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A10-48-3 and A7-32-10 two strawberry selections with well-balanced nutritional and organoleptic quality

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Abstract

In the last few years organoleptic and nutritional traits have become the major breeding targets in most strawberry breeding programs all over the world. Furthermore, the joint improvement of the nutritional value and the sensory key parameters of strawberry might increase the consumption rate of this fruit, which in turn will have a positive effect on consumer's health and a positive economic impact on the producing areas. New cultivars and strawberry selections bred in Huelva (Southwestern Spain) have been characterized in relation to their organoleptic and nutritional properties. Key metabolites associated to the strawberry flavor (sucrose, fructose, glucose, malic and citric acid and 20 main volatile compounds) as well as specific phytochemicals (nine phenolic compounds plus vitamin C) with relevant antioxidant capacities have been analyzed by high resolution gas chromatography coupled to mass spectrometry (HRGC-MS) and high performance liquid chromatography (HPLC). Data were analyzed using principal component analysis (PCA) and Pearson correlations to discriminate those strawberry selections that, besides good agronomic performances, produce fruits with high contents of key flavor components and health-promoting phytochemicals. PCA clearly distinguish selections A10-48-3 and A7-32-10 as having significantly different metabolic profiles and displaying the highest contents of sugars, organic acids, ethyl esters, mesifurane, terpineol, quercetin and vitamin C.

Keywords: *Fragaria × ananassa*, flavor, nutritional quality, sugars, vitamin C, phenolic compounds, volatile compounds.

INTRODUCTION

The continuous increase in the demand for fresh strawberry fruits may be attributed not only to the exceptional flavor of this fruit but also to its nutritional properties. Thus, strawberry breeders initially focused on the improvement of agronomical or qualitative traits, like size, number, yield, ripening, and firmness of fruit while in the last few years, organoleptic and nutritional traits have become the major breeding targets for the commercial strawberry (Diamanti et al., 2012). The development of new berry varieties thorough new breeding programs specifically focused on improving

organoleptic and nutritional quality, requires the identification of appropriated chemical markers to measure those quality parameters. In these sense, strawberry taste, aroma and nutritional value are the most valuable factors in terms of satisfaction of consumers (Pérez and Sanz, 2008; Leon et al., 2009). Strawberry taste is basically determined by soluble sugars and organic acids (Kruger et al., 2012). These compounds could also be used as fruit maturity indices or quality markers during postharvest handling. The characteristic aroma of strawberry is determined by a complex mixture of volatile compounds and depends essentially on the particular balance between the green and fruity notes associated to six carbons aldehydes, alcohols and volatile esters, and the floral and caramel like notes provided by compounds such as benzaldehyde, linalool, terpineol or mesifurane (Pérez and Sanz, 2010; Ubeda et al., 2012). From a nutritional point of view the strawberry is an excellent source of dietary fiber and fructose, which may contribute in regulating blood sugar levels, it is one of the richest fruits in vitamin C and folate and it also has a very complex phenolic profile which includes anthocyanins, flavonols and hidroxycinnamic acids (Giampieri et al., 2014). Over the past ten years Spain has occupied the third place among strawberry top producers, with the area of Huelva accounting for 95% of the national production. A few years ago a strawberry breeding program was initiated by FNM in Huelva (Spain) with the aim of obtaining strawberry varieties which could meet the needs of the local sector and the increasing quality requirements of the European markets. The aim of the present work was to evaluate the organoleptic and nutritional quality of two selections recently obtained in this program by comparing their metabolic profile (sugars, organic acids, phenolic compounds and volatile compounds) against Primoris and Sabrina, two control cultivars widely planted in Southwestern Spain which have been recently selected at FNM and Planasa's research nurseries in Huelva.

MATERIALS AND METHODS

Plant material and growing conditions

Strawberry plants were grown in open-field conditions at an experimental farm located in Gibraleón (Huelva). Plants, from high-elevation nurseries, were planted on October onto two-row raised beds covered with black polyethylene mulch (distance between the plants in the row 25 to 30 cm). In December the crop was covered either with small tunnels and transparent film. A randomized complete block design with three replicates (40 plants per replicate) was used. Fruit harvesting occurred at commercial ripeness (75% of red color). Three harvesting dates per season were established: (I) March; (II) April and (III) May.

Evaluation of agronomic and physicochemical quality parameters

For each cultivar total marketable yield (grams produced per plant from first January to end of May) and percentage of first class fruit in total yield were calculated. Additionally, three physicochemical quality indexes were also measured: fruit firmness with a 3 mm tip dynamometer, total soluble solids (Brix^o), and internal color according to the CIREF scale.

Extraction, fractionation and analysis of sugars, organic acids and phenolic compounds

Major sugars, organic acids, and phenolic compounds were extracted and fractionated according to a previously developed procedure slightly modified (Perez et al., 1997). Two replicates of approximately 700 g of fruit per cultivar were analyzed on each sampling date. Using the previously mentioned methodology two ethanolic extracts were obtained for each replicate. 5 ml aliquots of the ethanolic extract were evaporated in the dark to dryness at 50°C and the dry residue was redissolved in 1 ml of 0.2N H₂SO₄ containing 0.05%EDTA, and loaded onto a previously conditioned Cl8 Sep-Pak cartridge (Supelco, Bellefonte, PA). Sugars and organic acids were eluted with up to 4ml of the same

solution. After complete drying of the column phenolic compounds were eluted with 2 ml of methanol. Extracts were filtered through 0.45 µm Nylon filters before HPLC analysis.

Sugars and organic acids (including vitamin C) were analyzed according to Pérez et al., (1997) using a Beckman Coulter liquid chromatography system equipped with a System Gold 168 detector, a solvent module 126 and a Jasco RI 2031 differential refractometer connected in series. Isocratic separations of the compounds were done on a stainless steel Ion-300 (300 x 7.8 mm, 10 µm). On each sampling day, four extracts per treatment were analyzed.

Phenolic compounds were analyzed according to the method of Gil et al. (1997) slightly modified. Separation of the phenolic compounds was done in a Beckman Coulter liquid chromatography system equipped with a System Gold 168 detector, a solvent module 126 and a stainless steel Merck Superspher RP-18 (250 x 4 mm) column using water (2.5% formic acid) and methanol (2.5% formic acid) as mobile phases.

Analysis of volatile compounds

15-20 halves of strawberries were blended to obtain a fruit puree for each replicate, on each sampling date. Three vials containing 2 g of each strawberry puree and 2 ml of a saturated CaCl₂ solution added for enzyme inactivation were stored at -20°C until analysis. Volatiles were adsorbed on a SPME fiber DVB/Carboxen/PDMS 50/30 (Supelco Co., Bellefonte, PA) at 40°C and further analyzed using a HP-6890 gas chromatography apparatus with a FID detector (Agilent Technologies) equipped with a DB-Wax fused silica capillary column (60 m x 0.25 mm i.d., film thickness 0.25 µm; J&W, Scientific, Folsom, CA, USA). Compound identification was carried out on a Hewlett-Packard HRGC-MS 5975 series MSD equipped with the same column and oven program. Identification was done by matching against the Wiley/NBS Library and by GC retention time against standards.

Statistical analysis

Data were statistically evaluated using STATISTICA (Statsoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Agronomic and physicochemical quality parameters

Although the main goal of this study was to evaluate the nutritional and organoleptic properties of new strawberry cultivars bred in Southwestern Spain, basic agronomic and physicochemical quality indexes such as total yield, percentage of first class fruit, firmness, internal color and soluble solids content were also assessed. Sabrina, and A10-48-3 showed significantly ($p \leq 0.05$) higher yield per plant among the studied cultivars with 1269 g/plant and 1310 g/plant respectively. Among both cultivars A10-48-3 has a significantly higher percentage of first class fruit with 93% while the lowest value was found for Sabrina with 85% while intermediate values were found for A7-32-10 (88%) and Primoris (89%). Non-significant differences were found in terms of firmness, but very clear differences were found in the soluble solids content with the highest value found for A7-32-10 (10.6 °Brix), followed by A10-48-3 (10.4 °Brix), Primoris (10.4 °Brix) and Sabrina (9.4 °Brix).

Sugars, organic acids and phenolic profiles.

Data on the sugar and organic acids composition of the fruits are presented in figures 1 and 2. The maximum °Brix values obtained by A7-32-10 are confirmed by its high sugar content at the first harvesting date (75.1 mg/g FW). Only A10-48-3 had higher sugar content at this date (80.9 mg/g FW). On the second harvesting date when most strawberry cultivars suffer noticeable drop in sugars, A7-32-10 is again the second selection with higher sugar levels with 53.5 mg / g FW. The relationship between the content of sugars and organic acids is optimal in this selection and the vitamin C content of the highest among all selections tested at three harvesting dates (0.74, 0.62 and 0.64

mg/g FW, respectively). The phenolic content of the selection A7-32-10 is not very high, but extraordinarily balanced, without excessive concentration of anthocyanins, which provides an attractive orange-red color, and very high levels in some key phenolic components (Chiva-Blanch and Visioli, 2012) such as quercetin or kaempferol (Table 1).

Sugars values analyzed in the selection A10-48-3 are extraordinarily high in the early season (80.9 mg / g fruit) with sucrose content (40.6 mg / g FW) that almost duplicate those found in other cultivars. The sugar/acid ratio is excellent, surpassed only by the selection A7-32-10, and so does vitamin C content (0.63, 0.38 and 0.62 mg /g FW).

The pronounced decline of sugar content observed on the second harvesting date it is probably the large production of fruits per plant of this strawberry selection. The phenolic content of A10-48-3 is very low at the first harvesting date, mainly due to its low content of pelargonidin-3-glucoside (5.81 mg/100 g FW), but the anthocyanins (cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside) content rise to high levels in the second and third sampling date, in which this selection is among those with higher phenolic content. On the second harvesting date, it is especially remarkable the high content of ellagic acid derivatives (free ellagic acid and ellagic acid glycoside) found in this selection.

Aroma profiles

As it has been previously mentioned aroma profile of the strawberry fruit is determined by an important number of volatile compounds in which two groups of key components could be easily identified, one group (alcohols, aldehydes and short volatile esters) provides green fruity notes while a second group of compounds eluting in the final part of the chromatographic analysis imparts sweet and floral aromatic notes. In this study, 23 volatile compounds were quantitated in the studied strawberry cultivars. Data obtained are presented in Table 2 in which individual compounds are grouped according to their chemical classes. The volatile composition of the selections A7-32-10 and A10-48-3 are very complex, with a good balance of green-acid and sweet-fruity notes. In both cases, the content of compounds such as terpineol and mesifurane is very high. In this sense, it is especially remarkable that the highest contents of the key strawberry flavor component, mesifurane (Perez and Sanz, 2010), were found in A10-48-3 and A7-32-10.

CONCLUSIONS

Data obtained from the organoleptic and nutritional quality assessment of the cultivars Sabrina, Primoris, A10-48-3, A7-32-10 and six other selections provided by FNM (data not shown) were analyzed using principal component analysis (PCA) and Pearson correlations to obtain information on the variables that mainly influence samples differences and similarities. The first two principal components PC1 and PC2 gave Eigenvalues greater than 1.0 (5.0 and 3.57, respectively) and explained 47.5 % of the total variability. PCA score and loading plots for the two principal components clearly distinguish selections A10-48-3 and A7-32-10 as having significantly different metabolic profiles. By overlapping graphs A and B in figure 3 (projection of the cases and projection of the variables on the factor plane PC1x PC2) it is evident that A10-48-3 and A7-32-10 display the highest contents of key metabolites related to the organoleptic and nutritional quality of the strawberry such as sugars, ethyl esters, mesifurane, vitamin C and quercetin.

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Table 1. Phenolic composition.

		Mean values (mg/ 100g FW)						
	Harvest date	Ellagitannins	Cyan-3-G ²	Pelarg-3-G	Pelarg-3-R	Quercetin	Ellagic der.	Kaempferol
Sabrina	I	13.49±0.75 ¹	0.31±0.04	17.92±0.75	1.21±0.10	1.91±0.04	1.3±0.01	0.83±0.12
	II	8.69±0.38	0.21±0.10	28.10±2.25 ¹	2.36±0.08	1.97±0.63	1.69±0.12	1.12±0.29
	III	13.81±1.84	0.26±0.08	28.67±1.50	2.97±0.23	2.10±0.22	1.20±0.05	1.23±0.18
Primoris	I	8.20±0.10	0.95±0.24	15.12±1.97	0.83±0.14	1.81±0.48	1.51±0.11	0.71±0.02
	II	8.82±0.40	1.95±0.14	31.42±2.11	1.85±0.13	2.54±0.24	1.92±0.12	1.31±0.03
	III	9.24±0.16	1.69±0.05	24.81±0.68	1.70±0.03	3.53±0.04	1.74±0.09	1.61±0.02
A10-48-3	I	5.05±1.19	0.52±0.15	5.81±1.58	0.31±0.18	2.24±0.56	1.68±0.24	0.65±0.09
	II	4.90±0.82	1.15±0.04	24.16±0.94	1.89±0.19	1.65±0.22	2.01±0.14	0.82±0.13
	III	4.95±0.46	2.03±0.62	25.10±3.97	2.42±0.43	2.47±0.37	1.61±0.10	1.02±0.21
A7-32-10	I	3.38±0.34	1.04±0.13	14.95±0.11	1.17±0.01	2.82±0.57	2.05±0.03	1.14±0.15
	II	1.72±0.09	1.11±0.15	17.86±0.92	1.37±0.10	2.78±0.49	1.65±0.16	1.02±0.16
	III	1.44±0.09	0.67±0.16	14.22±1.27	1.47±0.23	2.21±0.42	1.47±0.05	0.94±0.14

¹Values represent the mean and standard deviation of four independent analyses

²Nine phenolic compounds were analyzed: ellagitannins, cyaniding-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, quercetin, ellagic acid and ellagic acid glycoside (expressed as ellagic derivatives), and kaempferol

Table 2. Volatile composition.

		Volatile components (area)										
	Harvest date	ethanol	hexanal	isoamyl acetate	butyl esters	methyl esters	hexyl esters	ethyl esters	benzaldehyde	linalool	mesifurane	terpineol
Sabrina	I	13085	380609	36548	2765303	513681	161447	223589	31804	142350	47365	134450
	II	393827	380250	43175	2378774	662301	352131	199928	19949	164456	25870	43431
	III	30210	469500	45312	3803106	1201520	510370	543027	51055	377174	87406	418392
Primoris	I	10940	378596	18073	3293915	803707	206353	446127	26810	220833	79063	76496
	II	19446	400133	25200	2622231	1034145	448175	224171	15049	233053	41197	45775
	III	79607	207256	43780	3496548	994502	397931	1060596	39578	334860	241314	250495
A10-48-3	I	40041	303411	28524	2474979	317885	204905	1315041	29272	198147	98482	136539
	II	33525	268183	14475	2140312	651185	319626	336964	1000	157514	43035	60427
	III	83592	136809	32123	2074741	784185	232102	3710809	33042	172947	176108	411068
A7-32-10	I	18163	511900	29296	3112339	593823	305849	945215	25000	143494	104075	100338
	II	19695	524806	20150	2365303	561931	499877	226885	22782	155118	53696	48821
	III	31805	838247	44277	3633260	946715	527383	775733	22937	195738	305246	190819

¹Values represent the mean of four independent analyses

²twenty three individual volatile compounds were quantitated. Volatile esters are grouped according to their alcohol moiety.

Figure 1

Figure 1: Sucrose, fructose and glucose content (mg/ g FW) at three different harvesting dates: (I) March, (II) April and (III) May

Figure 2

Figure 2: Content of Malic and citric acids (principal “Y” axis) and vitamin C (secondary “Y” axis) (mg/ g FW) at three different harvesting dates: (I) March, (II) April and (III) May.