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Research Article

Tomato Quality during Short-Term Storage Assessed by Colour and Electronic Nose

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An assay based on an electronic olfactory system was set to evaluate tomato fruits by sensing the aromatic volatiles during postharvest storage of 21 days at 19 ± 0.5°C in darkness. Olfactory system measurements were coupled with colour values. Odour profile and senescence parameters were carried out at 7-day intervals. Discriminant function analysis applied to electronic nose data showed three components, accounting for 99.2% of the total variance. In the present assay, separation among groups according to storage time (0, 7, and 14 days) was observed for wildtype. Overexpressed (Money Maker) lines/plants of tomato showed difference between odour profile for day 0 and day 21, even tough a no clear discrimination between 7 and 14 days was observed. Fruit lost weight almost linearly with shelf life ($P < 0.001$) presenting an averaged loss of 21% ($r^2 = 0.98$) for over-expressed (Money Maker) lines/plants, 13% ($r^2 = 0.97$) for silenced (Money Maker), and 14% ($r^2 = 0.98$) for wild type during 21 days of storage. Colour values L^*, a^*, and b^* data showed that colour properties changed during storage for all the lines considered. Correlations between odour profiles and colour parameter were obtained showing that the electronic nose is a useful technique for monitoring short-term storage of tomato.

1. Introduction

Flavour is defined as the aroma and taste perceived by the human senses and as such is an important food quality attribute [1]. The flavour of tomato results mainly from a combination of volatile compounds for aroma and of sugars and acids for taste. The aroma composition of fresh tomatoes has been studied and over 400 components have been identified, but only a limited number are useful to explain the global fresh tomato aroma. Several studies report aroma composition by cultivars [2], stages of ripeness [3], different culture conditions [4], and treatments [5] suggesting that these parameters influence the aroma composition of tomato.

Many efforts are made to maintain optimal visual quality (e.g., uniform colour, absence of decay, etc.) to attract customers. As a consequence, internal quality attributes, such as flavour, texture, and nutritive value, which are not readily detectable during sorting operations, receive less attention.

Visual appearance is a critical factor driving the initial choice for purchase, but subsequent purchases are influenced greatly by eating quality. Colour in tomato is the most important external characteristic to assess ripeness and postharvest life. Degree of ripening is usually estimated by colour charts. Colorimeters, on the other hand, express colour in numerical terms along the L^*, a^*, and b^* axes. However, most of the tomato literature, mainly express colour changes in terms of different mathematical combinations of b^* and a^* on the chromatic equatorial plane. As referred by López Camelo and Gómez [6], different colours are present during tomato ripening simultaneously. Chlorophyll is degraded from green to colourless compounds at the same time that carotenoids are synthesized from colourless precursor (phytoene) to ξ-carotene (pale yellow), lycopene (red), β-carotene (orange), and xanthophylls and hydroxylated carotenoids (yellow) in a kind of parallel biosynthetic pathway.

On the other hands, the odour of a food product is detected when its volatiles enter the nasal passages at the back of the throat and are perceived by receptors of the olfactory system [7]. Currently, the most common methods for measuring tomato flavour include sensory and instrumental studies. In sensory analysis, taste and aroma aspects of food products are evaluated by panels of specially trained people. Consumer studies provide unique information about the acceptance levels of a food, which is also widely used for the determination of overall quality.

The most important problems affecting sensory analysis include standardisation of measurements, correctness of training, stability, accuracy, and reliability.

The introduction of the electronic nose (E-nose) approach that employs an array of chemical sensors based on conducting polymers, metal oxides, surface acoustic wave devices, quartz crystal microbalances, or combination of these devices has provided an alternative to classical instrumental analysis [8]. Basically, the sensor elements give a signal pattern characteristic of the mixture of volatiles in the headspace of the sample.

This signal pattern is then evaluated using pattern recognition techniques such as neural networks and multivariate statistical techniques [9]. In horticulture, the electronic nose has been successful in monitoring pears [10], apples [11], and other fruits and vegetables [12].

The aim of this work was to study the organoleptic maturation of different transgenic lines of tomato plants using an electronic nose composed of metal oxide sensors and senescence parameters techniques. Short term of storage was analyzed using multivariate techniques to monitor quality of the fruit.

2. Materials and Methods

2.1. Fruit Material. Wild-type tomato plants cv. Money Maker and tomato plants overexpressing and silencing Asr1 gene under the control of promoters 35S and B33 were grown under controlled conditions in a greenhouse (200 μmol PAR s-1 m-2, 60% RH, 23°C).

Fruits were harvested manually from plants grown in the National Institute of Agropecuary Technology, during the summer at the ripening stage 5 (light red) (USDA colour chart, 1975). Fruits of uniform shape and size and free from fungal infection were selected. After harvest, fruits were washed with a solution of hypochlorite (150 ppm de Cl_2 as hypochlorite of sodium), air-dried at atmospheric temperature, and individually labelled and weighed. Samples were kept at 19 ± 0.5°C and 85% RH and analyzed weekly (7 days) for three weeks (21 days).

2.2. Measurement of Senescence Parameters. The loss in weight (Scout-Pro OHAUS, USA) of individual fruit was determined at weekly intervals as a percentage of initial weight at harvest. A mean of four fruits was used for each sampling period. Skin fruit colour was monitored using a ByK Gardner Spectro guide 45/0 Gloss. Colour values were measured at four points of the Ecuador line of the fruit, and CIELab system was used.

Among the several existing colour scales, CIELAB colour space is a three-dimensional spherical system defined by three colorimetric coordinates. The coordinate L^* is called the lightness. The coordinates a^* and b^* form a plane perpendicular to the lightness. The coordinate a^* defines the deviation from the achromatic point corresponding to lightness, to red when it is positive and to green if negative. Similarly, the coordinate b^* defines the turning to yellow if positive and to blue if negative.

Colour index (CI) was calculated according to

$$\mathrm{CI} = \frac{2000a^*}{L^*\sqrt{(a^{*2} + b^{*2})}}. \quad (1)$$

2.3. Electronic Nose. An electronic nose (EN) comprising 18 semiconductor oxide metallic sensors pure and doped semiconductor (MOS), coupled with a mass spectrometer system (NE-MS, Alpha Prometheus, Alpha MOS) was used to discriminate odours of the fruits.

The used device is equipped with two types of sensors: P and T sensors and LY ones. P and T are metal oxide sensors based on tin dioxide SnO_2 (n-type semiconductor), the difference between them resides in the geometry of the sensors. The LY sensors are metal oxide ones based on chromium titanium oxide (p-type semiconductor) and on tungsten oxide (n-type semiconductor). Table 1 presents an overview of the sensors of the electronic nose, as well as the chemical compounds to which they are sensitive. In the presence of a reducing gas, there is absorption with an electronic exchange of gas towards the sensors: the conductance of the n-type increase while for the p-type the resistance will increase, due that n-type are based on tin dioxide SnO_2 and p-type are based on chromium titanium oxide [13].

Doping with different elements increases SnO_2 selectivity for different gases. The adopted configuration results are very flexible for general purposes and convenient for a wide range of applications. Sensors are relatively nonspecific and can combine the signals of all the sensors in a unique signal (Figure 1). Each curve represents a different sensor. The curves represent the sensor conductivity (y-axis) over time (x-axis) when the volatiles from the fruit reach the measurement chamber, with respect to its value measure when carrier gas reaches the sensor.

Electronic nose data is analyzed by multivariate methods like principal component analysis and discriminate function analysis. The result obtained using these method are bi-dimensional plots, were axes are determinate by the sensors that contribute most to discriminate odour. On the other hand, similar odours tend to be grouped in clusters.

2.3.1. Samples for Electronic Nose. Each individual fruit belonging to wild-type (nontransgenic) plants and to transgenic plants was macerated in a stomacher machine for 30 s and 60 g pulp was mixed with 15 mL of saturated $CaCl_2$

Table 1: Sensors selectivity for different gases (Reference Manual, Alpha MOS, France).

Gas/Odour description		Sensors			Applications
		P-type	T-type	LY-type	
Flammable gases	Hydrocarbons	X	X		Cooking, roasting Petrochemistry Dairy products Food freshness Pet food
	Methane	X	X		
	Propane/butane	X		X	
	Hydrogen	X	X		
Organic compounds	Aldehydes	X	X		Rancidity odour Alcohol beverages Perfumes Fermentation Paints & Polymers industry (PE, PP)
	Solvents	X	X		
	Alcohol	X	X	X	
	Aromatic compounds (toluene, xylene)	X	X		
Toxic gases	Ammonia, Amines	X	X	X	Food freshness Environment
	Hydrogen sulphide		X	X	
	Carbon monoxide			X	
Oxidizing gases	Flour	X	X	X	Environment Packaging Trichloroanisol
	Chlorine	X	X	X	
	Nitrogen oxide			X	
	Ozone			X	
Cooking control	General purpose	X	X		Food aroma Natural aroma Volatiles Petrochemistry
	Humidity	X	X		
	Combustion gas monitoring	X	X		
Air quality control	General air pollution monitoring	X	X		Environment Air quality control
	Cigarette smoke	X			
	Carbon monoxide and gas monitoring	X		X	

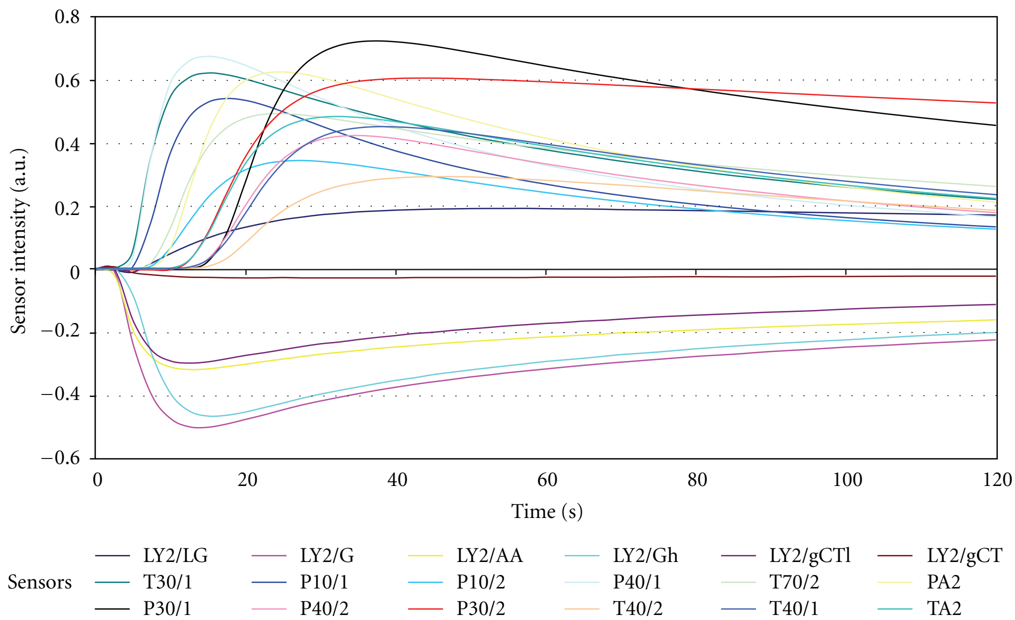

Figure 1: Signals of the 18 semiconductor oxide metallic sensors pure and doped semiconductor.

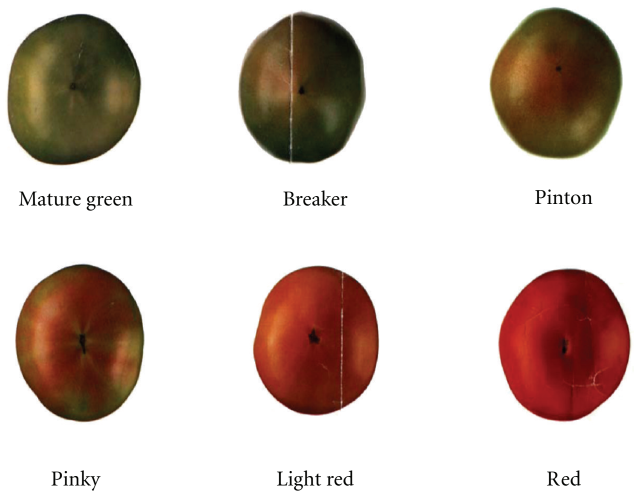

FIGURE 2: Different colour stages of mature process in tomato (California Tomato Board, USDA, 1976).

TABLE 2: Colour data for tomato cultivar wild type during 21 days of storage.

Samples	Colour values	Storage time (days)			
		0	7	14	21
Wild type	L^*	46.86 ± 4.97^a	36.06 ± 2.52^b	33.46 ± 3.48^c	33.43 ± 2.26^b
	a^*	5.95 ± 3.30^c	25.62 ± 3.13^a	26.8 ± 4.12^a	21.12 ± 3.16^b
	b^*	34.55 ± 5.6^a	21.36 ± 3.73^b	19.95 ± 2.14^{bc}	17.40 ± 4.33^c
	CI	8.05 ± 4.30^c	42.73 ± 2.34^b	48.28 ± 6.7^a	46.28 ± 2.01^{ab}

Small letters in the same row indicate that samples are significantly different ($P < 0.05$).

solution (added all at once) in the stomacher for another 5 s [14]. For electronic nose measurement, samples of 5 ± 0.1 g were placed in five 10 mL glass vials equipped with a screw cap and silicon septum.

The experimental part was divided into two steps. In the first step, sensors response of electronic nose was evaluated and experimental conditions of electronic nose were optimised using wild-type tomato. Once the experimental conditions and methodology of electronic nose was established, sensory evaluation was performed using overexpressing tomato plants. It was not possible to analyse silenced plants due to the fact that the amount of fruit was not enough.

2.3.2. Parameters Used for Electronic Nose Analysis. Samples were stabilised at 40°C for 10 min (incubation time) and shaked (500 rpm). Then, 1 mL of headspace sample was injected, the acquisition time being 120 s with a frequency of 0.5 s. Synthetic air was employed as carrier gas with a flow of 30 mL min^{-1}. Samples were analyzed thrice.

2.4. Statistical Analysis. In this work, statistic analysis was done under two approaches: univariate analysis with a completely randomized design and Pearson correlation; and multivariate discriminant and principal components analysis. The statistical software used was SPSS v. 12 (Illinois, USA).

3. Results and Discussion

3.1. General Senescence Parameters

3.1.1. Loss of Weight. With increasing the storage time fruit lost weight almost linearly ($P < 0.001$), and averaged loss of 21% ($r^2 = 0.98$) for overexpressed (Money Maker), 13% ($r^2 = 0.97$) for silenced (Money Maker), and 14% ($r^2 = 0.98$) for wild type was obtained after 21 days of storage. Maharaj et al. [5] reported that mature green tomato fruit (var. Capello), stored at 16°C and under high relative humidity for a period of 35 days, represented a loss of weight of 16% during 21 days of storage. Similar results reported Maharaj were observed, but in different mature stages.

3.1.2. Colour. Initially all fruits were light red (rating 5) in colour (Figure 2). The effects of processing and storage time on lightness L^*, a^*, and b^* coordinates and colour index for each sample are shown in Tables 2, 3 and 4.

Significant differences in L^* and b^* values were obtained for tomato wild-type samples. A decrease in values due storage was observed. Short term storage demonstrated that tomatoes colour were darker and less yellow than fresh samples. On the other hand, a^* values showed an increase, reaching the highest value at day 14 and then a decrease in red colour. CI increased during storage, having the highest value at day 14 (Table 2).

Table 3: Colour data for tomato silenced (Money Maker) at 7, 14, and 21 days of storage.

Samples	Colour values	Storage time (days)			
		0	7	14	21
Silenced plants	L^*	45.51 ± 3.89^{a}	32.89 ± 2.22^{b}	31.49 ± 1.82^{b}	31.55 ± 1.82^{b}
	a^*	13.62 ± 3.13^{c}	28.40 ± 2.51^{a}	26.92 ± 3.87^{ab}	25.47 ± 2.64^{b}
	b^*	34.59 ± 3.19^{a}	26.32 ± 5.79^{b}	22.37 ± 4.63^{b}	21.80 ± 3.53^{b}
	CI	16.28 ± 4.67^{b}	44.93 ± 1.31^{a}	49.04 ± 2.37^{a}	48.41 ± 1.85^{a}

Small letters in the same row indicate that the samples are significantly different ($P < 0.05$).

Table 4: Colour data for tomato overexpressed at 7, 14, and 21 days of storage.

Samples	Colour values	Storage time (days)			
		0	7	14	21
Overexpressed	L^*	47.14 ± 2.52^{a}	34.77 ± 2.27^{b}	31.32 ± 0.99^{c}	30.83 ± 1.78^{c}
	a^*	18.32 ± 3.95^{c}	27.00 ± 2.98^{b}	29.72 ± 2.70^{ab}	30.01 ± 2.43^{a}
	b^*	34.74 ± 2.33^{a}	26.92 ± 2.43^{b}	25.82 ± 3.01^{bc}	24.02 ± 2.24^{c}
	CI	19.91 ± 2.33^{d}	40.70 ± 2.59^{c}	48.28 ± 1.86^{b}	50.62 ± 2.66^{a}

Small letters in the same row indicate that the samples are significantly different ($P < 0.05$).

For silenced (Money Maker) tomato, significant differences in L^* and b^* values due storage were observed. Fresh tomato had the highest L^* and b^* values. The a^* value averaged after 7 days in storage indicated a significant loss in green colour. Colour index showed an increase during storage (Table 3).

L^* and b^* values decrease during storage, reaching the lowest values at day 14 for over- expressed samples. Parameter a^* increases during storage. CI shows the highest difference between initial and day 7 (Table 4).

Colour development in tomato is sensitive to temperature, having a better plastid conversion when temperature is above 12°C and below 30°C [6]. Tijskens and Evelo [15] demonstrated that b^* suffered big changes if tomatoes were ripened at high temperatures (over 30°C) and yellowing took place due to the inhibition of lycopene synthesis and the accumulation of yellow/orange carotenoids. On the other hand, at low temperatures (below 12°C), chlorophyll is not degraded and lycopene accumulation does not take place.

When red colour pigments started to be synthesized, a decreasing L^* value indicated the darkening of the red colour. This behaviour was observed in all samples between 7 and 14 days of storage.

In this research a significant decrease in b^* parameter is observed after day 7. On the other hand, Brunink et al. [8] reported that b^* values changed very little during ripening. This could be related to the fact that ζ-carotenes (pale-yellow colour) reach their highest concentration before full ripening, where lycopene (red colour) and β-carotene (orange colour) achieve their peaks [16, 17].

3.2. Electronic Nose

3.2.1. Wild-Type and Overexpressed Samples. E-nose data was analyzed applying discriminant function (DFA); analysis was performed using Wilks' lambda stepwise method for variable selection.

DFA was chosen because it considers the relation of data points for the specified classes. On the other hand, DFA takes into account the distribution within classes and the distances between them. Therefore, it allows us to collect information from all sensors in order to improve the resolution of classes.

The criterion used was the significance of F with a maximum of 0.05 to enter and a minimum of 0.10 to exit. The sensors that allow the classification of odour profiles over time were LY2/LG, LY2/G, LY2/AA, LY2/gCTl, LY2/Gct, P10/2, and T40/1.

Three discriminant functions (DFs) were found for the wild-type and overexpressed (Money Maker) lines/plants samples, accounting for 99.2% of the total variation (Figure 3). For wild-type samples three groups were obtained according to storage time (0, 7, and 14 days). On the other hand, three groups were obtained according to storage time (0, 7, 14, and 21 days) for the overexpressed tomato samples. Storage at days 7 and 14 did not show discrimination in odour for overexpressed samples. After 21 days of storage, the overexpressed samples showed difference in odour. These results can be attributed to differences in the volatile-fraction composition during storage that impacts on their odour profiles. Berna et al. [18] reported similar results with tomato (*L. esculentum* Mill.).

3.2.2. Correlation between E-Nose Data and Colour. In order to observe the electronic nose performance for monitoring the behaviour of fruit quality during storage time, olfactory measurements were related with colour parameters. Principal component analysis was applied to colour and electronic nose data considering only the sensors selected by DFA (LY2/LG, LY2/G, LY2/AA, LY2/gCTl, LY2/Gct, P10/2, and T40/1). Two components were obtained that explained 88.2% of the total variance (Figure 4). PC_1 is correlated positively with colour parameters L^* and b^* and sensors LY2/G, LY2/AA, LY2/gCTl, and LY2/Gct. Only samples at storage time T0 (initial time) were correlated positively with PC_1.

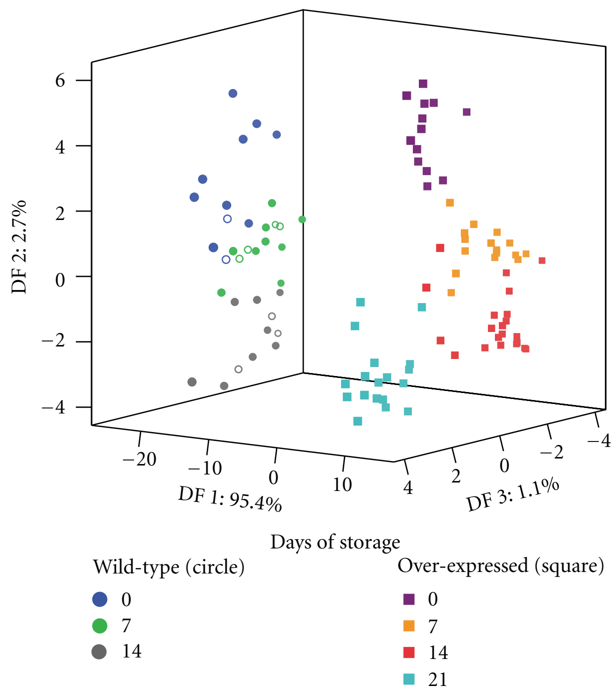

Figure 3: Discriminant function analysis of electronic data for organoleptic mature of wild-type tomato during 0 (blue circle), 7 (green circle), 14 (grey circle) days of storage and overexpressed tomato during 0 (violet square), 7 (orange square), 14 (red square) and 21 (aquamarine square) days of storage.

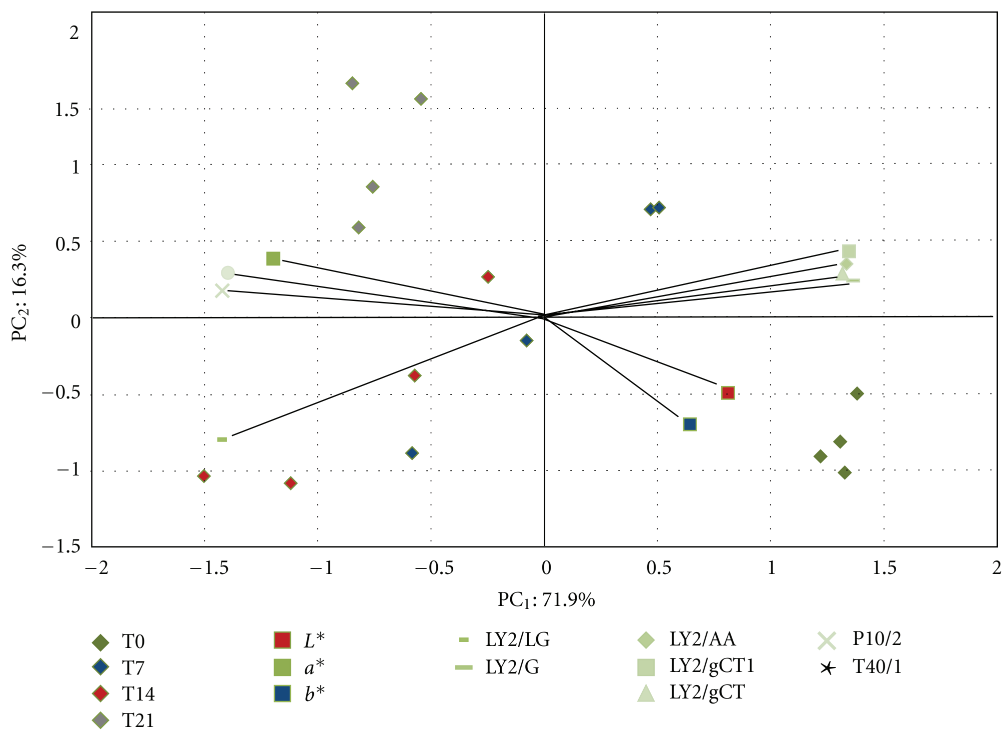

Figure 4: Principal component analysis (PCA) of electronic data (LY2/G(−), LY2/LG(-), LY2/AA(◇), LY2/gCTl(□), LY2/gCT(△), P10/2(×), and T40/1(⋆)) for organoleptic mature overexpressed tomato during 0 (T0, green rhombus), 7 (T7, blue rhombus), 14 (T14, red rhombus) and 21 (T21, grey rhombus) days of storage and colour parameters (a^* (green square), b^* (blue square) and L^* (red square)).

On the other hand, PC_1 was correlated negatively with colour parameters a^* and sensors LY2/LG, P10/2, and T40/1. Samples at storage time T21 were correlated negatively with PC_1. This result suggests that the different storage time of tomatoes could be monitored by means of the electronic nose.

4. Conclusions

The study of organoleptic mature plants using transgenic lines of tomato Money Maker with the gene ASR1 overexpressed and silenced under the constitutive 35S promoter and the patatin B33 promoter of potato showed changes in colour during storage.

Electronic nose showed differences in odour profiles during short-term storage for either overexpressed or wild-type (Money maker) tomatoes. Future research is needed in order to compare tomato lines response, focusing the attention on 7 and 14 days of storage.

In the last decade, odour research was focused principally on the identification of potent odorants, the determination of their odour relevance, and their release in different foods. Nowadays, the development of the electronic nose methodology, with a chemical sensory array, provides a powerful tool to analyze odour as a set of odorants present within a given sample. Sensory analysis, as a branch of the food industry, will be benefited with the adoption of this methodology.

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