacterias mesófilas aerobias después de 8 min de tratamiento a 30 W y 850 V con gas Helio. No se observó alteración en las propiedades sensoriales del puré de jaca tratado. El plasma atmosférico frío es una tecnología no térmica prometedora para su uso en productos de frutas procesadas.

Palabras clave: plasma frío atmosférico, puré de jaca, *Aspergillus niger*, parámetros de calidad.

INTRODUCTION

Nayarit, Mexico is an important producer of tropical fruits, such as jackfruit (*Artocarpus heterophyllus* Lam.), mainly commercialized as fresh fruit. Its high moisture content allied to the typical weather characteristics in the country (high relative humidity $94.56 \pm 9.68\%$ and high temperature $28.12 \pm 4.06\text{ }^\circ\text{C}$) produce great postharvest losses. These conditions promote the attack of fruit by fungi that could produce mycotoxins, causing important sanitary risks (Ragazzo-Sánchez, Gutiérrez-Escatel, Luna-Solano, Gómez-Leyva & Calderón-Santoyo, 2011; SAGARPA, 2019).

Recent studies have demonstrated contamination of dried vegetable products by fungi genera like *Aspergillus* and *Penicillium*. Consequently, mycotoxins such as ochratoxin A or aflatoxins B1 and B2 have been detected in this kind of products (Hell, Gnonlonfin, Kodjogbe, Lamboni & Abdourhamane, 2009). In addition, Ragazzo *et al.* (2011) identified *Aspergillus niger* strain ATCC1688 as a postharvest pathogen in jackfruit from Nayarit, Mexico. In this sense, some saprophytic fungi of the genera *Aspergillus* and *Penicillium* were detected in fresh jackfruit, then, the presence of these phytopathogens in jackfruit processed products is a latent risk.

Currently, Cold Atmospheric Gas Plasma (CAP) is an emerging non-thermal food technology that has recently drawn considerable interest in food and food processing surfaces decontamination (Han *et al.*, 2016). Plasma is the fourth state of matter and is defined as an ionized quasi-neutral gas. Plasma can be generated by applying an electrical field to an initially electrical neutral gas (Hertwig, Meneses & Mathys, 2018). Plasma is a source of different antimicrobial substances, including UV photons, charged particles, and reactive species. Some major reactive species generated from Helium-based plasma in an air atmosphere are O, O₃, O*, He⁺, He₂⁺, N, N₂⁺, NO⁺, and N (Torres-Segundo *et al.*, 2019). The main factors considered in the application of cold plasma for lethal effect on the microorganisms are the type of microorganism, duration of treatment, number of cells, the gas flow and mixture, physiological state of the cells, active species produced by plasma, and type of food (Bourke, Ziuzina, Han, Cullen & Gilmore, 2017).

Several studies indicate that reactive oxygen species are the most important agents in the microbial inactivation by CAP (Hähnel, von Woedtke & Weltmann, 2010). The gas used for plasma generation determines the type of active species formed (Lu *et al.*, 2016). CAP can inactivate a range of microorganisms including Gram-positive and Gram-negative bacteria, bacterial endospores, fungi and viruses on different surfaces (Li *et al.*, 2019). On the other hand, active species are present only when the discharge is driven during the gas plasma treatments and disappear some milliseconds after the discharge has been

turned off. Such features assure that CAP is an almost harmless operation for operators, consumers and materials. Some authors reported that no toxic residues remain on pasteurized foods (Moisan *et al.*, 2001).

Then, the objective of this study was to evaluate the effect of coldplasma on microbiological, physicochemical, aromatic, and sensorial parameters of jackfruit (*A. heterophyllus* Lam.) puree.

MATERIALS AND METHODS

Sample preparation

Jackfruits (*A. heterophyllus* Lam.) were collected from El Llano, San Blas, Nayarit, Mexico (21° 22' 30" North latitude, 105° 07' 30" West longitude and 40 masl Altitude). Fruits were selected with yellow and orange pulp as an index of ripening state and apparently uninfected. These samples were disinfected by immersion in a sodium hypochlorite solution at 2% v/v for 1 min and then left to dry at room temperature in a biological safety cabinet (Ragazzo-Sánchez *et al.*, 2011). They were cut open and seeds extracted from the juicy sheath and fleshy pericarp. Jackfruit puree (90:10 jackfruit:water) was dehydrated in a conventional oven (60 °C/10 h) up to $a_w \approx 0.76$.

Microbial contamination and decimal reduction time (D-value) of *Aspergillus niger*

A. niger was previously isolated and identified from jackfruit (Ragazzo-Sánchez *et al.*, 2011). The pathogen was grown on Potato Dextrose Agar (PDA) at 25 °C for 5 days. The spores suspension was prepared with 10 mL of a sterile saline solution at 0.9% NaCl and 0.1% Tween 80. The suspension was filtered in sterile gauze; spores were collected in a sterile flask and counted with a Neubauer chamber. 5 µL of a spores' suspension conveniently diluted (sterile water) was inoculated on the center of 0.1 g of jackfruit puree and gently homogenized to a final concentration at 7.55×10^5 spores/g.

After plasma treatment, 0.1 g of jackfruit sample was diluted in 1 mL of 0.1% peptone water in Stomacher closure bags 6041/CLR (Seward, UK). Decimal dilutions were performed and plated on potato dextrose agar (PDA, Bioxon, Mexico) containing 2% ampicillin (Bayer, México) and 0.6% Bengal rose (Analytyka, Mexico). Plates were incubated at 30 °C for 48 h for molds enumeration as an indicative of spores survival. Enumeration of total microbiota was performed on TSA at 37 °C for 48 h. Results were reported as log CFU/g on a dry basis. Microbial analysis was performed immediately after plasma treatment for D-value calculation and during the storage at 25 °C and 60% of relative humidity for 0, 7, 15, 30, and 60 days.

The decimal reduction time (D-value, min) considered the time required to inactivate 90% of the microbial population in the logarithmic scale. This was calculated for the yeasts and molds population from the slope of the inactivation plot obtained after

plasma treatments applied at different times. The D-value was obtained using the following Equation (1) (Fernández, Shearer, Wilson & Thompson, 2012):

$$\text{Log}[N/N_0] = -t/D \quad (1)$$

Where N: microorganism population at any time, t; N₀: initial microorganism population; D: decimal reduction time.

Plasma treatment and storage conditions

The plasma generator was designed and built at the Laboratory of Plasma Physics of the National Nuclear Research Institute in Toluca, Mexico, and described by Solís-Pacheco *et al.* (2013).

Jackfruit puree samples were inoculated as described above. Samples without inoculation were treated in order to determine the inhibition of *A. niger* spores and natural microbiota, respectively. Samples (0.1 g) with approximately 1 mm of thickness were placed on a Petri plate and then treated with plasma energy at atmospheric pressure. Plasma was generated using commercial Helium gas (He, 99.999% purity Praxair, Mexico) at a constant flow of 1.5 L/min, applied at different time intervals (0, 1, 2, 4, 6, 8, 10, 12, and 16 min) and 30 Watts input power and output voltage of 850 Volts. Before and after each plasma treatment, the enumeration of molds and microbiota (log CFU/g) were determined. Independent samples were used for each time treatment. Experiments were conducted in triplicate, and the samples were in duplicate.

After plasma treatments, samples of 60 g of jackfruit were packaged in low-density polyethylene (LDPE - 7" x 5") bags stored at 25 ± 1 °C and 60% of relative humidity. Quality attributes and microbiological parameters were analyzed in puree in duplicate immediately after the process and after 7, 15, 30, and 60 days of storage. Samples for aroma compounds determination were stored for seven months at 25 °C and analyzed in duplicate.

Microbiological analysis

Jackfruit puree was evaluated in order to know the inherent microbiota. 0.1 g of puree was homogenized in 10 mL of peptone water (2%) for 30 s in a peristaltic blender (Stomacher-400, Seward, UK). The samples were diluted 1:100, 1:500, and 1:1000. Subsequently, 100 µL of each dilution were spread on the following culture mediums: nutrient agar (NA), trypticase soy agar (TSA), potato dextrose agar (PDA), MacConkey agar (MAC), Eosin methylene blue agar (EMB). The number of colony-forming units (CFU/g) was obtained after 24 h at 37 °C for bacteria and 72 h at 27 °C for fungi. Colonies of bacteria were identified by biochemical assays (Gram staining, catalase, oxidase-fermentative, and Analytical Profile Index, API20E). Fungi were pre-identified using traditional morphological methods. Macroscopic (mycelium type, color, and growth

type), as well as microscopic (mycelium type, conidiophores morphology, and spore morphology) characteristics, were considered for identification.

Physicochemical analyses

Physicochemical analyses were performed during the storage period.

pH analysis. 0.3 g sample of jackfruit puree was diluted 1:10 with distilled water and pH measured using a Mettler Toledo digital pH meter, Model HI 120 (Mettler-Toledo GmbH) with a glass electrode (AOAC, 2000).

Total Acidity. Acidity was measured by diluting 1.5 g of sample in 7.5 mL distilled water and titrated against 0.1 N NaOH to a pH 8.2 end-point using a Mettler Toledo digital pH meter, Model HI 120 (Mettler-Toledo GmbH). Total acidity was expressed in g citric acid per 100 mL (AOAC, 2000).

Surface colour. Colour assessment was evaluated with a Hunter Lab MINOLTA Chroma Meter CR300 colorimeter (Hunter Laboratory) in the reflectance mode for jackfruit puree. Colour was expressed as L (brightness), a (redness) and b (yellowness) values. Results were expressed as the average of four measurements. In addition, the total colour difference (ΔE) was calculated as below (Equation 2) (Kovačević *et al.*, 2016):

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (2)$$

Where L₀, a₀ and b₀ were the values of the control (time 0 of storage), and L, a, and b were the values of CAP treated and untreated jackfruit puree.

Total soluble solids. 0.5 g of sample was analyzed in a refractometer Atago NAR-ITSOLID according to (AOAC, 2000).

Water activity (a_w). Water activity was measured by means of an Aqualab series 3 (Decagon Devices, Inc). Calibration was carried out with a solution of saturated NaCl with a_w = 0.755 and with a saturated solution of KCl with a_w = 0.432.

Aromatic compounds Analysis (GC-FID)

The flavour compounds quantification was performed in the headspace of jackfruit puree 0.5 g of non-treated and treated jackfruit puree were put in 1 g NaCl serum vial and capped airtight with Teflon-faced rubber septum and aluminum cap. The vial was sealed tightly and incubated at 30 °C for 10 min. The syringe SPME needle was then inserted, and the fiber (Carboxen/PDMS de 65 µm, Supelco, Bellefonte, PA) was exposed in the headspace inside the vial for 30 min. Desorption was finally made by exposing the fibre for 4 min in the injection port (split / splitless) gas chromatograph Varian 3,800 GC

equipped with a flame ionization detector (FID) (Solís-Solís, Calderón-Santoyo, Gutiérrez-Martínez, Schorr-Galindo & Ragazzo-Sánchez, 2007). Hydrogen was used as carrier gas at a flow 2.0 mL/min, in a column DB-WAX fused silica capillary (30 m x 0.25 mm i.d., film thickness 0.25 μ m) mark Varian (J & W Scientific, Folsom, CA, USA). The injector temperature was at 250 °C, and the detector temperature was 270 °C. The column temperature program was increased from 40 to 60 °C at 2 °C/min, then from 150 to 240 °C at 3 °C/min and maintained for 5 min at this final temperature. The transfer line temperature was 250 °C.

For volatile compounds identification, ethanol, geraniol, isoamylacetate, linalool, 2-nonanol, benzaldehyde, menthol, ethyl methyl phenylglycidate, and gamma undecalactone were used as reference standards. 2- nonanol (50 mg) was used by the standard internal method for quantification.

Sensory evaluation

A sensory panel composed of 22 untrained judges did the evaluation of taste, colour, odour, and texture parameters. A visual analog scaling anchored on one end with the verbal label “Disliking” and the other end with “Liking” was performed. The sensory results were analyzed by a two-factor analysis (judge and product) with an ANOVA test (Pedrero, Daniel & Pangborn, 1989) and differences were established by LSD test.

Statistical Analysis

Statistical analysis was performed using STATGRAPHICS Centurion XV software version 2.15.06. Analysis of variance (ANOVA) and multiple comparison procedures LSD test were conducted to determine significant differences ($p < 0.05$) among treatments.

RESULTS AND DISCUSSION

Detection and identification of jackfruit puree microbiota

Jackfruit puree samples without plasma treatment were analyzed in order to obtain the natural occurring microbiota. Seven bacteria strains were isolated from the jackfruit puree samples, one isolate was identified as *Escherichia coli*, and six strains were identified as *Enterobacter cloacae* according to the API20E test. Additionally, *Aspergillus* sp. was detected in the jackfruit puree according to the macroscopic characteristics that a colony presented on PDA. The size of the colony was 60.11 ± 1.34 mm after 7 days at 25 °C. The *Aspergillus* colonies showed rapid growth, irregular texture, plane, and velvety; colourless reverse and covered with a dense layer of dark brown-to-black conidial heads. Microscopic characteristic showed conidiophores were dark brown to black and rough – walled. Conidia were globose to subglobose. Phialides radially covered the vesicle (Pitt & Hocking, 2009). Then, it was evidenced that *Aspergillus* spp. spores can survive at puree preparation conditions.

Inactivation of *Aspergillus niger* spores and microbiota by cold plasma treatment

First, jackfruit puree was inoculated with *A. niger* spores at 7.55×10^5 spores/g. After the CAP treatments at 30 W and 1.5 L/min of Helium gas, jackfruit puree was analyzed for spore survival, and inhibition curves for *A. niger* spores were performed as a function of the treatment time (Figure 1A). *A. niger* spores survival curve showed two slopes, the first one comprised from time 0 to 2 min and the second slope from 2 to 8 min. *A. niger* spores were not detected after 8 min of CAP treatment. The decimal reduction time value (time for reduction of spores in 90%) for the first slope was $D_1 = 5.94$ min and for the second slope was $D_2 = 1.71$ min. The statistical analysis showed a significant difference between treatment times from 0 to 8 minutes ($p < 0.05$).

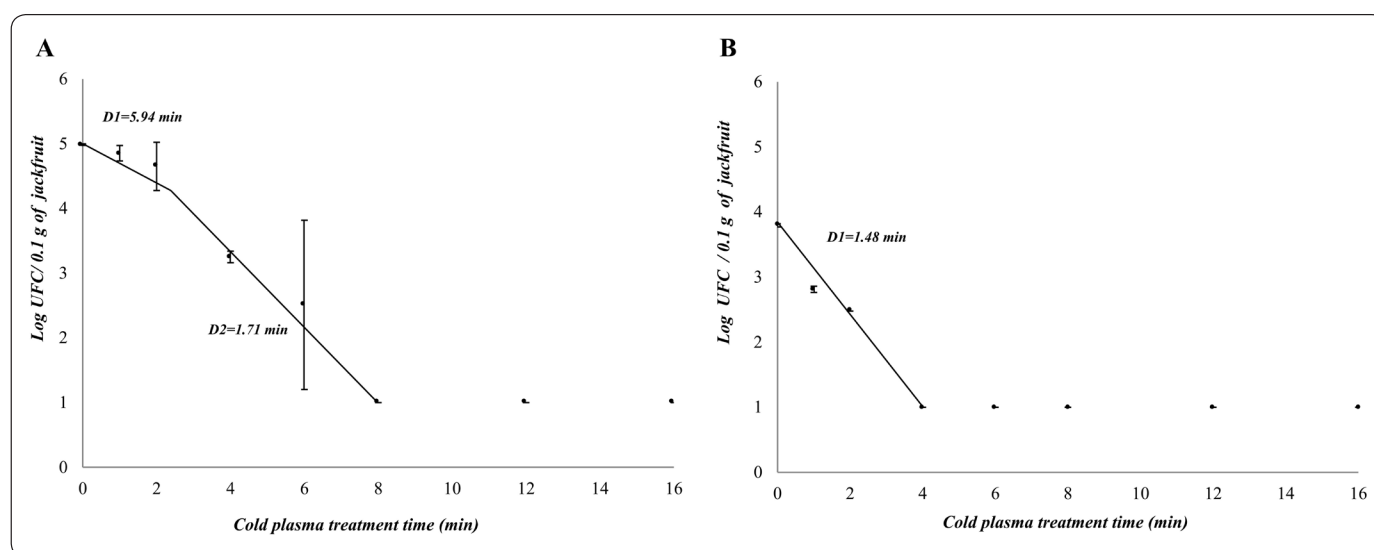


Figure 1. Survival curve of (A) *Aspergillus niger* spores and (B) total microflora in jackfruit puree sample treated with cold plasma.

A possible hypothesis for having two slopes, according to Moisan *et al.* (2001), is the existence of the first phase of exposition to the cold plasma where the toxic species are being generated and accumulated. These species cause slight alterations to the cellular membrane, and high values of D_1 were observed. At this state, the differences in D_1 values and resistance to the reactive species for the different microorganisms are attributed to the differences in the structures and composition of the membrane surface. Concerning the spores, a thick wall protecting the spores from the environment surrounds them. That outer cell wall contains lipids and proteins that confer it a hydrophobic nature. The inner wall of the cell is similar to the mycelial wall and consists of a β (1-3) glucan matrix reinforced with chitin fibrils (Eduard, 2009). It is noteworthy that the spores contain a cell wall thicker than the mycelia; also, the nuclear membrane that protects the DNA is composed of small acid soluble proteins ($\alpha\beta$ -type SASP), low-core water content and an increased amount of dipicolinic acid (Muranyi, Wunderlich & Heise, 2007). During the second phase, the concentration in these species is enough to the lethality. The irreversible damage and lysis in the microbial cells produce high inhibition rates and extremely short D_2 values (Laroussi, 2002). Plasma conditions in the experiment resulted in the most effective inactivation of *A. niger* on jackfruit puree with a reduction of just under 4 log CFU in only 8 min.

The active species density are important action mechanisms of cold plasma, which are responsible of inactivation, mycelial deformation tip, cellular protein destruction, cell apoptosis (lipid body accumulation), fragmentation and release on DNA, loss of permeability and cell leakage against fungi cells (Misra, Yadav, Roopesh & Jo, 2019). For example, atomic oxygen or metastable oxygen molecules, cause the drastic attack of atomic oxygen and singlet oxygen molecules and may contribute to the erosion and finally to the rupture of the cell wall. Some other reactive species, such as OH^\cdot , O_2^{-1} , O_2 , ozone (O_3) and nitrogen dioxide (NO_2) can be formed during the plasma treatment. OH^\cdot can easily attack unsaturated fatty

acids on the cell membrane, O_2^{-1} can mediate the generation of more reactive radicals, namely OH^\cdot and HOO^\cdot and the latter can also initiate lipid peroxidation and DNA mutation. O_2^{-1} can oxidize unsaturated fatty acids and membrane proteins, while O_3 can interfere with cellular respiration. All of these reactive species may interfere with the cell, DNA damage, and break chemical bonds (López *et al.*, 2019).

In the case of reduction of natural microbiota in jackfruit puree, the survival curve under CAP treatment at 30 W and Helium 1.5 L/min with respect to the exposure time gives a unique slope (Figure 1B). The total microbiota was inhibited after 4 min (reduction of 4 Log CFU), with $D = 1.48$ min. Contrary to eukaryotic, typical prokaryotes cells have single double-stranded molecule of DNA and not organized into multichromosomal structures. Moreover, they possess a complex cell wall, which consists of a peptidoglycan structure (Harvey, Cornelissen & Fisher, 2007). The application of cold plasma induces oxidative processes on the membrane lipid peroxidation and hydroxyl radical protein damage, leading to cell death (Guo, Huang & Wang, 2015; Montic, Kelly-Wintenberg & Roth, 2000; Mendis, Rosenberg & Azam, 2000). Because the jackfruit puree contains mostly Enterobacteria species, a rapid inhibition and a D value inferior to that obtained for *A. niger* spores were obtained

Effect of CAP processing technology on microbiological quality during storage

No mesophilic aerobic bacteria were detected along the 60 days of storage of jackfruit puree treated with CAP, conversely counts around 2 Log CFU/g were obtained for non-treated jackfruit puree (Table I). The mesophilic aerobic bacteria count must not exceed 50 CFU/g according to the Official Mexican Standards (NOM-130-SSA1-1995 in accordance with CAC/RCP23-1979 Rev. 1989). Then, cold plasma treatment is useful to reach this requirement. Additionally, mesophilic aerobic bacteria can grow at temperatures between 25 and 45 °C, and the storage at room temperature favours their growth in jackfruit puree (Ramírez, Serrano & Sandoval, 2006).

Table I. Microbiology counts for jackfruit puree treated with cold plasma treatment and stored at 25 °C for 60 days.

Jackfruit		Storage (day) Log UFC/g				
		0	7	15	30	60
Mesophilic Aerobic Bacteria	Control	2.58 ± 0.02 Aa	1.98 ± 0.16 Ab	1.83 ± 0.23 Ab	ND Ac	1.67 ± 0.96 Ab
	8 min treatment	ND Ba	ND Ba	ND Ba	ND Ba	ND Ba
Mold and Yeast	Control	2.63 ± 0.06 Aa	2.17 ± 0.07 Ab	2.09 ± 0.023 Ab	2.2 5 ± 0.07 Ab	2.10 ± 0.14 Ab
	8 min treatment	ND Ba	ND Ba	ND Ba	ND Ba	ND Ba

ND = Not detectable. a = Similar letters lowercase indicate no significant difference ($p < 0.05$) between days of storage. A = Similar letters uppercase indicate no significant difference ($p < 0.05$) between treatments.

Total coliforms were detected neither in CAP jackfruit puree treated nor in non-CAP treated puree, at any time of the storage period (Data not shown). This is probably because the jackfruit puree is a product with low water activity (approx. 0.6) and coliforms are reported to grow with difficulty at values of a_w lower than 0.91 (Damodaran & Parkin, 2019).

Molds and yeasts were not detected during the whole period of storage in the CAP treated jack fruit puree. Concerning the untreated puree, molds and yeast were detected in all samples at counts around 2.3 Log CFU/g, and the counts remained constant during the storage period (Table I). According to the NOM-130-SSA1-1995 less than 10 CFU/g of molds and yeast are permitted; untreated samples do not comply with this standard. Molds and yeasts grow at water activities as low as $a_w = 0.60$.

Effect of CAP processing technology on physicochemical properties during storage

Even if CAP treatments are efficient in terms of microbial inactivation it is important to test the impact in quality parameters of food products.

Colour measure

Jackfruit puree samples treated with cold plasma showed minimal changes in colour (ΔE) after 60 days stored at ambient temperature, but higher changes were noted in samples of untreated jackfruit puree during the same storage period (Table II). Colour changes in untreated samples can be caused by oxidative enzymes. Diamanti *et al.* (2016) have established that polyphenol oxidase and peroxidase enzymes are responsible for oxidation in a strawberry puree thermally processed and stored at ambient temperature. On the other hand, samples treated with cold plasma showed a low change in colour during storage, which can be related to a denaturalization of browning enzymes by cold plasma exposition (Tappi *et al.*, 2014; Surowsky, Fischer, Schlueter & Knorr, 2013).

Table II. Changes on colour values of cold plasma treated and untreated jackfruit puree during storage at 25 °C.

Treatment	Storage period (days)	ΔE
Untreated	0 – 7	15.87 ± 0.05 Ab
	0 – 15	16.26 ± 0.07 Ac
	0 – 30	16.12 ± 0.03 Aa
	0 – 60	16.15 ± 0.02 Aa
Cold plasma	0 – 7	6.90 ± 0.08 Ba
	0 – 15	7.00 ± 0.03 Bb
	0 – 30	7.76 ± 0.05 Bc
	0 – 60	7.85 ± 0.03 Bd

a = Similar letters lowercase indicate no significant difference ($p < 0.05$) between days of storage. A = Similar letters uppercase indicate no significant difference ($p < 0.05$) between treatments.

pH and total acidity

The pH values for cold plasma treated puree and the untreated control were statistically different ($p < 0.05$) along the storage period (Table III). This difference (0.1 to 0.5 pH units) had no impact in sensorial quality because the sensory panel did not observe variations in flavour. Besides, these minimal differences can be explained in terms of organic acids production because of the fermentative metabolism of microbial growth in untreated controls (Table III).

Total soluble solids (TSS, °Bx)

TSS were not affected by CAP nor changed during storage at 25 °C ($p > 0.05$) (Table III). On the subject of sugars, it has been demonstrated that glucose, fructose and sucrose, the major fruit sugars, are sensible to the UV light at 254 nm and

Table III. pH, Total acidity, a_w and TSS values of cold plasma treated and untreated jackfruit puree during storage at 25 °C.

	Storage days (d)	pH	Total acidity values	a_w	TSS
Untreated	0	4.26 ± 0.03 Ba	3.26 ± 0.02 Aa	0.76 ± 0.02 Aa	87 ± 0.0 Aa
	7	4.30 ± 0.01 Ba	2.96 ± 0.03 Ab	0.45 ± 0.03 Ab	87 ± 0.32 Aa
	15	4.35 ± 0.06 Bb	2.82 ± 0.01 Ac	0.34 ± 0.01 Ac	87 ± 0.29 Aa
	30	4.51 ± 0.02 Bc	2.45 ± 0.01 Ad	0.44 ± 0.02 Ab	87 ± 0.58 Aa
	60	4.98 ± 0.01 Bb	2.40 ± 0.03 Ae	0.53 ± 0.07 Ad	85 ± 0.07 Aa
Cold plasma	0	4.70 ± 0.02 Aa	2.71 ± 0.01 Ba	0.61 ± 0.01 Ba	87 ± 0.0 Aa
	7	4.70 ± 0.01 Aab	2.77 ± 0.02 Ab	0.53 ± 0.01 Aa	87 ± 0.78 Aa
	15	4.86 ± 0.03 Ab	2.41 ± 0.01 Bc	0.60 ± 0.04 Ba	87 ± 0.26 Aa
	30	4.64 ± 0.08 Ac	2.45 ± 0.01 Bc	0.55 ± 0.06 Ba	86 ± 0.84 Aa
	60	4.89 ± 0.01 Ad	2.41 ± 0.01 Bc	0.53 ± 0.08 Aa	85 ± 0.76 Aa

a = Similar letters lowercase indicates no significant difference ($p < 0.05$) between days of storage. A = Similar letters uppercase indicates no significant difference ($p < 0.05$) between treatments.

hydroxyalkyl and acyl radicals are formed as a result of its photodegradation (Tikekar, Anantheswaran & LaBorde, 2011). Nevertheless, it is known that UV radiation produced during plasma treatment is not high enough to produce these effects on sugars. It has only been reported during cold plasma treatments that those hydrophilic properties are modified in starch without hydrolysis of the molecule structure (Han, Manolach, Denes & Rowell, 2011).

Water activity (a_w)

There was a significant ($p < 0.05$) difference in the water activity in jackfruit puree treated and untreated during the storage period. Water activity decreases in the untreated samples through time storage, probably due to the gradual breakdown of proteins, carbohydrates, and lipids in the dough (Table III) (Maltini, Torreggiani, Venir & Bertolo, 2003). Nevertheless, at day 15th of storage rehydration occurs, the water found in the environment could be incorporated into the dough until equilibrium in jackfruit puree (Marín, Lemus, Flores & Vega, 2006). In contrast, puree treated with cold plasma presented an inferior value of water activity comparing with that found for untreated puree at time zero. This behavior can be attributed to the air flow through the plasma device (1.5 L/min) and to the slightly increased temperature in the plasma chamber ($33\text{ }^\circ\text{C} \pm 3$) because of the process. These factors cause acceleration in the evaporation of water on the surface of the jackfruit puree. However, during the storage period, the a_w remained constant due to a possible film formed during the CAP process on the surface that formed a barrier in the jackfruit puree by preventing the release of water

molecules. Wang *et al.* (2012) found a moisture loss of less than 5% for three species (carrot, cucumber, and pears) after 8 min of cold plasma treatment. Thus, a moisture loss of less than 5% will not significantly influence the sensory quality and is considered acceptable.

Volatile organic compounds

The aroma compounds detected in the untreated puree and cold plasma treated samples turned out to have minimal differences that were not statistically significant (Table IV). A total of 13 volatile compounds were identified in untreated as well as CAP treated puree. The major compounds were identified as 2-methyl propyl 3-methylbutanoate and (2E,4E)-hexa-2,4-dienoic acid, these compounds were found in treated and untreated jackfruit puree samples (Table IV). Particularly, 2-methyl propyl 3-methylbutanoate is in approx. 64% abundance with respect to the total identified aromatic compounds. This compound is an important contributor to the exotic flavour in jackfruit (Bicas *et al.*, 2011). Thus, plasma treatment did not affect the covalent bonds responsible for the integrity of aroma compounds. The effect of plasma treatments in aroma compounds has not been clarified before. However, the effect of UV radiation which is a component of the plasma has been investigated and only slight differences have been found in aroma compounds in apple slices irradiated at 0.5 and 1 KGy (Song *et al.*, 2012).

Effect of CAP processing technology on sensory properties

Two samples were hedonic evaluated: jackfruit puree treated with cold plasma at 30 W, Helium 1.5 L/min during 8 min and

Table IV. Volatile compounds identified on CAP treated and untreated jackfruit puree by gas chromatography.

No.	Compound	Retention time (min)	Untreated Sample control ($\mu\text{g/L}$)	CAP treated sample ($\mu\text{g/L}$)
1	Ethyl butyrate	7.15	3.40 ± 1.40 a	1.50 ± 0.53 a
2	Ethyl isovalerate	7.45	1.77 ± 1.61 a	3.23 ± 3.04 a
3	Propyl pentanoate	7.85	1.40 ± 0.09 b	2.27 ± 0.74 a
4	2-methylpropyl butanoate	8.76	0.07 ± 0.01 a	0.15 ± 0.07 a
5	2- Methylpropyl 3-methylbutanoate	9.18	45.97 ± 21.04 a	45.35 ± 6.28 a
6	Hexyl pentanoate	10.67	0.17 ± 0.14 a	0.22 ± 0.18 a
7	(3-methyl-1-butyl) 3-methylbutanoate	11.04	0.51 ± 0.09 a	0.36 ± 0.01 b
8	Isoamyl acetate	11.80	1.57 ± 0.25 a	1.10 ± 0.25 a
9	Butyl pentanoate	12.03	1.92 ± 0.14 a	0.77 ± 0.56 b
10	Pentyl hexanoate	29.18	4.62 ± 5.61 a	2.06 ± 2.11 a
11	Propyl benzoate	37.28	2.16 ± 0.01 b	2.31 ± 0.04 a
12	Butyl benzoate	37.68	0.83 ± 0.16 a	0.54 ± 0.01 b
13	(2E,4E)- hexa-2,4-dienoic acid	45.65	7.73 ± 0.31 a	11.00 ± 8.36 a

a = Similar letters lowercase indicates no significant difference ($p < 0.05$) between CAP treated and untreated samples.

untreated jackfruit puree. According to the scale used, the sensory panel evaluated jackfruit puree samples from “disliking” (one) to “liking” (nine). There were no significant differences between CAP treated and untreated samples evaluated in the sensory test ($p < 0.05$) and plasma treated jackfruit samples were found organoleptically acceptable for the whole attributes (Figure 2). These results are in accordance with the physicochemical properties obtained in this study.

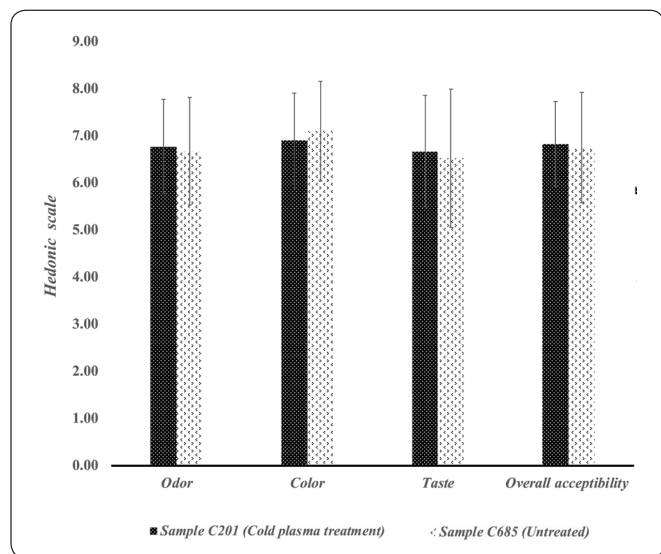


Figure 2. Sensory evaluation of puree treated with cold plasma and untreated samples.

In our study, the low content of lipids makes jackfruit a low susceptible product to sensorial changes during plasma treatments. The products with low lipid and fat content (i.e., dried herbs and spices and other horticultural products) are not significantly affected. In the case of jackfruit, the content of lipids is minimal (0.3 g of lipids/100 g) (<http://edis.ifas.ufl.edu/hs283>), and the UV application and radical exposure would have minimum impact on oxidation or other chemical changes, offering in this manner a good choice of treatment.

CONCLUSIONS

The cold plasma treatment effectively inhibited *Aspergillus niger* spores and total microbiota applying short times of processing, 8 and 4 min, respectively. The treatments did not affect the sensory, physicochemical and aromatic properties. Therefore, the plasma is a promising emerging technology for use in jackfruit puree because of their effectiveness in the inactivation of microorganism compared with thermal technologies and because this technology operates at low temperature and short time making it effective and profitable. Additionally, the capacity of maintaining low temperatures during operations as well as the use of nontoxic gases reduce both environmental impacts and safety risks, while maintaining high efficiency. Then, cold

plasma treatment can be considered as a friendly technology for the treatment of jackfruit puree.

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