Influence of high lycopene varieties and organic farming on the production and quality of processing tomato

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Abstract

The effect of conventional integrated pest management and organic farming production systems on the agronomic performance and quality of standard and high lycopene tomato cvs. has been evaluated for two years in two of the main processing tomato producing areas of Spain (Extremadura and Navarra). As an average, the production under organic farming was on average 36% lower than in conventional integrated pest management. Organic farming tended show reduced contents of citric and glutamic acid. Although the contents in sugars were not significantly affected, the ratios sucrose equivalents to citric and glutamic acid increased. Nevertheless, a strong influence of the environment and interactions were detected and under certain conditions (e.g. Extremadura), organic farming may increase the contents in glucose and fructose. The levels of lycopene were not affected by the cultivation system, while beta-carotene contents were higher under organic farming. High lycopene cvs. 'Kalvert' and 'ISI-

24424' registered the highest lycopene levels, but with 27.6% and 28.1% lower production levels compared to 'H-9036', the cv. with the best agronomic performance. 'Kalvert', with high accumulation of sugars and high ratios sucrose equivalents to citric and glutamic acid and high lycopene contents would be an ideal material for supplying quality markets. 'H-9997' with intermediated levels of lycopene accumulation proved to be a good material combining production levels and functional quality. 'CXD-277' offered the higher values in variables related with organoleptic quality with intermediate lycopene accumulation but with lower production.

Keywords sugar, acid, quality, carotenoid, lycopene, *Solanum lycopersicum*

Abrreviations SEq: Sucrose equivalents

1. Introduction

Consumers are increasingly concerned about the capacity of food to improve health and prevent diseases, accordingly there is an increasing demand for foods with an improved functional value (Granato, 2010). It is not clear, though, if marketing efforts have spurred this interest or vice versa, but the industry has made a clear emphasis in the promotion of 'healthy' agricultural food products and in the improvement of the contents of functional compounds (Goldman, 2011). In the case of tomato, one of the vegetables with the highest levels of economic value and consumption, the functional value is mainly determined by the contents in the carotenoids beta-carotene and lycopene, vitamin C and polyphenols.

It has been a long time since the cultivars with high vitamin C content such as 'Doublerich' were released, though with a limited success due to reduced fruit size (Stevens and Rick, 1987). More recently, and with higher success, high lycopene cultivars have been developed.

Those with higher efficiency include mutations such as *high pigment*, which increase the global content of carotenoids (up to 2- 3-fold the content of a standard cultivar). Additionally, these materials may have the collateral effect of increasing the levels of flavonoids and vitamin C, though at the expense of a reduced yield (reviewed by Cebolla-Cornejo et al., 2013).

Nevertheless, the commercialization of products with high functional value cannot ignore taste, one of the general success factors for the marketing of foods (Menrad, 2003). In the case of tomato, taste is mainly determined by sugars, organic acids and the relation between them. Among the key sugars, fructose and glucose represent up to 65% of the total soluble solids (TSS) content (Stevens et al., 1977), while the content in sucrose at the red stage is very low (Thakur et al., 1996). Among the key organic acids, as in other fruits, citric and malic acids are the most important, especially the former.

Traditionally, organoleptic quality in tomato has been evaluated using basic determinations such as TSS content or total titratable acidity, but during the last decade, the individual determination of specific compounds, or the use of derived variables such as sucrose equivalents or its relation with acid contents, has shown higher correlations with acceptability or sweetness (Baldwin et al., 1998, Cebolla-cornejo et al., 2011). The possible role of glutamic acid and its ratio with sucrose equivalents has also been considered (Bucheli et al., 1999).

Consumer interests are not only focused on organoleptic and functional quality, but the concern on environmental quality or the minimization of the effects of agriculture on the environment and on the produces is also growing. In fact, the European Union is clearly supporting the development of production systems based on a reduced use of chemicals, such as integrated pest management or organic farming, and consumers are concerned not only with the final characteristics of food, but also with the way in which it has been produced (Biguzzi et al., 2014).

At the moment, quite a lot of the cultivars used in organic farming or other low input systems have been bred under conventional high input systems. These varieties are not expected to have the ideal characteristics of materials targeted to a low input agriculture (Lammerts et al., 2011), though in fact little is known about the performance of this type of material under organic farming conditions (Döring et al., 2012). It is necessary to advance in the knowledge of the performance of available high input cultivars under these agricultural systems and on the effects of this type of agriculture on characteristics such as the organoleptic or functional value.

In this context, this paper analyses the performance and quality of standard and high lycopene tomato cultivars under conventional integrated pest management (IPM) and organic farming conditions in two of the main processing tomato growing areas of Spain (Extremadura and Navarra) with clearly differentiated environmental conditions. This information will be valuable in order to establish the conditions that maximize the consumer demands of higher organoleptic and functional quality in order to develop quality markets.

Material and methods

Plant material and experimental design

Six processing tomato cultivars were grown under conventional integrated pest management and under organic farming conditions in two sites, Extremadura (at the Southwest of Spain) and Navarra (in the Northeast of Spain), during two consecutive years (2012 and 2013). The cultivars were ‘CXD-277’ (Campbell’s seeds), ‘Heinz(H)-9661’, ‘H-9997’, ‘H-9036’ (Heinz Seed), ‘ISI-24424’ (Diamond seeds S.L.; Isi Sementi S.P.A.) and ‘Kalvert’ (Esasem S.P.A.). ‘H-9036’ and ‘H-9661’ are highly demanded by local farmers due to their agronomical performance and were considered as standard controls. The cultivation under organic farming and conventional IPM of Extremadura was carried out in the fields of the research center Finca “La Orden-

Valdesequera" in Badajoz (Spain) and in the case of Navarra conventional management was applied in the research fields of INTIA in Cadreita (Navarra, Spain). In the case of Navarra, conventional IPM was carried out in the research fields of INTIA, in Cadreita (Spain), whereas the organic farming was located in a field provided by the local organic farming business GUMENDI, in Lodosa (Spain). The edaphoclimatic conditions of the fields in Cadreita and Lodosa were as similar as possible in the area. In both sites, we have tried to use fields with the maximum similarity in soil characteristics (supplementary table 1) and as close geographic proximity as possible.

Plants were planted with four true leaves and good sanitary conditions. In Navarra the crop was planted on May 10th in 2012 and May 23rd in 2013, under polyethylene plastic mulching of 15 µm, a plant density of 35,714 plants ha^{-1} in the conventional system. For the organic farming system, the plants were planted on May 4th in 2012 and May 17th in 2013, with a biodegradable plastic Mater-Bi® of 15 µm and the same plant density. In Extremadura planting dates were April 24th in 2012 and May 2nd in 2013, with a plant density of 33,333 plants ha^{-1} with bare soil. For each cultivation system, a randomized complete block design with 3 blocks per condition was used, with 25 plants per block and condition.

Standard conventional IPM growing and organic farming practices were followed in each cultivation site. In both sites, drip irrigation was used. Hydric requirements were calculated as a function of crop evapotranspiration following FAO56 methodology (Allen et al., 1998).

A single harvest was made for each variety and cultivation system, considering commercial practices. The field was sampled sequentially until 85% of the fruits reached the red-ripe fruit stage in a sample, upon which the decision to harvest was made. Then, the harvest decision was taken. In Extremadura all the varieties were harvested on August 21st in the conventional system and on August 6th and 10th in the case of organic farming. In 2013 the plants were harvested on August 20th (conventional) and August 23rd (organic). In Navarra, for both systems, plants were harvested between August 21st and 29th and, in 2013, between September 16th and 26th (conventional) and on September 18th (organic).

Climate conditions were recorded using a HMP45C temperature and relative humidity probe (Vaisala, Helsinki, Finland) in Navarra and Extremadura, and a CMP3 pyranometer (Kipp&Zonen, Delft, the Netherlands) in Extremadura and a 110/S pyranometer (Skye, Powys, United Kingdom) in Navarra.

Analysis of organoleptic and functional quality

Two representative red-ripe fruits were collected from each of the 25 plants of the replicates. Fruits were pooled and homogenized obtaining a single sample, thus obtaining a biological mean of the replicate that was kept at -80°C until analysis.

On each homogenate the following basic quality parameters were determined: pH, TSS estimated by refractometry of the juice (average of two determinations) using a digital refractometer (ATAGO PR-1, Tokyo, Japan) with 0.1° Brix precision (results expressed as °Brix at 20°C) and Hunter a and b parameters (results expressed as Hunter a/b rate) using a digital colorimeter (CR 300, Minolta, Japan).

The contents of the carotenoids beta-carotene and lycopene were determined using reversed phase HPLC. A 1200 Series HPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a quaternary pump, a degasser, a thermostatic autosampler and a diode array detector (DAD), was used to separate the analytes. The method followed was developed by García-Plazaola and Becerril (1999) with small modifications (Cortés-Olmos et al., 2014).

Samples were thawed in the dark at 4°C and 100 mg of the homogenate were extracted with 14 ml of a 8:6 v/v, ethanol/hexane solution at 4°C, during 24 hours at 200 rpm using an horizontal shaker (Platform Rocker STR6, Viví, Stuart). Hexane was complemented with 0.05% butylated hydroxytoluene (BHT). Hexane supernatant was separated and concentrated using a

SpeedVacSPD-121P and refrigerated vapor trap RVT-4104 (Thermo Scientific, Waltham, USA) to complete dryness, and then re-suspended in 500 µl of hexane. The processed sample was then filtered using a hydrophobic filter of 0.20 µm (MS® PTFE, Membrane Solutions). During the whole process samples were protected from light. A reserved phase Zorbax ODS (250 x 4.6 mm i.d., 5 µm particle size) column protected by a guard column (12.5 x 4.6 mm i.d., 5 µm particle size) was used. The mobile phase consisted of two components: solvent A, with 84:9:7 v/v/v, acetonitrile/methanol/water and solvent B, with 68:32 v/v, methanol/ethyl acetate. The injection volume was 40 µl. The sample was then eluted using a lineal gradient from 100% of solvent A to 100% of solvent B for 12 minutes, followed by an isocratic elution of 100% of solvent B for 7 minutes. Then, a lineal gradient was established from 100% of solvent B to 100% of solvent A for 1 minute. Finally, an isocratic elution of 100% of solvent A for 6 minutes was performed to allow the column to re-equilibrate. The integrations of beta-carotene and lycopene were performed at 445 nm and 470 nm respectively. Two analytical replicates per sample were made. The results were reported as mg kg^{-1}fw.

Sugar and acid profile was obtained determining the contents in malic, citric and glutamic acids and the fructose, glucose and sucrose sugars. An Agilent 7100 capillary electrophoresis system (Agilent Technologies, Waldbronn, Alemania) was used following the method described by Cebolla-Cornejo et al. (2012). Fused silica capillaries (Polymicro technologies, Phoenix, AZ, USA) with 50 µm internal diameter, 363 µm external diameter, 67 cm total length and 60 cm effective length were used. The capillaries were initially conditioned with rinses at 50°C of NaOH 1N (5 minutes), NaOH 1N (5 minutes) and deionized water (Elix 3, Millipore, Billerica, MAS, USA) (10 minutes), followed by a rinse with running buffer at 20°C for 30 minutes. Between runs, the capillary was flushed with 58mM SDS (2 minutes) and running buffer (5 minutes). The conditions for the analysis were: hydrodynamic injection (20 seconds, 0.5 psi); -25 kV fixed voltage separation at 20°C (running buffer: 20 mM 2,6-piridin dicarboxilicacid (PDC) and 0.1% w:v hexadimethrine bromide, pH=12.1). Sucrose equivalents (SEq) and the ratios SEq/citric acid and SEq/glutamic acid were also calculated (Cebolla-Cornejo et al., 2011).

Processed tomato

Processed crushed tomato was obtained using the installations of a small industry in Navarra (Conservas Perón, Lodosa). For this purpose, 100 kg of tomato were obtained from the blocks under conventional management. Raw tomato was flushed with water and transported with water to the sorting table. After manual selection, a hotbreak thermic treatment was applied (100°C during 2 minutes). Tomatoes were crushed in a food mill and peels and seeds discarded. Salt was added following commercial practices. Citric acid was not added to adjust pH. Cristal jars were filled and they were sterilized at 110°C during 5 minutes. Only one sample per cv. was obtained.

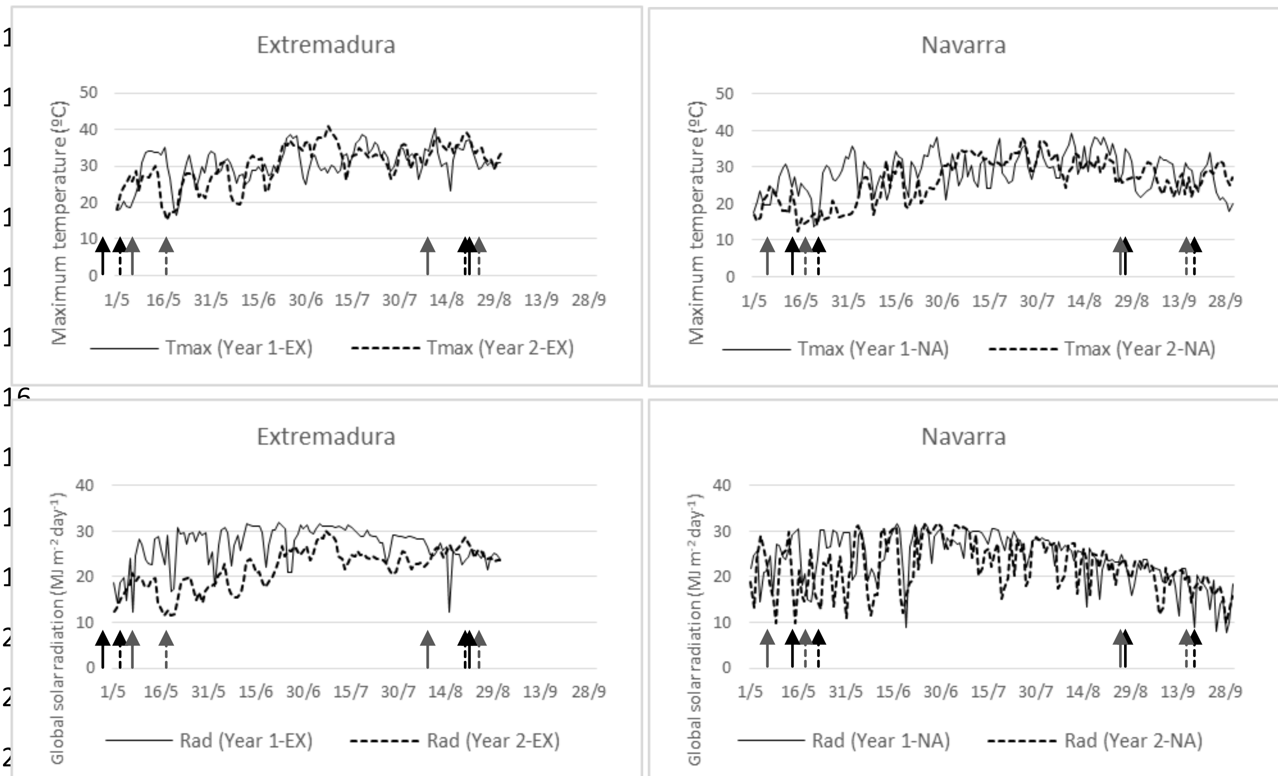
Statistical analysis

The effects of year, site of cultivation, cultivar (cv.) and cultivation system and their interactions were evaluated with a MANOVA test complemented with individual ANOVAs. The effect of cv. and cultivation system for each year and site was also evaluated using MANOVA biplots. This graphical methodology enables a rapid evaluation of similitudes using distance on the biplot and the angle between variables is related with the correlation between them. Bonferroni confidence circles represent an approximation to confidence intervals. The superposition of the projection of Bonferroni circles on each variable enables the identification of significant differences between groups. This analysis was performed using MultBiplot, a freeware licensed by Universidad de Salamanca Proff. Vicente-Villardon (2014 version).

Results

Multivariate analysis confirmed that all the studied factors (year, site, cultivation system and cv.), as well as double interactions, had a significant effect on agronomical performance ($p<0.01$). Conventional cultivation offered higher productions (Table 1). As an average, organic farming presented a 36% lower production. The environmental conditions of Navarra (in general) and those of the first year also maximized production. The environmental conditions of the second year were less favorable for tomato production, with a delay in planting dates due to abundant rainfalls that hindered the preparation of the field. In addition, the lower temperatures registered during the initial stages of development (Fig. 1) also delayed the growth of plants and resulted in lower vegetative growth and consequently lower productions. Furthermore, in Navarra, the incidence of *Alternaria solani* at the end of the cycle accelerated the ripening process in the organic farming site.

Fig 1. Maximum temperatures (°C) and global solar radiation (MJ m-2) during the growing seasons in Extremadura (EX) and Navarra (NA). Arrows represent approximate planting and harvest dates for conventional (back) and organic farming (grey) in 2012 (solid line) and 2013 (dashed line).



The environmental conditions of the first year and, in general, those of Navarra, as well as conventional cultivation offered a more intense red fruit color (higher Hunter a/b values). Higher lycopene contents were also detected in these conditions, though the effect of cultivation system was not significant. The contents in beta-carotene were higher in organic farming (Table 1).

Regarding variables with a direct relation with organoleptic quality, all the studied factors and interactions had a significant effect on the accumulation of sugars and organic acids and the derived variables (MANOVA, $p<0.01$). The second year was more favorable for the accumulation of sugars and acids, with higher mean contents in most of them, as well as higher values of sucrose equivalents (SEq) and of the SEq to citric and SEq to glutamic acid ratios (Table 2). Only the glutamic content was not affected by the year factor. The conditions of Extremadura were also more favorable for the accumulation of sugars and malic acid and the SEq to citric and SEq to glutamic acid ratios, while the conditions of Navarra increased the contents of citric and glutamic acids (Table 2).

Table 1. Effect of environment (year and site), cultivation system and cultivar on marketable production, basic quality aspects and carotenoid content.

		Marketable production (10^3 kg ha^{-1})	TSS (°Brix)	%DM	Hunter a/b	Lycopene mg kg^{-1}	Beta carotene mg kg^{-1}
Year (Y)	*p value*	<0.001	0.028	0.891	<0.001	<0.001	<0.001
	1	130.7	4.7	4.80	2.39	156.9	1.23
	2	83.7	4.6	4.80	2.09	135	1.44
Site (S)	*p value*	<0.001	0.120	0.007	<0.001	<0.001	<0.001
	Extremadura	85.6	4.6	4.87	2.17	126.1	1.15
	Navarra	128.8	4.7	4.73	2.30	165.8	1.52
Cultivation system (C)	*p value*	<0.001	0.404	<0.001	<0.001	0.362	<0.001
	Conventional	136.7	4.6	4.89	2.31	148.0	1.25
	Organic	77.6	4.6	4.71	2.16	143.9	1.42
Cultivar (V)	*p value*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	'CXD-277'	99.6^{ab}	5.2^{c}	5.15^{d}	2.33^{bc}	153.3^{bc}	0.76^{a}
	'H-9661'	107.1^{ab}	4.4^{ab}	4.80^{ab}	1.99^{a}	122.1^{ab}	1.27^{b}
	'H-9997'	116.3^{b}	4.6^{b}	4.86^{b}	2.43^{cd}	149.6^{bc}	0.68^{a}
	'H-9036'	131.1^{c}	4.4^{a}	4.62^{ab}	1.94^{a}	113.6^{a}	1.05^{b}
	'ISI-24424'	94.2^{a}	4.3^{a}	4.52^{a}	2.31^{b}	170.1^{cd}	2.00^{c}
	Kalvert	94.9^{a}	4.9^{b}	4.85^{c}	2.43^{d}	167.1^{d}	2.27^{c}
YxS	*p value*	<0.001	0.042	<0.001	<0.001	<0.001	<0.001
YxC	*p value*	0.008	0.183	<0.001	<0.001	<0.001	0.576

SxC	*p value*	<0.001	<0.001	<0.001	<0.001	<0.001	0.010
YxV	*p value*	0.002	0.826	0.326	<0.001	0.098	0.843
SxV	*p value*	0.156	0.732	<0.001	0.027	<0.001	<0.001
CxV	*p value*	0.001	0.228	0.022	<0.001	0.096	0.028

[1]Total soluble solids. [2] Dry matter.

Different letters for each cultivar indicate significant differences (Tukey test)

Organic farming tended to reduce the accumulation of citric and glutamic acids. The effect on the accumulation of sugars was not significant, and in organic farming higher SEq to citric and SEq to glutamic acids were observed.

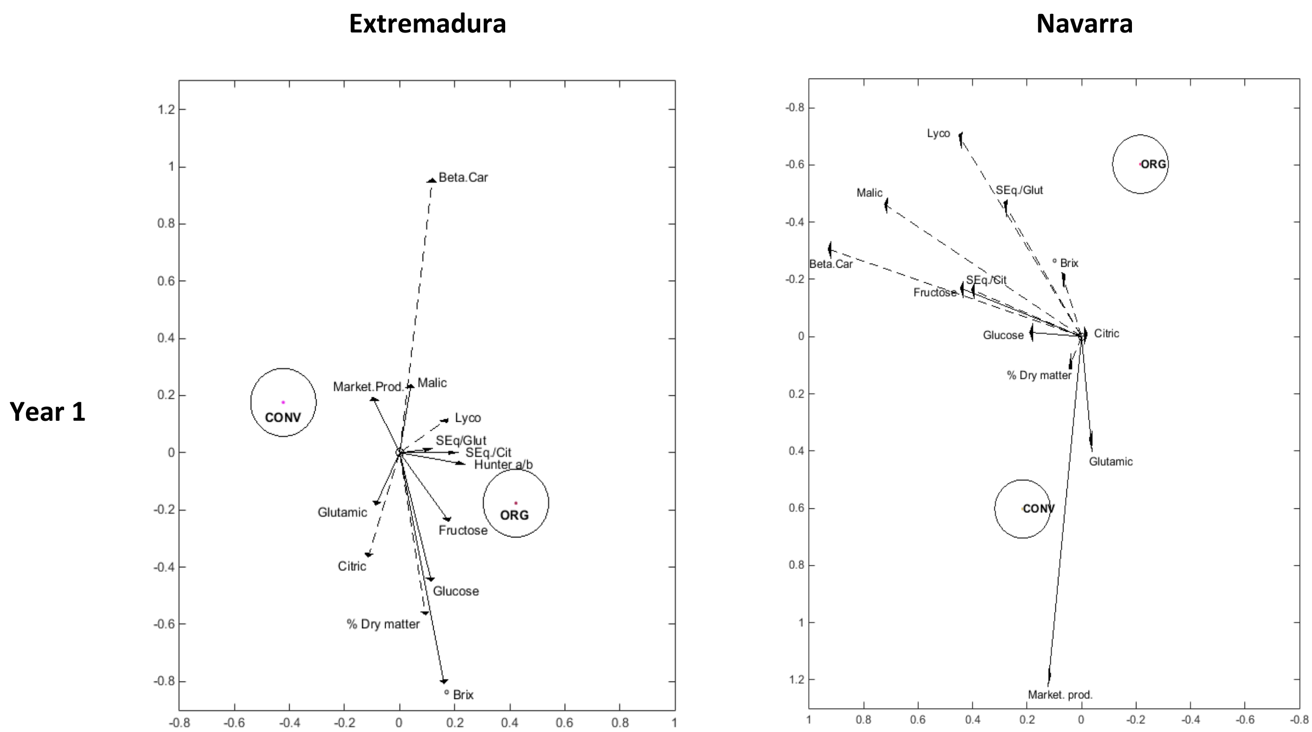
Table 2. Effect (mean value) of environment (year and site), cultivation system and cultivar on compounds and their derived variables related to organoleptic quality.

		Citric acid g kg^{-1}	Malic acid g kg^{-1}	Glutamic acid g kg^{-1}	Glucose g kg^{-1}	Fructose g kg^{-1}	Sucrose equivalents (SEq) g kg^{-1}	Ratio SEq/citric acid	Ratio SEq/glutamic acid
Year (Y)	*p value*	*<0.001*	*<0.001*	*0.38*	*0.016*	*<0.001*	*<0.001*	*0.001*	*<0.001*
	1	3.62	0.81	1.36	12.44	12.24	30.38	8.52	24.74
	2	3.99	1.27	1.41	13.12	14.50	34.79	9.14	34.61
Site (S)	*p value*	*<0.001*	*<0.001*	*<0.001*	*0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
	Extremadura	3.49	1.22	1.09	13.78	14.15	34.67	10.11	40.42
	Navarra	4.12	0.87	1.68	11.78	12.59	30.51	7.55	37.48
Cultivation system (C)	*p value*	*0.033*	*0.12*	*<0.001*	*0.53*	*0.51*	*0.76*	*0.001*	*<0.001*
	Conventional	3.88	1.06	1.62	12.87	13.27	32.48	8.52	21.87
	Organic	3.73	1.03	1.15	12.69	13.47	32.69	9.14	37.48
Cultivar (V)	*p value*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.003*
	'CXD-277'	3.65^{b}	0.98^{a}	1.64^{b}	14.96^{b}	15.07^{b}	37.14^{b}	10.35^{c}	27.68^{ab}
	'H-9661'	4.31^{c}	0.93^{a}	1.43^{ab}	12.20^{a}	12.36^{a}	30.41^{a}	7.22^{a}	30.37^{ab}
	'H-9997'	4.04^{c}	0.98^{a}	1.24^{a}	11.94^{a}	11.98^{a}	29.57^{a}	7.40^{a}	29.64^{ab}
	'H-9036'	3.71^{b}	0.96^{a}	1.51^{b}	12.54^{a}	13.04^{a}	31.84^{a}	8.80^{b}	25.40^{a}
	'ISI-24424'	3.31^{a}	1.20^{b}	1.34^{ab}	11.81^{a}	13.06^{a}	31.32^{a}	9.69^{bc}	29.44^{ab}
	Kalvert	3.82^{b}	1.20^{b}	1.16^{a}	13.23^{a}	14.71^{b}	35.25^{b}	9.52^{bc}	35.54^{b}
YxS	*p value*	*<0.001*	*0.021*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
YxC	*p value*	*0.63*	*0.287*	*<0.001*	*0.002*	*0.11*	*0.039*	*0.019*	*<0.001*
SxC	*p value*	*0.73*	*0.041*	*0.019*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
YxV	*p value*	*0.02*	*0.001*	*<0.001*	*0.032*	*0.003*	*0.006*	*0.514*	*0.124*
SxV	*p value*	*0.04*	*0.034*	*0.081*	*0.042*	*0.023*	*0.030*	*0.036*	*0.015*
CxV	*p value*	*0.17*	*0.026*	*0.032*	*0.14*	*<0.001*	*0.003*	*0.003*	*0.618*

Different letters for each cultivar indicate significant differences (Tukey test)

Effect of the cultivation system

The high influence of year and site of cultivation, as well as the interactions, made a detailed analysis of each year and area recommendable. MANOVA biplot showed that in Extremadura, in the first year, conventional management offered higher productions, while organic farming resulted in increased TSS, fructose, glucose and higher SEq to citric and SEq to glutamic acid ratios (Fig. 2). Although the Hunter a/b ratio was higher in these conditions, the effects on carotenoid contents were not significant (dashed line in the figure).



Year 2

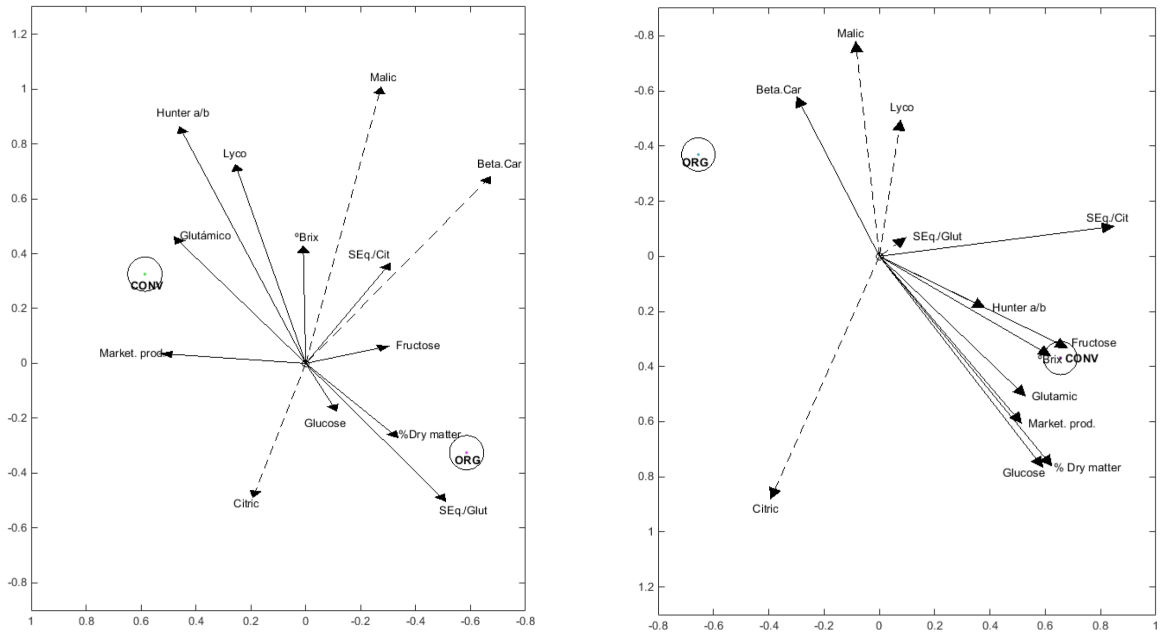


Fig. 2. MANOVA biplots considering the effect of cultivation system (CONV:conventional, ORG: organic) in the different years and sites of cultivation. Dashed lines indicate a non-significant effect (ANOVA, p=0.05).

During the second year in Extremadura, conventional management again yielded higher production values, this time with higher lycopene (and Hunter a/b ratio), TSS and glutamic acid. In organic farming higher fructose and glucose contents were detected again, as well as increased SEq to citric and SEq to glutamic acid ratios (especially the former).

In Navarra, during the first year, higher productions were obtained under conventional management, with higher dry matter (%DM) and higher glutamic, fructose and glucose contents (Fig. 2). During the second year the differences were more pronounced, probably as a consequence of the incidence of *Alternaria*. Consequently, under conventional management, even higher values were obtained for all the variables. However, citric and malic acid contents, the SEq to glutamic ratio and lycopene content showed no significant differences. Beta-carotene was preferentially accumulated under organic farming conditions

Although from a general point of view TSS were not affected by cultivation system, some differences were identified when sites and year of cultivation were analyzed independently. In Extremadura higher TSS were observed in organic farming during the first year, whereas in the second year higher values were obtained under conventional management. In Navarra the low TSS levels observed in the second year in organic farming may be related to the incidence of *Alternaria*, as the fruits affected with over-ripening tend to reduce TSS due to respiration.

Higher levels of %DM were detected under conventional management. However, when a detailed analysis is performed for each site and year, in Extremadura the effect of cultivation system was not significant in the first year, and higher levels were detected in organic farming during the second one. In Navarra the opposite effect was observed. In general, the Hunter a/b ratio associated with the redness of the fruit showed a strong relation with lycopene content (both vectors appear orientated in the same direction). The general analysis showed that conventional management would tend to increase this value. This might be explained by the higher lycopene contents observed in this cultivation system, though with a strong environmental effect, as in Navarra the effect of cultivation system was not significant.

Regarding organoleptic quality, in Extremadura during both years organic farming resulted in higher levels of sugars and higher SEq to citric and glutamic acid ratios (Fig. 2). Only during the second year an increased accumulation of glutamic acid was detected under conventional management. In Navarra, during the first year, higher levels of sugars and glutamic acid were obtained under conventional management. During the second year, higher values were obtained with this system for all the variables but for citric acid (with no significant effect).

Effect of the cultivar

In general, cv. ‘H-9036’ clearly offered the highest productions (Table 1), followed by ‘H-9997’ (with 11% lower production). Regarding basic quality parameters, ‘CXD-277’ stood out for higher %DM and TSS, followed by ‘Kalvert’, which showed a more intense red color. ‘Kalvert’ and ‘ISI-24424’ showed the highest carotenoid contents, though with a reduction in the production of 27.6% and 28.1%, respectively, when compared to ‘H-9036’. ‘H-9997’, with a relatively good agronomic performance, also showed intermediate levels of lycopene (though relatively low for beta-carotene) and it proved a successful combination of production levels and quality.

When analyzing the performance in each area and year of cultivation, in the first year in Extremadura, the MANOVA biplot showed that, in agreement with the global analysis, cvs. ‘Kalvert’ and ‘ISI-24424’, and to a lower extent ‘CXD-277’ (with low beta-carotene levels) offered the highest carotenoid accumulation (Fig. 3). On the other hand, ‘CXD-277’and ‘H-9997’ stood out for basic quality parameters (TSS and %DM) and higher fructose contents. ‘H-9661’ and ‘H-9036’ offered the highest production levels, but at the expense of a lower quality profile. The results obtained in the second year confirmed these trends, with higher quality in

'ISI-24424', 'Kalvert' and 'CXD-277' (the first two especially for functional quality and the latter for organoleptic quality).

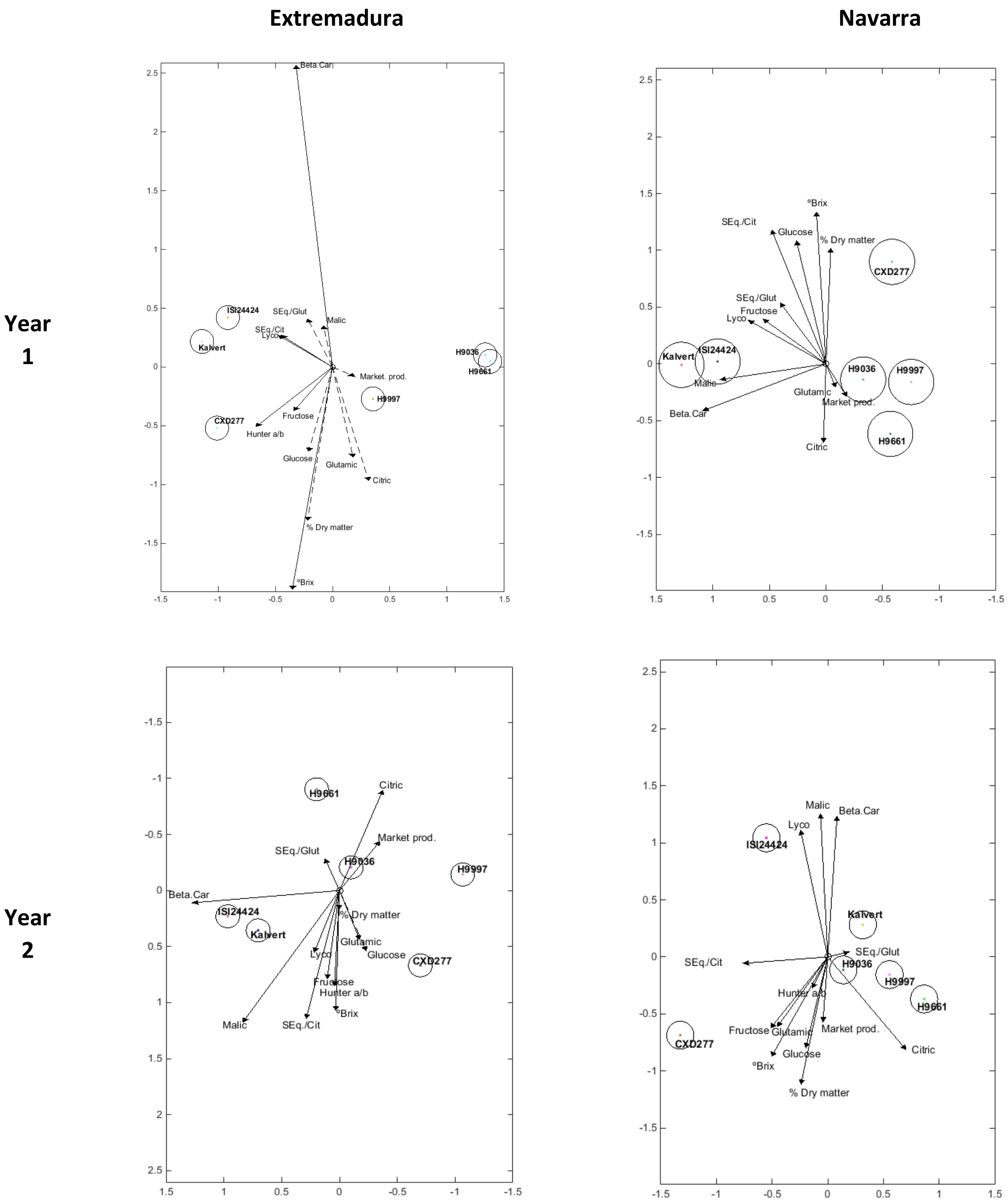


Fig. 3. MANOVA biplots considering the effect of cultivar in the different years and sites of cultivation. Dashed lines indicate a non-significant effect (ANOVA, p=0.05).

In Navarra, during both years the cv. ‘H-9036’ stood out for production levels, again at the expense of fruit quality (Fig. 3). On the other hand, ‘Kalvert’ and ‘ISI-24424’ stood out for carotenoid accumulation at the expense of productivity. Both cvs. also stood out during the first year for the accumulation of compounds related with organoleptic quality, though with a low acidic profile. The cv. ‘CXD-277’ showed an intermediate position, with moderate lycopene accumulations, a good accumulation of compounds related with organoleptic quality during the two years and a lower decrease in productivity.

The relative orientation of the vectors for yield and lycopene accumulation in all the years and sites of cultivation (with almost a 180° angle in most of them) evidenced the difficulty in combining high functional and agronomic performance in the same material. Something similar is observed in the case of organoleptic quality, though in some conditions the angle between variable vectors is not so pronounced. In Extremadura, during the first year, ‘H-9997’ proved to be a good material for both characteristics. Apart from this cv., during the second year, ‘CXD-277’ also achieved a balanced equilibrium between those variables. In Navarra it was more difficult to find a similar balance. In either case, ‘CXD-277’ would offer the best compromise, though its production during the first year was quite limited.

Considering all the results, ‘CXD-277’and ‘Kalvert’ stood out for sugar accumulation, especially the former. Acidic profile was highly cv. dependent. ‘H-9661’and ’H-9997’ tended to accumulate higher levels of citric acid, ‘ISI-24424’ and ‘Kalvert’of malic acid and ‘CXD-277’ of glutamic acid. ‘CXD-277’ was also noticeable due to its high SEq to citric acid ratio and ‘Kalvert’ for high SEq to glutamic acid ratio.

Processed tomato

A sample before the entrance to the processing plant could not be obtained. Nevertheless, we compared the mean results of the raw samples obtained under conventional cultivation and the samples collected after the whole process, resulting in canned crushed tomato. Despite this limitation, relatively high regression coefficients (0.49-0.93) were calculated for most compounds (Table 3). Only the contents of citric acid showed a lower correlation (0.22). Considering the positive relationship obtained in the regression models, the values of the raw tomatoes would represent a good indication of the relative composition of the processed product.

Table 3. Regression models between raw and processed (crushed) tomato contents.

Compound	**R^2**	**Equation**
Malic acid	0.69	$Y_P = 0.39 + 0.59\ X_F$
Citric acid	0.22	$Y_P = 2.24 + 0.37\ X_F$
Glutamic acid	0.86	$Y_P = 0.18 + 1.17\ X_F$
Fructose	0.49	$Y_P = 6.69 + 0.49\ X_F$
Glucose	0.93	$Y_P = 0.52 + 0.82\ X_F$

Discussion

Trends in plant breeding during the last decades have confirmed a tendency towards the development of new cvs.s with increased accumulation of functional compounds. The possible role of carotenoids on the prevention of certain types of cancer and cardiovascular diseases (Fiedor and Burda, 2014) has spurred the development of high lycopene cvs. Two mutations have been especially used for this purpose (reviewed by Cebolla-Cornejo et al., 2013). *Old gold* (*og*) and *old gold crimson* (og^c) represent two different alleles codifying a defective CYC-B enzyme that blocks the cyclisation of lycopene to beta-carotene, thus increasing the levels of the former at the expense of the latter. On the other hand, *high pigment* mutations (*hp-1*, *hp-2* and $hp\text{-}2^{dg}$), among other effects, affect a light dependent regulation of the carotenoid pathway resulting in increased contents in both lycopene and beta-carotene. In our case, different carotenoid profiles were observed in the cvs. with higher lycopene levels, suggesting the use of both strategies. Nevertheless, breeding companies do not declare the genes used during the development of each cv.

Independently of the strategy followed in the breeding process, our results demonstrate the difficulty of developing materials with increased carotenoid content and a good agronomical performance. As an example, high lycopene cv. 'Kalvert' has been reported in other works as a high lycopene tomato cv. In our work, higher mean contents have been obtained compared to previous studies (167 mg kg-1 vs. approx. 150 mg kg-1 in Ilahy et al., 2011). However, its commercial use may be affected by its relatively low productivity. In fact, the lower yield of high lycopene cvs. such as 'Kalvert' is compatible with the undesirable pleiotropic effects of high pigment mutations resulting in reduced yields (reviewed by Stommel, 2006). When compared to 'H-9036', the best commercial cv. being grown in the area, 'Kalvert' shows a 27.6% lower yield. Until the processing industry and the market become convinced of the added value of the accumulation of functional compounds and start paying a premium for contents in raw tomato (as it already does for TSS) it would be difficult to promote the cultivation of these cvs. Meanwhile, the commercialization of high lycopene cvs. may be achieved following two approaches. One of them would imply the use of cvs. with intermediate lycopene levels. As example, 'H-9997' offered only 12% lower lycopene content compared to cv. with the highest mean values 'ISI-24424', while the yield was on average only an 11.3% lower than 'H-9036'. The alternative strategy would imply the promotion of the side effects of the best high lycopene cvs. involving an extra added value. Following the case of 'Kalvert', this cv. stood out for glucose and fructose accumulation, SEq and SEq to citric and SEq to glutamic acid ratios, variables related with improved acceptability by sensory panels (Baldwin et al., 1998; Bucheli et al., 1999). Consequently, materials like this may be targeted to specific markets valuing both organoleptic and functional value. In previous studies 'Kalvert' already showed high levels of sugar accumulation. Lenucci et al. (2008) reported mean values of fructose plus glucose of 23 g kg^{-1}. Our results even improve this value with an average combined content of 27.9 g kg^{-1}.

Regarding breeding efforts, it should be considered that the results achieved in this work strengthen the idea that classic measurements of tomato quality should be replaced with specific determination of individual compounds. In fact, TSS usually used for selection processes in breeding programs due to its relation to overall flavor intensity (Stevens et al., 1977) showed no statistical differences between 'H-9661' and 'Kalvert', while the second offered significantly higher fructose contents and higher levels of SEq, variables with a better correlation with sweetness and acceptability (Baldwin et al., 1998).

Lenucci et al. (2008) described in their study of high lycopene cvs. the existence of variation for the fructose:glucose ratios. In our case, the ratios observed between both sugars are quite similar (1-1.1) and close to the standard values in tomato (Davies and Hobson, 1981). Nonetheless, a clear variation was found among the varieties analyzed for the acidic profile. As stated in the results section, 'H-9661' and 'H-9997' tended to accumulate higher levels of citric acid, 'IS-I24424' and 'Kalvert' of malic acid and 'CXD-277' of glutamic acid. Malic acid has more sour potential (14%) than citric acid. Although Bucheli et al. (1999) in their regression model for tomato fruitiness linked negatively malic acid contents, the high TSS and SEq to glutamic acid ratio values may compensate the high malic values obtained in 'Kalvert'.

Regarding the effect of the environment (year and site) on quality, among the different factors affecting sugar accumulation, solar radiation has a more important effect (Davies and Hobson, 1981). This may explain the higher levels obtained in Extremadura, considering the earlier harvesting dates and radiation levels during the ripening stage (Fig.1). The higher levels obtained during the second year may have another explanation, as the radiation levels were not higher. In this case, the slower growth rate and the lower productions obtained in this year may explain this effect. Bertin et al. (2000) proved that a lower fruit load involves a higher accumulation of sugars and an increase in the sugar to acid ratio. Regarding the environmental (year and site) effect on the accumulation of acids, the higher effect on malic compared to citric acid is in agreement with the environmental effects reported by Cebolla-Cornejo et al. (2011), where citric acid was not affected by environment (field vs. protected cultivation), while the contents in malic acid where significantly higher under protection (and thus under lower solar radiation levels). Nevertheless, it should be considered that the environmental factor in this case also includes different soils, plant densities (following commercial practices in each area) and farmers.

In the case of lycopene, the higher values obtained in Navarra might be related with the possibly saturating conditions of Extremadura during the ripening stage. Lycopene accumulates in a range of average day temperatures between 12°C to 32°C (Dumas et al., 2003). With temperatures higher than 30-32°C lycopene accumulation ceases and its cyclisation is promoted (Tomes et al., 1963; Dumas et al., 2003; Brandt et al., 2006). Additionally, high radiation levels have a negative effect on lycopene accumulation (Adegoroye and Jolliffe, 1987). The higher temperatures and radiation levels of Extremadura, with an earlier harvest (Fig 1.), may therefore explain the lower accumulation obtained at this site.

Apart from the possible benefits from a quality point of view of the use of high pigment varieties, the possible role of organic farming as a way to improve quality was also studied. Our results point out that in fact organic farming may improve the contents in glucose and fructose, but at levels that are highly dependent on the variety and environmental conditions (year and site effect). Under high radiation and temperature levels typical of Extremadura, the benefits would be obvious, but in milder climates, the differences may be attenuated or may even favor conventional management.

It should be considered that organic farming dramatically reduces production. As stated before, the lower fruit loads obtained under organic farming may explain higher contents of sugars (Bertin et al., 2000). In fact, in the MANOVA biplots an angle between 90-180° was observed between the vectors for production and reducing sugars. The second year in Navarra represents an exception. But in our opinion, the incidence of *Alternaria* resulted in an acceleration of the ripening process, and the over-ripening has been already linked with reductions in TSS due to respiration (Mejía-Torres et al. 2009).

Few works make a detailed comparison of specific sugars, but most of them use TSS as a general parameter related with tomato quality. Chassy et al. (2006) observed higher levels of TSS under organic farming in a 3-year study, as well as Barrett et al. (2007). In cherry tomatoes, a completely different material, Pinho et al. (2011) observed higher TSS levels under conventional management, but in the later harvest dates no differences were observed. With another related variable, Caris-Veyrat et al. (2004) observed higher levels of %DM under organic farming in processing tomato. In this case, the authors related the behavior not with lower fruit load, but with the absorption of mineralized nitrogen in organic farming, that would not force the growth of plants. Hallmann (2012) did not find differences in the global content of reducing sugars, though in one of the two years total sugars were higher. Migliori et al. (2012) did not find differences between conventional and organic farming in soluble sugars.

Toor et al. (2006) comparing the effect of organic vs. mineral nutrition over different quality parameters in tomato observed no difference in TSS, but a trend towards lower acidity in solutions based on nitrates and higher acidity with one of the organic fertilizers assayed. Riahi et al. (2009) agreed with these authors that in order to keep the C/N ratio stable, plants under organic farming may derive extra C towards the production of organic acids. On the other hand, Migliori et al. (2012) did not observe differences in the content of organic acids between both systems, though in one out of three years the pH of one of the cvs. was higher under conventional farming. Hallman et al. (2012) observed higher levels of acidity under organic farming, but only in one of the years assayed, and Barrett et al. (2007) obtained higher titratable acidity in organic farming, though not for all the growers. Our results tend to support a trend towards higher acid accumulation under conventional management. In this sense, global analysis significantly pointed out a slightly higher level in citric acid and a clearly higher glutamic acid content under this system. Under certain circumstances the content in malic acid may be affected towards higher contents under conventional management.

Regarding color and carotenoid accumulation Chassy et al. (2006) observed that in general the Hunter a/b parameter was not affected by cultivation system, though in each year a tendency towards higher values in conventional farming was detected. In our case, in general, higher values were obtained under conventional management, though with a strong interaction. For example, during the first year in Extremadura higher values were obtained under organic farming. Nevertheless, lycopene content was not significantly affected by cultivation system, though higher beta-carotene contents were obtained under organic farming. Contradictory results have been reported in the literature regarding carotenoid accumulation. Caris-Veyrat et al. (2004) obtained higher carotenoid levels under organic farming, while Riahi et al. (2009) found no effect of the cultivation system, and Rossi et al. (2008) obtained lower lycopene contents under organic farming and no significant differences in the case of beta-carotene. Many parameters are changed between conventional and organic cultivation and it is impossible to implement controlled factorial designs. Thus, it is complicated to obtain generalizable results. For example, we cannot rule out, in our case, uncontrolled factors, as different farmers took charge of organic and conventional production in Navarra, and soil textures were not exactly equal. This inconsistency is reflected in the results by Barrett et al. (2007) and Juroszek et al. (2009). These authors suggested that the higher or lower levels of lycopene under organic farming depended, in fact, on the grower considered, rather than the cultivation system.

Conclusions

Organic farming tended to reduce the contents in organic acids while it has non-significant effect on the contents of either fructose or glucose. This situation leads to an increase in the ratio SEq to citric and glutamic acid. The levels of lycopene were not affected by the cultivation system, while beta-carotene contents were higher under organic farming. The high lycopene cv. 'Kalvert' offers high values for the compounds and derived variables related with organoleptic quality. This type of cv. represents a good material targeted to high quality markets that may compensate with higher prices its lower yields. While the best high lycopene cvs. experience an important decrease in marketable production, it is possible to identify cvs. with intermediate contents and relatively high productions. Considering the good and positive correlation obtained in regression models between raw and processed (crushed) tomato, the analysis of raw material would be a good indicator of the effects of different factors on the contents in sugars and acids.

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Supplementary Table 1. Soil characteristics of the experimentation fields.

	Depth (cm)	*Sand*[1]	*Silt*[1]	*Clay*[1]	*M.O.*[2]	*pH*[3]	*E.C.*[4] *(dS m^{-1})*	*Texture*[1]
Characteristics of soil (conventional) in Navarra	*0-30*	*42.6*	*43.7*	*13.7*	*1.73*	*8.10*	*1.05*	*Loam*
	30-60	*38.1*	*46.7*	*15.2*	*0.92*	*8.20*	*0.77*	*Loam*
	60-90	*43.6*	*41.5*	*14.8*	*1.05*	*8.34*	*0.61*	*Loam*
Characteristics of soil (organic) in Navarra	*0-30*	*11.3*	*60.5*	*28.1*	*1.96*	*8.06*	*0.73*	*Silty Clay Loam*
	30-60	*6.8*	*77.4*	*15.8*	*1.36*	*8.21*	*0.54*	*Silt Loam*
	60-90	*11.7*	*74.2*	*14.0*	*0.84*	*8.11*	*0.54*	*Silt Loam*
Characteristics of soil (conventional and organic) in Extremadura	*0-30*	*69.9*	*14.9*	*15.2*	*0.90*	*6.68*	*0.16*	*Sandy Loam*
	30-90	*69.2*	*15.8*	*15.0*	*0.90*	*6.87*	*0.12*	*Sandy Loam*

[1]*USDA*
[2]*Oxidizable organic matter*
[3]*H2O (1:5)*
[4]*Electrical conductivity*