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Fungi Associated with Spoilage of Tomato (*Solanum Lycorpersicum* L.) in Koung-Khi Division of Cameroon and Effect of Plasma Activated Water on Fruit Rot

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Abstract **– The aim of this study was to ameliorate the conservation time of tomato fruit using Plasma Activated Water (PAW). Four tomato varieties (Rio Power, Vikina, Maruti and Nadira) collected in Koung-Khi Division and producers of Dschang at different maturity degrees (green physiological maturity, 25%, 50%, 75% and 100% maturity degree) were used. Disinfected fruits and soaked in PAW (at 0; 5; 10; 20; 40 and 80 min). The treated fruits at different times were incubated at Lab temperature (22±2°C) for 14 days. Each treatment was repeated five times and the rate of fruit rot was evaluated daily. The results showed that most frequent and pathogenic fungi isolated from all tomato varieties in the two sites were *Phytophthora infestans* (19.04 and 20.54%, respectively) and the lowest was *Verticillium albo-atrum* (4.71 and 3.52%). The soaking time (5 min) reduce significantly ($p<0.05$) the rot rate of fruit at green physiological maturity and 25% maturity degree in all varieties compared to other treatments, 14 days after soaking (DAS). The 50 and 75% maturity degrees of harvested fruits were best preserved at 5 to 10 min soaking. At 100% maturity degree (14 DAS), the suitable soaking time in PAW was 40 and 80 min for all varieties. This investigation showed that PAW could be used as a new approach to increase the preservation time of tomato in post-harvest due to its antimicrobial properties, notably presence of the NO˙ radicals in the discharge in contact with air leads to the formation of NOx which can hydrolyze into nitrous, nitric and peroxonitric acids in solution.**

Keywords **– Gliding Arc Plasma Activated Water, Tomato Varieties, Maturity Degrees, Preservation, Soaking Time.**

I. INTRODUCTION

Tomato is the most extensively cultivated fruity vegetable in Cameroon, which is grown in all the different ecological zones in the country. The country's production is consumed locally and exported to neighbouring countries like Equatorial Guinea, Nigeria, Gabon [1]. Cameroon is among the major producers of tomatoes in the Central African sub-region with almost 1068,495 tons in 2018 [2]. However, the full potential of the crop has been untapped because of many biotic and abiotic constraints. At harvest, tomato fruits are highly susceptible to biological constraints such as diseases which reduce its quality and the quantity in the markets, causing shortages and inducing high prices [3]. Due to its high contain of carbohydrates, protein, fat, vitamins and minerals that are essential for human health, tomato fruits are also suitable environment for microorganisms' growth. In addition, the poor preservation of fruits and vegetables leads to the development of

fungi, most of which are mycotoxigenic. These mycotoxigenic fungi belong to the genera of *Aspergillus*, *Fusarium* and *Penicillium* are harmful to mammals [4], leading to diseases such as hepatitis, haemorrhage, oedema, immunosuppression, oesophageal cancer and renal failure [5]. To overcome these problems, an alternative method to chemical control, the Plasma Activated Water (PAW) was used to preserve post-harvest tomato fruit against fruit rot in order to increase their shelf life. Plasma activated water is obtained from tap water, distillate water or demineralized water exposed to the action of a plasma-generating electrical discharge in vacuum, air, inert gases or other, with different reactor configurations. Previous studies have shown the beneficial effect of PAW on the post-harvest preservation of fruits and legumes [6]. The generated reactive oxygen species (ROS) and reactive nitrogen species (RNS) during process are the main reasons for preservation of food products [7-8]. PAW has proven to have anti-microbial properties because it has a high oxidation reduction potential so it could possibly to be applied as a food preservation method using the inactivation method because it can deactivate microorganisms which causes spoilage of food. The aim of this study was to ameliorate the conservation time of tomato fruit using Plasma Activated Water (PAW).

II. Material and Experimental Methods

2.1. *Location of the Study Site*

The study was conducted at the Applied Physical and Analytical Chemistry Laboratory of the University of Yaounde I for the Gliding Arc Plasma activation of water and its characterization at the Dschang branch of the Institute of Agricultural Research for Development research laboratory in terms of growth and physiological parameters of tomatoes plants. Dschang locality is located in the West region of Cameroon, more precisely between 5°25'-5°30' North Latitude and 10°-10°5' East Longitude, altitude of 1380 m. During the trial, the average temperature was around 21°C, and rainfall from 160 mm to 155 mm. The study was conducted in laboratory of Plant Pathology and Agricultural Zoology Research Unit, Faculty of Agronomy and Agricultural Sciences, University of Dschang.

2.2. *Collection of Plant Material*

The healthy fruits of four tomato varieties at different maturity degrees (green physiological maturity, 25%, 50%, 75% and 100% maturity degree) (Figure 1) were used for post-harvest conservation tests. Three varieties (RIO POWER, VIKIMA, MARUTI) were collected in the same site from the farms of tomato producers in Koung-Khi Division, and the NADIRA variety (Fig. 1) were collected on the experimental site of the Application and Research Farm of the Faculty of Agronomy and Agricultural Sciences of Dschang University. These varieties are the most tomato varieties grown by the farmers because of their high yields, their organoleptic qualities and their high demand in the market.

2.3. *Isolation and Identification of Fungi Associated with Spoilage of Tomato Fruits*

Tomato fruits showing disease symptoms were disinfected for 2 min in 5% sodium hypochlorite solution then, sliced into 2 mm² pieces. The slices were rinsed in three successive changes of sterile distilled water and transferred on sterile potato dextrose agar medium (PDA) supplemented with chloramphenicol (1 g/L) as antibacterial, for fungal isolation and purification [9]. The cultures were incubated in an inverted position at 24ºC for five days. The pure colonies that developed were identified based on their morphological characteristics (mycelium structure and spore morphology) using keys of fungi identification [10-11]. The occu-

-rrence (O) of each fungus was calculated using the following formula :

$$O = \frac{NF}{NT} \times 100 \quad (1)$$

where NF is the total number of samples from which a particular fungus was isolated and NT is the total number of samples considered [12].

2.4. *Pathogenicity Test of Spoilage Fungi*

The pathogenicity test was carried out on healthy disinfected with bleach solution (5%, 2 min) mature fruits. For each fungus isolated and identified, a 7-day-old culture was used as inoculum. The wound inoculation technique was used [13]. Inoculation consist of harvested a 5 mm fragment of the mycelium in the Petri dish and introduce in the wound; the wound was then covered with dry sterile cotton. After inoculation, fruits were kept in laboratory conditions (22 ± 2 °C) for seven days [14]. The length and width of the lesions developed were measured and the infected area covered by the mycelium estimated.

2.5. *Plasma Activated Water Production (PAW)*

The experimental apparatus used is a cylinder containing 430 mL of tap water, a glass reactor equipped with a water cooling jacket, and a magnetic stirrer (Fig. 2). The sliding discharge device held by the lid has two divergent aluminum electrodes, symmetrically arranged around a wet air nozzle according to the founding father Czernichowski *et al*. [15]. The moist air is obtained by bubbling the air flow supplied by a compressor through a Durand bottle filled with water. The gas flow rate is controlled by a flow meter set at Q = 800 L/h. Energy is supplied by a 40 kHz, 9-10 kV HV Generator. For this purpose 430 mL of tap water was exposed to the plasma for 30 min at the end of which the water enriched with oxidising chemicals, also known as Plasma Activated Water (PAW), is ready for use [16].

Fig. 1. Tomato fruits (variety RIO POWER) at different degrees of maturity: mature fruits at green degree (A), 25% (B), 50% (C), 75% (D) and totally ripe fruits at 100% (E).

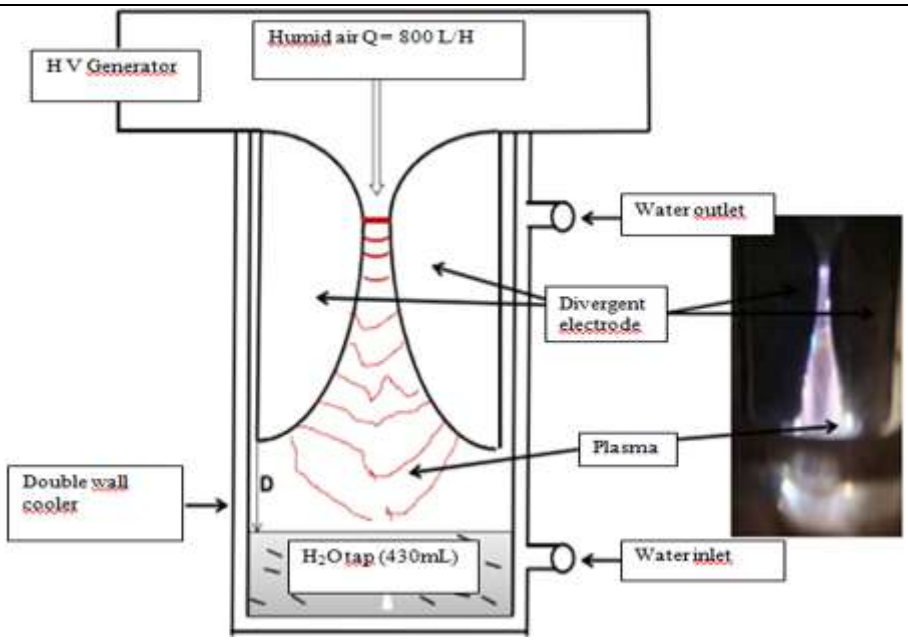

Figure 2. Experimental-processing system to have plasma activated water.

2.6. *Physicochemical Analysis of Plasma Activated Water*

The determination of pH, Conductivity and TDS was carried out using a HANNA HI 9811-5 pH/°C/EC/TDS multimeter with digital display. The colorimetric method has been used for the determination of nitrates (515 nm) and hydrogen peroxide [17].

2.7. *Evaluation of the Effect of Plasma Activated Water on the Tomato Fruit Rot Rate*

Four fruits varieties harvested from farmers farm were washed with tap water and introduced separately in a beaker of 1L according to ripening stage and variety. They were disinfected with bleach (5%, 2 min), then rinsed three times to remove the disinfectant. These fruits at different maturity degrees were soaked in Plasma Activated Water at different times (5; 10; 20; 40; 80 min). The control was not soaked in PAW. The observation of rotten fruits was recorded for 14 days and the rot percentage rate was assessed according to Ruonan *et al*. (2016) [18]. This percentage is the ratio of number of rotten fruits and total number of fruits expressed in percentage.

2.8. *Data Analysis*

The data collected on the rate of fruit rot were previously submitted to Arcsine transformation and then analysed using the software R version 4.0.1. The Kruskal Wallis test was used for means separation at 5%.

III. RESULTS

3.1. *Physicochemical Properties of Plasma Activated Water*

Data from the physicochemical analyses of plasma activated water are presented in Table 1. It is shown that after 30 min of exposure to humid air plasma, the pH of the tap water decreases from 6.4 to 3.2. The conductivity and total dissolved solids (TDS) in tap water increase after 30 min of exposure to the electrical discharge. Nitrate ions increasing with exposure time. It is found that the concentration of hydrogen peroxide (H_2O_2) in solution dincreases after 30 min of exposure to the electric discharge.

Table 1. Physicochemical analysis of plasma-activated tap water.

Treatments	pH	Conductivity (µs.cm^{-1})	TDS (mg/L)	[NO_3^-] (mmol/l)	[H_2O_2] (mmol/l)
Control	6.4	70	30	0.002	0
30 min	3.2	270	140	0.156	0.012

3.2. *Fungi Associated with Spoilage of Tomato Fruits and their Pathogenicity*

The fungi associated with tomato fruit deterioration in Koung-Khi Division and Dschang were: *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cercospora* sp., *Colletotrichum gloeosporioides*, *Fusarium oxysporum, Fusarium solani, Phytophthora infestans, Trichoderma viridae, Verticillium albo-atrum* (Table 2). The pathogenicity test showed that among the fungi isolated and identified from tomato fruits, *Trichoderma viridae et Verticillium albo-atrum* were not pathogenic. The most pathogenic fungus was *Phytophthora infestans. Cercospora sp.*was less pathogenic among pathogenic fungi.

Table 2. Occurrence of fungi, pathogenicity and infected area of fungi isolated on tomato fruits.

Fungi Isolated	Occurrence (%)		Pathogenicity	Infected Area (cm^2) ^{z}			
	Koung Khi	Dschang		RIOPOWER	VIKIMA	NADIRA	MARUTI
Alternaria alternata	9.52 ± 1.11^{c}	8.26 ± 1.44^{bc}	++	2.6 ± 0.7^{ab}	4.5 ± 0.9^{ab}	5.1 ± 0.3^{a}	3.1 ± 1.3^{ab}
Aspergi llus flavus	10.43 ± 1.15^{c}	10.34 ± 1.45^{b}	++	2.1 ± 1.2^{ab}	3.3 ± 1.1^{ab}	3.8 ± 1.3^{ab}	2.5 ± 0.3^{ab}
Aspergillus niger	8.61 ± 1.12^{cd}	9.32 ± 1.23^{b}	++	1.6 ± 0.5^{ab}	3.1 ± 0.6^{ab}	3.3 ± 0.7^{ab}	2.7 ± 1.4^{ab}
Cercospora sp.	4.86 ± 0.81^{e}	6.02 ± 1.55^{c}	+	1.01 ± 0.2^{b}	2.2 ± 0.4^{b}	2.8 ± 1.1^{b}	2.2 ± 0.1^{b}
Colletotrichum gloeosporioides	11.57 ± 1.16^{c}	12.43 ± 1.02^{b}	++	1.5 ± 0.5^{ab}	3.5 ± 0.6^{ab}	3.9 ± 0.7^{ab}	2.8 ± 0.1^{ab}
Fusarium oxysporum	14.28 ± 2.20^{b}	13.02 ± 2.02^{b}	++	1.1 ± 1.6^{ab}	3.9 ± 1.60^{ab}	4.7 ± 1.2^{ab}	1.7 ± 0.3^{ab}
Fusarium solani	9.41 ± 1.13^{c}	8.12 ± 1.23^{b}	++	1.2 ± 1.6^{ab}	3.23 ± 1.65^{ab}	3.6 ± 1.4^{ab}	2.3 ± 1.1^{ab}
Phytophthora infestans	19.04 ± 3.12^{a}	20.54 ± 3.02^{a}	+++	3.1 ± 1.5^{a}	5.4 ± 2.4^{a}	5.5 ± 2.7^{a}	3.8 ± 2.6^{a}
Trichoderma viridae	7.47 ± 1.11^{cd}	8.43 ± 1.21^{b}	-	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
Verticillium albo-atrum	4.71 ± 0.77^{e}	3.52 ± 0.45^{d}	-	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
Control	/	/	/	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}

^{a,b,c} The means in the column followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test. ^{z} = infected area of tomato varieties at 50% maturity stage, 5 days after inoculation.

3.3. *Potential of Gliding Arc Plasma Activated Water on the Preservation of Tomato Fruits at Green Physiologically Maturity*

At the green physiological maturity degree of tomato fruit RIO POWER variety, there was a significant difference ($p<0.05$) between the different soaking times and 7th and 14th day after soaking (DAS). At 7 day after soaking (DAS), RIO POWER gave the highest rate of fruit rot at 5 min soaking, compared to the other durations and control (0.00%). At 14 DAS, the control and 20 min soaking gave the highest rate of rot (40%) in the same variety. The 5 and 10 min soaking showed the lowest rot rates (8.1 and 10% respectively) (Table 3).

Table 3. Tomato fruit rot (%) at green physiologically maturity at different soaking time in Gliding Arc Plasma Activated Water.

Soaking Time (min)	RIO POWER		VIKIMA		NADIRA		MARUTI	
	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
Control	0.0 ± 0.0^{b}	42.4 ± 6.7^{a}	30 ± 5.5^{a}	48 ± 6.44^{a}	22.2 ± 4.1^{c}	56.3 ± 5.1^{b}	27.7 ± 6.1^{a}	48.7 ± 2.1^{a}
5	2.0 ± 1.3^{a}	8.1 ± 4.4^{c}	10 ± 3.5^{c}	20 ± 3.54^{c}	11.32 ± 3.7^{d}	21.44 ± 4.6^{d}	10.9 ± 4.2^{b}	27.3 ± 3.7^{b}
10	0.0 ± 0.0^{b}	10 ± 2.4^{c}	28 ± 4.4^{a}	33 ± 4.8^{b}	55.5 ± 2.6 ^{a}	66.6 ± 4.8^{a}	25.0 ± 5.2^{a}	31.3 ± 5.5^{ab}
20	0.0 ± 0.0^{b}	40 ± 3.8^{a}	20 ± 2.5^{b}	35 ± 6.4^{b}	39.1 ± 5.7^{b}	55.2 ± 3.6^{b}	7.7 ± 2.3b^{c}	43.6 ± 6.1^{a}
40	0.0 ± 0.0^{b}	25 ± 3.5^{b}	28 ± 4.4^{a}	40 ± 5.2^{ab}	33.3 ± 2.9^{b}	51.5 ± 4.4^{b}	4.5 ± 1.2^{c}	36.4 ± 2.8^{a}
80	0.0 ± 0.0^{b}	22 ± 2.2^{b}	25 ± 3.7^{a}	43 ± 4.3^{a}	5.2 ± 0.7^{c}	33.1 ± 2.6^{c}	10.3 ± 3.4^{b}	52.3 ± 3.3^{a}

^{abc} The means in the column followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test. DAS: Day after soaking.

VIKIMA variety at the green physiological maturity degree, was significantly difference ($p<0.05$) between the soaking times and between the 7th and 14th day after soaking. At 7 day after soaking, the control and the soaking time 10, 40 and 80 min showed the highest rot rates (30; 28; 28; 25% respectively). The 5 min soaking time showed the lowest rate of fruit rot (10%). At day 14, the control and the 80 min treatment had the highest rates of rot (48 and 43% respectively), while the 5 min treatment had the lowest (20%) (Table 3).

NADIRA variety at green degree physiological maturity, was showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 day after soaking, the soaking time 10 min gave the highest rot rate of fruit (55.5). The 80 min soaking time showed the lowest rot rate (5.2%). At 14 day after soaking, the 10 min soaking time gave the highest rot rate (66.6%) and the 5 min soaking time the lowest (21.44%) (Table 3).

MARUTI variety at the degree of physiological maturity green fruit, there was a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 day after soaking, the control and the 10 min treatment gave the highest rates of fruit rot (27.7 and 25% respectively). The 20 and 40 min treatments gave the lowest rot rates (7.7 and 4.5%). At day 14, the 5 min treatment gave the lowest rate of rot (27.3%).

3.4. *Potential of Gliding Arc Plasma Activated Water on the Preservation of Tomato Fruits at 25% Maturity Degree*

At 25% maturity degree, the rote rate of RIO POWER was significantly difference ($p<0.05$) between the different soaking times and between the 7th and 14th day after soaking. At the 7th day after soaking, the unsoaked and soaking fruits at 40 and 80 min treatments gave the highest fruit rot rates (18.3; 20.1 and 18.3% respectively). The other treatments (5;10 and 20 min) gave the lowest rot rates (0%). At 14 DAS in same variety, the control and soaking time 10 and 40 min gave the highest rot rates (50, 40.6 and 46.6% respectively). The soaking time 5 and 20 min gave the lowest rot rates (17. 3 and 20.4 % respectively) (Table 4).

Table 4. Tomato fruit rot (%) at 25% maturity degree at different soaking time in Gliding Arc Plasma Activated Water.

Soaking Time (min)	RIO POWER		VIKIMA		NADIRA		MARUTI	
	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14DAS
Control	21.3 ± 1.3^{a}	50.0 ± 4.6^{a}	29.4 ± 5.5^{a}	63.6 ± 5.4^{a}	33.33 ± 2.9 ^{a}	66.66 ± 2.8^{a}	20.1 ± 3.2^{a}	47 ± 4.3^{a}
5	0.0 ± 0.0^{b}	17.3 ± 2.5^{c}	7.5 ± 2.5^{b}	25.3 ± 3.1^{c}	11.31 ± 6.7^{b}	33.44 ± 1.6^{c}	12.3 ± 2.8^{b}	19 ± 2.7^{c}
10	0.0 ± 0.0^{b}	40.6 ± 2.2^{a}	14.7 ± 2.1^{b}	37.3 ± 4.2^{b}	35.6 ± 4.6 ^{a}	64.72 ± 3.5^{a}	15.8 ± 1.9^{b}	24 ± 3.5^{c}
20	0.0 ± 0.0^{b}	20.4 ± 2.4^{c}	5.6 ± 1.4^{c}	50.5 ± 6.4^{b}	18.2 ± 6.6^{b}	47.6 ± 3.8^{b}	22.4 ± 2.2^{a}	29 ± 1.6^{b}
40	20.1 ± 3.0^{a}	46.6 ± 5.7^{a}	20 ± 5.3^{a}	35 ± 7.8^{b}	36.7 ± 5.7^{a}	62.1 ± 6.9^{a}	17.6 ± 4.3^{ab}	42 ± 2.4^{a}
80	18.3 ± 1.1^{a}	29.3 ± 3.1^{b}	25 ± 6.2^{a}	42 ± 8.1^{b}	17.5 ± 0.0^{b}	44.33 ± 3.1^{b}	16.4 ± 4.6^{b}	20 ± 3.4^{c}

^{abc} The numbers in the columns followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test. DAS: Day after soaking.

At 25% maturity degree of VIKIMA variety, there was a significant difference ($p<0.05$) between the soaking times and between the 7th and 14th day after soaking. At 7 day after soaking, the control and soaking time 40 and 80 min gave the highest fruit rot rates (29.5; 20 and 25% respectively). The 20 min soaking time gave the lowest rate of fruit rot (5.6%). At 14 days after soaking in the same variety, the control gave the highest rate rot (63.6%). The 5 min soaking time gave the lowest rate rot (25.3%) (Table 4).

At 25% maturity degree of NADIRA fruits, a significant difference ($p<0.05$) between the soaking times and between the 7th and 14th day after soaking was observed. At 7th day after soaking, the control and 10 and 40 min soaking time treatments gave the highest fruit rot rates (33.33; 35.6 and 36.7% respectively). The other treatments (5; 20 and 80 min) gave the lowest rot rates. At 14 days after soaking in same variety, the control and the 10 and 40 min soaking time treatments gave the highest rot rates (66.66, 64.72 and 62.1% respectively). The 5 min treatment gave the lowest rate of rotten fruit (33.44%) (Table 4).

At 25% maturity degree of MARUTI fruits varieties, a significant difference ($p<0.05$) between the soaking times and the 7th and 14th day after soaking was showed. At 7 day after soaking, the control at 20 min soaking time treatments gave the highest rot rates (20.1 and 22.4% respectively). The 5, 10 and 80 min soaking time treatments gave the lowest rot rates (12.3, 15.8 and 16.4% respectively). At 14th DAS, the control and 40 min soaking time treatment gave the highest rot rates (47 and 42% respectively). The 5, 10 and 80-min soaking time treatment gave the lowest rot rates (19, 24 and 20% respectively) (Table 4).

3.5. *Potential of Gliding Arc Plasma Activated Water on the Preservation of Tomato Fruits at 50% Maturity Degree*

RIO POWER variety fruits at the 50% maturity degree, showed a significant difference ($p<0.05$) between the soaking time and between the 7th and 14th day after soaking. At 7 days after soaking (DAS), the control and 40 min soaking time treatment gave the highest rates of fruit rot (19.2 and 16.4 % respectively). The 10 min soaking time treatment gave the lowest rot rate (0%). At 14 DAS, the control and the 20 min soaking time treatment gave the highest rot rates (56 and 51% respectively). The 10 min soaking time treatment gave the lowest rot rate (5.2%) (Fig. 3A) and (Table 5).

Fig. 3. Tomato fruits treated with Plasma Activated Water. A: RIO POWER (10 min at 50% maturity), B: MARUTI (80 min at 100% maturity), C: VIKIMA (10 min at 50% maturity), D: Control (RIO POWER at 50% maturity), E: Control (MARUTI at 100% maturity), 14 days after soaking.

Table 5. Tomato fruit rot (%) at 50% maturity degree at different soaking time in Gliding Arc Plasma Activated Water.

Soaking Time (min)	RIO POWER		VIKIMA		NADIRA		MARUTI	
	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
Control	19.2 ± 4.0^{a}	56 ± 4.1^{a}	25 ± 6.5^{a}	76.2 ± 3.58^{a}	27.16 ± 3.66^{a}	82.2 ± 2.2^{a}	20.7 ± 2.3^{a}	65.3 ± 4.33^{a}
5	4.0 ± 1.9^{c}	20 ± 3.2^{d}	9.3 ± 2.5^{c}	20.2 ± 5.58^{c}	12.09 ± 2.3^{c}	42.3 ± 2.3^{b}	23.1 ± 3.3^{a}	48.7 ± 4.66^{b}
10	0.0 ± 0.0^{d}	5.2 ± 0.3^{e}	5.1 ± 2.5^{d}	10.5 ± 3.09^{d}	17.32 ± 3.66^{bc}	21.44 ± 2.5^{d}	7.1 ± 2.3^{c}	17.3 ± 1.5^{d}
20	5.0 ± 2.2^{c}	51 ± 1.2^{a}	22 ± 5.5^{a}	45.6 ± 6.89^{b}	21.26 ± 1.7^{b}	29.62 ± 3.1^{c}	17.8 ± 2.2^{b}	40.3 ± 6.6^{c}
40	16.4 ± 3.1^{a}	44 ± 2.9^{b}	12 ± 2.2^{bc}	44.4 ± 3.36^{b}	18.22 ± 2.66^{bc}	39.06 ± 3.2^{b}	14.6 ± 3.2^{b}	20.4 ± 2.7^{d}
80	8.0 ± 2.2^{b}	36 ± 5.8^{c}	14 ± 4.5^{bc}	39.2 ± 4.6^{b}	11.19 ± 1.33^{c}	37.03 ± 2.1^{b}	11.2 ± 2.3^{bc}	18.8 ± 1.9^{d}

[abc] The numbers in the columns followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test. DAS: Day after soaking.

VIKIMA variety at the 50% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control and the 20 min treatment gave the highest fruit rot rates (25% and 22% respectively). The 10 min treatment gave the lowest rate of rot (5.1%). At 14 DAS, the unsoaked control gave the highest rate of rot (76.2%). The 10 min treatment gave the lowest rate of rot (10.5%) (Fig. 3C) and (Table 5).

NADIRA variety at the 50% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the unsoaked control gave the highest rate of fruit rot (27.16%). The 5 and 80 min treatments gave the lowest rot rates (12.09 and 11.19%). At 14 DAS, the control gave the highest rate of rot (82.2%). The 10 min treatment gave the lowest rate of rot (21.44%) (Table 4).

MARUTI variety at the 50% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control and the 5 min treatment gave the highest fruit rot rates (20.7 and 23.1%). The 10 min treatment gave the lowest rate of fruit rot (7.2%).

At 14 days, the control showed the highest rate of fruit rot (65.3%). Treatments 10, 40 and 80 min gave the lowest fruit rot rates (17.3, 20.4 and 18.8% respectively) (Table 5).

3.6. *Potential of Gliding Arc Plasma Activated Water on the Preservation of Tomato Fruits at 75% Maturity Degree*

RIO POWER variety at the 75% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control and the 40 min treatment gave the highest fruit rot rates (22.3 and 25.4% respectively). The soaking times 5 and 10 min gave the lowest fruit rot rates (0%). At 14 DAS, the control gave the highest rate of fruit rot (100%). The soaking times 5 and 10 min gave the lowest fruit rot rates (9.5 and 5.8 respectively) (Table 6).

Table 6. Tomato fruit rot (%) at 75% maturity degree at different soaking time in Gliding Arc Plasma Activated Water.

Soaking Time (min)	RIO POWER		VIKIMA		NADIRA		MARUTI	
	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS	7 DAS	14 DAS
Control	22.3 ± 3.2^{a}	100 ± 0.0^{a}	37.2 ± 5.5^{a}	100 ± 0.0^{a}	48.33 ± 4.1^{a}	100 ± 0.0^{a}	25 ± 2.2^{a}	90.3 ± 3.9^{a}
5	0.0 ± 00^{c}	9.5 ± 4.4^{d}	10.4 ± 4.5^{c}	32 ± 3.77^{d}	22.22 ± 3.3^{c}	65.3 ± 3.5^{c}	5.3 ± 1.1^{c}	10.1 ± 1.8^{d}
10	0.0 ± 00^{c}	5.8 ± 2.2^{d}	10.1 ± 4.5^{c}	25 ± 2.72^{d}	24.2 ± 1.6^{c}	60.2 ± 5.0^{c}	8.1 ± 3.5^{c}	15.4 ± 1.2^{d}
20	15.7 ± 3.3^{b}	60 ± 3.3^{b}	23.2 ± 5.5^{b}	40 ± 5.1^{c}	33.11 ± 3.8^{b}	70.6 ± 2.6^{b}	18 ± 4.5^{b}	36.9 ± 2.7^{b}
40	25.4 ± 5.1^{a}	66 ± 5.4^{b}	35.3 ± 6.5^{a}	55 ± 4.3^{b}	43.6 ± 3.9^{a}	73.3 ± 2.4^{b}	18 ± 3.5^{b}	32.2 ± 3.4^{c}
80	13 ± 3.3^{b}	32 ± 3.1^{c}	26.1 ± 5.5^{b}	57 ± 2.7^{b}	35.5 ± 4.6^{b}	77.2 ± 4.3^{b}	5.9 ± 2.2^{c}	10.6 ± 1.7^{d}

^{abc}The numbers in the columns followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test. DAS: Day after soaking.

VIKIMA variety at the 75% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control and the soaking time 40 min gave the highest rot rates (37.2 and 35.3%). The soaking times 5 and 10 min gave the lowest fruit rot rates (10.4 and 10.1%). At 14 days, the control gave the highest rates of rot (100%). The soaking times 5 and 10 min gave the lowest fruit rot rates (32 and 25% respectively) (Table 6).

NADIRA variety at the 75% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control and the soaking time 40 min gave the highest fruit rot rates (48.33 and 43.6%). The soaking times 5 and 10-min gave the lowest fruit rot rates (22.22 and 24.2%). At 14 days, the control gave the highest rate of fruit rot (100%). The soaking times 5 and 10 min gave the lowest fruit rot rates (65.3 and 60.2%) (Table 6).

MARUTI variety at the 75% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control gave the highest rate of fruit rot (25%). The soaking times 5, 10 and 80 min gave the lowest fruit rot rates (5.3, 8.1 and 5.9 % respectively). At 14 days, the control gave the highest rate of fruit rot (90.3%). The soaking times 5, 10 and 80 min gave the lowest fruit rot rates (10.1, 15.4 and 10.6%) (Table 6).

3.7. *Potential of Gliding Arc Plasma Activated Water on the Preservation of Tomato Fruits at 100% Maturity Degree*

RIO POWER variety at 100% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 days after soaking, the unsoaked control gave the highest rot rate (15%). The soaking times 40 and 80 min gave the lowest rates of rot (0%). At 14 DAS, the

control showed the highest rate of rot (96.5%). The soaking times 40 and 80 min gave the lowest rot rates (16 and 13% respectively) (Table 7).

Table 7. Tomato fruit rot (%) at 100 % maturity degree at different soaking time in Gliding Arc Plasma Activated Water.

Soaking Time (min)	RIO POWER		VIKIMA		NADIRA		MARUTI	
	7 DAS	14 DAS	7 DAS	14DAS	7 DAS	14 DAS	7 DAS	14 DAS
Control	15 ± 2.6^{a}	96.5 ± 4.2^{a}	46 ± 4.5^{a}	100 ± 0.0^{a}	55.55 ± 3.5^{a}	100 ± 0.0^{a}	26 ± 3.6^{a}	98.4 ± 2.3^{a}
5	5.0 ± 1.7^{b}	40 ± 3.0^{b}	36 ± 3.5^{b}	65.55 ± 3.6^{b}	42.22 ± 2.8^{b}	75.5 ± 6.6^{b}	19 ± 2.2^{b}	42 ± 2.58^{c}
10	5.5 ± 1.3^{b}	32 ± 4.2^{c}	32 ± 3.4^{b}	34.66 ± 2.8^{d}	33.33 ± 2.8^{c}	66 ± 5.88^{bc}	17 ± 2.5^{b}	55 ± 3.24^{b}
20	7.2 ± 1.9^{b}	28 ± 2.3^{c}	23 ± 2.5^{c}	42.44 ± 4.6^{c}	32.11 ± 3.6^{c}	57.2 ± 4.6^{bc}	11.2 ± 3.3^{c}	35 ± 4.74^{d}
40	0.0 ± 0.0^{c}	16 ± 5.1^{d}	22 ± 2.5^{c}	34.44 ± 4.5^{d}	22.2 ± 3.7^{d}	40.33 ± 5.6^{c}	0.0 ± 0.0^{d}	10 ± 2.04^{e}
80	0.0 ± 0.0^{c}	13 ± 4.6^{d}	21 ± 3.5^{c}	28.31 ± 1.7^{d}	11.11 ± 1.7^{e}	26.52 ± 1.8^{d}	0.0 ± 0.0^{d}	9.3 ± 1.09^{e}

^{abc}The means in the column followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test. DAS: Day after soaking.

VIKIMA variety at 100% maturity degree, showed a significant difference ($P<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control gave the highest rate of fruit rot (46%). The soaking times 20, 40 and 80 min gave the lowest fruit rot rates (23, 22 and 21%). At 14 days, the control gave the highest rate of fruit rot (100%). The soaking times 10, 40 and 80 min gave the lowest fruit rot rates (34.66, 34.44 and 28.31% respectively) (Table 7).

NADIRA variety at 100% maturity degree, showed a significant difference ($p<0.05$) between the fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control gave the highest rate of fruit rot (55.55%). The soaking times 40 and 80 min gave the lowest fruit rot rates (22.2 and 11.11% respectively). At 14 DAS, the control gave the highest rate of fruit rot (100%). The soaking times 40 and 80 min gave the lowest fruit rot rates (40.33 and 26.52% respectively) (Table 7).

MARUTI variety at 100% maturity degree, showed a significant difference ($p<0.05$) between fruit soaking times and between the 7th and 14th day after soaking. At 7 DAS, the control gave the highest rate of fruit rot (26%). The soaking times 40 and 80 min gave the lowest fruit rot rates (0%). At 14 DAS, the control gave the highest rate of fruit rot (98.4%). The 40 and 80 min treatments gave the lowest fruit rot rates (10 and 9.3%) (Fig. 3B and Table 7).

3.8. *Potential of Gliding Arc Plasma Activated Water on Tomato Fruit Rot Rate (%) at the different Maturity Degrees during 10 and 80 min, 14 days after Soaking*

There is a significant difference ($p<0.05$) between the different degrees of fruit maturity of different tomato varieties and the soaking times of 10 and 80 min. At the degree of green fruit physiological maturity, the RIO POWER tomato variety presented the lowest fruit rot rate (10.1%) at the soaking time of 10 min, compared to the varieties VIKIMA, NADIRA and MARUTI which presented the highest rot rates (33.1; 66.1 and 52.0%, respectively). At the soaking time of 80 min at the degree of physiological maturity of green fruit, the tomato varieties RIO POWER and NADIRA presented the lowest fruit rot rates (22.1 and 33.1%, respectively),

compared to the VIKIMA and MARUTI varieties which presented the highest rot rates (43.6 and 31.5%) at this duration (Table 8).

Table 8. Tomato fruit rot rate (%) at the different maturity degree soaked in Gliding Arc Plasma Activated Water during 10 and 80 min, 14 days after soaking.

Maturity Stage	RIOPOWER		VIKIMA		NADIRA		MARUTI	
	10 min	80 min	10 min	80 min	10 min	80 min	10 min	80 min
Greenripe	10.1 ± 2.4^{c}	22.1 ± 2.2^{c}	33.1 ± 4.8^{a}	43.6 ± 4.3^{b}	66.6 ± 4.8^{a}	33.1 ± 2.6^{c}	52.0 ± 5.2^{a}	31.5 ± 5.5^{a}
25%	40.6 ± 2.2^{a}	29.3 ± 3.1^{b}	37.3 ± 4.2^{a}	42.3 ± 8.1^{b}	64.72 ± 3.5^{a}	44.33 ± 3.1^{b}	24.2 ± 3.5^{b}	20.7 ± 3.4^{b}
50%	5.2 ± 0.3^{d}	36.3 ± 5.8^{a}	10.5 ± 3.09^{c}	39.2 ± 4.6^{b}	21.44 ± 2.5^{b}	20.03 ± 2.1^{d}	17.3 ± 1.5^{c}	18.8 ± 1.9^{b}
75%	5.0 ± 2.1^{d}	32.6 ± 3.1^{a}	25.5 ± 1.72^{b}	57.3 ± 2.7^{a}	60.2 ± 5.2^{a}	77.2 ± 4.3^{a}	15.4 ± 1.2^{c}	10.6 ± 1.7^{c}
100%	32 ± 4.2^{b}	13.3 ± 4.6^{d}	34.6 ± 2.8^{a}	56.4 ± 1.7^{a}	66 ± 5.8b^{a}	37.52 ± 1.8^{c}	55 ± 3.24^{a}	9.3 ± 1.1^{c}

^{abc}The numbers in the columns followed by the same letter are not significantly different at the 5% probability threshold according to the Kruskal Wallis test.

At the maturity degree of 25%, the MARUTI variety presented the lowest rate of fruit rot (24.1%) at the soaking time of 10 min compared to the varieties RIO POWER, VIKIMA and NADIRA which presented the rates of rot the highest (40.6; 37.3 and 64.72%, respectively). At the soaking time of 80 min, the tomato varieties RIO POWER, VIKIMA, NADIRA and MARUTI presented the lowest rot rates (Table 8).

At the maturity degree of 50%, the RIO POWER tomato variety presented the lowest fruit rot rate (5.2%) at the soaking time of 10 min, compared to the VIKIMA, NADIRA and MARUTI varieties which had presented high rot rates (10.5; 21.44 and 17.3%, respectively). At the soaking duration of 80 min, the NADIRA variety presented the lowest rot rate (20.03%); the RIO POWER variety presented the highest rot rate (36.3%) at this duration (Table 8).

At the maturity degree of 75%, the RIO POWER variety showed the lowest fruit rot rate (5.0%) at the soaking time of 10 min. The NADIRA variety presented the highest rot rate (60.2%) at this duration. At the soaking time of 80 min, the MARUTI variety presented the lowest rot rate (10.6%). The varieties RIO POWER, VIKIMA and NADIRA presented the highest rot rates (32.6; 57.3 and 77.2%, respectively) (Table 8).

At 100% maturity, the RIO POWER variety showed the lowest rot rate (32%) at the soaking time of 10 min. The varieties VIKIMA, NADIRA and MARUTI presented the highest rot rates (34.6; 66 and 55%, respectively). At the soaking time of 80 min, the RIO POWER and MARUTI varieties presented the lowest rot rates (13.3 and 9.3%, respectively). The VIKIMA variety presented the highest rot rate (56.4%) (Table 8).

IV. Discussion

The physicochemical analyses of Plasma activated water shown that after 30 minutes of exposure to humid air plasma, the pH of the tap water decreases. This result is in line with those obtained by Benstaali *et al*. [19] who explained the gradual decrease in pH during plasma treatment due to the increase in treatment time, which further promotes the production of active species that diffuse into the aqueous solution. Indeed, the presence of the NO•radicals in the discharge in contact with air leads to the formation of NOx which can hydrolyze into nitrous, nitric and peroxonitric acids in solution [17]. The conductivity and total dissolved salts in tap water only

increase significantly after 30 min of exposure to the electrical discharge. The relative variations of these two parameters can be explained on the one hand by the change in pH. Indeed, the decrease in pH is accompanied by an increase in conductivity and total dissolved solids, as these two parameters are closely related. Furthermore, the study of the effect of ionizing radiation in the liquid phase [30], electrolysis of the discharge at liquid-gas interfaces [20] and electronic collisions with water vapor in moist gases [21] suggest the following reactions [22]:

$$H_2O + e^- \rightarrow e^- + H\bullet + HO\bullet \quad (2)$$

$$H^\bullet + O_2 \rightarrow HO_2^\bullet \quad (3)$$

$$HO_2^\bullet + HO_2^\bullet \rightarrow H_2O_2 + O_2 \quad (4)$$

$$H_2O_2 + hy \rightarrow 2HO\bullet \quad (5)$$

$$N_2 + O_2 \rightarrow 2NO^\bullet \quad (6)$$

$$4NO^\bullet + O_2 + 2H_2O \rightarrow 4NO_2^- + 4H^+ \quad (7)$$

These reactions lead to the formation of $^\bullet OH$ and H_3O^+. Also the high mobility of oxonium ion H_3O^+ in water contributes to the increase in conductivity due to these cations compared to other cations of similar size [23]. Table 1 shows that the release of ions and NO_3^- increases by 15.4 compared to the control after 30 minutes of tap water exposure to plasma discharge. Nitrate ions significantly increase after 30 minutes of treatment. This phenomenon could be explained by the oxidation of nitrite ions into nitrate ions according to the following equation [24-25]:

$$3NO_2^- + 2H^+ \rightarrow NO_3^- + 2NO + H_2O \quad (8)$$

Based on the obtained value of hydrogen peroxide concentrations in solution, it is observed that the concentration of H_2O_2 decreases over time of exposure. These results were similar to some previous work showing that the radicals formed upstream in the landfill ($HO^\bullet$ and $HO_2^\bullet$) responsible for the formation of H_2O_2 would not recombine sufficiently or that these radicals would preferentially participate in other reactions [26]. Also, the high solubility of hydrogen peroxide in water would contribute kinetically to make it react spontaneously in aqueous solution as soon as it is formed in the gas phase in the landfill [27].

The isolation of post-harvest fungi on tomato fruits has helped to highlight a panoply of fungal species most of which have already been reported by certain authors. Fungal isolates were: *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Cercospora* sp., *Colletotrichum gloeosporioides*, *Fusarium oxysporum, Fusarium solani, Phytophthora infestans, Trichoderma viridae, Verticillium albo-atrum*. Onuorah *et al*. [28] showed that *Aspergillus niger, Alternaria alternata, Fusarium oxysporium* were the fungi responsible for the post-harvest deterioration of tomato fruits. Baker et *al*. [29] also showed that *Aspergillus niger* was pathogenic to tomato fruits post-harvest. Fatih *et al*. [30] reported the presence of fungi *Alternaria alternata* and *Fusarium oxysporum* spoiled tomatoes. Grira *et al*. [31] showed that *Fusarium, Alternaria, Aspergillus niger* and *Trichoderma* fungi were pathogenic on tomato varieties SALIMAH, FARIDA, PETRA et TIMGAD. Benselimane et *al*. [32] showed that *Alternaria alternata*, *Aspergillus niger* fungi are responsible for the deterioration of tomatoes. The pathogenicity test was negative on *Trichoderma viridae* et *Verticillium albo-atrum,* which shows that these fungi were not pathogenic. On the other hand, this test was positive on the other fungi, which shows that they

were pathogenic to tomato fruits. The lesion diameter varied depending on the fungal species, showing that some fungi were more virulent than others. *Phytophthora infestans* was more pathogenic compared to other fungi. *Cercospara sp* less pathogenic. The deterioration of tomato fruits by these fungi would be attributed to the high water content of the fruits, the condition of storage facilities, which would favor the cultivation and development of the mycelia of these species [33].

4.1. *Potential of Plasma Activated Water on the Preservation of Tomato Fruits at different Maturity Degrees*

Plasma-activated water was tested on the spoilage rate of tomato fruits at different stages of ripeness. The results showed a reduction in the fruit spoilage rates. These results can be explained, on one hand, by the reduction in the concentration of H_2O_2 (Table 1). Indeed, the radicals formed upstream in the discharge (HO° and HO_2°), responsible for the formation of H_2O_2, may not recombine sufficiently or these radicals may prefer to participate in other reactions [34]. Similarly, Brisset et *al*. [22] observed that the high solubility of hydrogen peroxide in water would contribute to its spontaneous reaction in aqueous solution as soon as it forms in the gas phase in the discharge. On the other hand, the reactivity of the species (ROS (HO°, H_2O_2, °O_2H) and RNS (NO°, NO_2, HNO_2, NO_2-, NO_3-, ONOOH, ONOO-)) remains for several hours, or even several days after their production, as shown by the post-discharge effect of Glidarc plasma [35-36]. The presence of these species is thought to be responsible for this inhibition of pathogens on these fruits, which would help reduce the rate of spoilage. Joshi et *al*. [37] reported that during the generation process of plasma-activated water, reactive species such as hydrogen peroxide (H_2O_2), nitric acid (HNO_3), and peroxynitric acid (HNO_4) are formed, resulting in a low pH (due to the presence of the NO° radical in the discharge) and, consequently, antibacterial activity [17]. Gaeul et *al.* [39] showed that treatment with plasma-activated water after immersion washing of cherry tomatoes inoculated with Bacillus cereus, Salmonella sp., and Escherichia coli for 5 minutes reduced the presence of pathogens compared to untreated cherry tomatoes fruits immersed for 2 minutes in plasma-activated water are better preserved [40]. Malahela et *al*. [41] have shown that plasma-activated water extends the shelf life of fruits and vegetables. Plasma activated water is a potential microbial disinfectant to increase the shelf life of various food products [42]. In addition, plasma activated water help to promote food security, improve public health and foster a more sustainable and resilient food system [43-44-45]; it has also shown positive effects in the decontamination of spices (Hertwig et al., 2015a [46-47]). At 100% fruits maturity, the longest soaking times exhibited the lowest rot rates. This could be explained by the fact the antimicrobial effect of plasma activated water increases with the soaking time. These results are rather contrary to those of Ruonan et *al.* [18] who showed that the rot rate of tomato fruits did not vary with treatment times. However, the storage conditions and the varieties used were not the same, which could therefore create variability in the rate of fruit rot.

V. CONCLUSIONS

The aim of this study was to ameliorate the conservation time of tomato fruit using Plasma Activated Water. The results showed that Plasma Activated Water reduced the rate of tomato fruit rot at the 5 min soaking time, 14 days after soaking, at the green fruits' maturity degree and 25% on the RIO POWER, VIKIMA, NADIRA and MARUTI tomato varieties. At the 50% fruit maturity degree on the same varieties, it reduced the rate of fruit rot at the 10 min soaking time. At the 75% fruit maturity degree, it reduced the rate of rot at the 5 and 10 minute fruit soaking times. At 100% fruit maturity, Plasma Activated Water reduced the rate of tomato fruit rot

on the same varieties at 40 and 80 min soaking times, 14 days after soaking. The reduction in the rate of fruits rot by Plasma Activated Water is thought to be linked to the antimicrobial properties of this water. It could therefore be a new approach to the post-harvest preservation of tomato fruits.

Author Contributions:

Conceptualization, Aimé Magloire Tenkap Njopkou, Gertrude Keegoui and Jean Paul Kamseu-Mogo.; methodology, Jean Paul Kamseu-Mogo, Aime Magloire Tenkap Njopkou and Gertrude Keegoui.; software, Joseph Djeugap-Fovo.; validation, Jean Paul Kamseu-Mogo., and Joseph Djeugap-Fovo.; formal analysis, Jean Paul Kamseu-Mogo and Gertrude Keegoui.; investigation, Madou Chantal and Joliesse Koagne Nouteka.; resources, Joliesse Koagne Nouteka and Madou Chantal.; data curation, Aime Magloire Tenkap Njopkou and Jean Paul Kamseu-Mogo.; writing-original draft preparation, Jean Paul Kamseu-Mogo and Aimé Magloire Tenkap Njopkou.; writing-review and editing, Joliesse Koagne Nouteka, Madou Chantal, Joseph Djeugap-Fovo and Jean Paul Kamseu Mogo; visualization, Aimé Magloire Tenkap Njopkou and Jean Paul Kamseu-Mogo.; supervision, Joseph Fovo Djeugap.; project administration, Aime Magloire Tenkap Njopkou.; funding acquisition, Aime Magloire Tenkap Njopkou All authors haveread and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare that there are no conflict of interests.

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