Trade Science Inc.
ISSN : 0974-7419 Volume 11 Issue 3
Analytical CHEMISTRY
An Indian Journal
Full Paper
ACAIJ, 11(3) 2012 [123-130]

Physico-morphological and biochimical characterisation of tomato varieties cultivated in Morocco

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Received: 19th December, 2011 ; Accepted: 20th January, 2012

ABSTRACT

Tomato is an important crop in terms of its economic and nutritional value. Tomato fruit quality for fresh consumption is determined by external (size, colour, firmness) and internal fruit properties (taste, aroma) which are prone to physiological changes related to fruit development and ripening. The aroma profiles of three hybrid tomato varieties cultivated in Morocco were quantitatively and qualitatively determined by gas chromatography mass spectrometry at two stages of ripeness. Different physicochemical properties which define fruit organoleptic quality were evaluated. Eight dominant volatile compounds were detected. Inter-cultivar differences in biochemical composition and morphological features (shape, colour, etc) were demonstrated. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Tomato;
Hybrid varieties;
Physicochemical properties;
Aroma;
Volatile compounds.

INTRODUCTION

Tomato (*Solanum Lycopersicon*) is one of the most important and widely cultivated vegetables in Morocco, with an annual production which reaches 1 312 310 tons (FAO, 2008). In addition, several attributes have been proposed to characterize the tomato aroma, such as fruity, green, floral, grapefruit or pharmaceutical aromas[6, 12, 18]. These aromas are related to the volatiles contained in fruits. More than 400 aroma volatile compounds have been identified in tomato fruit[33], among which about 30 seems to be the most important for tomato aroma[4, 6]. Organoleptic quality is a very complex characteristic of fruits. During harvesting, the fruit may be subject to a range of environments, treatments and conditions which can have a profound influence on its eating quality. Besides, the assessors of quality, the consumers, are themselves highly variable with regard to their response to a particular foodstuff either because social conditioning or because of genetic variations in their sensory systems.

Consumption of tomato fruit has regularly increased over recent years, but consumers have become more and more concerned by its quality[11, 25]. Tomato fruit quality for fresh consumption is determined by external (size, colour, firmness) and internal fruit properties (taste, aroma).

Tomato taste is usually described by sweetness and sourness. It is mostly related to the fruit content in reducing sugars and organic acids[27, 31, 38], and to their ratio[12, 37]. Tomato fruit is primarily composed of sugars and acids, which represent about 60 % of the dry matter weight[20]. In mature tomato, glucose and fructose constitute the major sugars, and citric and malic acids are the major organic acids.

Hybrids between modern and old lines seemed thus to benefit from the qualities, being sweeter, with a stronger aroma intensity, and having juicer and less mealy fruits. Nevertheless a large variability was detected among hybrids.

Flavour of tomato, includes both taste and odour, is mainly composed of sweetness, sourness, and aroma, which corresponds to sugars, acids, and volatile compounds. The improvement of organoleptic and nutritional quality of food products to meet the expectations of consumers is a strategic research.

Breeders aim to develop varieties of fruit and vegetables that meet public expectations of shelf life, appearance, flavour, and agriculturists' expectations of robustness and variability. Thus, breeding programs focused on using appropriate and efficient selection criteria are needed.

In the present work, three tomato cultivars, representing three types of varieties encountered in the Moroccan market with different levels of aroma volatiles, were investigated: Daniela, Tilila and Piccolo. These varieties have excellent taste and flavour production, round fruit, or presented on beautiful bunches regular, vigorous plants with good disease resistance. The objective of this research is to compare the Physico-morphological and biochemical characters of three Moroccan tomato varieties and to identify their volatile compounds. Two major factors related to the fruit quality were investigated: the influence of variety and fruit ripeness. The relative influence of these two factors on the quality of tomato was also assessed.

MATERIEL AND METHODS

Sample collection

The study focused on three tomato varieties naming Piccolo, Daniela, Tilila (hybrid varieties of tomato cultivated in southern Morocco). Daniela was obtained in Israel by the Faculty of Agriculture of Rehovot. In addition, Daniela has introgressed gene 'rin' of non-ripening. Piccolo, a cherry tomato, is an achievement of Gauthier and Tilila is a normal tomato obtained by Royal Sluis (Seminis). The choice of these varieties is justified mainly by their higher frequency observed in domestic markets. They were collected from Souss region in the south of Morocco. The fruits were sorted visually (size, colour, etc.) to establish uniform lots for the analysis. The biophysical and chemical analyses of individual tomato fruit (pH, total titratable acidity, ash, sugar and protein contents) was conducted on half fruit and the other half of each tomato fruit was concurrently assessed for volatile analyses.

GC-MS

The method of Buttery et al.[13] was followed for the determination of volatile aroma compounds in the studied tomatoes. 30 g of tomatoes frozen in liquid nitrogen have been crushed, and hold for 180 s, a saturated solution of $CaCl_2$ (9g, 1mol) was added to the mixture in presence of an internal standard (2-octanol).

The bottle containing the mixture was sealed. Then, the solid phase micro-extraction (SPME) fibre was left in the mixture for 4 hours before its injection into GC-MS.

Physic-morphological characteristics: weight, number of lobes and shape factor

The morphological characteristics of fruits were expressed by the number of lobes counted for each fruit sample, the average fruit weight, and the shape factor (Sf) given by the following formula[21]:

$$Sf = \frac{\text{Average height of the fruit}}{\text{Average diameter of the fruit}}$$

The shape factor is used to classify the varieties into three categories of shape, including: Sf <0.8: flattened; 0.8 <Sf <1: round and Sf> 1: elongated.

Physicochemical characteristics: dry matter, firmness and coloration

Samples were analyzed in triplicate using the official methods of the Association of Official Analytical Chemists[2]. Ash content were determined by incinerating 2 g of each variety at 550°C over night. The penetrometer was used for measurement of tomato firmness. The colour measurements are made using. The skin colour of intact fruit was measured directly through a Minolta Chroma CR 400/410. The colour was recorded using the L*a*b* uniform colour space

(CIELAB), where L* indicates lightness, a* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis[16, 26]. Colour of each sample was measured at three different points on the fruit equator.

pH, titratable acidity, soluble sugars and proteins content

The pH is determined after centrifugation of crushed tomatoes to 7500 rpm for 15 min using a pH meter (pH 211, Hanna Instruments). The titratable acidity (TA) is obtained by titration of the juice of an aliquot of 5 g of fresh pulp with sodium hydroxide 0.1 N using a phenolphthalein method[1]. The determination of soluble sugars was performed by spectrophotometer at 485nm. Their concentrations are determined from a standard range of 0-0.1 mg glucose / ml. The Bradford assay is a protein determination method used for tomato proteins concentrations[9]. Bovine Serum Albumin (BSA) was used as standard.

RESULTS AND DISCUSSION

We have selected and compared three hybrids, with or without the rin mutation at the heterozygous level.

TABLE 1 : Metabolic origin of main volatile compounds involved in tomato fruit flavour

Pathway	Component	Enzymes
Fatty acids oxidation	Cis-hexenal Hexanal 1-penten-3-one Trans-2-hexenal, trans-2- pentenalpentenol- penten-3-ol, trans-2- heptenal 2- isobutylthiazole	Phospholipase Lipoxygenase Hydroperoxyde lyase Alcool deshydro- genase
Aminoacids	2-phenylethanol 3-methyl-butanol, 1- nitro-3-methyl-butane, 2 + 3methyl-butanal	Aminoacid decar- boxylase AADC1A,AADC1B, AADC2
Carotenoid related	6-methyl-5-hepten-2- one Geranyl-acetone pseudoionone β-ionone	Carotenoid cleavage Dioxygenase1
Terpenes pathway	Geranial Linalool Neral	Linalool synthase
Shikimate pathway	Methylsalicylate	Unknown

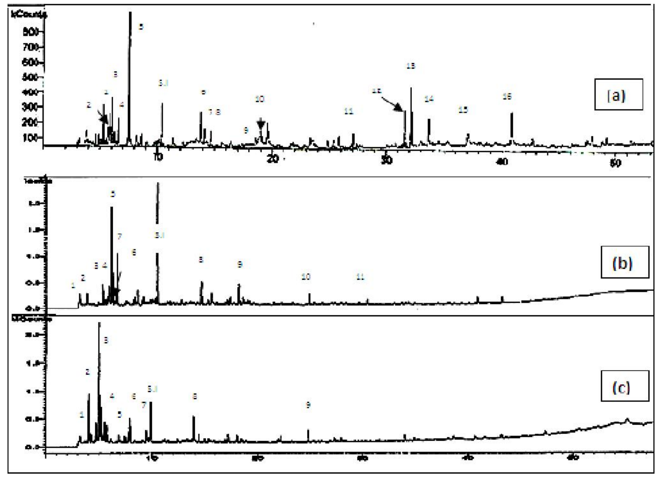

Figure 1 : GC profile of volatile compounds from the extracts of pulp microtome and Moroccan varieties. (a): variety Piccolo, 1: 2-Heptenal (E) 2: 1-One Octen -3, 3: 6-methyl-5-hepten-2-one, 4: Furan, 2-pentyl, 5: Limonene , 6: Naphthalene; 7: Methyl solicylate; 8: 6-Dodecenol, 9: 1-methoxy-4-(2-propenyl) 10: 6-Diéthylphénol; 11: 2,6-bis (1,1-dimethylethyl) -4 - (1-oxopropylphénol) 12: 3 - (methylthio) propyl acetate; 13: ∂-hexalactone; 14: 3- (methyltio) propanol 15: nerol; 16: β-ionone, (b): variety Tilila , 1: 2 - methyl-butanol, 2: 2-methyl-1-butanol, 3: 2-Heptenal (E) 4: 1-Octen -3-one; 5: 6-methyl-5-hepten -2 - one, 6: 3-hexen-1-ol, 7: 1-methoxy-4-(2-propenyl) 8: phenylacetaldehyde 9: 6-Diéthylphénol 10: Methyl solicylate;; 11: α-ionone, (c): Daniela variety; 1: 2 - methyl-butanol, 2: 2-methyl-1-butanol, 3: 2-Heptenal (E) 4: 1-One Octen -3, 5: 6-methyl-5-hepten -2 -one, 6: ethyl butyrate; 7: hexanal; 8: limonene; 9: ethyl 2 - (methylthio) acetate.

Tomato, like most vegetables contain a large number of potential flavour compounds, both volatile and non-volatile, as well as sugars.

Volatile compounds

Aroma analysis is done by means of gas chromatography-mass spectrometry (GC-MS). Tomato volatile compounds are usually grouped into five main classes[4, 14, 17, 19, 30, 32, 35, 36, 39], based on their metabolic origin (TABLE 1). It is the aroma profile that differentiates among cultivars because of the higher sensitivity of the olfactory system and the large number of volatile compounds produced in fruits. Well, but without forgetting the existence of a close interaction between taste and aroma, with aroma components affecting taste perceptions and vice versa. The compounds such as aldehydes, alcohols and terpenoids are responsible for the different bouquet of the various cultivars[34]. Carotenoids are the precursors of C14 and

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C13 volatile compounds that contribute to flavour and aroma of many fruit, such as β-ionone. For instance, tomato mutants rich in ∂-carotene produce high amounts of α-ionone, while those rich in β-carotene produce more β-ionone. Three tomato cultivars (Daniela, Tilila and Piccolo) were analyzed using the GC-MS (Figure 1). In fact, the results of the volatiles shows that 2-Heptenal, 6-methyl-5-heptene-2-one and 1-octen-3-one are shared by the three hybrid varieties of tomato, the hexanol is present either in Piccolo and Daniela. Methyl salicylate and 1-methoxy-4-(2-propenyl) are existing in Piccolo and Tilila. In addition, each variety displays some specific compounds like hexanal, ethyl butyrate and the ethyl 2- (methylthio) acetate for Daniela. Phenylacetaldehyde, 6-Diéthylphénol, 2-methyl-butanol, 3-hexen-1-ol in Tilila and 2-pentyl furan, naphthalene, 1-dodecanol, benzyl acetate, β-ionone, ∂ hexalactone-, 3 - (methyltio) propanol, 3 - (methylthio) propyl acetate, 2,6-bis (1,1-dimethylethyl) -4- (1-oxopropylphénol) in Piccolo. Therefore, these aromatic compounds are derived from different pathways like lipid derivatives (hexanol and hexenal). They can also be derivatives from amino acids such as 3 methylbutanol-(Leucine) and phenylacetaldehyde (phenylalanine) and derivatives of carotenoids: the β-ionone (from β-carotene) and 6-methyl-5-heptene-2-one (from lycopene)[14]. These compounds of tomato flavour are influenced by major factors: (1) Genetic control (cultivar): in tomatoes, the TA/SSC and levels of flavour volatiles were found to vary significantly among cultivars[7, 8]. (2) Physiological maturity: it has been proved that a poor flavour of tomatoes is found when they are harvest at mature green-ripened stage. (3) Postharvest handling: the synthesis of aroma compounds in ripe tomatoes is influenced by low concentration (or the absence) of oxygen, high carbon dioxide levels and temperature storage. The ability to form volatiles upon disruption, however, changes during ripening[4]. Levels of hexanal, cis-3-hexenal and trans-2-hexenal, formed after tissue homogenization, increase as the fruit ripens[7]. Alternatively, volatile aldehydes such as phenylacetaldehyde and 3-methylbutanal could be formed from enzymatic oxidation of corresponding alcohols that are released by enzymatic hydrolysis of glycosides during ripening.

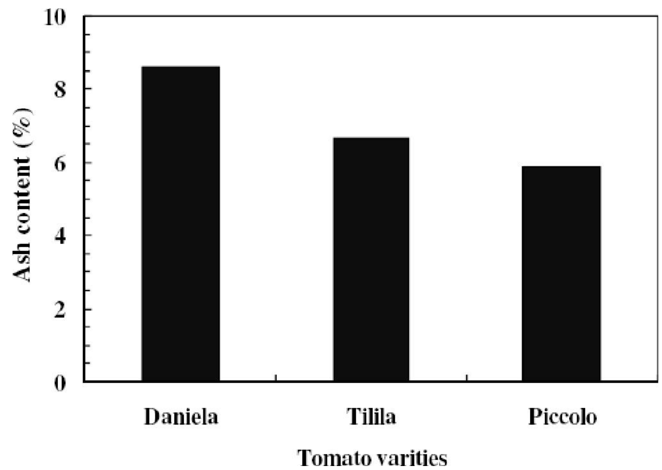

Figure 2 : Ash content of different studied tomato cultivars

Ash content

Non-volatiles such as sucrose, glucose, amino acids, organic acids and a host of other compounds derived from both primary and secondary metabolism may all play a role in flavour quality, it is apparent that the total system must be understood and taken into account when trying to implement improvements. The ash content (mineral content) of tomato fruits from the three studied cultivars was determined.

The Piccolo variety presents the highest percentage of mineral content compared to other varieties (Figure 2). Average ash content of ripe fresh fruit is between 5.0 and 7.5%[33]. It was reported that minerals have an effect on pH and titratable acidity and have buffering capacity as well; thus, they influence the taste of tomatoes[33].

pH, titratable acidity, soluble sugars and proteins content

The levels of sugars in vegetables can be high, but sweetness is frequently masked by other flavours. Level of soluble sugars appears to be the most closely correlated with the genotype of the different cultivars.

Like all vegetables, tomato sweetness is determined by its sugar and acidity. One tomato can have less sugar than a second yet taste sweeter because its acidity is lower than the second. Depending on the study, acidity is either related to the fruit pH or to the titratable acidity[3, 5]. Both sugars and acids contribute to the sweetness and to the overall aroma intensity[5].

High tomato-like aroma intensity and sweetness, but intermediate acidity are the most important

TABLE 2 : pH and titratable acidity of the different tomato cultivars at two stages of ripeness.

Parameter	Stage of ripeness	Tomato varieties		
		Daniela	Tilila	Piccolo
Titratable acidity (eq/100g)	orange	4.00	4.27	7.47
	red	4.67	4,12	5.87
pH	orange	5.08	4.99	4.94
	red	5.21	5.18	5.18

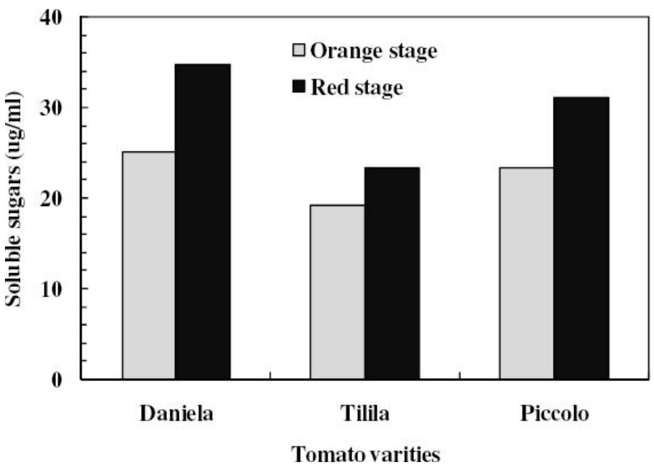

Figure 3 : Soluble sugars concentration in tomatoes cultivars of two stages of ripeness (orange and red)

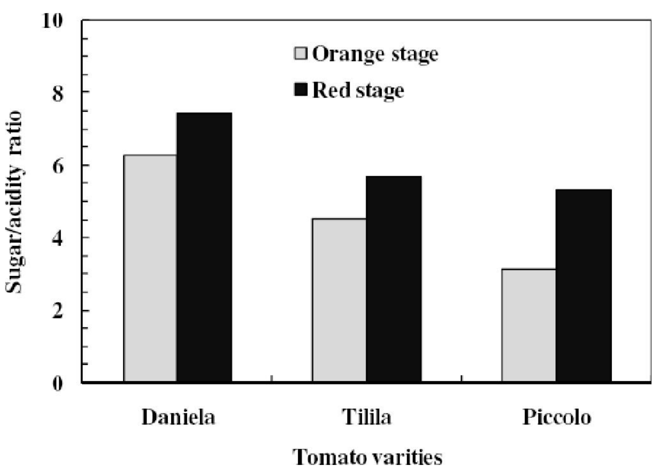

Figure 4 : Sugar content ratio to the titratable acidity in the studied tomato varieties at two stages of ripeness (red and orange).

characteristics for consumer preferences[5,28]. Malundo et al.[31] showed that given levels of sweetness correspond to optimal acid concentrations, beyond which acceptability decreases. Baldwin et al.[5] related the overall acceptability to the ratio of sugars to titratable acidity and to the concentration of several aroma compounds. The sweetest tomatoes are those with the largest sugar and highest pH (lowest acidity). The results are shown in TABLE 2 and Figures 3-4: The pH of different hybrid varieties studied, for both stages of maturity, like any pH tomato, has a value <7. It varies from one variety to another. The acidity or pH is a nonlinear scale which means that a small increase in pH leads to many times sweeter tomato. Daniela in the orange stage of ripeness exhibits thelowest acidity. In ripe red tomato, the ratio of malic and citric acid is lower. At higher levels of citric acid, the sweetness effect of glucose was found to be more than that of fructose[33]. Stevens et al.[37] observed high correlations between sourness, TA and pH. Malic acid has been reported to be 14 % more sour than citric acid, but it has less influence on tomato taste because of its lower concentration. Malundo et al.[31] found a negative correlation between acid concentration and pH, and positive correlation between pH and TA. Baldwin et al.[5] found significant correlations between TA and overall acceptability, pH with sweetness and sourness, and SSC2/TA (SSC=soluble solids, TA=titratable acidity) content with overall taste. Sugars, especially fructose, glucose and sucrose are the main carbohydrate (descending order) existing in fruits[22,23]. Sugar content of Daniela is higher than the other cultivars and its extremely high sweetness is enough to counteract its acidity. Positive correlations between sweetness, reducing sugar content and soluble solids have been shown[37]. In contrast, Jones and Scott[29] could not demonstrate any correlation between sugar or dry matter and sweetness of the F1 hybrids which were high sugar and acid varieties. Sugar/TA and levels of flavour volatiles were found to vary significantly among cultivars[7,8].

The protein concentrations of tomato juice decreased from one point to another more advanced and those of supernatant after centrifugation of the juice varieties increased.

Firmness, coloration and physico-morphological characteristics of tomato varieties

Consumers often have diverse preferences for foods. What is liked by one consumer can be disliked by another consumer. For this reason, we can analyse consumer preference in terms of defined sensory texture and flavour attributes. The main biochemical and physiological changes occurring in during fruit ripening

TABLE 3 : The main physico-morphological characteristics of the three studied tomato varieties cultivars

Tomato cultivar	Weight (g)	Shape factor (Sf)	Number of lobes	Firmness (10kg x 100g)		Coloration (medial 'a')	
				Orange stage	Red stage	Orange stage	Red stage
Daniela	17 ± 1.58	0.95	2	1.19	0.81	23.48	30.06
Piccolo	175 ± 7.03	0.87	3-4	0.91	0.75	23.65	29.99
Tilila	190 ± 5.61	0.89	3-4	1.24	0.85	23.78	30.18

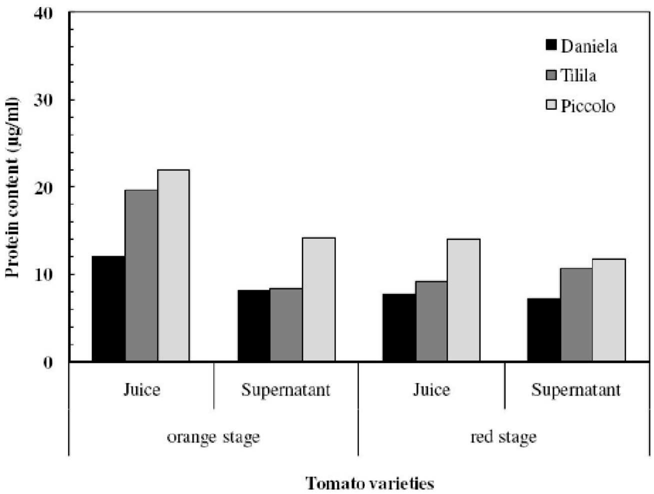

Figure 5 : Protein content in the supernatant and juice of the three studied tomato varieties stages at two stages of ripeness (red and orange stages)

regard colour, composition and structure. Weight is among the physical characteristics that were determined (TABLE 3). Piccolo variety is distinguished by its weight which is significantly low compared to other varieties (Daniela and Tilila) that are more or less close. TABLE 3 presents also the main physical and morphological characteristics of the studied tomato varieties. Texture changes during fruit softening and the associated development of flavour are bound together by the metabolic processes that direct fruit ripening.

Firmness is one of the most important factors for determination of tomatoes quality. As shown in TABLE 3 the relation between firmness and varieties were illustrated. The results show that there was significant difference between varieties and deformation values. Piccolo had required lower firmness. In comparison, the other both varieties (Daniela and Tilila) had approximately the same deformation values. In contrast deformation decreases with maturity. To optimise characteristic flavour it is important to use fruit that are fully ripe. Colour change in ripening tomato fruit varieties, from orange to red (TABLE 3). The characteristic colour of ripe tomato fruit is caused by accumulation of lycopene and β-carotene, concomitantly with the decrease in chlorophyll content during the transition from chloroplast to chromoplast. As photosynthetic membranes are degraded, chlorophyll is metabolized and carotenoids, including β-carotene and lycopene were accumulated. At the breaker stage of ripening, the red colour of lycopene begins to appear, the chlorophyll content decreases and the organoleptic properties of the fruit change[10, 16]. Previous studies have shown that, during normal ripening of pericarp in intact tomato fruit, tissue colour changes from green through orange to red[16], ethylene biosynthesis and respiration undergo a climacteric rise, tissues decrease in firmness, extractable activities of specific hydrolytic enzymes increase, and various fractions of the cell wall change in composition[16]. The presence of the "rin" mutation reduced the consumer preference. Differences were detected by all the analysis. These results confirmed the negative influence of the "rin" mutation on consumer preference, but also indicated that, when transferred into a hybrid with high flavour, the negative influence of the mutation is reduced. Selection could thus be carried out to obtain much sweeter and perfumed lines combined with shelf life in "rin" hybrids.

CONCLUSION

The individual as well as complex interactions with volatile and non-volatile constituents of flavour were studied. Given the rising competition from emerging producers and rising production costs in Morocco, producers are forced to think about the quality and traceability of their products. This quality determines the future of the Moroccan tomato on international markets. Recently, breeders have become increasingly aware of the need to provide consumers with fruits that have achieved a degree of ripeness and with specific organoleptic characteristics of the product and the variety concerned. As measured by objective criteria, the internal quality of fruit displays a high enough degree of development and introduced the minimum acceptable level of maturity by the consumer. The samples for the three southern Morocco tomato cultivars were done in accordance with the operational framework for controlling product quality. Progressive changes in colour, texture, flavour, and aroma in each tissue appear to result from changing local patterns of gene expression and metabolic activity. These processes are induced and coordinated, in part, by the biosynthesis and diffusion of ethylene, by local differences and changes in sensitivity to ethylene and, perhaps, by other regulatory signals[16]. This study was devoted to evaluation of physicochemical and morphological characteristics of three varieties of Moroccan tomatoes. The results of various analyses demonstrate the ability of the studied tomato varieties to offer a pretty good nutritional quality. However, the aroma patterns of the three cultivars varied qualitatively and quantitatively. A remarkable result was reported concerning volatile compounds in the piccolo cultivar which show a very specific and typical aroma profile. This finding can allow the identification of the cultivar.

ACKNOWLEDGMENT

Authors are thankful to the Prof. Romane abderrahman for providing necessary facilities for carrying out the GC-MS analysis.

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