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Research Article

Alleviating Chilling Injury in Stored Pomegranate Using a Single Intermittent Warming Cycle: Fatty Acid and Polyamine Modifications

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Pomegranate is a perishable superfruit with important human health-promoting phytochemicals. The use of cold storage is inevitable for its long-term preservation. As pomegranate is sensitive to temperatures below 5°C, it is therefore necessary and worthwhile to introduce a postharvest technique that is safe, applicable, and commercially acceptable to maintain the fruit quality under a cold storage condition. The efficacy of intermittent warming (IW) in the form of a single warming period (1 day at 20°C with 70% relative humidity (RH) before returning the treated fruit to storage) during the cold storage of 'Rabab-e-Neyriz' pomegranate (70 days at 2 ± 0.5°C and 90 ± 5% RH) was evaluated. To find the best treatment time, warming was performed at 4 temporary interruption points in storage (after 15, 25, 35, or 45 days of storage). For each interruption date, the treated fruit were compared to the controls twice, once immediately after treatment and once at the end of the storage period. It was founded that a single warming period at the right time during cold storage (before irreversible damage occurs) activated multiple mechanisms and physiological responses in pomegranate fruit peel that are significantly responsible for alleviating the severity of chilling damage to this commodity. In other words, warming on the 15th day was the most efficient treatment, resulting in better preservation of unsaturated fatty acids from peroxidation, lower malondialdehyde (MDA) production, and preservation of the unsaturated/saturated fatty acids (UFAs/SFAs) ratio (membrane integrity index) in the peel during storage and lower chilling injury symptoms. Moreover, the content of spermine (Spm) and putrescine (Put) (as important antioxidants acting as membrane safety agents) was significantly increased immediately after treatment, followed by a continuous increase in Spm and a higher level of Put compared to control until the end of storage.

1. Introduction

Nowadays, there is a worldwide and increasing notion that superfruits and their ingredients and extracts may have the ability to prevent diseases and/or be used as a cure for ailments. Pomegranate fruit (*Punica granatum* L.), known in many countries as the fruit of Eden [1], is a superfruit with excellent taste and great health benefits. Nearly 124 different phytochemicals can be found in pomegranate fruit; interestingly, not limited to the edible part of the fruit, which is likely to mediate in protective mechanisms against a wide range of oxidative and inflammatory human disorders, including cancer, type 2 diabetes, atherosclerosis and cardiovascular diseases [2].

Low-temperature storage is considered the most efficient way to preserve the postharvest quality of horticultural crops for an extended period of time. However, for some tropical or subtropical perishable products, such as pomegranate fruit, too long cold storage could result in a sequence of physiological

disorders collectively known as chilling injury (CI), leading to significant loss of quality [3]. Therefore, finding safe, effective, and preferably non-chemical treatments to reduce postharvest losses during cold storage of pomegranate is both worthwhile and inevitable.

Intermittent warming (IW) is a potential environmentally friendly postharvest technique to alleviate chilling injury (CI) in cold-stored products and refers to the periodic exposure of fruit to warm temperatures at 20–27°C during storage [3–7]. The timing of treatment is critical [8], i.e., the initial interruption of cold storage must occur before the chilling damages become irreversible [5, 9]. In addition, it is essential to understand the ideal temperature, duration, and frequency that could be specific to each product and cultivar [9]. There are several hypotheses regarding IW's mechanism of action in alleviating chilling damage, e.g., an induced change in unsaturated fatty acids (UFAs) concentration which is considered involved in membrane safety at low temperatures. It was assumed that shifting the temperature from low to high and then from high to low would probably result in an increment of saturated fatty acids (SFAs) followed by desaturation leading to accumulation of UFAs and a greater degree of unsaturation. This change was suggested to affect membrane fluidity and result in increased tolerance to low temperatures [6, 7, 10]. In addition, it has been reported that IW promotes polyamines (PAs) production in treated tissues [11]. It has been proposed that the mechanism of action for the alleviation of CI by exogenous PAs or treatments that enhance their endogenous values may be related to their ability to bind membranes and to have antioxidant activity [12] which mitigates changes in membrane fluidity and solute leakage [13] and results in membrane stability and delayed disintegration [14].

In contrast to achieving consensus on IW as a potential postharvest method, related research efforts in the new millennium have decreased. It is so important to keep in mind that there are challenges in choosing IW for commercial applications. It means that repeatedly increasing and decreasing the storage temperature is a slow and energy-intensive process. In addition, as an alternative, shifting the product from cold to warm rooms for several times is labor-intensive and requires the accessibility of specific spaces [3]. On the one hand, it is recommended that IW for crops with short shelf life, such as cucumbers, sweet peppers, and zucchini squash, be used more frequently [7, 11, 15]. On the other hand, if the warming is applied too frequently or for too long, an increased loss of quality may occur [16]. The authors believe it could be beneficial, more applicable, and commercially acceptable if only one cycle of IW is adequate to alleviate the incidence of CI and should be investigated for commercially important cultivars. In the other part of our research on the cold storage of 'Rabab-e-Neyriz' pomegranate fruit, the beneficial effect of one cycle of IW was revealed and related to the promotion of enzymatic and non-enzymatic antioxidant responses [17]. However, as stated by Biswas et al. [3], more research is needed to investigate the mechanisms by which IW alleviates CI, which can lead to alternative novel methods with similar advantages. To the best of our knowledge, more studies are required to evaluate the effects of IW on modifications in membrane fatty acids (FAs) and its fluidity and stability in cold-stored sensitive crops such as pomegranate fruit. Moreover, there is no literature available on the possible beneficial effect of IW via modification of endogenous PAs in pomegranate.

Pomegranate cv. 'Rabab-e-Neyriz' is a late ripening, exportable and commercial Iranian cultivar. The aim of the present research was to investigate the effects of a single warming period on modifications in FAs and PAs in fruit peel and its relationship with the mitigation of chilling damage to this product.

2. Materials and Methods

2.1. Plant Material, Experimental Design, and Treatments. Fully mature pomegranates cv. 'Rabab-e-Neyriz' were picked from a commercial orchard in Neyriz (Fars province, Iran). On the day of collection, the fruit were placed in vented plastic crates and transported to the laboratory in a cold room set to 5°C. Upon arrival at the laboratory, fruit with defects were discarded and the remaining were stored at 2 ± 0.5°C (chilling temperature) and 90 ± 5% RH for 70 days. IW was performed by exposure of the fruit to only one period of high temperature (1 day at 20°C with 70% RH) by shifting the fruit to a warm room during the storage period. To find the best treatment time, 4 temporary interruption points were dedicated to warming, i.e., 15th, 25th, 35th, or 45th days of storage.

The experimental design was factorial based on a complete randomized design with three replications. It included 4 temporal points of interruption in storage (15th, 25th, 35th, or 45th days of storage) ×2 levels of warming regime (warming and control) ×2 levels of sampling time (immediately after treatment and at the end of storage). In other words, there was 4 distinct groups of fruit for each interruption day and 4 abbreviations were assigned: WI, WE, NI, and NE. The first letter explains the warming regime; whether the fruit were warmed (W) or not (N), and the second letter shows the sampling time; whether the samples were taken immediately after the warming regime (I) or at the end of the storage period (E). These groups included: Group A: fruit were removed from storage and immediately warmed by shifting to the warm room, and then sampled without delay (abbreviated to WI; as 15WI, 25WI, 35WI and 45WI). Group B: fruit immediately sampled after removal from storage without warming treatment (abbreviated to NI; as 15NI, 25NI, 35NI, and 45NI). Group C: fruit were removed from storage and immediately shifted to the warm room for warming treatment, then were returned to cold storage and kept until the end of the storage period and sampled at the end (abbreviated to WE; as 15WE, 25WE, 35WE and 45WE). Group D: non-treated fruit without removal from storage, which were sampled at the end of cold storage (was abbreviated to NE; as 15NE, 25NE, 35NE, and 45NE). In other words, for each interruption date, groups B and D served as control for groups A and C, respectively. From this point of view, for each interruption date, the treated fruit were compared with the controls twice, immediately after treatment or at the end of the storage period. The content and composition of the FAs and the amount of PAs and malondialdehyde (MDA) in the peel samples

were analyzed. In addition, chilling injury (CI) index in different time-treated fruit was evaluated at the end of the storage followed by a shelf life of 3 days and compared with control. Intact fruit were used for this purpose.

For the preparation of the sample, each husk was carefully cut in the equatorial zone and the peels were manually separated. Peel tissues from 10 fruit in each replicate were combined, frozen in liquid N_2, and stored at −80°C for later analytical determinations.

2.2. MDA Content and CI Index. The level of lipid peroxidation in the peel tissue was measured in terms of MDA content (a product of lipid peroxidation) determined by the thiobarbituric acid (TBA) reaction with minor modification of the method of Heath and Packer [18]. Briefly, 0.25 g of peel sample was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenated sample was centrifuged at 10000×g for 5 min. To 250 μL aliquot of the supernatant, 1 mL 20% TCA containing 0.5% TBA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After being centrifuged at 10000×g for 10 min, the absorbance of the supernatant was read at 532 nm and the value for the nonspecific absorption at 600 nm was subtracted. The concentration of MDA was calculated using its extinction coefficient (ε) of 155 mM^{-1} cm^{-1} reported as μmol per g of peel fresh weight.

The CI index was assessed separately in each fruit with a 4-point hedonic scale based on the proportion of the peel surface impacted by CI symptoms (dehydration, browning, and pitting) [19]: 0 (no symptoms), 1 (1–25% of damaged area), 2 (26–50% of damaged area) and 3 (>50% of damaged area). The results were expressed as the mean±SD of CI calculated using the following formula:

$$\mathrm{CI} = \sum(\text{value of hedonic scale} \times \text{number of fruit with the corresponding scale number})/(4 \times \text{total number of fruit in the sample}). \quad (1)$$

2.3. Fatty Acids (FAs) Quantification. Total lipids were extracted according to the method of Rui et al. [20]. A gas chromatograph (GC, HP-model 6890) equipped with a flame ionization detector was used to separate and quantify fatty acids according to Mirdehghan et al. [21]. At first, two g of skin was homogenised in 10 mL of chloroform:-methanol:0.1 N HCl (200 : 100 : 1). Then, 10 mL of 0.1 N HCl was added before centrifugation at 4000 × g for 10 min. It was allowed to the organic phase to be dried. By adding 1 mL of boron trifluoride/methanol at boiling temperature for 10 min, fatty acid methylation was done. Using hexane, methylated fatty acids were extracted and then allowed to be dried and redissolved in 200 μL chloroform before injection. For fatty acid separation and quantification, a HP-Innowax polyethylene glycol capillary column (30 m × 250 μm × 25 μm) and a gradient of temperature (initially 120°C for 2 min and then a rate at 4°C/min to 190°C which was held for 5 min, and final rate at 4°C/min to 242°C) were used. Fatty acids were identified and quantified by comparing retention times and peak areas with authentic standards (Sigma–Aldrich, USA). Results were expressed as mg 100 g^{-1} fresh weight.

2.4. Polyamines (PAs) Quantification. Sample preparation and HPLC analysis of PAs was performed according to Mirdehghan et al. [21] with some modifications. For each replicate, 1 g of fresh tissue was homogenized with 10 mL of 5% cold perchloric acid. The homogenate was then centrifuged for 30 min at 20000 × g. The resulted supernatant was filtered through a 0.45 μm filter (Millipore) and used to determine free PAs by benzoylation, and derivatives analysed by HPLC (UnicamCrystal-200, UK). A 10 μL of filtered supernatant was used for this purpose. The elution system consisted of $MeOH/H_2O$ (64 : 36) solvent, running isocratically with a flow rate of 0.8 mL min^{-1} through a reversed-phase column (LiChroCart 250-4.5 μm) and detection was based on UV absorbance at 254 nm. PAs were identified and quantified by comparing retention times and peak areas with authentic standards (Sigma–Aldrich, USA). Results were expressed as nmol g^{-1} fresh weight.

2.5. Statistical Analysis. All data were subjected to two-way analysis of variance (ANOVA) performed with the SAS 9.1.3 service pack 4 software (SAS Institute, Cary, NC, USA), and the means were separated by the least significant difference (LSD) test at $P \leq 0.05$.

3. Results

3.1. MDA Content and CI Index. Mean comparisons showed that the MDA content increased significantly during storage. Generally, the warming resulted in a significantly lower MDA content compared to untreated fruit. Moreover, the mean value at the end of storage was significantly higher than the time of interruption (Table 1).

The amount of MDA in the peel of control fruit was nearly similar to the harvesting time until the 25th day of cold storage, and there was no significant difference between the cold-stored fruit sampled on the 15th and 25th days of storage. Subsequently, there was a significant increasing trend along with storage time. Fruit warming at 15th and 45th days instantly reduced the MDA content of the peel compared to the controls (Table 2). On the other hand, at the end of the storage period, the fruit treated at interruption dates had significantly lower MDA (Table 2) and CI index (Table 3) compared to the controls with the lowest levels recorded for the fruit treated on the 15th and 25th days.

Table 1: Effects of experimental factors on the malondialdehyde content; total saturated, monounsaturated, polyunsaturated, and unsaturated fatty acids; total fatty acids and the ratio of unsaturated/saturated fatty acids in the peel of cold-stored pomegranate.

	MDA (μmol g^{-1} fresh weight)	Total SFAs (mg 100 g^{-1} fresh weight)	Total MUFAs (mg 100 g^{-1} fresh weight)	Total PUFAs (mg 100 g^{-1} fresh weight)	Total UFAs (mg 100 g^{-1} fresh weight)	Total FAs (mg 100 g^{-1} fresh weight)	UFAs/SFAs
Temporal point of interruption in storage (day)							
15	19.70 ± 0.072^{d}	48.76 ± 0.048^{b}	28.00 ± 0.028^{a}	29.15 ± 0.030^{a}	57.15 ± 0.058^{a}	105.90 ± 0.019^{a}	1.21 ± 0.002^{a}
25	21.80 ± 0.058^{c}	49.27 ± 0.045^{b}	27.60 ± 0.028^{a}	28.70 ± 0.029^{a}	56.30 ± 0.057^{b}	105.57 ± 0.021^{a}	1.18 ± 0.002^{b}
35	28.26 ± 0.035^{b}	49.00 ± 0.046^{b}	24.65 ± 0.016^{b}	25.66 ± 0.016^{b}	50.31 ± 0.031^{c}	100.26 ± 0.025^{b}	1.05 ± 0.001^{c}
45	32.37 ± 0.024^{a}	53.84 ± 0.044^{a}	22.74 ± 0.010^{c}	23.68 ± 0.011^{c}	46.42 ± 0.020^{d}	99.32 ± 0.028^{b}	0.88 ± 0.001^{d}
Warming regime							
Warming	22.50 ± 0.012^{b}	46.59 ± 0.006^{b}	27.12 ± 0.005^{a}	28.24 ± 0.005^{a}	55.36 ± 0.010^{a}	101.95 ± 0.010^{b}	1.20 ± 0.001^{a}
Not warming	28.57 ± 0.016^{a}	53.84 ± 0.012^{a}	24.37 ± 0.007^{b}	25.36 ± 0.007^{b}	49.73 ± 0.014^{b}	103.57 ± 0.005^{a}	0.96 ± 0.001^{b}
Sampling time							
Immediately	20.54 ± 0.013^{b}	46.33 ± 0.007^{b}	27.73 ± 0.005^{a}	28.87 ± 0.006^{a}	56.59 ± 0.011^{a}	102.91 ± 0.008^{a}	1.24 ± 0.001^{a}
At the end of storage	30.53 ± 0.012^{a}	54.10 ± 0.011^{a}	23.76 ± 0.006^{b}	24.73 ± 0.006^{b}	48.50 ± 0.012^{b}	102.60 ± 0.008^{a}	0.92 ± 0.001^{b}

Data are means of replicates ($n = 12$ for each interruption date in storage and 24 for each warming regime and sampling date) ± standard error. Fruit were stored for 70 days at 2 ± 0.5°C and 90 ± 5% relative humidity. Warming was performed as 1 day at 20°C with 70% relative humidity. MDA: malondialdehyde; SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; UFAs: unsaturated fatty acids; FAs: fatty acids and UFAs/SFAs: unsaturated/saturated fatty acids ratio. For each experimental factor and evaluated index, means followed by the same letter are not significantly different by least significant difference (LSD) test, $P < 0.05$.

Table 2: The content of malondialdehyde and fatty acids in pomegranate fruit peel under one intermittent warming cycle during 70 days of cold storage.

	MDA (μmol g^{-1} fresh weight)	SFAs (mg 100 g^{-1} fresh weight)							MUFAs (mg 100 g^{-1} fresh weight)		PUFAs (mg 100 g^{-1} fresh weight)	
		C10	C12	C14	C15	C16	C17	C18	C16:1	C18:1	C18:2	C18:3
At harvest	18.60 ± 0.74	1.46 ± 0.11	5.36 ± 0.20	8.67 ± 0.45	1.49 ± 0.13	16.39 ± 0.66	1.92 ± 0.27	12.10 ± 1.53	17.78 ± 1.07	11.26 ± 1.63	12.44 ± 0.67	17.75 ± 1.47
Treatments												
15NI	13.44 ± 0.17^{g}	1.35 ± 0.09^{bc}	5.02 ± 0.06^{cd}	8.18 ± 0.14^{cd}	1.36 ± 0.14^{bc}	15.47 ± 0.37^{de}	1.83 ± 0.9^{bc}	11.37 ± 0.49^{cde}	18.56 ± 0.49^{a}	11.74 ± 0.60^{ab}	12.99 ± 0.77^{a}	18.56 ± 0.59^{a}
15WI	10.22 ± 0.16^{h}	1.30 ± 0.19^{c}	4.77 ± 0.21^{d}	7.81 ± 0.56^{d}	1.30 ± 0.13^{c}	14.76 ± 0.85^{e}	1.73 ± 0.16^{c}	10.85 ± 0.75^{e}	18.63 ± 0.77^{a}	11.79 ± 0.69^{ab}	13.04 ± 0.71^{a}	18.65 ± 0.63^{a}
15NE	35.93 ± 1.69^{a}	1.82 ± 0.07^{a}	6.72 ± 0.19^{a}	10.98 ± 0.41^{a}	1.80 ± 0.05^{a}	20.78 ± 0.59^{a}	2.41 ± 0.16^{a}	15.29 ± 0.39^{a}	12.97 ± 0.33^{e}	8.25 ± 0.37^{g}	9.13 ± 0.22^{d}	12.92 ± 0.46^{d}
15WE	19.22 ± 0.58^{e}	1.48 ± 0.21^{b}	5.40 ± 0.38^{cd}	8.83 ± 0.58^{c}	1.47 ± 0.13^{bc}	16.70 ± 0.52^{c}	1.96 ± 0.17^{b}	12.27 ± 0.52^{c}	18.40 ± 0.56^{a}	11.66 ± 0.46^{ab}	12.89 ± 0.82^{a}	18.41 ± 0.80^{a}
25NI	15.21 ± 0.57^{fg}	1.37 ± 0.13^{bc}	5.01 ± 0.44^{cd}	8.21 ± 0.79^{cd}	1.37 ± 0.16^{bc}	15.50 ± 1.06^{de}	1.82 ± 0.12^{bc}	11.40 ± 0.91^{cde}	18.90 ± 1.01^{a}	11.95 ± 0.69^{a}	13.21 ± 0.68^{a}	18.88 ± 0.71^{a}
25WI	16.74 ± 0.62^{f}	1.40 ± 0.13^{bc}	5.16 ± 0.41^{cd}	8.45 ± 0.44^{cd}	1.40 ± 0.17^{bc}	15.94 ± 0.69^{cd}	1.87 ± 0.11^{bc}	11.72 ± 0.88^{cde}	18.45 ± 0.28^{a}	11.67 ± 0.60^{ab}	12.91 ± 0.90^{a}	18.44 ± 0.50^{a}
25NE	35.34 ± 1.19^{a}	1.81 ± 0.08^{a}	6.69 ± 0.22^{a}	10.99 ± 0.33^{a}	1.83 ± 0.09^{a}	20.74 ± 0.66^{a}	2.42 ± 0.11^{a}	15.18 ± 0.40^{a}	12.98 ± 0.47^{e}	8.20 ± 0.33^{g}	9.03 ± 0.30^{d}	12.95 ± 0.25^{d}
25WE	19.95 ± 0.25^{e}	1.36 ± 0.04^{bc}	5.01 ± 0.29^{cd}	8.74 ± 0.08^{c}	1.49 ± 0.02^{b}	16.31 ± 0.09^{cd}	1.92 ± 0.04^{bc}	11.97 ± 0.35^{cd}	17.22 ± 0.23^{b}	11.03 ± 0.13^{bc}	12.31 ± 0.09^{a}	17.09 ± 0.58^{b}
35NI	23.84 ± 1.01^{d}	1.39 ± 0.09^{bc}	5.54 ± 0.35^{bc}	8.46 ± 0.38^{cd}	1.40 ± 0.09^{bc}	15.97 ± 0.45^{cd}	1.87 ± 0.09^{bc}	11.75 ± 0.52^{cde}	16.25 ± 0.38^{c}	10.28 ± 0.36^{cd}	11.36 ± 0.55^{b}	16.23 ± 0.51^{b}
35WI	24.11 ± 0.81^{d}	1.38 ± 0.07^{bc}	5.15 ± 0.41^{cd}	8.45 ± 0.37^{cd}	1.39 ± 0.10^{bc}	15.96 ± 0.76^{cd}	1.86 ± 0.06^{bc}	11.69 ± 0.62^{cde}	16.18 ± 0.45^{c}	10.24 ± 0.61^{cde}	11.32 ± 0.42^{b}	16.17 ± 0.42^{b}
35NE	35.72 ± 1.22^{a}	1.81 ± 0.07^{a}	6.71 ± 0.34^{a}	10.97 ± 0.40^{a}	1.84 ± 0.10^{a}	20.70 ± 0.48^{a}	2.43 ± 0.10^{a}	15.27 ± 0.49^{a}	12.96 ± 0.53^{e}	8.26 ± 0.31^{g}	9.06 ± 0.24^{d}	13.07 ± 0.31^{d}
35WE	29.36 ± 0.86^{c}	1.37 ± 0.09^{bc}	4.93 ± 0.48^{cd}	8.09 ± 0.46^{cd}	1.37 ± 0.09^{bc}	15.26 ± 0.53^{de}	1.78 ± 0.08^{bc}	11.22 ± 0.42^{de}	14.95 ± 0.40^{d}	9.48 ± 0.36^{ef}	10.47 ± 0.31^{bc}	14.97 ± 0.65^{c}
45NI	33.21 ± 1.99^{b}	1.71 ± 0.07^{a}	6.31 ± 0.51^{a}	10.33 ± 0.41^{ab}	1.72 ± 0.07^{a}	19.52 ± 0.56^{b}	2.30 ± 0.21^{a}	14.30 ± 0.45^{ab}	13.74 ± 0.72^{e}	8.72 ± 0.61^{fg}	9.64 ± 0.62^{cd}	13.77 ± 0.73^{d}
45WI	27.55 ± 1.31^{c}	1.35 ± 0.05^{bc}	4.98 ± 0.39^{cd}	8.17 ± 0.49^{cd}	1.36 ± 0.05^{bc}	15.43 ± 0.52^{de}	1.81 ± 0.07^{bc}	11.34 ± 0.55^{cde}	15.14 ± 0.54^{d}	9.59 ± 0.41^{de}	10.60 ± 0.64^{b}	15.15 ± 0.55^{c}
45NE	35.87 ± 2.46^{a}	1.82 ± 0.08^{a}	6.70 ± 0.60^{a}	10.97 ± 0.69^{a}	1.82 ± 0.10^{a}	20.73 ± 1.32^{a}	2.43 ± 0.26^{a}	15.24 ± 0.73^{a}	12.97 ± .67^{e}	8.22 ± 0.64^{g}	9.08 ± 0.32^{d}	12.98 ± 0.50^{d}
45WE	32.85 ± 2.07^{b}	1.66 ± 0.07^{a}	6.18 ± 0.42^{ab}	10.11 ± 0.50^{b}	1.68 ± 0.08^{a}	19.10 ± 0.59^{b}	2.24 ± 0.09^{a}	14.05 ± 0.40^{b}	13.82 ± 0.81^{e}	8.76 ± 0.38^{fg}	9.68 ± 0.62^{cd}	13.83 ± 0.65^{d}
LSD	2.109	0.161	0.649	0.783	0.178	1.112	0.200	0.994	0.858	0.793	0.916	0.928

Fruit were stored at 2 ± 0.5°C and 90 ± 5% relative humidity. Intermittent warming cycle was 1 day at 20°C with 70% relative humidity before returning the treated fruit to storage. Temporal points of interruption were 15th, 25th, 35th, or 45th days of storage. WI stands for warmed fruit which were immediately sampled after warming on the date of interruption, NI stands for not warmed fruit which were sampled on the date of interruption, WE stands for warmed fruit, which were returned to storage and sampled at the end of the storage period, and NE stands for not warmed fruit sampled at the end of the storage period. MDA: malondialdehyde; SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; C10: Capric acid; C12: Lauric acid; C14: Myristic acid; C15: Pentadecylic acid; C16: Palmitic acid; C17: Margaric acid; C18: Stearic acid; C16:1: Palmitoleic acid; C18:1: Oleic acid; C18:2: Linoleic acid and C18:3: Linolenic acid. Data are means of 3 replicates ± standard deviation. Means within a column followed by the same letter are not significantly different by least significant difference (LSD) test, $P < 0.05$.

Table 3: Differences in chilling injury index between control and intermittently warmed fruit after 70 days of cold storage and additional 3 days shelf life at 20°C.

	Warming day during storage				
	C	15	25	35	45
CI index	0.48 ± 0.04^{a}	0.18 ± 0.04^{d}	0.21 ± 0.03^{d}	0.27 ± 0.03^{c}	0.33 ± 0.02^{b}

Fruit were stored at 2 ± 0.5°C and 90 ± 5% relative humidity. Intermittent warming was conducted in the form of a single warming period (1 day at 20°C with 70% relative humidity) before returning the treated fruit to storage. Temporal points of interruption were 15th, 25th, 35th, or 45th days of storage. CI: Chilling injury; and C: control fruit. Data are means of 3 replicates ± standard deviation. Means with the same letter are not significantly different by least significant difference (LSD) test, $P < 0.05$.

3.2. FAs Modifications

3.2.1. SFAs, Mono and Poly UFAs. The following FAs have been identified and quantified in the peel samples (Figure 1(a) and Table 2): capric acid (C10), lauric acid (C12), myristic acid (C14), pentadecylic acid (C15), palmitic acid (C16), margaric acid (C17) and stearic acid (C18) as saturated (SFAs); palmitoleic acid (C16:1) and oleic acid (C18:1) as monounsaturated (MUFAs); and linoleic acid (C18:2) and linolenic acid (C18:3) as polyunsaturated (PUFAs) ones.

3.2.2. Total SFAs (Membrane Saturation Index). The fruit had significantly higher total SFAs at the last interruption time (45th day) of storage than other times. In addition, statistically lower mean values were recorded for the warmed fruit and those sampled at the interruption dates compared to the untreated fruit and those sampled at the end of the experiment, respectively (Table 1).

Up to 35 days of cold storage, the total SFAs did not change. Subsequently, a significant increasing trend was detected, with the highest amount recorded at the end of storage. The instant effect of warming on the membrane saturation index was detected only on the fruit treated on the 45th day, leading to a statistical decrease in the index compared to the control. However, treating the fruit at any time of interruption in storage resulted in significantly lower total SFAs recorded at the end of the experiment compared to the controls (Figure 2(a)).

3.2.3. Total MUFAs, PUFAs, and UFAs (Membrane Unsaturation Index). The results showed a significant decrease in all unsaturation indices during cold storage. On the other hand, the mean values of the warmed fruit and fruit immediately sampled at interruption dates were significantly higher than those of the untreated fruit and fruit sampled at the end of the cold storage period, respectively (Table 1).

Total MUFAs, PUFAs, and therefore membrane unsaturation index increased to the 25th day of storage, even more than the harvesting time. Subsequently, a significant decreasing trend was identified until the end of storage. By warming the fruit on the 45th day of cold storage, all unsaturation indices were increased significantly, even though at the end of the experiment, the fruit treated at any time during the storage period had significantly higher levels of MUFAs, PUFAs and UFAs than the controls (Figures 2(b), 2(c) and 2(d)).

3.2.4. Total FAs. Mean comparisons showed that the total FAs content at the first and second interruption dates was significantly higher than at the later dates, with no significant difference between the fruit sampled at the 15th and 25th or 35th and 45th days. Generally, the treated fruit had significantly lower total FAs than the untreated fruit, and there was no significant difference between the fruit sampled at interruption dates or at the end of the experiment (Table 1).

On the one hand, the total FAs increased to the 25th day of storage, even more than the harvesting time. On the other hand, the index subsequently decreased significantly, without a statistical difference between the fruit sampled on the 35th day and later. Fruit warming at interruption dates had no immediate impact on total FAs content except on the 45th day, which resulted in a significant decrease in the index compared to the control. Moreover, only fruit treated during the first month had higher total FAs compared to controls at the end of the storage period. From this point of view, there was a significant difference between the fruit treated on the 15th day and the control fruit at the end of the experiment (Figure 2(e)).

3.2.5. UFAs/SFAs Ratio (Membrane Integrity Index). It was observed a significant decreasing trend in the UFAs/SFAs ratio with progress in the storage period. In addition, a significantly higher mean value was found for treated fruit and fruit immediately sampled at interruption dates compared to untreated fruit and those sampled at the end of the storage period (Table 1).

The membrane integrity index improved to the 25th day of storage, even better than the harvesting time. Subsequently, a significant decreasing trend was detected up to the end of storage. On the other hand, at the end of the storage period, all fruit treated at interruption dates had a statistically higher UFAs/SFAs ratio compared to controls (Figure 2(f)).

3.3. PAs Modifications. The three main polyamines, putrescine (Put), spermidine (Spd), and spermine (Spm) in their free forms, were identified and quantified in pomegranate peel (Figure 1(b)).

3.3.1. Put Content. Mean comparisons showed that the advancement in storage from the 25th day to the end was followed by a significant decrease in the concentration of Put. Treated fruit had significantly higher content of Put than untreated fruit. In addition, the amount of Put was

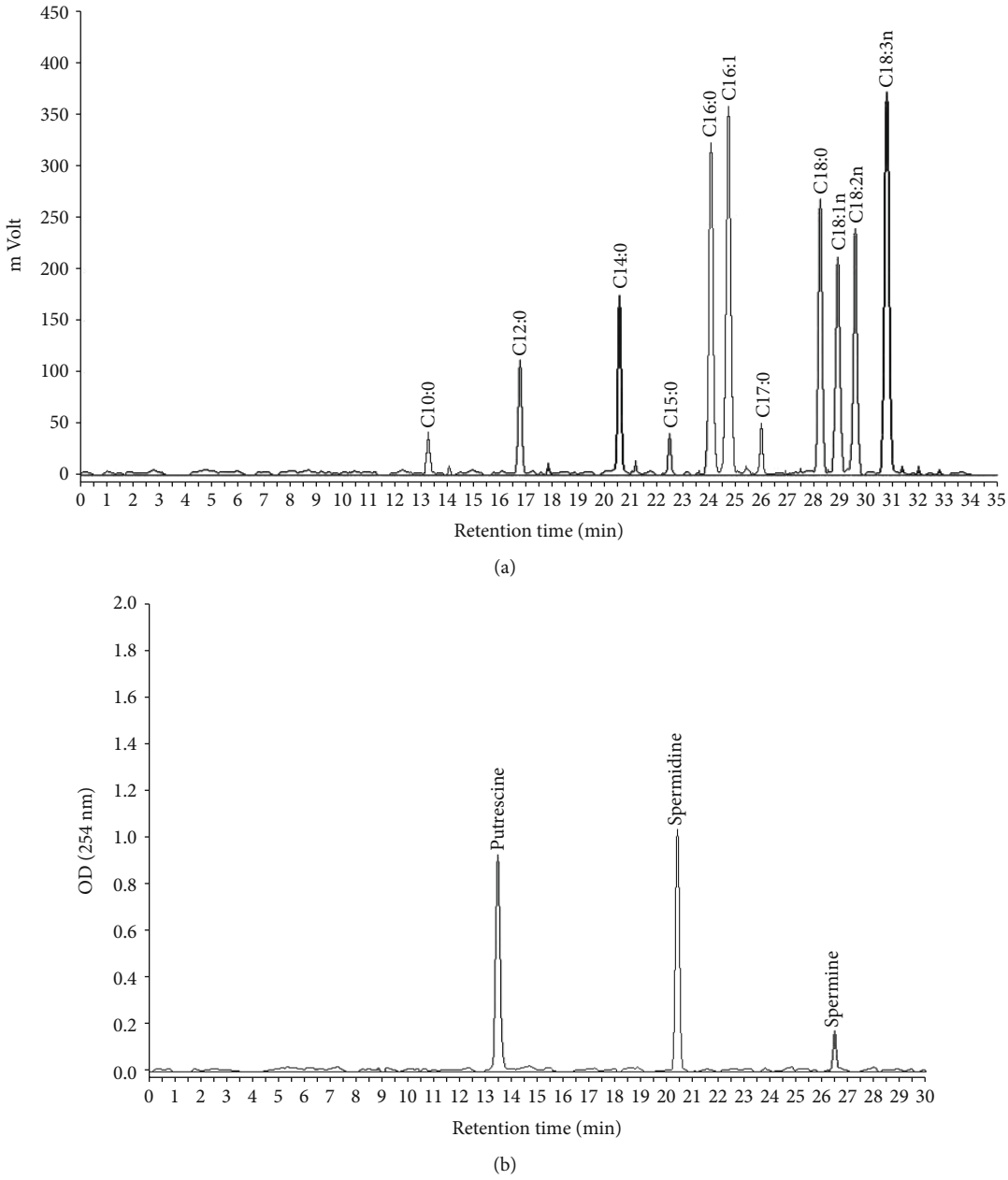


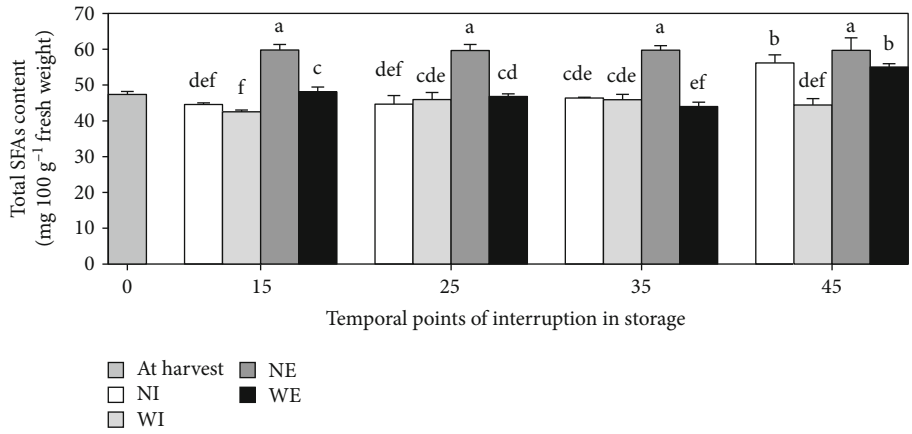
Figure 1: GC chromatogram of Fatty Acids (a), and HPLC chromatogram of Polyamines (b) in pomegranate peel. C10: Capric acid; C12: Lauric acid; C14: Myristic acid; C15: Pentadecylic acid; C16: Palmitic acid; C17: Margaric acid; C18: Stearic acid; C16:1: Palmitoleic acid; C18:1: Oleic acid; C18:2: Linoleic acid and C18:3 Linolenic acid.

statistically lower at the end of storage than during storage (Table 4).

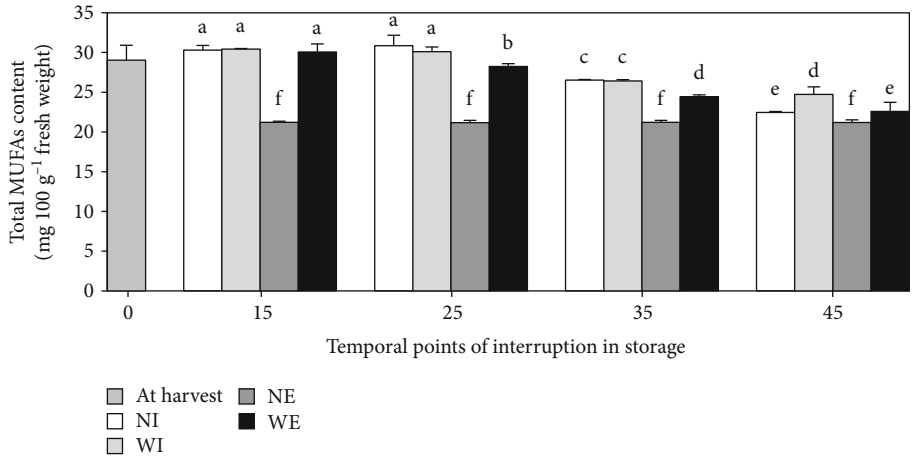
During cold storage, the content of Put decreased significantly and continuously until the 45th day, with no statistical difference between the 45th and 70th days. Warming at interruption dates resulted in an immediate increase and higher final contents compared to controls, except for the fruit treated on the 45th day which had the final amount of Put the same as the control (Figure 3(a)).

3.3.2. Spd Content. According to the mean comparison, the concentration of Spd increased significantly as the storage time progressed. In addition, the treated fruit had statistically higher levels of Spd than the untreated fruit and the amount of Spd at the end of storage was significantly higher than what was recorded during that period (Table 4).

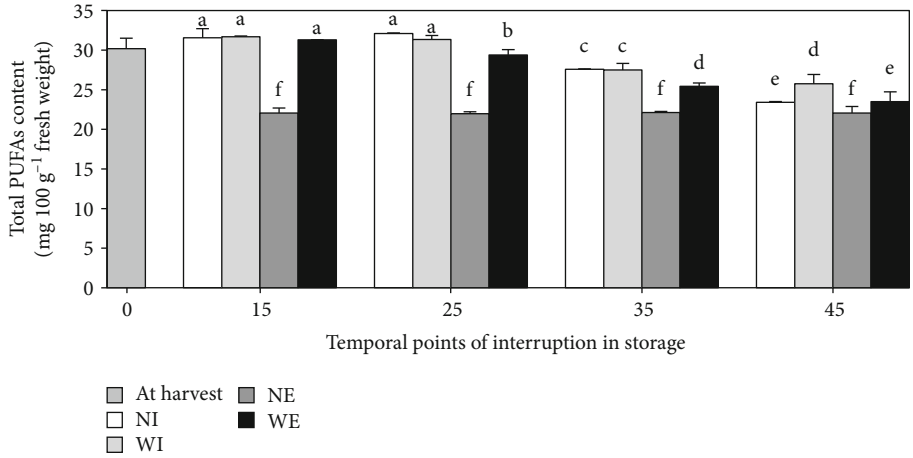
Statistically, the internal concentration of Spd was continuously increased until the 35th day of cold storage, with no differences between the fruit sampled on the 35th and 45th days. Subsequently, a significant decrease in Spd content was recorded for the last 25 days of cold storage. Warming at all interruption dates, except for the 15th day, resulted in a significant instant increase and a statistically higher final value of Spd at the end of storage compared to the control fruit (Figure 3(b)).



(a)

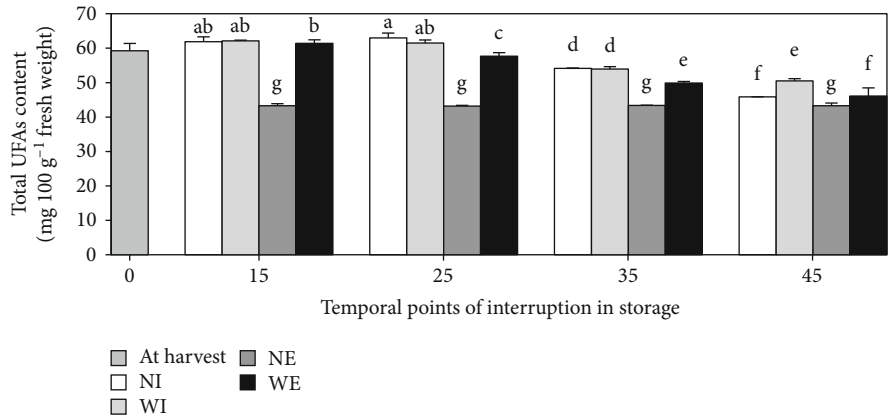


(b)

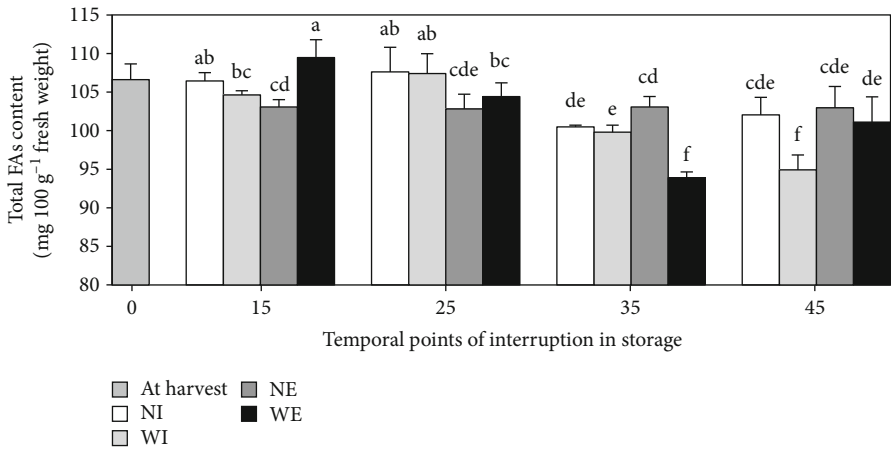


(c)

Figure 2: Continued.



(d)



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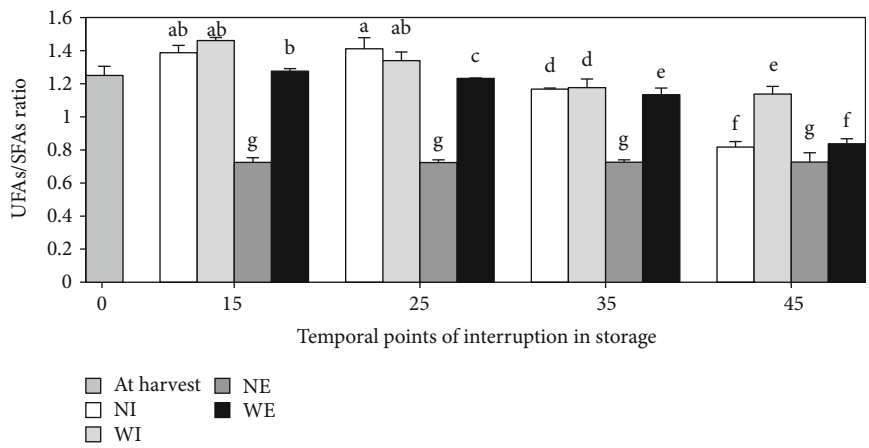

(f)

Figure 2: Modifications in the total content of saturated (a), monounsaturated (b), polyunsaturated (c), and unsaturated (d) fatty acids, total fatty acids (e), and unsaturated/saturated fatty acids ratio (f) in pomegranate fruit peel during 70 days storage (at 2 ± 0.5°C and 90 ± 5% RH). Intermittent warming was conducted in the form of a single warming period (1 day at 20°C with 70% RH) before returning the treated fruit to storage. Temporal points of interruption were 15th, 25th, 35th, or 45th days of storage. WI stands for warmed fruit which were immediately sampled after warming on the date of interruption, NI stands for not warmed fruit which were sampled on the date of interruption, WE stands for warmed fruit, which were returned to storage and sampled at the end of the storage period, and NE stands for not warmed fruit sampled at the end of the storage period. Data are means of 3 replicates ± SD. All statistical differences (by LSD test, $P \leq 0.05$) throughout the storage period are shown in different letters.

Table 4: Effects of experimental factors on the content of polyamines (PAs) in the peel of cold-stored pomegranate.

	Put (nmol g^{-1} fresh weight)	Spd (nmol g^{-1} fresh weight)	Spm (nmol g^{-1} fresh weight)
Temporal point of interruption in storage (day)			
15	269.50 ± 0.84^{a}	221.00 ± 0.34^{d}	26.65 ± 0.04^{b}
25	248.38 ± 0.79^{a}	295.00 ± 0.38^{c}	29.94 ± 0.05^{a}
35	194.50 ± 0.51^{b}	346.00 ± 0.45^{b}	19.25 ± 0.03^{c}
45	122.33 ± 0.22^{c}	374.00 ± 0.53^{a}	17.06 ± 0.03^{d}
Warming regime			
Warming	263.56 ± 0.16^{a}	350.30 ± 0.16^{a}	26.57 ± 0.01^{a}
Not warming	153.79 ± 0.16^{b}	267.76 ± 0.09^{b}	19.88 ± 0.01^{b}
Sampling time			
Immediately	260.38 ± 0.18^{a}	293.79 ± 0.16^{b}	22.19 ± 0.01^{b}
At the end of storage	156.98 ± 0.14^{b}	324.25 ± 0.13^{a}	24.26 ± 0.01^{a}

Data are means of replicates ($n = 12$ for interruption date in storage and 24 for warming regime and sampling date) ± standard error. Fruits were stored for 70 days at 2 ± 0.5°C and 90 ± 5% relative humidity. Warming was performed as 1 day at 20°C with 70% relative humidity. Put: putrescine; Spd: spermidine; Spm: spermine. For each experimental factor and evaluated index, means followed by the same letter are not significantly different by the least significant difference (LSD) test, $P < 0.05$.

3.3.3. Spm Content. Mean comparisons showed that more Spm level was detected in the fruit sampled on the 25th day of storage and those sampled on the 15th, 35th, and 45th days were at the next position, respectively, and all differences were significant. Generally, the warming resulted in statistically higher Spm content and the value detected at the end of storage was significantly higher than that measured at the time of interruption (Table 4).

Modifications in the concentration of Spm during storage could be categorized into three different parts: an increase up to the 25th day, subsequent decrease up to the 45th day, and then increase up to the end of storage. Most of the differences were almost significant. Warming during storage immediately led to a significant increase in the content of Spm. Although only fruits treated on the 15th and 25th days had statistically more Spm than controls at the end of the storage period, the other time-treated fruit were the same as the controls. The fruit sampled on the 25th day had a statistically higher endogenous content of Spm compared to other different time-sampled fruit and had a significantly higher instant increase and final content of Spm in response to treatment than the fruit treated at the other interruption dates (Figure 3(c)).

4. Discussion

4.1. MDA Content and CI Index. The investigation of changes in the MDA content (Table 2) revealed that lipid peroxidation increased after one month of chilling stress. Examining the physiological changes and the incidence of chilling injury in the same storage condition as we did earlier, Taghipour et al. [8] showed that pomegranate fruit cv. Rabab-e-Neyriz could be stored in cold temperature without significant CI for up to 30 days. From this point of view, our finding was consistent with them. At the end of the storage period, all different time-treated fruit had a significantly lower MDA and CI index compared to the controls, with the lowest levels recorded for the fruit treated on the 15th and 25th days (Tables 2 and 3). In accordance with our finding, Taghipour et al. [8] suggested that the warming should be carried out during the first month of cold storage, predicting it with the possible desired effect of extending the shelf life of this commercial cultivar. These data suggested IW's prominent efficacy in alleviating the incidence of pomegranate CI at low temperatures, which part of the related mechanisms are discussed based on our findings in the following parts of discussion.

4.2. FAs Modifications. It has been accepted that chilling-resistant subtropical and tropical horticultural crops have greater levels of UFAs and UFAs/SFAs ratio in cell membrane lipids [22, 23]. Higher levels of UFAs lead to higher membrane fluidity, identified as part of low-temperature tolerance mechanisms [24–26]. It means that the intensity of the lipid phase shift from flexible liquid crystalline to solid gel is lower in the membrane with greater fluidity, thus maintaining the membrane permeability and greater tolerance to low temperatures [5, 25]. For example, it has been indicated that chilling-tolerant cultivars of loquat fruit have a greater linoleic (C18:2) and linolenic (C18:3) acid content and lower palmitic (C16) and stearic acid (C18) concentrations, resulting in a greater UFAs/SFAs ratio [27]. On the one hand, higher activity of phospholipase D (PLD) and lipoxygenase (LOX) enzymes could be liable for UFAs degradation at chilling temperatures leading to decreased cell membrane integrity and increased adverse effects of CI [28]. On the other hand, non-enzymatic oxidation of UFAs by reactive oxygen species (ROS) associated with MDA production could result in reduced membrane integrity and increased adverse effects of CI [29]. The integrity of the membrane plays a crucial role in fruit pericarp browning as one of the CI symptoms [29].

Results indicated that the values of all SFAs (Table 2) and the membrane saturation index (Figure 2(a)) of the fruit sampled at any time of interruption up to the 35th day of cold storage were statistically the same and, in most cases,

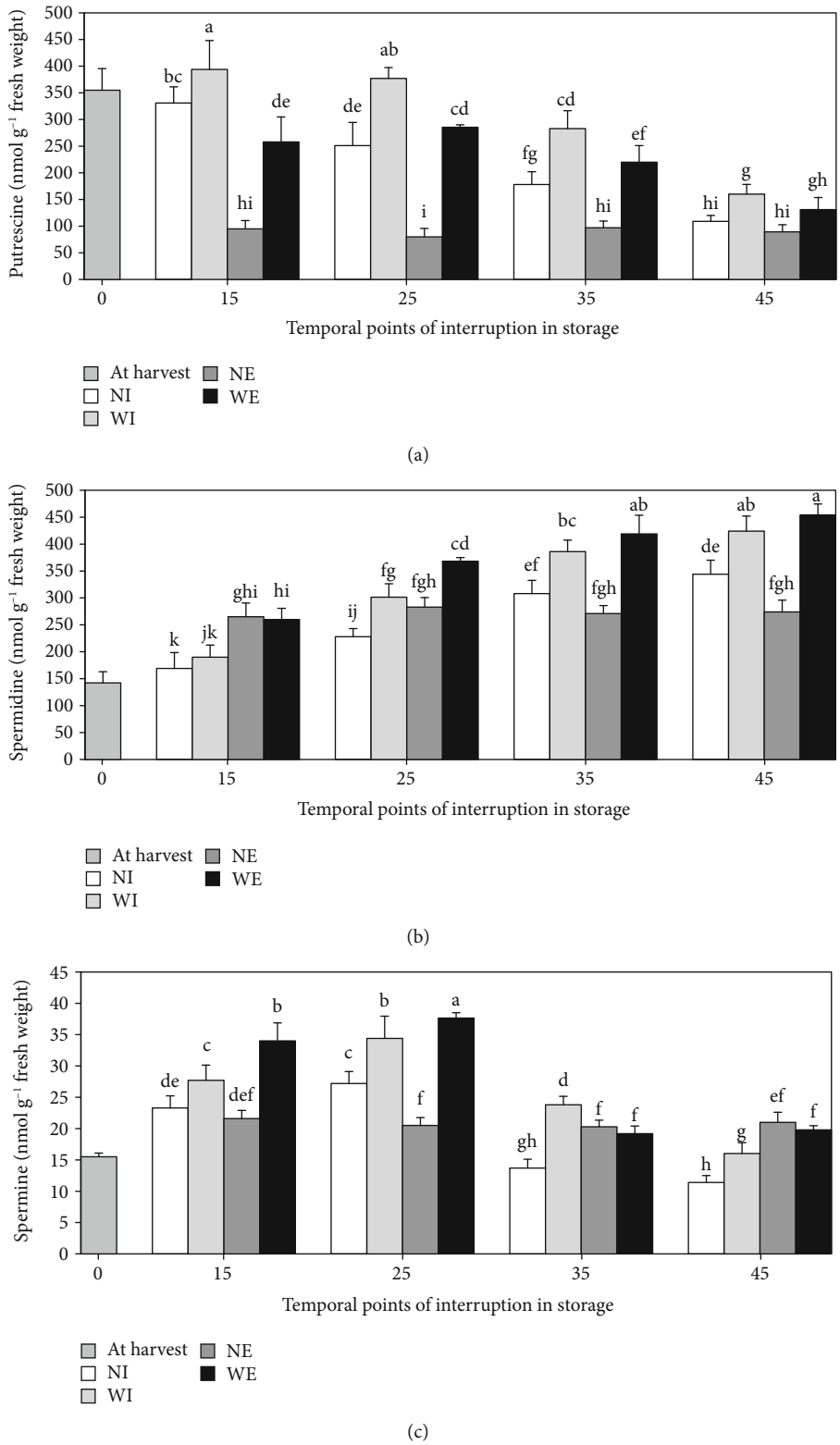

Figure 3: Modifications in the content of putrescine (a), spermidine (b), and spermine (c) in pomegranate fruit peel during 70 days storage (at 2 ± 0.5°C and 90 ± 5% RH). Intermittent warming was conducted in the form of a single warming period (1 day at 20°C with 70% RH) before returning the treated fruit to storage. Temporal points of interruption were 15th, 25th, 35th, or 45th days of storage. WI stands for warmed fruit which were immediately sampled after warming on the date of interruption, NI stands for not warmed fruit which were sampled on the date of interruption, WE stands for warmed fruit, which were returned to storage and sampled at the end of the storage period, and NE stands for not warmed fruit sampled at the end of the storage period. Data are means of 3 replicates ± SD. All statistical differences (by LSD test, $P \leq 0.05$) throughout the storage period are shown in different letters.

even lower than the harvest time value. Afterwards, as shown in Table 2, there was a significant increasing trend for all SFAs up to the 45th day of storage, which was continued only for palmitic acid during the last 25 days of the cold storage period. On the other hand, all MUFAs and PUFAs have risen up to the 25th day of storage, even more than the harvest time. However, a declining trend with significant differences between the fruit sampled on the 25th, 35th, and 45th days was subsequently founded, the latter having statistically the same contents of all MUFAs and PUFAs as detected at the end of the storage period. These data indicated that the fruit had significant changes in peel fatty acids following the expected adverse effects of CI on cell membrane integrity after approximately 1 month of cold storage. The stability of the membrane saturation index up to the 35th day (Figure 2(a)) and the stability of the unsaturation index up to the 25th day (Figure 2(d)), without an increase in the MDA level during the first month of storage (Table 2), could be associated with low-temperature fruit acclimatization to prevent chilling injury. This is similar to what was reported by Antunes and Sfakiotakis [30], as a major increase in the UFAs/SFAs ratio during the first days of kiwifruit storage as a response to tissue adaptation to new stress storage conditions.

Transferring cold-stored fruit to an elevated temperature and returning it to the previous temperature has been reported to regulate and readjust the metabolic processes leading to improved UFAs synthesis. In other words, the warming appears to result in an increase in the synthesis and elongation of the SFAs chains, which can act as a substrate for UFAs synthesis and increase the degree of unsaturation of the cell membrane after returning to cold temperature [6, 7, 10]. It has been noted, for instance, that peach and cucumber warming caused an increase in C18, while subsequent exposure to cold temperature resulted in an increase in C18:1, C18:2 and C18:3. It was suggested that IW could result in the enhanced amount of C18 as a prerequisite for UFAs synthesis, thus improving the degree of unsaturation in membrane lipids [6]. Rapid changes in membrane lipids with altered temperatures have also been reported in soybean roots. As the temperature was raised, C16 and C18 increased, but C18:1, C18:2, and C18:3 decreased in plasma membranes and mitochondrial membrane; the reverse trend happened as the temperature was lowered [31].

Moreover, the data collected at the end of storage showed that fruit treated for up to 35 days of cold storage had significantly higher amounts of all MUFAs and PUFAs (Table 2) and, as a result, higher levels of total MUFAs, PUFAs and UFAs (Figures 2(b), 2(c) and 2(d)) compared to control. Notably, in the fruit treated on the 15th day, these indices remained unchanged until the end of the experiment. Indeed, the fruit treated on the 45th day had a significant immediate increase in all MUFAs, PUFAs (Table 2) and total MUFAs, PUFAs and UFAs (Figures 2(b), 2(c) and 2(d)), leading to constant higher values compared to the control at the end of the storage period. On the other hand, the results showed that treatment at any time of interruption during storage did not result in immediate changes in the concentration of SFAs (Table 2) and the membrane saturation index (Figure 2(a)), except for a significant reduction in the indices on the 45th day. It was concluded that the statistically higher levels of UFAs and the unsaturation rates recorded at the end of the cold-warm-cold period could be mainly independent of an instant increase in SFAs in response to warming as a mandatory prerequisite. It could well be associated to the warm-induced mechanisms responsible for protecting UFAs at chilling temperatures. The warming on the 45th day led to an immediate and statistical increase in all UFAs, and a decrease in all SFAs and MDA content simultaneously (Table 2), indicating the possibility of a direct impact of treatment as an increase in UFAs, UFAs/SFAs ratio and a reduction in the peroxidation rate of UFAs. It means that the desaturation of SFAs could be a direct result of warming. This finding contrasts with the previous suggestions that the desaturation of SFAs is triggered by the return of the treated fruit to low temperatures [6, 7, 10]. The activity of LOX and PLD enzymes as a direct effect of warming may also be likely to decrease. To be proved, this claim needs to be studied.

4.3. PAs Modifications. Based on some of the scientific evidence, the protective role of PAs in alleviating different types of stress in plants has been proposed. Reducing endogenous polyamines by applying their biosynthesis inhibitors [32–34] or by using polyamine biosynthesis mutants [35, 36] has been shown to result in increased sensitivity to stress and increased injury to affected tissues. Moreover, exogenous application of polyamines before or during stress [33, 37–40] could lead to an increase in their endogenous content, leading to a reduction in stress-related injuries to varying degrees, depending on a number of factors, such as individual polyamines, plant species or cultivars, type of stress and its duration and intensity [41].

The relationship between CI and damage to the membranes has been proven [5]. On the one hand, it has been suggested that PAs, due to their antioxidant activity, are potential for membrane binding via interactions with phospholipids [12, 42] and triggering enzymatic antioxidant activities [41, 43–45] could be responsible for lower changes in membrane fluidity and solute leakage [13], and play an important protective role in the safety of membranes under conditions of stress [14]. On the other hand, PAs rise concomitantly with CI incidence has been reported in chilling-sensitive horticultural crops [46], but it remains unclear whether PAs rise is due to chilling stress or a defensive mechanism against CI [47]. For example, increases in the concentration of Put in conjunction with CI incidence for lemon, orange, lime, grapefruit, pepper, zucchini, and pepino fruit have been detected [48–52]. Moreover, it has been reported that IW promotes the production of PAs in treated tissues during the warming phase [11]. It was therefore of great interest for the authors to explore possible associations between the incidence of CI, natural changes in endogenous PAs and their modifications in response to IW treatment of pomegranate fruit as a sensitive commodity.

Expression of PA biosynthetic genes under different stresses could be regulated in a disparate manner, including

immediate induction with continuous increase or minor changes during stress periods, or induction only in response to certain stress periods [53–56]. In addition, different cultivars of the same species might also have different patterns of PAs under stress [41]. The endogenous Spm in the control fruit was significantly increased during the first month of cold storage (Figure 3(c)). This could be related to the activation of the PA biosynthesis pathway as part of the hypothesized ability of the 'Rabab-e-Neyriz' fruit to acclimate to stress. By increasing the internal content of Spd alongside the advancement in storage time (Figure 3(b)), it was concluded that Spd could play a more prominent role in alleviating the incidence of CI by extending the exposure time to chilling temperature.

Existing literature shows that postharvest techniques associated with high levels of PAs reduce CI in many sensitive horticultural commodities. For example, exogenous prestorage applications of PAs could decrease CI in commodities such as apple [57], zucchini [58], and mango [59, 60]. Likewise, pomegranate pre-storage treatment by Put or Spd significantly reduced the incidence of CI at chilling temperature; this effect was associated with enhanced endogenous PAs [47]. In addition to IW, the pre-storage hot water dip is known as the main method for postharvest heat treatment of horticultural commodities, which could reduce the incidence of CI associated with rises in endogenous PAs in sensitive crops [48, 61–66]. Accordingly, by prestorage hot water dip (at 45°C for 4 min), Mirdehghan et al. [21] reported a reduction in CI and an increase in PAs in the peel of the pomegranate with such a value were always higher than in control fruit, even more than that measured at harvest. One notable finding was an instantly and statistically enhanced biosynthesis of Put and Spm in fruit treated during the first month of storage compared to control or later treated fruit (Figures 3(a) and 3(c)). In contrast to the control fruit, in the case of fruit treated on the 15th or 25th day, Spm increased steadily and significantly until the end of the storage period (Figure 3(c)). It was concluded that the rate of immediate increase and the final content of Spm in response to treatment was time-dependent and that the warming treatment was assumed to have a synergistic effect with the inherent potential of Spm biosynthesis in the fruit peel. On the one hand, only fruit treated during the first month of storage had significantly higher final Spm content than the control fruit (Figure 3(c)). On the other hand, postponing the warming treatment to more than one month of cold storage significantly lowered the rate of treatment effect on Put biosynthesis and its final value (Figure 3(a)). These findings could emphasize the importance of performing IW treatment at the right time during storage to achieve optimum performance in extending the shelf life of the treated fruit. By warming on or after the 25th day, Spd was also immediately and significantly increased, resulting in statistically higher levels both at interruption dates and at the end of the storage period in the treated fruit compared to the control fruit (Figure 3(b)). As the storage period increased, the content of Put decreased significantly in the control fruit. Furthermore, the different time-treated fruit had a final Put content lower than that recorded after harvest (Figure 3(a)). Accumulation of Put is a general reaction to stress [43], although it could be converted into Spd via spermidine synthase (SPDS, EC 2.5.1.16) [41]. It was likely that part of the continuous decrease in the content of Put in the peel of cold-stored fruit was due to the conversion to Spd. In the same way, it could be assumed that part of the increased content of Put in the treated fruit has also been transformed into Spd, with a higher rate of transformation coinciding with the progress in storage time. This hypothesized mechanism could contribute to the significantly higher levels of Spd detected in fruit treated on or after the 25th day compared to control fruit both at interruption dates and at the end of storage time.

As stated earlier, it was established from the other part of our research [17] that IW, as a single cold-warm-cold cycle, could have beneficial effects on the cold storage of 'Rabab-e-Neyriz' pomegranate by inducing enzymatic and non-enzymatic antioxidant reactions in the fruit peel. It was concluded that maintaining higher levels of antioxidant activity is likely to be responsible for reducing lipid peroxidation following warming. It was founded that fruit treated for up to 35 days had significantly higher final activity of superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) than controls at the end of storage, with earlier warming resulting in higher activity. Furthermore, peel browning was not related to POD activity and this enzyme had only a beneficial antioxidant function. By investigating the mode of change in the content of 13 phenolic acids (as non-enzymatic antioxidants), it was also founded that the total phenolic content of the fruit treated at any time of interruption was statistically higher compared to the control, with a value higher than the time of harvest for the fruit treated during the first month of cold storage. In addition to being an important protective mechanism against CI incidence in fruit, higher total phenolic content can be regarded as an indicator of natural antioxidant sources available in the food industry.

On the basis of the overall findings of our research, it can be asserted that a single warming period at the right time during cold storage of pomegranate (before irreversible chilling damage occurs) triggers multiple mechanisms and physiological responses in fruit peel which are significantly responsible for alleviating the severity of chilling damage to this valuable horticultural commodity.

5. Conclusions

Using one cycle of IW by warming the cold-stored pomegranate fruit on the 15th day of storage led to an immediate and significant increase in the endogenous content of Spm and Put, followed by a continuous increase in the level of Spm and a higher level of Put up to the end of storage for treated fruit compared to control fruit. Furthermore, the cell membrane integrity index remained unchanged for the treated fruit until the end of the storage period. It was concluded that warming was likely to induce the protective mechanisms responsible for preserving UFAs from peroxidation, including modifications to endogenous PAs as membrane safety agents. The beneficial effect of the treatment was adversely affected by postponing during storage. Our findings could be crucial for industrial IW applications. It is highly recommended that the efficacy of a single warming

period during cold storage in maintaining the postharvest quality of other perishable horticultural crops with beneficial effects on human health be evaluated. It is predictable that the use of this safe and nonchemical postharvest treatment will result in a longer period of time for consumers to have access to high-quality, health-promoting horticultural crops for use.

Data Availability

All data have been placed in the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Research Article

Quality Evaluation of the Ready-to-Eat Avocado cv. Hass

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Consumer interest in avocado fruit has increased in the last decade in Europe. Nutritional and quality attributes affect the choice of these fruits, whose characteristics must also be maintained in the postharvest period. The preference regarding the feasibility of eating ripe fruits can assure and improve the success of the emerging marketing of avocados. The exposure of fruits to exogenous ethylene (C_2H_4) treatment can accelerate the process of fruit ripening. The aim of this work was at improving the existing knowledge about the quality traits of avocado cv. Hass fruits at the ready-to-eat stage. The most important qualitative traits (weight loss, dry matter content, hardness pulp, and external and internal fruit colour) were evaluated up to 96 hours, maintaining the fruit at two different temperatures, T1 (+8°C) and T2 (+17°C). A trained sensory panel was conducted at 96 hours to confirm the quality of avocado cv. Hass ripened with exogenous C_2H_4.

1. Introduction

Avocado (*Persea americana Mill*) belongs to the Lauraceae family and represents one of the four most important tropical fruits with global production and trade in expansion across Europe. The increase in consumption is related to different factors (the ready availability of the product through various sales channels and its versatility of use and consumption as well as a taste appreciable for the different products) [1]. In particular, its consumption is independent of that of traditional fruits but it is strongly correlated to the *food neophilia* trend. Recently, marketing researches have shown that consumer's preferences are much more affected by lifestyle and fashion trends than economic factors such as income and education [2]. Considering lifestyle changes over the course of a costumer's life, consumer preference and quality requirements are permanently evolving. Therefore, both intrinsic and extrinsic factors are constantly interacting rather than being separate and complementary to each other. Fashion trends and companies' marketing strategies, for instance, repeatedly affect consumer preference to create new food trends and quality standards, which will then result in the formulation of new intrinsic requirements requested by retailers and industries. Avocado can be considered a medicinal fruit due its high antioxidant levels and other nutritional properties [3], and different results regarding consumption of the nutrients in avocado in association with cardiovascular benefits have been reported recently [4]. Healthy properties due the high content of fatty monounsaturated acids, secondary metabolites such as carotenoids and tocopherols, and several bioactive compounds would classify avocado as a superfood. Studies reported that avocado oil is performing thanks to the nutritional and technological characteristics [5], showing stability at high temperatures similar to olive oil [6, 7]. Avocado proteins processed from the oil waste have been shown to have greater emulsifying stability than soy proteins [8] and are therefore suitable for use as functional ingredients in food systems. Then, the use of avocado seed then could have interesting application in the pharmaceutical [9] and food [10, 11] industries. Among the various cultivars, Hass, Arad, Fuerte, and Pinkerton are the main ones commercially known in Europe and the shape and colour of their peels are the main qualitative traits differentiating them. The acceptability of flesh firmness and the consumer intent to purchase are related to the buttery and creamy consistency and, consequently, to the maintenance of high levels of fatty acids [12]. Like other exotic fruits, avocados are transported from the main growing countries (Latin America,

the Caribbean, and South Africa) to European markets when they are unripe to avoid injury, product losses, and mechanical damage [13], but to achieve consumer satisfaction, pulp-ripening procedures are required and necessary [14]. As recently reported by Mpai and Sivakumar [15], the time necessary to reach the ready-to-eat stage differs as a function of the variety. The main commercial avocado varieties are "Hass," "Fuerte," "Lamb Hass," "Pinkerton," and "Ryan," and the influence of the growing season on their composition and the concentrations of peel epicatechin, phenolics in the pulp, and fatty acids could affect the ripening procedure. The ripening processes in *Persea americana Mill* affect the oil and dry matter (DM) contents, which are inversely related. Different products, such as calcium carbide (CaC_2), ethylene glycol ($C_2H_5O_2$), ethylene (C_2H_4), methyl jasmonate ($C_{13}H_{20}O_3$), and ethephon ($C_2H_6ClO_3P$), are commercially available to induce the artificial ripening of climacteric fruit, but it is well known that C_2H_4 exposure accelerates softening safely without possible hazards to human health [16, 17]. The stage of maturity at harvest time and the temperature affect the rate of ripening of avocado. The industrial application of the ripening agent with a catalytic generator must be performed in artificial ripening chambers in a range that should be between 10 and 1000 ppm at the optimum temperature of 15.5°C [18]. The time of exposure to the ripening agent is a function of the DM content of avocado that is the most important maturity index for avocado fruits. Avocado with a DM content in the range of 23–26% generally is exposed to a ripening agent in artificial ripening chambers for 1–2 days [19]. Actually, the ripening treatment of avocado fruits is adopted by many picking houses to have *ready-to-eat* fruits, which have shown significant increases in sales by retailers in the market scenario [20, 21]. Generally, *ready-to-eat* means fruits with a high level of service for the consumer (washed, peeled, cut, and packaged), but in some case, such as tropical fruits, they are not processed; this means that they are ready for consumption in terms of ripening but they are not precleaned or cut. The fresh ready-to-eat stage normally describes fruits with a high service level (washed, peeled, and cut) presented at the retail point of sale packaged, but, as in the case of tropical fruits, they can also be displayed at the point of sale whole with the peel and pulp already mature. Previous studies focused their attention on improving the postharvest of fresh avocado fruits by managing the temperature or the use of an edible coating or 1-methylcyclopropene (1-MCP), but limited are those that evaluated the quality of avocado during the ripening stage [22–24]. Nutritional and quality attributes affect the choice of these fruits, whose characteristics must also be maintained in the postharvest period. The eating quality remains the key of the quality concept as it is the baseline for consumer acceptance of fruit before a costumer formulates an idea of preference, and therefore, it is vital for the successfulness of a product. The aim of this work was at improving the existing knowledge about the quality traits of avocado fruits at the ready-to-eat stage. The most important qualitative traits were evaluated up to 96 hours, maintaining the fruit at two different temperatures, T1 (+8°C) and T2 (+17°C). A trained sensory panel was conducted at 96 hours to confirm the quality of avocado cv. Hass ripened with exogenous C_2H_4 comparing it with other commercial varieties.

2. Materials and Methods

2.1. Sampling Procedures and Qualitative Analysis. Persea americana Mill cv. Hass fruits were imported from Peru according to the storage and transport conditions of one of the most important ripening companies of Northern Italy. Fruits were sampled at the green stage of size 14 (258–313 g). The edible ripeness stage was reached at levels 3 and 4 according to the ripening chart (3.17–1.87 kg of pressure) (Figure 1).

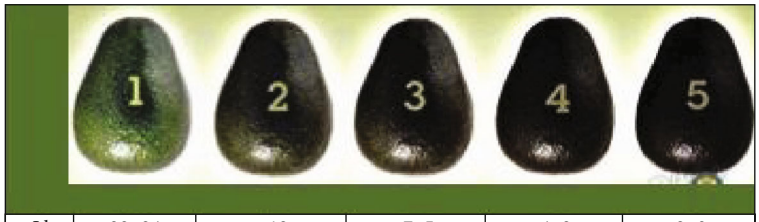
Avocado were experimentally forced to ripen by exposure to C_2H_4 (100 ppm) applied at 18°C for 24 h followed by storage at 5°C in a storage room and immediately transported to the laboratory of the University of Turin, Department of Agricultural, Forestry, and Food Sciences (DISAFA). Fruits were stored for up to 96 hours at two different temperatures T1 (+8°C) and T2 (+17°C). For each sample (T1 and T2) and control time (24, 48, 72, and 96 hours), 12 fruits were selected and analysed regarding weight losses, dry matter (DM), skin and pulp colour parameters (L, a, and b), firmness, and texture profile analysis (TPA). Weight loss (%) was determined using an electronic balance (model SE622), VWR Science Education, Radnor, Pennsylvania, (USA) with a 10^{-2} g accuracy. The weight was monitored for the entire storage time and the loss was calculated as the difference between the initial and final weights.

Dry matter was estimated by drying three replicates of approximately 20 g of material in an oven at 70°C for 24 hours. The fresh and dry weight data were used to calculate the respective DM percentages. Colour measurement was performed in the middle of the peel and pulp using a tristimulus CR-400 chromameter (Konica Minolta, Langenhoven, Germany) according to the Commission International declaring (CIE) $L^*a^*b^*$ system. L^* refers to the lightness and ranged from $L^* = 0$ (black) to $L^* = 100$ (white). Negative and positive values of a^* indicate green and red colours, respectively, while positive and values of negative b^* indicate yellow and blue colours, respectively.

The firmness and texture profile analysis (TPA) was performed with the Texture Analyser TA.XT. PLUS (Stable Micro Systems, USA) (30 kilo load cell). Since the shape and dimensions of the samples may strongly influence compression tests, the fruits were cut longitudinally into small pieces (3 cm height, 3 cm width, and 3 cm thickness) and each half was laid down and compressed at a pretest speed of 5 mm·s^{-1}, test speed of 10 mm·s^{-1}, and posttest speed of 10 mm·s^{-1}. The distance was set to 8.0 mm, and the trigger force was 5 g.

2.2. Sensory Evaluation of Ready-to-Eat cv. Hass

2.2.1. Sampling Procedures and Sensory Analysis. After 96 hours of storage at T1 and T2, samples of *ready-to-eat* cv. Hass were evaluated for their sensory properties. Stored



Lb	22–24	12	7–5	4–3	2–0
kg	14,51–10.88	5,44	3,17–2.27	1,81–1,36	0,91–0

Figure 1: Ripening chart for avocado.

fruits were compared with *ready-to-eat* avocado cv. Hass bought directly from the retail point of sale (sample named "competitor"). Two other varieties, Arad and Pinkerton, displayed as *ready-to-eat*, were bought at the same retail point to better proceed with the projective mapping (PM) analysis. In total, a typology of five samples, all from Perù, was considered.

The PM procedure as described by da Silva et al. [25] was applied to verify similarities and differences among samples. Ten panelists—six female and four male—ranging from 22 to 35 years old, from SATA S.r.l. (Alessandria, Italy), with previous experience in sensory evaluation of fresh fruit, were subjected to specific training prior to sensory evaluation. All fruits were cut into halves, divided lengthwise into two pieces, and served to the panelists. Each panelist received four pieces of fruit of each sample codified with a 3-digit code and presented simultaneously, in random order, as requested by the PM procedure. Panelists were asked to score a sensory sheet composed of descriptors using a continuous-intensity scale of 1–9, 1 being "extremely low intensity" and 9 being "extremely high intensity." The descriptors were chosen based on previous works and included firmness, creaminess, sweetness, bitter, intensity of flavour, intensity of aroma, hazelnut aroma, rancid aroma, and herbaceous aroma.

2.3. Statistical Analysis. All the pooled data were analysed using SPSS Statistics 24 (2017, IBM, Milan, Italy) for MAC. Analysis of variance (ANOVA) was performed followed by Tukey's post hoc test, when the differences were significant. Results from the PM analysis were performed with the multivariate multiple factor analysis (MFA). The coordinates x and y from each assessor of each product were treated as a group of two active variables to build the first two dimensions. Data were not scaled. Furthermore, 95% confidence ellipses were applied around the sample mean points, letting the bootstrap sequence iterate on the assessor's partial (rotated) coordinates instead of the original assessor's data, as suggested by other authors [25]. Using this approach, the confidence intervals do not include the assessor's variability, since the objective is to compare the avocado products. The mean scores obtained for each sample and for each descriptor were used as supplementary variables in the MFA analysis in order to enrich the sample description. Data obtained from the descriptors were classified as continuous and not scaled. A scree plot was made in order to decide how many dimensions to keep. Only variables with a cos2 value higher than 0.25 were plotted in the correlation map in order to select only variables that were significant differentiators of the products.

Table 1: Weight losses (%) of avocado cv. Hass during storage time.

Samples	24 h	48 h	72 h	96 h
T1	0.20%	0.30%	0.54%	0.57%
T2	0.87%	1.49%	2.19%	3.46%

3. Results and Discussion

3.1. Qualitative Analysis. The role of water loss in the ripening of avocado cv. Hass was studied by Lallu et al. in 2004 [26] who reported that the water content can initiate rot development. The water content is considered as a maturity indicator, and when avocado matures, the moisture content decreases. The effect of the relative humidity on the water loss and ripening rate of the Fuerte and Hass varieties has been investigated by Adato and Gazit [27]. The authors underlined the negative correlation between the daily rate of water loss from fruits and their ripening index, which increase by up to 40% for avocado with 2.9% water losses. The results reported in Table 1 indicated that the water content of avocado cv. Hass in *ready-to-eat* fruits is still high. For all the samples, water losses increased daily up to 96 hours of storage, but as expected, T2 mainly affects the weight loss content. Avocado fruits maintained at +8°C (T1) can maintain a good level of hydration, thus limiting water losses; at 96 hours of storage, in fact, fruit stored at a low temperature can contain up to six times more water than fruit stored at a temperature of +17°C (T2).

The change in DM content is linked to the fatty acid content, which, in turn, varies among avocado varieties. The dry matter content is well known to be influenced by the respiration rate [28], and with increased ripening, high levels of oil are concentrated in the pulp at the expense of DM [29]. Storage temperatures between 5°C and 10°C are reported to considerably slow down the metabolic activities of avocado fruits, thus slowing down the decrease in dry matter in the same storage period [30]. The results reported in Table 2 confirm those reported in previous studies [30]; in fact, samples maintained at +17°C (T2) have an important decrease showing at the end of the storage time (96 hours) −3.15% loss of DM, compared with the start value (0.87%).

Table 2: Dry matter (DM) content (%) of avocado cv. Hass during the storage period.

Samples	Start	24 h	48 h	72 h	96 h
T1	25.1% ± 0.02	23.73% ± 0.05	23.37% ± 0.03	23.32% ± 0.01	23.17% ± 0.02
T2		22.79% ± 0.02	22.33% ± 0.06	22.15% ± 0.03	21.95% ± 0.01

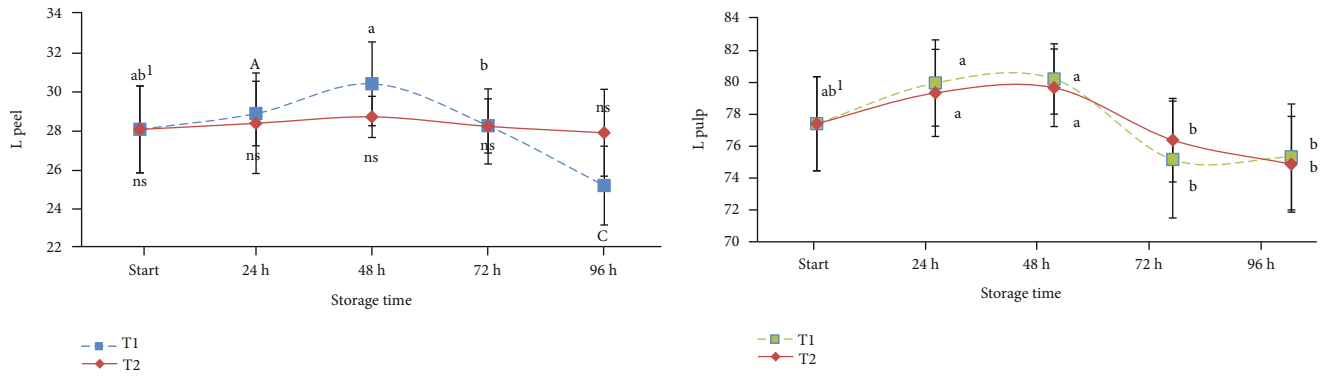


Figure 2: Evolution of the luminosity (L) colour parameter of stored cv. Hass (peel and pulp). Values followed by different letters are significantly different at $P \leq 0.05$ (Tukey's post hoc test).

For the same storage time, it can be observed that the sample maintained at +8°C (T1) showed a decrease of −1.93% compared with the starting value.

Different studies report the effect of storage treatments on colour evolution in avocado, and this qualitative parameter is considered, along with the firmness of the pulp, one of the most important in the evaluation of the ripening stage of avocado fruits and the efficiency of the applied technique during the storage period [31–34]. The degradation of chlorophyll and the synthesis of cyanidin 3-O-glucoside are the main factors that promote the browning of the avocado's peel, which also affects the marketability of the fruit. *Ready-to-eat* avocado fruits are characterized by a dark-green to deep purplish colour, but the storage temperature can especially modify the lightness of the fruit. In Figure 2, the evolution of the luminosity parameter (L) of the peel and pulp of both samples is reported. At the beginning of storage (start), samples of cv. Hass showed a value of L of 27.9. Over time, no statistically significant differences were observed for fruits maintained at +17°C (T2), while the lowest temperature of +8°C (T1) seemed to mainly affect the evolution of the skin colour in terms of brightness. This could be due the higher water content of samples maintained at +8°C as observed in Table 1. At the end of the storage time (96 hours), the losses in the L value were greater in the *ready-to-eat* samples, T1 achieving 25.1 compared with 27.8 for samples at T2. Considering instead the L value of pulp, the evolution up to 96 hours was similar for both samples stored at the two different temperatures. At the start time, all samples showed 76.9 for the L value, achieving 75.0 and 74.6, at T1 and T2, respectively.

The evolution of the greenness (a) and yellowness (b) of the peel and the pulp is reported in Figures 3 and 4, respectively. Considering the peel of ready-to-eat avocado, no statistically significant differences were observed during the storage period for samples maintained at T1 in terms of a and b values, while the highest temperature (T2) seemed to influence the green level of the peel with a value of 0.16 (start) to 2.02 at 96 hours. The influence of time at T1 and T2 was similar for the values observed concerning the evolution of the pulp colour (Figure 2), while yellow colour development in pulp seemed to be best maintained by the lowest temperature; in fact, after 96 hours of storage, samples showed a similar colour at the beginning of the shelf-life period (Figure 4).

The texture properties of fruits are strongly related to the judgment and taste evaluation of the final consumer. This expression is very important for avocado fruits, of which texture properties are strongly connected to the content of fatty acids and their distribution within the pulp [35]. Limited data are available on the texture profiles of *ready-to-eat* avocado fruits. In Table 3, some of the most important texture parameters are reported. At both storage temperatures (T1) and (T2), fruit firmness of *ready-to-eat* samples decreased significantly with storage time, although the rate of decrease differed. Samples which demonstrated the highest water losses (T2) also showed the highest hydrolysis of cellulose and hemicellulose, losing 44% of the initial pulp firmness after 96 hours. At the same storage time, samples stored at +8°C (T1) had lost 25% of their initial firmness.

The adhesiveness parameter shows the adhesion of the probe of the instrument used to analyse the sample. Negative values are related to the negative force area measured for the first simulated bite. No statistically significant differences in the time were observed for samples stored at T1. Increasing negative values were observed for T2 samples, which achieved the highest values after 96 hours; this means that the avocado pulp was difficult to remove from the probe due to its pasty and creamy appearance. Gumminess can be considered as the work necessary to disintegrate the sample to a consistency suitable for swallowing, and the decrease in gumminess values was observed to be in agreement with the sample water

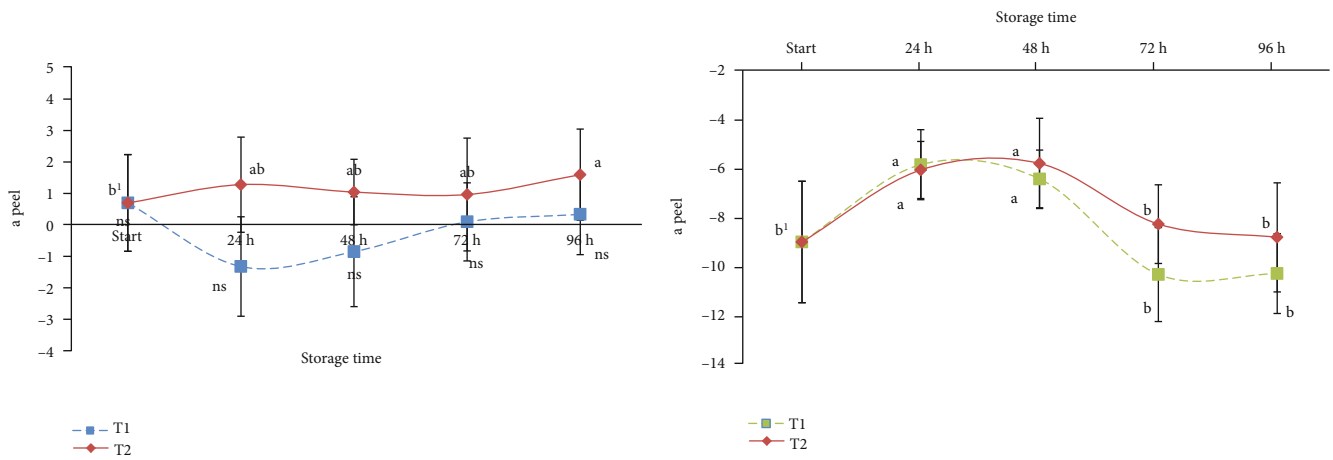


Figure 3: Evolution of greenness (a) colour parameter of stored cv. Hass (peel and pulp). Values followed by different letters are significantly different at $P \leq 0.05$ (Tukey's post hoc test).

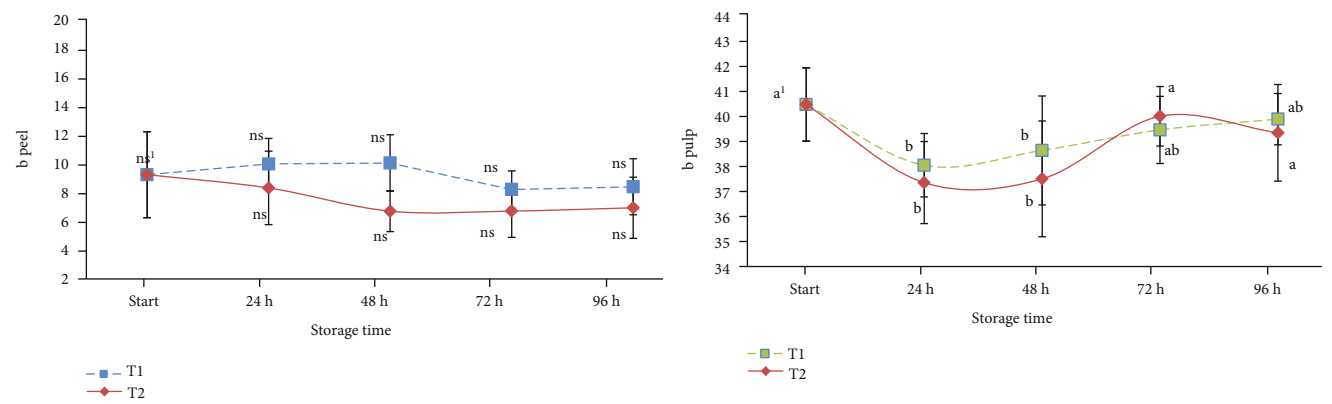


Figure 4: Evolution of the yellowness (b) colour parameter of stored cv. Hass (peel and pulp). Values followed by different letters are significantly different at $P \leq 0.05$ (Tukey's post hoc test).

Table 3: Evolution of texture parameters of avocado cv. Hass during storage.

Samples	Storage time				
	Start	24 h	48 h	72 h	96 h
			Firmness (N)		
T1	3.29 ± 0.39*a*[1]	3.23 ± 0.22*a*	3.10 ± 0.22*ab*	2.78 ± 0.64*ab*	2.46 ± 0.26*b*
T2	3.29 ± 0.39*a*	2.55 ± 0.50*b*	2.40 ± 0.21*bc*	1.95 ± 0.34*c*	1.83 ± 0.34*c*
			Adhesiveness (g∗sec)		
T1	−74.4 ± 38.9 ns	−59.1 ± 34.6 ns	−95.1 ± 36.4 ns	−69.4 ± 34.8 ns	−63.2 ± 22.1 ns
T2	−74.4 ± 38.9*ab*	−63.7 ± 38.9*a*	−75.6 ± 39.9*ab*	−104.6 ± 21.8*ab*	−127.1 ± 35.4*b*
			Gumminess (g∗sec)		
T1	7250.5 ± 2194.4*a*	5035.3 ± 1952.4*ab*	4921.6 ± 2439.6*ab*	4617.6 ± 2513.8*ab*	3316.7 ± 1263.3*b*
T2	7250.5 ± 2194.4*a*	2082.8 ± 1182.3*b*	2290.1 ± 1126.2*b*	3424.8 ± 1014.1*b*	2593.8 ± 814.5*b*
			Resilience		
T1	0.437 ± 0.122 ns	0.309 ± 0.105 ns	0.315 ± 0.084 ns	0.312 ± 0.085 ns	0.288 ± 0.031 ns
T2	0.437 ± 0.122*a*	0.296 ± 0.191*ab*	0.292 ± 0.088*ab*	0.268 ± 0.061*b*	0.257 ± 0.053*b*

Values followed by different letters in the same line are significantly different a $P \leq 0.05$ (Tukey's post hoc test).

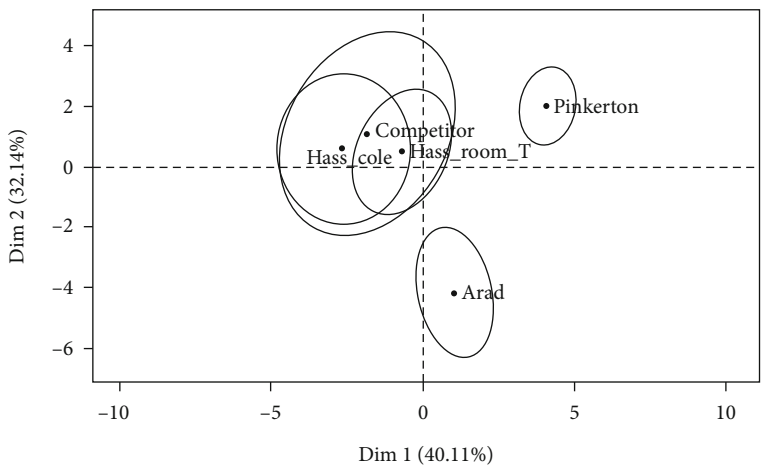


Figure 5: Dimension 1 (Dim 1) and dimension 2 (Dim 2) of the multiple factor analysis individual plot of avocado samples and confidence ellipses.

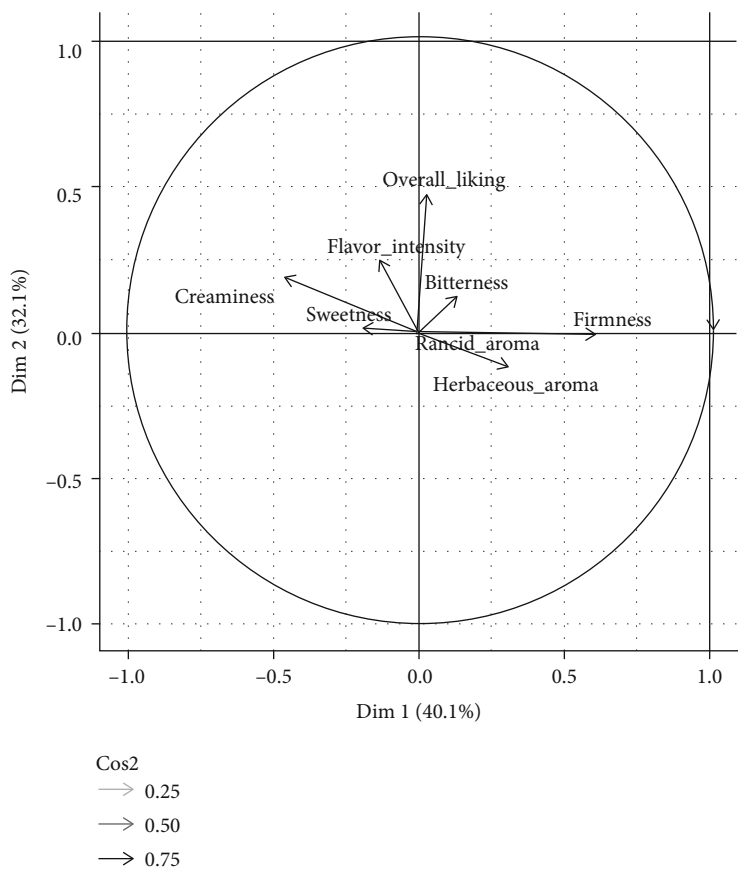


Figure 6: Biplot of sensory evaluation of different *ready-to-eat* avocado varieties, in dimensions 1 and 2.

content loss. Statistically significant differences among samples were already detectable after 24 hours of storage at both temperatures. The resilience parameter measures the elastic recovery of the sample and shares a similar trend with adhesiveness in describing the avocado texture. In fact, no statistically significant differences were observed for samples stored at +8°C (T1). Samples stored at +17°C (T2) showed lowest values and they decreased in time. Statistically significant differences were observed during the storage time.

3.2. Sensory Evaluation of Ready-to-Eat cv. Hass. To evaluate the quality of avocado cv. Hass ripened and stored after 96 hours, T1 and T2 samples were compared with other *ready-to-eat* commercial varieties. The scree plot suggests that only the first two dimensions should be kept in the analysis as they accounted for almost for 75% of the variance displayed by the samples.

It is clear that all avocado varieties are widely dispersed, as the confidence intervals displayed by the ellipsis drawn in

the MFA map do not overlap (Figure 5). It is also clear that none of the Hass samples (T1, T2, and control) were considered different from each other by the assessors. Hass and Pinkerton samples were positioned on opposite sides when considering the first dimension, and with respect to Arad, the same occurred when the second dimension was taken into account. This means that the quality differentiators among Hass and the two varieties are probably different. In order to gain a deeper understanding considering the quality differences among samples, the sensory descriptors used in the quantitative test need to be plotted.

In Figure 6 it is clear that texture attributes were the most discriminatory, as shown by the higher variance explained by the attribute firmness and creaminess. The PM test confirms that Pinkerton is harder and less creamy than the Hass samples. Pinkerton was also associated with a herbaceous aroma, while the Hass varieties were considered to have a more intense flavour. However, it is important to note that the hazelnut aroma was not displayed in the correlation graphic, despite its common association with the aroma profile of the Hass variety. This means that the samples tasted in this work were not very different from each other considering this important attribute. The overall preference attribute is indicated in a vector close to the Hass and Pinkerton varieties but far away from Arad, indicating that the latter variety was less appreciated by the panel. Probably, this variety was considered tasteless as demonstrated by its opposite position in relation to the flavour intensity attribute.

4. Conclusion

Globalization has led to a marked improvement in commercial exchanges, in particular those relating to the fruit and vegetable world, opening the way to new types of products that in the past, it would have been unthinkable to be able to buy in our markets. Consumption of a tropical fruit such as avocado is continuously increasing; this is due to a set of related factors such as the easy availability of the product in the different sales channels and their versatility of use and consumption as well as a taste appreciable for the different products. Among the different fruits, avocado benefits positively from exposure to ethylene to improve the ripening process. The current study was focused on improving the existing knowledge about the quality traits of avocado cv. Hass. Based on physicochemical and organoleptic evaluation, it was found that the cv. Hass can be ripened with ethylene and the fruit quality can be maintained up to 96 hours at +8°C. This new trend is the subject of novel research, especially in the management and the postharvest supply chain, because quality properties of *ready-to-eat* avocado are difficult to monitor considering only the external appearance.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Conflicts of Interest

The authors declare no conflict of interest.

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Review Article

Bioactive Compounds from Agricultural Residues, Their Obtaining Techniques, and the Antimicrobial Effect as Postharvest Additives

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Agricultural vegetable products always seek to meet the growing demands of the population; however, today, there are great losses in supply chains and in the sales stage. Looking for a longer shelf life of fruits and vegetables, postharvest technologies have been developed that allow an adequate transfer from the field to the point of sale and a longer shelf life. One of the most attractive methods to improve quality and nutritional content and extend shelf life of fruits and vegetables is the incorporation of bioactive compounds with postharvest technologies. These compounds are substances that can prevent food spoilage and the proliferation of harmful microorganisms and, in some cases, act as a dietary supplement or provide health benefits. This review presents an updated overview of the knowledge about bioactive compounds derived from plant residues, the techniques most used for obtaining them, their incorporation in edible films and coatings, and the methods of microbial inhibition.

1. Introduction

At present, agricultural problems are based on the adequate food production to satisfy global consumers. However, such production must focus on sustainable processes, thus avoiding generating pollution and waste derived from the agricultural process. It is useless to implement intensive and organic production technologies, if there are postharvest losses within the supply chain during transport, at the sales stage, or during the storage process. Much of the food waste occurs during the postharvest stage, when the product of interest is removed from the soil or separated from the mother plant and its deterioration begins. The routes of products from the countryside to urban areas, complex marketing systems, and the international trade are the main points of attention for the implementation of postharvest techniques, in order to preserve the quality of the products for as long as possible. The plant tissues when exposed to air, accompanied with changes in temperature, solar radiation, and some cuts or blows, begin a process of physiological deterioration. Some crops are even affected at the preharvest stage, where the limitation of nutrients or the impact of some conditions of the crop in its final phase limits the shelf life of the product in its postharvest stage [1].

The global agenda established by the United Nations towards 2030 has determined various objectives for sustainable development. In its objective number 12-Responsible Consumption and Production, it seeks a joint effort among nations to reduce food loss and the generation of food waste. Food losses are quantified from production to the retail level, while food waste comprises the retail and consumption levels [2]. In a study carried out by FAO et al. [2] during

2016, the percentage of food losses by region was analyzed, generating an average global loss of 13.80%, representing losses above 400 billion USD per year.

The significance of postharvest processes does not only lie in the conservation of agricultural products but also in the nutritional quality provided by having food in good condition or even with the addition of beneficial compounds for health. As is well known, fresh fruits and vegetables are a source of vitamins and minerals that can benefit human health. Postharvest deterioration decreases the benevolent capacity of these compounds, in addition to the generation of substances harmful to health.

Due to the importance of the application of postharvest technologies, the obtaining of quality food and enough quantity arises. Postharvest technologies are mainly based on controlling the storage environment or handling conditions, including temperature and humidity. Bioactive compounds are substances that can interfere with cell senescence; the reason why is they have been incorporated into postharvest technologies allowing to extend the shelf life of storage and transport stages. These bioactive compounds are generally found in the same foods or plants from where they are extracted by various techniques [3]. Bioactive compounds mainly exhibit antioxidant and antimicrobial properties [4], which make them attractive for incorporation into minimally processed and horticultural product preservation technologies.

2. Main Bioactive Compounds Present in Plant Waste

The circular economy is a system for the use of resources where the reduction of elements prevails, which applies different mechanisms with the aim of minimizing the generation of waste, thus releasing the economic growth of natural resources. Every year, it is estimated that a third of all food produced is wasted [5], whereof 20.4 million tons is wasted in Mexico [6]. In the fruit and vegetable sector, 45% of the total produced is lost in the production chains (postharvest, processing, and distribution) and consumption [7, 8]. Currently, world postharvest losses of fruits and vegetables caused by microorganisms are of the order of 5 to 25% in developed countries and between 20 and 50% in developing countries. This waste represents the origin of contamination of the soil, air, and water sources, affecting both the environment and public health [9].

In Mexico, the fruit processing industries (lemon, pears, tomato, apple, papaya, pineapple, banana, and oranges), cereals (corn), and vegetables (beans, cabbage, carrots, lettuce, and potatoes) generate around 76 million tons of annual waste [10]. Therefore, it is necessary to find new technological and environmentally friendly solutions, to use fruit and vegetable waste as new raw materials, in order to develop and expand the production of bioproducts with high added value.

Agroindustrial waste has a great potential to generate food additives that can be beneficial to ensure global food sustainability [11]. Considering their high removal rates, by-products of plant sources can be used to obtain commercially valuable bioactive products [12]. A bioactive compound is a substance that has biological activity. In a broader sense, it is a substance that has an effect or can trigger a physiological response in a living organism. As they come from plant sources, bioactive compounds are phytoconstituents that are part of the food chain and are responsible for numerous beneficial changes in health. For example, antioxidant, anticancer, anti-inflammatory, antiallergic, antiatherogenic, and antiproliferative properties are the main bioactivities of the bioactive compounds [13].

Bioactive compounds are synthesized from some plant primary metabolites (e.g., amino acids) or from intermediate compounds obtained by primary metabolism in specialized cell types only during a particular growth stage, or under specific conditions, making their extraction and purification difficult [14]. Commercially useful bioactive compounds (secondary metabolites) are terpenoids, polyphenols, vitamins, alkaloids, etc. [15], which have been used to prevent the risk of diseases and in the treatment of a wide range of illness. In plants, they have a protective role against biotic and abiotic stress [16]. Given that bioactive compounds are present in different amounts, it is important to develop their production, in order to obtain as much as possible, and find new sources cheaper and alternative [17, 18]. Therefore, the materials considered waste can be a valuable source of bioactive compounds, able to be extracted and implemented in new processes and products for the replacement of chemical food additives.

The by-products of the fruit processing industry such as bark, peel, seeds, and pomace represent the largest amount of waste, which is made up of a wide variety of bioactive compounds with multiple biological properties. The exploitation of residues for the development of food products with added value could allow the generation of additional benefits. It is currently known that the cost of technologies for the purification of bioactive compounds exceeds the cost of reprocessing, so that the full use of waste with functional properties as additives could lead the food industry to reduce waste and increase its profitability [14].

Fruit and vegetable waste is mainly composed of lignocellulosic biomass, which is made up of cellulose (30–50%), hemicellulose (15–35%), and lignin (10–20%) [19], being a viable source of sugars and phenolic compounds. Phenolic compounds are classified as primary antioxidants that are free radical scavengers that inhibit lipid oxidation, reducing the formation of volatile compounds (aldehydes and ketones) that cause rancidity [20]. Phenolic compounds are important for their antioxidant properties and their protection against degenerative diseases such as cancer and heart disease [21]. Phenolic acids are a group of derivatives of benzoic and cinnamic acids such as capsaicin, ellagic, salicylic, tannic, vanillin, gallic, syringic, p-coumaric, o-coumaric, m-coumaric, caffeic, ferulic, sinapinic, and chlorogenic acids occurring in both forms, free and bound [9]. Flavonoids are compounds that consist of two aromatic rings joined by a three-carbon bond. Furthermore, flavonoids belong to different subclasses such as anthocyanins, flavonols, flavanones, flavones, and isoflavones. The structure of stilbenes is represented by two phenyl rings linked together by a bridge of two carbon atoms

such as resveratrol and viniferine [20]. On the other hand, lignans are a complex, heterogeneous polymer, formed mainly by phenylpropanoid derivatives that correspond to the so-called monolignols (p-coumarilic, coniferyl, and sinapyllic alcohols) [16]. Coumarins are also simple phenolic compounds that have their chain side forming a cyclic structure (lactones or phenylpropanoids). Finally, tannins are grouped into two main classes: hydrolysable tannins, which are characterized by having a glycoside structure, and condensed tannins, which are polymers whose structures are related to flavonoid compounds (also called proanthocyanins, proanthocyanidins, or leucoanthocyanidins). Table 1 presents the main bioactive compounds in various plant residues with antimicrobial, anti-inflammatory, anticancer, antiallergic, antithrombotic, cardioprotective, and vasodilator activities.

3. Overview of the Methods for Obtaining Bioactive Compounds Derived from Plant Residues

The discovery of the extraction of bioactive compounds and their applications as active principles for the treatment of diseases has led to an accentuated development of human society. Natural bioactive compounds play an important role in daily activities, associated with many health benefits and low toxicity or negative effects. In recent years, a high demand for natural bioactive compounds has been generated, creating the need to develop suitable methods, equipment, or strategies for the extraction of such compounds from natural sources [50–52].

The selection of the extraction method and the operational parameters of the process is always based on the overall performance of bioactive compounds. However, it is necessary to determine the family of compounds or the specific compound to be extracted, in order to optimize the operating conditions [51].

The strategy for the extraction of bioactive compounds from agroindustrial waste should aim to maximize the removal performance of bioactive compounds and create the minimum residue. The selected method should be adapted to satisfy the demands of industrial processing, such as bioactive compound purity and its conservation conditions to avoid deterioration and oxidation of the compounds, as well as ensure the quality characteristics of the final product and the sustainability of the process [53].

The bioactive compounds from agroindustrial residues such as fruits, vegetables, and plants can be extracted by different methodologies, which are described below. The extraction processes can be influenced by many factors including the solvent and matrix properties, particle size, process pressure, time, temperature, and the solvent-matrix ratio [54].

3.1. Solid-Liquid Extraction. The solid-liquid extraction, commonly called maceration, is the oldest technique to obtain nonvolatile compounds from plants, by using different solvents in contact with the vegetal matrix. This removal technique is the basis for many other new technologies to enhance extraction yields of bioactive molecules and reduce the time of separation, inducing to solvent and energy saving [53–55]. The yield of a solid-liquid extraction depends on the type of the polarities of the solvents, the mixture pH, the extraction time, and temperature, as well as on the chemical composition of the sample and the particle size [51].

The new technologies are based in solid-liquid extraction and are named "assisted" technologies such as microwave, ultrasonic, pressure, electrical technologies (ohmic heating), enzymatic, and mechanical (pressurized hot water extraction and subcritical fluid extraction) treatments. These new technologies are suitable for improving the recovery of valuable compounds, with different properties and bioactivities, since they use green solvents and allow plant cell disruption with better compound extraction, while minimize the impact on bioactive compounds [55].

3.2. Microwave-Assisted Extraction. Microwave-assisted extraction (MAE) is a solid-liquid extraction with microwave heating. The microwaves are electromagnetic waves whose frequency varies between 300 MHz and 300 GHz. The microwave heating is produced by the capacity of the samples to absorb the microwave energy and convert it into heat. The conversion of microwave energy into heat occurs due to dipolar and ionic mechanisms. The presence of polar molecules is the cause of microwave heating due the dipolar nature of water. Microwave generates an oscillating electric field, and the permanently polarized dipolar molecules try to realign in the direction of the electric field, causing internal friction of the molecules resulting in the heating of the material [56–58].

The principal advantages of MAE are the high yields of extraction, compatibility with the environment, short extraction time, rapid temperature increase, high efficiency, better monitoring of the process, and low energy consumption and cost [59]. MAE efficiency for bioactive compounds extraction depends on particle size, irradiation time, power, and solid-to-liquid ratio. MAE has been applied to extract many natural compounds from agroindustrial residues from vegetables and fruits to obtain bioactive compounds, mainly antioxidants, polysaccharides, and oils rich in carotenoids and polyphenolic compounds [60].

3.3. Ultrasonic-Assisted Extraction. Ultrasonic-assisted extraction is a widely used technology to extract bioactive compounds from many agroindustrial wastes. The principle is based on the effects of cavitation, which intensify the mass transfer between the solvent and the matrix. The collapse of cavitation bubbles near tissue surfaces produces microjet impingement, resulting in tissue disruption and deep penetration of the solvent into the tissue matrix, increasing the extraction yield. Ultrasonic extraction offers an advantage in terms of shorter processing time and enhanced quality, and it is considered an environmentally friendly technology [46, 61].

3.4. Subcritical Water Extraction. Extraction with subcritical water is a recent method to extract bioactive compounds

Table 1: Major bioactive compounds present in various plant residues.

Source	Residue	Bioactive compound	Reference
		Fruits	
Citrus	Peel	Limonene, γ-terpinene	[22]
		Polyphenols, carotenoids, limonoids	[23]
	Seed	Catechin, epicatechin gallate, flavonoids	[24]
		Anthocyanins, phenolic compounds	[25]
Grape		Phenolic compounds	[26]
	Peel	Anthocyanins	[27]
		Flavonoids	[28]
Pineapple	Husk	Phenolic compounds	[29]
Acerola	Waste	Flavonoids and anthocyanins	[30]
Papaya	Seeds	Sulforaphane and phenolic compounds	[31]
Avocado	Seeds, husk, and damaged pulp	Phenolic compounds, acetogenic, carotenoids	[32]
Strawberry and raspberry		Phenols, sugars, uronic acids, and anthocyanins	[33]
Apple	Pomace	Flavonoids, hydroxycinnamic acid, dihydroxy alkaloids	[34]
	Peel	Phenolic compounds	[35]
Kiwi	Peel, seed	Gallic acid, catechin, rutin, quercetin, ferulic acid, vanillin	[36]
Orange and mango	Peel	Carotenoids, limonene, ascorbic acid, flavonoids, phenylpropanoids	[37]
Purple eggplant	Peel and pulp	Anthocyanins and phenolic compounds	[38, 39]
Jabuticaba	Peel	Anthocyanins and phenolic compounds	[40]
Pomegranate	Husk	Phenolic compounds	[41]
Passion fruit	Rinds and bagasse	Scirpusin B, piceatannol	[42]
		Vegetables	
Cardamom, radish, turnip	Aerial part and leaves	Phenolic compounds	[43]
Opuntia spp.		Phenols, betalain	[44]
Lettuce	Waste	Antioxidant extracts	[45]
Tobacco	Tobacco industrial waste	Alkaloids, phenolic compounds, terpenes, and terpenoids	[46]
Carrot	Pulp	Phenolic compounds	[35]
Tomatoes	Waste	Sterols, tocopherols, carotenes, terpenes, and polyphenols	[47]
Beet	Leaves and stems	2-Ethyl-1-hexanol, 2,2-dimethoxy-1,2-diphenyl-ethanone, 1,1,3-trimethoxypropane	[48]
Red cabbage Brussels sprout waste	Sprout residue	Phenolic acids, glycosylated flavonoids, acetylated flavonoids, anthocyanins	[49]

that uses solvents under high temperature (100-374°C) and critical pressure (1–22.1 MPa), to dissolve active substances from the raw materials. Due to these extreme conditions, the solubility of the compounds of interest is increased, making it possible to use water instead of organic solvents. The addition of ethanol helps reducing the extraction temperature and the concentration of toxic compounds, generated by the Maillard reaction in agroindustrial waste. Subcritical extraction has the advantages of obtaining high extraction yields, with an extremely low number of solvents and short operating times. However, there are some disadvantages: the need to raise the temperature, and for the extraction of several heat-sensitive compounds, high temperatures are not suitable and they result degraded [51, 62, 63].

3.5. Supercritical Fluid Extraction. Supercritical fluid extraction is a green extraction method that has been widely used to obtain bioactive compounds from different raw materials, with the application of carbon dioxide as a renewable and green solvent. The low price of extraction, low temperature of extraction, and no toxicity of carbon dioxide make this method of great option to extract bioactive compounds from agroindustrial residues [64, 65].

3.6. Membrane Technology. The final disposal of agroindustrial aqueous waste has become a major challenge for food processing industries, due to its negative impact on the environment. Pressure-driven membrane processes such as micro-, nano-, and ultrafiltration have several benefits in

Table 2: The main components of edible films and coatings (adapted from Espitia et al. [75]).

Components		Characteristics
Hydrocolloids	Proteins, such as casein, gelatin, wheat gluten, corn protein, soy, and whey Polysaccharides, such as starch, alginate, pectin, cellulose derivatives, chitosan, dextrin, carrageenan, and acacia	High permeability to water vapor, barrier to oxygen and carbon dioxide, mechanical strength, can be soluble or insoluble in water
Lipids	Waxes, fatty acids, acetylated monoglycerides, sucrose fatty acid esters, and shellac	High oxygen permeability, water vapor barrier, provide gloss, low structural strength, and durability
Mixtures	EF of casein and acetylated monoglycerides	Combination of hydrocolloid and lipid properties

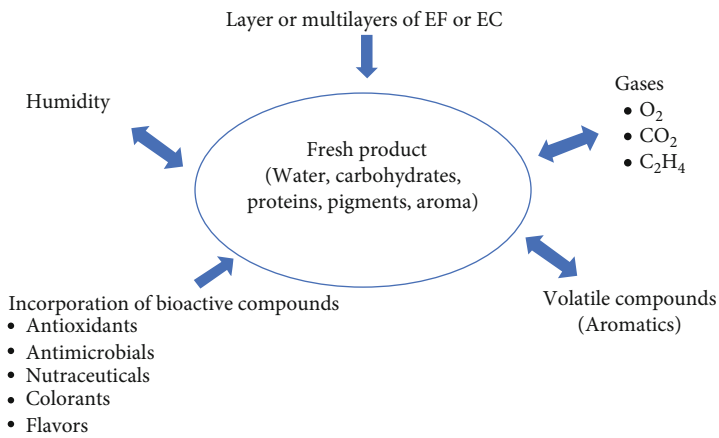

Figure 1: Barrier and transport effect of an edible coating (EC) and/or an edible film (EF) (adapted from Falguera et al. [76]).

the treatment of wastewaters: high separation efficiency, low energy requirements, easy scale-up, simple operation, high productivity, and the absence of phase transition. Many bioactive compounds have been recovered from agroindustrial wastes using membrane technology, such as antioxidant compounds, carbohydrates, pectin, proteins, sugars, and phenolic compounds [66].

3.7. Fermentation and Enzymatic Process. The production or biotransformation of bioactive compounds through fermentation or enzymatic processes from agroindustrial residues has increased in recent years. Solid-state fermentation (SSF) is a bioprocess where the enzymes released by a microorganism (bacteria, fungi, or yeast) act as biocatalysts and allow better release and extraction of bioactive compounds from different substrates. SSF is an economical biotechnological process, easy to implement as it requires small equipment and low capital investment. The enzymatic processes are more complex, are expensive, and have some limitations such as low stability, recovery, and temperature sensitivity that can be reduced using enzyme immobilization [55]. Most microorganisms used are filamentous fungi such as *Trichoderma* and *Aspergillus*, due to its high production of secondary metabolites, and enzymes that can be used to metabolize agroindustrial residues to produce different bioactive compounds [67].

4. Edible Films and Coatings as Postharvest Techniques

To avoid or reduce the adverse postharvest effects, different technologies have been implemented. Between the most used methods are storage at low temperatures, the application of gamma and ultraviolet radiation, conservation in controlled atmosphere, and use of plastic packaging, among others [68, 69]. Seeking for the improvement in the nutritional quality and commercial value of horticultural products, there is research focused on the development of edible films and coatings. They provide the possibility of enhancing the safety of the product by acting as a barrier against the transport of water vapor, oxygen, and compounds responsible for flavor, color, and aroma [70–73].

An edible film (EF) is a preformed matrix, obtained by molding, while an edible coating (EC) is a transparent, continuous, edible, and thin matrix, which is placed on the surface of the food by immersing or spraying the coating-forming solution, whose thickness is always less than that of EFs [74]. Both the EF and EC have the purpose of preserving the quality of the product, serving as packaging, and extending its useful life.

The EF and EC forming solutions are setup of components that are divided into three main categories, as indicated in Table 2. However, the formulation of both the

Table 3: Edible coatings and/or edible films that are applied to fruits and vegetables to prolong their postharvest quality.

Fruit and vegetable product	Formulation	Effect	Reference
Pear	Methylcellulose	Browning reduction	[78]
Strawberry	Cactus mucilage	Permanence of texture, color, and sensory attributes	[74]
Mango	Chitosan	Reduction in water loss, sensory properties, and inhibition of the growth of microorganisms	[79]
Apple	Alginate, gellan gum	Reduction of moisture loss, slowing down of respiration	[80]
Banana	Ascorbic acid + calcium chloride, cysteine + carrageenan	Reduction of enzymatic browning and maintenance of firmness	[81]
Strawberry	Flaxseed mucilage + chitosan	Barrier to gases, reduction of moisture loss, and antifungal effect	[72]
Broccoli	Chitosan	Reduction of mesophilic microbial load	[82]
Banana	Chitosan + Arabic gum	Control of the fungus that causes anthracnosis in the fruit (*Colletotrichum musae*)	[83]
Grape	Chitosan + carboxymethylcellulose	Decreased respiratory rate and increased mechanical resistance	[84]
Mango	Potato and cassava starch	Preservation of appearance, color, firmness, and reduction of respiration	[85]
Tomatoes	Cassava starch	Effect on the ripening process	[86]
Strawberry	Chitosan + starch with cinnamon essential oil	Preservation of total phenol content and antioxidant capacity, delay of microbial development	[87]
Mango	Chitosan + lemon and orange essential oils	Reduction of coliforms, psychrophiles, fungi, and yeasts	[88]
Pepper	Biopolymers (gums : Arabic, xanthan, and pectin) + candelilla wax + jojoba oil + tar extract	Permanence of quality physicochemical parameters, as well as an increase in shelf life	[89]
Carrot	Beeswax + gums (guar and xanthan) + canola oil + propolis tincture	Inhibition of mold and yeast growth; reduction of weight loss and color	[90]
Tomatoes	Bay wax + olive oil + Tween 80 + propylene glycol + glycerol + glucose	Good functional and mechanical characteristics; reduced weight loss, increased firmness, and good looks	[91]
Broccoli, cauliflower, carrot, and chayote	Low methoxyl pectin + carnauba wax + glycerol + ascorbic acid	Conservation of sensory quality	[92]
Pear	Candelilla wax + Arabic gum + jojoba oil + pomegranate polyphenols	Increased shelf life, maintaining product quality	[93]
Mango	Cassava starch + citrus pectin	Shelf life extension	[94]
Strawberry	Tara gum + lipids (beeswax, shellac) + glycerol	Reduction of the rate of respiration, delay of the senescence process, and loss of texture	[95]
Guava	Whey protein concentrate + glycerol + oregano extract	Delay of the ripening process	[96]
Strawberry	Aloe vera + sodium alginate	Improvement in quality parameters (less weight loss, greater firmness, greater color retention, greater titratable acidity)	[97]
Tihuixocote	Tejocote pectin	Delay of the rate of pulp and skin loss of firmness	[98]

films and the coatings will depend on the function that they will carry out. Additionally, both can act as supports that contain bioactive compounds such as antimicrobials, antioxidants, pigments, and compounds that provide flavor and aroma, preservatives, vitamins, and minerals, among others (Figure 1). Within the compounds that are usually used to provide stability to EF and EC are emulsifiers, surfactants, and plasticizers. The emulsifier holds the components

Table 4: Studies of the application of bioactive compounds on postharvest products for the inhibition of microorganisms.

	Bioactive compound/extraction source	Application technology	Inhibited microorganism	Microbiological technique used	Results obtained	Reference
Apple	Antioxidants, phenolic compounds, and flavonoids/ residues (shell flour) of *Acca sellowiana* or "guava de Brazil"	Films	*Escherichia coli* *Salmonella typhimurium* *Pseudomonas aeruginosa*	Disc broadcast	The highest inhibition of the microorganisms tested was achieved in the films with the highest concentration of shell residues (3%).	[109]
In vitro	Polyphenols, flavonoids/broccoli leaves, cauliflower, and cabbage	ND	*Alternaria* spp.	Poison plaque	Cauliflower extracts presented the highest percentage of inhibition ($24.14 \pm 0.58\%$) compared to the rest of the compounds.	[110]
Orange	Polyphenols/pomegranate husk	Edible coatings	*Penicillium digitatum*	Inoculation of the fungus in the fruit with subsequent coating; diameters of the areas affected by the fungus were measured five days after inoculation	Films with aqueous extract of the pomegranate peel (0.361 g) were able to inhibit the fungus *Penicillium digitatum* that causes the green fungus in oranges.	[111]
Fresh cut mango	Gallic acid/mango residues	Immersion in aqueous extracts	Aerobic mesophilic bacteria, fungi, and yeasts	Plate count	80% inhibition of aerobic mesophiles 79% inhibition of fungi and yeasts.	[112]
In vitro	Phenolic acids, flavonoids/ xoconostle (*Opuntia oligacantha*), orange essential oil, soy lecithin	Nanoemulsion with extracts of xoconostle, orange, and soy	*Colletotrichum gloeosporioides*	Well diffusion	The fungus was inhibited with the 4.15 mm nanoemulsion compared to the control, being the highest inhibition in the study.	[113]

together in the forming solution; the surfactant reduces the surface tension of the formulation, achieving greater uniformity; the plasticizer modifies its mechanical properties [75].

The use of edible films and coatings has proven to be an effective method of food preservation. However, when coating a fruit or vegetable, it is necessary that there should be a certain permeability to oxygen and carbon dioxide, in order to avoid anaerobic respiration. This anaerobic respiration could induce physiological disorders and a rapid loss of the quality and shelf life in food [77]. For this reason, the proper selection of the formulation is of utmost importance. Table 3 shows various studies of the components used as EC and/or EF, as well as their effects on fruit and vegetable products.

5. Microbial Inhibition Methods by Bioactive Compounds When Incorporated in the Postharvest Stage

The postharvest decomposition of fruits and vegetables by the action of pathogenic microorganisms represents significant levels in the losses of these products [99]. The incorporation of bioactive compounds in the postharvest of fruit and vegetable products has resulted in controlling the incidence of pathogenic microorganisms [100]. Table 4 shows some examples of antimicrobial activity of bioactive compounds recovered from plant residues and applied on plant products in the postharvest stage.

The exploration of natural plant products as a source of bioactive compounds for the protection of postharvest loss of fruits and vegetables is in its early stages. The information available on the mechanisms of action of plant extracts is scarce; its antimicrobial activity is probably attributable to more than one mode of action [101]. However, there are different sources that have cited the mechanisms by which these compounds exert their antimicrobial effects, mainly inhibiting deteriorating fungi and pathogenic bacteria.

Anibal et al. [102] reported that the antifungal effects of extracts of the pericarp and pomegranate peel with high amounts of punicalagin and tannins are attributed to changes in the structure and cell morphology of the *Candida* genus. When observed by electron microscopy, the treated cells presented irregular cell walls, with viscous material on the surface as well as hyphal rupture and desquamation.

It has also been described that aldehyde, a group of volatile compounds, interferes in fungal cell division by reacting and inactivating sulfhydryl, a functional group involved in the division of fungal cells. Compounds such as cinnamaldehyde (extracted from cinnamon), citral (extracted from lemon grass), and perillaldehyde (extracted from the perilla plant) are good electron acceptors. These compounds act by disrupting fungal metabolism by forming a charge transfer complex with electron donors present in fungal cells [103].

Besides, the polyphenols of green tea (*Camelia sinensis*) have been studied for their antifungal capacity against the fungus that affects rice. The study showed a change in the permeability of the membrane of the rice fungus when presenting an increase in the percentage of electrolyte leakage. Since phenolic hydroxyl groups can bind the hydrophilic end of the lipid bilayer to agglomerate the lipid of the membrane, thus, these damage the cell membrane and promote electrolyte leakage. Furthermore, green tea polyphenols have shown antimicrobial activity at high doses of epigallocatechin gallate (EGCG), damaging the liposome membrane in *E. coli* and *S. aureus*, leaking intramembrane materials and consequently the aggregation of liposomes. In addition, these polyphenols have been shown to strongly inhibit the biofilm formation of pathogenic *E. coli* strains, by reducing the expression of the regulatory protein "CsgD," a crucial activator in cellulose biosynthesis [104].

The antimicrobial capacity of some phenolic compounds extracted from mango residues, such as galangin, is attributed to the inhibition of cellular DNA gyrase in bacteria. The inhibition of the enzyme topoisomerase IV, as well as the anti-beta-lactamase activity (bind metals and proteins, affecting their bioavailability), generates nonspecific effects that trigger antioxidant and antimicrobial phenomena [105].

Organic acids have presented a mechanism of action in the inhibition of microorganisms that are related to acid-base balance, proton donation, and cell energy production. The cell of microorganisms in a normal state maintains equilibrium by establishing an internal pH close to neutrality. Cell homeostasis was defined as the establishment of a chemical balance in the face of environmental changes, damage to microbial cells, causing their alteration. When a change in pH occurs, proteins, nucleic acids, and phospholipids of the microbial cell can be structurally altered [106].

Various publications suggest that the main mechanism of the antimicrobial effects of volatile compounds extracted from different fruits indicates potential damage to the bacterial membrane, generating changes in permeability, polarization, and interruption of flow activity. Phenolic compounds have shown the ability to modify the regulation of genes associated with certain virulence attributes in bacteria, including hydrophobicity, adherence, motility, invasion, and biofilm generation. The above indicates the potential of phenolic compounds extracted from fruits as antimicrobials [107].

Other authors have described the mechanisms of action that some bioactive compounds generally present. Lauzardo et al. [108] mention that the toxicity effect of phenols on microorganisms can be attributed to enzymatic inhibition of compound oxidation. Terpenes and essential oils, although not fully studied, can cause membrane rupture by lipophilic compounds. The alkaloids are embedded in the DNA, while lecithins and polypeptides form ion channels in the cell membranes of microorganisms or, by competitive inhibition, cause adhesion of microbial proteins to the host's receptor polysaccharides.

6. Conclusions

Bioactive compounds are lined up to help revolutionize postharvest technologies and achieve improved increases in the shelf life of fruits and vegetables. The different protection mechanisms they provide make them versatile to

the various forms of application of current postharvest technologies. Phenolic compounds and their derivatives are to date the substances with the greatest protection properties against oxidative stress, as well as against the attack of microorganisms.

The use of residues of plant origin follows the ideology of circular bioeconomy, seeking sustainable processes that allow to earn an added value. Plant-based waste is a rich source for obtaining bioactive compounds. The challenge lies in the improvement of the current technologies or looking for new ones that allow a higher percentage of obtaining bioactive compounds, as well as methods for incorporation into fruit and horticultural products. Further research should be made focused on the exploration of novel sources of bioactive compounds from vegetal wastes, their green extraction methods, and the evaluation of microbial inhibition mechanisms. The literature indicates that the main mechanism of action for the compounds extracted from plant sources as antimicrobial agents is damage to the cellular structure. Although there is information that allows to explain these effects, more studies are necessary for a complete understanding of the different mechanisms of action by bioactive compounds on the presence of spoilage and pathogenic microorganisms in the postharvest stages.

Data Availability

The data supporting this systematic review are from previously reported studies and datasets, which have been cited.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Research Article

Effect of Different Modified Atmosphere Packaging on the Quality of Mulberry Fruit (*Morus alba* L. cv *Kokuso 21*)

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The control of temperature and gas composition is essential to maintain the fresh flavor and quality of perishable fruits like mulberry. This study presented a modified atmosphere experiment (MAP) for fresh fruit showing the potential benefits of innovative gas mixing with argon. The effects of MAP were studied on the physicochemical and qualitative attributes of mulberry preserved at $4 \pm 1^{\circ}C$ and $90 \pm 5\%$ R.H. Fresh mulberries were packaged with different gas combinations: MAP1 ($4\%O_2 + 6\%CO_2 + 90\%N_2$), MAP2 ($10\%O_2 + 5\%CO_2 + 85\%Ar$), CTR1 ($20.9\%O_2 + 0.04\%CO_2$), and CTR2 ($10\%O_2 + 5\%CO_2 + 85\%N_2$). Changes in quality parameters were evaluated after 0, 4, 8, and 12 days of storage. Mulberries packaged with MAP had a lower weight loss than CTR samples which lost more than 80% of their initial weight. Furthermore, the results showed that the argon treatment was the best in keeping the fruit juice content, preserving its structure. Despite not showing great differences with MAP1 treatment, Ar allowed to maintain high TSS up to 8 storage days, slowed CO_2 production. The sensory profile of mulberry fruit was not significantly affected by storage in modified atmospheres, and the production of potential unpleasant odors in MAP2 could not be perceived. The results of this study confirm that this innovative approach, using MAP technology, has a potential use in maintaining mulberry fruit quality for a longer time.

1. Introduction

Within the Italian sector of fruit production, small fruits are a niche produce. The consumption of blackberries, blueberries, mulberries, strawberries, and raspberries has increased during the last 10 years, due to the gradual raise of consumers' awareness of the high nutritional value of all types of small fruit [1] and of the related benefits for human health of their consumption, because of their bioactive compounds [2]. Small fruit are characterized by a high berry perishability, rapid quality decay, limited shelf life, even if stored under refrigerated conditions. This may cause high production costs, limitations for marketability and a consequent loss of commercial value [2].

Mulberry is a deciduous woody tree belonging to the genus *Morus* of the family *Moraceae* [3], globally distributed under varied climatic conditions ranging from tropical climate to the temperate one [4]. There are about 68 species of genus *Morus* worldwide [5], but only white mulberry (*Morus alba* L.) and black mulberry (*Morus nigra* L.) are cultivated in Italy [6, 7]. Mulberry is a climacteric fruit; it is rich in carotene; vitamins B1, B2, and C; glucose; sucrose; tartaric acid; and succinic acid [8, 9]; it also contains antioxidants, total phenolic, and anthocyans [10]. Moreover, due to its particular nutraceutical value, mulberry is considered as a functional food [10, 11]. Because of these reasons, mulberries are considered a high-end product. The cost in Italy and neighboring countries ranged from a minimum of 7 euros per kg of fresh product to a maximum of 13 euros due to the high costs of production, harvesting, storage, and distribution [12]. In fact, mulberry fruit is one of the most fragile and perishable fruits, both in harvesting and postharvest, having a very short shelf life of 2–3 days, which can be greatly reduced by storage temperatures above 0°C. The color of mulberry

fruit is related to the species: *Morus alba* L. has white and black fruit; instead, *Morus nigra* L. produces only black fruits. The mulberry fruit ripening is usually correlated to a skin color change due to an accumulation of anthocyans that implies a modification of pigments' concentration in surface tissues [13] and a degradation of chlorophylls and carotenoids with a consequent development of color from green to purple [14, 15].

Although, official statistics demonstrate that Italy imports small fruit from other countries, mulberry fruit is still poorly commercialized in Italy, compared to other countries, despite the increasing interest of consumers in this produce. Indeed, mechanical damage and microbial spoilage hamper their transport from the production to the processing/selling site [16, 17]. Therefore, there is much interest of stakeholders in developing preservation methods for all types of small fruit and particularly for the mulberry fruit which appears very interesting as a source of vitamins, minerals, and fiber in the human diet and for its use as a superfood or in the pharmaceutical industry. Different postharvest technologies, such as active packaging [18], edible coatings [19], oxidizing sanitation technologies [20], and modified atmosphere packaging and gaseous ozone prepackaging treatment [16, 21, 22], were proposed to preserve the quality of small fruit and extend their shelf-life. Chen et al. [23] investigated the effect of immersion in chlorine dioxide solutions (20-80 mg L^{-1}) on the shelf-life of mulberry fruits and observed a shelf-life extension of up to 14 days. Teng et al. [24] observed that chlorine dioxide and electrolyzed water solutions were effective in reducing microorganisms and extending the shelf-life of mulberries. Oz and Ulukanli [25] studied the effects of 1-methylcyclopropene (1-MCP) and calcium chloride on the postharvest quality and shelf life of mulberries. Hu et al. [26] treated the mulberry fruit with a hydrogen sulphide release compound (H_2S) and observed that the treatment increased the activity of some antioxidant enzymes and consequently reduced the production of superoxide anions in the fruit. Treatments of allyl isothiocyanate [27] and chitosan-caffeic acid [28] were found to have significant effects on the postharvest quality of the mulberry, and both treatments were proposed as potential methods to extend the shelf life of the fruit. More recently, Pinto et al. [16] and Tabakoglu and Karaca [29] reported that mulberry fruits subjected to a combined treatment with ozone had a lower rate of decay, respiratory intensity, and polyphenol-oxidase activity than control samples.

Modified atmosphere packaging (MAP) treatment effect on fruit quality was tested in strawberries, sweet cherries [30], pomegranate [31, 32], litchi [33], table grapes [34], kiwi [35], and blueberry [36]. MAP resulted in an effective method of fruit quality preservation to extend the shelf life of small fruits, with positive effects on physical-chemical parameters and a reduction in the development of fungi. For example, blueberries stored in MAP at 3°C showed a shelf-life extension of 9-15 days compared to air storage, depending on the packaging material [37], while for strawberries after storage at different temperatures [38], a shelf-life gain of more than 1 day was expected. Microbial growth control is achieved as a result of high CO_2 concentrations [39]; however, achieving a partial CO_2 pressure higher than 5 kPa in MAP could lead to the development of undesirable flavors in strawberries and raspberries [40] and/or may reduce the consistency of different types of small fruits [41].

The three traditional gases for modified atmosphere packaging are oxygen, carbon dioxide, and nitrogen [42, 43]. Recently, there was a great interest of researchers in the potential benefits of argon (Ar) and other noble gases in MAP applications [44, 45]. Moreover, argon was recently allowed to be used for MAP in the European Union as an alternative to nitrogen [46, 47], because it is inert, odorless, and tasteless [48]. Although inert, argon appeared to develop biochemical activities such as interference with oxygen receptor sites of enzymes and with protein conformation change. Furthermore, argon displaces oxygen more effectively than nitrogen. This is possibly based on its atomic size which is similar to molecular oxygen, its higher water solubility (0.034 vs. 0.016 g·L^{-1}), and its density which is higher than that of nitrogen (i.e., argon 1.650 kg/m^3 vs. nitrogen 1.153 kg/m^3) [49, 50]. With regard to the inhibitory activity against bacterial growth, argon was deemed to have a better solubility in fat, which results in improvement of membrane permeability of CO_2, salts, and acids to bacterial cells [51].

Several studies were conducted to investigate the effect of argon on enzyme activities and sensory characteristics of fruits and vegetables [52–56]. Various authors reported the effectiveness of Ar in limiting the growth of certain microorganisms, suppressing enzymatic activities, and controlling degradative chemical reactions in several perishable food products, such as mulberry fruits, which have been reported to show microbial activity, such as white spots of fungal hyphae growth, after just 1 or 2 days of shelf-life [39, 57, 58].

Despite there are many studies on postharvest use of MAP, to our knowledge, there are very few studies in literature regarding the influence of Argon and MAP on the shelf life and quality characteristics of small fruits. Particularly, the use of MAP in *Morus alba* fruit with Argon was not investigated yet. Stakeholders in the mulberry supply chain have raised concerns on the very short shelf life of fresh mulberry fruit sold in retail packages. In fact, because of high costs of production and wastes during postharvest storage, packaging, and transport, the mulberry supply chain is considered among the most unprofitable considering also high financial investments and labor required in the activities of the chain. For these reasons, in this study, several gas compositions were explored in order to ensure and maintain the quality characteristics of freshly harvested mulberries. Particularly, the objective of this research was to evaluate the effect of different MAP treatments (on quality parameters of mulberry fruit, stored at 4°C up to 12 d.

2. Materials and Methods

2.1. Plant Material. Fresh Italian white mulberry fruit (*Morus alba* L.) cv. 'Kokuso 21' grown in Sicily (Southern Italy) in a commercial orchard located in Partinico (38°06′ N, 13°07′ E, 103 m a.s.l.) consisting of 13-year-old trees trained to a vase shape were harvested at commercial maturity stage in the first week of June 2019. The commercial maturity stage of mulberry fruit cv. 'Kokuso 21' is reached when the fruit shows

a total soluble solid content of 13.0 (±0.4) °Brix [58]. Mulberry fruits that were over or under ripe and with slight injuries or defects were removed. After harvesting, the suitable fruit was immediately transferred by a refrigerated car within 2 hrs to the postharvest laboratory of the University of Palermo where it was processed within 3 hrs.

2.2. Experimental Design. To understand the effect of MAP treatments, the experiment was designed according to a full randomized block design with 4 main treatments: CTR1, CTR2, MAP1 and MAP2. Four storage times were tested: 0 (T_0), 4 (T_4), 8 (T_8), and 12 (T_{12}) days for each treatment and stored at 4 ± 1°C and 90 ± 5% RH.

Then, 2160 fruits were sampled as follows: 135 mulberry fruits per bag × 3 bags × 4 treatments × 4 times of storage were analyzed.

Therefore, the four treatments were as follows:

CTR1: 20.9% O_2+0.04% CO_2 (passive-MAP)

CTR2: 10% O_2+5% CO_2+85% N_2

MAP1: 4% O_2+6% CO_2+90% N_2

MAP2: 10% O_2+5% CO_2+85% Ar

Three hours after harvesting, the stem was cut to obtain uniform samples, and the fruits were irradiated for 30 minutes with ultraviolet germicidal light (UV-C; 180-280 nm with maximum at $\lambda = 254$ nm) to control microbial deterioration. Before packaging, fruits were washed with distilled water (5°C) and sanitized in 200 μLL-1 Ox-Virin (solution of hydrogen peroxide and peroxyacetic acid; 0.5% w/v) for 2 minutes.

Subsequently, the fruits were placed in low-density polyethylene bags (LDPE, Orved, S.p.A., Musile di Piave, Venezia, Italy -90 μ-80 mm-500 cm^3 films were the following: permeation to O_2 ($cm^3\,m^{-2}\,day^{-1}$): 4050; permeation to CO_2 ($cm^3\,m^{-2}\,day^{-1}$): 14000; OTR: 7500 $cm^3\,m^{-2}\,day^{-1}$; WVTR: 6.5 $g\,m^{-2}\,day^{-1}$under modified atmosphere packaging, using a digitally controlled packaging machine (VM 16 Orved S.p.A, Italy).

The relationship between the quantity of product and the gas mixture injected was 1 : 2 (v/v). Sensory and physicochemical analyses were carried out on three bags per treatment at each storage time to evaluate the shelf life of the fruit.

2.3. Weight Loss. The net weight loss of each bag was measured by a two-decimal precision digital scale (Gibertini EU-C 2002 RS, Novate Milanese, Italy), and the values were expressed as relative percentages of mean and standard deviation (1):

$$\textbf{Weight loss}(\%) = [(\mathrm{Wi} - \mathrm{Wd})/\mathrm{Wi}] \times 100, \quad (1)$$

where Wi is the initial weight and Wd is the weight measured at the end of each storage time.

2.4. Juice Content. The pulp of fruit was extracted using a centrifugal juicer (Centrika Metal, Mod. 0173, Ariete, Italy), and the juice extracted (J) was expressed as mL of juice per 100 g of pulp.

2.5. Total Soluble Solid. Total Soluble Solid (TSS) content was determined using a pocket refractometer Atago Pal-1 (Atago Co., Ltd, Tokyo, Japan). At each storage time, twenty fruits per bag were taken and squeezed together to obtain one homogeneous juice sample per treatment which was used for repeated readings. The results were expressed as °Brix.

2.6. Titratable Acidity. The titratable acidity (TA), expressed as malic acid (g malic acidL^{-1}), was determined by titration of 10 mL of juice with 0.1 M NaOH to an endpoint of pH 8.1, with a Crison Compact titrator pH-meter (Crison Instruments, SA, Barcelona, Spain).

2.7. Surface Color. Color was measured in terms of L^*, a^*, and b^* values under CIELab Color System using a portable colorimeter (Minolta CR-400 Chronometer; Konica Minolta Sensing, Osaka, Japan), using 10 fruit for each treatments. Two readings were taken for each fruit, for a total of 20 readings per treatment.

Results, expressed as chroma (C ∗) and hue angle (h°), were determined using a ∗ and b ∗ values according to Equations (2) and (3). The chroma value describes brightness, while the hue angle represents a coordinate in a standardised color space.

$$\boldsymbol{C}^* = \left(a^* + b^{*2}\right)0.5 \quad (2)$$

$$\boldsymbol{h}^\circ = \arctan\left(b^*/a^*\right) \quad (3)$$

2.8. Headspace Gas Analysis. At each sampling date, two bags per treatment were used to measure the CO_2 and O_2 content in the headspace of the bag using a Dansensor Checkpoint O_2 PBI analyzer and a CO_2 analyzer (Topac, Hingham, MS, USA) equipped with zirconium and infrared detectors, respectively. Gas samples were taken from the bags with a 20 mL syringe. Results were expressed as the average O_2 and CO_2 % for the three readings for each bag.

2.9. Sensory Profile. Sensory evaluation test was performed by an evaluation team of 30 panelists (sixteen men and fourteen women aged between 25 and 60 years) with a good background and knowledge of this type of evaluation [59–61]. During the preliminary meetings, 14 descriptors were selected on the basis of citation frequency (>60%) for the definition of the sensory profile, which are the following: Skin color (PC), Consistency (CN), Mulberry odor (MO), Fruity odor (FO), Off-odor (OO), Acid (AC), Sweet (SW), Bitter (BT), Juiciness (JUI), Astringent (AST), Mulberry flavor (MF), Fruity flavor (FF), Off-flavor (OF), and Total evaluation (TE).

The evaluation was carried out from 10.00 a.m. to 12.00 p.m. in a proper room with individual cabins under white lights. The samples were taken from the cold room 1 hour before being tasted and were presented in a white plastic plate [62]. Each panelist received in random order a sample of 2 anonymous mulberry fruits in each plate, labeled with numbers. Sparkling water was provided for rinsing between each sample.

The judges evaluated the intensity of each descriptor by assigning a score, each score represented a different level of intensity of the quality descriptors. The panelists scored the descriptors according to a 9-point intensity scale: 1 = no

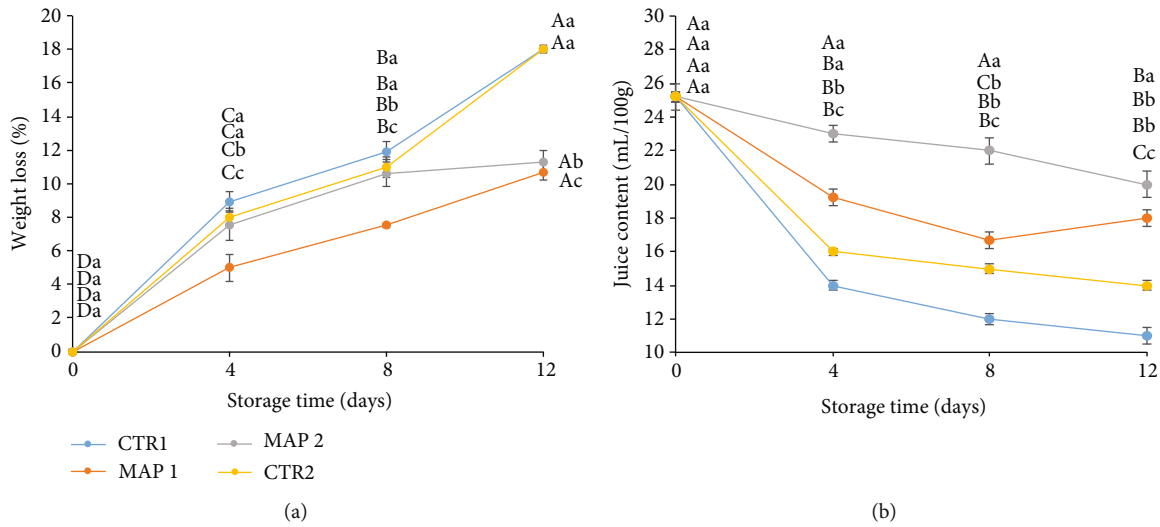

Figure 1: Pomological traits of mulberry fruit at 0, 4, 8, and 12 days of storage at 4 ± 1°C and 90 ± 5% RH (relative humidity) after all treatments. (a) Weight loss, %. (b) Juice content, mL/100 g. Means ± SD with different letters are significantly different at $p \leq 0.05$ using Tukey's HSD test. Different lowercase letter denotes significant differences ($p \leq 0.05$) among different treatments for the same sampling time. Letter "a" or "A" denotes the highest value. Different capital letters denote significant differences ($p \leq 0.05$) among different sampling times for the same treatment.

sensation, 2 = barely recognizable, 3 = very weak, 4 = weak, 5 = light, 6 = moderate, 7 = intense, 8 = very intense, and 9 = extremely intense [62].

2.10. Statistical Analysis. A Two-Way ANOVA was performed to evaluate the effect of the cold storage period and MAP on quality parameters using the univariate general linear model procedure. Statistical analysis was carried out using the SISTAT 13.2 statistical software (Systat Software Inc., CA, USA). Significant differences ($p \leq 0.05$) among treatments during each storage time and for each treatment over storage were evaluated by Tukey's multiple range test (Tukey HSD test). Pearson's correlation was also performed.

3. Results and Discussions

3.1. Physical Analyses

3.1.1. Weight Loss. One of the characteristics of small fruits like mulberry or strawberry which contributes to their highly perishable character is the rapid water loss [63]. Modified atmosphere packaging reduces water loss by maintaining a relatively high humidity in the headspace atmosphere [64].

In this study, all treatments showed a gradual loss of weight during storage due to transpiration (Figure 1(a)). However, significant differences were found ($p \leq 0.05$) in net weight loss for different treatments, particularly from day 8 of storage (Figure 1), when fruits treated with MAP1 and MAP2 had a lower weight loss: 10% and 10.5%, compared to CTR1 and CTR2 which had a weight loss of 18.09% and 18%, respectively. According to literature [65], the lower weight loss of samples treated with MAP1 and MAP2 could be a consequence of lower activity of the enzymes responsible for softening of pericarp (pectinesterase, polygalacturonase, and beta-galactosidase) in fruits subjected to these treatments and for loss of cellular juice. In particular, for fruits treated with Ar (MAP2), the lower weight loss compared to CTR1 and CTR2 could be due to the fact that argon has a higher capacity to form clathrate hydrate than nitrogen [66]. Argon combines with water at an appropriate pressure to form clathrate hydrate, which limits the fluidity of the water and thus reduces water loss in fruits and vegetables [67, 68]. In fact, in general, the effectiveness of nonconventional gases has been suggested in relation to their ability to lower the water activity of packaged food [69]. Therefore, the results obtained have revealed that the treatment with Argon maintained the weight of the fruit, and this is in agreement with previous studies [70].

From the data obtained, we note that fruits stored in MAP1 also lose less weight than CTR fruits, in agreement with several authors who state that nitrogen is used to safely balance shelf life extension with optimal organoleptic properties of the product [71].

3.1.2. Juice Content. Mulberry juice is a popular drink among consumers, because this fruit is considered healthy for its intrinsic characteristics [72]. Generally, consumers expect fruit to give the sensation of juiciness, and research on small fruits has shown that the juice content is influenced by several factors and not only related to water content [73].

In particular, as reported in the literature, mulberry juice is rich in biologically active compounds [74], and, therefore, keeping the mulberry fruit completely unaffected by any damage is very important from a nutritional and commercial point of view.

The decrease in juiciness is due to the depolymerization of pectins following the action of β-galactosidase and pectinesterases [75], which especially in fleshy fruits hydrolyze pectins to peptic acids and methyl alcohol, reducing their gelling power and making the pulp softer. In addition, Asrey et al.

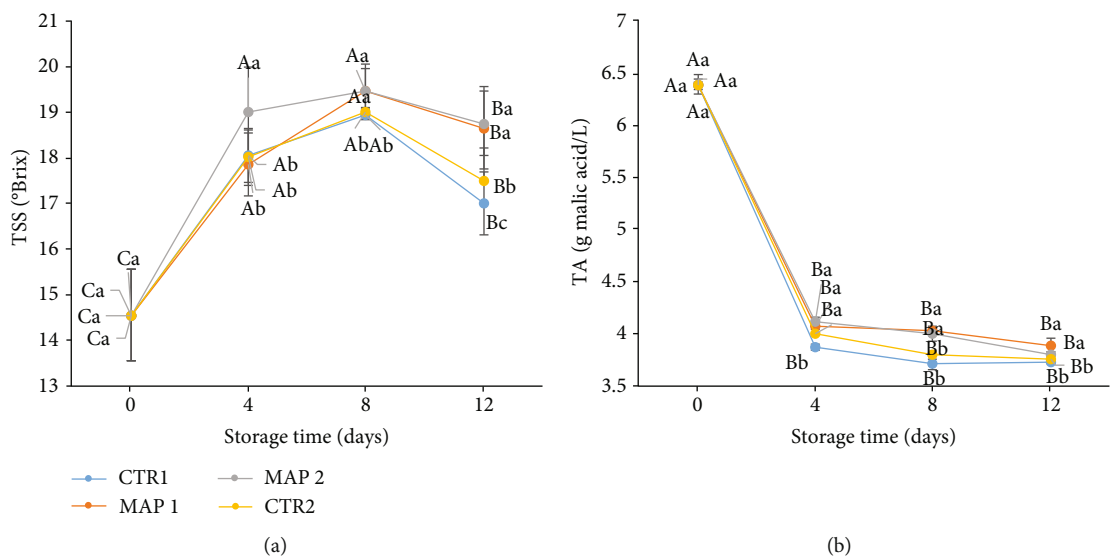

Figure 2: Pomological traits of mulberry fruit at 0, 4, 8, and 12 days of storage at 4 ± 1°C and 90 ± 5% RH (relative humidity) after all treatments. (a) TSS, °Brix. (b) TA, g malic acid/L. Means ± SD with different letters are significantly different at $p \leq 0.05$ using Tukey's HSD test. Different capital letter denotes significant differences ($p \leq 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p \leq 0.05$) among different treatments for the same sampling time. Letter "a" or "A" denotes the highest value. Different capital letters denote significant differences ($p \leq 0.05$) among different sampling times for the same treatment.

[76] studied the specific activities of polygalacturonase and pectinesterase which show higher levels at harvesting at commercial maturity, as in the case of the mulberry used in our experiment. At the eight days (T_8), we note that CTR1, CTR2, and MAP1 have reached the lowest value, confirmed by a visual loss of the fruit structure. MAP2 fruits, on the other hand, maintain the most constant juiciness values; for this reason, the MAP2 treatment appeared the most appropriate. In all our treatments, the juice content decreases in the first 4 days, probably due to low-temperature storage (Figure 1(b)); specifically, CTR1 decreases significantly, while MAP and CTR2 treatments show a less marked trend.

Furthermore, the results obtained for Ar-treated fruit can be explained by referring to what was reported by Zhang et al. [77] who state that argon has a better ability than nitrogen to reduce the level of dissolved oxygen, the presence of which is necessary for tyrosinase to catalyse the reaction, suggesting that argon can inactivate certain chemically active sites on the enzyme more effectively than nitrogen. Furthermore, Zhang et al. [77] state that argon treatment has a slightly greater effect on malic dehydrogenase than nitrogen treatment. This may be due to the fact that the higher solubility of argon compared to nitrogen may generate a higher affinity for the enzyme's inhibitory site.

Finally, argon appears to be a gaseous inhibitor for enzymes related to browning and respiration and therefore could play an important role in maintaining the quality of fresh fruit and vegetables to replace some chemical treatments that cause potential health risks.

The water solubility of Ar and N_2 is 0.034 and 0.016 g L^{-1} [78], respectively, so the greater capacity of Ar to delay the physiology of the mulberry compared to N_2 could be due to the greater capacity of these gases to dissolve in the aqueous layer of the fruit and then through the pulp cells. Therefore, argon may have inactivated certain chemically active sites on enzymes more effectively than N_2 by reducing the level of dissolved oxygen, the presence of which is necessary for oxidative enzymes to catalyse metabolic reactions.

3.1.3. Total Soluble Content and Titratable Acidity. Total soluble solids are a critical factor in determining fruit quality and consumer acceptability. Sugars are the main soluble metabolites and include glucose, fructose, and sucrose, accounting for 99% of the total sugar content [79]. TSS which mainly includes sugars and acids is closely related to taste and indicates the degree of maturity [80].

At harvest time, the values of TSS and TA were 14.55°Brix and 6.39 g malic acid/L, respectively. Observing the evolution of TSS and TA of mulberry, as expected, that after 4 days of cold storage, TSS increased and TA slightly decreased. TSS of our samples generally increased during the first 8 days of cold storage (24% CTR1, 25% CTR2, 27% MAP1, and 30% MAP2) and then decreased (Figure 2(a)). The TSS values decreased in the period from T_8 to T_{12} more for CTR1 and CTR2 fruits than for MAP1 and MAP2 fruits. This is due to the physiological ripening processes that determine the accumulation of sugars used as substrate in breathing processes [81] but is less pronounced in MAP-treated fruit due to the use of gas.

Fruits, due to the use of N_2 and Ar gases, consumed less O_2 (no hypoxic conditions were reached), as can be seen from Figure 3(a). Therefore, since we know that ripening is inversely proportional to the rate of respiration of the fruits, it is believed that the gases decreased the rate of respiration and therefore the consumption of organic substrates [82].

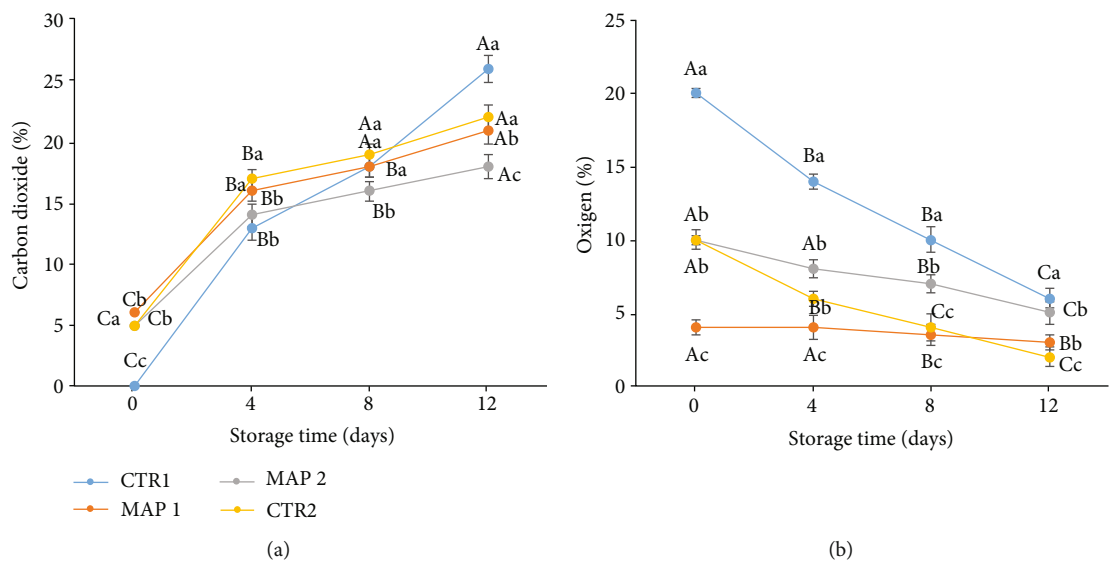

Figure 3: (a) Carbon dioxide content % (CO_2) and (b) oxygen % (O_2) inside packages with mulberry fruit stored at 4 ± 1°C and 90 ± 5% RH (relative humidity). Means ± SD with different letters are significantly different at $p \leq 0.05$ using Tukey's HSD test. Different capital letter denotes significant differences ($p \leq 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p \leq 0.05$) among different sampling times for the same treatment. Letter "a" or "A" denotes the highest value.

On the other hand, in agreement with the literature [39], we know that nitrogen is also used to replace oxygen in MAP products to prevent rancidity and inhibit the growth of aerobic organisms, and, on the other hand, Ar has some chemically active sites on enzymes and interferes in the evolution of soluble solids content during the ripening process. Rodriguez and Zoffoli [83] showed that in guava fruit, the significant increase in total sugars observed after the climacteric peak can be due to the increased activity of the enzymes responsible for the formation of complex sugars.

Moreover, as we can see from the data, the TSS values in mulberries preserved in MAP1 and MAP2 follow a more linear development than in fruit with CTR1 and CTR2 treatments.

In particular, fruits treated with MAP2 keep TSS content higher, until T_8, than other treatments, confirming that Ar interferes in the evolution of soluble solids [39].

The content of acid (TA), mostly malic acid, has gradually decreased over the storage period; as a result, the quality of the mulberries has decreased (Figure 2(b)). Before us, a similar behavior was observed by Caner [84], who attributes this to the dissolution of CO_2 in the water present on the surface of the fruit, generating carbonic acid and acidifying the fruit. The decrease in acidity was depending on the storage time ($p < 0.05$); this shows a high decrease in the values detected in all treatments during the first 4 days of storage and then stabilized at constant and established values up to 12 days of storage.

The sugar/acid ratio was evaluated, which is considered an index of fruit quality for fruit [85], and it is important to note that fruits treated with MAP show an increasing sugar/-acidity ratio during the storage period (2.28-4.40; 2.28-4.50; 2.28-5.45; 2.28-4.96 for CTR1; CTR2; MAP1; MAP2, respectively), thus suggesting a good quality characteristics for consumption even after storage [86].

3.2. Surface Color. Color is an important sensory characteristic of mulberry which affects the identification and recognition of the degree of maturity of the fruit and of the product quality; therefore, minimizing pigment losses during processing is one primary concern of the processor [45].

Maintaining the intrinsic color of fresh fruit is often used as a quality indicator and has a substantial impact on consumer acceptance [87]. Color development occurs as fruit matures, which is mainly due to the synthesis and degradation of anthocyanin, a pigment that contributes to the red color [88].

During the storage period, MAP1 and MAP2 were better at preserving luminosity, while CTR1 and CTR2 fruits became significantly darker with the lowest L ∗ value (data not shown).

Chroma values increased initially for all samples. However, since time T_8, they decreased, particularly the CTR1 samples, which were therefore less bright (lower chroma value). Moreover, as we can see from the data (Figures 4(a) and 4(b)), the highest chroma values were reported in both MAP treatments, probably due to the gas mixture that slowed down the oxidative processes [89], and this supports the observation that MAP preserved the color of mulberry fruit.

In the presence of oxygen, the fruit would suffer an enzymatic browning reaction [90, 91], which is undesirable as color is one of their most important parameters affecting consumer acceptance and purchase. Under normal conditions, in fruit, substrates and enzymes are distributed in different cell regions and enzymes do not promote browning. However, under adverse conditions such as senescence, the active oxygen metabolites are unbalanced, leading to lipidification of the cell membrane, destruction of the membrane structure, degradation of the cell structure, and the promotion of a large amount of contact between substrates and enzymes which leads to browning of the flesh and finally to loss of economic and nutritional value [92]. The oxidative lesion of the membrane, in fact, allows the mixing of the

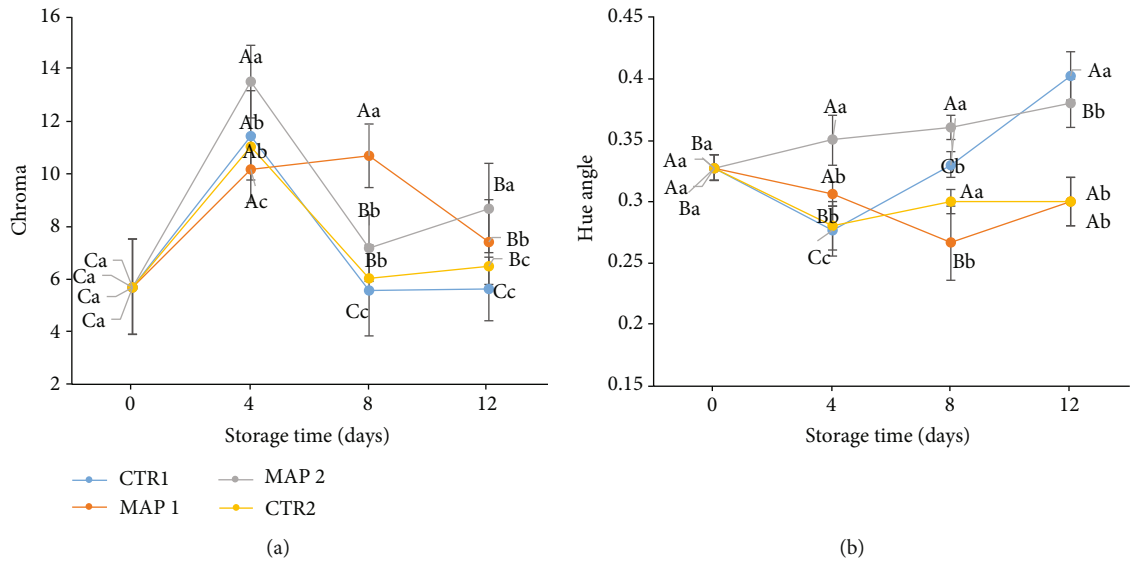

Figure 4: Color characteristics of mulberry fruit at 0, 4, 8, and 12 days of storage at 4 ± 1°C and 90 ± 5% RH (relative humidity) after all treatments. (a) Chroma value. (b) Hue angle. Means ± SD with different letters are significantly different at $p \leq 0.05$ using Tukey's HSD test. Different lowercase letter denotes significant differences ($p \leq 0.05$) among different treatments for the same sampling time. Letter "a" or "A" denotes the highest value. Different capital letters denote significant differences ($p \leq 0.05$) among different sampling times for the same treatment. Letter "a" denotes the highest value.

normally separated enzyme (PPO) and oxidisable substrates (polyphenols) [54].

3.3. Headspace Gas Analysis. The atmosphere in the packages depends on the initial gas added, the permeability of the packages, and the respiration of the fruit which produces CO_2 and consumes O_2 [93, 94]. Therefore, the composition of the gas is always in a state of dynamic change, and the concentration of O_2 decreases and CO_2 increases. The levels of O_2 and CO_2 detected in the headspace of the sample package during storage are shown in Figures 3(a) and 3(b). The initial gas composition changed rapidly for all treatments, and, as expected, we observe a decrease in O_2 concentration in the headspace, as well as an increase in CO_2 concentration during storage.

The final CO_2 content was 17% for MAP2 and 21% for MAP1, while it was 26% for CTR1 (passive-MAP) samples (Figure 3(a)). The changes observed can be attributed not only to fruit respiration but also to gas permeability through the LDPE film, as already reported by Hodges [95]. In general, the concentration of O_2 decreased rapidly during the first days of storage.

In particular, with regard to MAP1, the increase in CO_2 levels during storage caused a decrease in respiration and therefore in O_2 utilisation by the fruit [82].

With regard to CTR1, what we noticed was that the fruits consumed more than half of the initial O_2 concentration (Figure 3(b)) and simultaneously produced much more CO_2 than in the other treatments. This higher rate of respiration resulted in damage to the fruit which led to a greater loss of weight as they became very dehydrated and deliquescent.

In the MAP2 samples, CO_2 increased progressively during the storage period and at T_{12} was lower than in the other treatments. Similarly, the O_2 concentration decreased slightly from T_8, and this suggests that no undesirable changes occurred in the fruit, including the development of off-flavors [96], as also confirmed by the sensory analyses.

3.4. Sensory Profile. The fresh fruit has been much appreciated by panelists and specifically immediately after harvest (T_0) because of its intense flavor, aroma, and uniform epicarp color. After 4 days, it was possible to observe significant differences between all treatments. The highest score for the descriptors CN, PC, TE, and FF were given to fruit treated with MAP2, which maintained the values of the descriptors of fruit with almost no quality damages, followed by fruit treated with MAP1. In CTR fruit, it is possible to see a decrease of the organoleptic characteristics during the storage period. In general, in CTR, there was a decrease in the smell of mulberry with the appearance of off-flavor, probably due to the activation of fermentation processes. The fruit treated with MAP2 maintained high values of juiciness, sweetness, and consistency, which are very important parameters commercially. After 8 days (T_8), significant differences between CTR-, MAP1-, and MAP2-treated fruit begin to appear. CTR fruit had a general decrease of all descriptors, due to the normal physiological decay of the fruit in a passive atmosphere [97]. Fruit treated with MAP1 maintained the organoleptic characteristics, and those treated with MAP2 had the highest values for the attractiveness of the fruit, demonstrating the effectiveness of this gaseous mixture in maintaining the quality of the fruit. In fact, these fruits had very good values of consistency color, sweetness, and odor. At T_{12}, the descriptor that showed a significant decrease was the color. The fruit was opaque and with superficial deliquescence. The most appreciated fruits were those treated with MAP2 and the sensory analysis confirmed these results, highlighting that the

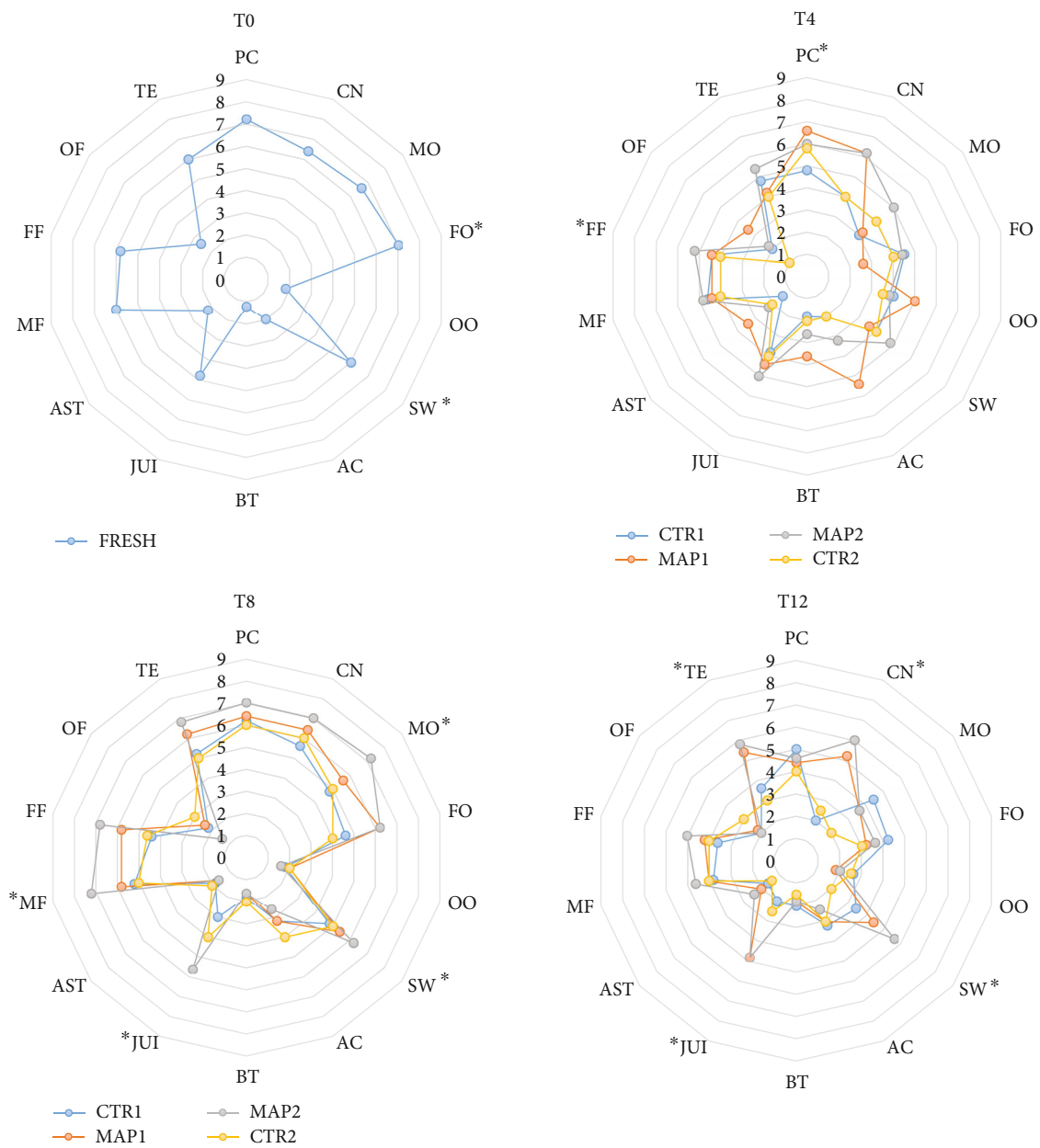

Figure 5: Sensory analyses of treated and untreated mulberry fruit at 0, 4, 8, and 12 (T0, T4, T8, T12) days of storage at 4° ± 1°C and 90% ± 5% RH. Descriptors legend: Peel color (PC), Consistency (CN), Mulberry odor (MO), Fruity odor (FO), Off-odor (OO), Sweet (SW), Acid (AC), Bitter (BT), Juiciness (JUI), Astringent (AST), Mulberry flavor (MF), Fruity flavor (FF), Off-flavor (OF), and Total evaluation (TE). For each descriptor, the values marked with * indicate significant differences between treatments. Data are the mean of 60 replications from one replicate of 60 fruit each.

MAP treatment with Argon MAP2 maintained all the organoleptic characteristics in terms of sweetness, flavor, and juiciness, providing valuable support in promoting the use of this treatment for prolonging the shelf-life of a fruit as delicate as these ones (Figure 5).

4. Conclusions

Modified atmosphere packaging (MAP) and argon treatments were found to be optimal storage treatments for mulberry fruit, maintaining their TSS but also their color, thus extending their shelf-life during refrigerated storage by up to 12 days. The results obtained show that the MAP1 (4% O_2+6% CO_2+90% N_2) and MAP2 (10% O_2+5% CO_2+85% Ar) treatments, combined with storage at low temperatures (4 ± 1°C), allowed to have fruit with good chemical-physical and organoleptic characteristics during 12 days. After 8 days of storage, MAP2 treatment showed optimal results in maintaining the juiciness, color, and TSS/TA ratio compared to fruit treated with MAP1 and CTR1 or CTR2. In particular, when comparing CTR2 and MAP2, it is possible to observe the positive effects of argon for the storage weight (over

80% for CTR2), the solid soluble content, the color, and the lower percentage of CO2 inside the bag.

It should be noted that although MAP with Ar prolongs the shelf life of the fruit after harvesting, the costs of the application are not low, and industrial use is useful only if the final product comes out with a high price on the market, but this is the case of mulberry fruit. Nevertheless, these findings clearly highlight that the use of Ar in the gaseous mixture, and the storage at low temperature provide a longer shelf life to this fruit of high intrinsic quality. Therefore, these findings may have useful implications for producers, stakeholders, and researchers, because a collaboration to allow the entrepreneurs of the mulberry supply chain to apply this technique will contribute to enhance the commercialization of Italian mulberry fruit in the foreign markets.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Willingness to Pay for Hexanal Technology among Banana Farmers in Meru County, Kenya

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From the perspective of food categories, fresh produce are the leading sources of food loss and waste globally. Their highly perishable nature shortens their shelf-lives leading to high postharvest losses if not properly handled. Currently, these losses are estimated at sixty-six percent based on total weight. Reduction of these losses will ensure constant supply of food along the supply chain as well as economic empowerment of the rural poor. Hexanal which is a naturally occurring compound has been developed as an intervention to prolong shelf-life of delicate tropical fruits such as bananas while also maintaining their quality. However, empirical evidence is still required on the usefulness of hexanal to farmers. It is envisaged that such evidence would inform scaling up of the technology in Kenya. This study assessed willingness to pay for hexanal and the factors influencing WTP amounts among banana farmers in Meru County, Kenya. Primary data was collected from 130 respondents who were grouped into aware and not aware of Hexanal. Results indicate that farmers who are aware of hexanal had a higher mean WTP Ksh 466.47 (US $4.66) compared to those not aware Ksh 331.86 (US $3.32). Factors such as age and income influenced the WTP amounts between subsamples. The major key policy implication of the study is the importance of stakeholders investing in the dissemination of information on hexanal among farmers to enhance uptake.

1. Introduction

Across the food categories, fruits and vegetables are the major causes of food loss and waste globally. According to literature, these losses are estimated at 66% based on total weight [1, 2]. Fresh produce has a very short shelf-life which predisposes them to deterioration when not adequately handled during harvesting, transporting, storage, marketing, and consumption. According to [3], postharvest loss is defined as the measurable qualitative and quantitative loss along the postharvest value chain.

These losses are higher in developing countries compared to the developed countries [4]. This is due to the lack or poor agricultural practices and specialized facilities such as cooling facilities, packaging materials, marketing systems, and infrastructure in developing countries [5] which delays produce reaching the markets on time and in good condition. In order to ensure increased availability of food along the supply chain from the existing production, it will be necessary that these losses and quality deterioration are reduced [6].

In Kenya, the fruit subsector is very important due to its tremendous contribution to the economy. In 2016, the subsector contributed Ksh 57 billion (US$ 570 million) which accounted for 27% Kenya's value of horticultural produce [7]. Bananas (*Musa* spp.) were ranked first in terms of production with 1.24 million tons being produced under 63074 Ha of land which was an increase from the 60743 Ha in 2015. The production was reported to be worth KSh 18.1 billion (US $ 180 million) accounting for 31.6% of the total fruits' production in the country [7]. The increased production has been attributed to the shift from backyard to commercial farming of bananas as a propoor agroenterprise [8, 9]. The major banana-producing regions in Kenya together with their percentage contributions are Meru (20%), Murang"a (11.7%), Kirinyaga (8.1%), Taita Taveta (6.6%), and Tharaka Nithi counties (5.6%) [10]. The most preferred

banana variety currently is the Cavendish (both the Dwarf and Giant) at 23%.

The banana enterprise is highly commercialized in Kenya as farmers sell 86% of their output [11]. Commercialization of bananas especially in Central and Eastern regions can be attributed to the decline in traditional cash crops such as coffee as well as the recent success in the introduction of high yielding tissue culture which includes Grand Nian, Williams, Chinese Cavendish, and Giant and Dwarf Cavendish varieties ([12], [13]). The market for banana is also rapidly expanding due to the demand for consumption of healthy foods.

Supply of the banana fruit is however lagging behind as the subsector is faced with high-post harvest losses which are estimated at 40% [5], the main cause being poor postharvest handling practices. A ripe banana is a delicate and perishable fruit [8] with a shelf-life of only 3-4 days [14]. The high-post harvest losses reduce the availability of the fruit in the supply chain as well as farmers' incomes. This is because farmers are forced to sell their produce at farm gate prices due to glut of the fruit in the market thereby making losses. It is therefore important to extend banana shelf-life to avert more postharvest and economical losses as well as enhance food and nutritional security which is one of the UN Sustainable Development Goals (SDGs) [15]. In addition, bananas are essential for human nutrition as they are a source of vitamins (B6 complex), calories, phytonutrients, and minerals (magnesium and potassium) [16].

1.1. Approaches and Technologies for Reducing Postharvest Losses in Bananas. Against this background, it is important to introduce and promote nonsophisticated technologies to farmers to delay ripening and prolong the shelf-life of bananas and other perishable farm produce at ambient conditions. This will ensure farmers benefit from the increased production and high demand of the bananas in the country. The locally available banana preservation methods/technologies include sun-drying, charcoal and brick coolers, and value addition into products such as banana flour.

Hexanal ($C_6H_{12}O$) which is a nanotechnology formulation that is organic in nature has been developed to prolong the shelf-life of mature green fruits such as strawberries, sweet cherries, and sweet bell peppers [17, 18]. Hexanal works by inhibiting the production of ethylene, thereby delaying ripening of fruits [19]. It has been found to be effective in increasing the shelf-life of fruits. In Kenya, efficacy trials carried out between 2014 and 2018 on bananas, mangoes, and pawpaw proved hexanal to be effective when used as either a spray or a dip. Bananas sprayed with hexanal solution remained on the trees for an extra 12 to 18 days before ripening based on peel color changes while dipping fruits in the solution prolonged their shelf-life by 9 days as well as improving their quality in terms of firmness and uniformity in color during ripening [20, 21].

Hexanal is insoluble in water, and to increase its solubility, a formulation is made known as the enhanced freshness formulation (EFF) that contains Tween 20, ethanol, and distilled water. Hexanal has been shown to have no negative effects on the human body. This is because it is oxidized after 48 hours to hexanoic acid which is further oxidized to water and carbon (IV) oxide during the respiration process [22]. Hexanal is also not traceable in treated tissues after 48 hours of treatment (http:/http://www.accessdata.fda.gov/). Therefore, fresh produce treated with hexanal do not require any special handling or cause any harmful effects. Currently, hexanal is not yet available in the Kenyan market and is only in use under experimental basis. However, efficacy trials have been successful and it is expected to be approved by the Kenya Plant Health Inspectorate Service (KEPHIS) for commercial purposes. In addition, hexanal is already in use and commercialized in other countries such as Canada and India.

To ensure broad and sustained adoption of hexanal technology, adequate information should be generated that will assist the product developers as well as other stakeholders to know how much farmers are willing to pay for the technology. Adoption of hexanal technology will ensure farmers have a little more time to look for premium markets thereby reducing their losses and increasing their incomes. The objective of this study was therefore to assess how much banana farmers are willing to pay for hexanal technology and the factors influencing their WTP.

1.2. Theoretical Framework. During the introduction of a new product in the market, the proponents are more interested in the production costs and consumer demand of the new technology. This is because these are the main considerations in the pricing of products and adoption by consumers. Estimating production costs is never a challenge unlike assessing the consumer demands for new products whose market prices are not yet set. This necessitates the need to create a hypothetical market scenario which is similar to real markets to enable economists assess consumer demands for new products [23] as well as their perceptions.

The current study was anchored on the random utility theory. The theory is based on the hypothesis that individuals are rational decision makers whose aim is to maximize utility relative to choices available. According to the theory, an individual will always select the alternative that maximizes his or her utility. Utility assigned to each alternative is determined by several attributes or characteristics, and since an individual's direct utility cannot be measured; their choices can be observed [24]. The utility of an alternative in this case depends on the attributes of the alternative as well as the individual whereby some are observable while others are unobservable to the analyst [25]. The observed attributes are represented as explanatory variables (deterministic component) while the unobserved attributes are treated as random variables (stochastic component) in the utility function [26].

According to [27], random utility models have demonstrated their usefulness over time in guiding of innovation development. [28] noted that survey responses from CVM are economically meaningful, as they are comprised of a utility maximizing response to a survey questions hence being consistent with the utility maximization economic model. Since utility maximization is subject to a budget constraint, a consumer can only choose a good that maximizes his/her utility but not above his/her budget as his/her demand will be constrained. With measurement of a good's quality being

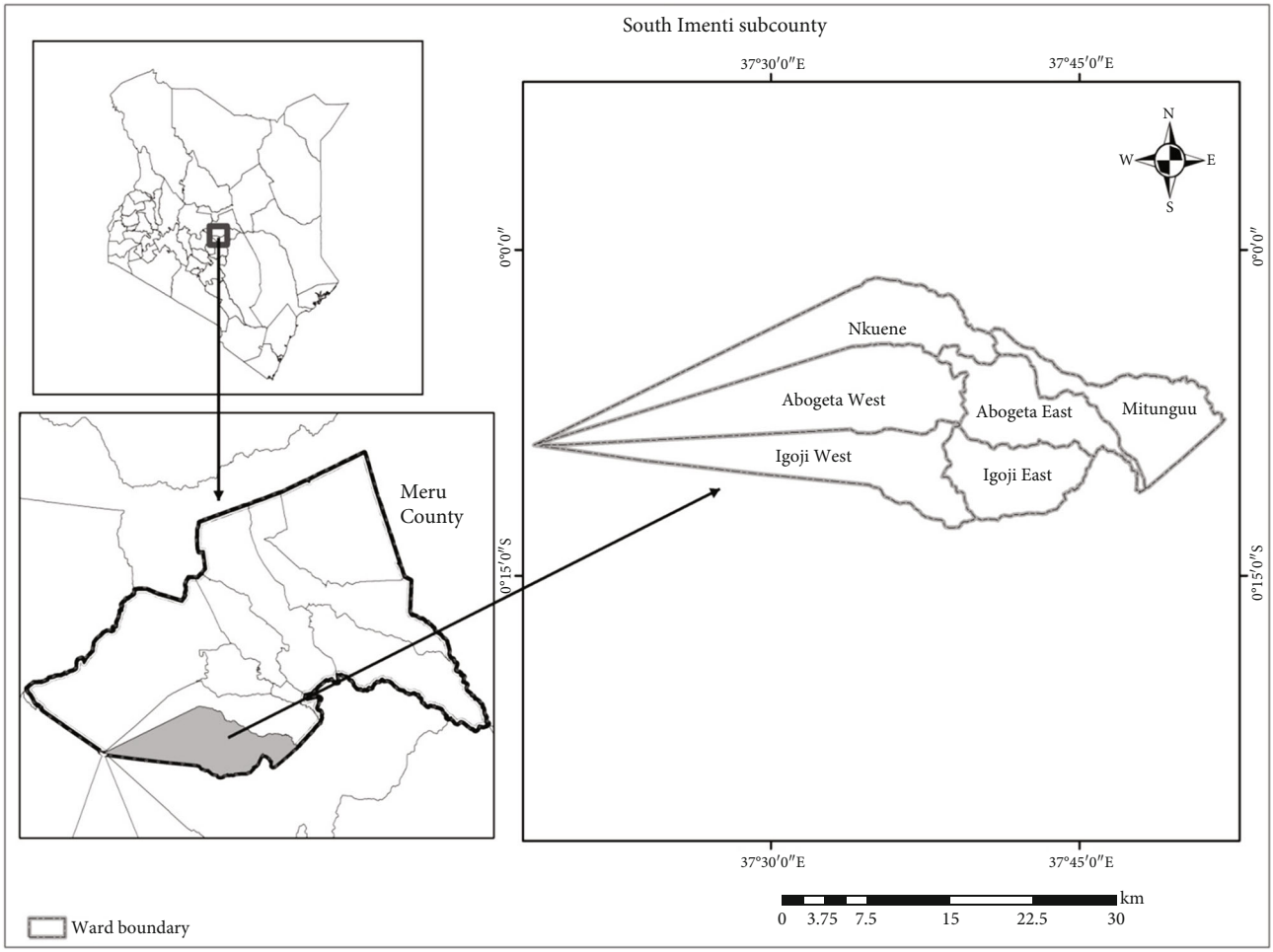

Figure 1: Map showing study areas in Imenti South subcounty. Source: created from Arc-GIS by Author.

represented by q a rational individual will always choose the level of market good represented by xm that maximizes their utility forming a Marshallian demand curve, xm (p, y, q); whereby (p is the current market price of the good and y is the individual's income). Therefore, WTP estimates are useful in agribusiness as they identify positions on the demand curve beyond which returns on investments are positive [29].

2. Materials and Methods

2.1. Study Area. Meru County (Figure 1), which is approximately 225 km northwest of Nairobi, is located on the eastern part of Mount Kenya covering an area of 6936 sq km. The county borders four other counties, namely, Laikipia to the west, Tharaka-Nithi to the southwest, Isiolo to the north, and Nyeri to the southwest. The area lies between altitudes of 300 and 5199 meters above the sea level. Climate is cool and warm with annual average temperatures ranging between 8°C in cold seasons and 32°C in hot seasons. The average annual rainfall received in the region is 1250 mm [30].

Agriculture is the main economic activity in the county with tea, coffee, and bananas being the main cash crops produced. Additionally, dairy and fish farming is also practiced mainly for local consumption. In 2014, 9715 tonnes of bananas were produced from the county which was an increase from 6884 tonnes in 2013 [30]. Tourism is also a major economic activity as the county has several tourist attraction sites such as the Lewa Conservancy, Meru Museum, Meru National Parks, and Mt. Kenya National Park. Despite the region being cosmopolitan, majority of the people are Meru speaking.

Meru County comprises of nine subcounties, and the current study was based in South Imenti subcounty. The subcounty which covers an area of 739 sq km is the most developed in Meru with a good and vast road network that facilitates transport of inputs and produce to markets. According to the 2009 household survey, population was at 179604 [31]. Furthermore, the subcounty was purposively selected as it is where efficacy trials of hexanal on bananas were conducted [21].

2.2. Sampling and Research Design. Data collection was conducted in April 2018 as a second follow-up survey on individuals interviewed during a baseline survey by the University of Nairobi in collaboration with the International Development

Research Centre (IDRC) in 2016. Criteria for selecting respondents for the current study were therefore grounded on the respondents from the baseline survey. The study only targeted farmers who produce bananas either for commercial or subsistence purposes. A household unit was used as the sampling unit.

The baseline study used a multistage sampling procedure in selecting respondents. In the first stage, Meru County was purposively selected based on empirical evidence as the region with the highest volume of bananas in terms of production and marketing ([32], [9]). The second stage involved mapping out banana producer groups in the area from which a sampling frame comprising banana producers in South Imenti subcounty was created. A sampling frame of 1800 households producing bananas was generated from banana farmer groups and cooperatives in the region. Given the known population from which sampling was conducted, Cochran (2007) formula for known population was used to generate the sample size as shown below:

$$\frac{n_0}{1 + (n_{0-1}/N)} = n, \tag{1}$$

$$\frac{384}{1 + ((384 - 1)/1800)} = 317. \tag{2}$$

From the formula, the ideal sample size for this study was 317 respondents. However, due to constraints in time and resources, systematic random sampling was used in the third stage to select every 10th respondent on the list which resulted in a representative sample of 180 households. Systematic random sampling eliminates bias by guaranteeing that each household has an equal opportunity of being selected [33]. The current study however managed to interview only 130 respondents which were occasioned by dropouts during the follow-up interviews.

Interviews were only conducted with the household head, spouse, or both. Absence of either the head or spouse in a household resulted in termination of the interview, and the household was systematically substituted. Since the households were specifically selected from banana production groups, replacement was only from a specific household list which greatly reduced the sample size. The main challenges encountered during data collection was absence of respondents, a tough terrain, and bad weather which made it difficult in accessing some households as well as increasing costs.

In the current study, the respondents were categorized into two groups comprising of the treatment and control groups. The treatment group (aware) ($n = 52$) attended a dissemination workshop where they were trained on the use and benefits of hexanal in February 2018. On the other hand, the control group (not aware) ($n = 78$) comprised of farmers that did not attend the dissemination workshop and were not aware of the existence of hexanal. Enumerators familiar with the local dialect collected data on sociodemographics of the respondents, farm characteristics, infrastructure, external support services, and WTP values.

2.3. Data Collection

2.3.1. Willingness to Pay Elicitation Format and Bidding Process. The study used contingent valuation method which is one of the stated preference (SP) approach used to elicit the maximum amounts farmers were willing to pay for hexanal technology. According to [34], CVM is the most common approach used to elicit information on the value of nonmarket goods using questionnaires. Despite its popularity, the approach harbors some concerns in relation to its associated bias hypothetical premise [35]. However, these concerns can be addressed by improving the design of the survey as well as the administration of the questionnaire [36]. There are four major elicitation techniques used in CVM, and they include the open-ended questions (OE) whereby respondents are directly asked for their maximum WTP, the payment card technique which involves a respondent picking a card containing their preferred maximum WTP value for the good in question, dichotomous choice (DC) technique whereby the respondent is asked to state 'yes' or 'no' to a predetermined bid that is set to reflect the maximum WTP, and the bidding game [37].

The choice of elicitation technique to use depends on the nature of good to be valued as well as the resources available for survey. In the case of this study, bidding game technique was used where the respondents were assigned a specific initial bid and were required to answer with a 'yes' or 'no' The difference between the dichotomous choice and the bidding game is that in the latter, the process is continuous whereby the interviewer increases the bid amount if the previous response was 'yes' until they obtain a negative response and reduces the initial bid amount if the previous response was 'no' up to a point a positive response is obtained and the highest amount the respondent is WTP is recorded [38].

Data was obtained on the WTP amounts for both groups of farmers who are aware as well as those not aware of the hexanal technology. Farmers who were already aware of the technology were given a brief reminder of the attributes of the technology, how to use the technology, and its' benefits as they had already attended a dissemination workshop on the same. As for the case of farmers not aware of the technology, a hypothetical scenario was provided in order enable the elicitation of the maximum WTP amount from the farmers. Information on mix ratios of Hexanal was explained of diluting 0.25 liters of hexanal with 12.5 liters of water. It was explained to them that the solution would be enough to spray 125 bunches of bananas or dip as many fruits till the solution is completely used. The hypothetical scenario was designed as follows: *"banana production supports many farmers economically in Kenya. However, lack of access to proper postharvest handling techniques contributes to great losses of up to 40% each year. There is an organic pre-harvest dip and spray known as Hexanal technology {which is an Enhanced Freshness Formulation (EFF)} that is capable of prolonging fruit shelf life by 21days on the trees and 17* days *in storage (at room temperature) to 26* days *in cold storage. Field trials carried out in Kenya show it is very effective in prolonging*

shelf life in mangoes and bananas while causing no harmful effects on humans. The product is currently not available in the market but considering the costs of importation it would cost Ksh.400 (US $4) per 0.25Litres. If the product was introduced in the market and you were required to pay for it, would you be willing to pay for it? Would you be willing to pay Ksh400 per 0.25L?"

Iterative bidding was then used to elicit the maximum WTP. First, the enumerator explained to the respondent that they would have to pay cash for the product or purchase it through credit from an agro-dealer and repay later after harvesting. A bid of ±Ksh 50 (US $0.5) was used whereby if the answer was "yes" to the initial amount of Ksh 400 an increment of the bid amount was added until the respondent said "no." In case the respondent responded "no" to the first amount an equal decrement of the bid used until the respondent revealed the amount they are willing to pay by answering with a "yes." The revealed amount was recorded as the maximum amount farmers are WTP. The base price of Ksh 400 of the hexanal technology was obtained from the aggregation of the components' current market value/prices used to formulate hexanal.

2.3.2. Data Analysis. The mean WTP amounts and the factors likely to influence farmers' WTP were all analysed using econometric software Statistical Package for Social Scientists (SPSS) version 20, and STATA version 14. SPSS was used for data entry and cleaning while STATA was used to estimate the mean amounts farmers are willing to pay for the hexanal technology as well as their determinants. Data was analysed separately for the two groups of farmers to obtain differences in WTP amounts and their determinants between the treatment farmers and control farmers.

2.3.3. Econometric Estimation. In the case of choice discrete response format as is the case in this study, it is assumed a farmer is interested in reducing postharvest losses of his fruits. Therefore, his/her corresponding indirect utility function would depend on q which is the novel product to be valued; p, prices of market goods; z which is the farmer's characteristics; y representing the farmer's income, and ε representing some stochastic components of preferences of the farmer which are unobservable to the researcher and hence treated as random [26]. Therefore, the farmer will be faced with the following indirect utility function $V(q^{\circ}, p, y, z, \varepsilon)$. With introduction of hexanal technology, a farmer is confronted with the opportunity of prolonging freshness of his/her fruits which will require a change from using product q° which is the traditional postharvest handling techniques to q^1, which is the hexanal technology that has proved to be effective in prolonging the shelf-life of mangoes, bananas, and pawpaw in Kenya. Hexanal technology is more effective and has greater benefits to the farmer than traditional techniques hence $q^1 > q^{\circ}$. It is assumed the farmer perceives the change as an improvement in terms of incomes from the reduced losses and hence his/her indirect utility is as follows;

$$V\left(q^1, p, y, z, \varepsilon\right) \geq V(q^{\circ}, p, y, z, \varepsilon) \tag{3}$$

However, when the farmer is informed that the change would cost Ksh A the farmer would only be willing to pay (by replying "yes") to the amount only if

$$V\left(q^1, p, y - A, z, \varepsilon\right) \geq V(q^{\circ}, p, y, z, \varepsilon), \tag{4}$$

and "no" otherwise, as his/her main objective is to maximize utility.

The maximum amount a farmer is willing to pay for a change from q° to q^1 can be expressed using the compensating variation measure whereby C satisfies

$$V\left(p.q^1, y - C, z, \varepsilon\right) = V\left(p.q^{\circ}, y, z, \varepsilon\right). \tag{5}$$

Thus, $C = C\left(p, q^1\, q^{\circ}, y, z, \varepsilon\right)$ is a farmer's maximum WTP for the change. If the stated price in the bid question is lower than the above WTP, a farmer will answer "yes" and "no" otherwise and hence

$$\text{Max WTP} = C = C\left(p, q^1 q^{\circ}, y, z, \varepsilon\right) \geq A. \tag{6}$$

Adoption of the hexanal technology is perceived as a farmer's way of improving the quality and freshness of his/her fruits by changing postharvest handling techniques from q° to q^1. Alternatively, the WTP for the change in this case is expressed as

$$\text{WTP} = \pi\left(q^1, p, w\right) - \left(q^{\circ}, p, w\right), \tag{7}$$

whereby w is the vector of input prices and p is the vector of output prices, which yields the following indirect restricted profit function $\pi\left(p, w, q\right)$. In reference to equation (7) above, WTP is the amount of profit the farmer would be ready to forego to obtain the hexanal technology q^1 rather than using traditional techniques q°. The farmer is likely to adopt the novel product which is the hexanal technology if he/she perceives it to provide higher utility. WTP in this case was evaluated using averaging the 'Yes' individual bid responses which resulted in the mean amount WTP in Ksh.

A two-limit tobit model was used to assess the factors influencing willingness to pay for hexanal with logWTP as the dependent variable. Tobit model was found to be superior to OLS and probit models due to the nature of the dependent variable which was scaled between 2 and 3. WTP was censored from above and below due to the presence of outliers within the data. Tobit model uses the maximum likelihood estimation that directly estimates σ and β.

Theoretically, the model is presented as follows [39]:

$$Y^* = X\beta + \varepsilon, \tag{8}$$

where Y^* is the latent (hidden) variable that is unobservable, β is the vector for some unknown coefficients, and X is the vector for independent variables while ε is the error term which is assumed to be independently distributed with a mean of zero and a variance of σ^2.

Two similar regressions for both the treatment and control groups were run using identical sets of independent variables. The estimating equations are as follows:

$$\begin{aligned}\mathrm{WTP}(Y^{*}\mathrm{treatment}) = {} & \beta_0 + \beta_1\mathrm{AGE} + \beta_2\mathrm{INC} + \beta_3\mathrm{SEX} + \beta_4\mathrm{EDU} \\ & \cdot + \beta_5\mathrm{CRDTACC} + \beta_6\mathrm{LANDSIZE} \\ & \cdot + \beta_7\mathrm{GRPMBRSHP} + \beta_8\mathrm{PERCACCEPT} \\ & \cdot + \beta_9\mathrm{INITIALBID} + \beta_{10}\mathrm{OCCP} \\ & \cdot + \beta_{11}\mathrm{DISTMKT} + \varepsilon_i \cdots ,\end{aligned} \tag{9}$$

$$\begin{aligned}\mathrm{WTP}(Y^{*}\mathrm{control}) = {} & \beta_0 + \beta_1\mathrm{AGE} + \beta_2\mathrm{INC} + \beta_3\mathrm{SEX} + \beta_4\mathrm{EDU} \\ & \cdot + \beta_5\mathrm{CRDTACC} + \beta_6\mathrm{LANDSIZE} \\ & \cdot + \beta_7\mathrm{GRPMBRSHP} + \beta_8\mathrm{PERCACCEPT} \\ & \cdot + \beta_9\mathrm{INITIALBID} + \beta_{10}\mathrm{OCCP} \\ & \cdot + \beta_{11}\mathrm{DISTMKT} + \varepsilon_i \cdots .\end{aligned} \tag{10}$$

3. Results and Discussion

3.1. Sample Statistics of the Respondents Are as Shown in Table 1 below

3.2. Households' Willingness to Pay for Hexanal

3.2.1. Estimation of Mean WTP between Categories of Farmers. The results show that both groups of farmers in Meru County are willing to pay positive amounts for the hexanal technology (Table 2). The minimum amounts farmers were willing to pay for hexanal was Ksh 100 (US \$1) for both aware and not aware farmers, respectively. Farmers who were aware of hexanal had higher mean WTP of Ksh 466.47 (US \$4.66) compared to Ksh 331.86 (US \$3.32) for farmers not aware of the technology. The mode for both categories of farmers was Ksh 400 (US \$ 4).

It was hypothesized that there would be no difference in the mean amounts farmers are willing to pay between the farmers aware and those not aware of the technology. However, results from t-test testing the hypothesis of equal WTP amounts was rejected at 1% level of significance ($p < 0.01$). The mean WTP amount for farmers who attended the dissemination workshop is statistically higher than for those who never attended. Specifically, farmers aware of hexanal are willing to pay Ksh 134.61 (US \$1.34) more than those ones not aware of the technology. Therefore, the results are an indication that access to information on existence of a new technology increases the acceptance and WTP amounts for the technology. These results are consistent with [40] who found out that farmers who had prior knowledge about a hermetic storage bag in Kenya had a higher WTP compared to those with no prior knowledge. In addition, the mean WTP for farmers aware of the technology is also higher than the initial bid value which is an indication of undervaluation of the hexanal technology which can happen in cases where prices for nonmarket goods are set with little or no consideration for farmers' preferences [41]. For both groups of samples, the median WTP was found to be lower than the mean WTP amounts for hexanal. The findings are consistent with literature whereby [42] used the CVM approach to study the WTP for ecotourism development in Hong Kong and found out that the median WTP was 16% lower than the mean WTP.

3.3. Factors Influencing WTP for Hexanal. Identical sets of independent variables were used in the tobit regression model for both groups of farmers, those aware and those not aware of hexanal. Results on Table 3 below indicate that LR chi^2 statistic for both groups were significant at 1% level of significance ($p < 0.01$) which is an indication variables included in this regression significantly contribute to the changes in the maximum amount households are willing to pay for hexanal. The pseudo-R^2 was 53.15 and 24.25 for the not aware and aware groups, respectively. The values indicate that independent variables included in this model could explain 53.15% and 24.25% variation in the maximum WTP, respectively.

Among the explanatory variables, the initial bid amount positively influenced ($p < 0.01$) the maximum WTP for both groups of farmers. This is an indication that increasing the bid amount results in increased mean WTP for hexanal.

Based on the economic theory by [43], increasing the bid amounts through iterative bidding approaches, such as in this case, increases the demand for the product thereby increasing prices. The findings are an indication that households in Meru County believed the initial bid amount to be the true value of the technology and based their maximum WTP on the amount. Additionally, the findings could be indicative of the likelihood of occurrence of a starting point bias that could explain the high influence of the initial bid on the WTP amounts.

Age of the respondent negatively influenced ($p < 0.1$) the mean WTP amounts among farmers not aware of the technology. The results are an indication that older farmers not aware of the technology were willing to pay lower amounts for hexanal compared to younger farmers. These findings are consistent with [44, 45] who also found out that farmers' age negatively influenced adoption of agricultural innovations. Several studies have also found out that younger farmers are more receptive towards innovations and hence more likely to adopt new agricultural technologies compared to older farmers. Older farmers have been reported to be more conservative compared to younger farmers as well as more risk averse [46, 47].

Gender of the respondent (being male) was found to negatively influence the mean WTP amounts among households aware of hexanal ($p < 0.01$). This means the mean WTP amounts were less for male farmers compared to female farmers. According to a study by [48] on gendered analysis of banana value chain in Meru County, the research found out that women dominated the retail marketing channel. The findings explain why the WTP for hexanal is higher for women compared to men as women view hexanal as a technology capable of reducing their losses thereby increasing incomes from their sales.

The explanatory variable 'main occupation of the respondent' negatively influenced WTP amount ($p < 0.05$) among

Table 1: Socioeconomic characteristics of sampled households.

Variable	Min	Max	Mean (SD)
Household characteristics			
Household size	1	6	3.36 (1.36)
Age of household head in years	25	90	60.6 (14.4)
Years of farming experience	0	70	30.98 (15.67)
Years of schooling of household head	0	18	9.04 (3.6)
Annual income from banana production (Ksh)	1400	720000	121536 (115159)
Annual total household income (Ksh)	23760	1979000	333793 (279737)
Marital status of household head (1 = married, 0 = otherwise)	0	1	0.79 (0.41)
Gender of household head (1 = male, 0 = female)	0	1	0.84 (0.37)
Main occupation of household head (1 = farming, 0 = otherwise)	0	1	0.78 (0.41)
Farm characteristics			
Total land size (acres)	1	40	2.9 (4.02)
Land tenure (1 = titled, 0 = otherwise)	0	1	0.72 (0.45)
Infrastructure			
Distance to input shop (km)	0	20	0.92 (2.02)
Distance to banana collection center (km)	0	11	2.5 (2.6)
External support services			
Access to credit (1 = yes, 0 = no)	0	1	0.14 (0.35)
Group membership (1 = yes, 0 = no)	0	1	0.59 (0.49)
Years of group membership	0	50	7.47 (10.84)
Access to extension (1 = yes, 0 = no)	0	1	0.23 (0.42)
Perception on social acceptability of hexanal	-1.59	2.49	5.04 (0.99)

Source: Survey data, 2018; SD: standard deviation; Ksh: Kenyan shillings; km: kilometer. Conversion Ksh 100 = US $1.

Table 2: WTP estimates (Ksh per 0.25 liters of hexanal).

Household category	Valid n	Mean	SD	Min	Max	Mode	Median	*t* value
Meru (aware)	78	466.47	203.7	100	1000	400	400	
Meru (not aware)	52	331.86	126.27	100	600	400	325	
								-4.6518 ***

Source: Survey data, 2018. Ksh: Kenyan shillings. Conversion Ksh 100 = US $1.

the farmers who attended the dissemination workshop and are aware of the hexanal. Farmers who practice farming as their main occupation had reduced WTP amounts compared to farmers engaging in other nonfarm activities. This could be explained in that farmers involved in other activities viewed hexanal a solution to save time spent on farm activities and looking for markets, which increased the demand of hexanal among them.

Marital status of the respondent (being married) positively influenced ($p < 0.05$) WTP amounts among famers aware of the technology. Married farmers were more likely to pay higher amount for hexanal compared to the unmarried if they perceived hexanal capable of increasing their incomes which would enable them take better care of their families.

Distance to the market center negatively influenced the WTP amounts ($p < 0.1$) among households not aware of the technology. Living far away from market centres reduced a farmer's WTP amount. The disincentive for this group of farmers could be from lack of information on the uses and benefits of hexanal in prolonging the shelf-life of the fruit and therefore causing farmers to incur more transaction costs in search of the information leading to low demand for the technology. This variable had no significant influence on the WTP among farmers aware of hexanal.

Land size which was measured in acres positively influenced ($p < 0.1$) the WTP amounts among farmers aware of the technology. Farmers with larger farm sizes were willing to pay higher amounts for hexanal to reduce postharvest losses due to their high production of bananas. A larger land size under banana production meant increased output which required good postharvest handling to avoid losses. This led to the increased demand for the technology among farmers already aware of the benefits of hexanal [49]. Farmers with small land sizes are in most case not able to invest in expensive technologies.

The variable income (income received from banana sales) had a positive influence ($p < 0.05$) on the WTP amounts

Table 3: Factors influencing WTP for hexanal.

Variable LogMAXWTP (Ksh)	Control Coefficient (robust SE)	Treatment Coefficient (robust SE)
Initial bid amount (Ksh)	0.225 (0.035)***	0.339 (0.052)***
Age of household head (years)	−0.003 (0.002)*	0 (0.002)
Gender of household head (1 = male, 0 = female)	0.016 (0.054)	−0.283 (0.043)***
Main occupation of household head (1 = farming, 0 = otherwise)	-0.032 (0.032)	−0.146(0.05)**
Marital status of household head (1 = married, 0 = otherwise)	0.035 (0.06)	0.089 (0.038)**
Years of schooling of household head	-0.005 (0.006)	-0.006 (0.007)
Distance to market (km)	−0.017 (0.0090)*	-0.04 (0.008)
Land size (acres)	0	0.025 (0.014)*
Annual income from banana sales (log)	-0.033 (0.024)	0.014 (0.006)**
Perception on social acceptability of hexanal	0.042 (0.017)**	0.11 (0.019)
Group membership (1 = yes, 0 = no)	0.043 (0.042)	-0.063 (0.04)
Constant	2.99 (0.327)***	2.487 (0.128)***
Log pseudolikelihood	26.01	27.3
LR chi^2 [40]	53.02***	56.95***
Pseudo-R^2	53.15	24.25

Note: *, **, and*** implies statistically significant at 10%, 5%, and 1%, respectively. Source: Survey data, 2018. Ksh: Kenyan shillings, Robust SE: robust standard errors.

among farmers who were aware of hexanal. Households obtaining higher incomes from banana production were willing to pay higher amount for hexanal. Income can be used as a proxy for a household's ability to purchase quality farm inputs. This is consistent with the broad range of literature which shows that households with higher incomes have higher chances of being early adopters of new technologies [50]. Therefore, increasing a household's income will increase demand for inputs such as hexanal if they perceive it to reduce their losses. [45] also found out that farmers with high incomes are more likely to pay more for quality and healthier foods while [51] who studied WTP for Aflasafe in Kenya found out that households with higher incomes were willing to pay higher prices for the biopesticide in order to produce maize free from aflatoxins.

The perception on social acceptance of the technology positively influenced the maximum WTP ($p < 0.5$) among farmers not aware of the technology. Households that perceived hexanal to be a socially acceptable product which they would be able to incorporate it as one of their post-harvest management practices were willing to pay higher amounts for it. The findings concur with [52] who found out that consumers' perceptions influenced their maximum WTP for genetically modified rice.

4. Conclusion

The study is aimed at assessing the WTP amounts for hexanal and the factors influencing them among banana farmers in South Imenti subcounty. Overall, farmers are willing to pay positive amounts for the hexanal technology. This provides sufficient guide on the pricing mechanisms for the technology developers and the extension providers. In addition, farmers who were aware of the technology had higher WTP amounts compared to those not aware of the technology which explains the critical role of information in enhancing the acceptability/adoption of a technology.

WTP was influenced by several factors between the two groups. Factors that positively influenced WTP included initial bid amount, marital status, land size, income, and perception on social acceptability of hexanal. On the other hand, age, gender, main occupation, and distance to market center were found to negatively influence WTP amounts. Therefore, product developers should ensure pricing of the technology takes into account the households' sociodemographic characteristics that were found to influence amounts farmers are willing to pay.

Therefore, stakeholders should provide sufficient information in order to enhance perception on social acceptability of the technology as it was found to positively influence the WTP. Since distance to market center negatively influenced WTP, extension providers should educate farmers living away from town centers through farmer field days and trainings to increase awareness on hexanal in order to enhance its adoption. Farmer's age negatively influenced WTP among farmers not aware of the technology; hence, there is a need for product developers to conduct more dissemination workshops among younger farmers not aware of the technology in order to demonstrate the effectiveness of the technology.

The current study only focused on the WTP amounts and did not consider if hexanal is actually profitable for use by the banana farmers. Assessing the cost-benefit analysis of the technology is therefore a potential area of future research. Output from the cost-benefit analysis could be useful in

providing more evidence for increased dissemination and commercialization of the technology as well as aiding farmers in making more informed investment decisions.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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