Journal of Food Quality

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Research Article

Changes in Vegetative and Reproductive Growth and Quality Parameters of Strawberry (*Fragaria* × *ananassa* Duch.) cv. Chandler Grown at Different Substrates

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Growth substrates (polyester wool, rice husk, and wheat straw), along with soil as control treatment, were compared for vegetative growth, yield, and quality of strawberry cv. Chandler. All growth substrates tested showed good results in terms of growth rate as compared with control. Strawberry plants grown in polyester wool showed the highest (89.50%) survival rate as compared to rice husk (70.50%), wheat straw (64.63%), and control (67.56%). Moreover, a significant increase was observed among number of flowers, fruits, and yield in plants grown in polyester wool. Besides, a significant high amount of total soluble solids (TSS) (12.38 oBrix), titratable acidity (TA) (1.21%), ash (0.72%), vitamin C (37.39 mg/100 g), total carotenoids (3.90 μg/100 g), and total anthocyanins (3.47 cyanidine-3-glucoside/100 g) was recorded in fruits grown in polyester wool as compared to control. From these results, it can be concluded that the use of polyester wool as a growth substrate for strawberries can give higher yield and better fruit quality.

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a high value crop which is well known due to its colour, taste, and nutritional value which is increasing its economic importance [1]. These small fruits serve as an important source of different minerals and vitamin C and are also rich in antioxidants [2, 3]. Because of their high nutritional contents and economic importance, cultivars and advanced cultivation techniques are gaining importance in order to meet growing market demand. Under traditional growing system, strawberries are grown from replanted runners season after season in the same fields. This technique causes vulnerability to soilborne diseases such as *Verticillium* wilt, *Phytophthora* crown and

root rot, black root rot, and charcoal rot caused by *Verticillium dahliae*, *Phytophthora cactorum*, *Cylindrocarpon destructans,* and *Macrophomina phaseolina*, respectively, in different strawberry cultivars [4, 5].

Different chemical disinfectants such as chloropicrin and methyl bromide fumigants have been used in the past to lessen the effect of soilborne pathogens and diseases [6]. However, these days people are becoming very conscious about side effects of synthetic fungicides and chemicals due to their hazardous effects on health. Further, these chemicals are dangerous for the overall environment as well. These concerns have resulted in efforts to phase out most of the chemicals around the world [7, 8]. The use of 1, 3-dichloropropene and chloropicrin or their mixtures has also been subjected to granting of exceptional uses depending on the crop, soilborne pathogen, and production area [8]. Therefore, there is a need to find alternatives to overcome these disease problems and maintain cultivation systems for strawberries with maximum yield.

Soilless culture is considered, the best candidate for production of strawberry because it tends to be less prone to diseases. This system can fit higher plant density in a small area than the traditional system with less use of water and fertilizer [9]. Moreover, this system also possesses high water-holding and retention properties when compared with mineral soils. Therefore, in recent past due to having large porosity, being lighter in weight, being free from pests and pathogens, and having less chemical complexes, many soilless substrates (peat, gravel, sand, rockwool, perlite, etc.) have been used for growing different horticultural and ornamental plants [10, 11].

Strawberry is also grown successfully in soilless culture; however, the dynamics of nutritional value of strawberries are highly dependent on the cultural media, environmental conditions, and cultivation techniques [12]. Several studies reported the role of diverse growing substrates on yield and overall quality of fruits [1, 5]. Four pepper cultivars grown in peat or in mixture media [peat + perlite + sand (1 : 1 : 1)] under greenhouse conditions showed that mixture media significantly improved fruit length, diameter, and weight in all cultivars as compared with peat. However, fruit quality parameters such as ascorbic acid content and total soluble solids were found higher in peat grown plants than the mixture media [13]. Similarly, an earlier study reported that when cucumber plants were grown at four different growing media, including peat, perlite, rice hull, and a mix substrate (perlite and rice-hull 50 : 50 *v*/*v*), results showed that substrates had significant effects on plant growth, total fruit yield, marketable fruits, and fruit weight, while no significant differences were observed among substrates in terms of fruit quality such as fruit length, diameter, and total soluble solids [14].

However, to our knowledge no studies have been done to evaluate the nutritional composition of strawberry fruits with respect to the nature of substrates. As growing substrate is a crucial feature in determining the nutrient and water uptake efficiency of plant which shows a significant role in manufacturing different biochemical compounds, we believe that this is an important question.

The current study was designed to evaluate the effect of four substrates on vegetative and reproductive growth and quality of fruits with special attention given to pH, total soluble solids, and titratable acidity which are responsible for flavour and taste development in strawberries. Further, secondary metabolites such as phenolics, flavonoids, carotenoids, and anthocyanins were also quantified during the experiment as these are associated with health promoting properties.

2. Materials and Methods

2.1. Experimental Details and Plant Material. The current study was done during 2018-2019 in the Department of Horticulture, Faculty of Agriculture, University of Poonch Rawalakot, Azad Jammu and Kashmir. Results reproducibility was confirmed by two experimental trials. The first trial took place from February to July 2018 and the second from February to July 2019 for all the parameters tested. In this study, different growth media were tested for cultivating strawberries. Strawberry runners of cv. Chandler were purchased from Mountain Agriculture Research Center Juglot, Gilgit-Baltistan, and transplanted to small plastic pots (12 inch). Based on the previous information reported, three different growing substrates were selected for growing of these runners. Each treatment was replicated three times and ten pots per replicate were filled in a ratio of 1 : 1 *v*/*v* (growing media: soil) with each of the growing media (polyester wool, rice husk, and wheat straw). Pots filled with only soil served as control. One runner per pot was planted. Physiochemical analysis of soil (preexperiment) which was used for filling of pots and pH of different growth substrates was done at Horticulture Lab, University of Poonch Rawalakot, and results showed that the soil was suitable for growing of strawberry plants (Table 1).

2.2. Field Site Description. Strawberry plants were planted in plastic pots and placed under shade at the Experimental Farm of the Faculty of Agriculture, University of the Poonch Rawalakot, Azad Jammu and Kashmir (latitude 33°51′32.18″N, longitude 73° 45′34.93″E). Shade was provided with polyethylene sheet (PE 200 μm). For this experiment, the pots were placed 15 cm apart. Fertilizers such as farm yard manure containing 0.5% N, 0.2% P_2O_5, and 0.5% K_2O were mixed with each growing media in a ratio (1 : 1) and urea 0.5 g in one liter of water was applied after one month of transplanting and then at fifteen days interval till harvest. Other agronomic practices such as irrigation were done twice in a week (200 ml per pot), and weeding was also done in order to ensure better growth and quality of strawberry plants. Data regarding vegetative growth, reproductive growth, and quality parameters of strawberry were recorded as mentioned below.

2.3. Vegetative and Reproductive Growth Parameters. Thirty plants from each treatment were used for data collection. Vegetative and reproductive growth were determined on the basis of various parameters. After one month of transplanting strawberry runners, survival rate was

Table 1: Physicochemical properties of soil before planting strawberry runners and pH of different growth substrates.

Parameters	Average content
Total N (%)	0.016
Available P (mg kg^{-1})	5.66
Available K (mg kg^{-1})	96.67
Soil organic matter (%)	0.33
Sand (g kg^{-1})	450
Silt (g kg^{-1})	260
Clay (g kg^{-1})	280
pH (soil)	7.4
pH (polyester wool)	8.5
pH (rice husk)	5.8
pH (wheat straw)	6.6

observed by calculating the number of plants from each treatment that survived divided by the number of plants originally planted. Leaf area was measured using a measuring scale at the time of harvest. The number of runners per plant was counted at the end of experiment. Days to first flower, fruits per plant, and days to harvest at commercial ripeness (>75% of the surface showing red colour) [1] were also recorded. Chlorophyll a, *b*, and total were measured by destructive sampling as given by Zahid et al. [15]. Overall yield per plant was measured after weighing harvested fruits from each plant and was expressed in grams. Fruit weight (01 fruit) expressed in grams (g) was determined using digital weighing balance (Model: Shimadzu A × 200, Japan) and average was taken. Diameter (01 fruit) expressed in millimeter (mm) was measured by using vernier calliper (Model: Insize SR44), and the average was taken.

2.4. Fruit Quality Parameters. A mixture of 300 g strawberry fruits from each treatment was used in triplicate to measure different fruit quality parameters. Fruits were kept in zip lock poly bags and were taken to Horticulture Lab, University of Poonch Rawalakot. Fruits were washed with purified water, air dried, and used for various analysis. Total soluble solids (TSS) were measured using method of Association of Official Analytical Chemists [16] by using hand refractometer (Model: Kyoto Company, Japan) and expressed in percentage. Titratable acidity (TA) was determined through titration method [17]. Briefly, the filtrate (5 ml) with 2-3 drops of 0.1% phenolphthalein solution as an indicator was titrated using 0.1 N NaOH to a pink endpoint. TA was measured in relation to ascorbic acid by using the following formula and expressed as the percentage:

$$\text{TA\%} = \frac{\text{ml of sodium hydroxide used} \times 0.1\,\text{N} \times \text{equivalent weight of ascorbic acid}}{\text{weight of sample} \times \text{volume of aliquot}}. \quad (1)$$

pH of extracted fruit juice was determined according to the method of AOAC [18] using a pH-meter (Model: WTW 82362 Inolab, Germany). pH-meter was calibrated with pH 4.0 and pH 9.0 buffers before taking observations. Electrode was cleaned and dried before recording data for each sample. Total phenolic contents were measured using spectrophotometer (Model: UV 4000, ORI, Reinbeker, Hamburg, Germany) and a method given by Maqbool et al. [19]. Mixture was prepared using 10% Folin–Ciocalteau's Reagent (0.5 ml), 7.0% sodium carbonate solution (1.5 ml), and aqueous extract (0.1 ml). Purified water was added to make volume of mixture up to 10 ml. This mixture was incubated at 40°C for 2 hours, and the absorbance was measured at 750 nm using spectrophotometer (Model: UV 4000, ORI, Reinbeker, Hamburg, Germany). Obtained results were presented as μg of gallic acid/g fresh weight (FW) of fruit. Total flavonoids were measured according to the method given by Maqbool et al. [19], and the results were expressed as mmol quercetin/100 g FW. Ash content was determined by burning 0.5 g of dried fruits at 600°C in electric muffle furnace (Model: SX-2-5-10) for three hours [20]. Ash content was calculated by the difference observed in weight [1]. Crude fiber was determined using the standard method given by AOAC [21]. Fruit sample (5 g) was collected and dried in an oven and then digested with 1.25% sodium hydroxide (NaOH) and sulfuric acid (H_2SO_4) solution. After that, samples were washed, dried, and then placed in a furnace at temperature of 500 or 550°C until samples were converted into white ash. The fiber content was determined by using the formula

$$\text{Crude fibre} = \frac{(c-b) - (d-b)}{(a)} \times 100, \quad (2)$$

where a is the sample weight, b is the crucible weight, c is the sample weight before ignition, and d is the sample weight after ignition.

Total carotenoids were measured by crushing strawberry fruit (2 g) using mortar and pestle followed by mixing with 10 ml of chilled acetone [22]. Filtration of reaction mixture was done followed by separating of carotenoids from acetone using petroleum ether. Absorbance was then taken at 450 nm using spectrophotometer (Model: UV 4000, ORI, Reinbeker, Hamburg, Germany). Standard calibration curve was prepared using all-trans-β-carotene (Sigma Chemical Co., USA). Vitamin C was determined by using 2,6-dichlorophenol indophenol dye. Fruit juice (5 ml) plus 4% meta phosphoric acid (5 ml) was mixed, and the solution was titrated by using dye until the persistence of light pink colour. The results were expressed as mg/100 g FW of fruit. Total anthocyanin was measured using spectrophotometer (Model: UV 4000, ORI, Reinbeker, Hamburg, Germany) by pH dilution method [23]. Two buffers, that is, sodium acetate (pH-4.5) and potassium chloride (pH 1.0), were used for dilution of samples. The absorbance of each dilution sample was observed at 510 nm and 700 nm, respectively,

using spectrophotometer. Anthocyanin pigment was recorded as mg of cyaniding-3-glucoside/liter using an extinction coefficient of 29,600 and molecular weight of 449.2. Antioxidant activity of strawberry samples was measured using the technique given by Molyneaux [24] with slight modifications. In this method, for measuring antioxidant activity, free-radical l, l-diphenyl-2-picrylhydrazyl (DPPH) was used. Mixtures of 50 μl-methanolic solution (diluted 1 : 6) of each extract was made by adding 0.1 mM methanolic solution of DPPH and placed in a dark place at room temperature to react with each other. Reduction in the absorbance of DPPH at 517 nm was recorded in gaps of time of 5 min until the absorbance stabilized for 30 min. DPPH radical scavenging activity of fruit extracts was measured by using the formula

$$\text{DPPH scavenging activity percentage} = \left(A_0 - \frac{A_s}{A_0}\right) \times 100, \quad (3)$$

where $-A_0$ is the absorbance of the treatment under control conditions and $-A_s$ is the absorbance of the sample taken for study.

2.5. Statistical Analysis. Experiment was set in a randomized complete block design (RCBD) with three replicates, and it was repeated twice, and results were pooled for analysis as the important outcomes of separate analysis showed almost parallel results across the trials. Collected data was subjected to analysis of variance (ANOVA) using statistical software (Statistix 8.1) [25] and means were compared using Tukey's test at P ˂ 0.05.

3. Results

3.1. Effect of Different Substrates on Vegetative and Reproductive Growth Parameters of Strawberries. Results regarding survival percentage of strawberry plants showed significant differences ($P < 0.05$) among different growing media (Figure 1). The lowest survival percentage (64.63%) was noted in strawberry plants grown in wheat straw, while the highest survival percentage (89.50%) was noted in plants grown in polyester wool. However, a nonsignificant difference ($P > 0.05$) was observed in survival percentage of strawberry plants grown in rice husk (70.50%) and soil (67.56%).

On the bases of survival percentage results soil, polyester wool, rice husk, and wheat straw were used for further studies.

Different growing media showed a significant ($P < 0.05$) difference in terms of vegetative growth of strawberry cv. Chandler (Table 2). Maximum leaf area was noticed in plants grown in polyester wool, while minimum leaf area was observed in plants grown in soil, whereas nonsignificant difference ($P > 0.05$) was recorded between plants grown in rice husk and wheat straw. Significantly ($P < 0.05$) higher number of runners per plant were observed in polyester wool.

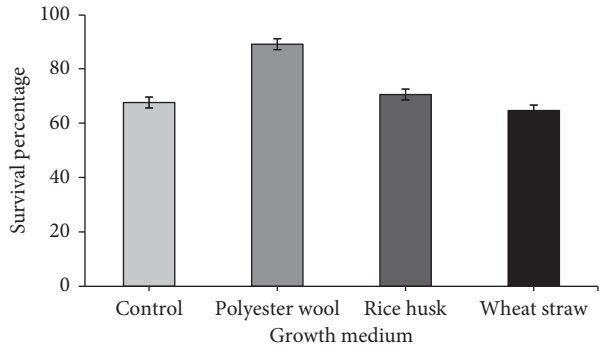

Figure 1: Effect of different growth substrates on survival percentage (%) of strawberry cv. Chandler. Vertical bars indicate standard error of means (±SEM) for three replicates.

Table 2: Effect of different growth substrates on vegetative growth of strawberry cv. Chandler.

Treatments	Leaf area (cm^2)	No. of runners
Soil (control)	40.63 ± 0.22 c	20.65 ± 0.40 bc
Polyester wool	61.46 ± 0.48 a	32.66 ± 0.46 a
Rice husk	53.30 ± 0.32 b	25.33 ± 0.18 b
Wheat straw	53.46 ± 0.26 b	22.34 ± 0.59 b

Note. Different letters in each column indicate significant ($P < 0.05$) difference among treatments, ±SD.

Results regarding days to first flower of strawberry plants revealed nonsignificant differences ($P > 0.05$) among different growing media (Table 3). All the plants took almost 52 to 57 days for appearance of first flower. Results regarding average number of flowers and average number of fruits per plant showed a significant ($P < 0.05$) difference among all treatments. Maximum number of flowers (29.73) and fruits (27.00) were recorded in plants grown in polyester wool, while minimum number of flowers (16.1) and fruits (14.60) were noted in plants grown in soil (Table 3). Rice husk and wheat straw statistically showed no difference for flowering and fruiting.

Results regarding number of days to harvest and yield per plant showed significant ($P < 0.05$) differences among treatments (Table 3). A minimum number of days to fruit harvest were recorded in polyester wool (115.6), whereas plants grown in soil took maximum number of days (132.4) for fruit harvesting. Similarly, maximum yield per plant (324.03 g) was found from plants grown in polyester wool, while minimum yield was recorded in plants grown in soil (163.52 g) (Table 3).

Results regarding chlorophyll showed significant ($P < 0.05$) differences among all substrates under study (Figure 2). The highest chlorophyll a, b, and total chlorophyll content were recorded in plants grown in polyester wool. In the case of chlorophyll b, plants grown in rice husk and wheat straw showed a nonsignificant ($P > 0.05$) difference with plants grown in soil.

Fruit characteristics in terms of weight of fruit and diameter of fruit were recorded for strawberries grown in different growth medium. A significant ($P < 0.05$) difference was recorded in weight of fruit and diameter of fruit of

Table 3: Effect of different growth substrates on reproductive growth of strawberry cv. Chandler.

Treatments	Days to first flower	No. of flowers	No. of fruits	Days to harvest	Yield (g)
Soil (control)	57.63 ± 0.15 a	16.10 ± 0.10 c	14.60 ± 0.21 c	132.40 ± 1.08 a	163.52 ± 1.70 c
Polyester wool	52.40 ± 0.37 a	29.73 ± 0.07 a	27.00 ± 0.08 a	115.60 ± 1.73 c	324.03 ± 4.34 a
Rice husk	50.90 ± 0.19 a	23.60 ± 0.21 b	21.80 ± 0.65 b	123.20 ± 1.67 b	283.03 ± 3.72 b
Wheat straw	52.46 ± 0.24 a	22.00 ± 0.17 b	20.43 ± 0.28 b	121.70 ± 1.75 b	275.13 ± 5.63 b

Note. Different letters in each column indicate significant ($P < 0.05$) difference among treatments, ±SD.

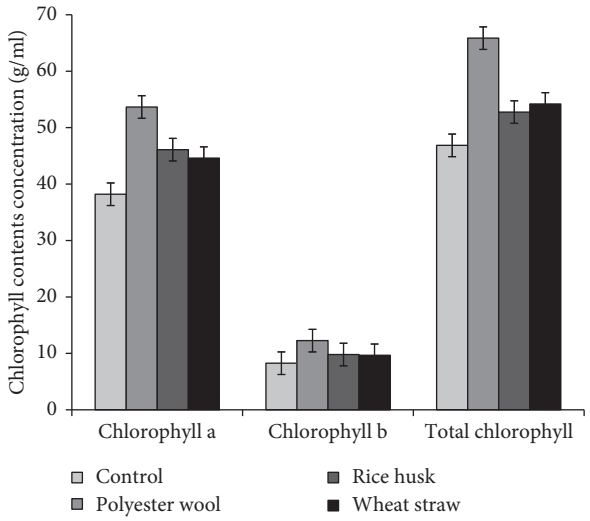

Figure 2: Effect of different growth substrates on chlorophyll contents concentration (g/ml) of strawberry cv. Chandler. Vertical bars indicate standard error of means (±SEM) for three replicates.

strawberries grown in different media (Figure 3). The highest fruit weight (19.2 g) was recorded in fruits grown in polyester wool, while the lowest weight of fruit was recorded in fruits grown in soil (13.9 g) (Figure 3(a)). Data regarding diameter of fruit showed that fruit grown in polyester wool had bigger sized fruits as compared to fruits grown in other growth medium (Figure 3(b)).

Our results are supported by correlation equation ($y = 0.2693x + 1.5783$; $R^2 = 0.9562$) between total chlorophyll content and weight of fruit which revealed that one unit increase in total chlorophyll content resulted in 0.2693 units increase in weight of fruit (Figure 4(a)). Similarly, correlation equation ($y = 0.296x + 2.674$; $R^2 = 0.8776$) between total chlorophyll content and fruit diameter revealed that one unit increase in total chlorophyll content resulted in 0.296 units increase in diameter of fruit (Figure 4(b)).

3.2. Effect of Different Substrates on Quality Parameters of Strawberries. Significant differences ($P < 0.05$) were observed among all treatments tested in terms of quality of strawberries (Table 4). Fruits obtained from plants grown in polyester wool showed higher TSS (12.38°Brix), TA (1.21%), pH (3.94), ash content (0.72%), and crude fiber content (2.69%), while fruits obtained from plants grown in rice husk and wheat straw were comparable with each other. The lowest amount of nutrients was recorded in fruits grown in soil (Table 4).

Significant differences ($P < 0.05$) were observed among treatments tested in terms of health-related compounds (Table 5). Fruits obtained from plants grown in polyester wool showed higher amounts of total carotenoids (3.90 μg/100 g FW), vitamin C (37.39 mg/100 g FW), total anthocyanins (3.47 mg cyaniding-3-glucoside/100 g FW), total flavonoids (8.08 mmol quercetin/100 g FW), total phenolics (8.60 μg gallic acid/g FW), and radical scavenging activity (94.3%) (Table 5), while fruits obtained from plants grown in rice husk and wheat straw were comparable with each other. The lowest amount of health-related compounds were recorded in fruits grown in soil (Table 5).

4. Discussion

4.1. Effect of Different Substrates on Vegetative and Reproductive Growth Parameters of Strawberries. The influence of growth media on the survival percentage of plants is highly dependent on bulk density and pH of growth media [26]. Low bulk density of soilless media results in low water holding capacity of media which results in low uptake of nutrients by the plants. Similarly, survival and growth of plants is also highly dependent on pH, aeration, and organic matter in growing media [27]. In this study, wheat straw possessed less pH (6.6) which had ultimately affected the uptake of nutrients and hence resulted in less growth. In case of polyester wool (pH: 8.5), the high survival percentage could be due to its high-water holding capacity which is helpful for better uptake of nutrients by the plants [5].

In a previous study, it was observed that vegetative growth of strawberry varied with the ecological conditions of substrate [28]. Therefore, the maximum leaf area and number of runners recorded in plants grown in polyester wool might be attributed to the fact that polyester wool had better water holding capacity than other substrates used during this study. Further, Ercisli et al. [27] also found similar results where they reported that good aeration and low water tension with high water holding capacity gave positive results on development and growth of strawberry roots. It has also been reported that strawberry can grow better in alkaline soils (pH: 7.2-8.8), whereas the pH of rice husk and wheat straw lied between 5.8 and 6.6 [29]. This low pH might be the reason for lower vegetative growth observed in strawberries grown in medium containing rice husk and wheat straw.

Chlorophyll is responsible for photosynthesis. Increase in chlorophyll content results in increased photosynthesis which has positive effects on crop growth and increases yield by reducing moisture loss [30].

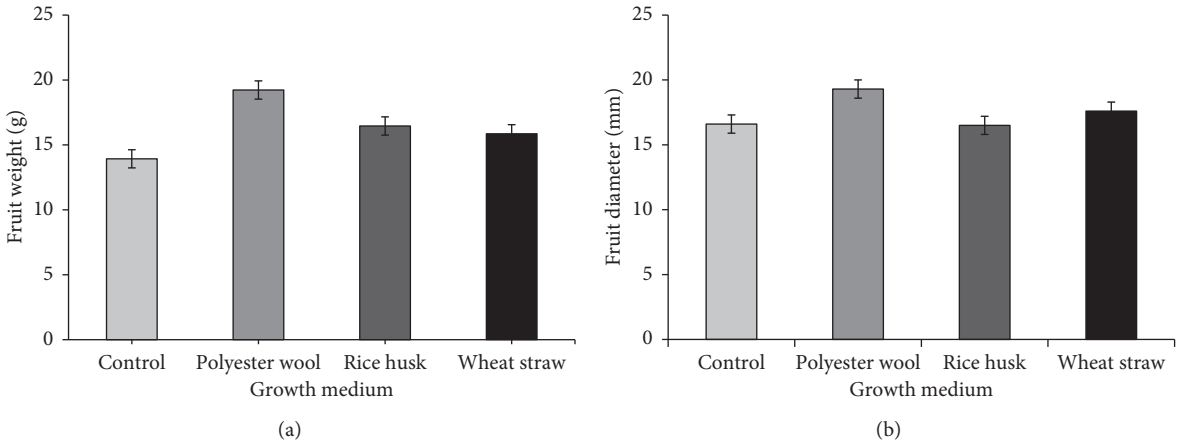

Figure 3: Effect of different growth substrates on (a) fruit weight g and (b) fruit diameter (mm) of strawberry cv. Chandler. Vertical bars indicate standard error of means (±SEM) for three replicates.

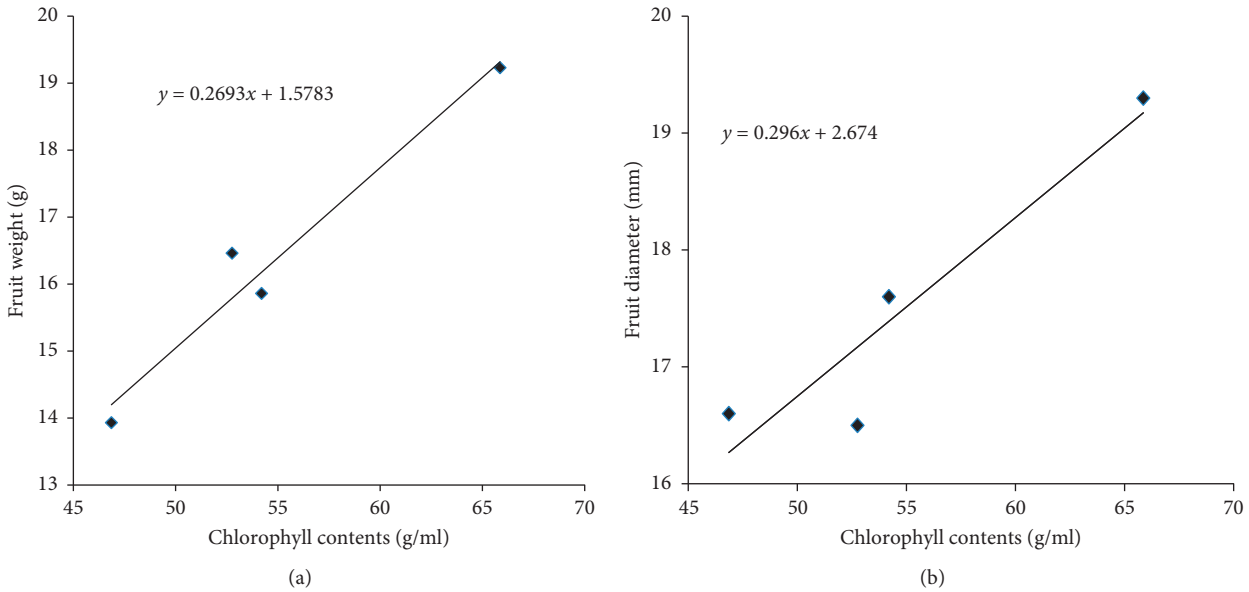

Figure 4: Relationship between chlorophyll contents: (a) fruit weight and (b) fruit diameter of strawberry cv. Chandler at $P < 0.05$.

Table 4: Effect of different growth substrates on proximate nutrients of strawberry cv. Chandler.

Treatments	TSS (Brix)	TA (%)	pH	Moisture (%)	Ash (%)	Fiber (%)
Soil (control)	7.36 ± 0.33 c	0.69 ± 0.03 c	3.94 ± 0.09 a	80.36 ± 2.06 b	0.54 ± 0.01 c	2.21 ± 0.06 c
Polyester wool	12.38 ± 0.36 a	1.21 ± 0.04 a	3.54 ± 0.06 c	91.76 ± 2.28 a	0.72 ± 0.01 a	2.69 ± 0.06 a
Rice husk	9.93 ± 0.41 b	1.07 ± 0.02 b	3.70 ± 0.11 b	85.00 ± 1.17 b	0.66 ± 0.02 b	2.43 ± 0.09 b
Wheat straw	9.16 ± 0.33 b	1.08 ± 0.07 b	3.73 ± 0.09 b	82.80 ± 1.55 b	0.65 ± 0.02 b	2.42 ± 0.06 b

Note. Different letters in each column indicate significant ($P < 0.05$) difference among treatments, ±SD; TSS = total soluble solids and TA = titratable acidity.

In our results, overall yield of strawberry depended on different growth substrates. The higher yield was obtained in plants grown in polyester wool. However, it is unclear that which chemical or physical factor had the most influencing role in plant growth and consequently the overall yield. Recent studies showed that microbial growth in soilless substrates showed a positive influence on roots of plants resulting in better plant growth [12].

Table 5: Effect of different growth substrates on health-related compounds of strawberry cv. Chandler.

Treatments	Phenolic (μg gallic acid/g FW)	Flavonoids (mmol quercetin/100 g FW)	Carotenoids (μg/100 g FW)	Vitamin C (mg/100 g FW)	Anthocyanins (mg cyanidine-3-glucoside/100 g FW)	Total antioxidants (% radical scavenging activity)
Control	4.97 ± 0.18 c	4.50 ± 0.19 c	1.16 ± 0.08 c	25.74 ± 0.99 b	0.98 ± 0.03 c	84.50 ± 2.90 c
Polyester wool	8.60 ± 0.70 a	8.08 ± 0.32 a	3.90 ± 0.44 a	37.39 ± 1.58 a	3.47 ± 0.18 a	94.30 ± 3.08 a
Rice husk	7.35 ± 0.32 b	6.22 ± 0.26 b	2.83 ± 0.15 b	28.55 ± 0.95 b	2.04 ± 0.14 b	89.90 ± 1.61 b
Wheat straw	6.90 ± 0.42 b	6.00 ± 0.58 b	2.86 ± 0.57 b	28.59 ± 1.98 b	2.01 ± 0.09 b	88.60 ± 2.89 b

Note. Different letters in each column indicate significant ($P < 0.05$) difference among treatments, ±SD; FW: fresh weight.

Fruit characteristics are highly correlated with chlorophyll content. It is generally believed that increase in leaf area which is active for photosynthesis is responsible for increase in fruit weight and size [9]. In our results, maximum weight and diameter of fruit could be due to the high chlorophyll pigment in plants grown in polyester wool.

4.2. Effect of Different Substrates on Quality Parameters of Strawberries. Different culture systems and phenotypes affect the nutrient quality of strawberries [31]. High acidity and high total soluble solids are responsible for good flavour [3]. Total soluble solids and acidity vary with change in genotypes of strawberries [9]. Difference in nutrient components of strawberry is highly dependent on growth media as the osmotic pressure in growth media affects the availability of nutrients to plants [5]. Results regarding ash and fiber in strawberry varied among different growth media which might be related to the availability of sugar content which are responsible for ascorbate synthesis. This precursor is necessary for enhancing the nutrient components in fruits [5].

Beneficial effects of fruits on the human body mainly depend on concentration of vitamin C, carotenoids, tocopherols, and flavonoids [32]. Differences in health-related compounds in strawberry grown in different growth substrates might be due to the fact that different bacteria grow in root zones of strawberries in soilless cultures [12]. These bacteria are responsible for the availability of wide range of substances (nitrogen, carbon, Fe, etc.) and enable plants to take nutrients from media in adequate amounts. Strawberries are a rich source of vitamin C and this content is 10 times higher than in apples and grapes [33]. Synthesis of vitamin C is genetic factor and tissue specific [34]. However, its synthesis is anabolic in plants and follow the $_{\text{L}}$-galactose pathway which is related to photosynthetic activity of plants [5]. Increased chlorophyll content in plants grown in polyester wool resulted in increased vitamin C content in strawberry fruits which could be due to the higher photosynthetic rate [9]. Increased leaf area and high chlorophyll content in our results are responsible for the increased photosynthetic rate, thus helping in accumulation of vitamin C in fruits.

Total carotenoids are one of the most important antioxidants found in strawberries. It is reported that increase in total carotenoids is enhanced with the process of photosynthesis [35]. The process of photosynthesis occurs mainly due to the presence of chlorophyll, and it has been reported that chlorophyll and carotenoids are positively correlated with each other. Moreover, both the chlorophyll and carotenoids are derived from the same pathway which is 2-C-methyl-D-erythritol 4-phosphate (MEP). Therefore, they are strongly dependent on each other [36]. It is also believed that total carotenoids are helpful in contributing colour to the fruits at final stages of ripening. A similar trend was observed in tomato fruits where de novo synthesis of carotenoids occurred mainly due to transformation of chloroplasts to chromoplasts [37]. In this way, increased amount of total carotenoids in strawberry fruits grown in polyester wool could be associated with the increased rate of photosynthesis, which helped in accumulation of carotenoids in harvested fruits.

Flavonoids are also responsible for different antioxidant activities in the human body. Besides their beneficial human health effects, flavonoids also help reduce over ripening in fruits. Moreover, they are important group of plant secondary metabolites which help in accumulation of flavonols and flavones which are used to protect plants from different biotic and abiotic stresses [38]. Some particular flavonoids can play vital role in regulation of climacteric ethylene biosynthesis resulting in stimulation of fruits ripening [39]. In many fruits, flavonols are the main flavonoids at the beginning of fruit development and several studies demonstrated that high light intensity affected the expression of genes responsible for biosynthesis of flavonoids and consequently increased the amount of flavonols [40–43]. In a recent study by Yuan et al. [42], it was observed that the biosynthesis of flavonoids and accumulation of secondary metabolites in soybeans was dependent on many factors including the rate of photosynthesis. With the increase in rate of photosynthesis, there was a great increase in flavonoid contents which influenced the fruit colour and nutritional quality. Thus, increased amount of flavonoids in fruits grown in polyester wool could be linked with the increased rate of photosynthesis, which helped in accumulation of flavonoids in strawberry fruits.

Anthocyanins and phenols are also very significant health-related compounds found in strawberry. Strawberry is a rich source of polyphenols [44]. Some researchers advocated that polyphenols account for more than 50% of total phenols in strawberry [45]. In our results fruits grown in

polyester wool showed the highest amount of total anthocyanins and total phenols which might be due to the increase in chlorophyll content. Similar results were obtained by Martínez et al. [9] where they observed higher amount of total anthocyanins and total phenols in strawberries grown in coir fiber. Further, they recorded that with the increase in SPAD values, there was an increase in total anthocyanins and total phenols. To confirm our findings, a study conducted by Pestana et al. [46] reported that lower SPAD values significantly reduced the amount of anthocyanins and total phenols in strawberry fruits. The changed amount of phenolic and anthocyanin compounds results in changed antioxidant activity which endorses the theory that the kind of growth substrate is vital for antioxidant capacity in strawberries [47]. In a recent study by Wysocki et al. [48], a higher yield as well as increased contents of polyphenols and anthocyanins were found in strawberry fruits from plants cultivated in the peat-coconut substrate. However, in this study, the increased contents in strawberry fruits obtained from plants grown in polyester wool growth substrate confirm that this substrate provided better conditions for plant development and assimilation of the assessed compounds.

5. Conclusions

Results of this study indicate that growth substrates had a great influence on vegetative and reproductive growth and quality indexes of strawberry cv. Chandler. These findings also suggest that polyester wool may serve as a valuable growth substrate for successful improvement in growth, yield, and quality of strawberry cv. Chandler. However, further studies should be carried out with different cultivars of strawberries to draw a general conclusion regarding the effect of growth substrates on vegetative and reproductive growth and quality. Consideration also needs to be given concerning the viability of expanding the use of polyester wool, the likely economic and environmental costs linked to its use and subsequent disposal, and sociocultural challenges to uptake.

Data Availability

All the relevant data have been provided in the manuscript. The authors will provide additional details if required.

Disclosure

Mehdi Maqbool is currently at Department of Horticulture, Faculty of Agriculture, University of Poonch Rawalakot, Azad Jammu and Kashmir, Pakistan.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

[1] A. Hossain, P. Begum, M. Salma Zannat, M. Hafizur Rahman, M. Ahsan, and S. N. Islam, "Nutrient composition of strawberry genotypes cultivated in a horticulture farm," *Food Chemistry*, vol. 199, pp. 648–652, 2016.

[2] J. Dragišić Maksimović, M. Poledica, D. Mutavdžić, M. Mojović, D. Radivojević, and D. Milivojević, "Variation in nutritional quality and chemical composition of fresh strawberry fruit: combined effect of cultivar and storage," *Plant Foods for Human Nutrition*, vol. 70, pp. 77–84, 2015.

[3] M. M. El-Mogy, M. R. Ali, O. S. Darwish, and H. J. Rogers, "Impact of salicylic acid, abscisic acid, and methyl jasmonate on postharvest quality and bioactive compounds of cultivated strawberry fruit," *Journal of Berry Research*, vol. 9, no. 2, pp. 333–348, 2019.

[4] K. V. Subbarao, Z. Kabir, F. N. Martin, and S. T. Koike, "Management of soilborne diseases in strawberry using vegetable rotations," *Plant Disease*, vol. 91, no. 8, pp. 964–972, 2007.

[5] P. Palencia, J. Giné Bordonaba, F. Martínez, and L. A. Terry, "Investigating the effect of different soilless substrates on strawberry productivity and fruit composition," *Scientia Horticulturae*, vol. 203, pp. 12–19, 2016.

[6] A. De Cal, A. Martinez-Treceño, T. Salto, J. M. López-Aranda, and P. Melgarejo, "Effect of chemical fumigation on soil fungal communities in Spanish strawberry nurseries," *Applied Soil Ecology*, vol. 28, no. 1, pp. 47–56, 2005.

[7] M. C. Fisher, D. A. Henk, C. J. Briggs et al., "Emerging fungal threats to animal, plant and ecosystem health," *Nature*, vol. 484, no. 7393, pp. 186–194, 2012.

[8] B. De los Santos, J. J. Medina, L. Miranda, J. A. Gómez, and M. Talavera, "Soil disinfestation efficacy against soil fungal pathogens in strawberry crops in Spain: an overview," *Agronomy*, vol. 11, no. 3, pp. 526–536, 2021.

[9] F. Martínez, J. A. Oliveira, E. O. Calvete, and P. Palencia, "Influence of growth medium on yield, quality indexes and SPAD values in strawberry plants," *Scientia Horticulturae*, vol. 217, pp. 17–27, 2017.

[10] W. R. Carlite, C. Cattivello, and P. Zaccheo, "Organic growing media: constituents and properties," *Vadose Zone Journal*, vol. 14, pp. 11539–11663, 2015.

[11] D. B. Dresboll, "Effect of growing media composition, compaction and periods of anoxia on the quality and keeping quality of potted roses (Rosa sp.)," *Scientia Horticulturae*, vol. 126, pp. 56–63, 2010.

[12] F. Martínez, S. Castillo, C. Borrero, S. Pérez, P. Palencia, and M. Avilés, "Effect of different soilless growing systems on the biological properties of growth media in strawberry," *Scientia Horticulturae*, vol. 150, pp. 59–64, 2013.

[13] F. Gungor and E. Yildirim, "Effect of different growing media on quality, growth and yield of pepper (*Capsicum annuum* L.) under greenhouse conditions," *Pakistan Journal of Botany*, vol. 45, no. 5, pp. 1605–1608, 2013.

[14] G. Peyvast, M. Noorizadeh, J. Hamidoghli, and P. Ramezani-Kharazi, "Effect of four different substrates on growth, yield and some fruit quality parameters of cucumber in bag culture," *Acta Horticulturae*, vol. 779, no. 779, pp. 535–540, 2008.

[15] N. Zahid, A. Ali, S. Manickam, Y. Siddiqui, P. G. Alderson, and M. Maqbool, "Efficacy of curative applications of submicron chitosan dispersions on anthracnose intensity and vegetative growth of dragon fruit plants," *Crop Protection*, vol. 62, pp. 129–134, 2014.

[16] AOAC, *Official Method of Analysis 960.20: Total Soluble Solids. Official Methods of Analysis of the Association of Chemists*, Analysis of the Association of Chemists, Washington, DC, USA, 1990.

[17] AOAC, *Official Method of Analysis 9720.21: Titratable Acidity. Official Methods of Analysis of the Association of Chemists*, Analysis of the Association of Chemists, Washington, DC, USA, 1990.

[18] AOAC, *Official Method of Analysis 994.16: pH. Official Methods of Analysis of the Association of Chemists*, Analysis of the Association of Chemists, Washington, DC, USA, 1990.

[19] M. Maqbool, N. Zahid, A. Hamid et al., "Evaluation of physico-nutritional and functional properties of indigenous pear cultivars grown in Rawalakot, Azad Jammu and Kashmir," *Pakistan Journal of Agricultural Sciences*, vol. 56, pp. 607–611, 2019.

[20] AOAC, "Official method of analysis 940.26: ash of fruits," in *Association of Official Analytical Chemists*, S. Williams, Ed., Publication by AOAC International, Rockville, MD, USA, 16th edition, 1998.

[21] AOAC, *Official Method of Analysis 978.10: Crude Fiber. Official Methods of Analysis of the Association of Chemists*, Analysis of the Association of Chemists, Washington, DC, USA, 1990.

[22] D. B. Rodrizues-Amaya and M. Kimura, *HarvestPlus Handbook for Carotenoid analysis. Harvest plus technical monograph series 2*, IFRI and CIAT., Washington DC , USA, 2004.

[23] Y. Zheng, S. Y. Wang, C. Y. Wang, and W. Zheng, "Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen treatments," *LWT - Food Science and Technology*, vol. 40, no. 1, pp. 49–57, 2007.

[24] P. Molyneux, "The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity," *Songklanakarin Journal of Science and Technolog*, vol. 26, pp. 211–219, 2004.

[25] R. G. D. Steel, J. H. Torrie, and D. A. Dickey, *Principles and Procedures of Statistic: A Biometrical Approach*, McGraw-Hill Publishing Co., Boston, MA, USA, 3rd edition, 1997.

[26] M. Raviv and J. H. Lieth, *Soilless Culture: Theory and Practice*, Elsevier, Amsterdam, Netherlands, 2008.

[27] S. Ercisli, U. Sahin, A. Esitken, and O. Anapali, "Effect of growing media on the growth of strawberry cvs.'Camarosa' and 'Fern'," *Acta Agrobotanica*, vol. 58, pp. 185–191, 2005.

[28] C. Paul, J. Gomasta, and M. M. Hossain, "Effects of planting dates and variety on growth and yield of strawberry," *International Journal of Horticulture, Agriculture and Food Science*, vol. 1, no. 4, pp. 1–12, 2017.

[29] D. Wang, M. Z. Gabriel, D. Legard, and T. Sjulin, "Characteristics of growing media mixes and application for open-field production of strawberry (Fragaria ananassa)," *Scientia Horticulturae*, vol. 198, pp. 294–303, 2016.

[30] J. Yu, M. Wang, C. Dong et al., "Analysis and evaluation of strawberry growth, photosynthetic characteristics, biomass yield and quality in an artificial closed ecosystem," *Scientia Horticulturae*, vol. 195, pp. 188–194, 2015.

[31] I. Ménager, M. Jost, and C. Aubert, "Changes in physicochemical characteristics and volatile constituents of strawberry (cv. Cigaline) during maturation," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 5, pp. 1248–1254, 2004.

[32] S. H. Häkkinen and A. R. Törrönen, "Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique," *Food Research International*, vol. 33, no. 6, pp. 517–524, 2000.

[33] B. M. Santos and C. K. Chandler, "Influence of nitrogen fertilization rates on the performance of strawberry cultivars," *International Journal of Fruit Science*, vol. 9, no. 2, pp. 126–135, 2009.

[34] P. Sun, N. Mantri, H. Lou et al., "Effects of elevated CO_2 and temperature on yield and fruit quality of strawberry (*Fragaria* × *ananassa* Duch.) at two levels of nitrogen application," *PLoS One*, vol. 7, Article ID e41000, 2012.

[35] V. Nour, M. E. Ionica, and I. Trandafir, "Bioactive compounds, antioxidant activity and color of hydroponic tomato fruits at different stages of ripening," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 43, no. 2, pp. 404–412, 2015.

[36] T. C. Barickman, D. A. Kopsell, and C. E. Sams, "Abscisic acid increases carotenoid and chlorophyll concentrations in leaves and fruit of two tomato genotypes," *Journal of the American Society for Horticultural Science*, vol. 139, no. 3, pp. 261–266, 2014.

[37] Z. Pék, L. Helyes, and A. Lugasi, "Color changes and antioxidant content of vine and postharvest-ripened tomato fruits," *HortScience*, vol. 45, no. 3, pp. 466–468, 2010.

[38] Y.-W. Ni, K.-H. Lin, K.-H. Chen, C.-W. Wu, and Y.-S. Chang, "Flavonoid compounds and photosynthesis in *Passiflora* plant leaves under varying light intensities," *Plants*, vol. 9, no. 5, p. 633, 2020.

[39] J. Aghofack-Nguemezi and W. Schwab, "Differential accumulation of flavonoids by tomato (*Solanum lycopersicum*) fruits tissues during maturation and ripening," *Journal of Applied Biosciences*, vol. 84, pp. 7674–7681, 2015.

[40] L. Zoratti, K. Karppinen, A. Luengo Escobar, H. Haggman, and L. Jaakola, "Light-controlled flavonoid biosynthesis in fruits," *Frontiers in Plant Science*, vol. 5, p. 534, 2014.

[41] Y. Xu, G. Wang, F. Cao, C. Zhu, G. Wang, and Y. A. El-Kassaby, "Light intensity affects the growth and flavonol biosynthesis of ginkgo (*Ginkgo biloba* L.)," *New Forests*, vol. 45, no. 6, pp. 765–776, 2014.

[42] M. Yuan, X. Jia, C. Ding et al., "Effect of fluorescence light on phenolic compounds and antioxidant activities of soybeans (*Glycine max* L. Merrill) during germination," *Food Science and Biotechnology*, vol. 24, no. 5, pp. 1859–1865, 2015.

[43] X. Ma, L. Song, W. Yu et al., "Growth, physiological, and biochemical responses of *Camptotheca acuminata* seedlings to different light environments," *Frontiers in Plant Science*, vol. 6, p. 321, 2015.

[44] M. R. Williner, M. E. Pirovani, and D. R. Güemes, "Ellagic acid content in strawberries of different cultivars and ripening stages," *Journal of the Science of Food and Agriculture*, vol. 83, no. 8, pp. 842–845, 2003.

[45] C. J. Atkinson, P. A. A. Dodds, Y. Y. Ford et al., "Effects of cultivar, fruit number and reflected photosynthetically active radiation on fragaria × ananassa productivity and fruit ellagic acid and ascorbic acid concentrations," *Annals of Botany*, vol. 97, no. 3, pp. 429–441, 2006.

[46] M. Pestana, F. Gama, T. Saavedra, P. J. Correia, S. Dandlen, and M. G. Miguel, "Evaluation of Fe deficiency effects on strawberry fruit quality," *Acta Horticulturae*, vol. 868, no. 868, pp. 423–428, 2010.

[47] A. M. Panico, F. Garufi, S. Nitto et al., "Antioxidant activity and phenolic content of strawberry genotypes fromFragariaxananassa," *Pharmaceutical Biology*, vol. 47, no. 3, pp. 203–208, 2009.

[48] K. Wysocki, J. Kopytowski, A. Bieniek, and J. Bojarska, "The effect of substrates on yield and quality of strawberry fruits cultivated in a heated foil tunnel," *Zemdirbyste-Agriculture*, vol. 104, no. 3, pp. 283–286, 2017.

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Research Article

Preharvest Factors Affecting Quality on "Abate Fetel" Pears: Study of Superficial Scald with Multivariate Statistical Approach

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Although superficial scald (SS) is well characterized on apples, there are only a few insights concerning the influence that agronomic and management variability may have on the occurrence of this physiological disorder on pears. In this study, we aimed to improve our understanding of the effect of different preharvest factors on SS development using a multivariate statistical approach. Pears (*Pyrus communis* L.) cv "Abate Fetel" were picked during two consecutive seasons (2018-2019 and 2019-2020) from twenty-three commercial orchards from three growing areas (Modena, Ferrara, and Ravenna provinces) in the Emilia-Romagna region of Italy. Bioclimatic indices such as weather and soil, agronomic management such fertilization and irrigation, orchard features such as rootstock and training systems, and SS incidence were carried out at harvest and periodically postharvest in all producers. Two different storage scenarios (regular atmosphere and use of 1-MCP) were also evaluated. Our data in both seasons showed high heterogeneity between farms for SS symptoms after cold storage either in the regular atmosphere or with 1-MCP treatment. Nevertheless, in 2018, all the producers showed SS at the end of the storage season, but in 2019 some of them did not exhibit SS for up to 5 months. In fact, some preharvest factors changed considerably between the two seasons such as yield and weather conditions. Indeed, some factors seem to affect SS in both growing seasons. Some can increase its occurrences such as physiological and agronomical factors: high yields, late date of blooming, heavy downpours, improper irrigation management (low watering frequency and high volumes), nitrogen (included that deriving from organic matter), soil texture (presence of clay), orchard age, and canopy volume in relation to training system and rootstock. Others can decrease SS such as climatic and management factors: late harvest dates, rain, gibberellins, calcium, manure, absence of antihail nets or use of photoselective nets, and site (probably related to better soils toward the Adriatic coast). Initial preharvest variability is an important factor that modulates physiological plant stress and, subsequently, the SS after cold storage in "Abate Fetel" pears. Multivariate techniques could represent useful tools to identify reliable multiyear preharvest variables for SS control in pear fruit different batches.

1. Introduction

The need to investigate fruit quality and postharvest management during long-term storage is accentuated by the fact that many Italian farmers have increased their fall productions (e.g., apples, pears, and kiwifruits), notoriously more demanding in terms of their management in the postharvest phase to avoid relevant economic losses [1]. Superficial scald (SS) is one of the main causes of product loss in winter pears such as the "Abate Fetel" variety inside storage cold chambers [2, 3]. The symptom appears as a general browning of the skin, due to the necrosis of the superficial tissues of the epidermis [4]. The study of aetiology is complex, but two main factors can be identified: cold damage and oxidative stress [5–7]. Over the years, attention has also been focused on the volatile α-farnesene compound and its oxidation process, considered to be the main responsible for this disorder [8–10]. Nowadays, the rapid and

nondestructive analysis of the VOC array carried out by PTR-ToF-MS identified 6-methyl-5-hepten-2-one (MHO) significantly associated with the development of the SS [11]. To prevent this disorder, until a few years ago, it was possible to treat in postharvest with ethoxyquin [12]. The ban on its use by the EU has raised considerable concerns. Nowadays, chemical treatments with 1-MCP to prevent SS provide results that are not fully acceptable: the main issue is the very slow poststorage maturation and a lack of consistency against SS [13]. "Abate Fetel" pears are affected by low oxygen and ethylene inhibitors, which can cause soft scald or inhibit its maturation even after normal shelf life, respectively [14]. Other methods of disorder mitigation include controlled atmosphere (CA) storage, dynamic CA regulated by chlorophyll fluorescence, oil wraps, and temperature conditioning [4]. However, relatively little is known about crop protectant postharvest practices [15], such as intermittent warming early in the cold storage period, which can be as effective as conventional chemicals [16]. Furthermore, the physiological development and subsequent ripening of the fruit of "Abate Fetel" can change dramatically depending on the previous years based on yields [17] and agronomic, climatic, or orchard management factors [18–20]. It is therefore evident that it is difficult to put in practice consolidated guidelines for the postharvest management of this variety. Horticultural researchers often must measure complex traits and develop relationships with treatments or associated variables. SS symptoms are just the final expression of a physiological disorder, which is multifactorial [20]. Identifying a single variable may not be possible, so we are forced to test many related variables. If the researcher uses univariate statistics to quantify differences or relationships, then the number of separate analyses required will equal the number of individual variables measured. Multivariate analysis reduces a large dataset to a small number of components, which can be scored along independent, linear axes. Variables strongly associated may share some underlying biological relationship. These associations are often useful for generating hypotheses or for understanding the behaviour of complex traits [21]. Many examples exist in the literature where multivariate analysis has been used successfully in plant sciences to develop novel hypotheses, to simplify large datasets, or to understand the response of complex traits [22, 23]. Hence, this research aims to investigate SS occurrence in "Abate Fetel" pears, which represents a model for postharvest storage disorders, to better understand the preharvest factors leading to a strong susceptibility of some producers' batches during storage and after shelf life. Advanced statistical tools are used to describe and identify best practices under the control of farmers or predictable variability of weather or soil that can help to manage, store, and sell "Abate Fetel" pears depending on their potential for storage in healthy conditions.

2. Materials and Methods

2.1. Plant Materials and Experimental Design. Twenty-three orchards of "Abate Fetel" pear located in the Emilia-Romagna region (Italy) in two consecutive seasons (2018-2019 and 2019-2020) were identified, characterized by a variable incidence, in the past, of poststorage SS. For each of them, we proceeded to a detailed characterization of the different crop management parameters, such as nitrogen and microelement application, irrigation regimes, application of plant growth regulators, soil texture and organic matter content, soil cover management, rootstock, training system, and presence/absence of hail nets. The productivity of the orchards, the temperatures and rain events, and the flowering and harvesting dates were also considered. To improve the characterization, surveys about preharvest factors were carried out with each producer considering their field diaries and experience about their orchards. At harvest, appropriate quantities of the product were then placed in a regular atmosphere (−0.5°C and >90% of relative humidity (RH)): eighteen boxes for each farm in the first year and twelve boxes in the second year. Thereafter, six boxes for each producer each year were treated with 1-MCP (Smart-Fresh^{TM}, AgroFresh Inc., Springhouse, PA, USA) and stored in a different room in the same company. After 3, 4, and 5 months of storage, the room was opened, following the calendar normally applied by the company.

2.2. Superficial Scald Assessment in "Abate Fetel" Pears. After 3, 4, and 5 months of cold storage plus 7 days at room temperature (20°C) and controlled humidity (60% of RH), the presence and extent of SS were assessed in 30 fruits per farm. We defined four classes depending on the severity of symptoms in the skin of pears: class 0 where there was no peel browning, class 1 from 0% to 25% of SS, class 2 from 25% to 50% of SS, and class 3 over than 50% of SS after shelf life. A SS index was computed as follows [24]:

$$\text{scald index} = \sum_{0}^{4} \frac{(\text{index level}) x\, (\text{fruits at this level})}{\text{total number of fruits}}. \quad (1)$$

2.3. Data Treatment and Statistical Analysis. All the data collected were subjected to multivariate analysis, to highlight which—among the factors considered—appears to be more related to the onset of SS. Multivariate statistical analyses, including canonical analysis (CA) and canonical correspondence analysis (CCA), were performed using the statistical software R [25] by addition of packages "candisc" [26] and "vegan" [27]. CA was applied to describe the evolution of SS through epochs and years and the effect of 1-MCP treatment. Afterwards, CCA was used to estimate the interactions between the frequencies of SS classes and the quantitative and qualitative variables. In the first case, the blue vector indicates the increase of the factor in a certain direction while in the second analysis the arrow means the presence of the factor (value 1; e.g., pear orchard with antihail nets). On the contrary, we have its absence or an opposite factor on the other side (value 0; e.g., training system such as fruit wall vs. spindle). Finally, we considered the total variability explained by two components (CCA1 and CCA2) and how each variable affects the first and the

second component. Factors and SS data of each epoch (3, 4, and 5 months) and year (2018 and 2019) were combined in CCAs to elaborate the overall picture. In the latter algorithm, 1-MCP treatment was not considered because of its extremely different behaviour between two consecutive seasons.

3. Results and Discussion

3.1. Superficial Scald Development in "Abate Fetel" Pears. Although "Abate Fetel" pears certified by Protected Geographical Indication (IGP) should guarantee consistent quality levels, the unavoidable variability arises from growing environment and production systems, which influence major preharvest factors [28]. Similarly, our data in seasons 2018-2019 and 2019-2020 showed a high heterogeneity between farms after cold storage concerning SS development (Figure 1). In general, we observed that damage of SS increases with time during storage up to 5 months (from 1.1 and 1.2 to 3.1 and 3.2) in a cold room for all the producers in both seasons (Figure 1). However, in 2018 after 5 months almost all the batches had symptoms of SS, but in 2019 some of them did not yet have important SS symptoms after 5 months (Table 1). Regarding 1-MCP treatment (4.1 and 4.2), we noticed that it helped to prevent SS in season 2019-2020, but it was not effective in season 2018-2019 (Figure 1). Moreover, some producers (e.g., 451 in the first season and 222 or 242 in the second season) lost their ripening capacity after 5 months of cold storage or with 1-MCP treatment (data not shown). Some findings reported that "Abate Fetel" pears stored in a normal atmosphere after 4 months become sensitive to SS, can lose their ripening ability and remaining firm, and become dry and unable to reach a buttery and juicy texture, satisfactory for consumption [29, 30]. Nevertheless, some farms showed more symptoms than others with different SS indexes (Table 1), probably because of different locations and the current heterogeneity of preharvest factors. For instance, Moggia et al. (2015) [31] reported high variation in apple across sites in the occurrence of internal browning, reaching up to 48% in some locations. As evidence, multivariate analysis allowed a classification of orchards according to their geographical coordinates and incidence of physiological issues [31].

3.2. Environmental and Agronomic Factors Affecting Superficial Scald in "Abate Fetel" Pears. Some preharvest factors changed considerably between the two seasons such as yield and weather conditions (Table 1), which are reported to affect SS symptoms [32–36]. High variability of fruit peel browning was observed between different years and between producers with different geographic and climatic variables (Table 1). Nevertheless, over six years, the same types of disorder can appear within the same orchard [31] and, consequently, it could be forecasted.

Crop load management is particularly important because it can affect plant physiological status and susceptibility to storage disorders [34]. In both seasons, high yield increases SS, probably due to an unbalance between source-sink ratio and reduction of element concentration. In the first CCA, the high yield projection to the first axis is 0.61 in the direction of class 2 and class 3 (Figure 2), while in the second multivariate analysis production of less than 30 tons per hectare contributes to axis 1 of 0.57 to class 0 (Figure 3). Crop load, associated with warmer climate conditions at harvest, can affect the balance of carbohydrates and calcium in the fruit and leaves and, subsequently, affect postharvest secondary metabolism and, possibly, susceptibility to SS [37, 38]. Indeed, high vegetative vigour can lead to transpiration imbalances and fewer elements being allocated to the developing fruit [39]. On the other hand, other researchers reported that "Passe Crassane" pear from less productive trees have also been shown to be more susceptible to browning disorders [40].

Considering both seasons, we found that late harvest dates, expressed as days after full bloom (DAFB), can prevent the occurrence of SS (contribution to component 1 is 0.43 against SS; Figure 2). As evidenced, the harvest of "Abate Fetel" pears in 2019, when we had a low SS occurrence in fruit, was delayed by 14 days (Table 1). In contrast with this work, SS of "Abate Fetel" pears grown in Argentina in the same orchard and in the same season affected 18% and 33% of the fruit harvested on 23 January and 6 February, respectively [41]. On the other hand, many other papers reported that SS is more severe on early-harvested fruit than on later-picked apples and pears [42, 43]. Therefore, we assume that growing conditions modulating fruit biochemistry would contribute to the effect of the harvest day over SS occurrence, which may justify contrasting findings from different authors with fewer orchards and seasons [44]. In fact, it seems that late-harvested "Abate Fetel" pears have a biochemical profile, such as sugar ratio and antioxidant compounds, enabling them to address the stressful storage condition. Therefore, the increased susceptibility to SS of immature fruit may be attributed to low antioxidants [45].

Bloom date is the first important information to roughly predict the commercial harvest time for a variety. Anyway, a 3- to 4-week variation in bloom date for the same cultivar in the same environment has been reported, showing how phenology in every single year is affected by temperature [46, 47]. In Figure 2, we observed that a late full bloom, expressed as days from the beginning of the year, can increase the occurrence of SS during storage (contribution to component 1 is 0.78 toward SS). In fact, in 2019, we registered an earlier blooming of 10 days compared to 2018. On the other hand, the harvest day between producers was just 3 days delayed in the second year (in 2018 was 03/09 and in 2019 was 06/09). The earlier the bloom time, the longer generally the fruit growing season [48]. So, in 2018, we had a shorter season (146 DAFB) than 2019 (160 DAFB), when we evaluated just a few SS symptoms (Table 1), and, probably, the stock of cold protectant compounds such as secondary metabolites and sugar alcohols with osmoregulatory properties could increase in the fruit. Based on the 30- to 40-year data, global warming is affecting fruit quality; in general, earlier blooming and increase of temperatures (in particular close to maturity) led to more storage disorders [47, 49].

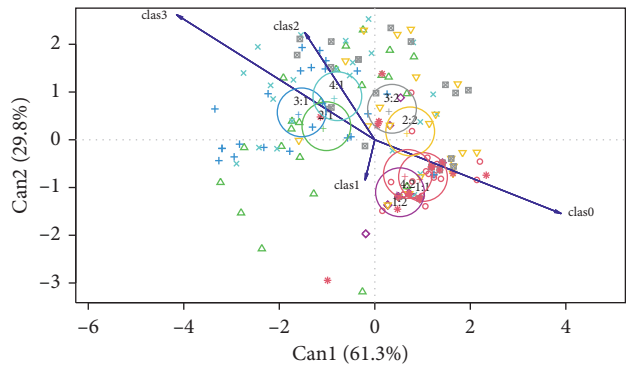

Figure 1: Discriminant canonical analysis (DCA) that describes the behaviour of superficial scald in "Abate Fetel" pears (the blue vectors are clas0 0%, clas1 1%–25%, clas2 26–50%, and clas3 51–100% of peel symptoms) and farm scores (coloured points) in two seasons (2018-2019 and 2019-2020). Percentages in parentheses represent the variance of each component (Can1 and Can2). The following abbreviations have been used for the epochs of sampling from cold rooms after 3 months in 2018 (1.1) and 2019 (1.2), 4 months in 2018 (2.1) and 2019 (2.2), 5 months in 2018 (3.1) and 2019 (3.2), and 5 months with 1-MCP in 2018 (4.1) and 2019 (4.2).

Table 1: Changes in preharvest factors (kilograms of nitrogen from fertilization, millimetres of rain during the growing season, yield in tons per hectare, harvest day expressed as day after full bloom (DAFB) and SS index after 5 months of storage of "Abate Fetel" producers between the first season (2018) and the second season (2019).

Producers	Nitrogen (kg)		Rain (mm)		Yield (t/ha)		Harvest day (DAFB)		SS index	
	1°Y	2°Y	1°Y	2°Y	1°Y	2°Y	1°Y	2°Y	1°Y	2°Y
111	70	151	236	279	45	29	146	162	40.8	14.2
121	31	219	302	281	52	28	146	160	50.0	8.3
131	79	45	226	313	54	41	145	151	61.7	45.8
212	128	82	287	347	40	27	152	165	37.1	25.0
222	67	43	320	354	22	11	152	160	41.7	0.8
242	141	44	152	467	23	5	149	163	35.8	3.3
262	84	110	188	406	40	20	149	164	58.8	16.7
272	9	37	356	416	20	15	147	159	25.0	2.5
282	49	115	124	387	29	21	147	163	50.4	31.7
292	44	20	185	405	15	19	145	163	20.4	48.3
311	339	150	281	339	23	4	146	162	45.8	4.2
321	58	80	248	319	17	10	146	162	58.8	20.0
331	94	73	196	368	41	12	146	162	56.3	39.2
341	98	148	217	332	16	8	153	150	55.4	41.7
351	135	209	256	309	24	20	143	150	20.8	30.8
412	83	168	197	408	38	13	144	157	37.5	23.3
432	62	71	276	448	45	13	146	161	62.9	23.3
442	107	64	194	411	33	13	140	159	54.6	15.8
451	3	79	244	293	40	28	141	165	2.5	5.0
461	75	70	209	301	57	22	143	168	21.3	5.0
472	99	100	375	454	35	16	144	154	45.0	28.3
482	55	120	403	327	35	6	145	151	28.3	7.5
492	133	103	248	354	30	11	146	160	57.1	12.5
Average	89	100	249	362	34	17	146	160	42.1	19.7

Indeed, DAFB cannot always be a precise indicator of fruit maturity, worsened by the fact that a broader bloom window can make fruit maturity even more heterogeneous and lead to errors in predicting optimal harvest dates to avoid SS [48]. The lack of fruit maturation uniformity impacts postharvest operations leading to the necessity to sort fruit in a more homogeneous way to tailor specific storage programs depending on the maturity at harvest to avoid storage issues [50].

Regarding preharvest factors, many studies on fruit quality at harvest highlighted the importance of weather conditions during fruit development [51]. Indeed, soil moisture and precipitation, higher than 120 mm, especially during cellular expansion, encourage vegetative growth and affect fruit maturation, explaining 39% of firmness variation [33, 52]. In the same way, SS in our work was affected by heavy downpours during summer (projection to component 2 is 0.19 toward class 3; Figure 3), which led to flooding and

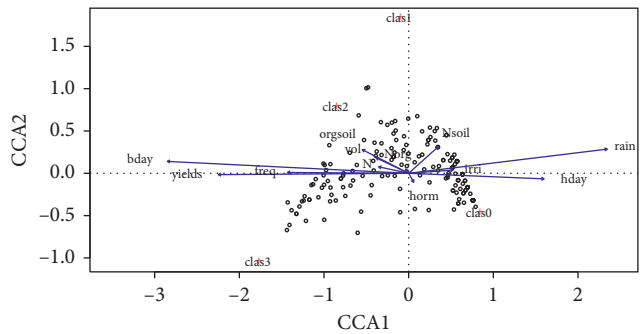

Figure 2: Canonical correlation analysis (CCA) of superficial scald classes of "Abate Fetel" pears (clas0 0%, clas1 1%–25%, clas2 26–50%, and clas3 51–100% of peel symptoms) against quantitative orchard features (blue vectors) and the scores of producers (black circles) in two seasons (2018-2019 and 2019-2020). Total variability explained (23%): CCA1 (95%); CCA2 (3%). The following abbreviations have been used: date of harvest (hday), full bloom date (bday), millimetres of rain (rain) and irrigation (irri) during the growing season, frequency of watering in days (freq), total (N) and organic nitrogen (Norg) expressed in kilograms in fertilizers, nitrogen in soil in percentage (Nsoil), organic matter in soil in percentage (orgsoil), volume of the canopy in m^3 (vol), yields of the orchards in tons (yields), and quantity in grams of gibberellins (horm).

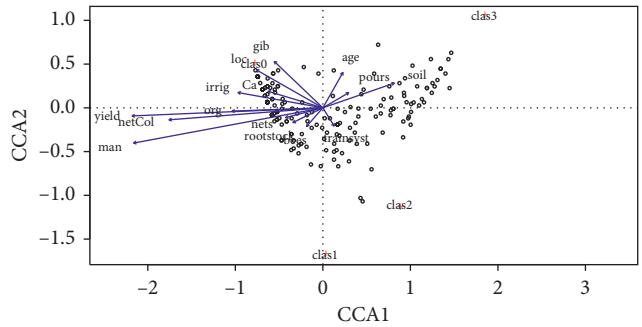

Figure 3: Canonical correlation analysis (CCA) of superficial scald classes of "Abate Fetel" pear (clas0 0%, clas1 1%–25%, clas2 26–50%, and clas3 51–100% of peel symptoms) against qualitative orchard features (blue vectors) and the scores of producers (black circles) in two seasons (2018-2019 and 2019-2020). Total variability explained (18%): CCA1 (93%); CCA2 (4%). The following abbreviations have been used: geographical position (loc) near the Adriatic sea (1) or far from Adriatic sea (0), use bees or pollinators (bees) yes (1) or no (0), use of organic matter (org) yes (1) or no (0), age of the orchard (age) <10 years (1) ≥ 10 years, use of gibberellins (gib) yes (1) or no (0), presence antihail nets (nets) yes (1) or no (0); rootstock (rootstock) weak (1) or strong (0), type of soil (soil) with abundant clay (1) or sand (0), type of irrigation (irrig) below canopy (1) or above canopy (0), training system (trainsyst) such as fruit wall (1) or spindle (0), use of calcium (Ca) yes (1) or no (0), origin of the organic matter used (man) cow manure (1) or poultry manure (0), heavy downpours (pours) during the season yes (1) or no (0), orchard average productivity (yield) ≤30 t/ha (1) or >30 t/ha (0), and presence of coloured nets or no-nets (netCol) yes (1) or no (0).

unbalance between fruit development and vegetative growth. Nevertheless, high soil moisture conditions are either the result of high irrigation or intense rainfall. Without considering fruit quality, the contribution of precipitations and irrigation against SS to component 1 is 0.64 and 0.14, respectively. To explain that, low precipitation conditions may be involved with calcium deficiency and, thereby, loss of cell turgor [53], as a result of reduced transportation of ions to and from cells [54]. In addition, it is possible that proteins specifically induced by low temperature and, associated with tolerance of cold damage, may have a longer-term effect [55]. It was postulated that preharvest temperatures below 10°degrees 2-3 weeks before harvest increase the content of unsaturated fatty acids such as oleic (C18 : 1) and linoleic (C18 : 2) acids to cope with this stress [44, 56]. Contrarily, it is also reported that low air temperature conditions reduce storability at harvest by diluting the calcium concentrations absorbed by the fruit [36]. However, precipitation events are closely related to low light intensities and low air temperatures and, as consequence, can affect the early drop of fruitlets between 30 and 60 DAFB [52] and, consequently, yield. In particular, in the second season (2019) which was rainy and with low SS, in general, yields were below average (Table 1).

Low density and larger trees, with lower temperature and light penetration inside the canopy [55], increase SS occurrence in stored pear fruit. In Figure 2, the contribution toward class 2 in components 1 and 2 of canopy volume is 0.10 and 0.25, respectively. Indeed, many fruit characteristics such as skin colour, flesh firmness, titratable acidity, soluble solids, and fruit size are influenced by the light [32, 57, 58], and therefore, defining the ripening distribution of fruit in the whole canopy could impact the storage process.

The ability of light to penetrate tree canopies and, therefore, to affect microclimate of fruit, is influenced by several factors including tree spacing, canopy architecture [59], rootstock, orchard management practices, such as pruning and thinning [60], and presence of antihail nets [61]. Regarding training system and rootstock, a slight correlation in Figure 3 was found in our trial where weak rootstocks and spindle-shaped trees, with small canopies, showed reduced SS. In particular, "Abate Fetel" trees grafted on quince contribute to components 1 and 2 of 0.08 and 0.19 against SS. On the other hand, the projection on component 2 of the variable represented by expanse tree architecture is 0.23 toward class 2. A study on the effect of training system and rootstock on poststorage fruit quality of "d'Anjou" pear found that the effect of training system on vigour and fruit nutrient content could contribute to poststorage concerns [62]. Indeed, fruit characteristics can greatly vary within expanse canopy that characterized orchards prone to SS in our study (Figure 3). In the Emilia-Romagna region, the harvest of pears is generally carried out with a single pick, and fruits are pooled together in the same bins. Consequently, high variability in quality exists in a single bin, which can impact postharvest fruit quality and storability and often results in repacking issues [32] and, therefore, fruit damages.

As far as the presence and colour of antihail nets, in Figure 3, we found that the shading effect of standard antihail nets can enhance the occurrence of SS after long-term storage. The contribution of antihail nets to component 2 towards class 1 is 0.12 and the projection of coloured or no-nets to component 1 is 0.46 against SS. It was shown that orchards with more exposure to sunlight produce better colour [63] and increased anthocyanins in fruit [64], offering better resistance to SS development [56]. Moreover, a study with multivariate analysis about within-tree factors of peach highlighted that lower light interception experienced under nets may have a detrimental effect on the flavour and may contribute to the variation in fruit quality at harvest [65]. Concerning the shade effect in fruit, it is often reported that the shaded side of the apple is more susceptible to storage disorders such as SS [66]. Superior SS protection on the sun-exposed side of the fruit may be related to elevated xanthophyll and anthocyanin levels and diminished susceptibility to photoinhibition, relative to the shaded side [67–69]. However, cultural practices such as antihail or anti-insect netting are employed in pear orchards by several growers in the Emilia-Romagna region. On the other hand, shading treatments with kaolin against sunburn affected fruit quality of "Packham's Triumph" pears increasing fresh weight and chlorophyll contents [70], as evidence of a slower climacteric outset.

Together with environmental factors, planting year and orchard site play an important role on SS in "Abate Fetel" pears, affecting vegetative self-shading and soil properties, respectively. In Figure 3, their contributions to component 2 are 0.46 and 0.49 towards class 3 and class 0, respectively. Indeed, not only weather and soil features can affect it, but also farm practices. Hence, in our research, young orchards appear to have more SS symptoms than older ones (Figure 3). Young trees, which probably have the characteristics of light-cropping trees, are considered to be more susceptible to storage disorders before harvest or during conservation [71, 72]. On the other hand, it is reported that high crop loads, especially in young orchards, can dramatically affect future productivity and fruit quality [72]. Moreover, in young apple trees, nitrogen is used to stimulate growth and excessive levels of this element can reduce fruit quality with fruits that are larger, greener, softer, more prone to drop, and more affected by storage issues [73]. Moreover, in our study, pear orchards near the Adriatic Coast (Ravenna and Ferrara provinces) had lower SS incidence than producers from inland areas (Modena and Bologna provinces). Agar et al. (1999) [74] observed that differences in ripening behaviour and response to ripening inhibitors might occur in the fruit of the same cultivar grown in different environments. They found that "Bartlett" pears from growing locations with cooler preharvest temperatures and/or from later harvests within a growing location had a different ability to ripen. By contrast, Chiriboga et al. (2013) [75] found that the variability of fruit quality of "Conference" pears after 105 days of cold storage was explained by the 1-MCP effect, followed by the shelf-life duration, harvest date, and to a lesser extent the orchard location.

Orchard location may act on the fruit sensitivity to SS in terms of availability of organic matter to spread in the orchard and texture of the soil. In the first case, it seems to prevent SS, and in the second case, the presence of clay apparently promotes it. In Figure 3, the projections of the organic matter and soil texture on the first component are 0.27 and 0.21. Soil factors determine in part fruit physiology and especially the ability of the fruit to regulate the initial physiological maturity at harvest [76], which can result in differences in ripening behaviour for fruit grown in different environments [77]. In fact, we observed that abundant clay in the soil can induce SS and, likely, increases organic matter content and soil moisture (Figures 2 and 3). This is partially in contradiction with the effects of precipitations and irrigation on SS (Figure 2). On the other hand, water stress may be a more critical factor than soil texture by the combination of high temperatures and low soil moisture aggravating SS development [36].

Nonetheless, soil texture affects the capacity of roots to extract, from the soil solution, nutrients such as nitrogen, which could be provided by fertilization or naturally occurring organic matter, affecting on the one hand vegetative growth and on the other hand soil fertility. Studies concerning the relationship between increased levels of nitrogen fertilizer and the incidence of storage disorders have reported variable results depending on the general nitrogen status of the orchard and the availability of other soil nutrients [71]. In Figure 2, nitrogen fertilization seems to promote SS (its contribution to the first axis is 0.09). We considered not only nitrogen from chemical fertilizers but also from organic matter, such as poultry manure spread in the orchards, which seems to have the same effect on SS with lower importance (Figure 2). Nitrogen fertilization promotes vegetative growth with consequent higher self-shading and chlorophyll content in fruit [78], but it can

reduce flesh firmness and TSS content [79]. Thus, we assume that high nitrogen soil fertilization would increase fruit size but reduces storage quality and crop value. Furthermore, low N availability impacts fruit quality: organic "Abate Fetel" pears had more total polyphenols and higher ascorbic acid stimulated by hexoses than conventionally grown ones. Also, the sugar profile differed, with a higher ratio of monosaccharides/disaccharides [80]. Nevertheless, nutrients needed for trees are in the soil so the orchard floor represents a substantial portion of the orchard agroecosystem, and if properly managed, it can reduce fertilizer costs [81]. In our experience, the slower release of the naturally occurring nitrogen humidified inside an organic matter of soil seems to prevent the occurrence of SS in "Abate Fetel" pears (0.09 of contribution to the principal component; Figure 2). The total organic matter in soil has not the same effect on SS (Figure 2). Pears demand 50–60 kg ha^{-1} to retain good fruit quality and production. So, the role of reserves and N resorption in the fall from leaves or cover crops were key findings that led to a more efficient and sustainable N management for each region and orchard in order to optimize nutrient uptake and minimize leaching [81, 82].

Any given fertilizer program cannot be successful without an efficient irrigation program. Water is scarce in most pear districts for both quantity and quality [81]. Water availability was identified as the major factor controlling tree growth globally in the current climate change scenario [83]. In Figure 2, the amount of water supplied in "Abate Fetel" orchard in 2018 and 2019 was considered (0.14 of importance in component 1). As we discussed before, watering has the same effect on SS of precipitation (Figure 2), but the modality of water application had an additional effect (Figures 2 and 3). Where the time interval (days between two watering) was considered, we observed that larger volumes at the same time (i.e., longer time interval) induced higher SS (0.39 of importance in component 1). Along the same line, microirrigation helped to prevent SS after storage (0.25 of importance in component 1). In apples, water stress lowered the rate of firmness loss, indicating an alteration in the physiological mechanism of fruit ripening [84]. Nevertheless, extended dry periods increased the risk of storage disorders when followed by heavy rains or irrigation [85], such as heavy downpours and high irrigation volumes (Figures 2 and 3). In addition, increasing irrigation frequency may lessen high temperature effects on foliage plant growth [86]. At elevated temperatures, the oxygenating reaction of RUBISCO (ribulose bisphosphate carboxylase-oxygenase) increases more than the carboxylating one because CO_2 declines more rapidly with increasing temperature than does O_2 [39, 87]. Thus, photorespiration becomes proportionally more important [87], generating an unbalance between carbohydrate rates. Concerning the application of deficit irrigation during the season, early stressed "Nijisseiki" Asian pear had a higher concentration of sugars such as sucrose, glucose, fructose, and sorbitol with a cold protectant activity than nonstressed fruit. Nevertheless, early stressed fruit tended to have higher flesh spot decay although it was reduced in the late-stress treatment [34, 88].

Calcium is transported in the transpiration stream [89]. In "Niitaka" pear, water stress decreased the concentration of calcium in flesh during the early stage of fruit growth. Moreover, it increased peroxidase activity and may be due to limited calcium absorption [90]. As we can notice in Figure 3, producers that spray calcium in "Abate Fetel" pears during the vegetative season have less SS in pear fruit and its contribution in the first component is 0.16. This insight agrees with Drake et al. (1979) [37] and Bramlage et al. (1985) [38] who negatively correlated fruit calcium level and SS development and found SS to be more prevalent when peel calcium was <700 mg L^{-1} [91]. In "Abate Fetel" pears, 53% of untreated fruits and only 4.6% of those treated with 4.5% $CaCl_2$ were affected by soft scald after 210 days of storage [92]. By the way, Gerasopoulos and Richardson (1997) [93] suggested that fruit calcium concentration increases the chilling requirement for induction of ripening capacity.

Parthenocarpic fruits tend to have lower calcium concentrations [94], and, in general, fruits sprayed with gibberellins have lower seed numbers, lower calcium concentrations, and an increased incidence of storage disorders [95–97]. However, as we can notice in Figure 3, it seems that the use of gibberellins, instead of pollinators, during the blooming can prevent SS; eventually, by postponing fruit harvest maturity and extending growth season cause parthenocarpic behaviour (its contribution to the second axis is 0.60). However, we can notice in Figure 2 that the quantity of hormones such as gibberellins does not affect SS after storage, and its projection on component 2 is 0.14. In "Forelle" pears, gibberellins produced fruit with a large cell diameter (140.3 μm) and resulted in a low mealiness percentage and, consequently, with higher fruit quality [98]. Nevertheless, the incidence of storage issues in larger celled fruit was explained arguing that cell contact area between neighbouring cells is reduced making the cells prone to cell-to-cell debonding during ripening [99], resulting in tissue breakdown [100].

4. Conclusions

The pear industry in Italy is currently threatened by many issues and among them storage-related problems and fruit quality concerns. This works shows the extreme variability among producers and seasons in terms of appearance and severity of superficial scald (SS) as a physiological disorder in "Abate Fetel" pears with either normal atmosphere or 1-MCP cold storage. Weather patterns, soil characteristics, bloom date, and location, outside grower's control, have an impact, but also yield, irrigation regime and volumes, fertilization, growth regulators, rootstock, and training system, which growers have a handle on, can affect SS in pears during storage. Our approach, using multivariate statistical techniques, has highlighted several key preharvest factors which could be grouped considering their biological relationships. In general, SS seems to be induced by several plant physiological stresses resulted from an improper yield management, without considering tree resources and weather conditions, an unbalance between reproductive and

vegetative growth and self-shading effect, a short season and, consequently, few cold protectant compounds, and, finally, a soil deficiency caused by a not efficient governance of water supply and organic matter fertility. In the future, a widespread application of such statistical tools will be recommended to describe complex traits that impact fruit storage, with the goal of predicting and improving it. The major conclusion, however, is that pear batches from different orchards should be sorted for their potential to develop SS after shelf life before applying storage technologies or placing them in cold rooms where they become, perforce, all equal. The technology to do so is available but has not yet been tested with this goal. We hope to be able to continue developing effective, predictive approaches to fulfil this achievement.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] A. A. Saquet, "Storage of pears," *Scientia Horticulturae*, vol. 246, pp. 1009–1016, 2019.

[2] B. D. Whitaker, M. Villalobos-Acuña, E. J. Mitcham, and J. P. Mattheis, "Superficial scald susceptibility and α-farnesene metabolism in "Bartlett" pears grown in California and Washington," *Postharvest Biology and Technology*, vol. 53, no. 1–2, pp. 43–50, 2009.

[3] P. Eccher Zerbini, P. Cambiaghi, M. Grassi, and A. Rizzolo, "The effect of 1-MCP on the quality of Conference and Abbé Fétel pears," *Acta Horticulturae*, vol. 671, pp. 397–403, 2005.

[4] S. Lurie and C. B. Watkins, "Superficial scald, its etiology and control," *Postharvest Biology and Technology*, vol. 65, pp. 44–60, 2012.

[5] D. Zhanyuan and J. B. William, "Peroxidative activity of apple peel in relation to development of poststorage disorders," *HortScience*, vol. 30, no. 2, 1995.

[6] N. A. Abbasi, M. M. Kushad, I. A. Hafiz, and M. Maqbool, "Relationship of superficial scald related fruit maturity with polyphenoloxidase and superoxide dismutase activities in red spur delicious apples," *Asian Journal of Chemistry*, vol. 20, no. 8, pp. 5986–5996, 2008.

[7] F. J. P. Silva, M. H. Gomes, F. Fidalgo, J. A. Rodrigues, and D. P. F. Almeida, "Antioxidant properties and fruit quality during long-term storage of "rocha" pear: effects of maturity and storage conditions," *Journal of Food Quality*, vol. 33, no. 1, pp. 1–20, 2010.

[8] B. D. Whitaker, T. Solomos, and D. J. Harrison, "Quantification of α-farnesene and its conjugated trienol oxidation products from apple peel by C18-HPLC with UV detection," *Journal of Agricultural and Food Chemistry*, vol. 45, no. 3, pp. 760–765, 1997.

[9] D. D. Rowan, M. B. Hunt, S. Fielder, J. Norris, and M. S. Sherburn, "Conjugated triene oxidation products of α-farnesene induce symptoms of superficial scald on stored apples," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 6, pp. 2780–2787, 2001.

[10] B. D. Whitaker, J. F. Nock, and C. B. Watkins, "Peel tissue α-farnesene and conjugated trienol concentrations during storage of 'White Angel'×'Rome Beauty' hybrid apple selections susceptible and resistant to superficial scald," *Postharvest Biology and Technology*, vol. 20, no. 3, pp. 231–241, 2000.

[11] B. Farneti, N. Busatto, I. Khomenko et al., "Untargeted metabolomics investigation of volatile compounds involved in the development of apple superficial scald by PTR-ToF-MS," *Metabolomics*, vol. 11, no. 2, pp. 341–349, 2015.

[12] P. M. Chen, D. M. Varga, E. A. Mielke, T. J. Facteau, and S. R. Drake, "Control of superficial scald on 'anjou' pears by ethoxyquin: effect of ethoxyquin concentration, time and method of application, and a combined effect with controlled atmosphere storage," *Journal of Food Science*, vol. 55, no. 1, pp. 167–170, 1990.

[13] M.-A. Chiriboga, M. Saladié, J. Giné Bordonaba, I. Recasens, J. Garcia-Mas, and C. Larrigaudière, "Effect of cold storage and 1-MCP treatment on ethylene perception, signalling and synthesis: influence on the development of the evergreen behaviour in 'Conference' pears," *Postharvest Biology and Technology*, vol. 86, pp. 212–220, 2013.

[14] A. Rizzolo, M. Grassi, and M. Vanoli, "Influence of storage (time, temperature, atmosphere) on ripening, ethylene production and texture of 1-MCP treated 'Abbé Fétel' pears," *Postharvest Biology and Technology*, vol. 109, pp. 20–29, 2015.

[15] A. Folchi, P. Bertolini, and D. Mazzoni, "Preventing ripening blockage in 1-mcp treated 'abate fetel' pears by storage temperature management," *Acta Horticulturae*, vol. 1079, pp. 215–221, 2015.

[16] L. A. Honaas, H. L. Hargarten, S. P. Ficklin et al., "Co-expression networks provide insights into molecular mechanisms of postharvest temperature modulation of apple fruit to reduce superficial scald," *Postharvest Biology and Technology*, vol. 149, pp. 27–41, 2019.

[17] S. Serra, R. Leisso, L. Giordani, L. Kalcsits, and S. Musacchi, "Crop load influences fruit quality, nutritional balance, and return bloom in 'honeycrisp' apple," *HortScience*, vol. 51, no. 3, pp. 236–244, 2016.

[18] F. Rapparini, E. Gatti, S. Predieri, and L. Cavicchi, "Effect of pear production system on volatile aroma constituents of fruits," *Acta Hortic*, vol. 800, no. 2, pp. 1061–1067, 2008.

[19] T. Gomila, G. Calvo, and A. P. Candan, "Factors affecting sensitivity of 'abate fetel' pears to friction discoloration," *Acta Horticulturae*, vol. 909, pp. 687–692, 2011.

[20] S. Musacchi and S. Serra, "Apple fruit quality: overview on pre-harvest factors," *Science Horticulture (Amsterdam)*, vol. 234, pp. 409–430, 2018.

[21] A. F. Lezzoni and M. P. Pritts, "Applications of principal component analysis to horticultural research," *HortScience*, vol. 26, no. 4, pp. 334–338, 1991.

[22] B. H. Wu, B. Quilot, M. Génard, J. Kervella, and S. H. Li, "Changes in sugar and organic acid concentrations during fruit maturation in peaches, P. davidiana and hybrids as

analyzed by principal component analysis," *Scientia Horticulturae*, vol. 103, no. 4, pp. 429–439, 2005.

[23] N. Busatto, B. Farneti, A. Tadiello et al., "Wide transcriptional investigation unravel novel insights of the on-tree maturation and postharvest ripening of 'Abate Fetel' pear fruit," *Horticulture Research*, vol. 6, no. 1, pp. 1–15, 2019.

[24] E. Pesis, S. E. Ebeler, S. T. de Freitas, M. Padda, and E. J. Mitcham, "Short anaerobiosis period prior to cold storage alleviates bitter pit and superficial scald in Granny Smith apples," *Journal of the Science of Food and Agriculture*, vol. 90, no. 12, pp. 2114–2123, 2010.

[25] R core team, *R: A Language and Environment for Statistical Computing*, R Project for Statistical Computing, Indianapolis, IND, USA, 2020.

[26] M. Friendly and J. Fox, *Candisc: Visualizing Generalized Canonical Discriminant and Canonical Correlation Analysis*, 2020, https://cran.r-project.org/package=candisc.

[27] J. Oksanen, F. G. Blanchet, M. Friendly et al., *Vegan: Community Ecology Package*, 2019, https://cran.r-project.org/package=vegan.

[28] D. Sugar, D. G. Richardson, P. M. Chen, R. A. Spotts, R. G. Roberts, and T. Chand-Goyal, "Advances in improving the postharvest quality of pears," in *Acta Horticulturae, 475*, pp. 513–526, International Society for Horticultural Science, Leuven, Belgium, 1998.

[29] M. Vanoli, M. Grassi, and A. Rizzolo, "Ripening behavior and physiological disorders of 'Abate Fetel' pears treated at harvest with 1-MCP and stored at different temperatures and atmospheres," *Postharvest Biology and Technology*, vol. 111, pp. 274–285, 2016.

[30] H. Murayama, T. Katsumata, O. Horiuchi, and T. Fukushima, "Relationship between fruit softening and cell wall polysaccharides in pears after different storage periods," *Postharvest Biology and Technology*, vol. 26, no. 1, pp. 15–21, 2002.

[31] C. Moggia, M. Pereira, J. A. Yuri et al., "Preharvest factors that affect the development of internal browning in apples cv. Cripp's Pink: six-years compiled data," *Postharvest Biology and Technology*, vol. 101, pp. 49–57, 2015.

[32] J. Zhang, S. Serra, R. Leisso, and S. Musacchi, "Effect of light microclimate on the quality of 'd'Anjou' pears in mature open-centre tree architecture," *Biosystems Engineering*, vol. 141, pp. 1–11, 2016.

[33] M. Lachapelle, G. Bourgeois, J. R. DeEll, and J. R. Deell, "Effects of preharvest weather conditions on firmness of 'McIntosh' apples at harvest time," *HortScience*, vol. 48, no. 4, pp. 474–480, 2013.

[34] T. Robinson and S. Lopez, "Cropload and nutrition affect honeycrisp apple quality," *New York Fruit Q*, vol. 17, pp. 25–28, 2009.

[35] T. M. Mills, M. H. Behboudian, and B. E. Clothier, "Preharvest and storage quality of 'Braeburn' apple fruit grown under water deficit conditions," *New Zealand Journal of Crop and Horticultural Science*, vol. 24, no. 2, pp. 159–166, 1996.

[36] V. E. Emongor, D. P. Murr, and E. C. Lougheed, "Preharvest factors that predispose apples to superficial scald," *Postharvest Biology and Technology*, vol. 4, no. 4, pp. 289–300, 1994.

[37] M. Drake, W. J. Bramlage, and J. H. Baker, "Effects of foliar calcium on McIntosh apple storage disorders," *Commun. Soil Sci. Plant Anal.*vol. 10, no. 1–2, pp. 303–309, 1979.

[38] W. J. Bramlage, S. A. Weis, and M. Drake, "Predicting the occurrence of poststorage disorders of "McIntosh" apples from preharvest mineral analyses," *Journal of the American Society for Horticultural Science*, vol. 110, pp. 493–498, 1985.

[39] J. N. Wünsche and I. B. Ferguson, "Crop load interactions in apple," *Horticultural Reviews*, vol. 31, pp. 231–290, 2005.

[40] P. Eccher Zerbini, F. L. Gorini, and L. Gasperetti, "Internal browning of passacrassana pears in relation to the tree's productivity," *Acta Horticulturae*, vol. 69, pp. 267–274, 1977.

[41] G. Calvo, A. P. Candan, and T. Gomila, "Post-harvest performance of 'abate fetel' pears grown in Argentina in relation to harvest time," *Acta Horticulturae*, vol. 909, pp. 725–730, 2011.

[42] M. Ingle, "Physiology and biochemistry of superficial scald of apples and pears," in *Horticultural Reviews*, pp. 227–267, John Wiley & Sons, Oxford, UK, 2001.

[43] N. Isidoro and D. P. F. Almeida, "α-Farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine," *Postharvest Biology and Technology*, vol. 42, no. 1, pp. 49–56, 2006.

[44] C. A. Torres, G. Sepulveda, N. Mejía, B. G. Defilippi, and C. Larrigaudière, "Understanding the key preharvest factors determining "Packham's Triumph" pear heterogeneity and impact in superficial scald development and control," *Postharvest Biol. Technol.*vol. 172, Article ID 111399, 2021.

[45] F. E. Huelin and K. E. Murray, "α-Farnesene in the natural coating of apples," *Nature*, vol. 210, no. 5042, pp. 1260-1261, 1966.

[46] J. L. Anderson and S. D. Seeley, "Bloom delay in deciduous fruits," *Journal of American Society for Horticultural Science*, vol. 15, pp. 97–144, 1993.

[47] T. Sugiura, H. Ogawa, N. Fukuda, and T. Moriguchi, "Changes in the taste and textural attributes of apples in response to climate change," *Scientific Reports*, vol. 3, pp. 2418–2427, 2013.

[48] C. M. Kingston, "Maturity indices for apple and pear," *Horticultural Reviews*, vol. 13, no. 32, p. 402, 1992.

[49] M. Blanke and A. Kunz, "Einfluss rezenter Klimaveränderungen auf die Phänologie bei Kernobst am Standort Klein-Altendorf-anhand 50-jähriger Aufzeichnungen," *Erwerbs-Obstbau*, vol. 51, no. 3, pp. 101–114, 2009.

[50] M. S. Krishnaprakash, B. Aravindaprasad, C. A. Krishnaprasad et al., "Effect of apple position on the tree on maturity and quality," *Journal of Horticultural Science*, vol. 58, no. 1, pp. 31–36, 1983.

[51] G. Calderón-Zavala, A. N. Lakso, and R. M. Piccioni, "Temperature effects on fruit and shoot growth in the apple (Malus domestica) early in the season," *Acta Horticulturae, 636*, International Society for Horticultural Science, Leuven, Belgium, pp. 447–453, 2004.

[52] W. J. Bramlage, "Interactions of orchard factors and mineral nutrition on quality of pome fruit," in *Proceedings of International Symposium on Pre- and Postharvest Physiology of Pome-fruit*, Truiden, Belgium, February 1993.

[53] M. Saure, "Reassessment of the role of calcium in development of bitter pit in apple," *Functional Plant Biology*, vol. 23, no. 3, pp. 237–243, 1996.

[54] M. N. Westwood, "Temperate-zone pomology," in *Physiology and Culture*Timber Press Inc., Portland, OR, USA, 1993.

[55] I. Ferguson, R. Volz, and A. Woolf, "Preharvest factors affecting physiological disorders of fruit," *Postharvest Biology and Technology*, vol. 15, no. 3, pp. 255–262, 1999.

[56] T. M. Ramokonyane, H. Sciences, and J. A. V. D. Merwe, *Effects of Dynamic Controlled Atmosphere and Initial Low Oxygen Stress on Superficial Scald of 'Granny Smith' Apples*

and ' Packham ' S Triumph ' Pears, Stellenbosch University, Stellenbosch, South Africa, 2016.

[57] J. W. Palmer, *Canopy Manipulation for Optimum Utilization of Light*, Butterworth & Co. (Publishers) Ltd, Oxford, UK, 1989.

[58] S.-K. Jung and H.-S. Choi, "Light penetration, growth, and fruit productivity in 'Fuji' apple trees trained to four growing systems," *Scientia Horticulturae*, vol. 125, no. 4, pp. 672–678, 2010.

[59] T. L. Robinson and A. N. Lakso, *Light Interception, Yield and Fruit Quality of "Empire" and "Delicious" Apple Trees Grown in Four Orchard Systems*, International Society for Horticultural Science (ISHS), Leuven, Belgium, 1989.

[60] F. Kappel and G. H. Neilsen, "Relationship between light microclimate, fruit growth, fruit quality, specific leaf weight and N and P content of spur leaves of "Bartlett" and "Anjou" pear," *Scientia Horticulturae (Amsterdam)*, vol. 59, no. 3–4, pp. 187–196, 1994.

[61] E. J. Seeley, W. C. Micke, and R. Kammereck, ""Delicious" apple fruit size and quality as influenced by radiant flux density in the immediate growing environment," *Journal of the American Society for Horticultural Science*, vol. 105, pp. 645–647, 1980.

[62] E. A. Mielke, "Effects of rootstock and training system on fruit quality and peel nutrient content in 'd'Anjou' pears," *Journal of Tree Fruit Production*, vol. 3, no. 2, pp. 57–74, 2004.

[63] C. Brooks and J. S. Cooley, *Apple Scald and its Control*, pp. 83–205, Farmers' Bulletin, Washington, DC, USA, 1970.

[64] J. E. Jackson and R. O. Sharples, "The influence of shade and within-tree position on apple fruit size, colour and storage quality," *Journal of Horticultural Science*, vol. 46, no. 3, pp. 277–287, 1971.

[65] M. Génard and C. Bruchou, "Multivariate analysis of within-tree factors accounting for the variation of peach fruit quality," *Scientia Horticulturae (Amsterdam)*, vol. 52, no. 1–2, pp. 37–51, 1992.

[66] S. A. Rodikov, "New data on the effect of solar radiation and α-farnesene on development of apple superficial scald during storage," *Russian Agricultural Sciences*, vol. 34, no. 3, pp. 162–164, 2008.

[67] Z. Ju, Y. Yuan, C. Liu, S. Zhan, and M. Wang, "Relationships among simple phenol, flavonoid and anthocyanin in apple fruit peel at harvest and scald susceptibility," *Postharvest Biology and Technology*, vol. 8, no. 2, pp. 83–93, 1996.

[68] F. Ma and L. Cheng, "The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate-glutathione pathway than the shaded peel," *Plant Science*, vol. 165, no. 4, pp. 819–827, 2003.

[69] P. Li and L. Cheng, "The shaded side of apple fruit becomes more sensitive to photoinhibition with fruit development," *Physiologia Plantarum*, vol. 134, no. 2, pp. 282–292, 2008.

[70] G. M. Colavita, V. Blackhall, and S. Valdez, "Effect of Kaolin particle films on the temperature and solar injury of pear fruits," *Acta Horticulturae*, vol. 909, pp. 609–615, 2011.

[71] R. O. Sharples, *Orchard and Climatic Factors*, The Biology of Apple and Pear Storage, vol. 3, 1973.

[72] D. A. Rosenberger, J. R. Schupp, S. A. Hoying, L. Cheng, and C. B. Watkins, "Controlling bitter pit in `Honeycrisp' apples," *Horttechnology*, vol. 14, no. 3, pp. 342–349, 2004.

[73] D. Boynton and G. H. Oberly, *Temperate to Tropical Fruit Nutrition*, N. F. Childers, Ed., pp. 1–50–489–503, Somerset Press, Somerville, NJ, USA, 1966.

[74] I. T. Agar, W. V. Biasi, and E. J. Mitcham, "Exogenous ethylene accelerates ripening responses in Bartlett pears regardless of maturity or growing region," *Postharvest Biology and Technology*, vol. 17, no. 2, pp. 67–78, 1999.

[75] M.-A. Chiriboga, W. C. Schotsmans, C. Larrigaudière, E. Dupille, and I. Recasens, "Responsiveness of 'Conference' pears to 1-methylcyclopropene: the role of harvest date, orchard location and year," *Journal of the Science of Food and Agriculture*, vol. 93, no. 3, pp. 619–625, 2013.

[76] E. A. Curry, "Regulating ripening of 'bartlett' pears using preharvest plus postharvest aminoethoxyvinylglycine (AVG)," *The Open Horticulture Journal*, vol. 1, no. 1, pp. 21–25, 2008.

[77] M. Villalobos-Acuña and E. J. Mitcham, "Ripening of European pears: the chilling dilemma," *Postharvest Biology and Technology*, vol. 49, no. 2, pp. 187–200, 2008.

[78] H. Daugaard and J. Grauslund, "Fruit colour and correlations with orchard factors and post-harvest characteristics in apple cv. Mutsu," *The Journal of Horticultural Science and Biotechnology*, vol. 74, no. 3, pp. 283–287, 1999.

[79] G. Nava, A. R. Dechen, and G. R. Nachtigall, "Nitrogen and potassium fertilization affect apple fruit quality in southern Brazil," *Communications in Soil Science and Plant Analysis*, vol. 39, no. 1–2, pp. 96–107, 2008.

[80] G. Bertazza, G. Cristoferi, and C. Bignami, "Fruit composition and quality of organically and conventionally grown apple, apricot and pear in the Veneto region (Northern Italy)," *Acta Horticulturae*, vol. 873, pp. 309–316, 2010.

[81] E. E. Sánchez, "Nutrition and water management in intensive pear growing," *Acta Horticulturae*, vol. 1094, pp. 307–316, 2015.

[82] E. E. Sánchez, A. Giayetto, L. Cichón, D. Fernández, M. C. Aruani, and M. Curetti, "Cover crops influence soil properties and tree performance in an organic apple (Malus domestica Borkh) orchard in northern Patagonia," *Plant Soil*, vol. 292, no. 1–2, pp. 193–203, 2007.

[83] I. Paudel, H. Gerbi, A. Zisovich et al., "Drought tolerance mechanisms and aquaporin expression of wild vs. cultivated pear tree species in the field," *Environmental and Experimental Botany*, vol. 167, Article ID 103832, 2019.

[84] R. C. Ebel, E. L. Proebsting, and M. E. Patterson, "Regulated deficit irrigation may alter apple maturity, quality, and storage life," *HortScience*, vol. 28, no. 2, pp. 141–143, 1993.

[85] C. Brooks and D. F. Fisher, "Irrigation experiments on apple spot diseases," *Journal of Agricultural Research*, vol. 12, no. 3, pp. 109–137, 1918.

[86] R. T. Poole, "Influence of Maximum Air Temperatures and Irrigation Frequencies during High Temperature Periods on Growth of Four Foliage Plants," *HortScience*, vol. 16, no. 4, pp. 556-557, 1981.

[87] H. Lambers, F. S. Chapin, and T. L. Pons, *Plant Physiological Ecology*, Springer, New York, NY, USA, 1998.

[88] M. H. Behboudian and G. S. Lawes, "Fruit quality in 'Nijisseiki' Asian pear under deficit irrigation: physical attributes, sugar and mineral content, and development of flesh spot decay," *New Zealand Journal of Crop and Horticultural Science*, vol. 22, no. 4, pp. 393–400, 1994.

[89] K. Mengel and E. A. Kirkby, "Principles of plant nutrition. Bern," Springer, Amsterdam, Netherlands, 2001.

[90] S.-H. Lee, J.-H. Choi, W.-S. Kim, T.-H. Han, Y.-S. Park, and H. Gemma, "Effect of soil water stress on the development of stone cells in pear (Pyrus pyrifolia cv. 'Niitaka') flesh," *Scientia Horticulturae*, vol. 110, no. 3, pp. 247–253, 2006.

[91] W. J. Bramlage, M. Drake, and J. H. Baker, "Relationships of calcium content to respiration and postharv est condition of apples," *Journal of the American Society for Horticultural Science*, vol. 99, no. 4, pp. 376–378, 1974.

[92] P. Bertolini, M. Guizzardi, C. Casadei, and V. Agroalimentare, "Influence of calcium and oxygen levels on soft scald of stored abbe fetel pears," *Acta Horticulturae*, vol. 596, pp. 851–856, 2002.

[93] D. Gerasopoulos and D. G. Richardson, "Fruit maturity and calcium affect chilling requirements and ripening of `d'Anjou' pears," *HortScience*, vol. 32, no. 5, pp. 911–913, 1997.

[94] F. Bangerth, "A role for auxin and auxin transport inhibitors on the Ca content of artificially induced parthenocarpic fruits," *Physiologia Plantarum*, vol. 37, no. 3, pp. 191–194, 1976.

[95] W. J. Lord and W. J. Bramlage, "Effects of gibberellins A4+7 and 6-benzylamino purine on fruit set, fruit characteristics, seed content, and storage quality of "McIntosh" apples [Malus domestica, Daminozide, fruit firmness]," *Amreican Society for Horticultural Science*, vol. 17, 1982.

[96] W. J. Bramlage, S. A. Weis, and D. W. Greene, "Observations on the relationships among seed number, fruit calcium, and senescent breakdown in apples," *HortScience*, vol. 25, no. 3, pp. 351–353, 1990.

[97] K. Tomala and D. R. Dilley, "Calcium content of McIntosh and Spartan is influenced by the number of seeds per fruit," in *Proceedings of 5th International CA Research Conference*, pp. 53–61, Wenatchee, WA, USA, June 1989.

[98] T. Muziri, K. I. Theron, and E. M. Crouch, "Mealiness development in 'forelle' pears (pyrus communis L.) is influenced by cell size," *Acta Horticulturae*, vol. 1094, pp. 515–523, 2015.

[99] E. M. Crouch and M. Huysamer, "Cell wall compositional differences between mealy and non-mealy "Forelle" pear during ripening," *Acta Hortic*, vol. 877, pp. 1005–1010, 2011.

[100] B. B. Buchanan, W. Gruissem, and R. L. Jones, *Biochemistry and Molecular Biology of Plants*, Wiley, Rockville MD USA, 2015.

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Research Article

Effect of *Opuntia ficus-indica* Mucilage Edible Coating in Combination with Ascorbic Acid, on Strawberry Fruit Quality during Cold Storage

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Strawberry fruit is a nonclimacteric fruit and is one of the most consumed berries in the world. It is characterized by high levels of vitamin C, folate, vitamin E, β-carotene, and phenolic constituents as well asanthocyanins that are strictly related to health benefits. Strawberries are highly perishable fruit with a very short postharvest life due to their susceptibility to mechanical injury, rapid texture softening, physiological disorders, and infection caused by several pathogens (yeast and mold) that can rapidly reduce fruit quality. The aim of the present study was to evaluate the effect of the application of *Opuntia ficus-indica* mucilage in combination with ascorbic acid, as edible coating, on quality, sensorial parameters, and microbiological characteristics of strawberry fruit during cold storage at $4 \pm 0.5^{\circ}$C and 85% RH. Strawberries were characterized by a linear increase of weight loss during the storage at 4°C that was significantly higher (+11.3% on average) in the uncoated strawberries. The coating affected the ascorbic acid content of the strawberries that increased by 36.0% in coated strawberries; total soluble solid content and color of the strawberries were only affected by storage. Visual quality and sensorial analysis recorded higher scores in the coated samples at the end of the cold storage period. Furthermore, the mucilage coating did not negatively affect the natural taste of strawberries. The application of *O. ficus-indica* gel-based edible coating in combination with ascorbic acid, although not able to inhibit the microbial growth, limited significantly their development in coated strawberry fruits. Our results suggest that *Opuntia* mucilage plus 5% ascorbic acid could be a useful biochemical way of maintaining strawberry fruit quality and extending their postharvest life.

1. Introduction

Strawberry fruit (*Fragaria* × *ananassa* Duch.) is a nonclimacteric fruit and is one of the most consumed berries in the world, characterized by a peculiar and highly appreciated taste and flavor. Strawberry is a relevant source of bioactive compounds because of its high levels of vitamin C, folate, vitamin E, β-carotene, and phenolic constituents, as well as anthocyanins, substances strictly related to health benefits [1, 2]. Strawberries are highly perishable fruit with a very short postharvest life due to their susceptibility to mechanical injury, rapid texture softening, physiological disorders, and infection caused by several pathogens (yeast and mold) that can rapidly reduce fruit quality [1, 3].

Several technologies have been developed for strawberry preservation during postharvest, such as controlled atmospheres, hot water treatments, UV, and chemical treatments, but most of them have negative effects on color, flavor, aroma, and texture [1, 4]. In the past, fungicides were used to control mold and yeast growth, but several studies confirm that they can leave residues that could be dangerous for human health and the environment [5]. For this reason, consumers prefer natural and eco-friendly fruit products without any chemical additives [1]. A common technology used to control mold growth and reduce fruit senescence is cold storage under controlled/modified conditions, but high CO_2 concentrations can cause off-flavor development on fruit [6].

Among different postharvest management strategies of environment-friendly fresh fruit handling, the application of edible coatings has been reported to be very effective [7].

Edible coatings can act as a semipermeable barrier against gases and water vapor. It can modify fruit tissue metabolism by affecting respiration rate, decreasing moisture and firmness loss, preserving the color, transporting antimicrobial, antioxidant, and other preservatives, controlling microbial growth and maintaining fruit quality for a longer period [6, 8]. Several studies reported that the applications of edible coatings improved quality, extended storage, and shelf life of various fruit such as papaya [9], kiwifruit [10], and strawberries [11].

A novel edible coating for fruit storage based on the mucilage or polysaccharides extracted from cladodes of *Opuntia ficus-indica* was recently investigated on kiwifruit slices [10], breba fig [12], strawberry [13], banana [14], tomato [15], and mandarin [16].

Those studies reported that *O. ficus-indica* edible coating positively affected fruit quality, reducing water transpiration and browning, maintaining fruit fresh weight, visual score values, fruit firmness, nutraceutical traits, and controlling microbial growth, resulting in a longer storage period [10, 12–16].

O. ficus-indica mucilage is a complex carbohydrate composed of variable amounts of l-arabinose, d-galactose, l-rhamnose, and d-xylose, as well as galacturonic acid, which is a potential ingredient for the food industry, due to its nutritional and technological properties, such as viscosity [17]. Mucilage is, in fact, a hydrocolloid with a great water retention capacity that makes it interesting for the production of natural edible coatings with a high nutraceutical value, useful for fruit and food preservation [18].

Another compound widely used in fruit products during storage is ascorbic acid (AA) in concentrations ranging from 0.5 to 5% w/v [11]. Several authors reported that AA has an antibrowning effect in fruit fresh-cut products under different storage conditions [9, 19] and reduces vitamin C degradation during storage [11]. Other studies reported that AA has an antimicrobial effect on fresh-cut fruit such as jackfruit [20], apple [21], and papaya [9] and it can be added to the edible coating to improve the shelf-life of the fruit.

Sogvar et al. [11] reported that the application of Aloe vera in combination with AA on strawberry fruit improves the postharvest life by maintaining fruit quality attributes as well as firmness, solid soluble content, titratable acidity, vitamin C, anthocyanin, total phenolic, and total antioxidant activity and reduces decay by suppression of total aerobic mesophilic bacteria, yeasts, and mold growth. Other studies showed that coating with chitosan and ascorbic acid is an effective solution for inhibiting the growth of microorganisms, retarding enzymatic browning reactions, and reducing the weight loss of fresh-cut apples during refrigerated storage [22].

Those studies suggest that the addition of AA to the edible coating could be a useful biochemical way of maintaining strawberry fruit quality and extending their postharvest life. However, further studies are necessary to study how the addition of AA to different edible coatings, such as *O. ficus-indica* mucilage, could affect microbial growth and physicochemical traits of strawberry fruits during cold storage. The aim of the present study was to evaluate the effect of the application of *O. ficus-indica* mucilage in combination with ascorbic acid, as edible coating, on quality, sensorial parameters, and microbial growth of strawberry fruits during cold storage at $4 \pm 0.5^{\circ}$C and 85% RH.

2. Materials and Methods

2.1. Strawberry Fruits. The strawberry fruits (cv. Florida Fortuna) used in the trial were collected in the experimental farm of the Department of Agricultural, Food and Forest Science (SAAF), University of Palermo, located in Marsala (37°45'02.8"N 12°32'32.10"E, 50 m a.s.l.) and immediately transported to the research laboratory of the SAAF Department, University of Palermo, where they were graded for their uniformity in size, shape, and bright red color. A bulk of homogeneous fruits free from defects was selected for the coating treatment and subsequent cold storage. The selected fruits were divided into two treatment groups of 20 replicates (4 replicates for each of 5 storage sampling times).

2.2. Fresh Mucilage Extraction. One-year-old cladodes were collected from *O. ficus-indica* (OFI) plants grown in the germplasm collection of the SAAF Department of the University of Palermo (38°7'N 13°2'E, 29 m a.s.l). Harvested cladodes were moved to the laboratory, where they were processed for mucilage extraction, using a modified version of Du Toit and De Wit's patented method, developed in South Africa [23].

Cladodes were washed with chlorinated water to improve mucilage shelf life and to remove impurities and spines. Cladode chlorenchyma was removed with a peeler to obtain very pure mucilage from the parenchyma. Cladodes were then sliced into squares and cooked in a microwave oven (900 W) for 3–5 min, until soft. The cooked, soft cladode pieces were then mixed using an Omni Mixer Homogenizer (mod. Omni-Mixer, 17107, Dupont Instruments Sorvall, Kennesaw GA, USA) to aid the mucilage extraction. The obtained pulp was then centrifuged using a Sigma centrifuge (mod. 6K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at $8{,}117x$ g for 15 min at 4°C, to separate the liquid mucilage from the solids. The mucilage was then decanted and weighed while the solid material left in the falcon tubes was discarded. No chemicals have been used during this extraction process.

2.3. Fresh Mucilage Edible Coating Application. Half of the selected strawberries were treated with OFI mucilage in combination with 5% of ascorbic acid [11] and the remaining strawberries were treated with distilled water and used as control. Mucilage edible coating and distilled water were applied by using an atomizing spray system (flow rate: $1\ \mathrm{L \cdot h^{-1}}$; air pressure: 50 kPa). Soon after coating, all fruits were air-dried at room temperature for

15', then placed in polyethylene (PE) boxes (each a 10 fruit replicate), and stored at 4 ± 0.5°C and 85% RH for 12 days.

2.4. Quality Parameters: Weight Loss, Color, Overall Quality, Firmness, Soluble Solid Content, Titratable Acidity, and Ascorbic Acid Content. The quality of strawberries was assessed soon after coating (0 d) and at 3, 6, 9, and 12 d of storage at 4°C. For each storage time and experimental treatment, four samples of five strawberries were randomly collected and analyzed. The fruits were weighed at each sampling time to evaluate weight loss. Strawberries' external color was measured at two opposite points on each strawberry using a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan). CIE $L^*a^*b^*$ coordinates were recorded as L^* (lightness), a^* (positive values for reddish colors and negative values for greenish colors), and b^* (positive values for yellowish colors and negative values for bluish colors). From these components, chroma (C^*) and hue angle (h°) were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h^\circ = \arctan(b^*/a^*)$ [24]. Overall visual quality (OQ) was evaluated by a panel made of 10 people (5 females and 5 males, aged between 25 and 50 years) using a 1 to 5 scale, where 5 = excellent product with a fresh appearance and optimal sensory acceptability (e.g., typical odor and color, no defects), 3 = fair/limit of sensory acceptability and marketability (e.g., minor alterations), and 1 = poor/unmarketable, with altered odor, extended discolored or decayed area, or other severe defects.

Fruit firmness was measured using a digital penetrometer (model 53205, TR Snc., Forlì, Italy) equipped with flat stainless steel cylinder probes of 6 mm diameter. Each fruit was punched with constant force to a depth of 0.5 cm and the mean peak force was calculated in Newton. After firmness analysis, the fruits of each sample were cut into small pieces and homogenized. Ten grams of the homogenate was suspended in 100 mL of distilled water and then filtered. The extracts were used to determine the soluble solid content (SSC expressed as °Brix) using a digital refractometer (MTD-045nD, Three-In-One Enterprises Co., Ltd., Taipei, Taiwan) and to measure the titratable acidity (TA) by potentiometric titration with 0.1 M NaOH up to pH 8.1 (TA expressed as meq 100 g^{-1} fresh weight [f.w.]). Ascorbic acid content was determined by extracting 10 g of a blended strawberry sample from each sampling date (0, 3, 6, 9, and 12 days of storage) in 100 mL metaphosphoric acid (HPO_3) and then filtered through Whatman no. 1 filter paper. A volume of 10 mL from the filtered solution was determined volumetrically with the 2–6 dichlorophenol-indophenol reagent until a slightly pink coloration was observed and persisted for 15 s [25]. The reading of ascorbic acid content was expressed in mg 100 g^{-1} fruit sample.

2.5. Sensory Analysis. For each sampling date, a sample of fruit for each treatment was subjected to sensory evaluation. The sensory profile was constructed by a semitrained panel made of 10 judges (5 females and 5 males, aged between 25 and 50 years) who generated a list of descriptors by using commercial fruit in a few preliminary meetings [26]. The semitrained panel was made up of people normally familiar with strawberries. This type of panel can discriminate differences and communicate their reactions, though the individuals may not have been formally trained; furthermore, a semitrained panelist judgment is likely to be closer to that of the average consumer [27]. Sensory analysis was focused on 6 principal descriptors: firmness, sweetness, acidity, aroma, taste, and off-flavor [28].

Strawberries sample was dispensed into a small plastic tray with a 3-digit code on the side and served to the judges, and the different descriptors were measured using an eight-point intensity scale where the digit 1 indicates the descriptor absence while the digit 8 indicates the full intensity. Among the judges, the order of presentation of the fruit was randomized; water was used to rinse the mouth [27].

2.6. Microbiological Analyses. O. ficus-indica mucilage added with ascorbic acid and the fruit samples from the different trials and storage times were subjected to the microbiological analyses. Cell suspensions of mucilage were directly subjected to decimal serial dilutions in Ringer's solution (Sigma-Aldrich, Milan, Italy), while fruit samples were first homogenized in Ringer's solution (Sigma-Aldrich) to a ratio 1 : 10 (fruit: diluent) by the Bag-Mixer 400 stomacher (Interscience, Saint Nom, France) for 2 min at the highest speed (blending power 4) and then serially diluted in the same isotonic solution.

Decimal dilutions were plated on several agar media: Plate Count Agar (PCA), incubated aerobically for 72 h at 30°C for total mesophilic microorganisms (TMM); PCA incubated aerobically for 7 d at 7°C for total psychrotrophic microorganisms (TPM); Pseudomonas Agar Base (PAB) added with Cetrimide Fucidin Cephaloridine (CFC) supplement, incubated aerobically for 48 h at 25°C for pseudomonads; double-layered Violet Red Bile Glucose Agar (VRBGA), incubated at 37°C for 24 h for members of the Enterobacteriaceae family; Dichloran Rose Bengal Chloramphenicol (DRBC) agar, incubated aerobically for 48 h at 28°C for yeasts; Yeast extract Peptone Dextrose (YPD) agar supplemented with 0.1 g·L^{-1} of chloramphenicol to avoid bacterial growth, incubated aerobically for 7 d at 25°C for molds.

All media and supplements were purchased from Biotec (Grosseto, Italy). All plate counts were carried out in triplicate.

2.7. Statistical Analysis. Statistical analyses of quality and sensory parameters were performed using the GLM procedure of the SPSS 14.0 software package (SPSS, Inc., Chicago, IL) and the means were compared by Tukey's test at a significance level of 0.05.

Microbiological data were subjected to One-Way Variance Analysis (ANOVA) using XLStat software version 2020.3.1 for Excel (Addinsoft, New York, USA). The level of significance and the differences between control and experimental trials were achieved by Tukey's test. $P < 0.05$ was considered significant.

3. Results and Discussion

3.1. Quality Parameters: Weight Loss, Color, Overall Quality, Firmness, Soluble Solid Content, Titratable Acidity, and Ascorbic Acid Content. Strawberries were characterized by a linear increase of weight loss during the storage at 4°C (Table 1). A higher weight loss (+11.3% on average) was recorded in the uncoated strawberries. The effect of OFI mucilage coating on weight loss was similar to other edible coatings tested on strawberry fruits [29] and a reduction of weight was also found in sliced or entire fruits (kiwi, fig, strawberry) coated with cactus mucilage [10, 12, 13, 30] and could be ascribed to the increased retention of water due to the reduction of transpiration and respiration determined by the hydrophilic character of mucilage coating that closed the opening of stomata and lenticels [31]. After harvest, the weight of the fruits is likely to change due to several factors, including loss of extracellular and intracellular water, sugar consumption determined by cellular respiration and degradation of the cell wall, and consequent loss of water by the cell breakdown [32]. These factors and loss in cell turgidity pressure and loss of extracellular and vascular air may also lead to the modification of tissue texture. Measuring the mechanical properties of fruits like strawberries can be challenging, as their structure is very inhomogeneous. They vary a lot in shape and size, so it can be very difficult to take a uniform sample from tested strawberries. Puncture testing has often been used to measure and follow changes in texture and firmness of fruits and vegetables at different preharvest and postharvest stages. The use of the puncture penetration method allows evaluating both the skin toughness and the flesh firmness of strawberries [33]. The firmness of strawberries was affected by storage that determined a significant reduction of this parameter after 6 days at 4°C for control fruit, with further significant changes recorded after 9 days and at the end of the storage period so that strawberry decreased from 10.4 N at day 0 to 7.3 N at day 12 for both coated and uncoated samples (Table 1). Nevertheless, the coated strawberries retained a slightly higher firmness after 6 and 9 days of storage at 4°C, so that this treatment determined a higher firmness than control (+5.1% on average). The decrease in fruit firmness during cold storage might be due to the breakdown of cell wall components such as insoluble pectin and also due to cellular collapse [34] that could have been reduced in the coated fruits. A lower decrease of firmness during cold storage was also found in strawberries and other fruits coated with OFI mucilage [10, 12, 13]. This could be ascribed to the presence of calcium in OFI mucilage [35] that interacts with the pectic acid in the cell walls to form calcium pectate, thus retaining the integrity of cell walls in fruit tissues.

Storage also affected the soluble solid content of the strawberries (Table 1). This parameter was almost constant during the first week of storage (7.5 °Brix on average) and significantly increased at the end of the experiment (8.6 °Brix). A similar trend was recorded for the titratable acidity (TA) that increased significantly at the end of the storage period (+26.1%) (Table 1). The increase in soluble solid content might be due to hydrolysis into sugars and to the capacity of cold storage to slow down the metabolic activities of strawberries during storage [29]. The effect of cold storage in combination with the edible coating application on metabolic activities could have also reduced the utilization of organic acid during the respiratory process thus explaining their increase during cold storage, as reported by Hazarika et al. [29].

The coating affected the ascorbic acid content of the strawberries that increased by 36.0% in coated strawberries (Table 1). This parameter showed small variations during storage that were significant only at day 3. Ascorbic acid is an important phytochemical with powerful antioxidant activity and can scavenge the reactive oxygen species (ROS) produced in the body thus protecting from many severe diseases [36]. The cactus mucilage coating showed to be useful in increasing the nutritional value of strawberries during the entire storage period.

The color of the strawberries was only affected by storage (Table 1). Color lightness decreased from 41.5 at day 0 to 37.6 at day 3 and showed no further reduction until the end of the experiment. Chroma and hue decreased more severely during storage so that the color of strawberries got less vivid and turned to the darkest red. Visual quality is of paramount importance in affecting strawberry acceptance and marketability. Appearance changes that strawberries may suffer during storage can be symptoms of freshness loss and microbiological decay [37]. The overall appearance of coated and uncoated strawberries changed significantly during storage, but control strawberries had a more severe descending trend of the OQ scores that dropped below the limit of marketability after seven days of storage, whereas the strawberries coated with cactus mucilage recorded OQ scores above the limit of marketability throughout the storage period (12 days) (Figure 1).

3.2. Microbiological Counts. Strawberry is a highly perishable fruit and its shelf-life is usually influenced by different microbial infections determined by bacteria, yeasts, and molds [38]. Table 2 shows the results of the microbiological analyses of the strawberry fruit samples collected during the experimentation. The results of viable counts performed on *O. ficus-indica* mucilage are not reported in the table because they were below the detection limit for all microbial groups objects of investigation. Statistically significant differences were found according to Tukey's test for the levels of TMM, TPM, and molds between coated and uncoated strawberry fruits. The initial concentrations of TMM, TPM, and molds in control fruits (C) were 3.04, 2.81, and 3.34 Log CFU g^{-1}, respectively, and increased during refrigerated storage. Even in coated strawberry fruits (MA), these microbial populations increased during storage but showed lower microbial cell densities than the untreated fruits. The same trend was reported by Sogvar et al. [11] on strawberry fruits treated with Aloe vera gel-based edible coating in combination with ascorbic acid. No colonies of pseudomonads and yeasts responsible for the microbial spoilage of fruits and vegetables [39] were detected in any of the samples analyzed. Members of the

Table 1: Chemical and physical parameters of strawberries coated with cactus mucilage + ascorbic acid (MA) or uncoated (C) during storage at 4°C.

Source of variance		Weight loss (g 100 g^{-1} fw)	Firmness (N)	SSC (°Brix)	TA (meq 100 g^{-1} fw.)	Ascorbic acid (mg 100 g^{-1})	L^*	Chroma	Hue
Coating									
C		9.0b	9.2b	7.7	10.6	36.9B	37.5	46.5	37.7
MA		8.1a	9.7a	7.9	11.0	50.2A	38.0	46.6	38.3
Storage									
0		—	10.2d	7.4c	10.1b	44.6A	41.5A	48.8A	42.1a
3		3.6d	10.6bc	7.5c	10.5ab	37.7B	37.6B	47.0B	38.4b
6		6.8c	9.9c	7.7c	11.3a	43.9A	36.7B	45.5Bc	37.1bc
9		9.8b	9.2a	8.0bc	11.2a	44.0A	36.1B	46.0Bc	36.9bc
12		13.9a	7.3ab	8.6a	11.0a	47.7A	36.8B	45.2C	35.3c
Coating x *Storage*									
	0	—	10.2	7.4	10.1	40.1	41.5	48.8	42.1
	3	3.9	10.6	7.5	9.9	30.7	37.5	46.9	37.2
C	6	7.2	9.4	7.8	10.7	38.5	36.2	45.0	36.5
	9	10.3	8.7	7.7	11.4	35.6	35.1	46.4	36.0
	12	14.5	7.2	8.3	11.0	39.8	37.2	45.1	36.5
	0	—	10.2	7.4	10.1	49.0	41.5	48.8	42.1
	3	3.3	10.6	7.5	11.0	44.7	37.8	47.2	39.6
MA	6	6.4	10.4	7.7	11.8	49.3	37.2	46.0	37.8
	9	9.3	9.7	8.3	11.0	52.5	37.1	45.6	37.9
	12	13.2	7.5	8.9	11.1	55.7	36.3	45.4	34.2
Significance[x]									
Coating		*	*	ns	ns	***	ns	ns	ns
Storage		***	***	***	*	***	***	***	***
Coating x Storage		ns	ns	ns	ns	ns	ns	ns	ns

Each value is the mean of 4 replicated samples. Values in a column followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$. [x]Significance: ns = not significant; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$. [y]Titratable acidity expressed as meq/100 g fresh weight.

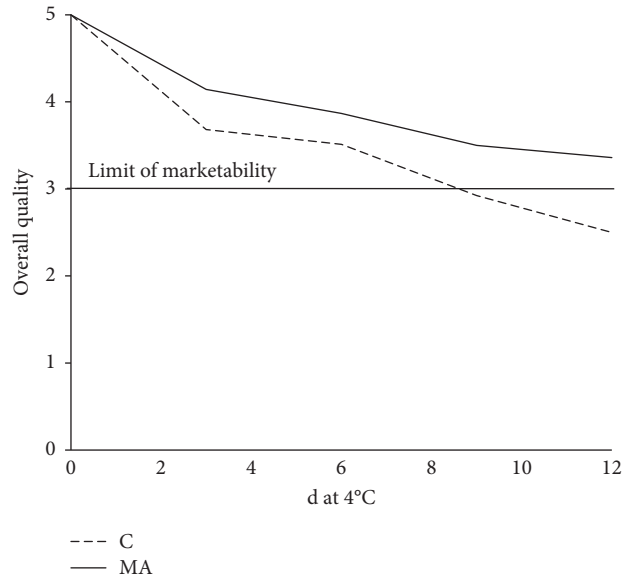

Figure 1: Influence of coating and time of storage on the overall visual quality of strawberry fruits (C: control fruits; MA: fruits coated with cactus mucilage + ascorbic acid; scores: 5, excellent or having a fresh appearance; 3, average limit of marketability; 1, unmarketable).

Enterobacteriaceae family, responsible for gastroenteritis and even chronic infections [40, 41], were below the detection limit in both coated and control strawberry fruits during the entire period of analysis.

3.3. Sensory Analysis. Uncoated (C) and coated (MA) strawberry fruit samples were subjected to sensory evaluation on each sampling date. Strawberry fruits were positively affected by mucilage coating in combination with the ascorbic acid treatment; indeed panelists preferred MA samples in each sampling date (data not shown).

MA samples showed mean scores 0.5 higher in terms of sensory evaluation than C samples after 3 days of storage at 4°C (Figure 2). In particular, judges perceived the largest difference in the aroma descriptor in MA samples with scores 1.6 higher than C ones (Figure 2).

At the end of the storage (12 days), MA samples were preferred by judges showing the highest scores in all sensorial parameters, and MA samples obtained sensory evaluation mean scores 1.2 higher than C ones (Figure 3). Panelists perceived off-flavor in C samples after 9 (data not shown) and 12 days at 4°C (Figure 3), while the perception of

Table 2: Microbial loads of control (C) and coated (*O. ficus-indica* mucilage in combination with ascorbic acid-MA) strawberry fruit samples during storage at 4°C.

Samples	Microorganisms		
	TMM	TPM	Molds
C 0 d	3.04 ± 0.22^{a}	2.81 ± 0.15^{a}	3.34 ± 0.23^{a}
MA 0 d	2.99 ± 0.19^{a}	2.88 ± 0.23^{a}	3.26 ± 0.12^{a}
C 3 d	3.19 ± 0.17^{a}	3.30 ± 0.27^{a}	3.49 ± 0.21^{a}
MA 3 d	2.74 ± 0.12^{b}	2.72 ± 0.11^{b}	2.89 ± 0.15^{b}
C 6 d	3.59 ± 0.21^{a}	3.47 ± 0.25^{a}	3.65 ± 0.26^{a}
MA 6 d	3.01 ± 0.15^{b}	2.95 ± 0.20^{b}	2.96 ± 0.17^{b}
C 9 d	3.75 ± 0.18^{a}	3.70 ± 0.24^{a}	3.81 ± 0.19^{a}
MA 9 d	3.15 ± 0.21^{b}	3.07 ± 0.22^{b}	3.10 ± 0.24^{b}
C 12 d	4.08 ± 0.24^{a}	4.12 ± 0.21^{a}	4.23 ± 0.21^{a}
MA 12 d	3.49 ± 0.19^{b}	3.38 ± 0.19^{b}	3.59 ± 0.25^{b}

Units are log CFU g^{-1}. Results indicate mean values ± S.D. of three plate counts. Data within a column followed by the same letter between the C and MA on the same day of refrigerated storage are not significantly different according to Tukey's test at $p < 0.05$.

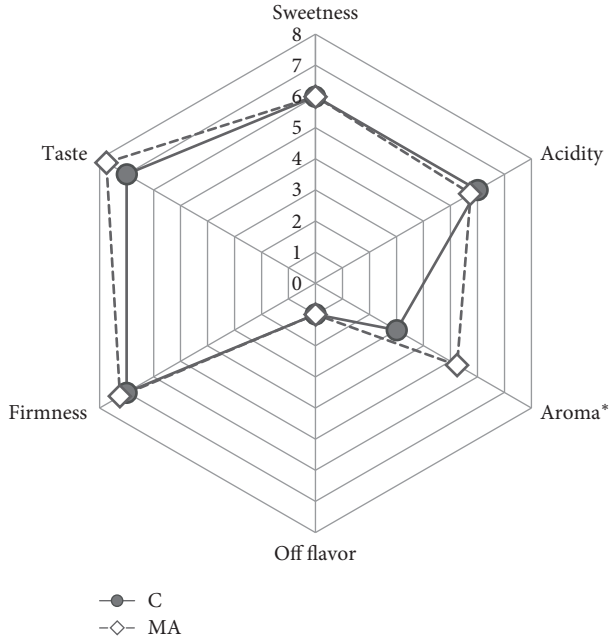

Figure 2: Sensory analysis of untreated (C) and treated (*O. ficus-indica* mucilage in combination with ascorbic acid-MA) strawberry "Fortuna" fruit, after 3 days of storage at 4°C. Data are mean of 15 fruit for each treatment. *Significant differences for values (Tukey's test at $p < 0.05$).

this descriptor was almost absent in MA samples in each sampling date (Figures 2 and 3).

The sensory analysis showed that judges had a higher preference for coated samples at the end of the cold storage period, as reported by Del-Valle et al. [13]. The mucilage

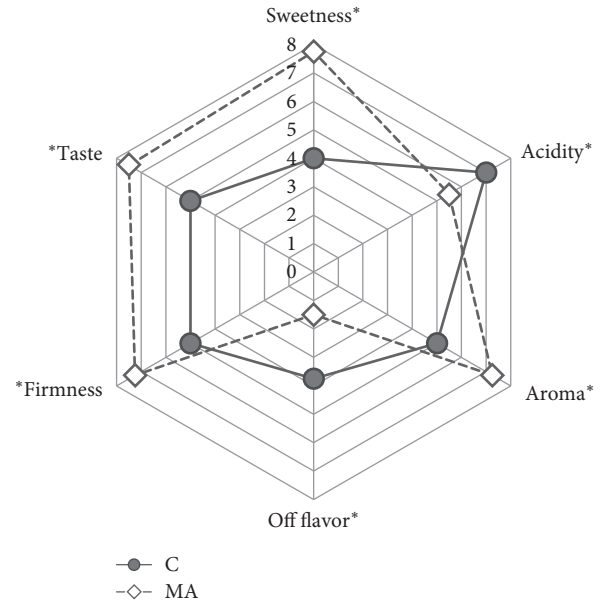

Figure 3: Sensory analysis of untreated (C) and treated (*O. ficus-indica* mucilage in combination with ascorbic acid-MA) strawberry "Fortuna" fruit, after 12 days of storage at 4°C. Data are Mean of 15 fruit for each treatment. *Significant differences for values (Tukey's test at $p \leq 0.05$).

coating did not negatively affect the natural taste of strawberries, which is an important aspect regarding the use of edible coatings when taste modification is undesirable. Indeed, MA coating has exalted some important parameters, as well as aroma, sweetness, and taste that are particularly appreciated by consumers.

4. Conclusions

Our data showed that *Opuntia* mucilage had a barrier effect on strawberries after harvest, reflected by the lower weight loss and a slightly higher firmness of coated samples than uncoated ones, after 9 days of storage at 4°C. This factor could reduce economic losses due to spoilage caused by mechanical damage during the handling and transportation of strawberries. The addition of ascorbic acid in the cactus mucilage coating showed to be useful in increasing the nutritional value of strawberries during the entire storage period. Soluble solid content and color were affected only by storage and not by the coating treatment.

Visual quality and sensorial analysis showed that judges had a higher preference for coated samples at the end of the cold storage period. Furthermore, the mucilage coating did not affect negatively the natural taste of strawberries, which is an important aspect regarding the use of edible coatings

when taste modification is undesirable. Indeed, MA coating has exalted some important parameters and aroma, sweetness, and taste that are particularly appreciated by consumers.

From the microbiological point of view, the application of *O. ficus-indica* gel-based edible coating in combination with ascorbic acid is not able to inhibit the microbial growth but slowed down significantly their development in coated strawberry fruits.

In conclusion, our data suggest that *O. ficus-indica* mucilage plus 5% of ascorbic acid could be a useful biochemical way of maintaining strawberry fruit quality and extending its postharvest life.

Data Availability

The data are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] N. B. Gol, P. R. Patel, and T. V. R. Rao, "Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan," *Postharvest Biology and Technology*, vol. 85, pp. 185–195, 2013.

[2] F. Giampieri, S. Tulipani, J. M. Alvarez-Suarez, J. L. Quiles, B. Mezzetti, and M. Battino, "The strawberry: composition, nutritional quality, and impact on human health," *Nutrition*, vol. 28, no. 1, pp. 9–19, 2012.

[3] K. D. Vu, R. G. Hollingsworth, E. Leroux, S. Salmieri, and M. Lacroix, "Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries," *Food Research International*, vol. 44, no. 1, pp. 198–203, 2011.

[4] V. Kuchi and C. Sharavani, *Fruit Physiology and Postharvest Management of Strawberry*, Intechopen Books, London, UK, pp. 1–20, 2019.

[5] H. Li and T. Yu, "Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit," *Journal of the Science of Food and Agriculture*, vol. 81, no. 2, pp. 269–274, 2000.

[6] A. G. Pérez and C. Sanz, "Effect of high-oxygen and high-carbon-dioxide atmospheres on strawberry flavor and other quality traitsistry," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 5, pp. 2370–2375, 2001.

[7] K. Pushpendra and S. Shruti, "Edible coating for fresh fruit: a review," *International Journal of Current Microbiology and Applied Sciences*, vol. 7, no. 5, pp. 2619–2626, 2018.

[8] B. A. Rahimi, T. H. Shankarappa, N. A. Sahel et al., "Effective edible coatings on control of microbial growth in strawberry fruits," *Indian Journal of Ecology*, vol. 46, no. 7, pp. 91–95, 2019.

[9] M. S. Tapia, M. A. Rojas-Graü, A. Carmona, F. J. Rodríguez, R. Soliva-Fortuny, and O. Martin-Belloso, "Use of alginate- and gellan-based coatings for improving barrier, texture and nutritional properties of fresh-cut papaya," *Food Hydrocolloids*, vol. 22, no. 8, pp. 1493–1503, 2008.

[10] A. Allegra, P. Inglese, G. Sortino, L. Settanni, A. Todaro, and G. Liguori, "The influence of *Opuntia ficus-indica* mucilage edible coating on the quality of "Hayward" kiwifruit slices," *Postharvest Biology and Technology*, vol. 120, pp. 45–51, 2016.

[11] O. B. Sogvar, M. K. Saba, and A. Emamifar, "Aloe vera and ascorbic acid coatings maintain postharvest quality and reduce microbial load of strawberry fruit," *Postharvest Biology and Technology*, vol. 114, pp. 29–35, 2016.

[12] A. Allegra, G. Sortino, P. Inglese et al., "The effectiveness of *Opuntia ficus-indica* mucilage edible coating on postharvest maintenance of "Dottato" fig (*Ficus carica* L.) fruit," *Food Packaging Shelf Life*, vol. 12, pp. 135–141, 2017.

[13] V. Del-Valle, P. Hernández-Muñoz, A. Guarda et al., "Development of a cactus-mucilage edible coating (*Opuntia ficus-indica*) and its application to extend strawberry (*Fragaria ananassa*) shelf-life," *Food Chemistry*, vol. 91, no. 4, pp. 751–756, 2005.

[14] L. V. Aquino, J. Rodriguez, L. L. Mendez et al., "Inhibicion del oscurecimiento con mucilago de nopal (*Opuntia ficus-indica*) en el secado se platano roatan," *Informacion Tecnologica*, vol. 20, no. 4, pp. 15–20, 2009.

[15] D. R. Olicón-Hernández, Á. Acosta-Sánchez, R. Monterrubio-López, and G. Guerra-Sanchez, "Chitosan and *Opuntia ficus-indica* mucilage as the base of a polymeric edible film for the protection of tomatoes against *Rhizopus stolonifer*," *TIP Revista Especializada en Ciencias Químico-Biológicas,*vol. 22, pp. 1–9, 2019.

[16] S. Riaz, M. T. Sultan, M. Sibt-e-Abass et al., "Extraction of polysaccharides from *Opuntia cactus* for its potential application in edible coating to improve the shelf life of citrus (Kinnow Mandarin) fruit," *Journal of Microbiology, Biotechnology and Food Science*, vol. 8, pp. 745–750, 2018.

[17] C. Sáenz, E. Sepúlveda, and B. Matsuhiro, "*Opuntia* spp. mucilage's: a functional component with industrial perspectives," *Journal of Arid Environments*, vol. 57, pp. 275–290, 2004.

[18] C. M. Messina, R. Arena, M. Morghese et al., "Seasonal characterization of nutritional and antioxidant properties of *Opuntia ficus-indica* [(L.) Mill.] mucilage," *Food Hydrocolloids*, vol. 111, p. 106398, 2021.

[19] M. I. Gil, J. R. Gorny, and A. A. Kader, "Responses of "Fuji" apple slices to ascorbic acid treatments and low-oxygen atmospheres," *HortScience*, vol. 33, pp. 305–309, 1998.

[20] J. Z. Acedo, D. A. C. Varron, I. C. Emnace et al., "Antimicrobial effects of ascorbic acid and calcium lactate in freshcut jackfruit (*Artocarpus heterophyllus* Lam.)," *Acta Horticulturae*, vol. 989, pp. 199–208, 2012.

[21] H. Qi, W. Hu, and A. Jiang, "Extending shelf-life of fresh-cut "fuji" apples with chitosan-coatings," *Innovative Food Science and Emerging Technologies*, vol. 12, pp. 62–66, 2011.

[22] K. S. Özdemir and V. Gökmen, "Effect of chitosan-ascorbic acid coatings on the refrigerated storage stability of fresh-cut apples," *Coatings*, vol. 9, no. 8, p. 503, 2019.

[23] A. Du Toit and M. De Wit, "A process for extracting mucilage from *Opuntia ficus-indica* and *Aloe barbadensis*," South Africa Patent No. PA153178/P, 2011.

[24] R. G. McGuire, "Reporting of objective color measurements," *HortScience*, vol. 27, pp. 1254-1255, 1992.

[25] G. Liguori, P. Inglese, O. Corona et al., "Effects of 1-methylcyclopropene on postharvest quality traits, antioxidant activity and ascorbic acid content of mature-ripe mango fruits," *Fruits*, vol. 72, no. 4, pp. 238–246, 2017.

[26] C. Ruiz-Capillas and A. Moral, "Use of semitrained panel members in the sensory evaluation of hake (*Merluccius*

merluccius L.) analyzed statistically," *Journal of Food Quality*, vol. 26, pp. 181–195, 2003.

[27] G. Liguori, C. Gentile, G. Sortino et al., "Food quality, sensory attributes and nutraceutical value of fresh "osteen" mango fruit grown under mediterranean subtropical climate compared to imported fruit," *Agriculture*, vol. 10, no. 4, p. 103, 2020.

[28] E. Y. Gil-Giraldo, A. L. Duque-Cifuentes, and V. D. Quintero-Castano, "Obtaining minimally processed strawberry (*Fragaria x ananassa*) products and their physicochemical, microbiological, and sensory characterization by using edible coatings," *Revista DYNA*, vol. 85, no. 207, pp. 183–191, 2018.

[29] T. K. Hazarika, Lalrinfeli, Lalthanmuani, J. Lalchhanmawia, and D. Mandal, "Alteration of quality attributes and shelf-life in strawberry (*Fragaria* × *ananassa*) fruits during storage as influenced by edible coatings," *Indian Journal of Agricultural Science*, vol. 89, pp. 28–34, 2019.

[30] A. d. J. Cenobio-Galindo, J. Ocampo-López, A. Reyes-Munguía et al., "Influence of bioactive compounds incorporated in a nanoemulsion as coating on avocado fruits (*Persea americana*) during postharvest storage: antioxidant activity, physicochemical changes and structural evaluation," *Antioxidants*, vol. 8, p. 500, 2019.

[31] R. Das and G. Medhi, "Physio-chemical changes of pineapple fruits under certain postharvest treatments," *South Indian Horticulture*, vol. 44, pp. 5–7, 1996.

[32] M. Martinez-Ferrer, C. Harper, F. Perez-Muntoz et al., "Modified atmosphere packaging of minimally processed mango and pineapple fruits," *Journal of Food Science*, vol. 67, pp. 3365–3371, 2002.

[33] M. Suutarinen and K. Autio, "Improving the texture of frozen fruit: the case of berries," *Texture in Food*, Elsevier, Amsterdam, Netherlands, pp. 388–409, 2004.

[34] B. V. C. Mahajan, D. Kumar, and W. S. Dhillon, "Effect of different polymeric films on shelf-life and quality of pear fruits under supermarket conditions," *Indian Journal of Horticulture*, vol. 70, pp. 309–312, 2013.

[35] E. Sepúlveda, C. Sáenz, E. Aliaga et al., "Extraction and characterization of mucilage in *Opuntia* spp." *Journal of Arid Environment*, vol. 68, pp. 534–545, 2007.

[36] N. Patel, A. G. Naik, and S. S. Arbat, "Response of postharvest chemical treatments on shelf-life and quality of custard apple cv. Balanagar," *Indian Journal of Horticulture*, vol. 68, pp. 547–550, 2011.

[37] G. La Scalia, G. Aiello, A. Miceli et al., "Effect of vibration on the quality of strawberry fruits caused by simulated transport," *Journal of Food Processing Engineering*, vol. 39, pp. 140–156, 2016.

[38] A. L. Wszelaki and E. J. Mitcham, "Effect of combinations of hot water dips, biological control and controlled atmospheres for control of gray mold on harvested strawberries," *Postharvest Biology and Technology*, vol. 27, no. 3, pp. 255–264, 2003.

[39] M. Barth, T. R. Hankinson, H. Zhuang et al., "Microbiological spoilage of fruits and vegetables," in *Compendium of the Microbiological Spoilage of Foods and Beverages*, pp. 35–183, Springer, New York, NY, USA, 2009.

[40] G. Francis, C. Thomas, and D. O'Beirne, "The microbiological safety of minimally processed vegetables," *International Journal of Food Science and Technology*, vol. 34, pp. 1–22, 1999.

[41] J. Y. D'Aoust, "Current foodborne pathogens: salmonella," in *Food Safety Handbook: Microbiological Challenges*, M. Storrs, M.-C. Devoluy, and P. Cruveiller, Eds., pp. 128–141, BioMérieux Education, Marcy-l'Étoile, France, 2007.

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Research Article

Increasing Lignification in Translucent Disorder Aril of Mangosteen Related to the ROS Defensive Function

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Mangosteen fruit has a high potential on the global fruit market, but some disorders, including translucent flesh, are major problems of fruit quality, limiting the marketability. The present study was conducted to compare physiological changes of reactive oxygen species (ROS), cellular lignification between translucent and normal aril, and elucidate the relation. Mangosteen fruits at purple peel color were collected from eastern Thailand during the middle of the rainy season of 2019. Translucent aril accumulated higher lignin content in the tissues, expressing firmer texture ten times higher than normal aril. Lignification was increased in translucent aril by 740% and 25% higher coniferyl alcohol dehydrogenase (CAD) and peroxidase (POD) activity, respectively, induced by high H_2O_2. Healthy aril performed higher activities of superoxide dismutase (SOD) (8.5 times) and ascorbate peroxidase (APX) (1.3 times) to those in translucent aril. Furthermore, the higher flavonoid content, ascorbic acid content, and antioxidant capacities detected in normal aril could significantly reduce oxidative stress. Although containing high antioxidant systems, healthy aril was found to accumulate higher malonaldehyde content (MDA). This study provides intensive evidence of oxidative stress and the defensive systems between normal and translucent tissues.

1. Introduction

Mangosteen (*Garcinia mangostana*) belongs to the Guttiferae family. It is a unique tropical fruit, expressing prominent calyx on top of the fruit looking like a crown and so-called the 'Queen of fruit.' Mangosteen fruit consists of a thick pericarp derived from the floral ovary wall and 5–7 aril segments. The edible part (aril) is developed from the seed integument. The periodical cycle of fruit growth from flower opening until maturity takes about 11–12 weeks [1]. The fruit ripening starts by the pericarp color turning from pale green to purple followed by aril softening [2]. When mature green fruit starts ripening with the peel showing a red pad sign, mangosteen is classified as a climacteric fruit according to its respiratory pattern [3]. However, mangosteen fruit shows uneven ripening among aril segments in the fruit [4]. Furthermore, abnormal symptoms, including aril translucency and stiff texture of the aril, could be operated during ripening maturation. These disorders are severe obstacles in mangosteen marketability.

Translucent flesh disorder usually occurs during on-tree fruit ripening during the rainy season. The previous study showed that the number of fruits generating flesh translucency increased when the tree was applied by water sprinkling over the canopy for 2–3 h [5]. On the other hand, a high level of underground water did not stimulate the induction of translucent flesh disorder [6]. Moreover, an application of water supplied at the harvested fruit peduncle did not induce translucent flesh disorder in ripe fruit [3]. Since capillary water (lenticel-occupied water) in the

pericarp of on-tree mature green fruit induced a hypoxic condition, this incident enhanced translucent flesh disorder which found a remarkable accumulation of lignin in the ripe aril [7]. Furthermore, our previous study found a significantly high proportion of Na_2CO_3-SP between differential pectin fractions of translucent disorder aril [7]. A comparison of respiratory patterns indicates that translucent disorder aril conducted a higher respiration rate than healthy aril [8]. Typically, translucent disorder initially occurs in the largest aril segment, which showed high vigorous energies in both aril and seed than healthy arils [9]. As hypoxic cellular conditions are stimulated by alternative catabolic pathways to produce more energy for maintaining systematic survival, these stress phenomena could be encouraged by the overemission of reactive oxygen species (ROS) in several plant parts [10, 11]. The ROS directly attacks the cell membrane or is transformed into a harmful hydroxyl radical (OH), enhancing the lipid membrane's peroxidation. However, ROS can be subsequently reduced by some biological pathways, including cellular defensive mechanisms. Plants under stress drive their defensive resilience through the alternative antioxidant systems, both enzymatic and nonenzymatic shuttles. The enzymatic system includes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). On the other hand, phenolics, flavonoids, and ascorbates are involved in preventing stress. Some abiotic stresses inducing the defensive mechanisms in plants through ROS metabolisms have been recently reported [12–15]. Thus, the purposes of this research were to investigate ROS generations, phenolic accumulation, and lignification and to explore the relation of enzymes associated with ROS removal in ripe mangosteen aril between the healthy and translucent tissues.

2. Materials and Methods

2.1. Plant Materials. Fresh ripe mangosteen fruits at the peel purple stage were obtained from a commercial orchard in Chanthaburi Province, eastern Thailand, in July 2019, and were transported to the Applied Science Laboratory at King Mongkut's University of North Bangkok (KMUTNB) within a day. Fruits were then sorted by the uniformity of size (ca. 70–90 g/fruit), peel color, and absence of defects. For a preliminary classification of translucent flesh disorder, fruits were floated onto water containing 1.0% sodium chloride. The fruit that sank in the solution was presumed as having translucent flesh disorder, comprising higher gravity. After air-drying at the ambient room temperature (23–28°C), the fruit pericarp was carefully removed by a sharp knife without aril damage. In the present study, the fruit largest aril segment of normal white (Supplementary Figure 1(a)) and translucent disorder flesh (Supplementary Figure 1(b); pointing arrow) was collected from one hundred mangosteen fruits for investigating the ROS stress and lignification.

2.2. Firmness Measurement. Aril firmness was measured in the middle of the segment by using a TA-XT 21 texture analyzer (Stable Microsystems, UK) using a 2 mm spherical plunger with 5 mm distance depth and 1.0 $mm{\cdot}s^{-1}$ test speed. The maximum force was recorded in Newton unit.

2.3. Cross Section and Staining. A thin piece of free-hand cross section (40 microns) from normal and translucent disorder arils was stained with 0.1 M potassium phosphate buffer (pH 6.8) containing 0.05% (w/v) Toluidine Blue O-dye for 2 min and then washed with distilled water [16]. The stained tissues were observed under a light microscope (EMZ, Meiji Techno, Japan).

2.4. Lignin Determination. Lignin content in the flesh was investigated according to the method of Bruce and West [17]. Four g of aril flesh was homogenized in 16 mL of methanol by using a homogenizer (IKA Ultrarax T 25, Germany). Homogenate was filtered through Whatman GF/Ag filter No. 1. The remaining residual was washed twice by methanol and dried in an oven at 60°C for 24 h. Fifty mg of dried residual was mixed in 5 mL of 2 M hydrochloric acid and 0.5 mL of thioglycolic acid. The mixture was boiled at 100°C for 4 h. After cooling, the suspension was centrifuged at $12{,}000 \times g$ for 30 min. The pallet was rinsed with 5 mL of distilled water. The pallet was mixed in 5 mL of 0.5 M sodium hydroxide for lignin thioglycolate extraction and left for 18 h. The mixture was centrifuged at $12{,}000 \times g$ for 30 min. The supernatant (1 mL) was added with 1 mL of concentrated hydrochloric acid and incubated for 4 min. The reaction was centrifuged at $10{,}000 \times g$ for 10 min. The radish-brown residual was collected and dissolved in 25 mL of 0.5 M sodium hydroxide. The dissolved lignin solution's absorbance was measured by using a spectrophotometer (Shimadzu UV-1800, Japan) at 280 nm. The content was reported in Abs_{280} nm/50 mg DW.

2.5. ROS Generation and Its Transformation Determination. Superoxide anion ($O_2^{\cdot-}$) was extracted and measured according to Chaitanya and Naithani [18]. Two g of aril flesh was homogenized in 5 mL of 0.05 M potassium phosphate buffer (pH 7.8) including 1 mM diethyl-dithiocarbamate (a SOD inhibitor). The homogenate was centrifuged at $12{,}000 \times g$ at 4°C for 15 min. The supernatant (1 mL) was added with 3 mL of a reaction mixture containing 0.1 M phosphate (pH 7.8), 1 mM diethyl-dithiocarbamate, and 0.25 mM nitro blue tetrazolium (NBT). The absorbance of the reaction solution was spectrophotometrically measured at 540 nm within a min.

Hydrogen peroxide (H_2O_2), an $O_2^{\cdot-}$ transformation, was measured according to the work of Zouari et al. [19]. One g of aril was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at $12{,}000 \times g$ at 4°C for 15 min. The supernatant (1 mL) was then added to 2 mL of a reaction mixture containing 0.01 M potassium phosphate buffer (pH 7.0) and 1 M potassium iodide. The reaction absorbance was spectrophotometrically measured at 390 nm.

2.6. ROS Defensive Enzyme Assays. Extracts of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were performed according to

the methods of Dhindsa et al. [20] and Jiménez et al. [21]. For SOD, POD, and CAT, 1 g of aril flesh was homogenized in 10 mL of 0.05 M potassium phosphate buffer (pH 7) containing 1% (w/v) polyvinylpolypyrrolidone (PVPP). For APX extraction, 1 g of aril flesh was homogenized in 10 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 0.1% (v/v) Triton X-100. The homogenate was centrifuged at $12{,}000 \times g$ at 4°C for 15 min, and the supernatant was used for activity assay.

The extraction and assay of CAD were performed with the slightly modified Goffner et al. [22]. Briefly, 2 g of aril was homogenized in 10 mL of 100 mM phosphate buffer (pH 7.5) containing 1 mM EDTA, 5 mM magnesium chloride, 0.05% (v/v) Tween 20, and 2.5 mM 2-mercaptoethanol. The homogenate was high-speed centrifuged at $18{,}000 \times g$ at 4°C for 30 min. The supernatant was used for enzyme activity assay. One mL of the crude enzyme was incubated in 3 mL of 100 mM phosphate buffer (pH 7.5) containing 0.2 mM NADP and 0.1 mM coniferyl alcohol for 1 min. The activity of the enzyme was measured by using a spectrophotometer at 400 nm.

SOD activity assay was perfprmed according to the work of Dhindsa et al. [20]. The crude (100 μL) was mixed in 3 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 13 mM methionine, 75 mM NBT, 4 mM riboflavin, and 100 μM ethylene diamine tetra-acetic acid. The solution mixture was vortexed for 1 min and then left under fluorescent light (15 watts) for 30 min. The absorbance was spectrophotometrically measured at 560 nm. SOD activity was calculated from the reaction mixture's absorbency with the enzyme (sample) and without enzyme (control). One unit of SOD activity was represented by 50% NBT inhibition calculated from the following formula:

$$\mathrm{SOD}\left(\frac{\text{unit}}{g\mathrm{FW}}\right) = \frac{\left(\mathrm{Abs}_{\mathrm{Control}} - \mathrm{Abs}_{\mathrm{Sample}}/\mathrm{Asb}_{\mathrm{Control}} \times 100\right)}{2}. \quad (1)$$

POD and CAT activity assays were performed according to Song et al.'s method [23]. One hundred μL of the crude extract was mixed in 3 mL of 0.05 M potassium phosphate buffer (pH 7.0) containing 200 mM hydrogen peroxide and 20 mM guaiacol. The absorbance of POD was measured at 470 nm. One POD activity unit was represented by an increase in the absorbance (0.01) in 1 min. For CAT activity, 100 μL of the crude was mixed in 3 mL of 0.05 M potassium phosphate buffer (pH 7.0) containing 200 mM hydrogen peroxide. The absorbency was measured at 240 nm. One unit of CAT activity was represented by a decrease in the absorbance (0.01) for 1 min.

2.7. Malondialdehyde (MDA) Determination. Determination of MDA was performed according to Dipierro and Leonardis [24]. Two g of aril flesh was homogenized in 10 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at $12{,}000^\circ \times {}^\circ g$ for 10 min. The supernatant (1 mL) was reacted with 2 mL of 20% (v/v) TCA containing 0.5% (v/v) of thiobarbituric acid (TBA) and incubated in a hot bath at 100°C for 10 min and then cooled down on ice. The absorbances were spectrophotometrically measured at 532 and 600 nm, respectively. MDA content was calculated from the following formula with 155 $\mathrm{mM}^{-1}\,\mathrm{cm}^{-1}$ extinction coefficient value:

$$\mathrm{MDA}\left(\frac{\mu\mathrm{mol}}{g\mathrm{FW}}\right) = \left(\frac{\mathrm{Abs}_{532\mathrm{nm}} - \mathrm{Abs}_{600\mathrm{nm}}}{155\,\mathrm{mM}^{-1}\,1\mathrm{cm}^{-1} \times 1000}\right). \quad (2)$$

2.8. Phenolics Accumulation and Phenylalanine Ammonia Lyase (PAL) Activity Assay. Determination of aril phenolics was performed according to Recuenco et al.'s method [25]. One g of flesh was homogenized in 10 mL of methanol. After centrifugation at $10{,}000 \times g$ for 15 min, the supernatant (100 μL) was mixed in 3 mL of distilled water and 0.5 mL of Folin–Ciocalteu phenol reagent for 3 min. The mixture was added with 2 mL of 20% (w/v) of sodium carbonate. The absorbance of the reaction was recorded at 750 nm. Phenolic acid content was compared to the absorbance of gallic acid standard. For flavonoid determination, 2 mL of supernatant was mixed in 5 mL of distilled water and 0.15 mL of 5% (w/v) sodium nitrate for 5 min, and then, 0.15 mL of 10% (w/v) aluminium chloride was added. The reaction absorbance was measured at 510 nm. Flavonoid content was compared with catechin standard.

PAL assay was performed according to the work of Camm and Towers [26]. Five g of flesh was homogenized in 60 mL of cold acetone and filtered through Whatman filter paper No. 2. The remaining brown residual was rewashed with cold acetone until the residual color turned white. The residual was blended in 50 mL of cold 95% ethanol and filtered through Whatman filter paper No. 2. The residual was dried at room temperature (25°C) and then kept in a desiccant chamber. For PAL, 0.5 mg of the acetone powder was mildly stirred in 50 mL of 0.1 M sodium borate buffer (pH 8.8) at 4°C for 30 min. After centrifugation at $12{,}000 \times g$ at 4°C for 20 min, the supernatant (1 mL) was mixed in 1.5 mL of 0.1 M sodium borate buffer (pH 8.8) and 1 mL of 10 mg/mL phenylalanine and incubated in a hot bath at 30°C for 1 h. Five N HCl (0.5 mL) was added into the reaction to stop the enzyme activity. The reaction absorbance with and without phenylalanine was measured at 290 nm. One unit of PAL activity was represented by an increase in 1 μmol cinnamic acid per 1 h.

2.9. Ascorbic Acid Determination. An ascorbic acid determination was performed, according to Klein and Perry's method [27]. One g of aril flesh was homogenized in 10 mL of 5% (w/v) metaphosphoric acid and centrifuged at $12{,}000 \times g$ for 10 min. The supernatant (0.5 mL) was mixed in 4.5 mL of 0.1 mM 2, 6-DCIP. The absorbance of ascorbic acid in the mixture was then spectrophotometrically measured at 515 nm and compared to the ascorbic acid standard curve.

2.10. Antioxidant Ability Assays

2.10.1. Reducing power Assay. One g of aril flesh was homogenized in 9 mL of methanol with a homogenizer. The homogenate was made to stand at ambient temperature

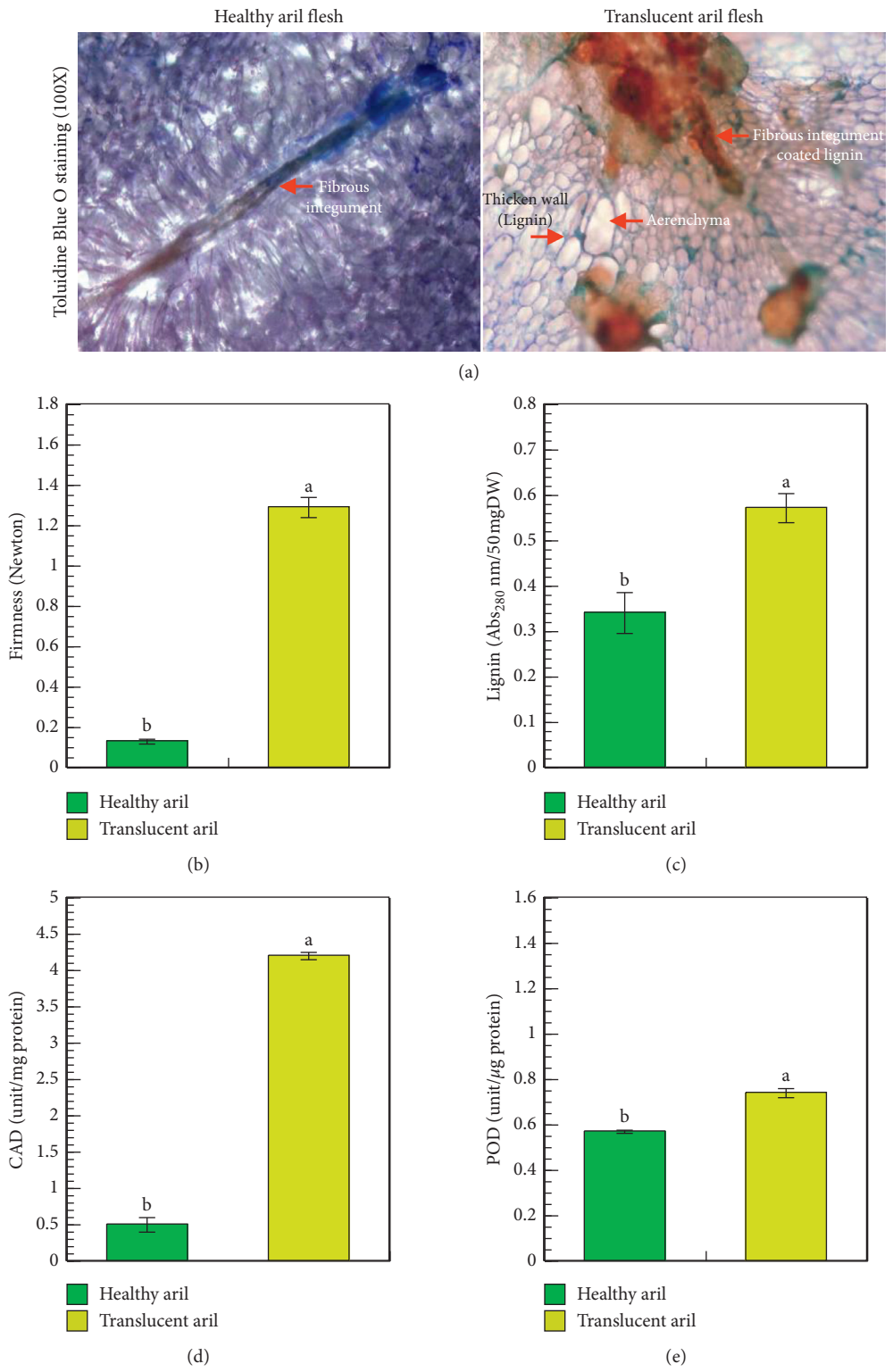

Figure 1: Toluidine Blue O-stained tissues (a), firmness (b), lignin content (c), coniferyl alcohol dehydrogenase activity (d), and peroxidase activity (e) in healthy and translucent arils. Means ($n = 4$) with different lower-case letters on the bars are significantly different.

(24–28°C) for 3 h and then suctioned through Whatman filter paper No. 1. The methanolic supernatant (5 mL) was mixed with 1.25 mL of 0.2 M potassium phosphate buffer (pH 6.6) and 1.25 mL of 1.0% potassium ferricyanide (w/v). The mixture was then incubated in a hot water bath at 50°C for 20 min. The mixture was cooled down on ice before adding 1.25 mL of 10% (w/v) trichloroacetic acid. The cleared zone fraction (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride and incubated at ambient temperature for 10 min. The absorbance of the solution mixture was spectrophotometrically measured at 700 nm [24].

2.10.2. DPPH Scavenging Inhibition Assay. DPPH scavenging ability was measured, according to Chang et al. [28]. One g of aril flesh was homogenized in 10 mL of methanol and centrifuged at $10{,}000 \times g$ for 15 min. The supernatant (0.1 mL) was added into 2.9 mL of 1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) and kept in the dark condition for 30 min. Then, mixture absorbance was monitored at 517 nm. The reaction without the methanol-extraction solution was demonstrated as a control. The percentage of scavenging inhibition was calculated from the following formula:

$$\text{DPPH inhibition (\%)} = \frac{\left(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}\right)}{\text{Abs}_{\text{Control}}} \times 100. \quad (3)$$

2.11. Protein Determination. Protein content in the enzyme reaction was measured according to Bradford's method [29]. The crude at 0.5 mL was mixed in a protein reagent composed of 100 mg of Coomassie Brilliant Blue G-250 (CBB) in 50 mL of ethanol and 100 mL of 85% (w/v) phosphoric acid. The absorbance was recorded at 595 nm and then compared to Bovine Serum Albumin (BSA) standard.

2.12. Statistical Analysis. Independent Sample t-test analysis was performed to compare the variation of the parameter mean values between both treatments (4 replicates each; 4 fruit/replicates) at $p < 0.05$ using an SPSS software version 26 (IBM, Chicago, IL, USA).

3. Results and Discussion

3.1. Lignification and Firmness. Translucent aril, behaving as a stiffening structure, exhibits firm-crispy texture and a translucent tissue character. The microscopic images of Toluidine Blue O-stained aril structure show that lignin was localized in the parenchyma cell wall and high in the protruding fibrous-chain of the seed coat of translucent flesh disorder (Figure 1(a)). Moreover, we found that some parenchyma cells in translucent tissues were either transformed into collenchyma cells or collapsed to form aerenchyma. The firmness of healthy aril was only 0.21 Newtons, which was 10 times lower than that of translucent aril (Figure 1(b)). This evidence was related to the higher lignin content detected in the translucent tissues, double the amount compared to healthy arils (Figure 1(c)). The translucent aril conducted high lignification, which was increased by the activity of two key enzymes in lignin biosynthesis, namely, CAD and POD. When the former increased by 740% (Figure 1(d)), the latter was 25% up (Figure 1(e)) in the translucent aril. From these two enzymes directly involved in the accumulation of lignins, CAD could be the rate-limiting step of lignification in mangosteen aril under the stress conditions. Translucent flesh disorder is usually generated in mangosteen fruit during fruit ripening developed on tree in the rainy season. Water covering the fruit could induce hypoxic and oxidative stress in the fruit [5]. Lignification induction was found in wheat under waterlogging conditions related to increasing CAD and POD activities [10]. The modification of lignins in the cell wall, simultaneously induced by abiotic stress, could modify the cell-wall matrix and the properties. Schopfer [30] reported that cell-wall stiffening of maize coleoptile was related to an intercellular coupling of monolignol residues by POD, a lignin bound-membrane enzyme, using H_2O_2 as an electron acceptor. As a result, changes in a high proportion of Na_2CO_3-SP in the pectin [7] and the high lignification could transform the white soft aril tissues into translucent crispy tissues under stress conditions.

3.2. ROS Generation and Its Transformation. Oxidative intermediates were investigated in both healthy and translucent arils of mangosteen fruit. Superoxide anion ($O_2^{\cdot-}$) was dramatically higher in translucent aril, which was 8.5-fold compared to a healthy one (Figure 2(a)). This harmful ROS was suddenly dismutated to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). When H_2O_2 content was 125% higher accumulated in translucent aril (Figure 2(b)), SOD activities were, however, equal in both healthy and translucent arils (Figure 2(c)). Levels of lipid peroxidation could imply oxidative stress and damage. Interestingly, malonaldehyde (MDA), a membrane-damaged end-product by lipid peroxidation, was slightly higher in healthy aril (Figure 2(d)). MDA is widely used as a marker of oxidative lipid injury in plant tissues under biotic and abiotic stress. However, this reaction in plant tissues could be potentially interfered by many biological compounds which vary according to the tissue types and stress conditions [31]. For oxidative stress in mangosteen fruit, H_2O_2 in healthy aril could be transformed into hydroxyl radical ($OH^{\cdot}$) via Fenton reaction in the presence of Fe^{2+} than translucent aril. Alternatively, H_2O_2 was contributed to be a cosubstrate of POD to produce lignins in translucent aril, which is higher than that in healthy aril. Oxidative intermediates such as $O_2^{\cdot-}$ and H_2O_2 were highly induced in okra pods [12] and asparagus shoots [32] during storage under abiotic stress. Furthermore, Jia et al. [11] found that cherry rootstock released a high ROS amount under waterlogging conditions. For survival, the harmful radical was discriminated by a scavenging mechanism using several enzymes and cosubstrates.

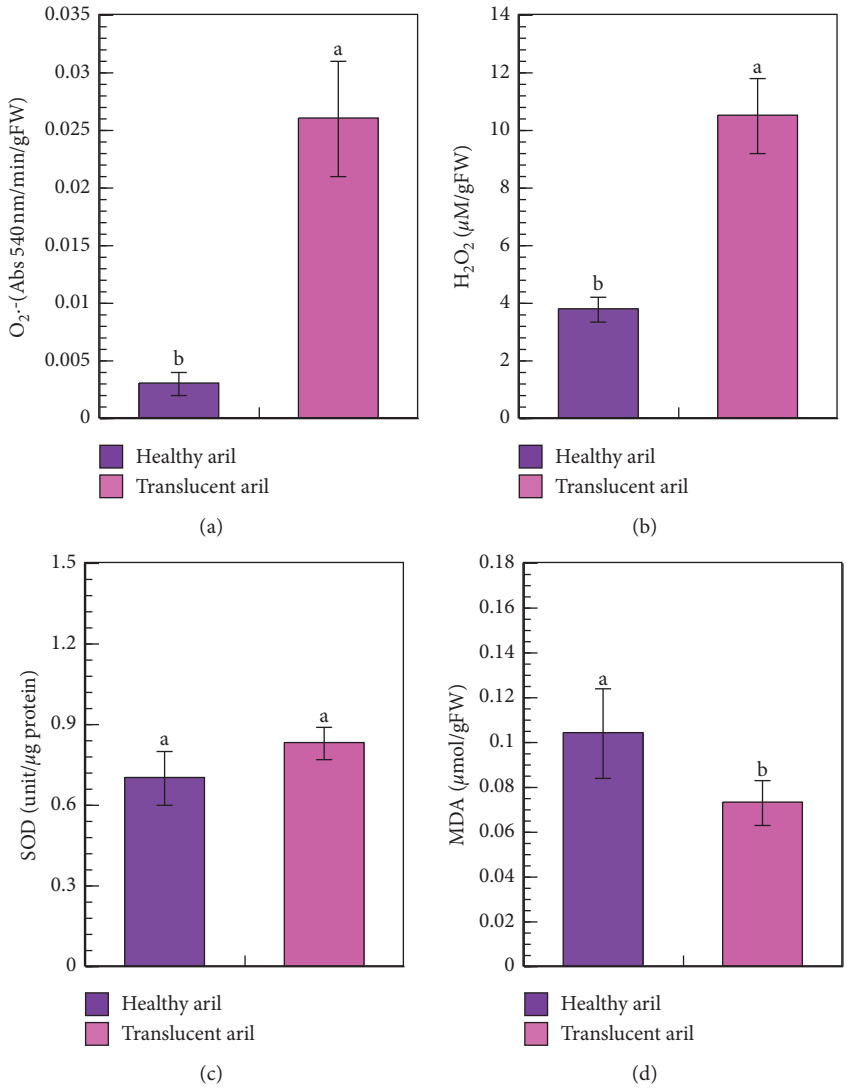

Figure 2: $O_2^{\cdot-}$ (a), H_2O_2 content (b), superoxide dismutase activity (c), and malonaldehyde content (d) in healthy and translucent arils. Means ($n = 4$) with different lower-case letters on the bars are significantly different.

3.3. The Differential ROS Defensive Mechanisms. The phenolics content was 54 mg GAE/g FW in translucent aril and 1.7 times higher than in healthy aril (Figure 3(a)). Since PAL activity was significantly 100% higher in translucent aril (Figure 3(b)), the phenolics were contributed to form lignin along with the lignified enzyme series. Phenolic biosynthesis could be stimulated by some abiotic stresses such as hypoxia and physical damage to produce phenylpropanoid intermediates as defensive mechanisms of plant cells [32]. Translucent flesh disorder is developed during on-tree fruit ripening in rainy season or when a fruit was covered with water for several hours, which could induce hypoxic conditions in the fruit [5]. Furthermore, the level of transformed H_2O_2 can alternatively be reduced by other plant defensive reactions. Although CAT activity, catalyzing H_2O_2 into water in peroxisome, was almost equal in both healthy and translucent arils (Figure 3(c)), APX activity in healthy aril was significantly higher than that in translucent aril (Figure 3(d)). Thus, APX could play a key role in ROS defense in the healthy mangosteen aril by utilizing ascorbic acid and H_2O_2 into water via the ascorbate-glutathione cycle [33]. When the lower content of ascorbate by 24% was detected in translucent aril (Figure 4(a)), the higher range of flavonoids by 35% was measured in healthy aril (Figure 4(b)). Flavonoids could be an effective antioxidant supplement to reduce the activity of ROS. The supportive evidence was shown by approximately 100% higher of the high reducing power ability (Figure 4(c)) and potent inhibition of DPPH scavenging in healthy aril (Figure 4(d)). Abiotic stresses inducing antioxidant systems' defensive mechanisms through redox-reaction via ROS metabolism were reported in some field crops [14, 15] and horticultural crops [12, 34].

We propose the comparative routes of ROS generation and the defensive mechanism, taking place in the healthy and translucent disorder of ripe mangosteen arils in Figure 5. Translucent aril is an abnormal symptom occurring during fruit ripening on the tree. The initial

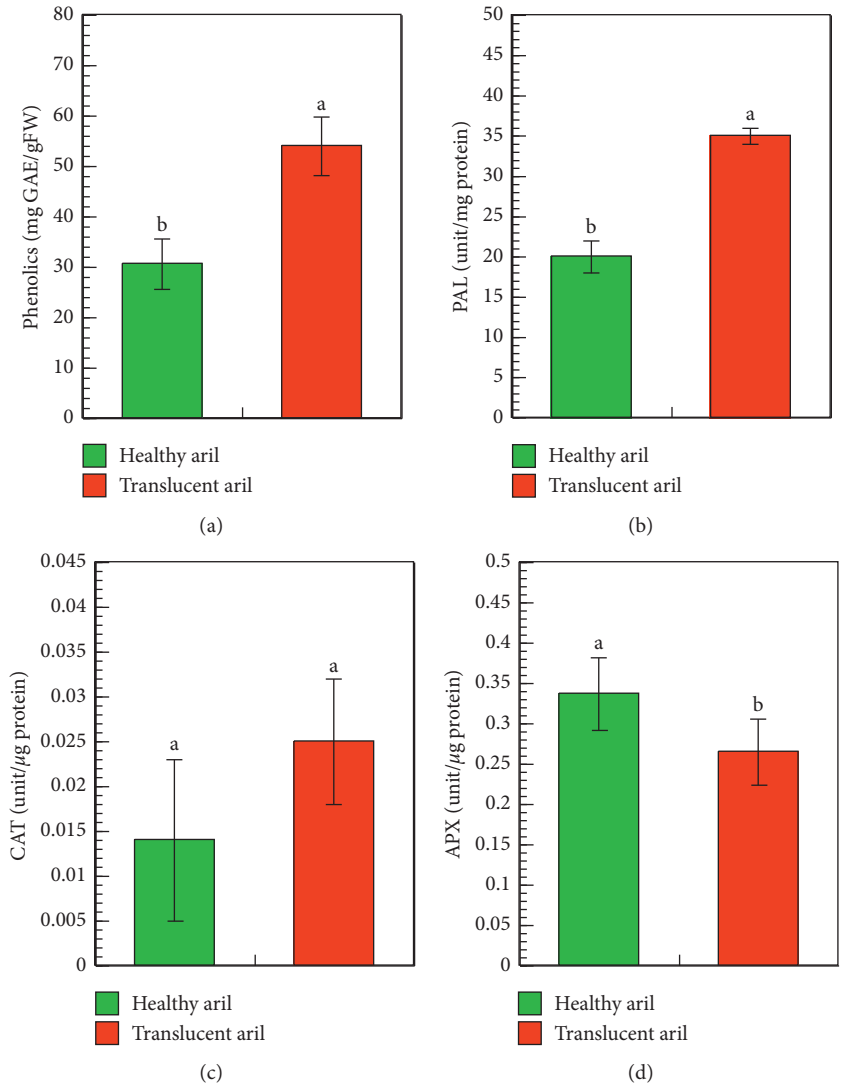

Figure 3: Phenolic content (a), phenylalanine ammonia lyase activity (b), catalase (c), and ascorbate peroxidase (d) in healthy and translucent arils. Means ($n = 4$) with different lower-case letters on the bars are significantly different.

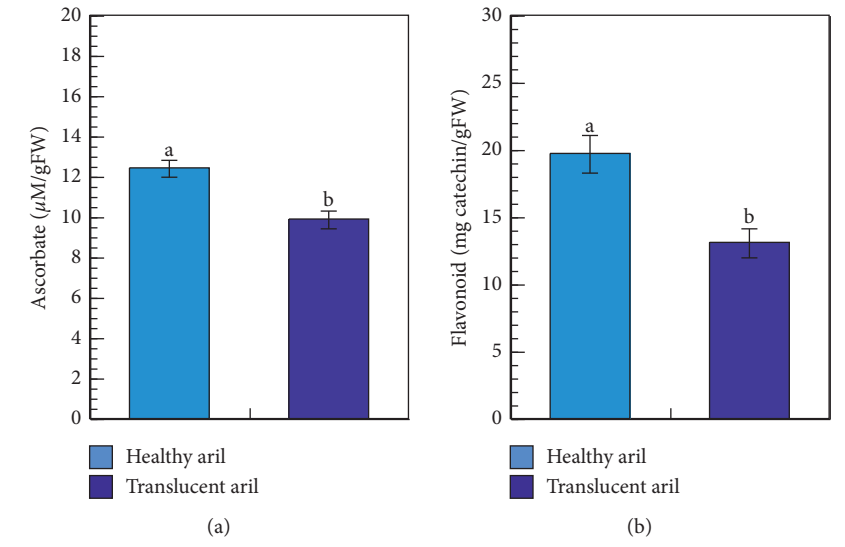

Figure 4: Continued.

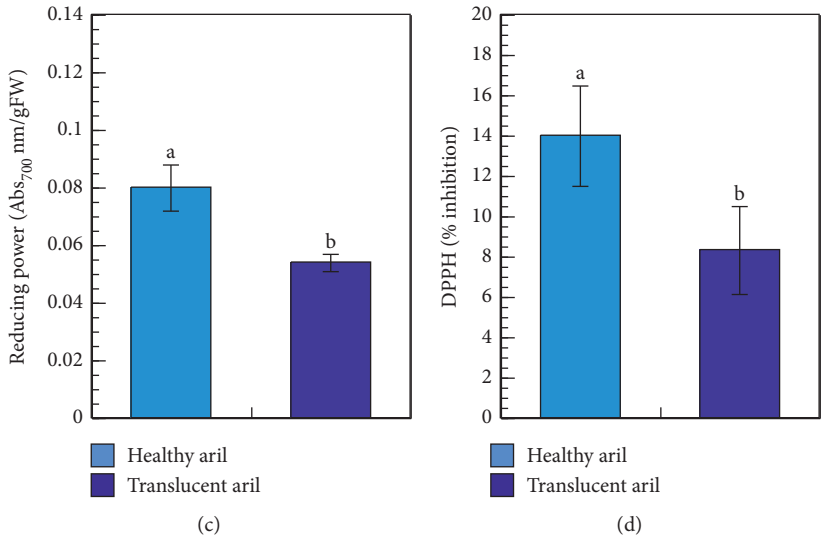

Figure 4: Ascorbic acid (a), flavonoid content (b), reducing power activity (c), and DPPH inhibition (d) in healthy and translucent arils. Means ($n = 4$) with different lower-case letters on the bars are significantly different.

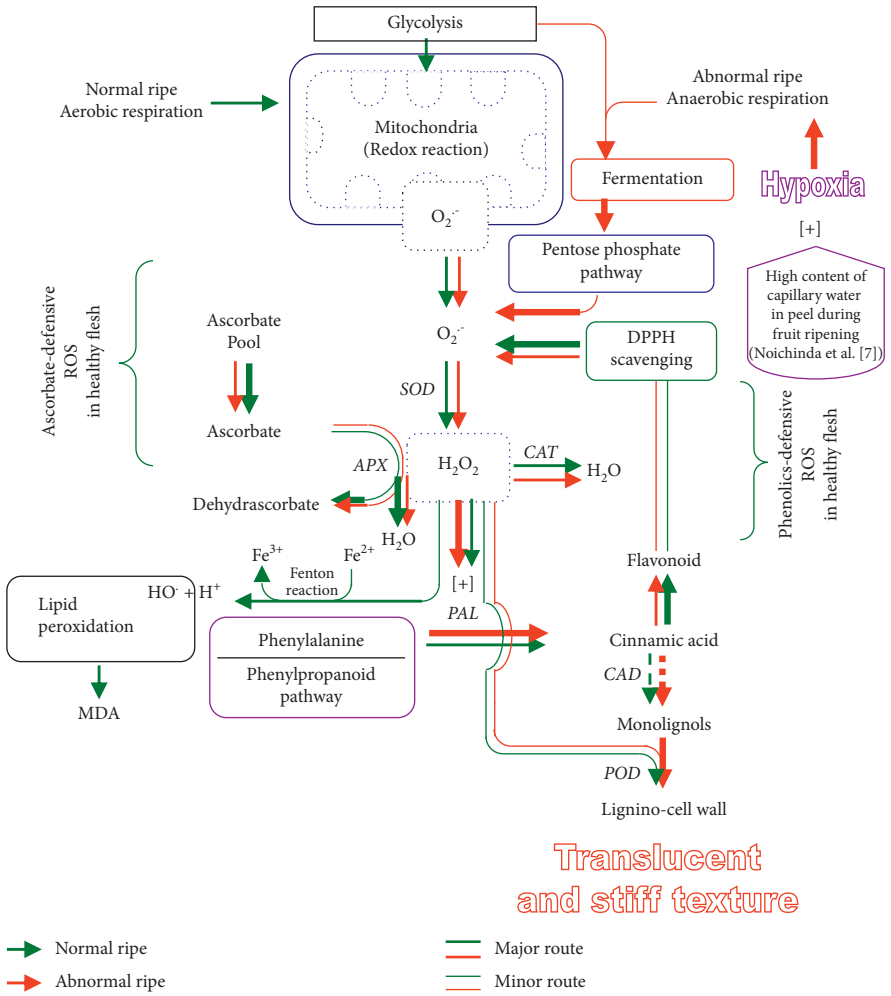

Figure 5: Putative pathways of lignin formation in translucent mangosteen aril related to an increase in phenolics accumulation and the failure of ROS defensive mechanism.

mechanism is induced under hypoxia by water by applying either rainfalls or artificial water supply over the fruits [7]. Under a hypoxic condition in fruit by full capillary water in the mangosteen pericarp, the living cells of aril lack metabolite-driving energy and so called 'an energy crisis' stress [35]. As a result of survival, cells must produce alternative energy via the fermentation route instead of the typical oxidative phosphorylation. Alternatively, glucose could be dehydrogenated to form ribose via the pentose phosphate pathway. This phenomenal stress is dramatically released of ROS content through the redox-reaction process. Since ROS could further react to lipid peroxidation in the cell membrane, plant cells comprise their defensive mechanism to defend the ROS. SOD can catalyze $O_2^{\cdot-}$ into H_2O_2 (a nonactive ROS form), and H_2O_2 is then detoxified into water by CAT. However, due to the low activity of CAT in both healthy and translucent arils instead, APX was important to oxidize H_2O_2 and ascorbate into the water in healthy aril, but not in translucent aril. Consequently, a high concentration of remaining H_2O_2 could induce the PAL activity to produce cinnamic acid via a translucent aril's phenylpropanoid pathway. This phenolic (cinnamic acid) was derived into two substances. The first was to produce flavonoids, which were potent inhibitors of DPPH in healthy aril. Secondly, cinnamate is dehydrogenated into monolignol by CAD, and the phenolic is then bound to the cell wall by POD. This evidence plays an outstanding role in defense ROS in translucent aril by increasing the cell wall's lignin. In normal aril, high activities of CAT and APX and high content of flavonoids and ascorbate could effectively reduce H_2O_2 in the cells. However, the remaining H_2O_2 in healthy aril could be further reacted to a vigorous lipid peroxidation form such as $OH^{\cdot}$ when presenting of Fe^{2+} via Fenton reaction that damaged membrane lipid as indicated by MDA concentration.

4. Conclusions

Translucent aril of mangosteen contained high oxidative supplements, whereas normal tissues comprised the better defensive mechanism. Normal aril conducted not only high activities of antioxidant enzymes of superoxide dismutase and ascorbate peroxidase but also high contents of antioxidants of flavonoids and ascorbic acid. Translucent aril reduced a high amount of H_2O_2 by inducing lignification of the cell wall through cinnamyl alcohol dehydrogenase and peroxidase, resulting in higher firmness with stiffening structure and crispy texture.

Data Availability

The supplementary figure data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Supplementary Materials

Supplementary Figure 1. Mangosteen fruit with healthy aril (a) and translucent aril (arrow point) (b). (*Supplementary Materials*)

References

[1] K. Wanichkul and S. Kosiyachinda, "Fruit development of mangosteen (*Garcinia mangostana* Linn.)," *Agricultural Science Journal*, vol. 13, pp. 63–72, 1979.

[2] S. Noichinda, K. Bodhipadma, S. Singkhornart, and S. Ketsa, "Changes in pectic substances and cell wall hydrolase enzymes of mangosteen (*Garcinia mangostana*) fruit during storage," *New Zealand Journal of Crop and Horticultural Science*, vol. 35, no. 2, pp. 229–233, 2007.

[3] S. Noichinda, Effect of Modified Atmosphere Condition on Quality and Storage Life Mangosteen (*Garcinia mangostana* L.) Fruit, MS Thesis, Department of Horticulture, Kasetsart University, Bangkok, Thailand, 1992, in Thai.

[4] C. Wongs-Aree and S. Noichinda, "Postharvest physiology and quality maintenance of tropical fruits," in *Postharvest Handling A Systems Approach*, W. J. Florkowski, R. L. Shewfelt, B. Brueckner, and S. E. Prussia, Eds., Academic Press is an imprint of Elsevier, Cambridge, MA, USA, 3rd edition, 2014.

[5] S. Noichinda, K. Bodhipadma, and C. Wongs-Aree, "Mangosteen," in *Postharvest Physiological Disorders in Fruits and Vegetables*, S. Tonetto de Freitas and S. Pareek, Eds., CRC Press, Boca Raton, FL, USA, 2019.

[6] P. Chuennakorn, P. Paiboon, and S. Yingjajaval, *Rate of Water Flow and the Occurrence of Gamboges and Translucent Flesh Disorders in Mangosteen Fruit*, Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Nakhon, Thailand, in Thai, 2011.

[7] S. Noichinda, K. Bodhipadma, and S. Kong, "Capillary water in pericarp enhances hypoxic condition during on-tree fruit maturation that induces lignification and triggers translucent flesh disorder in Mangosteen (*Garcinia mangostana* L.)," *Journal of Food Quality*, vol. 2017, Article ID 7428959, 7 pages, 2017.

[8] V. Luckanatinvong, The study on chemical composition, cell viability and influence of water on flesh translucent disorder in mangosteen (*Garcinia mangostana* L.), MS Thesis, Department of Horticulture, Kasetsart University, Bangkok, Thailand, 1996, in Thai.

[9] J. Siritikul, S. Noichinda, K. Bodhippadma, and C. Wongs-Aree, "Aril and seed viabilities related to the developmental stage of translucent pulp in mangosteen," *Agricultural Science Journal*, vol. 45, no. 2, pp. 561–564, 2014.

[10] T. Nguyen, S. Son, M. C. Jordan, D. B. Levin, and B. T. Ayele, "Lignin biosynthesis in wheat (*Triticum aestivum* L.): its response to waterlogging and association with hormonal levels," *MBC Plant Biology*, vol. 16, no. 28, 2016.

[11] L. Jia, X. Qin, D. Lyu, S. Qin, and P. Zhang, "ROS production and scavenging in three cherry rootstocks under short-term waterlogging conditions," *Scientia Horticulturae*, vol. 257, Article ID 108647, 2019.

[12] S. Phornvillay, N. Prongprasert, C. Wongs-Aree, A. Uthairatanakij, and V. Srilaong, "Physio-biochemical responses of okra (*Abelmoschus esculentus*) to oxidative stress under low temperature storage," *The Horticulture Journal*, vol. 89, no. 1, pp. 69–77, 2020.

[13] M. H. Siddiqui, S. Alamri, M. Nasir Khan et al., "Melatonin and calcium function synergistically to promote the resilience through ROS metabolism under arsenic-induced stress," *Journal of Hazardous Materials*, vol. 398, Article ID 122882, 2020.

[14] A. A. Alsahli, J. A. Bhat, M. N. Alyemeni, M. Ashraf, and P. Ahmad, "Hydrogen sulfide (H2S) mitigates arsenic (As)-Induced toxicity in pea (*Pisum sativum L.*) plants by regulating osmoregulation, antioxidant defense system, ascorbate glutathione cycle and glyoxalase system," *Journal of Plant Growth Regulation*, vol. 166, 2020.

[15] C. Kaya, M. Ashraf, M. N. Alyemeni, F. J. Corpas, and P. Ahmad, "Salicylic acid-induced nitric oxide enhances arsenic toxicity tolerance in maize plants by upregulating the ascorbate-glutathione cycle and glyoxalase system," *Journal of Hazardous Materials*, vol. 399, Article ID 123020, 2020.

[16] T. P. O'Brien, N. Feder, and M. E. McCully, "Polychromatic staining of plant cell walls by Toluidine-blue O," *Phytoplasma*, vol. 59, pp. 367–373, 1964.

[17] R. J. Bruce and C. A. West, "Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of Castor bean," *Plant Physiology*, vol. 91, no. 3, pp. 889–897, 1989.

[18] K. S. K. Chaitanya and S. C. Naithani, "Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of *Shorea robusta* Gaertn.f." *New Phytologist*, vol. 126, no. 4, pp. 623–627, 1994.

[19] M. Zouari, C. Ben Ahmed, W. Zorrig et al., "Exogenous proline mediates alleviation of cadmium stress by promoting photosynthetic activity, water status and antioxidative enzymes activities of young date palm (*Phoenix dactylifera* L.)," *Ecotoxicology and Environmental Safety*, vol. 128, pp. 100–108, 2016.

[20] R. S. Dhindsa, P. Plumb-Dhindsa, and T. A. Thorpe, "Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase," *Journal of Experimental Botany*, vol. 32, no. 1, pp. 93–101, 1981.

[21] A. Jiménez, J. A. Hernández, L. A. del Rio, and F. Sevilla, "Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of Pea leaves," *Plant Physiology*, vol. 114, no. 1, pp. 275–284, 1997.

[22] D. Goffner, I. Joffroy, J. Grima-Pettenati et al., "Purification and characterization of isoforms of cinnamyl alcohol dehydrogenase fromEucalyptus xylem," *Planta*, vol. 188, no. 1, pp. 48–53, 1992.

[23] H. Song, X. Gao, B. Feng et al., "Leaf senescence and physiological characters in different Adzuki bean (*Vigna angularis*) cultivars (lines)," *African Journal of Agricultural Research*, vol. 8, pp. 4025–4032, 2013.

[24] S. Dipierro and S. De Leonardis, "The ascorbate system and lipid peroxidation in stored potato (*Solanum tuberosum* L.) tubers," *Journal of Experimental Botany*, vol. 48, no. 3, pp. 779–783, 1997.

[25] M. C. Recuenco, M. S. Lacsamana, W. A. Hurtada, and V. C. Sabularse, "Total phenolic and total flavonoid contents of selected fruits in the Philippines," *Philippine Journal of Science*, vol. 145, pp. 275–281, 2016.

[26] E. L. Camm and G. H. N. Towers, "Phenylalanine ammonia lyase," *Phytochemistry*, vol. 12, no. 5, pp. 961–973, 1973.

[27] B. P. Klein and A. K. Perry, "Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States," *Journal of Food Science*, vol. 47, no. 3, pp. 941–945, 1982.

[28] C.-H. Chang, H.-Y. Lin, C.-Y. Chang, and Y.-C. Liu, "Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes," *Journal of Food Engineering*, vol. 77, no. 3, pp. 478–485, 2006.

[29] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[30] P. Schopfer, "Hydrogen peroxide-mediated cell-wall stiffening in vitro in maize coleoptiles," *Planta*, vol. 199, pp. 43–49, 1996.

[31] M. W. Davey, E. Stals, B. Panis, J. Keulemans, and R. L. Swennen, "High-throughput determination of malondialdehyde in plant tissues," *Analytical Biochemistry*, vol. 347, no. 2, pp. 201–207, 2005.

[32] C. Wongs-Aree and S. Noichinda, "Glycolysis fermentative by-products and secondary metabolites involved in plant adaptation under hypoxia during pre- and postharvest," in *Hypoxia and Anoxia*, K. Das and M. S. Biradar, Eds., IntechOpen, London, UK, 2018.

[33] T. Ushimaru, Y. Maki, S. Sano, K. Koshiba, K. Asada, and H. Tsuji, "Induction of enzymes involved in the ascorbate-dependent antioxidative system, namely, ascorbate peroxidase, monodehydroascorbate reductase and dehydroascorbate reductase, after exposure to air of rice (*Oryza sativa*) seedlings germinated under water," *Plant and Cell Physiology*, vol. 38, no. 5, pp. 541–549, 1997.

[34] W. W. Lwin, V. Srilaong, P. Boonyaritthongchai, C. Wongs-Aree, and N. Pongprasert, "Electrostatic atomized water particles reduces postharvest lignification and maintain asparagus quality," *Scientia Horticulturae*, vol. 271, Article ID 10948, 2020.

[35] T. Fukao and J. Bailey-Serres, "Plant responses to hypoxia - is survival a balancing act?" *Trends in Plant Science*, vol. 9, no. 9, pp. 449–456, 2004.

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Research Article

Polyphenol Content and Antioxidant Activity of Stevia and Peppermint as a Result of Organic and Conventional Fertilization

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Stevia rebaudiana Bertoni and *Mentha piperita* are plants that generate interest mainly due to the presence of bioactive compounds in their leaves, such as phenolics. Studies indicate that phenolics have pharmacological and therapeutic properties, including antioxidant activity. Phenolic compounds may be affected by the type of fertilization. For this reason, organic and chemical fertilization were evaluated along with antioxidant activity. Results showed significant differences for total phenols in organic peppermint (62% higher content). Also, DPPH test displayed differences for peppermint and stevia (572% and 16% greater in organic). Organic fertilization may be alternative for producing high added agricultural and commercial products.

1. Introduction

Dietetics products and natural food ingredient demand has increased over the last years. A lot of interest has emerged on sources of natural antioxidant since many health problems are associated with the action of toxic forms of oxygen responsible for oxidation processes. Antioxidants are capable of inhibiting reactive oxygen species (ROS) [1]. Phenolic compounds may represent approximately 19–23% of dry peppermint leave weight [2]. It has been reported that phenolics show beneficial health effects due to free radical scavenging properties. Mexican population consumes infusions in a regular manner, and one of the most popular is prepared from peppermint (*Mentha piperita*). Peppermint leaves contain a wide array of bioactive components (fatty acids, volatile compounds, carotenoids, and phenolic compounds). At the same time, an interest in natural sweeteners has emerged due to the increasing health consciousness and concern related to sugar consumption and the problems related to the safety of some artificial non-nutritive sweeteners [3]. Stevia is an herb of Asteraceae family, which grows wild in South America, such as Paraguay and Brazil. Leaves are the economic part of the plant with a high concentration of steviol glycosides [4]. *Stevia rebaudiana* Bertoni has been used as a natural sweeter; it is categorized by high concentration of steviol glycosides in its leaves, which are up to 200 to 400 times sweeter than sucrose. In addition to their sweeteners, stevia plants possess other compounds such as terpenes, sterols, volatile acids, vitamins, carotenes, organic acids, polysaccharides, hormones, microelements, and phenolic compounds (tannins and flavonoids) [5].

Particularly important for the antioxidant capacity in stevia and peppermint are phenolic compounds, which are

secondary metabolites. The promotion of product quality regarding secondary metabolites content may be of high relevance for a commercial expansion of stevia and peppermint. Phenolic compounds are involved in various plant processes such as growth and reproduction and are also synthesized as a defense mechanism to various stresses; therefore, their production can be enhanced by different conditions, among them, type of fertilization. Nowadays, consumers are more concerned of possible exposure to agrochemicals. Organic agricultural practices do not allow the use of chemical compounds for crop nutrition, synthetic compounds for pest, disease, and weed control. Many have suggested that the use of organic fertilizers is an essential source of nutrients for sustainable agriculture and in addition to cover the physiological requirements of crops, favoring the development of high-quality crops [6]. Due to a plethora of implications, among them, health benefits and the use of environmental friendly agriculture practices [7], the aim of this study was investigating the effect of organic and chemical (conventional) fertilization on the content of bioactive compounds in *S. rebaudiana* and *M. piperita*. The work is focused on the analysis of antioxidant capacity, phenolic compounds, and steviosides levels in those materials, to assess differences between both types of fertilization.

2. Materials and Methods

2.1. Plant Material. Conventional peppermint and stevia plants were obtained from a local supplier. The plants belonged to the same batch. Organic stevia plants were got from a commercial greenhouse with organic care located at San José Iturbide, Guanajuato, México. Organic peppermint was grown at the Universidad Autónoma de Querétaro, Amazcala campus. Leaves were collected and dried at 45°C for 24 h (Fisher Scientific, 650D, USA). Next, they were milled in a grinder (Krups GX4100, México).

2.2. Extract Preparation. Extraction for phenolics and antioxidant determinations were performed by placing 1 g (PRACTUM 224-1S; Sartorius, Göttingen, Germany) of fresh sample in a 50 mL tube and mixed with 10 ml of methanol. The tubes were protected from light and shaken at 200 rpm (Orbit 1000 model S2030-1000; Labnet, Woodbridge, NJ, USA) for 24 h at 25°C. After incubation, the samples were centrifuged (Sorvall Biofuge Primo *R* model 75005448; Thermo Scientific, Osterode, Germany) at $6,793 \times g$ for 10 min. Aliquots of the supernatant were taken for the assays. We followed the methods of Garcia-Mier, Jimenez-Garcia [8].

2.3. Quantification of Condensed Tannins. Condensed tannins expressed as milligrams of (+)-catechin equivalents per gram of dry sample were quantified according to the next procedure proposed by Garcia-Mier, Jimenez-Garcia [8]. Briefly, 200 μl of vanillin reagent (1% vanillin, 8% HCl in methanol) was added to 50 μl of methanolic extract and placed in a 96-well plate; each sample was tested in triplicate. Condensed tannins were quantified at 492 nm in a microplate reader (Multiskan Go model 51119300; Thermo Scientific, Vantaa, Finland) using (+)-catechin (up to 0.1 mg·mL^{-1}) as a reference standard. A blank sample was prepared by subjecting the original extract to the same conditions of reaction without the vanillin reagent.

2.4. Quantification of Flavonoids. Briefly, the method for the determination of flavonoids content was performed according to Garcia-Mier, Jimenez-Garcia [8]. It consisted of mixing 50 μl of the methanolic extract with 180 μl of distilled water and 20 μl of a solution 2-aminoethyldiphenylborate 1% in a 96-well plate. The absorbance of the solution was monitored at 404 nm with a microplate reader (Multiskan Go model 51119300; Thermo Scientific, Vantaa, Finland). A rutin standard was prepared in methanol. Extract absorption was compared with that of a rutin standard curve (up to 2 μg·ml^{-1}). Flavonoid content was expressed as mg of rutin equivalent per gram of dry sample.

2.5. Quantification of Total Phenols. Total phenols (expressed as mg of gallic acid equivalent per gram of dry sample) were determined by the Folin-Ciocalteu method with modifications. To 40 μL of extract were added 460 μL of distilled water, 250 μL of Folin Ciocalteau reagent, and 1250 μL of 20% sodium carbonate solution. After 2 hours in the dark, samples were read at 750 nm in a UV/Vis spectrophotometer (Genesys 10S UV-Vis, Themo Fischer Scientific, USA). Gallic acid was used for the calibration curve [9].

2.6. Antioxidant Activity by 1,1-Diphenyl-2-picrylhydrazyl Radical (DPPH) Inhibition Assay. Radical scavenging activity (RSA) was determined using stable radical DPPH method. The assay was performed following the next procedure. All reactions were conducted in 96-well microplates. Aliquot (20 μL) of methanolic extracts was mixed with 100 μM of DPPH (200 μl) in methanol. It was used a control and a blank. After 30-minute incubation at ambient temperature in darkness, absorbance was recorded at 515 nm in a microplate reader (Multiskan Go model 51119300; Thermo Scientific, Vantaa, Finland). It was prepared as a calibration curve with Trolox. The antioxidant activity was expressed as percent of inhibition [8].

2.7. Antioxidant Activity by 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic Acid) (ABTS) Inhibition Assay. The radical cation was prepared by mixing 7 mM ABTS stock solution with 140 mM potassium persulfate (1/1, v/v) and leaving the mixture for 12 h until reaction was completed and the absorbance was stable. The $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 nm at 730 nm for measurement. The photometric assay was conducted on 0.9 mL of ABTS solution and 0.1 mL of extract and mixed for 45 seconds, and measurements were taken at 730 nm after 15 minutes. The antioxidant activity of the sample was calculated by determining the decrease in absorbance. Trolox was used as standard substance. This assay was based on the ability of different substances to scavenge

radicals. The antioxidant activity was expressed as percent of inhibition [10].

2.8. HPLC Analysis. For extraction, purification, and quantification of stevia extracts, the methodology used was proposed by Mondal, Majumdar [11]. It consisted of *S. rebaudiana* freeze-dried leaves (1 g) that were mixed with 10 mL of a mobile phase (acetonitrile : water 80 : 20) for 20 minutes. Rebaudioside A was identified and quantified by the high-pressure liquid chromatography method (HPLC, Hewlett Packard 1100 model), in which 20 μL was taken and was injected into the chromatography equipment. The used column was Zorbax Carbohydrate with a flow of 0.1 mL/min.

2.9. Statistical Analysis. Data were subjected to analysis of variance (ANOVA) followed by Student's t-test (with $P \leq 0.05$) by JMP (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Phenolic Compounds. Phenolic compounds, flavonoids, and condensed tannins as well as antioxidant activity were analyzed in stevia and peppermint by spectrophotometric methods after the application of organic and conventional fertilization. A number of reports indicate that these compounds are involved in the prevention of non-transmissible chronic diseases by means of their antioxidant activity [12, 13]. The total phenol, flavonoid, and tannin content for stevia and peppermint are shown in Tables 1 and 2. Results indicate significant differences between organic and conventional fertilization in peppermint for total phenolics but not for flavonoids and condensed tannins; these variables do not display significant differences in stevia; nevertheless, it is relevant to mention that organic stevia has 33% more total phenols than the conventional one. In the high total phenolic content in peppermint, other phenolics may be implicated apart from flavonoids and condensed tannins such as phenolic acids, and caffeic acid derivatives were the most abundant phenolic compounds in stevia [14]; also, caffeic acid was the most abundant phenolic acid in peppermint [15]; hydroxycinnamate derivatives have been identified and quantified in stevia methanolic extracts [2, 16]. Various authors have stated that the use of organic fertilizer enhances the amount of secondary metabolites such as phenolic as well the antioxidant activity in crops [17, 18]. The amount of stevia phenolic compounds found in this study is considerably lower than those reported by other studies (e.g., total phenol: 80.13 mg gallic acid/g extract and total flavonoids: 111.16 mg quercetin/g extract) [5, 19, 20]. The same behavior occurs for peppermint, where total phenolic and total flavonoid in peppermint leaves were 360.04 ± 0.285 and 421.96 ± 0.25 mg $100\,g^{-1}$, respectively [21]. According to Gupta et al. [22], tannin detected content in stevia leaves is 5.68 mg $100\,g^{-1}$ what is higher than the amount found in this work. Sujana et al. [23] reported 2 mg·g^{-1} of tannins; this value is higher than the one found in this research. Higher levels of total phenolic content were consistently found in organic and sustainable marionberries, strawberries, and corn as compared to those produced by conventional agricultural practices [24]. According to the study of Faller and Fialho [25] made on fruits and vegetables, organic agriculture results in food products with similar or marginally higher polyphenol content and antioxidant capacity. The perception among consumers is that organic cultivars possess a higher nutritional quality than conventional; nevertheless, it is not easy to estimate compositional differences due to agricultural practices because of the vast number of variables such as crop, irrigation patterns, weather variations, handling, etc. Post-harvest management and also laboratory extraction techniques may be implicated in these differences [6].

3.2. Antioxidant Activity. Antioxidant capacity was determined in extracts from leaves of *S. rebaudiana* Bertoni and *M. piperita.* This activity was characterized by two different assays, consisting of measuring the ability to scavenge the DPPH radical. Results obtained are shown in Tables 3 and 4 for stevia and peppermint, respectively. A different behavior between these two tests can be observed. In the ABTS test, no significant differences were observed. However, DPPH test displayed significant differences for both plants. Stevia presented 0.880 ± 0.009 and 0.827 ± 0.16 mg Trolox equivalent g of extract^{-1} for organic and chemical fertilization, respectively. Peppermint has 0.889 ± 0.014 and 0.558 ± 0.028 mg Trolox equivalent g of extract^{-1} for organic and conventional fertilization, respectively. These results are lower than those presented in other studies [13]. This may be related to the lower amount of phenolic compounds founds in the material tested. Yildiz-Ozturk et al. [19] found DPPH radical scavenging activities of around 90% which are higher than the ones found; nevertheless, Singh et al. [26] reported radical scavenging activities (%) that ranged between 47.1% and 82.4% for leaf and flower, respectively. Both assays are classified into the group of single electron transfer based methods, which accounts for the similarity of results reported in many occasions. However, it must be considered that the conditions in which the tests are performed are different, so they must be considered complementary [27]. Total phenolic content correlates positively with antioxidant activity. Other studies have found the same relation. It is stated that antioxidant capacity characterizing stevia leaves is mainly attributable to the presence phenolic compounds, such as flavonoids [2] and steviosides [28]; Barroso et al. [14] stated the total flavonoid content was less strongly correlated with the antioxidant activity in comparison with the total phenolic acids, due to its higher concentration present in stevia. . The phenolic compounds present in the herbs and spices have been reported to show natural antioxidant activity and are applied as food preservatives. The predominant mode of antioxidant activity of phenolic compounds is believed to be a radical scavenging via hydrogen donation [5].

Organic fertilization may enhance some bioactive compounds that correlate with antioxidant activity in plants; however, more research is needed to ensure getting high

Table 1: Total phenolics, flavonoids, and condensed tannins in stevia[a].

Fertilization	Plant	Total phenolics[b]	Flavonoids[c]	Condensed tannins[d]
Organic	Stevia	0.948 ± 0.157^{a}	0.165 ± 0.030^{a}	0.006 ± 0.001^{a}
Conventional	Stevia	0.708 ± 0.089^{a}	0.186 ± 0.088^{a}	0.006 ± 0.002^{a}

[a]Each value is the mean of three replicates ± standard error. Different letters in each column express significant difference $P \leq 0.05$ by Student's t-test. [b]mg of gallic acid equivalent/g of dry sample. [c]mg equivalent of rutin/g of dry sample. [d]mg of (+) catequine equivalent/g dry sample.

Table 2: Total phenolics, flavonoids, and condensed tannins in peppermint[a].

Fertilization	Plant	Total phenolics[b]	Flavonoids[c]	Condensed tannins[d]
Organic	Peppermint	0.905 ± 0.055^{a}	0.112 ± 0.006^{a}	0.001 ± 0.000^{a}
Conventional	Peppermint	0.558 ± 0.047^{b}	0.379 ± 0.037^{b}	0.017 ± 0.005^{b}

[a]Each value is the mean of three replicates ± standard error. Different letters in each column express significant difference $P \leq 0.05$ by Student's t-test. [b]mg of gallic acid equivalents/g of dry sample. [c]mg equivalent of rutin/g of dry sample. [d]mg of (+) catequine equivalent/g dry sample.

Table 3: Radical scavenging activity of stevia as a result of type of fertilization[a].

Fertilization	Plant	% inhibition	
		ABTS	DPPH
Organic	Stevia	92.847 ± 2.394^{a}	72.780 ± 1.817^{a}
Conventional	Stevia	94.526 ± 0.077^{a}	62.681 ± 3.076^{b}

[a]Each value is the mean of three replicates ± standard error. Different letters in each column express significant difference $P \leq 0.05$ by Student's t-test.

Table 4: Radical scavenging activity of peppermint as a result of type of fertilization[a].

Fertilization	Plant	% inhibition	
Organic	Peppermint	93.783 ± 0.210^{a}	74.474 ± 2.801^{a}
Conventional	Peppermint	93.330 ± 0.406^{a}	11.815 ± 5.336^{b}

[a]Each value is the mean of three replicates ± standard error. Different letters in each column express significant difference $P \leq 0.05$ by Student's t-test.

added value agricultural products and nutraceutical products. In some cases, the strategy is not just organic or conventional but integrative, as the one exposed for peppermint where the application of chemical fertilizers with nano fertilizers can be an alternative to improve the quali-quantitative characteristics of peppermint [29]. More foods must be evaluated to solve the controversy of whether organic foods have a nutritional and/or sensory advantage when compared to their conventionally produced counterparts.

Due to their amount of steviol glycosides, which are of great medicinal and nutraceutical importance worldwide, two different varieties of stevia (Morita and Eriete) were evaluated in their amount of glycosides (stevioside, rebaudioside A, and rebaudioside C) under organic and conventional fertilization. For organic fertilization, in Morita, the stevioside, rebaudioside A, and rebaudioside C content expressed as mg/g of dry matter was 9.582, 1.184, and 7.496, respectively. For Eriete, it was 1.160, 24.719, and 5.748, respectively. For Morita cultivated under conventional fertilization, steviosides were not detected and rebaudioside A was found in an amount of 14.877 mg/g of dry matter and rebaudioside C in 5.893. Eriete under this same condition presented 25.710 mg/g of dry matter for rebaudioside A and 4.891 mg/g of dry matter for rebaudioside C. No differences in the steviol glycoside content were found between varieties or fertilization. Díaz-Gutiérrez et al. [30] found concentration of rebaudioside C and stevioside increased under organo-mineral fertilizer (compost from poultry manure with inorganic salts) under greenhouse conditions. These authors found that nutrients as nitrogen, calcium, magnesium, and sulfur presented a significant correlation with the production of steviol glycosides. The implication of nitrogen fertilization in the production of steviol glycosides and other stevia bioactives has been also stated by other authors [14, 28], implying that adequate N rate is important to significantly increase and optimize the bioactive compound levels in stevia; however, it was stated by Barbet-Massin et al. [31] that steviol concentration in the leaf decreased with increasing nitrogen concentration; then, the implication of nitrogen in the development of steviol glycosides needs further evaluation since it also implies that an N deficit could be switching between the production of steviol glycoside and the synthesis of key isoprenoids, such as gibberellins, chlorophylls, and carotenoids.

It is identified that, among steviol glycosides, stevioside and rebaudioside A are present in the highest concentrations. Stevioside is 300 times sweeter than sucrose; it has a slight licorice flavor and a bitter aftertaste. In contrast, rebaudioside A lacks the bitter aftertaste and is 250 up to 400 times sweeter than sucrose [31]; for this reason, the rebaudioside A/stevioside ratio is considered a parameter to measure the quality of stevia extract [32]. For Morita cultivated under organic condition, rebaudioside A/stevioside ratio was of 0.12, whereas for Eriete it was 21.31. In cultivars grown in conventional manner, no stevioside was detected. The results for Eriete are relevant since it will develop a better taste in steviol glycoside products. It is relevant to mention that the ratio reported here is higher than the one reported in a study where mannitol, an NaCl stress, was evaluated (0.75–1.53).

Results obtained in the present investigation suggest that stevia and peppermint grown under organic conditions display higher levels of polyphenols and antioxidant activity; nonetheless, additional research must be done to confirm possible differences between conventional and organic stevia

and peppermint in order to provide better guidance to agricultures and consumers.

Data Availability

The data are available from the corresponding author upon request.

Conflicts of Interest

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

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References

[1] M. K. Roy, M. Takenaka, S. Isobe, and T. Tsushida, "Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: effects of thermal treatment," *Food Chemistry*, vol. 103, no. 1, pp. 106–114, 2007.

[2] M. G. Figueroa Pérez, N. E. Rocha-Guzmán, E. Mercado-Silva, G. Loarca-Piña, and R. Reynoso-Camacho, "Effect of chemical elicitors on peppermint (*Mentha piperita*) plants and their impact on the metabolite profile and antioxidant capacity of resulting infusions," *Food Chemistry*, vol. 156, pp. 273–278, 2014.

[3] K. Amin, S. Ozgen, and Z. Selamoglu, "Stevia rebaudiana: a potential boon for human health," *SM Journal of Medicinal Plant Studies*, vol. 1, p. 1005, 2017.

[4] M. Reis, L. Coelho, G. Santos, U. Kienle, and J. Beltrão, "Yield response of stevia (Stevia rebaudiana Bertoni) to the salinity of irrigation water," *Agricultural Water Management*, vol. 152, pp. 217–221, 2015.

[5] J. C. Ruiz Ruiz, Y. B. Moguel Ordoñez, M. Á. Basto, and M. R. Segura Campos, "Antioxidant capacity of leaf extracts from two Stevia rebaudiana Bertoni varieties adapted to cultivation in Mexico," *Nutricion Hospitalaria*, vol. 31, 2015.

[6] A. Moreno-Reséndez, R. Parcero-Solano, J. L. Reyes-Carrillo et al., "Organic manures improved the phenolic content, antioxidant capacity and soluble solids in pepper," *Food and Nutrition Sciences*, vol. 07, no. 14, p. 1401, 2016.

[7] K. Umesha, G. R. Smitha, B. S. Sreeramu, and A. A. Waman, "Organic manures and bio-fertilizers effectively improve yield and quality of stevia (Stevia rebaudiana)," *Journal of Applied Horticulture*, vol. 13, no. 02, pp. 157–162, 2011.

[8] L. Garcia-Mier, S. N. Jimenez-Garcia, R. G. Guevara-González, A. A. Feregrino-Perez, L. M. Contreras-Medina, and I. Torres-Pacheco, "Elicitor mixtures significantly increase bioactive compounds, antioxidant activity, and quality parameters in sweet bell pepper," *Journal of Chemistry*, vol. 2015, Article ID 269296, 2015.

[9] V. Dewanto, X. Wu, K. K. Adom, and R. H. Liu, "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 10, pp. 3010–3014, 2002.

[10] M. Oh and K. Yoon, "Comparison of the biological activity of crude polysaccharide fractions obtained from Cedrela sinensis using different extraction methods," *Polish Journal of Food and Nutrition Sciences*, vol. 68, no. 4, pp. 327–334, 2018.

[11] S. Mondal, G. Majumdar, and S. De, "Clarifications of stevia extract using cross flow ultrafiltration and concentration by nanofiltration," *Separation and Purification Technology*, vol. 89, pp. 125–134, 2012.

[12] A. Carrera-Lanestosa, Y. Moguel-Ordóñez, and M. Segura-Campos, "Stevia rebaudiana Bertoni: a natural alternative for treating diseases associated with metabolic syndrome," *Journal of Medicinal Food*, vol. 20, pp. 933–943, 2017.

[13] M. J. Álvarez-Robles, A. López-Orenes, M. A. Ferrer, and A. A. Calderón, "Methanol elicits the accumulation of bioactive steviol glycosides and phenolics in Stevia rebaudiana shoot cultures," *Industrial Crops and Products*, vol. 87, pp. 273–279, 2016.

[14] M. R. Barroso, N. Martins, L. Barros et al., "Assessment of the nitrogen fertilization effect on bioactive compounds of frozen fresh and dried samples of Stevia rebaudiana Bertoni," *Food Chemistry*, vol. 243, pp. 208–213, 2018.

[15] J. Lv, H. Huang, L. Yu et al., "Phenolic composition and nutraceutical properties of organic and conventional cinnamon and peppermint," *Food Chemistry*, vol. 132, no. 3, pp. 1442–1450, 2012.

[16] M. Molina-Calle, F. Priego-Capote, and M. D. Luque de Castro, "Characterization of Stevia leaves by LC-QTOF MS/MS analysis of polar and non-polar extracts," *Food Chemistry*, vol. 219, pp. 329–338, 2017.

[17] N. F. Omar, S. A. Hassan, U. K. Yusoff, N. A. P. Abdullah, P. E. M. Wahab, and U. R. Sinniah, "Phenolics, flavonoids, antioxidant activity and cyanogenic glycosides of organic and mineral-base fertilized cassava tubers," *Molecules*, vol. 17, no. 3, pp. 2378–2387, 2012.

[18] M. Ibrahim, H. Jaafar, E. Karimi, and A. Ghasemzadeh, "Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (Labisia pumila Benth)," *Molecules*, vol. 18, no. 9, pp. 10973–10988, 2013.

[19] E. Yildiz-Ozturk, A. Nalbantsoy, O. Tag, and O. Yesil-Celiktas, "A comparative study on extraction processes of Stevia rebaudiana leaves with emphasis on antioxidant, cytotoxic and nitric oxide inhibition activities," *Industrial Crops and Products*, vol. 77, pp. 961–971, 2015.

[20] I.-S. Kim, M. Yang, O.-H. Lee, and S.-N. Kang, "The antioxidant activity and the bioactive compound content of Stevia rebaudiana water extracts," *LWT—Food Science and Technology*, vol. 44, no. 5, pp. 1328–1332, 2011.

[21] A. Dyab, A. Aly, and H. Matuk, "Enhancement and evaluation of peppermint (Mentha piperita L.) beverage," 2015.

[22] E. Gupta, S. Purwar, A. Singh, S. Sundaram, and G. Rai, "Evaluation of nutritional, anti-nutritional and bioactive compounds in juice and powder of stevia rebaudiana," *Indian Journal of Natural Sciences*, vol. 5, no. 28, 2015.

[23] P. Sujana, T. M. Sridhar, P. Josthna, and C. V. Naidu, "Antibacterial activity and phytochemical analysis of," *American Journal of Plant Sciences*, vol. 4, p. 77, 2013.

[24] D. K. Asami, Y.-J. Hong, D. M. Barrett, and A. E. Mitchell, "Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices," *Journal of Agricultural and Food Chemistry*, vol. 51, pp. 1237–1241, 2003.

[25] A. Faller and E. Fialho, "Polyphenol content and antioxidant capacity in organic and conventional plant foods," *Journal of Food Composition and Analysis*, vol. 23, pp. 561–568, 2010.

[26] S. Singh, V. Garg, D. Yadav, M. N. Beg, and N. Sharma, "In vitro antioxidative and antibacterial activities of various parts of Stevia rebaudiana (Bertoni)," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 4, pp. 468–473, 2012.

[27] J.-K. Moon and T. Shibamoto, "Antioxidant assays for plant and food components," *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 1655–1666, 2009.

[28] S. Tavarini, C. Sgherri, A. M. Ranieri, and L. G. Angelini, "Effect of nitrogen fertilization and harvest time on steviol glycosides, flavonoid composition, and antioxidant properties in Stevia rebaudiana Bertoni," *Journal of Agricultural and Food Chemistry*, vol. 63, pp. 7041–7050, 2015.

[29] A. Ostadi, A. Javanmard, M. A. Machiani, M. R. Morshedloo, M. Nouraein, and F. Rasouli, "Effect of different fertilizer sources and harvesting time on the growth characteristics, nutrient uptakes, essential oil productivity and composition of Mentha x piperita L," *Industrial Crops and Products*, vol. 148, Article ID 112290, 2020.

[30] C. Díaz-Gutiérrez, A. Hurtado, A. Ortíz, C. Poschenrieder, C. Arroyave, and C. Peláez, "Increase in steviol glycosides production from Stevia rebaudiana Bertoni under organo-mineral fertilization," *Industrial Crops and Products*, vol. 147, Article ID 112220, 2020.

[31] C. Barbet-Massin, S. Giuliano, L. Alletto, J. Daydé, and M. Berger, "Nitrogen limitation alters biomass production but enhances steviol glycoside concentration in Stevia rebaudiana Bertoni," *PLoS One*, vol. 10, no. 7, Article ID e0133067, 2015.

[32] S. Hajihashemi and J. M. Geuns, "Steviol glycosides correlation to genes transcription revealed in gibberellin and paclobutrazol-treated Stevia rebaudiana," *Journal of Plant Biochemistry and Biotechnology*, vol. 26, pp. 387–394, 2017.

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Review Article

Mycotoxins Analysis in Cereals and Related Foodstuffs by Liquid Chromatography-Tandem Mass Spectrometry Techniques

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In the entire world, cereals and related foodstuffs are used as an important source of energy, minerals, and vitamins. Nevertheless, their contamination with mycotoxins kept special attention due to harmful effects on human health. The present paper was conducted to evaluate published studies regarding the identification and characterization of mycotoxins in cereals and related foodstuffs by liquid chromatography coupled to (tandem) mass spectrometry (LC-MS/MS) techniques. For sample preparation, published studies based on the development of extraction and clean-up strategies including solid-phase extraction, solid-liquid extraction, and immunoaffinity columns, as well as on methods based on minimum clean-up (quick, easy, cheap, effective, rugged, and safe (QuEChERS)) technology, are examined. LC-MS/MS has become the golden method for the simultaneous multi-mycotoxin analysis, with different sample preparation approaches, due to the range of different physicochemical properties of these toxic products. Therefore, this new strategy can be an alternative for fast, simple, and accurate determination of multiclass mycotoxins in complex cereal samples.

1. Introduction

Most people of developed and developing countries use cereals and cereal-based products as their primary source of nutrients and energy [1–4]. Nevertheless, due to rich contents of fat, protein, and minerals, they are providing a great environment for fungal growth [5, 6]. Contamination of cereals in preharvest and postharvest stages with fungi can lead to the production of mycotoxins [7–9]. In this line, some environmental agents such as humidity, temperature, inadequate storage conditions, insect damage, and drought play important roles in the level and diversity of contamination by mycotoxins [10–12]. In addition, the incidence and mycotoxins concentration in cereal-based food products can be associated with some factors, such as physical and chemical food characterization (pH, composition, and water activity), production management (storage, harvesting, and conditions of processing), and weather status (humidity and temperature) [13–16].

These secondary toxic metabolites are secreted by some important fungal genera including *Aspergillus*, *Penicillium*, *Fusarium,* and *Alternaria* [17–22]. Naturally toxic compounds with a low molecular weight and a high bioaccumulation ability, mycotoxins, are thermally stable [23, 24]. According to literature, among more than 400 identified secondary compounds, deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEN), and aflatoxins (AFTs) were renowned as the most studied mycotoxins and are considered a hazard to human or animal health [25–32].

Currently, biologically modified mycotoxins, introduced due to plant metabolism and known as "masked mycotoxins," have also been described such as ZEA-14-sulfate (Z4S) and α- and β-zearalenol (α- and β-ZOL) [33–37]. The most common examples are 3-acetyl-deoxynivalenol (3-

ADON) and 15-acetyl-deoxynivalenol (15- ADON) which have been detected in *Fusarium*-contaminated cereals [38–41]. Both compounds ascend from 3,15-diacetyl deoxynivalenol [42, 43]. The occurrence of 3-ADON and 15-ADON in cereals has been described in some studies [44–47].

For the determination of mycotoxins in cereals and related foodstuffs, sampling of nonhomogeneous compounds and the analytical techniques are strongly important. In this line, proper sampling techniques must be put in place to obtain representative results. Therefore, sample selection, sample size, and number of incremental samples must be well recognized due to the mycotoxin heterogeneous distribution within the lots [48, 49]. Since fungal growth is limited to certain locations in the lot and arbitrarily distributed, fungi contamination and mycotoxin production are considered as "spot processes" [48]. According to the Commission Regulation (EC) No. 519/2014 [50], from lots ≥50 tonnes, incremental sample number must be a minimum of 100, with a total of 10 kg of aggregate samples. For lots <50 tonnes, 3 to 100 incremental samples should be collected, with a corresponding aggregate sample weight of 1 (minimum weight) to 10 kg. In the case of lots >500 tonnes, the representative sample should be at least 10% of the lot.

Analysis of mycotoxin in cereals and related foodstuffs is a decisive practice to approve food security. Several detection methods have been established, among the most common currently used are LC-MS/MS methods. When compared to other separation and detection techniques, LC-MS/MS methods present very high analytical sensitivity. Extraction procedures and suitable clean-up, providing good recoveries and reducing matrix effects, are consequently extremely important to analytical method development and optimization. In this way, aqueous solvents and/or acidic solvents are crucial for quantitative extraction of FBs or OTA, while high organic solvents are suitable for mycotoxins such as AFs, OTA, and ZEA [51–53]. On the other hand, clean-up procedures towards mycotoxin analyses are largely performed by solid-phase extraction (SPE) or immunoaffinity columns (IAC) [54, 55]. Based on solid samples such as cereals and related foodstuff samples, SPE was used as a clean-up and/or concentration step following a prior extraction procedure [56, 57]. Several SPE columns are commercially available, with different solid phases ranging from C18 materials (ion exchange) to more specific adsorbent materials [56, 58, 59]. IAC, a method based on the interaction between antigen and antibody, displays some advantages, including a minimal loss of mycotoxins and a maximal elimination of interfering substances [60–62]. Compared to SPE extraction, the utilization of IAC as a clean-up procedure could greatly improve the specificity of subsequent analysis [54]. Other comparable clean-up procedure includes the QuEChERS-like method, which offers the opportunity to extend the number of analytes to be analyzed by a less time-consuming approach [58, 63]. According to Amirahmadi et al. [64], this method involves extraction with acetonitrile and partitioning clean-up after the addition of a salt mixture ($MgSO_4$ and NaCl). Remarkably, QuEChERS is reliable with a number of advantages, such as simplicity, minimum steps, and effectiveness in cleaning-up complex samples [65].

For quantitative analysis of mycotoxin in cereal-based food samples, chromatographic techniques showed a group of techniques most commonly used which are highly selective, sensitive, and accurate [66–69]. For mycotoxin analysis, high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), gas chromatography (GC), and LC-MS/MS are commonly used chromatographic techniques [70, 71]. HPLC-UV, HPLC-diode array (DAD), HPLC-fluorescence detector (FLD), or mass spectrometry (MS) detector has been used to detect AFT, OTA, DON, ZEN, fumonisins (FUM), citrinin (CIT), and patulin (PAT). By using Liquid chromatography techniques to mass spectrometry (LC-MS/MS), the concurrent detection of multiple mycotoxins in various cereals and related foodstuffs products was established [70, 71]. TLC is cost-effective, simple, and suitable for rapid screening of common mycotoxin, but the lack of automation limits its use; moreover, GC coupled with electron capture (ECD), flame ionization (FID), or MS detector applied for volatile mycotoxins (trichothecenes (TCTs) and PAT) also limits its commercial use [72, 73].

Consecutively, the present review presents an emphasized overview on the development, optimization, and validation of LC-MS/MS-based methodologies towards the analysis of mycotoxins in cereals and related products. In addition, clean-up and extraction procedures and chromatographic and detection parameters, as well as the analytical method performance process, were well discussed.

2. Analytical Methods: Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

The basic principle of MS/MS is the selection and fragmentation of precursor ion and measurement of the m/z ratio of the product ions formed [73, 74]. There are two fundamentally different approaches to MS/MS: tandem mass spectrometry in space or in time [75]. The triple quadrupole (QqQ) was the frequently used space instrument tandem mass spectrometry in space. Equally, other examples of tandem mass spectrometers included quadrupole-time-of-flight (QqToF) and Orbitrap hybrid instruments [76–79]. However, tandem-in-time instruments are typically ion-trapping mass spectrometers, which comprise 3D quadrupole ion traps (QIT) [80], linear ion traps (LIT) [81, 82], and Fourier transform-ion cyclotron resonance (FT-ICR) instruments [83, 84].

After extraction with acetonitrile/water, QqQ LC-MS/MS methods were examined for the quantification of TCTs and ZEA in cereals by using electrospray ionization (ESI) [85] and atmospheric pressure chemical ionization (APCI) [86, 87] interfaces.

Cavaliere et al. [88] presented their method for the determination of 8 TCTs, three FUM, ZEA, and alpha-zearalenol in corn samples and used ESI QqQ MS in both polarity modes. A positive-ion mode ESI QqQ LC-MS/MS

method for the simultaneous determination of 16 mycotoxins on a cellulose filter was developed by Delmulle et al. [89].

In targeted mycotoxin determination LC-MS/MS, analytical methods using a QqQ and linear ion trap (QLIT) mass spectrometer are the most commonly used procedures [90]. The combination of QqQ MS (QqQ/QLIT) is valuable because this instrument retains the selective reaction monitoring mode (SRM) [75, 91, 92]. Rapid multimethods based on QqQ/QLIT approaches are able to analyze simultaneously up to 300 mycotoxins and also their metabolites or other related food contaminants depending on the length of the chromatographic run [92–95].

The sustained development of mass spectrometers, including Orbitrap-based systems as well as other instrument platforms such as the QTOF, was thus driven by aims of accelerating scan speed and increasing sensitivity [96, 97]. This instrument can be defined as a triple quadrupole where the last quadrupole is substituted by an oa-TOF or as the addition of a collision cell to a TOF analyser and a quadrupole analyser [97]. To perform fragmentation with higher-energy collisional dissociation (HCD), a gas-filled quadrupole (the HCD cell) was fitted directly after the C-trap [98]. Besides, it has been stated that TOF and Orbitrap analyzers, with resolving power of 10,000–100,000 and 140,000–240,000 (full width at half maximum defined at m/z), were used respectively [75]. These analyzers are very sensitive making easier the analytes identification giving accurate results even when we are dealing with very low levels of analytes. Some authors have exploited their potential in the quantitative analysis of mycotoxins showing higher significance for Orbitrap [99].

A new generation of hybrid techniques such as the Q-orbital ion trap (Q Exactive) instrument combines the advantages of high-performance quadrupole selection of precursor ions with those of high-resolution mass detection [100, 101]. The subsequently developed Q Exactive instrument allowed precursor ion isolation on an exactive-type mass spectrometer. For isolation of precursors, a mass filtering quadrupole was utilized [101, 102]. Thereafter, for detection, the HCD cell voltages are ramped and ions are conveyed back into the C-trap from where they are injected into the Orbitrap. In fact, structural information can be obtained on compounds of interest and fragment ions can be used for confirmation in targeted analyses [102].

Regarding identification, metabolite ions in a full scan spectrum (MS) are subsequently isolated to generate MS/MS spectra; data-dependent acquisition (DDA) approach is the most common strategy [103, 104]. Thereafter, metabolite structure is elucidated through MS/MS spectral similarity corresponding to the standard metabolite spectral library. In this context, Human Metabolome Database (HMDB) [105], METLIN [106], and MassBank [107] are frequently referred to as a spectrum-centric approach. MassBank is the first public source of mass spectra of small chemical compounds for life sciences (<3000 Da) [107], while METLIN includes an annotated list of known metabolite structural information that is easily cross-correlated with its catalogue of high-resolution Fourier transform mass spectrometry (FTMS) spectra, MS/MS spectra, and LC/MS data [106]. Application of DDA in analysis of mycotoxins was demonstrated in several recent studies [108, 109]. Nevertheless, DDA suffers from numerous limitations. For example, in one experiment, not a limited number of ions with highest abundance detected in the full MS scan are isolated and fragmented in a product ion scan experiment [110–112]. Also, the selected precursor ions may be derived from many adducted ions instead of molecular ions [113, 114]. If applied to the analysis of mycotoxin-contaminated foodstuff, these problems would be aggravated since these metabolites habitually occur at lower concentrations, and absolute quantification is critical for compliance with regulatory limits [109].

Technological advances have greatly increased the resolution, speed, and sensitivity of mass spectrometers. This has allowed for new types of nontargeted methods to become more practicable, precisely data-independent acquisition (DIA). It should be noted that the DIA approach depends on the width of the isolation window, and many ions can be cofragmented. Consequently, the product ion spectra are more complex compared with targeted methods and at each segment producing one multiplexed MS/MS spectrum derived from multiple precursor ions [115, 116]. DIA approaches have been established on each of the Orbitrap mass spectrometer platforms to take benefit of their specific architectures. Development in the area of DIA included methods such as wide isolation window SIM scan DIA (WiSIM-DIA) on the Orbitrap fusion mass spectrometer [117]. This approach utilized an ultrahigh-resolution SIM scan for quantification, complementary with classic DIA. In proteomics, several data MS analysis methods and programs, such as DIA-Umpire [118] and Skyline [119, 120], were used. In this line, DIA-Umpire, a comprehensive computational workflow and open-source software for DIA data, detects precursor and fragment chromatographic features and assembles them into pseudo-MS/MS spectra which can be identified using conventional database searching and protein inference tools without the need for a spectral library [119]. In the same way, Egertson et al. [120] described the use of DIA on a Q-Exactive mass spectrometer for the detection and quantification of peptides in complex mixtures using the Skyline Targeted Proteomics Environment.

The most promising feature of DIA analysis of mycotoxins is that the data generated is ideal for retrospective analysis. Newly characterized mycotoxins can be identified in archived data by high-resolution precursor mass, retention time, and multiple product ions. High-resolution MS alone has been used to collect data that can be retrospectively analyzed for the presence of mycotoxins [109, 120, 121]. In this vein, Renaud et al. [109] reported the development of a powerful LC-DIA analysis method on a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer for mycotoxin analysis produced by Fusarium graminearum in maize. On the contrary, Berthiller et al. [122] reported a method detection limit of 0.012 g/ml for D3G in purified sample extracts, corresponding to 0.02 g/g in contaminated cereals. These authors also estimated their LOD from the signal

intensity of their standards, based on the limited ion suppression they observed. The pigment LOQ and LOD were 4.3 and 0.0005 g/kg, respectively. Good linearity for the pigment standard curve (R^2 0.999) was also observed.

In LC-MS, the majority of multimycotoxin methods used ESI interface. In fact, positive-mode ESI is exclusively applied to couple high-performance liquid chromatography (HPLC) or ultrahigh-performance liquid chromatography (UHPLC) and MS detection [73, 123–126]. This technique has been effectively used for the synchronized quantification of mycotoxins with different chemical structures [54] in one single run [89, 126]. The LC/MS-MS technique has been reported by many studies in multimycotoxin determination, such as 17 different mycotoxins in barley and malt [127].

3. Current Methods Used for LC-MS/MS Determination of Mycotoxins in Cereals and Related Products

The approaches include those used for screening and quantification in both official control and research. It should be noted that the approaches discussed mostly have been developed for the determination of EU-regulated mycotoxins in various food matrices to strictly respond to the EU legislation [128]. Despite the interesting benefits that could procure MS/MS as a very selective technique, its signal could be overestimated and lost in the case of some challenging samples leading finally to false positive results. Also, although LC-MS is considered to be a highly sensitive analytical technique, trace detection levels of some analytes seem impossible especially when compromises related to sample preparation and LC-MS/MS conditions have to be made. These methods are developed based on the QuEChERS approach [129]. This approach was established for a very rapid extraction and purification with regard to multipesticide analysis. Its relevant principle relies on the partitioning of an acetonitrile-water mixture induced by addition of inorganic salts. In general, LC-MS/MS techniques including QuEChERS approach are ineffective for the AFs and OTA detection in baby foods at the EU limits. Therefore, for these particular metabolites, specific clean-up methods with immunoaffinity columns (IACs) or combinations with another clean-up technique are used [52]. The application of immunoanalysis for a rapid screening of mycotoxins represents an attractive analytical method commonly used nowadays. The main criteria for research of such approaches include simplification and rapidity of analysis, sensitivity improvement, and matrix effect reduction. Immunoassays generally applied for rapid detection of individual mycotoxins are summarized in a review concerning immunochemical assays [52]. The common immunomethods applied for mycotoxin detection rely on binding of specific antibodies to a solid support (direct competitive ELISA format) or coated antigens (indirect competitive ELISA format). These formats are used in all nonhomogenic methods: microtiter plate immunoassays and sensors. Homorganic methods implicate the fluorescent polarization and capillary electrophoretic immunoassays [52].

Lattanzio et al. [52] detected and quantified aflatoxins (B_1, B_2, G_1, and G_2), ochratoxin A, fumonisins (B_1, B_2), deoxynivalenol, zearalenone, T-2, and HT-2 toxins in maize. In fact, reversed-phase liquid chromatography coupled with electrospray ionization triple quadrupole mass spectrometry (LC/ESI-MS/MS) was used as chromatographic mobile phase, a linear gradient of methanol/water containing 0.5% acetic acid and 1 mM ammonium acetate. The method exhibited good linearity; also, matrix-coordinated calibration curves for all analytes were linear over the relevant working range with r (coefficient of correlation) values between 0.9980 and 0.9999 [52]. In addition, recoveries higher than 79% were obtained for all tested mycotoxins with relative standard deviations less than 13%. These authors reported that method performances were quite satisfactory for all tested mycotoxins at contamination levels close to or below the relevant EU maximum permitted or recommended levels. Limits of detection (LOD) in maize ranged from 0.3 to 4.2 μg/kg [52]. These LODs are similar with or slightly lower than those reported by other authors using MRM detection for the analyses of the same mycotoxins in maize or maize-based food extracts after SPE clean-up [86, 88, 89].

On the other hand, QuEChERS procedure has been used for the development of an LC-MS/MS assay for the determination of 17 mycotoxins in cereals for human consumption and infant cereals [129]. All tested matrices gave LOQs below the maximum levels except for AFLA B1 in infant cereals (maximum level = 0.1 μg/kg, LOQ = 1 μg/kg). Matrix effects were nevertheless more important in soya (LOQ for the aflatoxins B_1, B_2, G_1, and G_2 = 2 μg/kg) and even more in corn gluten (pet food material). Higher LOQs were thus obtained in corn gluten (pet food ingredient) for which no regulatory limits have been established [130]. These authors, also, have chosen the ESI^+ mode since the sensitivity of critical compounds with low maximum levels (i.e., aflatoxins B_1, B_2, G_1, and G_2 and OTA) was visibly enhanced. In contrast, an acceptable sensitivity for ZON, as $[M-H]^-$ ion, was only obtained in the ESI-mode. At the same time, the addition of ammonium formate to the aqueous mobile phase clearly enhanced the sensitivity for both type A and B TCTs detected under their ammonium adduct $[M+NH_4]^+$, whereas formic acid in both mobile phases increased the overall sensitivity, giving better peak shape for the acidic compounds, i.e., FB1, FB2, and OTA [131].

Modified QuEChERS which used acidified acetonitrile (ACN), $MgSO_4$, NaCl, and citrate buffer salts, combined with dispersive solid-phase extraction (d-SPE) clean-up and followed by LC-ESI-MS/MS method, was applied for the determination of EU-regulated mycotoxins in several cereals such as wheat, maize, and rice [132]. In cereals, aflatoxins, ochratoxins, fumonisins, trichothecenes, and zearalenone were detected and quantified. The performance of the method was assessed and compared to European Commission (EC) Regulations, by studying the selectivity, specificity, LOD, LOQ, linear dynamic range (LDR), matrix effect, accuracy, precision, and uncertainty. In this context, Fernandes et al. [132] reported a good linearity ($r^2 > 0.9713$)

for all mycotoxins investigated, and LODs ($S/N = 3$) and LOQs ($S/N = 10$) were below the tolerance levels of mycotoxins set by EC. Recoveries of the extraction process, obtained with different spiked concentrations, ranged from 72.9 to 120.6%, with relative standard deviations (RSD) lower than 23.0%.

Rubert et al. [133] reported the comparison of four different extraction techniques used in the determination of 32 mycotoxins in barley. These methods included QuEChERS modification, matrix solid-phase dispersion (MSPD: extraction MeCN/MeOH, 50/50, v/v), supported liquid extraction (SLE: extraction MeCN/water/acetic acid, 79/20/1, v/v/v), and solid-phase extraction (SPE, previous SLE extract). Accordingly, it has been shown that modified QuEChERS method was faster and easier than the other methods. Also, it enables to extract well all of the mycotoxins (from 64.1% DON-3-G to 93.4% T-2). These authors validated the method according to the directive and guide on that subject [134]. In this regard, confirmation of identity, specificity/selectivity, linearity, lowest calibration level (LCL), ranging between 1 and 100 μg/ kg for enniatin B (ENB) and NIV, respectively was done. The precision, process efficiency, and recovery were, also, studied [135]. Remarkably, Rubert et al. [133] reported that the UHPLC-HRMS was a robust technique for validation and routine mycotoxin analysis. This latter technique showed sensitivity and selectivity to identify simultaneously 32 mycotoxins.

Rubert et al. [135] developed a method to analyze simultaneously 14 mycotoxins (nivalenol (NIV), deoxynivalenol (DON), aflatoxin B_1 (AFB_1), aflatoxin B_1 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin (AFG_2), diacetoxyscirpenol (DAS), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), ochratoxin A (OTA), HT-2, T-2, ZEN, and beauvericin (BEA)) by LC-MS/MS. In this study, a comparison between eight sorbents (C18, C8, phenyl, amine-bonded phases, celite, silica, Florisil®, and alumina (acidic, neutral, and basic)) using an optimized solvent, MeCN/MeOH (50/50, v/v) 1 mM ammonium, to elute the desirable compounds, was performed. As a result, FMs were only extracted with C18 or C8, being the best recoveries for all mycotoxins obtained with C18 (from 72% of ZEN to 93% of deoxynivalenol (DON)) [135]. The sensitivity was evaluated by LOD and LOQ values and then was calculated analysing fortified flour sample. In all these cases, LOQs were always lower than the European maximum levels (MLs) established by EU. The authors commented that the precision, calculated as RSD, was between 3% and 14% for the intraday test and from 4% to 14% for the interday test. The recovery ranges in low and high spiked levels were 68.8–89.6% and 72.6–87.5%, respectively, for the intraday test and 68.7–88% and 72.8–87.6% for the interday test at LOQ and 10 times LOQ, respectively. Similar to matrix effects, recoveries and its repeatability were studied in the three varieties of cereal (wheat, corn, and rice) flour by three replicates. In all matrix tested, recoveries were satisfactory (between 70% and 120%).

Serrano et al. [136] studied the contents of 14 mycotoxins in samples of different cereal (rice, wheat, maize, rye, barley, oat, spelt, and sorghum) and cereal products (snacks, pasta, soup, biscuits, and flour) from four countries of the Mediterranean region (Spain, Italy, Morocco, and Tunisia). Samples were extracted with matrix solid-phase dispersion (MSPD) and determined by liquid chromatography-tandem mass spectrometry with a triple quadrupole mass analyser. The frequency of contaminated samples from Spain, Italy, Tunisia, and Morocco was 33%, 52%, 96%, and 50%, respectively. For legislated mycotoxins (AFs, FBs, DON, ZEN, and OTA), the LOQs were lower than the MLs established by the European Union (EC 401/2006) [137]. For fumonisins (FBs), the levels ranged from <LOQ-184 μg/kg for FB_1, and from 121 to 176 μg/kg for FB_2. The maximum FB_1 value (184 μg/kg) was found in a wheat pasta sample from Tunisia, and the maximum FB_2 value (176 μg/kg) was found in a rice grain sample from Morocco. These results were lower than those obtained in other studies for maize, wheat, rice, and barley products [138–140]. Recoveries of fortified cereal samples at two spiked levels ranged between 68.7–89.6% and 72.6–87.6%; in addition, the relative standard deviations varied from 3% to 14%. These values agree with EU criteria [141]. In addition, all mycotoxins exhibited good linearity over the working range (low concentration level at LOQ), and the regression coefficient of calibration curves was higher than 0.992 [136].

Otherwise, Lacina et al. [142] have performed different extraction methods for the simultaneous analysis of 288 pesticides and 38 mycotoxins. In fact, three different extractions were carried out for wheat and other products: aqueous acetonitrile extraction followed by a modified QuEChERS method (method A), aqueous acetonitrile extraction (method B), and pure acetonitrile extraction (method C). In these extraction procedures, different eluent modifiers were used for positive- and negative-mode ESI measurements to obtain high sensitivity and very sharp peaks. Then, it has been found that pure acetonitrile extraction (method C) did not show acceptable recoveries compared to QuEChERS approach and aqueous methanol extraction that present satisfactory recoveries ranging from 70% to 120% with RSD less than 20% for most of the analyte-matrix combinations. Despite the fact that QuEChERS-like method led to lower LOQ and more coherent results, the recoveries were low especially for polar analytes (DON 3-glucoside (DON-3-Glc), NIV, T2 tetraol) due to the partitioning step. On the other hand, extraction using QuEChERS approach was selected as the most suitable procedure for the tested analytes [142].

Juan et al. [143] tested several solvent mixtures: MeCN/MeOH (60/40, v/v), MeCN/MeOH (40/60, v/v), MeCN/water (84/16, v/v), and MeCN/water (16/84, v/v) to extract TRC and ZEN from grain cereal, flour, and bread. It has been found that the highest recoveries and the lowest matrix effects were shown with the MeCN/water (84/16, v/v) mixture. Analytes were determined by LC-MS/MS and relative recoveries obtained were higher than 70%. In this line, the obtained recoveries ranged for wheat were 73–98%; oat, 75–96%; barley, 73–99%; and spelt, 78–99%. In addition, the precision (RSDs) of theses samples ranged for wheat was 2.4–11; oat, 2.8–13; barley, 2.8–15; and spelt, 2.4–12. As well, a good linearity ($r^2 > 0.992$) was obtained and quantification limits (2.5–25 ng/g) were below European Regulatory levels.

Equally, sensitivity was high due to the low LOD and LOQ [143].

By using gradient RP-LC with atmospheric pressure chemical ionization triple quadrupole mass spectrometry (LC-APCI-MS/MS), Berthiller et al. [86] developed a novel method for the simultaneous determination of the Fusarium mycotoxins. Nivalenol, deoxynivalenol, fusarenon-X, 3-acetyl-deoxynivalenol, the sum of 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, and zearalenone in maize have been detected [86]. The swift clean-up of maize samples was performed with MycoSep® #226 columns, and the calibration curves for all analytes are linear over the working range of 30–1000 μg/kg, respectively. Depending on the mycotoxin, squared correlation coefficients (R^2) were in the range of 0.994–0.999 and LOD ranged from 0.3 to 3.8 μg/kg.

Barthel et al. [144] analyzed fifty nine samples of barley and barley products for 18 trichothecene mycotoxins by a sensitive LC-MS/MS. After sample extract clean-up on MycoSep®-226 columns, these authors confirmed that LODs were ranged between 0.062–0.70 μg/kg. Furthermore, the recovery was ranged between 75 and 104% for all mycotoxins with relative standard deviations (RSDs) between 2.1 and 17%. The results complied with the requirements of Commission Regulation (EC) 401/2006 [145].

Ren et al. [146] developed an analytical method for the simultaneous quantification of 17 kinds of *Aspergillus*, *Fusarium,* and *Penicillium* mycotoxin contaminants in foods and feeds by ultrahigh-performance liquid chromatography combined with ESI triple quadrupole tandem mass spectrometry (UPLC-MS/MS) under the multiple reaction monitoring (MRM) mode and especially focused on the optimization of extraction, clean-up. The 10 positive ions and 7 negative ions of mycotoxins were separated by gradient elution with the retention time of 6.5 and 4 min, respectively. The LOQ of selected analytes ranged from 0.01 to 0.70 $\mu g{\cdot}kg^{-1}$, which was lower than the criteria of EU, USA, and other countries on the determination of the minimum limiting level of various mycotoxins in foods including baby foods and feed stuffs. In this way, Amézqueta et al. [147] determined the OTA residue in cocoa beans by HPLC with the LOQ value of 0.1 $\mu g{\cdot}kg^{-1}$. Meanwhile, Sugita-Konsihi et al. [148] quantified the DON level using HPLC method and achieved reasonable LOQ value (100 $\mu g{\cdot}kg^{-1}$). Papp et al. [149] validated an analytical method for the determination of AT B_1, B_2, G_1, and G_2 in corns, wheat, fish, peanut products, rice, and sunflower seeds by HPLC with the LOD range of 2–10 $\mu g{\cdot}kg^{-1}$. Ren et al. [146], also, reported high correlation coefficients ($r^2 > 0.99$) of 17 mycotoxins which were obtained within their respective linear ranges (0.05–20 $\mu g{\cdot}kg^{-1}$ for 10 positive ions and 0.5–50 $\mu g{\cdot}kg^{-1}$ for 7 negative ions) and reasonable recoveries (70.6–119.0%) of them were also demonstrated in different spiked levels.

In 2012, Soleimany et al. [87] developed and used a LC-MS/MS method for simultaneous determination of aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), fumonisins (FB_1 and FB_2), T2, and HT2-toxin in cereals. One-step extraction using solvent mixtures of acetonitrile : water : acetic acid (79 : 20 : 1) without any clean-up was employed for extraction of these mycotoxins from cereals. The method exhibited good linearity over the relevant working range, and R^2 was between 0.950 for DON and 0.999 for AFB_1. There was significant difference among the LODs in the standard solution and in matrices. LODs of mycotoxins standard solutions were far lower than LODs in matrixes. The LODs and LOQs of standards and matrixes ranged between 0.01–20 ng/g and 0.02–40 ng/g, respectively, which are acceptable because they were far below the European Regulations for correspondent maximum levels of mycotoxins in foods. The LODs were lower than those reported by Sulyok et al. [53] and comparable to those reported by Ventura et al. [150]. Concerning recovery values, the study by Soleimany et al. [87] showed a range from 76.8% to 108.4% for all mycotoxins. The recovery results were better than those reported by Delmulle et al. [89] (52.6–89.2%), Sulyok et al. [53] (75–108%), Spanjer et al. [126] (46–115%), and Monbaliu et al. [151] (76–105%) for relevant mycotoxins. RSD% for this procedure was lower than 12.7% for all mycotoxins.

In another study, von Bargen et al. [99] described the first application of isotopically labeled $^{13}C_2$-moniliformin for the analysis of moniliformin (MON) in cereals. The use of high-resolution mass spectrometry was described to be a suitable alternative technique for the detection of this compound. The developed method is based on the use of strong anion exchange columns for cleanup prior to HPLC analysis. In fact, the recovery rate was equal to 75.3%, and the LOD and LOQ were 0.7 and 2.5 μg/kg, respectively.

On the other hand, Sirhan et al. [152] established a new method based on QuEChERS followed by LC-ESI-QTOF-MS/MS to determine eight type-A and type-B trichothecenes in cereal samples. The recoveries of fortified cereal samples ranged from 61.9% to 110.9%, and RSDs were lower than the acceptable 12% in all the cases. The sensitivity was determined by estimating the limit of detection (LOD) and limit of quantification (LOQ). Indeed, the LODs of type-A and type-B trichothecenes were 6.1–8.3 and 12.5–18.7 mg = kg, respectively.

Habler and Rychlik [95] developed a multimycotoxin stable isotope dilution LC-MS/MS method for 14 fusarium toxins. Linearity, intraday precision, interday precision, and recoveries were ≥0.9982, 1–6%, 5–12%, and 79–117%, respectively. Method accuracy was verified by analysing certified reference materials for deoxynivalenol, HT2-toxin, and T2-toxin with deviations below 7%. The recoveries range between 86 and 109% for all analytes with RSDs below 7% and between 79 and 117% for the matrix calibration with maximal RSD of 17%. The LODs range between 0.1 and 5 μg/kg and the LOQs range between 0.2 and 15 μg/kg, except for NIV and D3G, whose LODs and LOQs are 70 and 200 μg/kg and 10 and 30 μg/kg, respectively. The high LOD and LOQ of NIV with 70 and 200 μg/kg, respectively, are due to the low MS/MS sensitivity and are comparable with the limits reported by Ediage et al. [153]. The LODs and LOQs of the ENNs and BEA using the method presented here reveal 2–100 times higher sensitivity than those previously reported [154, 155].

Table 1: Overview on advanced LC-MS- and LC-MS/MS-based methods to study the multiple mycotoxins in cereals and cereal-based food.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Maize	NIV DON FUS-X ADONs 3ADON DAS HT2 T2 ZON/ZAN	CH_3CN-H_2O (84 : 16, v/v)	Clean-up columns, MycoSep® #226 and #227 from Romer Labs®	(i) Eluent A H_2O-CH_3OH (80 : 20, v/v), eluent B H_2O-CH_3OH (10 : 90, v/v), both containing 5 mM NH_4CH_3COO- (ii) Gradient: 0.5 min 0% eluent B, linear gradient to 100% eluent B to 4.5 min, 100% eluent B to 7 min, 7.1 min 0% eluent B, reequilibration 3 min, total run 10 min	Thermo Electronaquasil® RP-18 column	3.7 0.8 1.6 0.9 3.8 0.3 1.0 0.3 0.9	18.3 2.7 5.6 3.5 13.4 1.1 3.5 0.8 3.2	[86]
Wheat and maize	18 trichothecene mycotoxins (i) Type-A trichothecenes (T-2, HT-2, T-2 triol, T-2 tetraol, DAS, MAS, NEO, DacVOL, and VOL) (ii) Type-B trichothecenes (DON, 3-acDON, 15-acDON, NIV, and FUSX) (iii) Type-D trichothecenes (SG, SH, RA, and VA)	Dilute and shoot	MycoSep®-226	(i) Eluent A CH_3OH-H_2O-CH_3COOH (10 : 89 : 1, v/v/v), eluent B CH_3OH-H_2O-CH_3COOH (97 : 2 : 1, v/v/v), both containing 5 mM NH_4CH_3COO- (ii) Gradient 2 min at 100% eluent A, linear increase to 100% eluent B within 12 min, held at 100% eluent B for 3 min, reequilibration at 100% eluent A for 4 min, total run 19 min	Gemini® C18 column, 150 × 4.6 mm i.d., 5 μm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (all from Phenomenex, Torrance, CA, US)	[0.064–0.41] [0.092–0.70] [0.20–0.38]	[0.22–1.4] [0.31–2.2] [0.67–1.3]	[145]
Maize, durum wheat, corn flakes, and maize crackers	DON AFG_2 AFG_1 AFB_2 AFB_1 FB_1 FB_2 OTA HT-2 T-2 ZEA	2-step extraction: (1) H_2O; (2) CH_3OH. Evaporation and redissolution in PBS before IAC	AOFZDT2TM column at 1-2 drops per second; the column was then washed with 20 mL	(i) Eluent A H_2O, eluent B CH_3OH, both containing 0.5% CH_3COOH and 1 mM NH_4CH_3COO- (ii) Gradient 3 min at 20% eluent B, jump to 40% eluent B, linear increase to 63% eluent B within 35 min, 63% eluent B for 11 min, reequilibration at 20% eluent B for 10 min, total run 59 min	Gemini1 C18 column (150 mm, 2 mm, 5 mm particles; Phenomenex, Torrance, CA, USA), preceded by a Gemini C18 guard column (4 mm, 2 mm, 5 mm particles)	4.2 0.8 0.4 0.3 0.6 1.1 0.4 0.6 1.9 1.5 0.7	n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d	[52]

Table 1: Continued.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Various foods and feed	ATB_1 ATB_2 ATG_1 ATG_2 ATM_1 T-2 HT-2 VCG CTN OTA 3-ADON 15-ADON FX ZON NIV DON	CH_3CN-H_2O (84 : 16, v/v)	Mycosep #226 and #228 Aflazon+ multifunctional cartridges	(i) Eluent A ESI+ 10 mM NH_4CH_3COO-, ESI− 0.1% 0.1% (v/v) aqueous NH_3, eluent B CH_3OH (ii) Gradient initially 20% eluent B, linear increase from 5.5 to 85% eluent B, 100% eluent B within 0.3 min, reequilibration for 2 min at 20% eluent B, total run 10 min	UPLCBEH C18 column (1.7 μm, 100 mm × 2.1 mm i.d., Waters)	0.003 0.003 0.003 0.006 0.003 0.060 0.006 0.006 0.101 0.064 0.182 0.182 0.152 0.091 0.182 0.212	0.01 0.01 0.01 0.02 0.01 0.20 0.02 0.02 0.35 0.21 0.60 0.60 0.50 0.30 0.60 0.70	[146]
Rice, corn, wheat, rye, oat, barley, infant cereals, soya, and corn gluten	$AflB_1$ $AflB_2$ $AflG_1$ $AflG_2$ DON NIV 15-AcDON FUSX NEO HT-2 T-2 FB_1 FB_2 ZON OTA	QuEChERS	n-hexane (5 mL) under agitation and centrifugation	(i) Eluent A 0.15% (v/v) HCOOH+ 10 mM NH_4HCOO-, eluent B 0.05% HCOOH (v/v) in CH_3OH (ii) Gradient: 0% eluent B at 1 min, linear increase to 100% eluent B until 15 min, 100% eluent B for 5 min, reequilibration at 0% eluent B for 5 min, total run 25 min	ZorbaxBonus-RP column 150 mm 2.1 mm i.d., 3.5 μm, equipped with a ZorbaxRB C8 guard column 12.5 mm, 2.1 mm i.d., 5 μm (both from Agilent Technologies, Geneva, Switzerland)	n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d	[1–10] [1–10] [1–10] [1–10] [50–250] [100–500] [50–250] [25–125] [25–125] [25–125] [25–125] [5–25] [50–250] [50–250] [50–250] [0.50–2.5]	[130]
Barley	3ADON, 15ADON, DON, DON-3-Glc, FUS-X, NIV, HT2, T2, DAS, NEO, AFs, OTA, FBs, STER, ZEN, penitrem A, BEA, Alternaria toxins, ergot alkaloids	QuEChERS (2 g sample, 10 mL 0.1% HCOOH in H_2O, 3 min shaking, 10 mL CH_3CN, 3 min shaking, 4 g $MgSO_4$, 1 g NaCl, shaking)	C18-SPE clean-up procedure was performed with Oasis HLB cartridges (150 mg) from Waters (Milford, MA, USA)	(i) Eluent A H_2O with 5 mM NH_4HCOO- and 0.1% HCOOH, eluent B CH_3OH (ii) Gradient: start with 5% eluent B, increase to 50% eluent B in 6 min, increase to 95% eluent B within 4 min, keep until 15 min of the run, reequilibration at 5% eluent B for 3 min	Acquity UPLC HSS T3 analytical column (100 mm, 2.1 mm i.d., 1.8 mm; Waters, Milford, MA, USA)	n.d	n.d	[133]

Table 1: Continued.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Barley and barley products	T-2, HT-2, T-2 triol, T-2 tetraol, DAS, NEO, DON, NIV, 3-AcDON, 15-AcDON, FUS-X	Acetonitrile : water (84/16)	A MycoSep® column (no. 226, CoringSystem Diagnostix, Germany)	A binary linear gradient was applied which consisted of eluent A (methanol + 5 mmol/l ammonium formate) and eluent B (water + 5 mmol/l ammonium formate) with a total flow rate of 0.4 ml/min: 0 min 95% B, 11 min 95% B, 22 min 35% B, 26 min 35% B, 27 min 95% B, 35 min 95% B	Synergi™ polar-RP® 150 × 2 mm, 4 μm (Phenomenex, Aschaffenburg, Germany)	[0.02–2.25]	n.d	[53]
Cereal and cereal products	NIV DON AFB_1 AFB_2 AFG_1 AFG_2 DAS FB_1 FB_2 HT-2 T-2 OTA BEA	Extraction with matrix solid-phase dispersion (MSPD) method	—	The gradient that started at 100% A (5 mM ammonium formate in water) and 0% B (5 mM ammonium formate in methanol) increased linearly to 100% B in 10 min, followed by a linear decrease to 80% B in 5 min, then to 70% B in 10 min. Afterwards, the initial conditions were maintained for 5 min.	GeminiNX C18 (150 mm, 4.6 mm I.D., 5 μm particle size) analytical column supplied by Phenomenex (Barcelona, Spain), preceded by aguard column C18 (4 mm, 2 mm I.D.)	n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d n.d	85.24 31.25 0.25 1.50 0.25 0.75 5.00 83.33 83.75 35.5 12.50 3.00 1.00	[136]
Maize, wheat, rye, oat, oat flakes, and flours (maize, wheat, rye, and oat)	MON	Acetonitrile/water (84/16)	1M hydrochloric acid from *n* exchanger material (SAX). The SAX column (Bond Elut-SAX, 500 mg, 3 mL) (Agilent Technologies, Böblingen, Germany)	Solvent A: 1% formic acid in methanol, and solvent B: 1% formic acid in water. The detection was set to 260 nm. An isocratic run at 20% A was performed for 10 min at a flow rate of 250 μL/min. An isocratic run at 95% A was used for 10 min	A 150 mm × 2.1 mm i.d., 5 μm, Synchronis HILIC with a 10 mm × 2.1 mm i.d. guard column (Thermo Scientific, Dreieich, Germany),a 150 mm × 2.1 mm i.d., 3.5 μm	0.7	2.5	[99]

Table 1: Continued.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Maize	FMs	Water/methanol (30/70)	Sep-Pak C18 cartridges	Gradient elution was performed using bidistilled water (eluent A) and acetonitrile (eluent B), both acidified with 0.2% formic acid: initial condition at 100% A, 0–5 min isocratic step, 5–30 min linear gradient to 100% B, 30–35 min isocratic step, 35-36 min linear gradient to 100% A and reequilibration step at 100% A for 14 min (total analysis time: 50 min)	A 250 × 2.1 mm i.d., 5 mm, XTerra C18; the flow rate was 0.2 ml/min	20	n.d	[156]
Cereals and cereal products (wheat, wheat-based noodles, rice, rice-based noodles, and corn)	NEO DAS T-2 HT-2 DON NIV 15-ac-DON FUSX	QuEChERS method	—	Mobile phase A consists of 1% acetic acid and 5 mM ammonium acetate in water and mobile phase B consisted of 1% acetic acid and 5 mM ammonium acetate in methanol. The gradient was changed to 80% mobile phase B over 10 min, and then maintained for 3 min. After 13 min of run time, the gradient was returned to 30% mobile phase B over 1 min	ZORBAX Eclipse XBD-C18, 2.1 mm, 100 mm, 1.8 mm (P.N. 928700-902) column	0.02 0.02 0.05 0.02 0.045 0.05 0.02 0.04	n.d n.d n.d n.d n.d n.d n.d n.d	[152]
Cereals (rice, wheat, oat, barley, and maize)	Acetonitrile/water/acetic acid (79/20/1)	AFs, OTA, ZEN, DON, FB_1, FB_2, T-2, HT-2	—	Different proportions of mobile phase consisted of methanol or acetonitrile and acetic acid (0-1%), different flow rates (0.2-0.3 mL/min)	A column, 150 mm, 4.6, 3 μm particle size C18 columns (Thermo Scientific, CA, USA)	[10^{-5}–0.02]	[2×10^{-5}–0.04]	[87]

Table 1: Continued.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Wheat	NIV DON FUSX 15-AcDON 3-AcDON DAS NEO HT-2 T-2 ZEN α-ZOL β-ZOL	Acetonitrile/water (84/16)	A SecurityGuard™ cartridge C18 (4.0 3.0 mm i.d. 5 μm).	Mobile phase A consisted of an $H_2O/CH_3OH/CH_3COOH$ mixture (89 : 10 : 1, v/v/v) containing 5 mM ammonium acetate, while mobile phase B: $H_2O/CH_3OH/CH_3COOH$ mixture (2 : 97 : 1, v/v/v) containing 5 mM ammonium acetate. The following gradient was applied: initial condition 55% B; 0–3 min, 70% B; 3–8 min, 100% B; 8–11 min constant at 100% B; 11–13 min returning to the initial conditions and maintain during 2 min 55% B.	Phenomenex (Castel Maggiore, Italy) Gemini C18 (150 mm, 2.0 mm, i.d. 5 μm particle size, 110A)	5.5 1 5.5 2 2 2 5.5 2 3 1.5 1.5 2	15 10 20 10 10 10 20 6 8 5 5 2.5	[143]
Maize and other cereals (sorghum, millet, rice, sesame, wheat, infant food, cuscus, cornflakes, and cookies)	AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, FB_1, FB_2, FB_3, OTA, DON, NIV, ZEN, MON, CIT, ENA, ENA1, ENB, ENB1, ENB2, BEA, STC	Acetonitrile/water/acetic acid (79/20/1)	—	Both eluents contained 5 mM ammonium acetate and were composed of methanol/water/acetic acid 10 : 89 : 1 (v/v/v; eluent A) or 97 : 2 : 1 (eluent B), respectively. After an initial time of 2 min at 100% A, the proportion of B was increased linearly to 100% within 12 min, followed by a hold-time of 3 min at 100% B and 4 min column reequilibration at 100% A	A Gemini C18 column, 150 × 4.6 mm, 5 μm particle size, equipped with a C18 4 × 3 mm guard cartridge, all from Phenomenex (Torrance, CA, USA).	[0.005–250]	n.d	[157]

Table 1: Continued.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Breakfast and infant cereals	FB_1, FB_2, FB_3	Acetonitrile/water (85/15)	A C18 security guard cartridge (4 mm × 2 mm i.d., 5 μm), both Phenomenex (Madrid, Spain)	The sing gradient elution with water as mobile phase A and methanol as mobile phase B, both containing 0.5% formic acid. After an isocratic step of 65% B for 3 min, it was gradually increased to 95% B in 4 min and held constant for 3 min. Afterwards, the initial conditions were maintained for 10 min	A Luna C18 analytical column (150 mm × 4.6 mm i.d., 5 μm) Phenomenex (Madrid, Spain)	n.d	n.d	[158]
Barley, maize breakfast cereals, and peanuts	AFB_1 AFB_2 AFG_1 AFG_2 FB_1 FB_2 FB_3 DON ZEN HT2 T2 OTA	CH_3CN-H_2O-CH_3COOH (79.5 : 20 : 0.5, v/v/v). Evaporation and redissolution in PBS before IAC		(i) Eluents A H_2O, eluent B CH_3OH, both containing 5 mM NH_4CH_3COO- (ii) Gradient 5% eluent B increased to 50% eluent B in 1 min, linear increase to 100% eluent B within 6 min, 100% eluent B to 8 min, at 8.1 min initial conditions 5% eluent B, reequilibration at 5% eluent B for 2 min, total run 10 min		0.05 0.05 0.05 0.05 5 5 5 1 1 0.5 0.5 0.1	0.1 0.1 0.1 0.1 10 10 10 5 5 1 1 0.25	[159]
Maize and maize-beer	69 mycotoxins	Extraction with many solvents (ACN/water/glacial acetic acid 79 : 20 : 1, v/v/v)	—	(i) Two eluting solvents (eluent A and eluent B) that each contained 5 mM ammonium acetate were prepared using MeOH/water/glacial acetic acid (10 : 89 : 1, v/v/v) (eluent A) and (97 : 2 : 1, v/v/v) (eluent B) (ii) After an initial time of 2 min at 100% eluent A, the proportion of eluent B was increased linearly to 50% within 2–5 min and to 100% within 5–14 min, followed by a holding-time of 4 min at 100% eluent B and 2.5 min column reequilibration at 100% eluent A	A Gemini C18 column (Phenomenex, Torrance, CA, US).	[0.05–0.14]	[3–41]	[92]

Table 1: Continued.

Matrix	Analytes	Extraction	Clean-up step	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Maize and cereal-based products	AFB_1 AFB_2 AFG_1 AFG_2 DON FB_1 FB_2 HT2 OTA T2 ZEN	Raw extract	—	(i) Eluent A H_2O–HCOOH (99.9 : 0.1, v/v), eluent B CH_3OH–HCOOH (99.9 : 0.1, v/v) both containing 5 mM NH_4HCOO- (ii) Gradient: 0.5 min at 30% eluent B, linear increase to 100% eluent B in 7.5 min, hold at 100% eluent B for 1.5 min, at 9.6 min back to 30% eluent B, reequilibration at 30% eluent B for 2 min, total run 11.5 min	A ZORBAX RRHD Eclipse Plus C18 (100 × 2.1 mm, 1.8 μm) column from Agilent Technologies	0.04 0.04 0.2 0.1 3.4 1.4 1.3 0.8 0.1 0.1 1.2	0.1 0.1 0.1 0.4 1 4.3 3.9 2.5 0.4 0.2 2.9	[160]
Barley, malt, oat, wheat, and maize	DON 3-ADON 15-ADON HT2 T2 BEA FUSX NIV ZEA	CH_3CN-H_2O (84 : 16, v/v),	—	(i) Eluent A H_2O-HCOOH (99.9 : 0.1, v/v), eluent B CH_3OH-HCOOH (99.9 : 0.1, v/v); gradient ESI– 2 min at 10% eluent B, linear increase to 99% eluent B in 6 min, hold at 99% eluent B for 7.5 min, for 2 min back to 10% eluent B, reequilibration at 10% eluent B for 9.5 min, total run 25 min; ESI+ 2 min at 10% eluent B, linear increase to 87% eluent B in 6 min, hold at 87% eluent B for 7 min, increase to 100% eluent B in 5 min, hold at 100% eluent B for 3.5 min, for 2 min back to 10% eluent B, reequilibration at 10% eluent B for 9.5 min.	A Shimadzu LC-20A Prominence system (Shimadzu, Kyoto, Japan) using a Hydrosphere RP-C18 column (150 × 3.0 mm^2, S-3 μm, 12 nm, YMC Europe GmbH, Dinslaken, Germany).	0.9 1.7 4.6 0.1 0.2 0.3 5 5 0.5	2.6 13.5 13.5 0.2 0.5 0.7 3.1 2.9 1.5	[95]

Some of the most common methods used for both mycotoxin identification and quantification are summarized in Table 1 in terms of chromatographic conditions (mobile phase and gradient and analytical column), detection, and quantification in each method for different cereal matrices.

4. Hidden Mycotoxins Characterization

Mycotoxin derivatives that are undetectable by conventional analytical techniques are designated masked mycotoxins [161, 162]. Chemical transformations that generate masked mycotoxins are catalyzed by plant enzymes [38]. The group of masked mycotoxins comprises both extractable conjugated and bound (nonextractable) varieties. Bound mycotoxins are covalently or noncovalently attached to polymeric carbohydrate or protein matrices [38, 39]. Extractable conjugated mycotoxins can be detected by appropriate analytical methods when their structure is known and analytical standards are available. Bound mycotoxins, however, are not directly accessible and have to be unconventional from the matrix by chemical or enzymatic treatment before chemical analysis.

Among all modified mycotoxins, most occurrence data exist for deoxynivalenol-3-β-d-glucopyranoside (D3G), which was detected in naturally contaminated maize and wheat for the first time in 2005 [163]. Cereal contamination with D3G was reported to occur worldwide according to surveys from the UK, [164], the Czech Republic [165], China [166], and Canada [167]. Subsequent surveys showed intermittently high contaminations of D3G exceeding 1000 μg/kg in naturally contaminated wheat [122]. D3G also has been detected in oats and barley [122, 168].

D3G was noticed in wheat bread; nevertheless, the levels were below the LOQ (100 μg/kg). Using a more sensitive method, 80% of 116 flour, breakfast cereal, and snack samples from the Czech market analyzed were found to be contaminated with D3G at concentrations ranging from 5 to 72 μg/kg [164]. Interestingly, Sasanya et al. [169] reported that some wheat samples contained significantly higher values (up to 2.7 fold) of D3G compared to DON. The linearity (r^2) of D3G was 0.914; recovery was 70.0%, while LOQ and LOD were 1 and 0.5 1 g/kg, respectively [169]. On the other hand, Berthiller et al. [162] reported a method detection limit of 0.012 g/ml for D3G in purified sample extracts, corresponding to 0.02 g/g in contaminated cereals. Berthiller et al. [162] also estimated their LOD from the signal intensity of their standards, based on the limited ion suppression they observed. The pigment LOQ and LOD were 4.3 and 0.0005 g/kg, respectively. Good linearity for the pigment standard curve (R^2 0.999) was also observed [162].

Suman et al. [170] reported the development of a liquid chromatography/linear ion trap mass spectrometry method capable of determining D3G. Samples were extracted with a mixture of methanol/water (80 : 20; v/v) and cleaned up using immunoaffinity columns. Chromatographic separation was performed using a core-shell C_{18} column with an aqueous acetic acid/methanol mixture as the mobile phase under gradient conditions. The method was in-house validated on a bread matrix as follows: matrix-matched linearity ($r^2 > 0.99$) was recognized in the range of 10–200 μg/kg; trueness expressed as recovery was close to 90%; good intermediate precision (overall RSD < 9%) and adequate LOD and LOQ limits (4 and 11 μg/kg, respectively) were realized. The reliability of the method was finally demonstrated in bread, cracker, biscuit, and minicake commodities, resulting in relatively low levels of DON-3G, which were not higher than 30 μg/kg [170].

Dall'Asta et al. [171] developed an LC-ESI-MS/MS method for the simultaneous detection of the main fumonisins and their hydrolyzed derivatives allowing for a simplified sample preparation without previous clean-up. The method has a very low LOQ (10 μg/kg for FB_1, 12 μg/kg for FB_2 and FB_3, 70 μg/kg for HFB_1, HFB_2, and HFB_3 in maize flour) and a very good recovery for all the analytes. The sensitivity was good for all the considered analytes being the LOD and LOQ values comparable with those from other recently published LC-MS/MS methods, although those methods required a sample purification and preconcentration step [88, 89, 172]. Bound fumonisins were found to be present not only in thermally treated maize-based products but also in mild processed or even raw products (pasta, bread, cakes, crisps, and flour) and they were always present in almost similar or even higher amounts than the free forms [171]. Osborne fractions of maize proteins showed that fumonisins were particularly bound to prolamins and glutelins [171].

Hu et al. [173] investigated free and hidden fumonisins in raw maize and maize-based products from China. A total of 58 samples were analyzed using LC-MS/MS. Among all the samples, 66% were contaminated with free fumonisins above limits of quantitation, and a higher percentage of 86% was found for total fumonisins (free + hidden). The response functions for FB_1, FB_2, HFB_1, and HFB_2 showed that all the R^2 were greater than 0.99, suggesting good linearity. The LODs of FBs and HFBs were between 6 and 7 μg/kg, and the LOQs were between 23 and 28 μg/kg [173]; these results showed that the present method was about 4 times more sensitive than that reported by Oliveira et al. [174]. In comparison, by using isotope-labeled internal standards, Bryła et al. [175] found LOQs of 22 μg/kg for HFBs, which were similarly sensitive as the study of Hu et al. [173].

Andrade et al. [176] have validated multimycotoxin method based on extraction with acidified acetonitrile and LC-ESI^+-MS/MS analysis. The LOQs ranged from 0.5 to 121 μg/kg and proved to be suitable for the multimycotoxin analysis in wheat, maize, and rice products. Bound/hidden fumonisins were determined after extraction of the free forms using the multimycotoxin method, followed by a basic hydrolysis of the unextracted bound/hidden and solid-liquid extraction with low temperature purification (SLE-LTP). Recoveries for HFB_1, HFB_2, and HFB_3 were evaluated in six replicates fortified with the prepared standards at levels of 1.2, 1.8, and 2.5 g/kg, respectively. Recoveries were 75.6% (RSD of 6.6%) for HFB_1, 108.0% (RSD of 10.6%) for HFB_2, and 74.9% (RSD of 12.2%) for HFB_3.

Table 2: Overview on advanced LC–MS- and LC-MS/MS-based methods to study the multiple masked mycotoxins in cereals and cereal-based food.

Matrix	Extraction	Clean-up step	Analytes	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Cereal biscuits, Cocoa biscuits, minicake, crackers, wholemeal crackers, bread, and wholemeal bread	Methanol/water (80 : 20) mixture	2% v/v sodium hypochlorite solution treatment	DON-3G	Linear binary gradient with the following solvents: A was made by water (0.5% acetic acid), B was made by 100% methanol (0.5% acetic acid). Gradient elution 0–3 min, isocratic step 10% B; 3–21 min to 40% B; 21–27 min to 60% B; 27–30 min to 10% B; finally, a reequilibration step at 10% B	Kinetex C18 column (2.6 lm; 100 A;150 mm 2.10 mm; Phenomenex, Torrance, CA, USA)	4	11	[170]
Corn flakes, corn-based breakfast cereals , corn chips, and tortilla chips	Methanol-acetonitrile-water (25 : 25 : 50, v/v/v)	10 ml of a combined mixture of 10 ml filtrate and 10 ml phosphate-buffered saline (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 1.16 g Na_2HPO_4, 8 g NaCl, and 1 g NaN_3 in 1 L H_2O (pH 7.0 with 1N HCl) were added to a FumoniTest™ immunoaffinity column (IAC)	Hydrolyzed fumonisin B_1 Hydrolyzed fumonisin B_2	Acetonitrile-methanol (50 : 50, v/v) (Eluent A) and 0.02% aqueous formic acid (v/v) (Eluent B). Gradient elution: 0–1 min 35% A; 1% 10 min 60% A; and 10-16 min: maintained 60%.	HPLC MS: A Quatro II tandem mass spectrometer with electrospray ionization (ESI) in positive-ion mode (Micromass, Manchester, UK) interfaced with a HP 1100 LC system (Agilent Technologies, PaloAlto, CA, USA) equipped with Synergi Polar PR column (15 cm, 2 mm, 4 mm)	0.001 0.0015	n.d n.d	[177]
Maize and maize-based products	Acetonitrile : water 1 : 1	—	HFB_1 HFB_2 HFB_3	Water (eluent A) and methanol (eluent B), both acidified with 0.1% formic acid: 0–3 min, isocratic step 100% A, switched to the waste in order to wash out the salts and to focus the analytes on the C18 precolumn cartridge; 3–5 min to 45% B; 5–10 min isocratic 45% B; 10–25 min to 85% B, 25–35 min isocratic 85% B; finally, a reequilibration step at 100% A.	LC-MS/MSColumn C18 XTerra Waters narrow bore (250 × 2.1 mm, 5 μm) equipped with a C18 precolumn cartridge	20 25 20	70 70 70	[171]

Table 2: Continued.

Matrix	Extraction	Clean-up step	Analytes	LC conditions	Chromatographic column	Sensitivity (μg/kg)		References
						LOD	LOQ	
Gluten-free products	Water/methanol (30 : 70 v/v)		Hydrolyzed FB_1, FB_2 and FB_3	A: water and B: methanol, both acidified with 0.2% formic acid: 0–2 min, isocratic step 30% B, and to focus the analytes on the C18 precolumn cartridge; 2–5 min to 45% B; 5–25 min to 90% B; 25–35 min isocratic step 90% B, 35-36 min to 30% B; finally, a reequilibration step at 30% B (initial conditions) for 20 min	LC-MS/MS: C18 XTerraWaters narrow bore (250 mm, 62.1 mm, 5 lm) equipped with a C18 precolumn cartridge	20	70	[177]
Raw maize and maize-based products	10 mL of 2M NaOH		HFB_1 HFB_2	A (0.1% formic acid in water) and B (methanol). (i) 0~3.0 min, 40% B; 3.0~10.0 min, 40%~100% B; 10.0~11.0 min, 100% B; 11.0~12.0 min, 00%~40% B; 12.0~15.0 min, 40% B	Agilent EclipseXDB-C8 column (150 mm × 4.6 mm, 5 μm i.d., Agilent, Santa Clara, CA, USA)	6 6	23 23	[173]
Cereal and maize products	10 mL of KOH (2M)	—	HFB_1	Water (A) and methanol (B). The gradient started at 40% B; held for 1 min; increased to 86% B in 11 min, held for 2 min; increased to 95% B in 2 min and held for 4 min	LC-MS/MS Gemini C18 analytical column (150 × 4.6 mm, 5 m) preceded by a C18 security guard cartridge (4.0 × 3.0 mm, 5 m), both from Phenomenex®	n.d	[0.5–121]	[176]
Wheat	MeOH : dichlormethane, 50 : 50	Strata-X^R	DON-3-glucoside (D3G)	MeOH/water, 70 : 30	QP8000^R MS/MSSynergyR fusion 150 × 4.6 × 4	0.5	1	[169]
Malts	ACN : water, 84 : 16	Mycosep^R 226	D3G	MeOH/water	LCQ^R MS/MS:Synergy^R hydroRP 100 × 3 × 4	0.5	5	[168]
Malts	ACN : water, 84 : 16	—	D3G	MeOH/water	LCQ^R MS/MSSynergyR hydroRP 100 × 3 × 4	n.d	[1–2.5]	[178]

Table 2 presents a well-detailed description of the analytical method mentioned above for masked mycotoxin from cereals and related foodstuffs.

5. Conclusion

Cereals and related foodstuffs could be contaminated by diverse toxin-producing fungal species that are linked to severe and chronic toxic effects for both humans and animals. Consequently, many successful methods, such as LC-MS/MS, have been identified in this area. LC-MS/MS continues to play a central role in the determination of mycotoxins in cereals and related foodstuffs unless a drastically different approach to distinct complex mixtures is advanced. In this context, smaller amounts of samples can be processed faster than ever. To quantify free and masked mycotoxins in cereals and related foodstuffs, separation stayed as important as ever. The great increases in sensitivity and selectivity of LC-MS instruments have made a significant contribution in qualitative and quantitative determination of mycotoxins in in cereals and related foodstuffs. In this line, the increasing use of hybrid mass spectrometers, incorporating mass analyzers that are capable of high mass resolution and accurate mass measurements, mitigates some of the problems associated with selectivity and identification, but further technological development of LC-MS interfaces is required to minimize matrix effects. However, maintaining confidence in the assignment of identity and isobaric interference are still the major limitations for LC-MS methods used for the quantification and identification of mycotoxins in cereals and related foodstuffs. Eventually, interested chemists could keep continuing research and contribute to develop and suggest new and advanced analytical techniques to ensure higher sensitivity and obtain solutions to several issues related to mycotoxins.

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 no conflicts of interest.

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References

[1] H. J. Lee and D. Ryu, "Worldwide occurrence of mycotoxins in cereals and cereal-derived food products: public health perspectives of their co-occurrence," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 33, pp. 7034–7051, 2017.

[2] S. Mishra, K. M. Ansari, P. D. Dwivedi, H. P. Pandey, and M. Das, "Occurrence of deoxynivalenol in cereals and exposure risk assessment in Indian population," *Food Control*, vol. 30, no. 2, pp. 549–555, 2013.

[3] A. Nematollahi, M. Kamankesh, H. Hosseini, J. Ghasemi, F. Hosseini-Esfahani, and A. Mohammadi, "Investigation and determination of acrylamide in the main group of cereal products using advanced microextraction method coupled with gas chromatography-mass spectrometry," *Journal of Cereal Science*, vol. 87, pp. 157–164, 2019.

[4] A. M. Khaneghah, A. Farhadi, A. Nematollahi, Y. Vasseghian, and Y. Fakhri, "A systematic review and meta-analysis to investigate the concentration and prevalence of trichothecenes in the cereal-based food," *Trends in Food Science & Technology*, vol. 102, pp. 193–202, 2020.

[5] A. Heshmati, T. Zohrevand, A. M. Khaneghah, A. S. Mozaffari Nejad, and A. S. Sant'Ana, "Co-occurrence of aflatoxins and ochratoxin A in dried fruits in Iran: dietary exposure risk assessment," *Food and Chemical Toxicology*, vol. 106, pp. 202–208, 2017.

[6] N. N. A. Kyei, D. Boakye, and S. Gabrysch, "Maternal mycotoxin exposure and adverse pregnancy outcomes: a systematic review," *Mycotoxin Research*, vol. 36, no. 2, pp. 243–255, 2020.

[7] S. Agriopoulou, E. Stamatelopoulou, and T. Varzakas, "Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods," *Foods*, vol. 9, no. 2, pp. 137–184, 2020.

[8] S. Marin, A. J. Ramos, G. Cano-Sancho, and V. Sanchis, "Mycotoxins: occurrence, toxicology, and exposure assessment," *Food and Chemical Toxicology*, vol. 60, pp. 218–237, 2013.

[9] H. Kebede, H. Abbas, D. Fisher, and N. Bellaloui, "Relationship between aflatoxin contamination and physiological responses of corn plants under drought and heat stress," *Toxins*, vol. 4, no. 11, pp. 1385–1403, 2012.

[10] H. Hussein and J. M. Brasel, "Toxicity, metabolism, and impact of mycotoxins on humans and animals," *Toxicology*, vol. 167, no. 2, pp. 101–134, 2001.

[11] L. Aldars-García, M. Berman, J. Ortiz, A. J. Ramos, and S. Marín, "Probability models for growth and aflatoxin B1 production as affected by intraspecies variability in Aspergillus flavus," *Food Microbiology*, vol. 72, pp. 166–175, 2018.

[12] P. Rodrigues, A. Venâncio, and N. Lima, "Mycobiota and mycotoxins of almonds and chestnuts with special reference to aflatoxins," *Food Research International*, vol. 48, no. 1, pp. 76–90, 2012.

[13] H. Rastegar, S. Shoeibi, H. Yazdanpanah et al., "Removal of aflatoxin B1 by roasting with lemon juice and/or citric acid in contaminated pistachio nuts," *Food Control*, vol. 71, pp. 279–284, 2017.

[14] A. Reyneri, "The role of climatic condition on micotoxin production in cereal," *Veterinary Research Communications*, vol. 30, no. 1, pp. 87–92, 2006.

[15] A. M. Khaneghah, Y. Fakhri, H. M. Gahruie, M. Niakousari, and A. S. Sant'Ana, "Mycotoxins in cereal-based products during 24 years (1983–2017): a global systematic review," *Trends in Food Science & Technology*, vol. 91, pp. 95–105, 2019.

[16] I. Kamika, K.-t.-N. Ngbolua, and M. Tekere, "Occurrence of aflatoxin contamination in maize throughout the supply chain in the democratic republic of Congo," *Food Control*, vol. 69, pp. 292–296, 2016.

[17] L. Escrivá, S. Oueslati, G. Font, and L. Manyes, "Alternaria mycotoxins in food and feed: an overview," *Journal of Food Quality*, vol. 2017, Article ID 1569748, 20 pages, 2017.

[18] V. G. G. Uka, G. G. N. Moore, D. Arroyo-Manzanares, S. M. D. G. Nebija, G. De Saeger, and J. D. Di Mavungu,

"Secondary metabolite dereplication and phylogenetic analysis identify various emerging mycotoxins and reveal the high intra species diversity in Aspergillus flavus," *Frontiers in Microbiology*, vol. 10, p. 667 686, 2019.

[19] M. R. Greeff-Laubscher, I. Beukes, G. J. Marais, and K. Jacobs, "Mycotoxin production by three different toxigenic fungi genera on formulated abalone feed and the effect of an aquatic environment on fumonisins," *Mycology*, vol. 11, no. 2, pp. 105–117, 2020.

[20] F. B. Campagnollo, K. C. Ganev, A. M. Khaneghah et al., "The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: a review," *Food Control*, vol. 68, pp. 310–329, 2016.

[21] A. M. Khaneghah, L. M. Martins, A. M. von Hertwig, R. Bertoldo, and A. S. Sant'Ana, "Deoxynivalenol and its masked forms: characteristics, incidence, control and fate during wheat and wheat based products processing—a review," *Trends in Food Science & Technology*, vol. 71, pp. 13–24, 2018.

[22] V. Kagot, S. Okoth, M. De Boevre, and S. De Saeger, "Biocontrol of Aspergillus and Fusarium mycotoxins in Africa: benefits and limitations," *Toxins*, vol. 11, no. 2, pp. 109–117, 2019.

[23] A. Alshannaq and J.-H. Yu, "Occurrence, toxicity, and analysis of major mycotoxins in food," *International Journal of Environmental Research and Public Health*, vol. 14, no. 6, pp. 632–651, 2017.

[24] B. R. Rushing and M. I. Selim, "Aflatoxin B1: a review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods," *Food and Chemical Toxicology*, vol. 124, pp. 81–100, 2019.

[25] L. Gambacorta, D. Magistà, G. Perrone, S. Murgolo, A. F. Logrieco, and M. Solfrizzo, "Co-occurrence of toxigenic moulds, aflatoxins, ochratoxin A, Fusarium and Alternaria mycotoxins in fresh sweet peppers (Capsicum annuum) and their processed products," *World Mycotoxin Journal*, vol. 11, no. 1, pp. 159–174, 2018.

[26] S. W. Gratz, R. Dinesh, T. Yoshinari et al., "Masked trichothecene and zearalenone mycotoxins withstand digestion and absorption in the upper GI tract but are efficiently hydrolyzed by human gut microbiota in vitro," *Molecular Nutrition & Food Research*, vol. 61, no. 4, Article ID 1600680, 2017.

[27] L. Escrivá, G. Font, L. Manyes, and H. Berrada, "Studies on the presence of mycotoxins in biological samples: an overview," *Toxins*, vol. 9, no. 8, pp. 251–283, 2017.

[28] G. Winter and L. Pereg, "A review on the relation between soil and mycotoxins: effect of aflatoxin on field, food and finance," *European Journal of Soil Science*, vol. 70, pp. 882–897, 2019.

[29] S. A. O. Adeyeye, "Aflatoxigenic fungi and mycotoxins in food: a review," *Critical Reviews in Food Science and Nutrition*, vol. 60, no. 5, pp. 709–721, 2020.

[30] G. Conte, M. Fontanelli, F. Galli, L. Cotrozzi, L. Pagni, and E. Pellegrini, "Mycotoxins in feed and food and the role of ozone in their detoxification and degradation: an update," *Toxins*, vol. 12, no. 8, pp. 486–404, 2020.

[31] J. M. Oteiza, A. M. Khaneghah, F. B. Campagnollo et al., "Influence of production on the presence of patulin and ochratoxin A in fruit juices and wines of Argentina," *LWT*, vol. 80, pp. 200–207, 2017.

[32] R. Köppen, M. Koch, D. Siegel, S. Merkel, R. Maul, and I. Nehls, "Determination of mycotoxins in foods: current state of analytical methods and limitations," *Applied Microbiology and Biotechnology*, vol. 86, no. 6, pp. 1595–1612, 2010.

[33] I. Slobodchikova and D. Vuckovic, "Liquid chromatography—high resolution mass spectrometry method for monitoring of 17 mycotoxins in human plasma for exposure studies," *Journal of Chromatography A*, vol. 1548, pp. 51–63, 2018.

[34] K. Jiang, Q. Huang, K. Fan et al., "Reduced graphene oxide and gold nanoparticle composite-based solid-phase extraction coupled with ultra-high-performance liquid chromatography-tandem mass spectrometry for the determination of 9 mycotoxins in milk," *Food Chemistry*, vol. 264, pp. 218–225, 2018.

[35] S. A. Tittlemier, B. Cramer, C. Dall'Asta et al., "Developments in mycotoxin analysis: an update for 2017-2018," *World Mycotoxin Journal*, vol. 12, no. 1, pp. 3–29, 2019.

[36] V. Moreno, M. Zougagh, and Á. Ríos, "Hybrid nanoparticles based on magnetic multiwalled carbon nanotube-nano$C_{18}SiO_2$ composites for solid phase extraction of mycotoxins prior to their determination by LC-MS," *Microchimica Acta*, vol. 183, no. 2, pp. 871–880, 2016.

[37] H. Zhao, X. Chen, C. Shen, and B. Qu, "Determination of 16 mycotoxins in vegetable oils using a QuEChERS method combined with high-performance liquid chromatography-tandem mass spectrometry," *Food Additives & Contaminants: Part A*, vol. 34, no. 2, pp. 255–264, 2017.

[38] F. Berthiller, C. Crews, C. Dall'Asta et al., "Masked mycotoxins: a review," *Molecular Nutrition & Food Research*, vol. 57, no. 1, pp. 165–186, 2013.

[39] F. Berthiller, R. Schuhmacher, G. Adam, and R. Krska, "Formation, determination and significance of masked and other conjugated mycotoxins," *Analytical and Bioanalytical Chemistry*, vol. 395, no. 5, pp. 1243–1252, 2009.

[40] P. Kovalsky, G. Kos, K. Nährer et al., "Co-occurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished feed and maize-an extensive survey," *Toxins*, vol. 8, no. 12, pp. 363–391, 2016.

[41] A. M. Khaneghah, M. H. Kamani, Y. Fakhri, C. F. S. C. Coppa, C. A. F. de Oliveira, and A. S. Sant'Ana, "Changes in masked forms of deoxynivalenol and their co-occurrence with culmorin in cereal-based products: a systematic review and meta-analysis," *Food Chemistry*, vol. 294, pp. 587–596, 2019.

[42] B. Kluger, C. Bueschl, M. Lemmens et al., "Stable isotopic labelling-assisted untargeted metabolic profiling reveals novel conjugates of the mycotoxin deoxynivalenol in wheat," *Analytical and Bioanalytical Chemistry*, vol. 405, no. 15, pp. 5031–5036, 2013.

[43] E. Varga, G. Wiesenberger, C. Hametner et al., "New tricks of an old enemy: isolates of Fusarium graminearum produce a type A trichothecene mycotoxin," *Environmental Microbiology*, vol. 17, no. 8, pp. 2588–2600, 2015.

[44] M. De Boevre, J. D. Di Mavungu, P. Maene et al., "Development and validation of an LC-MS/MS method for the simultaneous determination of deoxynivalenol, zearalenone, T-2-toxin and some masked metabolites in different cereals and cereal-derived food," *Food Additives & Contaminants: Part A*, vol. 29, no. 5, pp. 819–835, 2012.

[45] M. De Boevre, L. Jacxsens, C. Lachat et al., "Human exposure to mycotoxins and their masked forms through cereal-based foods in Belgium," *Toxicology Letters*, vol. 218, no. 3, pp. 281–292, 2013.

[46] H. Nakagawa, K. Ohmichi, S. Sakamoto et al., "Detection of a new Fusarium masked mycotoxin in wheat grain by high-

resolution LC-Orbitrap™ MS," *Food Additives & Contaminants: Part A*, vol. 28, no. 10, pp. 1447–1456, 2011.

[47] H. E. Ok, S.-W. Choi, H. J. Chang, M.-S. Chung, and H. S. Chun, "Occurrence of five 8-ketotrichothecene mycotoxins in organically and conventionally produced cereals collected in Korea," *Food Control*, vol. 22, no. 10, pp. 1647–1652, 2011.

[48] B. Maestroni and A. Cannavan, "Sampling strategies to control mycotoxins," in *Determining Mycotoxins and Mycotoxigenic Fungi in Food and Feed*, pp. 3–36, Woodhead Publishing, Cambridge, UK, 2011.

[49] A. C. Weaver, N. Adams, and A. Yiannikouris, "Invited review: use of technology to assess and monitor multi-mycotoxin and emerging mycotoxin challenges in feedstuffs," *Applied Animal Science*, vol. 36, no. 1, pp. 19–25, 2020.

[50] European Union, "Commission regulation (EU) no. 519/2014 of 16 May 2014 amending regulation (EC) no. 401/2006 as regards methods of sampling of large lots, spices and food supplements, performance criteria for T-2, HT-2 toxin and citrinin and screening methods of analysis," *Official Journal of the European Union*, vol. 29, no. L147, pp. 29–43, 2014.

[51] D. Garon, E. Richard, L. Sage, V. Bouchart, D. Pottier, and P. Lebailly, "Mycoflora and multimycotoxin detection in corn silage: experimental study," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 9, pp. 3479–3484, 2006.

[52] V. M. T. Lattanzio, M. Solfrizzo, S. Powers, and A. Visconti, "Simultaneous determination of aflatoxins, ochratoxin A and Fusarium toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup," *Rapid Communications in Mass Spectrometry*, vol. 21, no. 20, pp. 3253–3261, 2007.

[53] M. Sulyok, F. Berthiller, R. Krska, and R. Schuhmacher, "Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize," *Rapid Communications in Mass Spectrometry*, vol. 20, no. 18, pp. 2649–2659, 2006.

[54] L. Zhang, X.-W. Dou, C. Zhang, A. Logrieco, and M.-H. Yang, "A review of current methods for analysis of mycotoxins in herbal medicines," *Toxins*, vol. 10, no. 2, pp. 65–103, 2018.

[55] G. P. Munkvold, S. Arias, I. Taschl, and C. Gruber-Dorninger, "Mycotoxins in corn: occurrence, impacts, and management," in *Corn*, pp. 235–287, AACC International Press, Saint Paul, MN, USA, 2019.

[56] V. L. Pereira, J. O. Fernandes, and S. C. Cunha, "Mycotoxins in cereals and related foodstuffs: a review on occurrence and recent methods of analysis," *Trends in Food Science & Technology*, vol. 36, no. 2, pp. 96–136, 2014.

[57] V. Scarpino, A. Reyneri, and M. Blandino, "Development and comparison of two multiresidue methods for the determination of 17 Aspergillus and Fusarium mycotoxins in cereals using HPLC-ESI-TQ-MS/MS," *Frontiers in Microbiology*, vol. 10, pp. 361–371, 2019.

[58] N. W. Turner, H. Bramhmbhatt, M. Szabo-Vezse, A. Poma, R. Coker, and S. A. Piletsky, "Analytical methods for determination of mycotoxins: an update (2009–2014)," *Analytica Chimica Acta*, vol. 901, pp. 12–33, 2015.

[59] I. Reinholds, I. Pugajeva, E. Bogdanova, J. Jaunbergs, and V. Bartkevics, "Recent applications of carbonaceous nanosorbents for the analysis of mycotoxins in food by liquid chromatography: a short review," *World Mycotoxin Journal*, vol. 12, no. 1, pp. 31–43, 2019.

[60] J. Singh and A. Mehta, "Rapid and sensitive detection of mycotoxins by advanced and emerging analytical methods: a review," *Food Science & Nutrition*, vol. 8, no. 5, pp. 2183–2204, 2020.

[61] R. Colombo and A. Papetti, "Pre-concentration and analysis of mycotoxins in food samples by capillary electrophoresis," *Molecules*, vol. 25, no. 15, pp. 3441–3459, 2020.

[62] N. Ünüsan, "Systematic review of mycotoxins in food and feeds in Turkey," *Food Control*, vol. 97, pp. 1–14, 2019.

[63] A. Malachová, M. Stránská, M. Václavíková et al., "Advanced LC-MS-based methods to study the co-occurrence and metabolization of multiple mycotoxins in cereals and cereal-based food," *Analytical and Bioanalytical Chemistry*, vol. 410, no. 3, pp. 801–825, 2018.

[64] M. Amirahmadi, S. Shoeibi, H. Rastegar, M. Elmi, and A. Mousavi Khaneghah, "Simultaneous analysis of mycotoxins in corn flour using LC/MS-MS combined with a modified QuEChERS procedure," *Toxin Reviews*, vol. 37, no. 3, pp. 187–195, 2018.

[65] B. Zhang, X. Chen, S.-Y. Han et al., "Simultaneous analysis of 20 mycotoxins in grapes and wines from hexi corridor region (China): based on a QuEChERS-UHPLC-MS/MS method," *Molecules*, vol. 23, no. 8, pp. 1926–1942, 2018.

[66] M.-Y. Wu, M. E. Mead, M.-K. Lee et al., "Systematic dissection of the evolutionarily conserved WetA developmental regulator across a genus of filamentous fungi," *MBio*, vol. 9, no. 4, Article ID e01130, 2018.

[67] C. Santos Pereira, S. C. Cunha, and J. O. Fernandes, "Prevalent mycotoxins in animal feed: occurrence and analytical methods," *Toxins*, vol. 11, no. 5, pp. 290–351, 2019.

[68] H. Peng, Y. Chang, R. C. Baker, and G. Zhang, "Interference of mycotoxin binders with ELISA, HPLC and LC-MS/MS analysis of aflatoxins in maize and maize gluten," *Food Additives & Contaminants: Part A*, vol. 37, no. 3, pp. 496–506, 2020.

[69] H.-Y. Wang, Y.-L. Yang, Y. Wang, S.-F. Zhang, and L. Li, "Development of disk-shaped monolithic microplates for detecting multiple mycotoxins," *Analytical Methods*, vol. 11, no. 48, pp. 6084–6091, 2019.

[70] H. Shi, S. Li, Y. Bai, L. L. Prates, Y. Lei, and P. Yu, "Mycotoxin contamination of food and feed in China: occurrence, detection techniques, toxicological effects and advances in mitigation technologies," *Food Control*, vol. 91, pp. 202–215, 2018.

[71] M. A. Haque, Y. Wang, Z. Shen, X. Li, M. K. Saleemi, and C. He, "Mycotoxin contamination and control strategy in human, domestic animal and poultry: a review," *Microbial Pathogenesis*, vol. 142, Article ID 104095, 2020.

[72] A. Roseanu, L. Jecu, M. Badea, and R. W. Evans, "Mycotoxins: an overview on their quantification methods," *Romanian Journal of Biochemistry*, vol. 47, no. 1, pp. 79–86, 2010.

[73] S. Agriopoulou, E. Stamatelopoulou, and T. Varzakas, "Advances in analysis and detection of major mycotoxins in foods," *Foods*, vol. 9, no. 4, pp. 518–540, 2020.

[74] M. Pascale, A. De Girolamo, V. Lippolis, J. Stroka, H. G. J. Mol, and V. M. T. Lattanzio, "Performance evaluation of LC-MS methods for multimycotoxin determination," *Journal of AOAC International*, vol. 102, no. 6, pp. 1708–1720, 2019.

[75] S. J. Hird, B. P.-Y. Lau, R. Schuhmacher, and R. Krska, "Liquid chromatography-mass spectrometry for the determination of chemical contaminants in food," *TrAC: Trends in Analytical Chemistry*, vol. 59, pp. 59–72, 2014.

[76] P. Herrero, N. Cortés-Francisco, F. Borrull, J. Caixach, E. Pocurull, and R. M. Marcé, "Comparison of triple quadrupole mass spectrometry and orbitrap high-resolution mass spectrometry in ultrahigh performance liquid chromatography for the determination of veterinary drugs in sewage: benefits and drawbacks," *Journal of Mass Spectrometry*, vol. 49, no. 7, pp. 585–596, 2014.

[77] A. Kaufmann, P. Butcher, K. Maden, S. Walker, and M. Widmer, "Quantitative and confirmative performance of liquid chromatography coupled to high-resolution mass spectrometry compared to tandem mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 25, no. 7, pp. 979–992, 2011.

[78] A. R. Fontana, I. Rodríguez, M. Ramil, J. C. Altamirano, and R. Cela, "Liquid chromatography time-of-flight mass spectrometry following sorptive microextraction for the determination of fungicide residues in wine," *Analytical and Bioanalytical Chemistry*, vol. 401, no. 2, pp. 767–775, 2011.

[79] A. Kaufmann, P. Butcher, K. Maden, S. Walker, and M. Widmer, "Quantification of anthelmintic drug residues in milk and muscle tissues by liquid chromatography coupled to orbitrap and liquid chromatography coupled to tandem mass spectrometry," *Talanta*, vol. 58, no. 2, pp. 991–1000, 2011.

[80] T. Rejczak and T. Tuzimski, "Recent trends in sample preparation and liquid chromatography/mass spectrometry for pesticide residue analysis in food and related matrixes," *Journal of AOAC International*, vol. 98, no. 5, pp. 1143–1162, 2015.

[81] J. Rubert, K. J. James, J. Mañes, and C. Soler, "Applicability of hybrid linear ion trap-high resolution mass spectrometry and quadrupole-linear ion trap-mass spectrometry for mycotoxin analysis in baby food," *Journal of Chromatography A*, vol. 1223, pp. 84–92, 2012.

[82] J. Tolosa, G. Font, J. Mañes, and E. Ferrer, "Natural occurrence of emerging Fusarium mycotoxins in feed and fish from aquaculture," *Journal of Agricultural and Food Chemistry*, vol. 62, no. 51, pp. 12462–12470, 2014.

[83] M. Ghaste, R. Mistrik, and V. Shulaev, "Applications of Fourier transform ion cyclotron resonance (FT-ICR) and orbitrap based high resolution mass spectrometry in metabolomics and lipidomics," *International Journal of Molecular Sciences*, vol. 17, no. 6, pp. 816–837, 2016.

[84] M. Rychlik, B. Kanawati, C. Roullier-Gall et al., "Foodomics assessed by Fourier transform mass spectrometry," in *Fundamentals and Applications of Fourier Transform Mass Spectrometry*, pp. 651–677, Elsevier, Amsterdam, Netherlands, 2019.

[85] S. Biselli and C. Hummert, "Development of a multicomponent method forFusariumtoxins using LC-MS/MS and its application during a survey for the content of T-2 toxin and deoxynivalenol in various feed and food samples," *Food Additives and Contaminants*, vol. 22, no. 8, pp. 752–760, 2005.

[86] F. Berthiller, R. Schuhmacher, G. Buttinger, and R. Krska, "Rapid simultaneous determination of major type A- and B-trichothecenes as well as zearalenone in maize by high performance liquid chromatography-tandem mass spectrometry," *Journal of Chromatography A*, vol. 1062, no. 2, pp. 209–216, 2005.

[87] F. Soleimany, S. Jinap, and F. Abas, "Determination of mycotoxins in cereals by liquid chromatography tandem mass spectrometry," *Food Chemistry*, vol. 130, no. 4, pp. 1055–1060, 2012.

[88] C. Cavaliere, P. Foglia, E. Pastorini, R. Samperi, and A. Laganà, "Development of a multiresidue method for analysis of major Fusarium mycotoxins in corn meal using liquid chromatography/tandem mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 19, no. 14, pp. 2085–2093, 2005.

[89] B. Delmulle, S. De Saeger, A. Adams, N. De Kimpe, and C. Van Peteghem, "Development of a liquid chromatography/tandem mass spectrometry method for the simultaneous determination of 16 mycotoxins on cellulose filters and in fungal cultures," *Rapid Communications in Mass Spectrometry*, vol. 20, no. 5, pp. 771–776, 2006.

[90] M. Castro-Puyana, R. Pérez-Míguez, L. Montero, and M. Herrero, "Reprint of: application of mass spectrometry-based metabolomics approaches for food safety, quality and traceability," *TrAC: Trends in Analytical Chemistry*, vol. 96, pp. 62–78, 2017.

[91] G. Hopfgartner, E. Varesio, V. Tschäppät, C. Grivet, E. Bourgogne, and L. A. Leuthold, "Triple quadrupole linear ion trap mass spectrometer for the analysis of small molecules and macromolecules," *Journal of Mass Spectrometry*, vol. 39, no. 8, pp. 845–855, 2004.

[92] W. A. Abia, B. Warth, M. Sulyok et al., "Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS)," *Food Control*, vol. 31, no. 2, pp. 438–453, 2013.

[93] A. Malachová, M. Sulyok, E. Beltrán, F. Berthiller, and R. Krska, "Optimization and validation of a quantitative liquid chromatography-tandem mass spectrometric method covering 295 bacterial and fungal metabolites including all regulated mycotoxins in four model food matrices," *Journal of Chromatography A*, vol. 1362, pp. 145–156, 2014.

[94] E. Varga, T. Glauner, F. Berthiller, R. Krska, R. Schuhmacher, and M. Sulyok, "Development and validation of a (semi-) quantitative UHPLC-MS/MS method for the determination of 191 mycotoxins and other fungal metabolites in almonds, hazelnuts, peanuts and pistachios," *Analytical and Bioanalytical Chemistry*, vol. 405, no. 15, pp. 5087–5104, 2013.

[95] K. Habler and M. Rychlik, "Multi-mycotoxin stable isotope dilution LC-MS/MS method for Fusarium toxins in cereals," *Analytical and Bioanalytical Chemistry*, vol. 408, no. 1, pp. 307–317, 2016.

[96] H. R. Morris, T. Paxton, A. Dell et al., "High sensitivity collisionally-activated decomposition tandem mass spectrometry on a novel quadrupole/orthogonal-acceleration time-of-flight mass spectrometer," *Rapid Communications in Mass Spectrometry*, vol. 10, no. 8, pp. 889–896, 1996.

[97] A. Leitner, M. Faini, F. Stengel, and R. Aebersold, "Crosslinking and mass spectrometry: an integrated technology to understand the structure and function of molecular machines," *Trends in Biochemical Sciences*, vol. 41, no. 1, pp. 20–32, 2016.

[98] J. V. Olsen, B. Macek, O. Lange, A. Makarov, S. Horning, and M. Mann, "Higher-energy C-trap dissociation for peptide modification analysis," *Nature Methods*, vol. 4, no. 9, pp. 709–712, 2007.

[99] K. W. von Bargen, L. Lohrey, B. Cramer, and H. U. Humpf, "Analysis of the Fusarium mycotoxin moniliformin in cereal samples using $^{13}C_2$-moniliformin and high-resolution mass spectrometry," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 4, pp. 3586–3591, 2012.

[100] A. Michalski, E. Damoc, J. P. Hauschild et al., "Mass spectrometry-based proteomics using Q exactive, a high-

performance benchtop quadrupole orbitrap mass spectrometer," *Molecular & Cellular Proteomics*, vol. 10, no. 9, 2011.

[101] A. M. Evans, B. R. Bridgewater, Q. Liu et al., "High resolution mass spectrometry improves data quantity and quality as compared to unit mass resolution mass spectrometry in high-throughput profiling metabolomics," *Metabolomics*, vol. 4, no. 2, Article ID 1000132, 2014.

[102] V. M. T. Lattanzio, S. D. Gatta, M. Godula, and A. Visconti, "Quantitative analysis of mycotoxins in cereal foods by collision cell fragmentation-high-resolution mass spectrometry: performance and comparison with triple-stage quadrupole detection," *Food Additives & Contaminants: Part A*, vol. 28, no. 10, pp. 1424–1437, 2011.

[103] G. J. Patti, O. Yanes, and G. Siuzdak, "Metabolomics: the apogee of the omics trilogy," *Nature Reviews Molecular Cell Biology*, vol. 13, no. 4, pp. 263–269, 2012.

[104] H. Li, Y. Cai, Y. Guo, F. Chen, and Z.-J. Zhu, "MetDIA: targeted metabolite extraction of multiplexed MS/MS spectra generated by data-independent acquisition," *Analytical Chemistry*, vol. 88, no. 17, pp. 8757–8764, 2016.

[105] D. S. Wishart, D. Tzur, C. Knox et al., "HMDB: the human metabolome database," *Nucleic Acids Research*, vol. 35, pp. D521–D526, 2007.

[106] C. A. Smith, G. O. Maille, E. J. Want et al., "METLIN," *Therapeutic Drug Monitoring*, vol. 27, no. 6, pp. 747–751, 2005.

[107] H. Horai, M. Arita, S. Kanaya et al., "MassBank: a public repository for sharing mass spectral data for life sciences," *Journal of Mass Spectrometry*, vol. 45, no. 7, pp. 703–714, 2010.

[108] W. Jia, L. Shi, F. Zhang, C. Fan, J. Chang, and X. Chu, "Multiplexing data independent untargeted workflows for mycotoxins screening on a quadrupole-orbitrap high resolution mass spectrometry platform," *Food Chemistry*, vol. 278, pp. 67–76, 2019.

[109] J. B. Renaud and M. W. Sumarah, "Data independent acquisition-digital archiving mass spectrometry: application to single kernel mycotoxin analysis of Fusarium graminearum infected maize," *Analytical and Bioanalytical Chemistry*, vol. 408, no. 12, pp. 3083–3091, 2016.

[110] C. Nerin, P. Alfaro, M. Aznar, and C. Domeño, "The challenge of identifying non-intentionally added substances from food packaging materials: a review," *Analytica Chimica Acta*, vol. 775, pp. 14–24, 2013.

[111] X. Zhu, Y. Chen, and R. Subramanian, "Comparison of information-dependent acquisition, SWATH, and MSAll techniques in metabolite identification study employing ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry," *Analytical Chemistry*, vol. 86, no. 2, pp. 1202–1209, 2014.

[112] X. Domingo-Almenara, J. R. Montenegro-Burke, H. P. Benton, and G. Siuzdak, "Annotation: a computational solution for streamlining metabolomics analysis," *Analytical Chemistry*, vol. 90, no. 1, pp. 480–489, 2018.

[113] J.-Y. Cho, H.-J. Lee, S.-K. Jeong, and Y. K. Paik, "Epsilon-Q: an automated analyzer interface for mass spectral library search and label-free protein quantification," *Journal of Proteome Research*, vol. 16, no. 12, pp. 4435–4445, 2017.

[114] A. Michalski, J. Cox, and M. Mann, "More than 100,000 detectable peptide species elute in single shotgun proteomics runs but the majority is inaccessible to data-dependent LC-MS/MS," *Journal of Proteome Research*, vol. 10, no. 4, pp. 1785–1793, 2011.

[115] A. Panchaud, A. Scherl, S. A. Shaffer et al., "Precursor acquisition independent from ion count: how to dive deeper into the proteomics ocean," *Analytical Chemistry*, vol. 81, no. 15, pp. 6481–6488, 2009.

[116] H. Tsugawa, T. Cajka, T. Kind et al., "MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis," *Nature Methods*, vol. 12, no. 6, pp. 523–526, 2015.

[117] W. Zhang, R. Kiyonami, Z. Jiang, and W. Chen, "Quantitative analysis of targeted proteins in complex sample using novel data independent acquisition," *Chinese Journal of Analytical Chemistry*, vol. 42, no. 12, pp. 1750–1758, 2014.

[118] J. A. Vizcaíno, E. W. Deutsch, R. Wang et al., "ProteomeXchange provides globally coordinated proteomics data submission and dissemination," *Nature Biotechnology*, vol. 32, no. 3, pp. 223–226, 2014.

[119] C.-C. Tsou, D. Avtonomov, B. Larsen et al., "DIA-umpire: comprehensive computational framework for data-independent acquisition proteomics," *Nature Methods*, vol. 12, no. 3, pp. 258–264, 2015.

[120] J. D. Egertson, B. MacLean, R. Johnson, Y. Xuan, and M. J. MacCoss, "Multiplexed peptide analysis using data-independent acquisition and skyline," *Nature Protocols*, vol. 10, no. 6, p. 887, 2015.

[121] B. J. A. Berendsen, R. S. Wegh, T. Meijer, and M. W. F. Nielen, "The assessment of selectivity in different quadrupole-orbitrap mass spectrometry acquisition modes," *Journal of The American Society for Mass Spectrometry*, vol. 26, no. 2, pp. 337–346, 2014.

[122] F. Berthiller, C. Dall'asta, R. Corradini et al., "Occurrence of deoxynivalenol and its 3-β-D-glucoside in wheat and maize," *Food Additives & Contaminants: Part A*, vol. 26, no. 4, pp. 507–511, 2009.

[123] S. Y. Woo, S. Y. Ryu, F. Tian, S. Y. Lee, S. B. Park, and H. S. Chun, "Simultaneous determination of twenty mycotoxins in the Korean soybean paste doenjang by LC-MS/MS with immunoaffinity cleanup," *Toxins*, vol. 11, no. 10, pp. 594–512, 2019.

[124] T. Bessaire, C. Mujahid, P. Mottier, and A. Desmarchelier, "Multiple mycotoxins determination in food by LC-MS/MS: an international collaborative study," *Toxins*, vol. 11, no. 11, pp. 658–675, 2019.

[125] F. Al-Taher, J. Cappozzo, J. Zweigenbaum, H. J. Lee, L. Jackson, and D. Ryu, "Detection and quantitation of mycotoxins in infant cereals in the U.S. market by LC-MS/MS using a stable isotope dilution assay," *Food Control*, vol. 72, pp. 27–35, 2017.

[126] M. C. Spanjer, P. M. Rensen, and J. M. Scholten, "LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs," *Food Additives & Contaminants: Part A*, vol. 25, no. 4, pp. 472–489, 2008.

[127] M. Bolechová, K. Benešová, S. Běláková, J. Čáslavský, M. Pospíchalová, and R. Mikulíková, "Determination of seventeen mycotoxins in barley and malt in the Czech Republic," *Food Control*, vol. 47, pp. 108–113, 2015.

[128] F. Soleimany, S. Jinap, A. Faridah, and A. Khatib, "A UPLC-MS/MS for simultaneous determination of aflatoxins, ochratoxin A, zearalenone, DON, fumonisins, T-2 toxin and HT-2 toxin, in cereals," *Food Control*, vol. 25, no. 2, pp. 647–653, 2012.

[129] M. I. da Costa Marinho, A. I. G. Costa, N. M. Vieira, M. C. G. Paiva, F. C. L. de Freitas, and A. A. da Silva, "Validation and application of a QuEChERS based method for estimation of half-life of imidazolinone herbicides in soils

by LC-ESI-MS/MS," *Ecotoxicology and Environmental Safety*, vol. 167, pp. 212–217, 2019.

[130] A. Desmarchelier, J.-M. Oberson, P. Tella, E. Gremaud, W. Seefelder, and P. Mottier, "Development and comparison of two multiresidue methods for the analysis of 17 mycotoxins in cereals by liquid chromatography electrospray ionization tandem mass spectrometry," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 13, pp. 7510–7519, 2010.

[131] C. Cavaliere, G. D'Ascenzo, P. Foglia, E. Pastorini, R. Samperi, and A. Laganà, "Determination of type B trichothecenes and macrocyclic lactone mycotoxins in field contaminated maize," *Food Chemistry*, vol. 92, no. 3, pp. 559–568, 2005.

[132] P. J. Fernandes, N. Barros, J. L. Santo, and J. S. Câmara, "High-throughput analytical strategy based on modified QuEChERS extraction and dispersive solid-phase extraction clean-up followed by liquid chromatography-triple-quadrupole tandem mass spectrometry for quantification of multiclass mycotoxins in cereals," *Food Analytical Methods*, vol. 8, no. 4, pp. 841–856, 2015.

[133] J. Rubert, Z. Dzuman, M. Vaclavikova, M. Zachariasova, C. Soler, and J. Hajslova, "Analysis of mycotoxins in barley using ultra high liquid chromatography high resolution mass spectrometry: comparison of efficiency and efficacy of different extraction procedures," *Talanta*, vol. 99, pp. 712–719, 2012.

[134] L. A. Berrueta, R. M. Alonso-Salces, and K. Héberger, "Supervised pattern recognition in food analysis," *Journal of Chromatography A*, vol. 1158, no. 1-2, pp. 196–214, 2007.

[135] J. Rubert, C. Soler, and J. Mañes, "Evaluation of matrix solid-phase dispersion (MSPD) extraction for multi-mycotoxin determination in different flours using LC-MS/MS," *Talanta*, vol. 85, no. 1, pp. 206–215, 2011.

[136] A. B. Serrano, G. Font, M. J. Ruiz, and E. Ferrer, "Co-occurrence and risk assessment of mycotoxins in food and diet from Mediterranean area," *Food Chemistry*, vol. 135, no. 2, pp. 423–429, 2012.

[137] EC (European Communities), "European commission regulation (EC) no. 1881/2006 of 19 December, 2006. Setting maximum levels for certain contaminants in foodstuff," *Official Journal of the European Union*, vol. 364/5, pp. 5–24, 2006.

[138] G. Castellá, M. R. Bragulat, and F. J. Cabañes, "Fumonisin production by Fusarium species isolated from cereals and feeds in Spain," *Journal of Food Protection*, vol. 62, no. 7, pp. 811–813, 1999.

[139] C. Cavaliere, P. Foglia, C. Guarino et al., "Mycotoxins produced by Fusarium genus in maize: determination by screening and confirmatory methods based on liquid chromatography tandem mass spectrometry," *Food Chemistry*, vol. 105, no. 2, pp. 700–710, 2007.

[140] M. Weidenbörner, *Encyclopedia of Food Mycotoxins*, Springer Science & Business Media, Berlin, Germany, 2001.

[141] Commission Decision, "Commission decision (EC) no. 2002/657 of 12 August 2002 implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results," *Official Journal of the European Communities*, vol. 221, pp. 8–36, 2002.

[142] O. Lacina, M. Zachariasova, J. Urbanova, M. Vaclavikova, T. Cajka, and J. Hajslova, "Critical assessment of extraction methods for the simultaneous determination of pesticide residues and mycotoxins in fruits, cereals, spices and oil seeds employing ultra-high performance liquid chromatography-tandem mass spectrometry," *Journal of Chromatography A*, vol. 1262, pp. 8–18, 2012.

[143] C. Juan, A. Ritieni, and J. Mañes, "Determination of trichothecenes and zearalenones in grain cereal, flour and bread by liquid chromatography tandem mass spectrometry," *Food Chemistry*, vol. 134, no. 4, pp. 2389–2397, 2012.

[144] J. Barthel, C. Gottschalk, M. Rapp, M. Berger, J. Bauer, and K. Meyer, "Occurrence of type A, B and D trichothecenes in barley and barley products from the Bavarian market," *Mycotoxin Research*, vol. 28, no. 2, pp. 97–106, 2012.

[145] European Commission, "Commission recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC)," *Official Journal of European Union*, vol. 229, pp. 7–9, 2006.

[146] Y. Ren, Y. Zhang, S. Shao et al., "Simultaneous determination of multi-component mycotoxin contaminants in foods and feeds by ultra-performance liquid chromatography tandem mass spectrometry," *Journal of Chromatography A*, vol. 1143, no. 1-2, pp. 48–64, 2007.

[147] S. Amézqueta, E. González-Peñas, M. Murillo, and A. López de Cerain, "Validation of a high-performance liquid chromatography analytical method for ochratoxin A quantification in cocoa beans," *Food Additives and Contaminants*, vol. 21, no. 11, pp. 1096–1106, 2004.

[148] Y. Sugita-Konsihi, T. Tanaka, S. Tabata et al., "Validation of an HPLC analytical method coupled to a multifunctional clean-up column for the determination of deoxynivalenol," *Mycopathologia*, vol. 161, no. 4, pp. 239–243, 2006.

[149] E. Papp, H. Klara, G. Záray, and E. Mincsovics, "Liquid chromatographic determination of aflatoxins," *Microchemical Journal*, vol. 73, no. 1-2, pp. 39–46, 2002.

[150] M. Ventura, D. Guillen, I. Anaya et al., "Ultra-performance liquid chromatography/tandem mass spectrometry for the simultaneous analysis of aflatoxins B1, G1, B2, G2 and ochratoxin A in beer," *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-To-The-Minute Research in Mass Spectrometry*, vol. 20, no. 1, pp. 3199–3204, 2006.

[151] S. Monbaliu, C. Van Poucke, C. Van Peteghem, K. Van Poucke, K. Heungens, and S. De Saeger, "Development of a multi-mycotoxin liquid chromatography/tandem mass spectrometry method for sweet pepper analysis," *Rapid Communications in Mass Spectrometry*, vol. 23, no. 1, pp. 3–11, 2009.

[152] A. Y. Sirhan, G. H. Tan, and R. C. S. Wong, "Simultaneous detection of type a and type b trichothecenes in cereals by liquid chromatography coupled with electrospray ionization quadrupole time of flight mass spectrometry (LC-ESI-QTOF-MS/MS)," *Journal of Liquid Chromatography & Related Technologies*, vol. 35, no. 14, pp. 1945–1957, 2012.

[153] E. N. Ediage, C. van Poucke, and S. De Saeger, "A multi-analyte LC-MS/MS method for the analysis of 23 mycotoxins in different sorghum varieties: the forgotten sample matrix," *Food Chemistry*, vol. 177, pp. 397–404, 2015.

[154] L. Hu and M. Rychlik, "Biosynthesis of $^{15}N_3$-labeled enniatins and beauvericin and their application to stable isotope dilution assays," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 29, pp. 7129–7136, 2012.

[155] J. L. Sørensen, K. F. Nielsen, P. H. Rasmussen, and U. Thrane, "Development of a LC-MS/MS method for the analysis of enniatins and beauvericin in whole fresh and ensiled maize," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 21, pp. 10439–10443, 2008.

[156] C. Falavigna, M. Cirlini, G. Galaverna, S. Sforza, A. Dossena, and C. Dall'Asta, "LC/ESI-MS/MS analysis outlines the different fumonisin patterns produced by *F. verticillioides* in culture media and in maize kernels," *Journal of Mass Spectrometry*, vol. 47, no. 9, pp. 1170–1176, 2012.

[157] B. Warth, A. Parich, J. Atehnkeng et al., "Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 36, pp. 9352–9363, 2012.

[158] N. Mahnine, G. Meca, M. Fernandez-Franzon, J. Manes, and A. Zinedine, "Occurrence of fumonisins B1, B2 and B3 in breakfast and infant cereals from Morocco," *Phytopathologia Mediterranea*, vol. 51, pp. 193–197, 2012.

[159] E. De Angelis, L. Monaci, M. Pascale, and A. Visconti, "Fate of deoxynivalenol, T-2 and HT-2 toxins and their glucoside conjugates from flour to bread: an investigation by high-performance liquid chromatography high-resolution mass spectrometry," *Food Additives & Contaminants: Part A*, vol. 30, no. 2, pp. 345–355, 2013.

[160] E. Varga, T. Glauner, R. Köppen et al., "Stable isotope dilution assay for the accurate determination of mycotoxins in maize by UHPLC-MS/MS," *Analytical and Bioanalytical Chemistry*, vol. 402, no. 9, pp. 2675–2686, 2012.

[161] T. I. Ekwomadu, T. A. Dada, N. Nleya, R. Gopane, M. Sulyok, and M. Mwanza, "Variation of Fusarium free, masked, and emerging mycotoxin metabolites in maize from agriculture regions of South Africa," *Toxins*, vol. 12, no. 3, pp. 149–168, 2020.

[162] S. T. Tran and T. K. Smith, "Determination of optimal conditions for hydrolysis of conjugated deoxynivalenol in corn and wheat with trifluoromethanesulfonic acid," *Animal Feed Science and Technology*, vol. 163, no. 2–4, pp. 84–92, 2011.

[163] L. Freire and A. S. Sant'Ana, "Modified mycotoxins: an updated review on their formation, detection, occurrence, and toxic effects," *Food and Chemical Toxicology*, vol. 111, pp. 189–205, 2018.

[164] O. Vendl, C. Crews, S. MacDonald, R. Krska, and F. Berthiller, "Occurrence of free and conjugated Fusarium mycotoxins in cereal-based food," *Food Additives & Contaminants: Part A*, vol. 27, no. 8, pp. 1148–1152, 2010.

[165] A. Malachova, Z. Dzuman, Z. Veprikova, M. Vaclavikova, M. Zachariasova, and J. Hajslova, "Deoxynivalenol, deoxynivalenol-3-glucoside, and enniatins: the major mycotoxins found in cereal-based products on the Czech market," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 24, pp. 12990–12997, 2011.

[166] F. Q. Li, W. Wang, J. J. Ma, C. C. Yu, X. H. Lin, and W. X. Yan, "Natural occurrence of masked deoxynivalenol in Chinese wheat and wheat-based products during 2008–2011," *World Mycotoxin Journal*, vol. 5, no. 3, pp. 221–230, 2012.

[167] S.-T. Tran, T. K. Smith, and G. N. Girgis, "A survey of free and conjugated deoxynivalenol in the 2008 corn crop in Ontario, Canada," *Journal of the Science of Food and Agriculture*, vol. 92, no. 1, pp. 37–41, 2012.

[168] K. Lancova, J. Hajslova, J. Poustka et al., "Transfer of Fusarium mycotoxins and "masked" deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer," *Food Additives & Contaminants: Part A*, vol. 25, no. 6, pp. 732–744, 2008.

[169] J. J. Sasanya, C. Hall, and C. Wolf-Hall, "Analysis of deoxynivalenol, masked deoxynivalenol, and Fusarium graminearum pigment in wheat samples, using liquid chromatography-UV-mass spectrometry," *Journal of Food Protection*, vol. 71, no. 6, pp. 205–1213, 2008.

[170] M. Suman, E. Bergamini, D. Catellani, and A. Manzitti, "Development and validation of a liquid chromatography/linear ion trap mass spectrometry method for the quantitative determination of deoxynivalenol-3-glucoside in processed cereal-derived products," *Food Chemistry*, vol. 136, no. 3-4, pp. 1568–1576, 2013.

[171] C. Dall'Asta, G. Galaverna, G. Aureli, A. Dossena, and R. Marchelli, "A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in maize and maize-based products," *World Mycotoxin Journal*, vol. 1, no. 3, pp. 237–246, 2008.

[172] A. Faberi, P. Foglia, E. Pastorini, R. Samperi, and A. Laganà, "Determination of type B fumonisin mycotoxins in maize and maize-based products by liquid chromatography/tandem mass spectrometry using a QqQ linear ion trapmass spectrometer," *Rapid Communications in Mass Spectrometry*, vol. 19, no. 2, pp. 275–282, 2005.

[173] L. Hu, H. Liu, J. Yang et al., "Free and hidden fumonisins in raw maize and maize-based products from China," *Food Additives & Contaminants: Part B*, vol. 12, no. 2, pp. 90–96, 2019.

[174] M. S. Oliveira, A. C. L. Diel, R. H. Rauber et al., "Free and hidden fumonisins in Brazilian raw maize samples," *Food Control*, vol. 53, pp. 217–221, 2015.

[175] M. Bryła, M. Roszko, K. Szymczyk, R. Jędrzejczak, and M. W. Obiedziński, "Fumonisins and their masked forms in maize products," *Food Control*, vol. 59, pp. 619–627, 2016.

[176] P. D. Andrade, R. R. Dantas, T. L. d. S. d. Moura-Alves, and E. D. Caldas, "Determination of multi-mycotoxins in cereals and of total fumonisins in maize products using isotope labeled internal standard and liquid chromatography/tandem mass spectrometry with positive ionization," *Journal of Chromatography A*, vol. 1490, pp. 138–147, 2017.

[177] J. W. Park, P. M. Scott, B. P.-Y. Lau, and D. A. Lewis, "Analysis of heat-processed corn foods for fumonisins and bound fumonisins," *Food Additives and Contaminants*, vol. 21, no. 12, pp. 1168–1178, 2004.

[178] M. Kostelanska, J. Hajslova, M. Zachariasova et al., "Occurrence of deoxynivalenol and its major conjugate, deoxynivalenol-3-glucoside, in beer and some brewing intermediates," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 8, pp. 3187–3194, 2009.

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Review Article

Effect of Cold Plasma on Quality Retention of Fresh-Cut Produce

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In recent years, the consumption of the fresh-cut products has been increased due to the consumers' lifestyle and awareness. However, maintaining the quality and nutritional value of these products during storage is being difficult in comparison to whole fruits and vegetables. In actual, the procedures used in the fresh-cut industry may intensify the deterioration caused by physical damage by minimal processing. Commonly, the quality degradation, discolouration, loss of moisture, loss of firmness, microbial load increase, and loss of nutrients and flavor occur in the fresh-cut product after minimal processing. To maintain the quality and increase the shelf-life of the fresh-cut product, it is necessary to use various techniques, including physical, chemical, and nondestructive processes. In this review, first, an introduction to minimal processing and its effect on fresh-cut product quality was expressed, and then, the methods used to maintain fresh-cut product quality after minimal processing were reviewed. Finally, the effect of cold plasma on the qualitative characteristics in some fresh-cut products was investigated. The review showed that cold plasma treatments can significantly inhibit microorganisms and extend the shelf-life of fresh-cut products. In addition, no or minimal impacts were observed on physicochemical and organoleptic quality attributes of the treated fresh-cut products. Therefore, the use of cold plasma is promising for the fresh-cut industry.

1. Introduction

Due to alteration in lifestyles, the consumption of the fresh-cut product (FCP) has been increased in the last decade [1, 2]. Ready-to-eat, appealing, nutritious, and safe are the prominent features of these products [3]. The International Fresh-Cut Produce Association (IFPA) defines fresh-cut products as fruits or vegetables that have been trimmed and/or cut into a 100% usable product which is packaged to offer consumers high nutrition, convenience, and flavor while still maintaining freshness [4]. FCP as a novel and attractive product has attracted the most attention and led to the ever-growing fresh-cut industry and need to develop a novel method to reduce the adverse effects of the processing and maintain their fresh-cut features [5]. These products are very vulnerable due to the minimal processes used in their preparations against corruption and microbial contamination. Minimal processing of fruits and vegetables includes different processing steps such as washing, peeling, cutting, grating or slicing, and disinfection (Figure 1), which are used to obtain the ready-to-eat product [6].

Minimal processing by removing the natural protection (skin) and destroying the internal compartmentalization led them to be much more perishable than when the original product is intact [7]. Minimal processing of the product stimulates biochemical reactions (surface browning, softening, and loss of texture), physiological ageing (dehydration, increasing respiration rate, increasing ethylene production, and loss of appearance), mechanical damage (dehydration and decay), and microbial growth (spoilage and pathogens). As a result of these reactions, the quality of the product reduced (Figure 2), which leads to a minimal shelf-life [3]. As FCP is highly perishable, storage in suitable packaging and cold conditions are essential. However, packaging and cold conditions were not enough to preserve FCP quality at optimum levels during the storage period. Therefore, the appropriate technologies, along with refrigeration, are needed. Some of the conventional methods such as modified atmosphere packaging (MAP) [8], edible coatings [9], and ozone [10] are used to maintain the FCP quality to prevent biochemical and biological complications due to physical damage. These techniques have

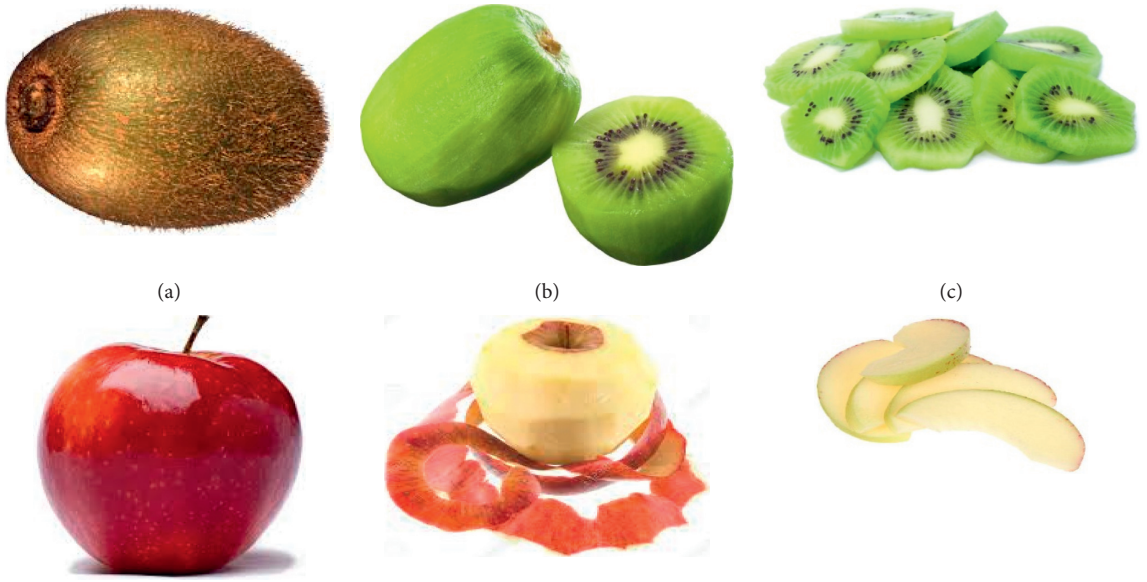

Figure 1: Minimal processing of fresh fruits and vegetables includes washing, peeling, and cutting.

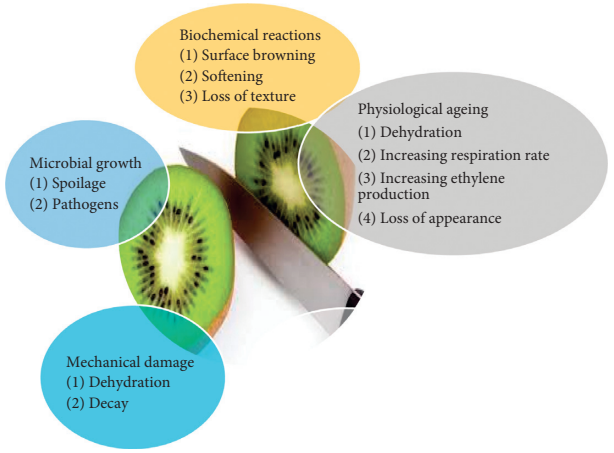

Figure 2: Effect of minimal processing on the decay and shelf-life of fresh fruits.

technological and economic disadvantages. These methods are primarily being used to preserve colour, texture, and delay of senescence phenomena caused by tissue-injury reactions; however, these methods are not able to disinfect the FCP.

The exponential growth in demand for FCP has led researchers to develop new nonthermal methods to maintain the desired fresh properties of FCP and increase shelf-life. Therefore, there is a vital need for advanced postharvest technology. Some of the nonthermal methods as disinfection technologies such as UV light and ultrasound have been applied to improve the shelf-life in FCP [11]. Nevertheless, these methods have fundamental disadvantages in cost of items, potential risks, and maintaining control, which limits their practical application on a large scale.

A new alternative that has recently been developed to improve shelf-life is cold plasma. Plasma is known as the fourth state of matter and is produced by the induction of energy into the gas mixture and includes the ionization of the gas and the formation of active components such as radicals, charged particles, and ultraviolet radiation [6]. Cold plasma is formed by microwave (MV), radio frequency (RF), direct current (DC), and alternating current (AC). It has been used in a variety of setups, including dielectric barrier, jet plasma, and corona discharges [12]. Among all the unique features of cold plasma, the most important feature seems to be the microbial inactivation by reactive oxygen and nitrogen species [13]. Researchers identified microbial decontamination of nonthermal plasma on the number of fruits and vegetables [14, 15]. Also, recently inactivate endogenous enzymatic activity in fresh-cut products by cold plasma has attracted the attention of researchers [16].

The application of cold plasma is a new pattern in the fresh-cut industry and a novel technology to reduce microbial load at the FCP [17, 18] (Figure 3). Therefore, the potential use of cold plasma for the fresh-cut industry is widespread, and it has unique potential for FCP treatment.

There has been no dedicated reference on the topic of cold plasma technologies and their potential applications for fresh-cut produce. This review aimed to introduce cold plasma for preservation of fresh-cut produce. First, the cold plasma technology for the increased shelf-life of fresh-cut produce was present, and then the effect of cold plasma on some quality parameters of the fresh-cut produce was reviewed.

2. Decontamination of FCP with Cold Plasma

Cold plasma has been used for fresh-cut product decontamination such as apples, melons, lettuce, and mangoes [6, 15, 16, 19]. Among the new application cold plasma for fresh-cut product decontamination summarized in Table 1, Ziuzina et al. [28] showed that the use of cold plasma for 120

Figure 3: The influence of cold plasma on the quality and safety of FCP.

Table 1: Various cold plasma systems used for decontamination of different FCP.

FCP	Cold plasma systems	Salient results	References
Cucumber slice Carrot slice Pear slice	Atmospheric pressure cold plasma, microjet	Effective inactivation was achieved on sliced fruits and vegetables after 1st plasma treatment	Wang et al. [20]
Mangos Melons	Cold atmospheric plasma system	Cold plasma has potential in decontaminating the skins of soft fruits such as mangoes and melons	Perni et al. [21]
Bananas	Atmospheric pressure pulsed cold plasma	This study demonstrated that the cold plasma technique has the potential to prolong the shelf-life of bananas	Trivedi et al. [22]
Fresh-cut cucumbers	Atmospheric-pressure low-temperature plasma	The results showed that the *E. coli* cell morphology was changed due to the charged particles	Sun et al. [23]
Fresh-cut kiwifruit	Atmospheric double-barrier discharge plasma	No significant changes in antioxidants content and antioxidant activity were observed among treated samples and control ones.	Ramazzina et al. [24]
Spinach Lettuce Tomato Potato	Nonthermal low pressure oxygen plasma	This study confirms that nonthermal oxygen plasma can be a new effective method of sanitization for fresh produce	Zhang et al. [25]
Lettuce Carrot	Atmospheric pressure cold plasma	Cold plasma represents a potential technology for disinfection of fruits and vegetables without important changes in physical characteristics	Bermúdez-Aguirre et al. [26]
Carrot Lamb's lettuce	Microwave cold plasma	The investigations showed a possible inactivation of seven different microorganisms with cold plasma	Schnabel et al. [27]
Fresh-cut apples	Dielectric barrier discharge plasma	Promising results have been obtained regarding enzymatic browning inhibition and a specific effect on the reduction of polyphenol oxidase activity on the apple slices has been found	Tappi et al. [16]
Fresh-cut melon	Direct-barrier discharge plasma	A significant increase in microbial shelf-life was achieved following the 15 + 15 min plasma treatment	Tappi et al. [6]

seconds lead to reduce *Salmonella*, *E. coli*, and *L. monocytogenes* to undetectable levels on cherry tomatoes.

Fresh strawberries were treated with in-package DBD plasma system, and the results showed a 2-log decrease in microbial load (aerobic mesophilic bacteria, yeast, and mold) due to cold plasma treatment [29]. Niemira and Sites [15] studied the inactivation of *Escherichia coli* and *Salmonella Stanley* pathogens using gliding arc cold plasma. The results of this study showed that these two pathogens were inactivated using cold plasma. The intensity of inactivation of these two pathogens increased with increasing gas flow rate. The growth of *Salmonella* spp., *E. coli*, and *Listeria monocytogenes* as spoilage microbes and pathogens has been reported in the fresh-cut melon during storage [6]. Tappi et al. [6] investigated the stabilization of fresh-cut melon using cold plasma. The results show that cold plasma is very efficient to maintain the quality of fresh-cut melons.

The effects of cold plasma on mango and melon with four different pathogens were investigated by Perni et al. [21]. The rate of inactivation of the four pathogens was different, but all four pathogens were inactivated after several minutes of applying the cold plasma.

Cold plasma significantly inactivated *Salmonella Stanley* and *E. coli O157:H7* (inoculated at apple surfaces), but it did not hurt the appearance of the apple [15]. Due to the high moisture content in the apple fruit, it has a concise shelf-life, and to increase its shelf-life, it is necessary to use different preservation methods. These results emphasize that cold plasma as a nonthermal process can effectively destroy human pathogens and make apple fruit healthy.

Trivedi et al. [22] studied shelf-life of bananas by cold plasma. Bananas are one of the best tropical fruits, and about 1.4 million bananas are wasted each day due to their high perishability. The results showed that cold plasma can increase the shelf-life of bananas by inhibiting the growth of pathogens compared to conventional methods [22].

FCP is very highly susceptible to loss of sensory, microbial, and nutritional quality, therefore, maintaining the quality characteristics of these products is a very significant challenge. Thus, the use of cold plasma has been proposing to preserve and improve the quality of FCP (Table 1). Cold plasma is a novel, nonthermal technology which has shown good potential for FCP decontamination. However, most of the research is largely focused on microbial inactivation studies, with limited emphasis on quality properties. Cold plasma processing has been shown to affect the quality attributes of the FCP during treatment as well as in storage. Therefore, in this review, we tried to investigate the effect of cold plasma on FCP quality characteristics.

3. Effect of Cold Plasma on Quality Parameters

The quality of fresh-cut products is a combination of parameters including appearance, texture, flavor, and nutritional value [30]. Consumers judge the quality of fresh-cut fruit and vegetables on the basis of appearance and freshness at the time of purchase. However, subsequent purchases depend upon the consumer's satisfaction in terms of texture and flavor of the product. Consumers are also interested in the nutritional quality and safety of fresh-cut products [31]. Cold plasma is a novel technology used for decontaminating fresh-cut produce. This technology may facilitate the maintenance of high-quality food products due to antimicrobial properties. However, due to the existence of reactive species, cold plasma can also react with nearly all food constituents, affecting physicochemical (for example, pH, carbohydrate, vitamins, anthocyanin, and respiration rate) and organoleptic (for example, taste, colour, and texture) attributes of various fresh produce [30, 31]. Therefore, it is important to study the effect of plasma on quality characteristics.

3.1. Respiration Rate. The respiration rate of fresh-cut produce is one of the most critical factors in maintaining quality, and one of the most common replies to the damage is respiration. It is considered as a main index of the product shelf-life [32]. The rate of respiration increased after cutting due to the energized state of all living tissues. Depending on the degree of cutting of the product and the storage temperature, the rate respiration in fresh-cut produce may vary between 1.2 and 7.0 [2]. Therefore, after minimal processing similar to the ripening process, the increased respiration creates energy and carbon skeletons for the anabolic reactions [33]. Cellular respiration is a set of metabolic reactions and processes that involves the oxidation of large organic molecules (starch, sugar, and organic acids) to smaller products (CO_2 and H_2O), along with releasing the energy (ATP and heat), and this energy is used by the cell for synthetic reactions [34]. Respiration rate is often a good indicator of the shelf-life of products, and as the respiration rate increases, the shelf-life of the product decreases [35]. Also, an increased rate of ethylene production in response to minimal processing may stimulate respiration and leads to faster senescence and deterioration of vegetative tissues. Therefore, the respiration rate should be reduced with appropriate methods to increase the shelf-life of fresh-cut produce. Misra et al. [34] reported the respiration rate decrease during the storage time for the control as well as the CP-treated tomatoes. At the end of storage period, the respiration rates were similar for the control and CP-treated tomatoes. The results of Tappi et al. [16] showed that the plasma treatment can cause an alteration of the cellular respiratory pathway. In addition, Misra et al. [36] explored cold plasma treatment for postharvest strawberries in modified atmosphere packaging and indicated no significant increase in respiration rate.

3.2. Firmness. The maximum force required to pierce the sample is used as a firmness indicator and consumers prefer to buy firm fresh-cut fruits. The textural characteristic, especially the firmness, is a critical quality parameter because it has a significant impact on product acceptance. The fresh agricultural product has a firm and tender texture and experiences softening of the texture during storage [37]. Firmness reduction is associated with fresh-cut product storage and is regarded as a sign of declining quality and deterioration [38]. Tappi et al. [16] reported that cold plasma treatments led to an increase of firmness in fresh-cut apples. The amount of firmness was 18.9 N for the control and 21.8 N for plasma-treated samples at 15 kV for 10 min (5 + 5). There was no difference between the firmness of fresh-cut melon and kiwifruit slices treated with cold plasma and the control sample. The highest firmness was related to the mushroom treated with cold plasma, which indicates the potential application of this innovative technology in increasing the shelf-life and quality after mushroom harvesting [37].

3.3. Colour Retention. Colour is a critical parameter in the quality of food because it can affect the consumer's decision to buy and is probably the first quality factor that is judged [27]. Colour plays a crucial role in food selection and is one of the most important parameters for the consumer [31]. The appearance of FCP is a determining agent in customer acceptance, and it strongly affects the decision to purchase the product [39]. After minimal processing of fresh produce, the metabolic reactions that stimulate respiration and/or ethylene production lead to some undesirable effects that

affect consumer acceptability. Browning is one of the most common adverse changes that affect the colour of the product and thus affects the visual quality of fresh products [39]. Enzymatic browning results from oxidation of phenolic compounds catalysed by polyphenol oxidase (PPO) followed by nonenzymatic formation of pigments. Cutting operation promotes the contact between enzymes and their substrates and leads to an immediate increase in the respiration rate and endogenous metabolic activity [40]. Therefore, the browning and discoloration effects are the most common undesirable changes that affect color and consequently affect the visual appearance of fresh-cut produces [39]. In the past, various chemical and physical methods have been used to control the enzymatic browning of fresh-cut fruits. The chemical methods used to destroy enzymes are based on the immersion process. Organic acids are the most common materials used for this study that is applied in combination with calcium salts [38]. The use of edible coatings as a carrier of antibrowning chemical agents to prevent browning has also been extensively investigated by researchers and several scholars have studied the use of modified atmosphere packaging (MAP) for controlling browning reactions [8]. In recent years, various innovative therapies have been tested to control browning reactions in fresh-cut fruits. Currently, among the innovative techniques, cold plasma is used for biological therapies. Tappi et al. [16] reported a significant reduction in PPO activity in fresh-cut Pink Lady apples due to cold plasma treatment. The researchers showed that the activity of a PPO enzyme in the treated samples was about 45% lower than the untreated sample (control). The reduction of PPO activity may be due to the reaction between enzymes and free radicals.

Table 2 shows the changes in $L*, a*, b*, \Delta E$, and chroma of cold plasma-treated samples. As seen in Table 2, the effect of cold plasma on the changes of colour was variable, which is due to the different types of fruits and vegetables. Cold plasma may stabilize the colour in fruits where the browning reaction occurs, and in fruits containing carotenoids, it may reduce the colour slightly. The accepted mechanism for the observed loss of enzymatic functionality upon plasma exposure is an oxidation of the side-chain amino acids that cause an alteration of the secondary structures of the protein operated by the reactive species. Bermúdez-Aguirre et al. [26] reported that the destruction of carotenoid pigments by reactive species led to significant changes in the colour of cold plasma-treated tomatoes [26].

3.4. pH and Acidity. These two parameters are important to evaluate the freshness in cut fruits and vegetables and strongly are correlated due to the pH depending on the presence of acidic compounds. Acid content in fresh-cut produce tends to decrease over time, probably due to the organic acid oxidation which occurs with fruit ripening [42]; as a consequence, a pH increase is expected during the storage time. Any drastic change could lead to an undesirable impact on the taste, texture, and shelf-life of the food. However, in the case of fresh fruits and vegetables, there are significant variations due to differences in cultivation practices, varietal differences, environmental parameters, etc. The pH and acidity changes after plasma treatment were mostly attributed to the interaction of plasma reactive gases with the moisture present in the fresh-cut products [19, 27]. Oehmigen et al. [43] reported the formation of nitric acid induced by reactive nitrogen species such as NO as the reason for acidification in air plasma treatments. However, many researchers also reported no pH effect of CP treatments in food products with buffering capacity [18, 29]. No significant changes in the acidity and pH after CP treatment were reported in cherry tomatoes, blueberry, mandarins, and melon [6, 18, 22, 34].

3.5. Vitamins. The sensitivity of vitamins to different processing techniques is essential to preserve the nutritional properties of the fresh-cut products [30]. While some vitamins, such as riboflavin (B2), pyridoxine (B6), and biotin, are usually stable, others, such as thiamin (B1) and vitamins A, C, and E, are relatively labile [44]. Most of the reported studies on cold plasma treatment of food products have only focused on vitamin C (ascorbic acid) stability. Most of the studies on cold plasma treatment of fresh-cut products have reported no significant reduction in ascorbic acid content after plasma treatment. Ramazzina et al. [24], Oh et al. [45], and Song et al. [46] reported no significant effect on ascorbic acid in kiwifruit, radish sprout, and lettuce, respectively. However, up to 4% reduction in ascorbic acid content was observed after plasma treatment of cut fruits and vegetables [22]. The degradation of ascorbic acid could be attributed to the reaction with ozone and other oxidizing plasma species during the processing. Sample type, processing time, and plasma gas were critical factors for ascorbic acid degradation. Misra et al. [29] reported a decrease in the ascorbic acid content of cold plasma-treated strawberries. Both applied voltage and treatment time were found to have a significant effect on the ascorbic acid content. There was a statistically significant difference between samples treated at 60 kV than those at 80 kV. The reaction of ozone and other oxidizing species of cold plasma with ascorbic acid is proposed to be the major mode of action for the observed loss during processing. Also, Wange et al. [20] have studied vitamin C loss of fresh fruit and vegetable slices (cucumber, carrot, and pear slices) during cold plasma treatment. The vitamin C loss for the cucumber, carrot, and pear slices was 3.6%, 3.2%, and 2.8%, respectively. The slight reduction in vitamin C content is most likely due to its oxidation by the cold plasma. In addition, vitamin C is light sensitive [47], so UV generated by plasma may also play an important role in the vitamin C degradation.

3.6. Antioxidant Activity. Although the antioxidant activity is not a direct quality attribute used in the fresh-cut industry, it is a close indicator of various polyphenols, flavonoids, and flavanols present in the fresh-cut products [30]. The reported results on the effects of CP treatment on the phenolic contents of the food products have a wide degree of variation. A decrease in the total phenols was reported in lamb's lettuce [48]. No significant effect in apples [32] but a significant increase in blueberries [41] was also reported. No significant changes in the antioxidant capacity after CP

Table 2: The $L*, a*, b*, \Delta E$, and chroma values for various FCP after cold plasma treatment.

FCP	CP treatment	$L*$	$a*$	$b*$	$\Delta E\Delta$E	References
Banana	*C*	50.2 ± 4.4	4.9 ± 1.2	34.8 ± 3.7	—	Trivedi et al. [22]
	PT	53.9 ± 3.9	5.5 ± 1.4	34.0 ± 3.0	—	
Cucumber 1	*C*	63.78 ± 0.25	−6.07 ± 0.10	16.61 ± 0.10	0	Sun et al. [23]
	PT	63.89 ± 0.23	−5.98 ± 0.15	16.63 ± 0.13	0.73 ± 0.25	
Cucumber 2	*C*	63.57	−6.25	20.53	0	
	PT	63.15	−6.17	19.76	0.67 ± 0.12	
Carrot	*C*	55.49	20.87	24.60	0	Wang et al. [22]
	PT	54.81	20.01	23.30	1.51 ± 0.63	
Pear	*C*	68.51	−0.55	6.69	0	
	PT	69.14	−0.25	7.83	1.34 ± 0.09	
Blueberries	*C*	48.90 ± 0.73	0.30 ± 0.02	−1.19 ± 0.03	—	Sarangapani et al. [41]
	PT	43.76 ± 0.86	0.23 ± 0.06	−1.43 ± 0.06	—	
Melon	*C*	57.9 ± 4.7	20.8 ± 1.7	46.6 ± 3.1	—	Tappi et al. [6]
	PT	54.4 ± 3.5	17.5 ± 2.7	34.0 ± 3.7	—	

C, control; PT, plasma treatment.

treatment was reported in radish sprouts, kiwifruits, and red chicory [32, 45, 49]. These studies show that the type of fresh-cut products, plasma generation source, mode of exposure, and treatment parameters are critical in controlling the effects of CP on the antioxidant activity of food products. Also, Ramazzina et al. [24] used ABTS, DPPH, and FRAP assays to observe the effect of DBD plasma on the antioxidant activity and antioxidant contents of kiwifruit. The result showed no alteration in all the assays conducted after the NTP treatment. Generally, plasma-ROS should have caused oxidation of the phenolic compounds responsible for the antioxidant activity; however, due to the counteractive effect of the tissue response mechanisms in the kiwifruit, the ROS-induced oxidation was impeded [24].

3.7. Sensory Properties. Consumer interest worldwide in the quality of fresh-cut products has increased in recent years. Product quality is a complex issue, since it includes visual characteristics, physical properties such as texture, mineral and vitamin contents, flavor, and other organoleptic characteristics [50]. Appearance, flavor, and freshness of a product can play a principal role in the consumer's decision to purchase it and can influence perception by other senses. However, it is very important to remember that maturity and postharvest treatments, such as cold plasma, can significantly affect the taste and aroma quality of fresh produce [24]. Sensory parameters such as taste, flavor, colour, and texture in cold plasma-treated cherry tomatoes remained at an acceptable level after treatment [51]. Similarly, Srey et al. [52] reported that cold plasma did not cause any statistically significant change in the sensorial quality (colour and texture).

Effects of cold plasma on some of the quality attributes of fresh-cut products are presented in this study. Among the quality attributes, the effect of cold plasma on colour parameters was the most widely studied, probably because colour is the most obvious parameter that directly influences the acceptance by consumers. Cold plasma has been applied for the decontamination of food products, as an alternative to washing procedures with chemicals. Since the temperature of the product during the treatment is very close to the ambient, this technique can be suitable for the processing of temperature sensitive products such as fresh-cut fruits and vegetables. Moreover, the potential direct application of packed products seems promising [29, 36].

4. Conclusions and Future Perspectives

Demand for FCF has risen sharply due to the growing consumer concern about their own diets and the supply of high-value fresh-cut products. However, the minimal process used to get ready the products will intensify the procedure of ripening and exposing the products to microbial contamination; therefore, shelf-life is shortened. In recent years, some studies have already been conducted to lessen the undesirable effects of minimal processing on the quality of FCP. The purpose of these studies would be to increase the shelf-life of the product by preserving the quality characteristics of the product. Therefore, enhanced substitute sanitization approaches and processing methods could be applied for making sure safeness and increasing the shelf-life of FCP. Cold plasma can be an innovative and emerging technology useful for disinfecting foodstuff surfaces. Cold plasma positively affected the visible quality preservation of FCP during storage. Cold plasma is promising for extending the storage life of FCP. Generally, cold plasma is a potential technology for treating FCP to improve their quality and prolong their shelf-life.

However, understanding the effects of cold plasma on the quality changes of FCP is still limited. Considering the high oxidative action of cold plasma, its effect on the bioactive compounds of the fresh-cut fruit tissue has to be deeply studied in order to highlight the nature of gas plasma effect on biochemical tissue response.

Data Availability

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

Ethical Approval

The study did not involve any human or animal testing.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

[1] O. Martin-Belloso and R. S. Fortuny, *Advances in Fresh-Cut Fruits and Vegetables Processing*, CRC Press, Cleveland, OH, USA, 2010.

[2] G. R. Velderrain-Rodriguez, A. E. Quirós-Sauceda, G. G. Aguilar, M. W. Siddiqui, and J. A. Zavala, "Technologies in fresh-cut fruit and vegetables," in *Minimally Processed Foods*, pp. 79–103, Springer, Berlin, Germany, 2015.

[3] U. De Corato, "Improving the shelf-life and quality of fresh and minimally-processed fruits and vegetables for a modern food industry: a comprehensive critical review from the traditional technologies into the most promising advancements," *Critical Reviews in Food Science and Nutrition*, vol. 60, no. 6, pp. 940–975, 2020.

[4] D. Rico, A. B. Martín-Diana, J. M. Barat, and C. Barry-Ryan, "Extending and measuring the quality of fresh-cut fruit and vegetables: a review," *Trends in Food Science & Technology*, vol. 18, no. 7, pp. 373–386, 2007.

[5] A. Y. Ramos-Villarroel, N. Aron-Maftei, O. Martín-Belloso, and R. Soliva-Fortuny, "The role of pulsed light spectral distribution in the inactivation of *Escherichia coli* and Listeria innocua on fresh-cut mushrooms," *Food Control*, vol. 24, no. 1-2, pp. 206–213, 2012.

[6] S. Tappi, G. Gozzi, L. Vannini et al., "Cold plasma treatment for fresh-cut melon stabilization," *Innovative Food Science & Emerging Technologies*, vol. 33, pp. 225–233, 2016.

[7] P. Sharma, V. P. Shehin, N. Kaur, and P. Vyas, "Application of edible coatings on fresh and minimally processed vegetables: a review," *International Journal of Vegetable Science*, vol. 25, no. 3, pp. 295–314, 2019.

[8] E. Aguayo, C. Requejo-Jackman, R. Stanley, and A. Woolf, "Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices," *Postharvest Biology and Technology*, vol. 57, no. 1, pp. 52–60, 2010.

[9] M. E. Martiñon, R. G. Moreira, M. E. Castell-Perez, and C. Gomes, "Development of a multilayered antimicrobial edible coating for shelf-life extension of fresh-cut cantaloupe (Cucumis melo L.) stored at 4°C," *LWT-Food Science and Technology*, vol. 56, no. 2, pp. 341–350, 2014.

[10] M. V. Selma, A. M. Ibáñez, M. Cantwell, and T. Suslow, "Reduction by gaseous ozone of *Salmonella* and *microbial flora* associated with fresh-cut cantaloupe," *Food Microbiology*, vol. 25, no. 4, pp. 558–565, 2008.

[11] M. P. Palekar, T. M. Taylor, J. E. Maxim, and A. Castillo, "Reduction of *Salmonella enterica* serotype Poona and background microbiota on fresh-cut cantaloupe by electron beam irradiation," *International Journal of Food Microbiology*, vol. 202, pp. 66–72, 2015.

[12] H. Bagheri, S. Abbaszadeh, and A. Salari, "Optimization of decontamination conditions for Aspergillus flavus inoculated to military rations snack and physicochemical properties with atmospheric cold plasma," *Journal of Food Safety*, Article ID e12850, 2020, In press.

[13] E. Takai, K. Kitano, J. Kuwabara, and K. Shiraki, "Protein inactivation by low-temperature atmospheric pressure plasma in aqueous solution," *Plasma Processes and Polymers*, vol. 9, no. 1, pp. 77–82, 2012.

[14] F. J. Critzer, K. Kelly-Wintenberg, S. L. South, and D. A. Golden, "Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces," *Journal of Food Protection*, vol. 70, no. 10, pp. 2290–2296, 2007.

[15] B. A. Niemira and J. Sites, "Cold plasma inactivates Salmonella Stanley and *Escherichia coli* O157: H7 inoculated on golden delicious apples†," *Journal of Food Protection*, vol. 71, no. 7, pp. 1357–1365, 2008.

[16] S. Tappi, A. Berardinelli, L. Ragni, M. Dalla Rosa, A. Guarnieri, and P. Rocculi, "Atmospheric gas plasma treatment of fresh-cut apples," *Innovative Food Science and Emerging Technologies*, vol. 21, pp. 114–122, 2014.

[17] B. A. Niemira, "Cold plasma decontamination of foods," *Annual Review of Food Science and Technology*, vol. 3, no. 1, pp. 125–142, 2012.

[18] M. Y. Won, S. J. Lee, and S. C. Min, "Mandarin preservation by microwave-powered cold plasma treatment," *Innovative Food Science and Emerging Technologies*, vol. 39, pp. 25–32, 2017.

[19] A. Sharma, G. Collins, and A. Pruden, "Differential gene expression in *Escherichia coli* following exposure to nonthermal atmospheric pressure plasma," *Journal of Applied Microbiology*, vol. 107, no. 5, pp. 1440–1449, 2009.

[20] R. X. Wang, W. F. Nian, H. Y. Wu et al., "Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation," *The European Physical Journal D*, vol. 66, no. 10, p. 276, 2012.

[21] S. Perni, D. W. Liu, G. Shama, and M. G. Kong, "Cold atmospheric plasma decontamination of the pericarps of fruit," *Journal of Food Protection*, vol. 71, no. 2, pp. 302–308, 2008.

[22] M. H. Trivedi, K. Patel, H. Itokazu et al., "Enhancing shelf life of bananas by using atmospheric pressure pulsed cold plasma treatment of the storage atmosphere," *Plasma Medicine*, vol. 9, no. 1, 2019.

[23] Y. Sun, Z. Zhang, and S. Wang, "Study on the bactericidal mechanism of atmospheric-pressure low-temperature plasma against *Escherichia coli* and its application in fresh-cut cucumbers," *Molecules*, vol. 23, no. 4, p. 975, 2018.

[24] I. Ramazzina, A. Berardinelli, F. Rizzi et al., "Effect of cold plasma treatment on physico-chemical parameters and antioxidant activity of minimally processed kiwifruit," *Postharvest Biology and Technology*, vol. 107, pp. 55–65, 2015.

[25] M. Zhang, J. K. Oh, L. Cisneros-Zevallos, and M. Akbulut, "Bactericidal effects of nonthermal low-pressure oxygen plasma on *S. typhimurium* LT2 attached to fresh produce surfaces," *Journal of Food Engineering*, vol. 119, no. 3, pp. 425–432, 2013.

[26] D. Bermúdez-Aguirre, E. Wemlinger, P. Pedrow, G. Barbosa-Cánovas, and M. Garcia-Perez, "Effect of atmospheric pressure cold plasma (APCP) on the inactivation of *Escherichia coli* in fresh produce," *Food Control*, vol. 34, no. 1, pp. 149–157, 2013.

[27] U. Schnabel, R. Niquet, O. Schlüter, H. Gniffke, and J. Ehlbeck, "Decontamination and sensory properties of microbiologically contaminated fresh fruits and vegetables by

microwave plasma processed air (PPA)," *Journal of Food Processing and Preservation*, vol. 39, no. 6, pp. 653–662, 2015.

[28] D. Ziuzina, S. Patil, P. J. Cullen, K. M. Keener, and P. Bourke, "Atmospheric cold plasma inactivation of *Escherichia coli*, *Salmonella enterica* serovar Typhimurium and Listeria monocytogenes inoculated on fresh produce," *Food Microbiology*, vol. 42, pp. 109–116, 2014.

[29] N. N. Misra, S. K. Pankaj, J. M. Frias, K. M. Keener, and P. J. Cullen, "The effects of nonthermal plasma on chemical quality of strawberries," *Postharvest Biology and Technology*, vol. 110, pp. 197–202, 2015.

[30] S. K. Pankaj, Z. Wan, and K. M. Keener, "Effects of cold plasma on food quality: a review," *Foods*, vol. 7, no. 1, p. 4, 2018.

[31] A. I. Muhammad, Q. Xiang, X. Liao, D. Liu, and T. Ding, "Understanding the impact of nonthermal plasma on food constituents and microstructure-A review," *Food and Bioprocess Technology*, vol. 11, no. 3, pp. 463–486, 2018.

[32] B. B. Surjadinata and L. Cisneros-zevallos, "Modeling wound-induced respiration of fresh-cut carrots (*Daucus carota L.*)," *Journal of Food Science*, vol. 68, no. 9, pp. 2735–2740, 2003.

[33] M. Helena Gomes, R. M. Beaudry, D. P. F. Almeida, and F. Xavier Malcata, "Modelling respiration of packaged fresh-cut "Rocha" pear as affected by oxygen concentration and temperature," *Journal of Food Engineering*, vol. 96, no. 1, pp. 74–79, 2010.

[34] N. N. Misra, K. M. Keener, P. Bourke, J. P. Mosnier, and P. J. Cullen, "In-package atmospheric pressure cold plasma treatment of cherry tomatoes," *Journal of Bioscience and Bioengineering*, vol. 118, no. 2, pp. 177–182, 2014a.

[35] G. L. Robertson, "Food packaging principles and practice second edition," *Food Science and Technology-New York-Marcel Dekker-*, vol. 152, 2006.

[36] N. N. Misra, T. Moiseev, S. Patil et al., "Cold plasma in modified atmospheres for post-harvest treatment of strawberries," *Food and Bioprocess Technology*, vol. 7, no. 10, pp. 3045–3054, 2014b.

[37] M. Cullen, F. H. Sheu, M. J. Tsai, and Y. H. Chu, "The effects of dielectric barrier discharge plasma gas and plasma-activated water on texture, color, and bacterial characteristics of shiitake mushroom," *Journal of Food Processing and Preservation*, Article ID e14316, vol. 44, no. 1, pp. 1–9, 2019.

[38] Z. U. Khan, G. Aisikaer, R. U. Khan et al., "Effects of composite chemical pretreatment on maintaining quality in button mushrooms (Agaricus bisporus) during postharvest storage," *Postharvest Biology and Technology*, vol. 95, pp. 36–41, 2014.

[39] P. M. A. Toivonen and D. A. Brummell, "Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables," *Postharvest Biology and Technology*, vol. 48, no. 1, pp. 1–14, 2008.

[40] R. C. Soliva-Fortuny and O. Martín-Belloso, "New advances in extending the shelf-life of fresh-cut fruits: a review," *Trends in Food Science & Technology*, vol. 14, no. 9, pp. 341–353, 2003.

[41] C. Sarangapani, G. O'Toole, P. J. Cullen, and P. Bourke, "Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries," *Innovative Food Science & Emerging Technologies*, vol. 44, pp. 235–241, 2017.

[42] O. Lamikanra, J. C. Chen, D. Banks, and P. A. Hunter, "Biochemical and microbial changes during the storage of minimally processed cantaloupe," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 12, pp. 5955–5961, 2000.

[43] K. Oehmigen, M. Hähnel, R. Brandenburg, C. Wilke, K. D. Weltmann, and T. Von Woedtke, "The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids," *Plasma Processes and Polymers*, vol. 7, no. 3-4, pp. 250–257, 2010.

[44] A. P. Dionísio, R. T. Gomes, and M. Oetterer, "Ionizing radiation effects on food vitamins: a review," *Brazilian Archives of Biology and Technology*, vol. 52, no. 5, pp. 1267–1278, 2009.

[45] Y. J. Oh, A. Y. Song, and S. C. Min, "Inhibition of *Salmonella typhimurium* on radish sprouts using nitrogen-cold plasma," *International Journal of Food Microbiology*, vol. 249, pp. 66–71, 2017.

[46] A. Y. Song, Y. J. Oh, J. E. Kim, K. B. Song, D. H. Oh, and S. C. Min, "Cold plasma treatment for microbial safety and preservation of fresh lettuce," *Food Science and Biotechnology*, vol. 24, no. 5, pp. 1717–1724, 2015.

[47] S. Helgadóttir, S. Pandit, V. R. Mokkapati, F. Westerlund, P. Apell, and I. Mijakovic, "Vitamin C pretreatment enhances the antibacterial effect of cold atmospheric plasma," *Frontiers in Cellular and Infection Microbiology*, vol. 7, p. 43, 2017.

[48] F. Grzegorzewski, J. Ehlbeck, O. Schlüter, L. W. Kroh, and S. Rohn, "Treating lamb's lettuce with a cold plasma - influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of Valerianella locusta," *LWT - Food Science and Technology*, vol. 44, no. 10, pp. 2285–2289, 2011.

[49] F. Pasquali, A. C. Stratakos, A. Koidis et al., "Atmospheric cold plasma process for vegetable leaf decontamination: a feasibility study on radicchio (red chicory, Cichorium intybus L.)," *Food Control*, vol. 60, pp. 552–559, 2016.

[50] D. M. Trevisani, J. C. Beaulieu, and R. Shewfelt, "Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing," *Critical Reviews in Food Science and Nutrition*, vol. 50, no. 5, pp. 369–389, 2010.

[51] T. Lee, P. Puligundla, and C. Mok, "Intermittent corona discharge plasma jet for improving tomato quality," *Journal of Food Engineering*, vol. 223, pp. 168–174, 2018.

[52] S. Srey, S. Y. Park, I. K. Jahid, and S. D. Ha, "Reduction effect of the selected chemical and physical treatments to reduce L. monocytogenes biofilms formed on lettuce and cabbage," *Food Research International*, vol. 62, pp. 484–491, 2014.

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Research Article

Exposure to Volatile Essential Oils of Myrtle (*Myrtus communis* L.) Leaves for Improving the Postharvest Storability of Fresh Loquat Fruits

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Fresh loquat (*Eriobotrya japonica* Lindl.) fruits easily lose their marketability because of fungal spoilage, browning, and weight loss after harvest. The use of essential oils as postharvest treatment is a talented alternative to fungicides mainly because of their ability to reduce respiration and transpiration of the fresh fruits during storage. However, the postharvest studies with the volatiles of essential oils are limited. The present study was conducted to determine the effectiveness of volatile essential oils of myrtle (*Myrtus communis* L.) leaves for preserving the postharvest storability of loquat fruits. Test fruits of the present study were exposed to 5 different treatments, which are (i) water vapor (2 min), (ii) myrtle leaves (3% w/w), (iii) myrtle leaf vapor (2 min), (iv) myrtle leaf vapor (10 min), and (v) untreated control. A total of 30 compounds were isolated from the essential oil, and the three highest amounts of compounds were determined as eucalyptol (39.38%), α-pinene (24.98%), and linalool (8.18%). Exposure to myrtle leaves (3% w/w) and myrtle leaf vapor (2 min) was also noted to provide higher efficacy for reducing the weight loss, decay incidence, and browning index.

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical plant which was originated from south-central China. It belongs to the Rosaceae family [1] and widely adapted to many regions of the world, including South Africa, the United States of America, Asia, and Mediterranean countries [2]. Loquat fruits are nutritious and have high amount of diverse phytochemicals [3]. Loquat fruits have also been reported as delicious fruits, which are highly appreciated by consumers and have been used for many purposes, including fresh consumption, syrups, jams, juices, and wines [2]. Loquat fruits also have a long history in traditional Chinese medicine [4]. One of the main disadvantages of loquat fruits for marketing is the short storability of the fruits due to enzymatic browning, weight loss, and pathogenic decay [5]. Weight loss and pathogenic decay were reported to be controlled by storing the fruits at low temperatures (5 to 7°C). However, low temperatures are known to adversely affect fruit quality due to chilling injury [6]. The main symptoms of chilling injury at the loquat fruits are the internal and external browning together with increased fruit firmness and reduced juice content. Heat treatment [7] and application of edible coatings [8–10] have been reported to reduce fruit browning.

Postharvest pathogens are the other important factors reducing the marketability of loquat fruits. Over the last 70 years, fungicides and other agrochemicals have been the

main methods for controlling postharvest pathogens. However, the acceptability of agrochemicals has been decreasing since the beginning of the 20th century, due to the confirmation of their possible adverse effects on human health and the environment [11]. Therefore, alternatives to the agrochemicals have been the subject of current studies, which mainly focus on the use of biomaterials [12]. The use of plant-derived biomaterials in postharvest handling of fruits had great interest since the 20th century, mainly because of their high acceptability by the consumers due to their extended threshold for toxicity [13]. The plant extracts [14–18], essential oils [19–21], and edible coatings and/or films [22–24] have been reported to have high potential for improving the postharvest storability of fresh fruits and vegetables and controlling postharvest pathogens.

The mode of action of the plant-derived biomaterials is mainly a result of the semipermeable barrier for water vapor, oxygen, and carbon dioxide which help to reduce transpiration and respiration. Biomaterials also include diverse phytochemicals which are known to activate some enzymes (ascorbate peroxidise-APX, catalase-CAT, and superoxide dismutase-SOD) and/or deactivate some others (polyphenoloxidase-PPO and peroxidise-POD), which help for preserving postharvest fruit quality and/or controlling fruit pathogens [17, 25]. Essential oils (EOs), with their diverse phytochemical compounds and biologically active characteristics, have high potential for preserving postharvest fruit quality [26–28]. Another advantage of the EOs is their ability to be applied as vapors [29]. Myrtle (*Myrtus communis* L.) plants grow wildly in the Mediterranean basin and are an important element in the maquis [30]. The essential oils of myrtle leaves are reported to be used in the perfume and flavor industry and have high antimicrobial activity [31]. Several studies have been performed about the chemical composition of the essential oils of myrtle leaves [31–34]; however, the postharvest studies with the EOs of myrtle leaves are limited. Therefore, this study was conducted to investigate the effectiveness of volatile essential oils of myrtle (*M. communis* L.) leaves for preserving the postharvest storability of loquat fruits. Parallel to the postharvest studies, the essential oils of the myrtle leaves of the present study were also determined.

2. Materials and Methods

2.1. Materials. Loquat fruits *Eriobotrya japonica* cv. "Morphitiki" of the present study were hand-collected at horticultural maturity (27 April 2020) based on skin color (fully yellow-orange) from a commercial plantation located in Yayla village in Northern Cyprus. The SSC: TA was also used to determine horticultural maturity. The SSC was between 11.00% and 12.00%, whereas the TA was between 0.95 g $100\,g^{-1}$ and 1.05 g $100\,g^{-1}$ [10]. Fruits were then immediately transferred to the laboratory and were selected to ensure homogeneous size and color and to eliminate any damaged fruits. At the same time, leaves of myrtle (*Myrtus communis* L.) were collected from the same village, about 400 m away from the loquat plantation. In the experiments, the young leaves of myrtle are used because their balsamic time (June) is very near and the old ones are eliminated. The use of the old leaves can be quite dangerous because normally they contain some specific pathogens which may aggravate the decay of the treated fruits.

2.2. Experimental Methodologies. The experimental studies were planned to continue for 42 days and quality parameters to be measured within a 7-day interval (7, 14, 21, 28, 35, and 42). For each measurement point, 3 replications with 10 fruits in each were assigned. Therefore, totally 180 fruits (6 * 3 * 10) were designed for each treatment. After selection, fruits were randomly divided into 5 groups (# of treatments) of 180 fruits in each group. These groups were then treated with one of the following treatments: (i) water vapor (2 min), (ii) myrtle leaves (3% w/w of total weight of fruits), (iii) myrtle leaf vapor (2 min), (iv) myrtle leaf vapor (10 min), and (v) untreated control. Fruits were all dipped in distilled water for 1 min duration and air dried for 30 min before exposing to the treatments explained below. Detailed explanation for the treatments is given in Table 1.

After exposing to the abovementioned treatments and packaging, all fruit trays were transferred to the storage conditions of $4 \pm 1^{\circ}C$ and 95% relative humidity. Studies were continued for 42 days, and a total of 3 trays from each group were taken out from cold rooms with 7-d intervals (7, 14, 21, 28, 35, and 42 d) to measure the quality characteristics. Study duration and measurement points were determined according to the several previous studies which used 5/7-day intervals for 35–40 d storage durations [7, 35].

2.3. Data Collection. Weight loss, decay incidence (DI), browning index (BI), fruit firmness, soluble solids concentration (SSC), and titratable acidity (TA) were all measured according to the following methods. Weight loss (%) was determined by subtracting the final weight from the initial weight and dividing the solution to the initial weight and multiplying with 100. Initial and final weights of the fruits were all measured with a digital scale (±0.01 g). DI of the fruits was assessed by using the four-point scale formula [36]. To do so, all fruits were individually graded from 0 to 3 where 0 means no decay, 1 represents slight decay (≤25%), 2 equals moderate decay (25% < 50%), and 3 means severe decay (>50%). The following formula was then used to calculate the DI. $DI = \{[(1 \times N_1) + (2 \times N_2) + (3 \times N_3)] \times 100/(3 \times N)\}$. Here, N equals to the total number of fruits where N_x represents the number of fruits with "x" score of decay severity.

The BI was used to measure the chilling severity of the fruits. The 0–4 scale of Rui et al. [37] was used to determine BI. In this scale, 0 equals no browning, 1 represents slight browning (less than <5%), 2 means moderate browning (5–25%), 3 represents moderately severe browning (25–50%), and 4 equals to severe browning (>50%). Scoring of the fruits was performed after 1 additional day at 20°C. Next, the following formula was used to calculate BI. BI index = $\{\sum[(\text{BI scale}) \times (\text{number of fruit at that BI})]\}/(4 \times \text{total number of fruit in each treatment})$. The limit of

Table 1: Detailed explanations of the treatments.

Treatments	Explanation
Water vapor (2 min)	Firstly, 20 L of water was boiled in a stainless steel cap. Then, a checkered wire (with 1 cm^2 sieve) was put over the cap. Hereafter, loquat fruits were lined up in a single row on the wire and waited for 2 min. The fruits were than air dried for 1 h and transferred to small PET trays (16.5 * 8.5 * 3.5 cm) with a volume of 490.8 mL. Ten fruits were placed in each tray, and the upper part was covered with a linear low-density polyethylene (LLDPE) stretch film with 10 micron thickness
Myrtle leaves (3% w/w)	Ten loquat fruits (equalling to 1 replication) were placed in small PET trays (described above) with myrtle leaves (3% w/w), and the upper part was covered with an LLDPE stretch film. The fruits were kept together with the myrtle leaves until the end of the planned storage durations
Myrtle leaf vapor (2 min)	Similar to the water vapor treatment, 20 L of water was boiled in a stainless steel cap. Then, 200 g of fresh myrtle leaves were added into boiling water and boiled for 5 minutes. Then, loquat fruits were lined up in a single row on the wire and waited for 2 min. Hereafter, the same procedure was followed for drying and packing of the fruits.
Myrtle leaf vapor (10 min)	This treatment is the same as that described above, "myrtle leaf vapor (2 min)" treatment, whereas the exposing duration of the loquat fruits to the myrtle leaf vapor is 10 min, instead of 2 min
Untreated control	Fruits of this treatment (untreated control) were dried for additional 30 min to make it equal with the abovedescribed treatments. No any other applications have been performed to these fruits

acceptability (marketability) of the fruits was defined as a BI index of 0.4 according to Ghasemnezhad et al. [8].

A hand penetrometer with a 5 mm-diameter probe was used at a speed of 1 mm s^{-1} to measure the fruit firmness (kg cm^{-2}). Then, SSC of each loquat fruits was assessed by using a hand refractometer. Weight loss, fruit firmness, decay incidence, browning index, and SSC were measured for 30 individual fruits for each treatment. Finally, TA of loquat fruits was assessed according to the method of AOAC [38] as g/100 g of malic acid. For TA determination, a mixture of the juice of 10 fruits belonging to the same replication of same treatment was used. Thus, a total of 3 replications were used for each treatment.

2.4. Isolation and Analyses of Essential Oil. The steam distillation method (by using Clevenger-type apparatus (European Pharmacopeia)) was used to separate volatile essential oils from the plant leaves, and the oil was kept at amber vials at +4°C until analysis. Essential oil components were analysed with a GC-MS (Gas Chromatography-Mass Spectrometry) device, Thermo Scientific ISQ Single Quadrupole. 5 μl of essential oil was diluted in 2 ml cyclohexane. The column model was TG-Wax MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter * 60 m length, and 0.25 μm film thickness). The ionization energy was calibrated as 70 eV, and the mass interval was m/z 1.2–1200 amu. The scan mode was used as the screening more in data collection. The MS transfer line temperature was 250°C, MS ionization temperature was 220°C, and column temperature was 50°C at the beginning; then, it was increased up to 220°C with 3°C min^{-1} rate of temperature increase. The structure of each component was defined by using mass spectrums (Wiley 9) with Xcalibur software. Retention indices were determined using retention times of n-alkanes (C8–C40) that were injected after the *M. communis* essential oil under the same chromatic conditions.

2.5. Data Analysis. Raw data of the experiments were subjected to the analysis of variance (ANOVA) by using SPSS 22.0 software. Comparison of the means of different treatments was assessed by Tukey's HSD test at $P = 0.05$. Microsoft excel was then used to prepare figures from the means and standard deviations.

3. Results

3.1. Chemical Composition of the Volatile Essential Oils. Volatile essential oil of myrtle leaves was determined to consist of 30 different compounds. The three most abundant compounds were determined as eucalyptol (39.38%), α-pinene (24.98%), and linalool (8.18%). The full list of the chemical compounds is given in Table 2. Eucalyptol is a colorless organic liquid compound, a cyclic ether, and a monoterpene. α-Pinene is a colorless terpenoid and liquid plant metabolite, which is substituted by methyl groups at 3 positions. Linalool is also a monoterpenoid, which is substituted by methyl groups at 2 positions and a hydroxy group at 1 position. Moreover, α-terpineol and limonene was found to have 6.94% and 6.16% in the essential oil of myrtle leaves.

3.2. Weight Loss and Fruit Firmness. Results of the present study showed that, as expected, the weight loss of the loquat fruits increased during the storage (Table 3.). Two (myrtle leaf (3% w/w) and myrtle leaf vapor (2 min)) of the four test applications were found to be highly effective in preventing the weight loss during storage (Figure 1(a).). Water vapor, which was tested as the second control of the present study was found to have slight influence on the weight loss prevention, but it was not statistically significant. Besides that, myrtle leaf vapor (10 min) application was also ineffective in preventing the weight loss, as compared with the myrtle leaf vapor (2 min). These results suggest that the increase in the exposing duration to the myrtle leaf vapor causes a reduction in the effectiveness of the application. At the end of the 42 days of storage, the minimum weight loss was noted from the myrtle leaf (3% w/w) treatment as 17.60% and was followed by the myrtle leaf vapor (2 min) treatment with 18.05%. No significant difference was noted between these two treatments. Moreover, the highest weight loss was observed from the control treatment with 23.20%.

Table 2: Chemical compositions of the volatile essential oils (%) of myrtle leaves.

RI*	Compound name	Percentages (%)
1035	α-Pinene	24.98
1135	β-Pinene	0.18
1163	Isobutylisobutyrate	0.28
1206	Limonene	6.16
1246	Sabinene	Trace
1255	β-Pinene	Trace
1281	Eucalyptol (1.8-cineol)	39.38
1303	p-Cymene	0.81
1535	Linalool	8.18
1605	Linalylacetate	1.96
1614	Elemene	Trace
1668	Caryophyllene	0.70
1673	Terpinen-4-ol	0.34
1688	Caryophyllene	Trace
1704	Isopinocarveol	0.14
1721	Estragole	0.40
1725	Humulene	0.29
1734	α-Terpineol	6.94
1738	trans-Caryophyllene	0.04
1743	α-Terpinylacetate	4.68
1745	Myrtenylacetate	0.99
1761	Geranylacetate	1.90
1768	Nerol	Trace
1778	Myrtenol	0.64
1796	trans-Carveol	Trace
1813	trans-Caryophyllene	Trace
1895	D-verbenone	Trace
2025	Methyleugenol	0.26
2085	Caryophyllene oxide	0.27
2090	Junipene	0.05

*Retention indices were calculated according to n-alkanes retention times and samples retention times.

Table 3: Effects of cold storage on the different quality attributes (average of five different treatments) of loquat fruits during 42 days of storage.

Storage duration	WL (%)	DI (%)	BI	Firmness	SSC	TA
At harvest	0.00 g	0.00 c	0.00 d	0.42 e	11.52 c	0.98 c
7 days	1.60 f	0.00 c	0.03 d	0.39 f	10.39 e	0.76 e
14 days	4.50 e	2.89 c	0.19 c	0.43 e	9.82 f	0.66 f
21 days	8.66 d	6.22 c	0.28 bc	0.47 d	10.99 e	0.59 f
28 days	11.88 c	10.22 c	0.31 bc	0.51 c	11.27 cd	0.89 d
35 days	14.96 b	48.67 b	0.39 ab	0.57 b	12.08 b	1.12 b
42 days	20.60 a	83.56 a	0.45 a	0.61 a	12.73 a	1.42 a

Means of quality attributes in the same column with the same letters showed no significant differences according to Tukey's HSD test at $P \leq 0.05$.

Firmness of the loquat fruits showed a slightly different tendency during storage as compared with the other quality parameters (Table 3.). In the first weeks of storage, a slight decrease was noted in the fruit firmness, and then, it increased continuously corresponding to the decline of fruit weight and increase in BI. Fruit firmness was significantly lower at the fruits treated with myrtle leaf vapor (2 min) and myrtle leaf vapor (10 min) as compared with the untreated control fruits (Figure 1(b).). However, this was higher than the initial fruit firmness. The initial firmness of loquat fruits in present study was 0.42 kg cm^{-2} and was found to increase until 0.72 kg cm^{-2} at the untreated control fruits after 42 days of storage. During this period of cold storage, the application of myrtle leaf vapor (2 min) and myrtle leaf vapor (10 min) was observed to keep fruit firmness at lower degrees, 0.52 kg cm^{-2} and 0.55 kgcm^{-2}, respectively.

3.3. Decay Incidence and Browning Index. As expected, DI (%) and BI were observed to have an increasing trend during cold storage. However, the decay incidence was not significant from the harvest date until the 28th day of storage (Table 3.). Similar with the results of weight loss, the myrtle leaf (3% w/w) and myrtle leaf vapor (2 min) treatments were found to be effective in preventing the DI (Figure 2(a)). At the end of the storage (42 days of storage), the minimum DI was observed from the myrtle leaf (3% w/w) treatment with 50%. Although it was effective in reducing the DI, this was not acceptable for marketing. Therefore, it can be concluded from the results that the loquat fruits can be stored for 35 days in cold rooms, with the application of either myrtle leaf (3% w/w) or myrtle leaf vapor (2 min).

The results about BI were slightly similar with the effects on the weight loss and DI. However, it was noted that the myrtle leaf vapor (2 min) application has higher influence than the myrtle leaf (3% w/w) (Figure 2(b).). In addition to that, myrtle leaf vapor (10 min) application was also successful in preventing the BI. At the end of the storage period (42 days), the lowest browning index was noted from the myrtle leaf vapor (2 min) application with a score of 0.225 and was followed by the myrtle leaf (3% w/w) and myrtle leaf vapor (10 min), with BI scores of 0.392 and 0.408, respectively. At that time, the BI was observed as 0.708 at the untreated control fruits.

3.4. Soluble Solids Concentration and Titratable Acidity. In present study, the fruit SSC and TA were observed to have a decreasing trend during the first 21 days of cold storage, and then, the trend was changed to reverse (Table 3.). The initial SSC was 11.52% and was noted to increase up to 12.91% at the untreated control fruits in 42 days of storage. Results suggested that the all applications have a significant influence on the SSC, and the lowest SSC (12.52%) was measured from the fruits treated with myrtle leaf vapor (2 min) (Figure 3(a)). A similar trend was observed for the TA. The initial TA content was 0.98 g 100 ml^{-1} malic acid. The TA of the untreated control fruits decreased to 0.55 g 100 ml^{-1} malic acid in 21 days of storage, and then, it showed an increasing trend. The final TA content of the untreated control fruits was 1.51 g 100 ml^{-1} malic acid. Results suggested that all treatments have significant effect on TA. Thus,

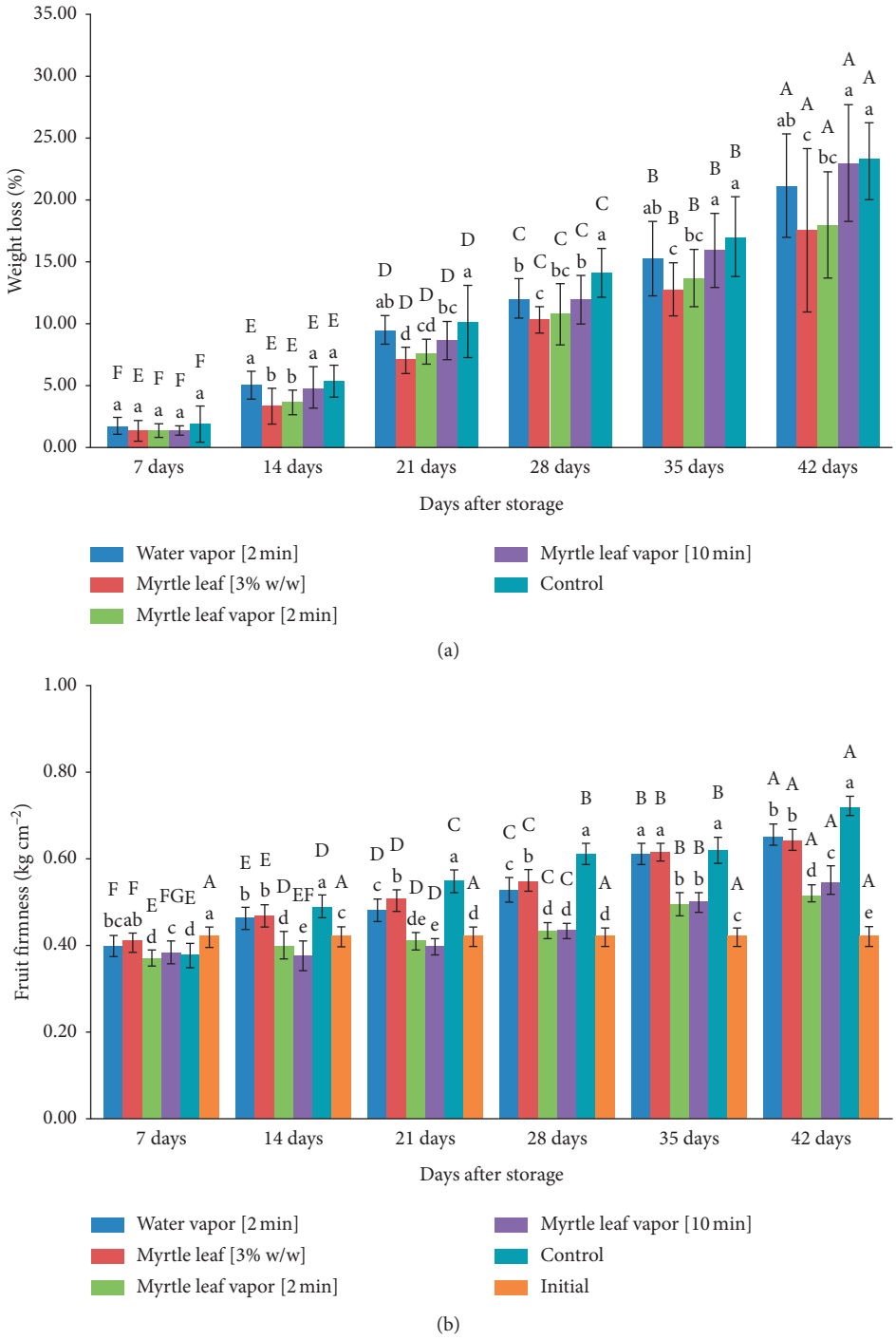

Figure 1: Effects of volatile essential oils of myrtle leaves on the (a) weight loss and (b) fruit firmness of loquat fruits during 42 days of storage. Different lowercase letter denote significant differences among different treatments for the same sampling time, and different capital letter denotes significant differences among different sampling times for the same treatment, according to Tukey's HSD test at $P \leq 0.05$.

the minimum TA content (1.32 g 100 ml^{-1} malic acid) was measured from the myrtle leaf vapor (2 min) (Figure 3(b)).

4. Discussion

Novel results of the current work suggested that exposing loquat fruits to the volatile essential oils of myrtle leaves, either by packing together in a concentration of 3% w/w or exposing to the myrtle leaf vapor for 2 min, improves the postharvest storability of fruits. Overall results about the quality parameters of untreated control fruits are in accordance with the findings of Song et al. [9]. The dominant chemical compounds of the volatile essential oils were found to be te eucalyptol, α-pinene, and linalool for the myrtle leaves in the present study. Similarly, eucalyptol and α-pinene compounds were noted to be the two most dominant

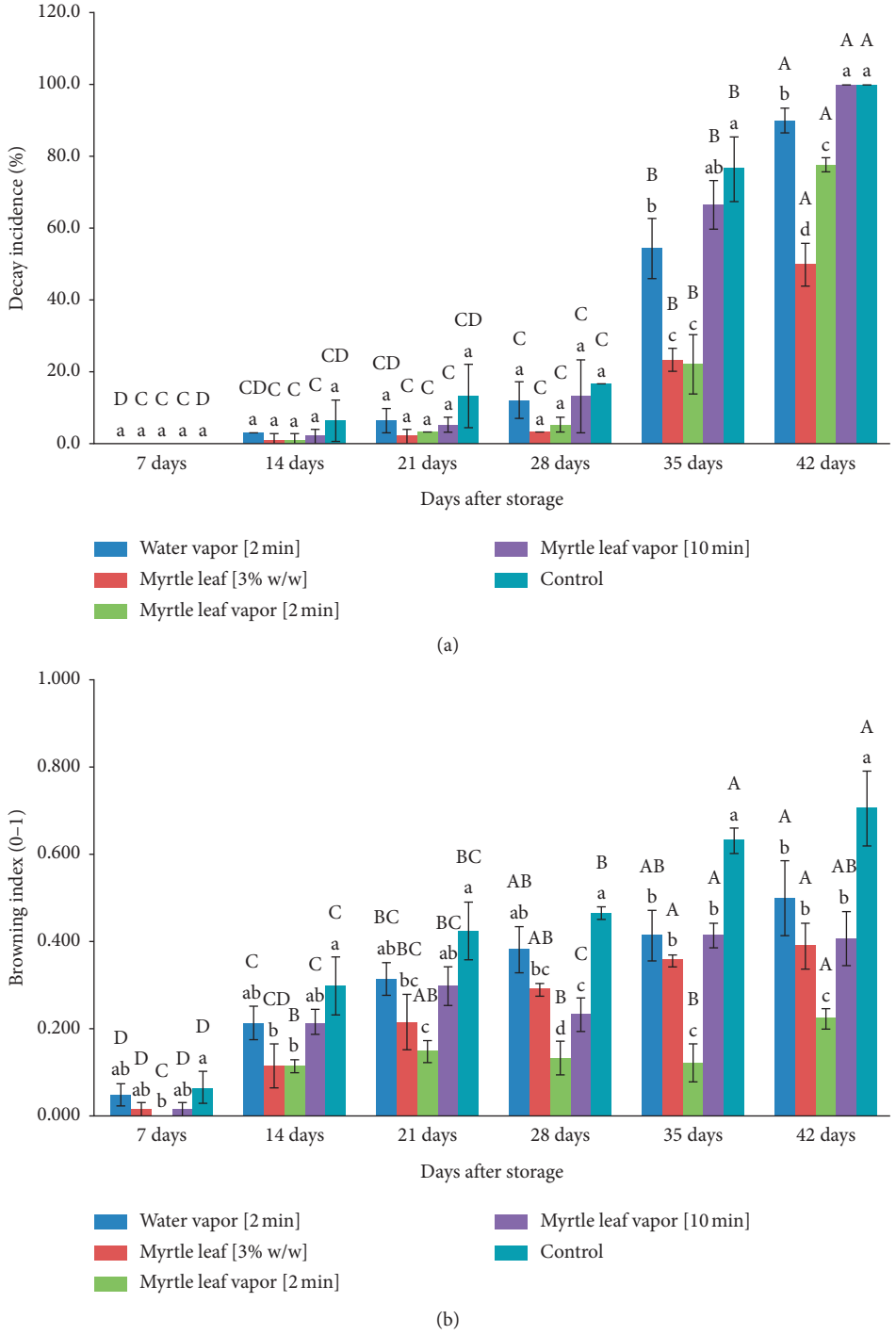

Figure 2: Effects of volatile essential oils of myrtle leaves on the (a) decay incidence and (b) browning index of loquat fruits during 42 days of storage. Different lowercase letters denote significant differences among different treatments for the same sampling time; and different capital letters denote significant differences among different sampling times for the same treatment, according to Tukey's HSD test at $P < 0.05$.

compounds of the essential oils extracted from *M. communis* L. leaves [33, 34]. Myszka et al. [34] reported 24 different chemical compounds from the essential oils of myrtle leaves, where 18 of them were similar to those of the current work. There is not much study about the effects of myrtle essential oils on the fruit weight, but similar results were noted for different essential oils including similar compounds in their chemical structures [15, 17, 19, 21, 24].

These terpenes were previously noted to have high bioactivity against several food spoilage microorganisms and pathogens [34, 39, 40]. The mechanism was reported be mainly disruption of the membrane functions by damaging

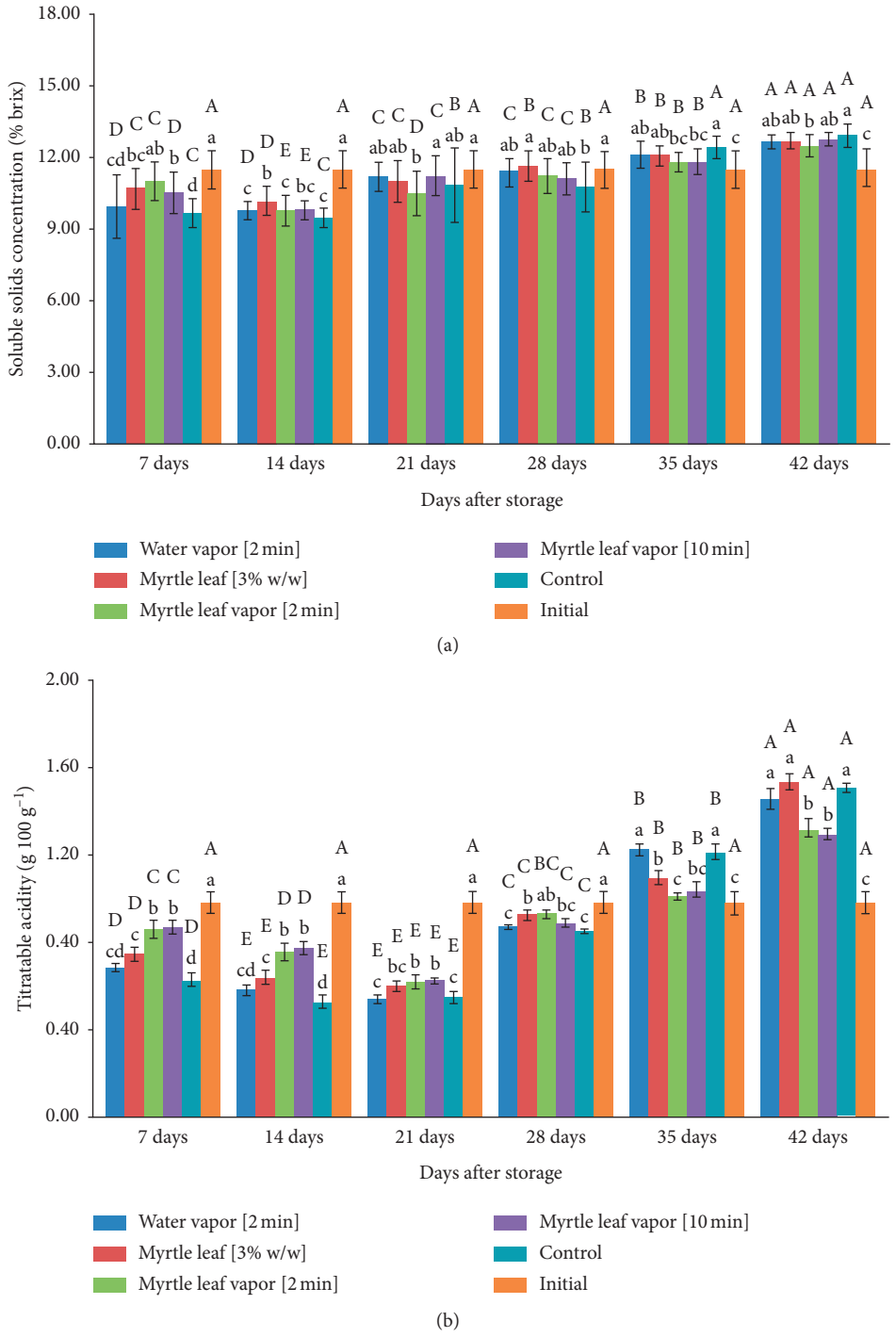

Figure 3: Effects of volatile essential oils of myrtle leaves on the (a) soluble solids concentration and (b) titratable acidity of loquat fruits during 42 days of storage. Different lowercase letters denote significant differences among different treatments for the same sampling time, and different capital letters denote significant differences among different sampling times for the same treatment, according to Tukey's HSD test at $P < 0.05$.

the cell wall and membrane structures [39]. The two most important functions were noted to be electron transfer or enzyme activity. Therefore, the high influence of the myrtle leaf and myrtle leaf vapor on the prevention of the decay incidence in the present study can be attributed to the high eucalyptol and α-pinene contents in the volatile essential oils of myrtle leaves. Similar effects were previously noted for the essential oils of myrtle leaves on the *Salmonella typhimurium* [41], Gram-positive and Gram-negative bacteria [42], and *Cryptococcus neoformans* (yeast) [43]. In the study of Fadil et al. [41], it is was noted that a mixture of *Thymus vulgaris L.* (55%) and *M. communis L.* (45%) essential oils provides high

antibacterial activity against *Salmonella typhimurium*. In another study carried out in Algeria, is was found that the *M. communis* L. essential oil has strong antifungal activity against *Cryptococcus neoformans* (yeast) and *Epidermophyton floccosum*, *Microsporum canis*, and *Trichophyton rubrum* (dermatophytes) [43]. Current results of this study also reveal the traditional use of the Myrtle plants as a disinfectant in Greece [44]. In a most similar study, the essential oil vapors of *M. communis* L. were tested against *Penicillium digitatum* in *in vitro* conditions, and the results showed that it elapsed time between the fungal inoculum and vapor contact [45]. However, in the mentioned study, the essential oils of *M. communis* L. leaves were noted to have lower efficacy than those of *Rosmarinus officinalis* L. The volatile essential oil of myrtle leaves was also tested *in vitro* against two stored-product insects, *Tribolium confusum* and *Callosobruchus maculates*, and the results of this research showed that the volatile essential oils of myrtle leaves might be used as a potential biocontrol agent these insects [46].

Fruit browning, in conjunction with chilling injury, is among the most important postharvest problems of loquat fruits [47]. Current work demonstrated that exposing fruits to the volatile essential oils of myrtle leaves either by packing together or exposing to leaf vapor is an effective method for reducing the fruit browning. Several studies suggested that the application of different hormones or chemicals, i.e., methyl jasmonate, salicylic acid, and 1-methylcyclopropene, was effective in reducing the browning index of loquat fruit [5, 48].

In agreement with the weight loss results, the results of the present study showed that the volatile essential oils of myrtle leaves are effective in preventing the changes in the fruit firmness. According to Song et al. [35], firmness of loquat fruits increases during storage, together with the lignin contents, and those are the typical symptoms of fruit browning. The main cause of the increase in fruit firmness was also attributed to the cell expansion and secondary lignification in cell wall [48]. Thus, the increase in lignin content causes a rigidity characteristic at the cell wall, which then causes an increase in fruit firmness [49]. Therefore, contrary to many of the fruits, increase in fruit firmness is not a desirable characteristic for the loquat fruits which has less extractable juice [50]. No measurement has been performed in the current work about the lignin content of the loquat fruits, but results showed that the essential oils of myrtle leaf provide favorable conditions for preventing the increase in fruit firmness. Furthermore, results of the present study showed that the volatiles of myrtle leaf have a slight influence on the SSC and TA contents of loquat fruits during storage. In a similar work, myrtle leaf oil application was noted to display similar effects on the SSC of strawberry fruits during storage [51].

5. Conclusions

To sum up, results of the current work suggested that the diverse composition of the volatile essential oils of myrtle leaf makes it a suitable agent for postharvest handling. Among the described 30 chemical compounds, eucalyptol, α-pinene, and linalool were found to be the most abundant compounds. Results also demonstrated that packaging of loquat fruits with myrtle leaves (3% w/w) or exposing them to the myrtle leaf vapor (2 min) provide higher efficacy for the reduction of weight loss, prevention of decay incidence, and reducing the fruit browning. Overall, results suggested that the fresh loquat fruits can be stored for 35 days at $4 \pm 1^{\circ}$C and 95% relative humidity by exposing to myrtle leaves (3% w/w) and myrtle leaf vapor (2 min). Thus, the current study would be a preliminary study to investigate the effects of volatile essential oils of myrtle leaf on fruit quality and pathogenic mechanisms, and the results would guide further studies with different fruits.

Data Availability

All data used to support the findings of this study are included within the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

"Conceptualization was carried out by N.P.B., İ.K., and C.W.; İ.K. and C.W. were responsible for the methodology; investigation was conducted by N.P.B. and İ.K.; data curation was performed by İ.K.; writing of the original draft was carried out by N.P.B. and İ.K.; C.W. was involved in writing the review and editing; visualization was performed by İ.K. All authors have read and agreed to the published version of the manuscript."

References

[1] J. Janick, "Predictions for loquat improvement in the next decade," *Acta Horticulture*, vol. 887, pp. 25–29, 2010.

[2] S. Lin, "*Eriobotrya japonica (loquat)*," in *The Encyclopedia of Fruit and Nuts*CABI, Wallingford, UK, 2008.

[3] X. Li, C. Xu, and K. Chen, "Nutritional and composition of fruit cultivars: loquat (*Eriobotrya japonica* Lindl.)," in *Nutritional Composition of Fruit Cultivars*, X. Li, Ed., pp. 371–394, Academic Press, Cambridge, MA, USA, 2016.

[4] D. D. He and Z. K. Luo, "Textual research on the herbal medicine of loquat," *Journal of Chinese Medicinal Materials*, vol. 7, pp. 1687–1690, 2019.

[5] Y. H. Zheng, S. Y. Li, Y. F. Xi, X. G. Xu, and Y. B. Yi, "Polyamine changes and chilling injury in cold-stored loquat fruits," *Acta Botanica Sinica*, vol. 42, no. 8, pp. 824–827, 2000.

[6] L. L. Shan, X. Li, P. Wang et al., "Characterization of cDNAs associated with lignification and their expression profiles in loquat fruit with different lignin accumulation," *Planta*, vol. 227, no. 6, p. 1243, 2008.

[7] Y. Zhang, P. Jin, Y. Huang et al., "Effect of hot water combined with glycine betaine alleviates chilling injury in cold-stored loquat fruit," *Postharvest Biology and Technology*, vol. 118, pp. 141–147, 2016.

[8] M. Ghasemnezhad, M. A. Nezhad, and S. Gerailoo, "Changes in postharvest quality of loquat (*Eriobotrya japonica*) fruits

influenced by chitosan," *Horticulture, Environment, and Biotechnology*, vol. 52, no. 1, pp. 40–45, 2011.

[9] H. Song, W. Yuan, P. Jin et al., "Effects of chitosan/nano-silica on postharvest quality and antioxidant capacity of loquat fruit during cold storage," *Postharvest Biology and Technology*, vol. 119, pp. 41–48, 2016.

[10] İ Kahramanoğlu, "Preserving postharvest storage quality of fresh loquat fruits by using different bio-materials," *Journal of Food Science and Technology*, vol. 57, no. 8, pp. 3004–3012, 2020.

[11] R. R. Sharma, D. Singh, and R. Singh, "Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: a review," *Biological Control*, vol. 50, no. 3, pp. 205–221, 2009.

[12] C. Silvestre, D. Duraccio, and S. Cimmino, "Food packaging based on polymer nanomaterials," *Progress in Polymer Science*, vol. 36, no. 12, pp. 1766–1782, 2011.

[13] E. Z. Tomazoni, M. R. Pansera, G. F. Pauletti, S. Moura, R. T. S. Ribeiro, and J. Schwambach, "In vitro antifungal activity of four chemotypes of Lippia alba (verbenaceae) essential oils against *Alternaria solani* (Pleosporeaceae) isolates," *Anais da Academia Brasileira de Ciências*, vol. 88, no. 2, pp. 999–1010, 2016.

[14] J. Chen, Y. Shen, C. Chen, and C. Wan, "Inhibition of key citrus postharvest fungal strains by plant extracts in vitro and in vivo: a review," *Plants*, vol. 8, no. 2, p. 26, 2019.

[15] İ. Kahramanoğlu, "Effects of lemongrass oil application and modified atmosphere packaging on the postharvest life and quality of strawberry fruits," *Scientia Horticulturae*, vol. 256, Article ID 108527, 2019.

[16] A. Moosa, S. T. Sahi, S. A. Khan, and A. U. Malik, "Salicylic acid and jasmonic acid can suppress green and blue moulds of citrus fruit and induce the activity of polyphenol oxidase and peroxidase," *Folia Horticulturae*, vol. 31, no. 1, pp. 195–204, 2019.

[17] K. Ncama, A. Mditshwa, S. Z. Tesfay, N. C. Mbili, and L. S. Magwaza, "Topical procedures adopted in testing and application of plant-based extracts as bio-fungicides in controlling postharvest decay of fresh produce," *Crop Protection*, vol. 115, pp. 142–151, 2019.

[18] İ. Kahramanoğlu, S. Usanmaz, V. Okatan, and C. Wan, "Preserving postharvest storage quality of fresh-cut cactus pears by using different bio-materials," *CABI A&B*, vol. 1, p. 7, 2020.

[19] R. Pavela and G. Benelli, "Essential oils as ecofriendly biopesticides? Challenges and constraints," *Trends in Plant Science*, vol. 21, no. 12, pp. 1000–1007, 2016.

[20] R. A. Hassanein, E. A. Salem, and A. A. Zahran, "Efficacy of coupling gamma irradiation with calcium chloride and lemongrass oil in maintaining guava fruit quality and inhibiting fungal growth during cold storage," *Folia Horticulture*, vol. 30, no. 1, pp. 67–78, 2018.

[21] İ. Kahramanoğlu, M. Aktaş, and Ş. Gündüz, "Effects of fludioxonil, propolis and black seed oil application on the postharvest quality of "wonderful" pomegranate," *PLoS One*, vol. 13, no. 5, Article ID e0198411, 2018.

[22] S. Saucedo-Pompa, R. Rojas-Molina, A. F. Aguilera-Carbó et al., "Edible film based on candelilla wax to improve the shelf life and quality of avocado," *Food Research International*, vol. 42, no. 4, pp. 511–515, 2009.

[23] C. Mannozzi, J. P. Cecchini, U. Tylewicz et al., "Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life," *LWT-Food Science and Technology*, vol. 85, pp. 440–444, 2017.

[24] S. C. Riva, U. O. Opara, and O. A. Fawole, "Recent developments on postharvest application of edible coatings on stone fruit: a review," *Scientia Horticulturae*, vol. 262, Article ID 109074, 2020.

[25] S. Ali, A. S. Khan, A. Nawaz et al., "Aloe vera gel coating delays postharvest browning and maintains quality of harvested litchi fruit," *Postharvest Biology and Technology*, vol. 157, Article ID 110960, 2019.

[26] N. Okut, M. Yagmur, N. Selcuk, and B. Yildirim, "Chemical composition of essential oil of mentha longifolia L. subsp. longifolia growing wild," *Pakistan Journal of Botany*, vol. 49, no. 2, pp. 525–529, 2017.

[27] A. Servili, E. Feliziani, and G. Romanazzi, "Exposure to volatiles of essential oils alone or under hypobaric treatment to control postharvest gray mold of table grapes," *Postharvest Biology and Technology*, vol. 133, pp. 36–40, 2017.

[28] A. A. Alemu, W. Garedew, and A. Gebre, "Essential oil yield and yield components of basil (Ocimum basilicum L.) as affected by genotype and intrarow spacing at Jimma, SW Ethiopia," *Acta Agrobotanica*, vol. 71, no. 3, 2018.

[29] P. Tripathi, N. K. Dubey, and A. K. Shukla, "Use of some essential oils as post-harvest botanical fungicides in the management of grey mould of grapes caused by *Botrytis cinerea*," *World Journal of Microbiology and Biotechnology*, vol. 24, no. 1, pp. 39–46, 2008.

[30] M. Nassar, E.-S. Aboutabl, R. Ahmed, E.-D. El-Khrisy, K. Ibrahim, and A. Sleem, "Secondary metabolites and bioactivities of myrtus communis," *Pharmacognosy Research*, vol. 2, no. 6, p. 325, 2010.

[31] K. Zomorodian, M. Moein, Z. G. Lori et al., "Chemical composition and antimicrobial activities of the essential oil from myrtus communis leaves," *Journal of Essential Oil Bearing Plants*, vol. 16, no. 1, pp. 76–84, 2013.

[32] A. Salimi Beni, M. K. Kocheki Shahmokhtar, A. Masoumias, and H. Khajehsharifi, "Phytochemical and biological studies of some myrtus (*Myrtus communis* L.) populations of south west region of zagros (Iran)," *Current Issue - Natural Products Chemistry and Research*, vol. 5, no. 290, p. 2, 2017.

[33] M. Usai, M. Marchetti, N. Culeddu, and M. Mulas, "Chemical composition of myrtle (Myrtus communis L.) berries essential oils as observed in a collection of genotypes," *Molecules*, vol. 23, no. 10, p. 2502, 2018.

[34] K. Myszka, N. Sobieszczańska, A. Olejnik et al., "Studies on the anti-proliferative and anti-quorum sensing potentials of Myrtus communis L. essential oil for the improved microbial stability of salmon-based products," *Lebensmittel-Wissenschaft & Technologie*, vol. 127, Article ID 109380, 2020.

[35] H. Song, X. Wang, W. Hu et al., "A cold-induced phytosulfokine peptide is related to the improvement of loquat fruit chilling tolerance," *Food Chemistry*, vol. 232, pp. 434–442, 2017.

[36] S. Cao, Y. Zheng, and Z. Yang, "Effect of 1-MCP treatment on nutritive and functional properties of loquat fruit during cold storage," *New Zealand Journal of Crop and Horticultural Science*, vol. 39, no. 1, pp. 61–70, 2011.

[37] H. Rui, S. Cao, H. Shang, P. Jin, K. Wang, and Y. Zheng, "Effects of heat treatment on internal browning and membrane fatty acid in loquat fruit in response to chilling stress," *Journal of the Science of Food and Agriculture*, vol. 90, no. 9, pp. 1557–1561, 2010.

[38] AOAC, *Official Method of Analysis of the Association of Official Analytical Chemistry*, AOAC, Arlington, VA, USA, 15th edition, 1990.

[39] V. Aleksic and P. Knezevic, "Antimicrobial and antioxidative activity of extracts and essential oils of myrtus communis L," *Microbiological Research*, vol. 169, no. 4, pp. 240–254, 2014.

[40] Y. Eguchi, A. Widiastuti, H. Odani et al., "Identification of terpenoids volatilized from Thymus vulgaris L. by heat treatment and their in vitro antimicrobial activity," *Physiological and Molecular Plant Pathology*, vol. 94, pp. 83–89, 2016.

[41] M. Fadil, K. Fikri-Benbrahim, S. Rachiq et al., "Combined treatment of thymus vulgaris L., Rosmarinus officinalis L. and myrtus communis L. essential oils against *Salmonella typhimurium*: optimization of antibacterial activity by mixture design methodology," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 126, pp. 211–220, 2018.

[42] M. Fadil, A. Farah, B. Ihssane, T. Haloui, S. Lebrazi, and S. Rachiq, "Intrapopulation variability of *Myrtus communis* L. growing in morocco: chemometric investigation and antibacterial activity," *Journal of Applied Research on Medicinal and Aromatic Plants*, vol. 7, pp. 35–40, 2017.

[43] A. Bouzabata, C. Cabral, M. J. Gonçalves et al., "*Myrtus communis* L. as source of a bioactive and safe essential oil," *Food and Chemical Toxicology*, vol. 75, pp. 166–172, 2015.

[44] S. Sumbul, M. A. Ahmad, M. Asif, and M. Akhtar, "Myrtus communis linn.-a review," *Indian Journal of Natural Products and Resources*, vol. 2, no. 4, pp. 395–402, 2011.

[45] G. Ladu, L. Cubaiu, G. d'Hallewin, G. Pintore, G. L. Petretto, and T. Venditti, "*Rosmarinus officinalis* L. and *Myrtus communis* L. essential oils treatments by vapor contact to control penicillium digitatum," *Journal of Food Processing & Technology*, vol. 6, no. 9, p. 1, 2015.

[46] A. Khani and F. Basavand, "Chemical composition and insecticidal activity of myrtle (myrtus communis L.) essential oil against two stored-product pests," *Journal of Medicinal Plants and By-Products*, vol. 2, pp. 83–89, 2012.

[47] S. Pareek, N. Benkeblia, J. Janick, S. Cao, and E. M. Yahia, "Postharvest physiology and technology of loquat (eriobotrya japonicaLindl.) fruit," *Journal of the Science of Food and Agriculture*, vol. 94, no. 8, pp. 1495–1504, 2014.

[48] S. F. Cao, Y. H. Zheng, K. T. Wang, H. J. Rui, H. T. Shang, and S. S. Tang, "The effects of 1-methylcyclopropene on chilling and cell wall metabolism in loquat fruit," *The Journal of Horticultural Science and Biotechnology*, vol. 85, no. 2, pp. 147–153, 2010.

[49] Q. Zhao and R. A. Dixon, "Transcriptional networks for lignin biosynthesis: more complex than we thought?" *Trends in Plant Science*, vol. 16, no. 4, pp. 227–233, 2011.

[50] C. Cai, C. Xu, X. Li, I. Ferguson, and K. Chen, "Accumulation of lignin in relation to change in activities of lignification enzymes in loquat fruit flesh after harvest," *Postharvest Biology and Technology*, vol. 40, no. 2, pp. 163–169, 2006.

[51] Z. Ulukanli and A. T. Oz, "The effect of oleum myrtle on the fruit quality of strawberries during MAP storage," *Journal of Food Science and Technology*, vol. 52, no. 5, pp. 2860–2868, 2015.