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Effect of autochthonous lactic acid bacteria starters on health-promoting and sensory properties of tomato juices

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ABSTRACT

Strains of *Lactobacillus plantarum*, *Weissella cibaria/confusa*, *Lactobacillus brevis*, *Pediococcus pentosaceous*, *Lactobacillus* sp. and *Enterococcus faecium/faecalis* were identified from raw tomatoes by Biolog System, partial 16S rRNA gene sequence and subjected to typing by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis. Ten autochthonous strains were singly used to ferment tomato juice (TJ) via a protocol which included fermentation at 25 °C for 17 h and further storage at 4 °C for 40 days. Unstarted TJ and TJ fermented with an allochthonous strain of *L. plantarum* were used as the controls. All autochthonous strains grew well in TJ reaching cell densities ca. 10,000 and 10 times higher than unstarted TJ and TJ fermented with the allochthonous strain. Viscosity of TJs fermented with autochthonous strains was generally the highest, especially when started with *W. cibaria/confusa* which synthesized exo-polysaccharides. Overall, unstarted TJ and TJ fermented with the allochthonous strain showed marked decreases of ascorbic acid (ASC), glutathione (GSH) and total antioxidant activity (TTA) during storage. On the contrary, several TJs fermented with autochthonous strains, especially with *L. plantarum* POM1 and POM 35, maintained elevated values of ASC, GSH and TAA. The variation of color indexes mirrored the above behavior. TJs fermented with the above two autochthonous strains were compared to controls based on volatile components through Purge and Trap or Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (PT or SPME-GC/MS) analysis. As shown by Principal Component Analysis a large number of volatiles belonging to various chemical classes markedly differentiated TJs fermented with autochthonous strains with respect to controls.

1. Introduction

Tomatoes are some of the most widely used and versatile vegetable crops. Tomatoes are consumed as fresh or as industrially processed. Processed tomatoes include canned and sun-dried tomatoes, juices, ketchup, pastes, purees, salads, sauces and soups (Shi and Le Mauger, 2000).

Tomatoes contain abundant health-promoting related components such as lycopene, provitamin A (Beecher, 1998), ascorbic acid (Sahlin et al., 2004), vitamin E, folate, flavonoids and potassium (Leonardi et al., 2000). Regular consumption of tomatoes has been associated with a reduced risk of various types of cancer (Weisburger, 1998) and heart diseases (Pandey et al., 1995). These effects are mainly attributed to the presence of antioxidants, especially carotenoids, flavonoids, lycopene and β-carotene (Lavelli et al., 2000). Furthermore, the American Cancer Society recommends to increase the daily intake of fruits and vegetables rich in carotenoids, and vitamins C and E to lower risk of cancer and cardiovascular diseases (World Cancer Research Fund, 1997). Epidemiological studies have shown that the increased consumption of tomato foods is consistently associated with low risk of a variety of cancers, especially prostate cancer (Giovannini, 2002).

Among processed tomatoes, juices may also be considered as health-promoting beverages (Suzuki et al., 2002). Nevertheless, processed fruits and vegetables have lower nutritional and health-promoting values than their fresh counterparts due to variable loss of antioxidants during processing (Murcia et al., 2000). Thermal processing (e.g., 90–100 °C for a few s to 10 min) is the most common technology used for inactivating microorganisms and enzymes, and for extending shelf-life of tomato juices (Vega-Mercado et al., 1997). Inevitably, heat treatments of tomato juices induce undesirable changes of color, flavor and nutritional value, and also decrease health-promoting properties (Goodman et al., 2002; Qin et al., 1996). Nowadays, consumer's demand for minimally processed foods is

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increasing remarkably. Preferences shift towards fresh-like and highly nutritional value, health-promoting and rich flavor ready-to-heat foods with enhanced shelf-life. Lactic acid fermentation of vegetables, currently used as the bio-preservation method for the manufacture of finished and half-finished foods, is an important biotechnology for maintaining and/or improving safety, nutritional, sensory and shelf-life properties of vegetables (Demir et al., 2006; Karovičová and Kohajdová, 2002). Three technology options are usually considered for lactic acid fermentation of vegetable juices: (i) spontaneous fermentation by autochthonous lactic acid bacteria, (ii) fermentation by starter cultures that are added into raw vegetables, and (iii) fermentation of mild heat-treated vegetables by starter cultures (Hammes, 1990). *Lactobacillus plantarum*, *Lactobacillus xylosus* and *Lactobacillus brevis* are the starter cultures most frequently used for fermentation of vegetable juices (Šulc, 1984). In most of the cases such commercial cultures do not correspond to autochthonous strains. To get desirable properties of fermented vegetable juices, lactic acid bacteria has to be adapted to the intrinsic characteristics of the raw materials.

To our knowledge, no studies have considered the use of selected autochthonous lactic acid bacteria for the fermentation of tomato juices. Studies only aimed at elucidating the effect of thermal processing, high hydrostatic pressure and high-intensity pulsed electric field on flavor, color, and physico-chemical and antioxidants properties of tomato juices (Hsu et al., 2008; Odriozola-Serrano et al., 2007; Servili et al., 2000). Yoon et al. (2004) showed the suitability of tomatoes as the raw material for the manufacture of probiotic juice. Several species of lactobacilli were shown to be viable at cell densities of 6.0–9.0 log CFU ml^{-1} during four weeks of storage.

After the isolation and identification of lactic acid bacteria from tomatoes, this paper describes the use of selected strains for fermentation of tomato juices and characterizes tomato juices for viscosity, color, antioxidants and volatile compounds.

2. Materials and methods

2.1. Isolation of lactic acid bacteria

Tomato fruits (*Licopersicon esculentum* Mill, cultivar Sunrise) at commercial maturity were purchased in triplicate from three local markets (Bari, Italy) and kept at 4 °C prior to use. Twenty grams of tomato were suspended in 180 ml of sterile sodium chloride (0.9%, w/v) solution and homogenized with a Classic Blender (PBI International Milan, Italy) for 2 min at room temperature. Serial dilutions were made and plated on MRS agar (Oxoid Ltd, Basingstoke, Hampshire, England), at 30 °C for 48–72 h under anaerobiosis, for isolating presumptive mesophilic lactic acid bacteria. At least 15 colonies, possibly with different morphology, were isolated from MRS plates of the highest dilution. Gram-positive, catalase-negative, non-motile rod and cocci isolates were cultivated in MRS broth at 30 °C for 24 h, and re-streaked into MRS agar.

2.2. Genotypic and phenotypic identification of lactic acid bacteria

Genomic DNA was extracted as described by De Los Reyes-Gavilán et al. (1992) from 2 ml of MRS culture broth of each isolates. Two primer pairs (Invitrogen), LacbF/LacbR and LpCoF/LpCoR (De Angelis et al., 2006), were used to amplify 16S rRNA gene fragment of lactic acid bacteria (Di Cagno et al., 2007). The expected amplicons of about 1400 and 1000 bp (after amplification with primers pairs LacbF/LacbR and LpCoF/LpCoR, respectively) were eluted from the gel and purified by the GFX™ PCR DNA Gel Band Purification Kit (GE Healthcare, Buckinghmshire, UK). PCR products were separated by electrophoresis and purified as described above, and subjected to sequencing. Taxonomic strain identification was performed by comparing the sequences of each isolate with those reported in the Basic BLAST database (Altschul et al., 1997). Strain showing homology of at least 97% were considered to belong to the same species (Goebel and Stackebrandt, 1994). Two primers (Invitrogen) recALb1F/recALb1R were used to amplify 16S rRNA gene fragment to differentiate *L. plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum* (Torriani et al., 2001).

Phenotypic identification was carried out by Biolog System (Biolog, Inc., Hayward, CA, USA) using 95 different carbon sources. Three days before the inoculation of Biolog AN plates (Biolog, Inc.), the isolates were streaked twice on MRS agar plates and incubated for 24 h at 30 °C. The wells of the Biolog AN plates were inoculated with 150 µl bacterial suspensions adjusted to 65% transmittance as recommended by the manufacturer. Positive reactions were automatically recorded using a microplate reader with a 590-nm wavelength filter.

2.3. Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis

Two primers (Invitrogen Life Technologies, Milan, Italy), with arbitrarily chosen sequences (M13, 5′-GAGGGTGGCGGTTCT-3′ and P4 5′ CCGCAGCGTT 3′) (Sahlin et al., 2004), were used singly in two series of amplification (Di Cagno et al., 2007). The molecular weight of the amplified DNA fragments was estimated by comparison with 1 Kb Plus DNA Ladder (Invitrogen). For RAPD analysis, the presence or absence of fragments was recorded as 1 or 0, respectively. Only reproducible well-marked amplified fragments were scored, with faint bands being ignored. The two series of RAPD-PCR profiles were evaluated and combined to obtain a unique dendogram, calculating an index of genetic similarity by the Simple Matching coefficient (Sokal and Michener, 1958).

2.4. Tomato juice fermentation

The protocol for fermentation and storage of tomato juices (TJs) is described in Fig. 1. Sucrose was added for enhancing the flavor of TJ by reducing the intrinsic flavor acidity of tomatoes. Twenty-four-hour-old cells of *L. plantarum* POM1, POM8, POM27, POM35 and POM43, *L. brevis* POM2, *Enterococcus faecium*/*faecalis* POM3, *Weissella cibaria*/*confusa* POM11, *Pediococcus pentosaceus* POM10 and *Lactobacillus* sp. POM44 were used as the single autochthonous starter. *L. plantarum* LP54, previously isolated from green olive fermenting brines and belonging to the Culture Collection of the Department of Plant Protection and Applied Microbiology, was used as the allochthonous starter. Twenty-four-hour-old cells cultivated in MRS broth at 30 °C were harvested by centrifugation (10,000 ×*g*, 10 min, 4 °C), washed twice in 50 mM sterile potassium phosphate buffer (pH 7.0), re-suspended in sterile distilled water to a final optical density at 620 nm (OD_{620}) of 2.5 (final cell number corresponding to ca. 9.0 log CFU ml^{-1}) and used to inoculate TJs (4%, v/v). TJ subjected to the same treatments (Fig. 1), except for the use of starters, was used as the control (unstarted).

2.5. Determination of pH, tritatable acidity and carbohydrates

The pH was measured by a Foodtrode electrode (Hamilton, Bonaduz, Switzerland).

Total titratable acidity (TTA) was measured on 10 ml of TJ homogenized with 90 ml of distilled water (Classic Blender, PBI International), and expressed as the amount (ml) of 0.1 M NaOH to achieve a pH of 8.3.

Sucrose, glucose and fructose were determined by enzymatic methods (DHIFFCHAMB Italy Srl).

2.6. Microbiological analyses

Samples of TJ (10 ml) were suspended in sterile 0.1% (w/v) peptone-water solution and homogenized with a Classic Blender 400 (PBI International) for 2 min at room temperature. Mesophilic lactic acid bacteria and yeasts were determined on MRS agar (Oxoid) at 30 °C for 48–72 h under anaerobiosis and on Yeast extract-Peptone-

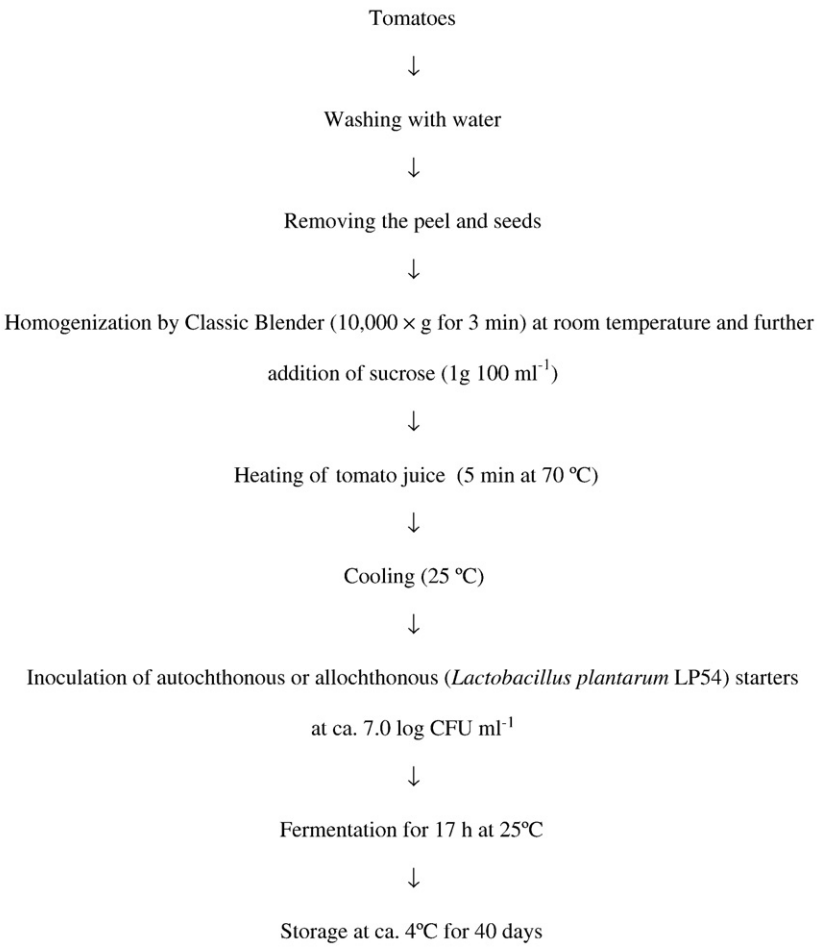

Fig. 1. Protocol for fermentation and storage of tomato juice.

Dextrose agar (YPD, Oxoid), added of 150 ppm chloramphenicol, at 30 °C for 72 h, respectively. The monitoring of the autochthonous and allocthonous strains was carried out by RAPD-PCR.

2.7. Kinetics of growth and acidification

Kinetics of growth and acidification were determined and modeled according to the Gompertz equation as modified by Zwietering et al. (1990): $y=k+A \exp\{-\exp[(\mu_{max} \text{ or } V_{max}\, e/A)(\lambda-t)+1]\}$; where y is the growth expressed as log CFU ml^{-1} min^{-1} or the acidification extent expressed as dpH dt^{-1} (units of pH min^{-1}) at the time t; k is the initial level of the dependent variable to be modelled (log CFU ml^{-1} or pH units); A is the difference in cell density or pH (units) between inoculation and the stationary phase; μ_{max} or V_{max} is the maximum growth rate expressed as Δlog CFU ml^{-1} h^{-1} or the maximum acidification rate expressed as dpH min^{-1}, respectively; λ is the length of the lag phase expressed in minutes; and t is the time. The experimental data were modeled through the non-linear regression procedure of the statistic package Statistica per Windows (Statsoft, Tulsa, Oklahoma, USA).

2.8. Synthesis of exo-polysaccharides

Colonies from cell suspensions of each strain, pre-cultivated in MRS broth, were allowed to grow in MRS agar with the addition of 292 mM sucrose, 146 mM glucose or 146 mM fructose. After incubation at 30 °C for 48 h, the synthesis of exo-polysaccharides (EPS) was determined by visual appearance of the mucoid colonies. The screening was carried out in three separated experiments.

2.9. Viscosity and color measurements

The apparent viscosity was measured on approximately 35 ml of tomato juice using the sine wave vibro-viscometer A&D SV-10 (A&D Company Ltd., Japan), which measures viscosity by detecting the driving electric current needed to resonate two sensor plates at a constant frequency of 30 Hz and amplitude of less than 1 mm. Viscosity measurements were carried out on the TJs, immediately after they had been fermented (17 h at 25 °C), and at the end of the storage (40 days at 4 °C) on TJs previously adapted at 25 °C for 30 min.

The color of tomato juices was measured using a Chromameter CR-200 tristimulus colorimeter (Minolta, Osaka, Japan). Samples were placed in Petri dishes and filled to the top. The values of a (green-red tonality) and b (blue-yellow tonality) were determined.

2.10. Ascorbic acid, glutathione and antioxidant activity

Ascorbic acid (ASC) and glutathione (GSH) were assayed in deproteinized extracts. An aliquot of tomato juice and 10% meta-phosphoric acid containing 1 mM EDTA (v/v, ratio 1:1) were mixed, vortexed and centrifuged at 20,000 ×g for 15 min at 4 °C. The supernatants were collected for the analysis of vitamin C and glutathione according to de Pinto et al. (1999).

Total antioxidant activity (TAA) was measured using the assay of ABTS radical scavenging activity according to Miller and Rice-Evans (1997). Trolox at a concentration of 0 to 500 μM was used as the standard. The free-radical-scavenging activity was expressed as mM of Trolox per liter of sample.

2.11. Determination of free amino acids

Total and individual free amino acids (FAA) of the water extracts of TJs were analyzed using a Biochrom 30 series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Na-cation-exchange column (20 by 0.46 cm inner diameter) (Di Cagno et al., 2007).

2.12. Determination of volatile compounds

Volatile compounds were determined after two methods of extraction: (i) Purge and Trap (PT), and (ii) Solid Phase Microextraction (SPME). Both methods were coupled with Gas Chromatography-Mass Spectrometry (PT/SPME-GC/MS). Prior to PT analysis, 10 ml of TJ were placed in a glass extractor connected to the PT apparatus (Tekmar 3000, Agilent Instruments, NY). Extraction was performed by helium at a flow rate of 40 ml min^{-1} on a tenax trap at 37 °C. Trap desorption was performed at 225 °C and injection into the chromatograph was performed with a cryo-cool-down. For SPME extraction, 2 ml of TJ were placed in a 10 ml sealed flask and placed in a bath at 40 °C for 30 min. A SPME polydimetylsiloxane fiber (Supelco, Sigma Chemical Co.) was introduced into the flask and held in the headspace for 30 min, then removed and desorbed in 5 min in a splitless chromatograph injector at 250 °C. The chromatograph (Agilent Instruments) was equipped with a DB5-like capillary column (RTX5 Restek, Agilent instruments), 60 m length, 0.32 μm internal diameter, and 1 μm thickness. The mass detector (MSD5973, Agilent Instruments) was used in scan mode, from 29 to 206 atomic mass volt at 70 electron volt. Chromatographic conditions and quantification of compounds was as reported by Di Cagno et al. (2007).

For extraction of volatile free fatty acids, 15 ml of TJ were mixed with valeric acid as internal standard and 50 ml of sulphuric acid 10% (v/v), homogenized using an ultraturrax and poured into a Jalade apparatus to the bottom of which was attached a balloon containing 60 ml of diethyl ether (Carlo Erba, Val de Reuil, France) and 60 ml of petroleum spirit (40 to 60 °C) (Normapur Prolabo, Fontenay S/Bois, France), and at the top was a refrigerator. Extraction was performed for 6 h by ebullition of the solvent. The solvent phase was separated from water, mixed with 50 ml of a solution ethanol/water (4:1) (v/v) and two drops of 1% phenolphthalein. The volatile free fatty acids were saponified by addition of 1% NaOH (w/v). The water phase was kept and desiccated by heating at 103 °C. GC apparatus (CE8160 Thermoquest, Agilent Instruments) equipped with a FFAP column (Stabilwax DA Restek, 30 m length, 0.53 μm diameter and 1 μm thickness) and a FID detector were used for analysis. Chromatographic conditions were as reported by Di Cagno et al. (2007).

2.13. Statistical analysis

All fermentations were carried out in triplicate and samples were analyzed in duplicate (total of six analyses for each sample). Data were subjected to one-way ANOVA (SAS, 1985); pair-comparison of treatment means was achieved by Tukey's procedure at $P<0.05$, using the statistical software, Statistica for Windows (Statistica 6.0 per Windows 1998). Cluster analysis was carried out on similarity estimates using the unweighted pair group method with arithmetic average, from which a dendogram representing the relationship between isolates was obtained. Analysis was performed using the statistical software Statistica for Windows. Viscosity, color index (a and b), ASC, GSH and TAA, FAA, and glutamic acid were analyzed by using multivariate statistical techniques. Factor reduction analysis was performed on the data by the covariance matrix for the determination of principal components using the statistical software (Statistica 6.0 per Windows 1998). The same analysis was carried out for volatile compounds on the basis of chemical classes calculated as the sum of all compounds within, taking for each data normalized areas as the area ration between fermented TJ and the unstarted control.

3. Results

3.1. Identification and typing of lactic acid bacteria

As estimated by plating on MRS agar, presumptive mesophilic lactic acid bacteria varied from 2.50 to 3.56 log CFU g^{-1}. Gram-positive, catalase-negative, non-motile cocci and rods, able to grow at 15 °C and

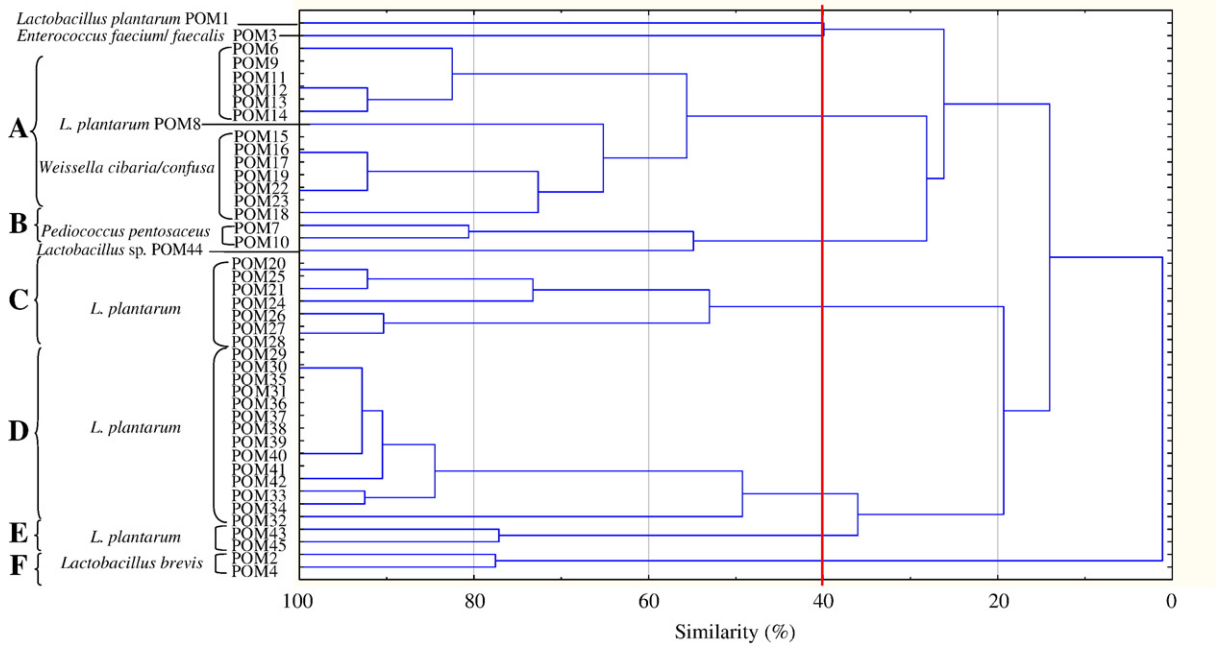

Fig. 2. Dendogram obtained by combined random amplification of polymorphic DNA patterns for the isolates from tomatoes (*Licopersicon esculentum* Mill, cultivar Sunrise), using primer M13 and P4. Cluster analysis was based on the simple matching coefficient and unweighted pair group with arithmetic average.

to acidify MRS broth were identified by partial sequencing of the 16S rRNA. The species were the following: *L. plantarum* (25 isolates), *W. cibaria/confusa* (13 isolates), *L. brevis* (2), *P. pentosaceus* (2), *Lactobacillus* sp. (1) and *E. faecium/faecalis* (1). All 44 isolates of lactic acid bacteria were subjected to RAPD-PCR analysis. Primers M13 and P4 generated different patterns (bands ranging from 5000 to 100 bp) and were used for clusters analysis (Fig. 2). The reproducibility of the RAPD fingerprints was assessed by comparing the PCR products obtained from three separate cultures of the same strain. At the similarity level of 40%, the highest percentage of the isolates was grouped in 6 cluster (clusters A–F) (Fig. 2). *L. plantarum* POM1, *E. faecium/faecalis* POM3, and *Lactobacillus* sp. POM44 which gave unique RAPD pattern did not belong to any cluster. Isolates of *L. plantarum* did not group in a unique cluster but were separated into different clusters (C, D and E). Clustering at 40% of similarity gave also the separation of cluster B (only *P. pentosaceus* isolates) and cluster F (only *L. brevis*). Only *L. plantarum* POM8 was grouped in cluster A that included isolates of *W. cibaria/confusa*.

L. plantarum POM8 and *W. cibaria/confusa* POM11 (cluster A), *P. pentosaceus* POM10 (B), *L. plantarum* POM27 (C), *L. plantarum* POM35 (D), *L. plantarum* POM43 (E), *L. brevis* POM2 (F) and *L. plantarum* POM1, *E. faecium/faecalis* POM3 and *Lactobacillus* sp. POM44, which gave unique RAPD patterns, were further subjected to phenotypic identification by using the Biolog System (Biolog, Inc.). Genotypic identification was confirmed. In particular, strains of *L. plantarum* mainly differed for the fermentation of glycerol, D-malic acid, D-galacturonic acid, inosine, D-sorbitol and D-ketobutyric acid. *L. brevis* POM2 was the only strain which fermented β-methyl D-galactoside and D-melibiose. None of the strains used sucrose as the carbon source. All the above ten strains were used as autochthonous starters to ferment tomato juice (TJ) following the protocol described in Fig. 1.

3.2. Kinetics of growth and acidification

All autochthonous strains grew from 7.0 log CFU ml^{-1} to cell densities which ranged from 9.2±0.39 to 9.8±0.58 log CFU ml^{-1}. Overall, the stationary phase of growth was reached after 15 h of fermentation at 25 °C. The lag phase ranged from 0.39±0.02 to 5.66±0.22 h as well as μ_{max} ranged from 0.16±0.011 to 0.32±0.018 log CFU ml^{-1} h^{-1}. After 17 h of fermentation, the unstarted TJ contained ca. 5.2±0.21 log CFU ml^{-1} of presumptive lactic acid bacteria while the allochthonous *L. plantarum* LP54 reached the cell density of 8.52±0.37 log CFU ml^{-1}. The lag phase for *L. plantarum* LP54 was 3.54±0.32 h and μ_{max} was 0.53±0.019 log CFU ml^{-1} h^{-1}. After 40 days of storage at 4 °C, all fermented TJs showed decreases of the cell number of lactic acid bacteria of ca. one log cycle. Autochthonous and allochthonous lactic acid bacteria were monitored by RAPD-PCR analysis throughout the process. All strains were detectable after 40 days of storage at 4 °C. After storage, only unstarted TJ and TJ fermented with allochthonous *L. plantarum* LP54 contained yeasts at cell numbers of 7.50±0.12 and 7.82±0.24 log CFU ml^{-1}, respectively. Clostridia were never found in 10 ml of TJs by culturing on Oxoid Thioglycolate Medium U.S.P. at 30 °C for 72 h (details omitted).

Due to the low initial pH of TJ (pH ca. 4.3), the lactic acidification caused a moderate decrease of pH. Except for *L. plantarum* POM27 which gave pH ca. 3.78, all the other autochthonous strains acidified to values of 3.95–4.05. Total titratable acidity (TTA) ranged from 30 to 35 ml 0.1 M NaOH ml^{-1}. Overall the kinetic of acidification was characterized by values of λ and V_{max} of ca. 2.58±0.18 and 0.029±0.0032, respectively. Unstarted TJ had pH of ca. 4.2±0.12 and TTA of 21±1.79 ml 0.1 M NaOH ml^{-1}. The allochthonous *L. plantarum* LP54 only slightly acidified: pH 4.23±0.22 and TTA 24.6±2.34 ml 0.1 M NaOH ml^{-1}. After 40 days of storage at 4 °C, TJs fermented by *L. plantarum* POM27 and POM35 had values of pH of 3.55±0.15 and 3.58±0.27. All the other autochthonous strains caused a decrease of pH in the range 3.62–3.87. TTA of TJs increased markedly during storage. It was the highest for *L. plantarum* POM1 and POM35, ca. 81 ml 0.1 M NaOH ml^{-1}. At the end of storage, unstarted and allochthonous *L. plantarum* LP54 TJs had pH of 4.01±0.37 and 3.90±0.48, respectively. TTA for both controls did not exceed 45 ml 0.1 M NaOH ml^{-1}. Except for TJ started with EPS-positive *W. cibaria/confusa* POM11 (ca. 12 mM of sucrose), all the other started TJs, at the end of storage, contained levels of sucrose ranging from 24 to 27 mM that almost corresponded to the initial concentration. All fermented TJs had residual concentrations of glucose and fructose less than 8 mM. On the contrary, allochthonous *L. plantarum* LP54 TJ and, especially, unstarted TJ contained residual concentrations of glucose and fructose above 15 and 40 mM, respectively.

Fig. 3 shows the representative kinetics of growth and acidification of unstarted TJ and TJs fermented with the autochthonous *L. plantarum* POM35 and allochthonous *L. plantarum* LP54.

3.3. Synthesis of EPS and viscosity and color measurements

As determined on MRS agar, all *W. cibaria/confusa* strains (cluster A), including *W. cibaria/confusa* POM11, showed the capacity of synthesizing EPS. The synthesis was found when sucrose was added to MRS agar. Isolates belonging to the other species did not synthesize EPS in culture medium.

According to the above findings, the highest value of viscosity was found in TJ fermented with *W. cibaria/confusa* POM11 (66.50±1.1 mP) (Table 1). After fermentation, all the other TJs started with autochthonous strains had values of viscosity ranging from 20.10 to 51.90 mP. Unstarted TJ had a viscosity of 20.75±0.4 mP. Fermentation with the allochthonous *L. plantarum* LP54 gave a value of 40.20±0.9 mP. Except for TJ fermented with *L. plantarum* POM27 that decreased to ca. 50% of the initial value (23.5±1.3 mP), all the other TJs maintained the values of viscosity almost constant during storage at 4 °C.

After fermentation, the green-red tonality (a) of TJs started with autochthonous strains ranged from 17.75 to 21.29 (Fig. 4). Values of 17.0±1.22 and 18.03±1.05 were found for unstarted TJ and TJ fermented with allochthonous *L. plantarum* LP54, respectively. Major differences were found at 40 days of storage at 4 °C. While (a) of the unstarted TJ markedly decreased to 12.5±0.56, those of TJs, especially fermented with *L. plantarum* POM1, POM8, and POM43, *Lactobacillus* sp. POM44 or *W. cibaria/confusa* POM11, remained almost constant. A similar trend was found for blue-yellow tonality (b), even though the decreases during storage were more pronounced for some TJs (e.g., started with *P. pentosaceus* POM10 or *L. plantarum* POM43) (data not shown).

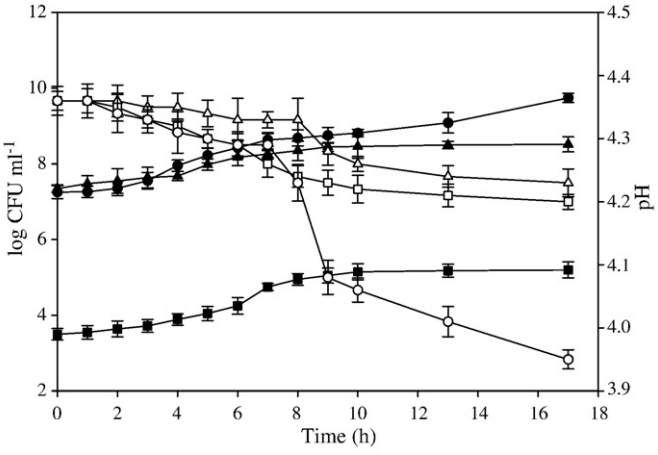

Fig. 3. Representative kinetics of growth (filled symbols) and acidification (empty symbols) of unstarted TJ (squares) and TJs fermented with the autochthonous *Lactobacillus plantarum* POM35 (circles) and allochthonous *Lactobacillus plantarum* LP54 (triangles). Data are the means of three independent experiments±standard deviations (n=3) analyzed in duplicate.

Table 1
Viscosity (mP) of tomato juices fermented for 17 h at 25 °C by autochthonous or allochthonous strains of lactic acid bacteria, and further stored until 40 days at 4 °C

Strains	Viscosity (mP)	
	17 h	40 days
Unstarted[a]	20.75±0.4 f	20.12±0.2 f
Lactobacillus plantarum POM8	44.10±0.9 c	44.50±0.7 c
Weissella cibaria/confusa POM11	66.50±1.1 a	67.80±0.7 a
Pediococcus pentosaceus POM10	30.75±0.8 e	30.45±0.8 e
L. plantarum POM27	47.40±1.4 bc	23.50±1.3 f
L. plantarum POM35	51.90±0.8 b	52.20±1.1 b
L. plantarum POM43	20.10±0.7 f	19.43±0.6 f
Lactobacillus brevis POM2	41.60±0.7 d	41.20±0.5 d
L. plantarum POM1	49.00±0.8 b	49.20±0.6 b
Enterococcus faecium/faecalis POM3	43.10±0.8 c	42.60±0.8 c
Lactobacillus sp. POM44	39.00±1.4 de	39.70±1.4 de
Allochthonous *L. plantarum* LP54	40.20±0.9 d	40.80±1.2 d

Each value was expressed as the mean±standard deviations ($n=3$) analyzed in duplicate. a–f, Means within the column with different superscript letters are significantly different ($P<0.05$).

[a] Tomato juice subjected to the same treatments, except for the use of the starter, was used as the control (unstarted).

3.4. Ascorbic acid, glutathione and total antioxidant activity

After fermentation, the concentration of ascorbic acid (ASC) of TJs started with autochthonous strains was in the range 119–144 mg l^{-1} (Fig. 5A). Values of 135±14 and 133±24 mg l^{-1} were found for unstarted TJ and TJ fermented with allochthonous *L. plantarum* LP54, respectively. The decrease of ASC during storage significantly ($P<0.05$) differentiated TJs. In particular, those fermented with *E. faecium/faecalis* POM3, *L. brevis* POM2 and *L. plantarum* POM35 decreased very slightly. In spite of the decrease occurring during storage, the concentration of ASC of TJ fermented with *L. plantarum* POM1 remained high also. This was due to the high level of ASC after fermentation. The concentration of ASC of unstarted TJ and TJ fermented with allocthonous strain markedly decreased from 136±14 and 134±24 mg l^{-1} to 57±3 and 63±12 mg l^{-1}, respectively.

After fermentation, all TJs started with autochthonous strains had a concentration of glutathione (GSH) which ranged from 260 to 397 mg l^{-1}. Unstarted TJ had a value of 330±42 mg l^{-1}. Fermentation with the allochthonous *L. plantarum* LP54 gave a value of 315±24 mg l^{-1} (Fig. 5B). After 40 days of storage at 4 °C, all fermented TJs showed a decrease of the concentration of GSH. The decrease was more pronounced for some TJs (e.g., those started with *L. brevis* POM2, *L. plantarum* POM8, *P. pentosaceus* POM10 and *L. plantarum* POM43). TJ fermented with *L. plantarum* POM1 showed the lowest decrease of GSH with a residual concentration ca. 59 and 69% higher those found in unstarted TJ and TJ fermented with allochthonous *L. plantarum* LP54, respectively.

Total antioxidant activity (TAA) mirrored the above findings. After storage, TJs fermented with *E. faecium/faecalis* POM3, *L. brevis* POM2 and *L. plantarum* POM1 and POM35 had the highest values of 237±16 to 283±19 TE l^{-1}. TAA of unstarted TJ and TJ fermented with allochthonous *L. plantarum* LP54 was 95±3 and 151±15 TE l^{-1}, respectively.

3.5. Concentration of free amino acids

After fermentation, the total concentration of free amino acids (FAA) was the highest for TJs fermented with *L. plantarum* POM1 and POM8, and *Lactobacillus* sp. POM44 (3365±292–3680±315 mg l^{-1}). Unstarted TJ had a concentration of 2225±237 mg l^{-1} and the TJ fermented with the allochthonous *L. plantarum* LP54 gave a value of 1776±345 mg l^{-1}. Nevertheless, for most of TJs the concentration of FAA markedly increased during storage, being in the range 4569±347–6101±368 mg l^{-1}. Only *L. plantarum* POM1 and POM35 maintained almost constant the initial concentration of FAA (3688±315 and 2392±288 mg l^{-1}, respectively). Glu, Asp, Ser and Phe were the FAA found at the highest concentrations in all TJs. Besides, TJs fermented with *L. plantarum* POM1 and POM35 had concentrations of glutamic acid (850±146 and 736±123 mg l^{-1}, respectively) lower than those found in the other TJs (1100±135–1560±105 mg l^{-1}). γ-Amino butyric acid (GABA) was found in all TJs fermented with autochthonous strains. The concentration ranged from 214±36 to 423±49 mg l^{-1}, being the highest in TJ fermented with *L. plantarum* POM1. Unstarted TJ and TJ fermented with the allochthonous *L. plantarum* LP54 had a concentration of GABA below 200 mg l^{-1}. After 40 days of storage at 4 °C, the concentration of GABA of all TJs slightly increased.

Principal component analysis (PCA) was applied to viscosity, a and b tonalities, ASC, GSH, TAA, glutamic acid and total FAA data of TJs stored for 40 days. The score plot of the first and second PC after PCA analysis is shown in Fig. 6. The two PC explained 70.3% of the total variance. TJs fermented with *L. plantarum* POM1 and POM35 were grouped in the same zone of the plane because they shared several features such as high viscosity, and a and b tonalities, high concentration of ASC and GSH, high TAA and lowest concentration of FAA and glutamic acid.

3.6. Volatile compounds

Based on the above findings, only TJs fermented with *L. plantarum* POM1 and POM35 were subjected to characterization for volatile components. The profiles were compared to unstarted TJ and TJ fermented with allocthonous *L. plantarum* LP54.

One hundred-ninety-six volatile compounds with molecular masses of 40–200 Da were identified, and grouped according to chemical class. The profile of volatile compounds after storage substantially amplified the differences already found after fermentation (data not shown). Esters and alcohols were the most numerous chemical classes. After 40 days, PCA analysis (Fig. 7) showed that unstarted TJ was characterized by the most aldehydes, furans and alkenes. TJ fermented with allochthonous *L. plantarum* LP54 was discriminated from autochthonous strains, especially by high levels of esters, alcohols and sulphur compounds and a few furans. Except for volatile fatty acids (VFAs) and ketones, the profiles of TJs fermented with autochthonous *L. plantarum* POM1 and POM35 were similar.

Unstarted TJ was the richest in aldehydes (Table 2), except for pentanal, hexanal and nonanal higher in TJ fermented with the allochthonous strain, and 2-butenal and 5-methyl-2-furancarboxaldehyde higher in TJ

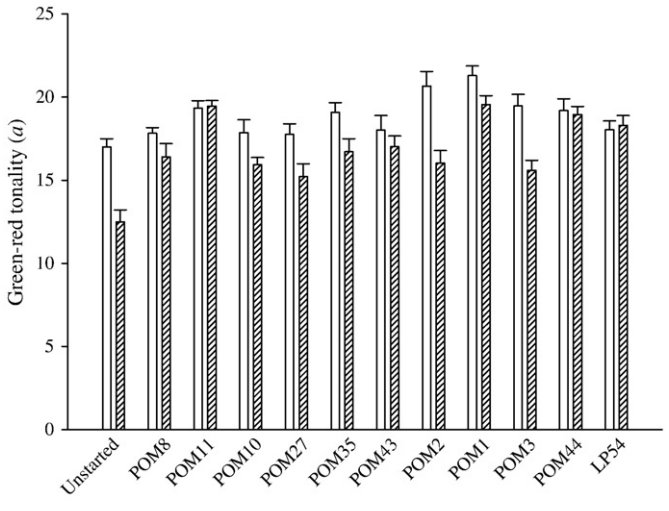

Fig. 4. Green-red tonality (a) of tomato juices fermented for 17 h at 25 °C (▨) by autochthonous or allochthonous strains of lactic acid bacteria, and further stored until 40 days at 4 °C (□). Data are the means of three independent experiments±standard deviations ($n=3$) analyzed in duplicate. Strains are indicated by codes, the name of the species is reported in materials and methods. Tomato juice not inoculated, subjected to the same protocol was used as the control (unstarted).

fermented with *L. plantarum* POM1. Butanal, octanal, 2,4-hexadienal (E,E), 2-methyl-propanal and 3-methyl-butanal were also high in TJ fermented with allochthonous strain. Overall, levels of aldehydes were low in TJs fermented with autochthonous strains.

TJ fermented with allochthonous *L. plantarum* LP54 was characterized by high levels of primary and branched alcohols, and by low level of secondary alcohols (Table 2). TJs fermented with autochthonous strains were similar and differed from both controls for primary saturated alcohols (ethanol to 1-pentanol) and unsaturated alcohols of more than six carbon atoms. TJ fermented with *L. plantarum* POM1 had the highest levels of secondary alcohols from 2-pentanol to 2-nonanol, and also 3-methyl-3-butan-1-ol.

Except for 2-propanone, the levels of 2-ketones were the lowest in unstarted TJ (Table 2). 2,3-Butanedione and, especially, 3-hydroxy-2-butanone were very low or absent in this TJ. TJ fermented with the allochthonous strain was very poor in 2-propanone and methyl-branched ketones. Overall, TJ fermented with *L. plantarum* POM35 had the highest levels of ketones.

Except for methyl octanoate and 2-hexen-1-yl acetate, high levels of esters differentiated TJ fermented with the allochthonous strain from the other TJs (Table 2). Besides, ethyl decanoate, ethyl laurate, hexyl propanoate, heptyl acetate, 3-methyl-butyl propanoate and x-methyl-1-pentyl acetate were present in the above TJ only. Lower values of ethyl and propyl esters differentiated TJs fermented with autochthonous strains from both the controls.

Among sulphur compounds, TJ started with allochthonous *L. plantarum* LP54 was differentiated from the other TJs by higher levels of carbon disulphide, thiophene and 2-methyl thiazole, and by lower

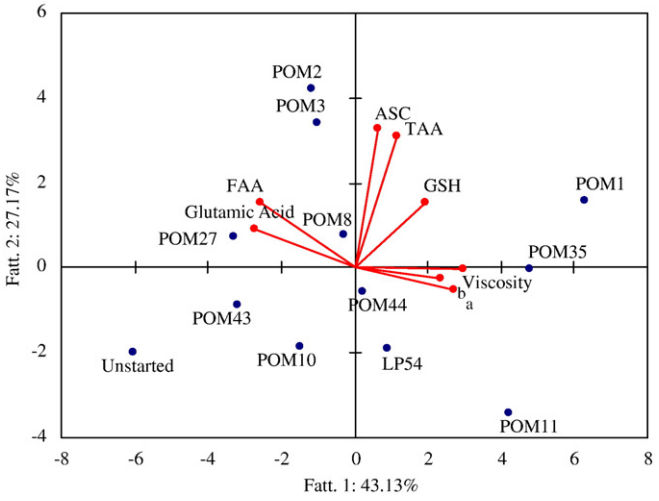

Fig. 6. Score plot of the first and second principal components (PC) after PC analysis based on viscosity (mP), green-red (a) and blue-yellow (b) tonalities, ascorbic acid (ASC, mg l^{-1}), glutathione (GSH, mg l^{-1}), total antioxidant activity (TAA, TE ml^{-1}), glutamic acid (mg l^{-1}) and total free amino acids (FAA, mg l^{-1}) of tomato juices fermented by autochthonous or allochthonous strains of lactic acid bacteria after 40 days of storage at 4 °C. Strains are indicated by codes, the name of the species is reported in materials and methods. Tomato juice not inoculated, subjected to the same protocol was used as the control (unstarted).

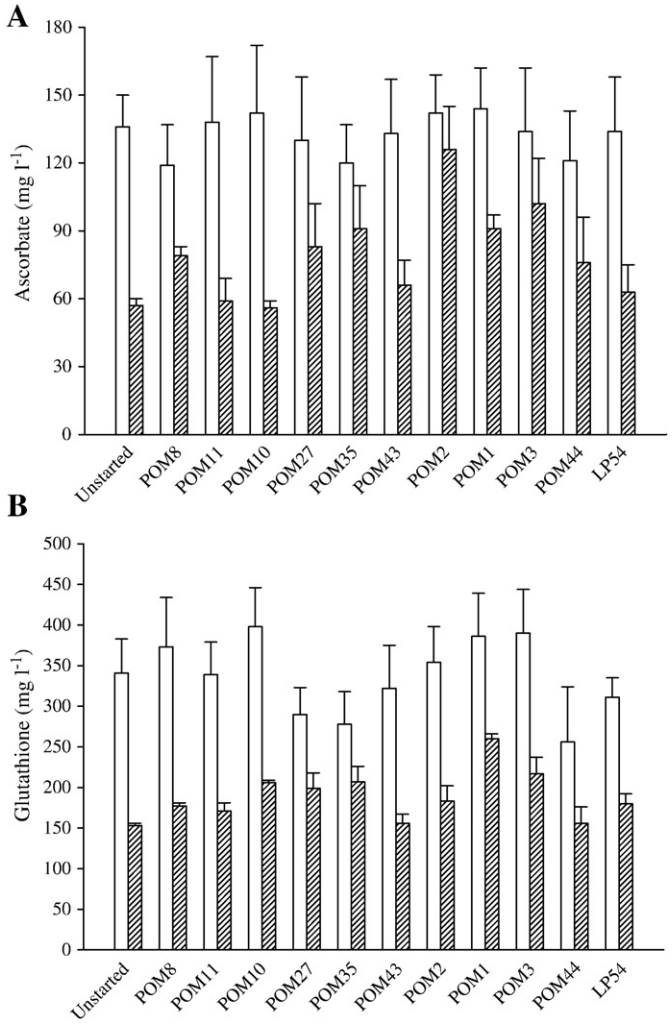

Fig. 5. Concentration of ascorbate (A) and glutathione (B) (mg l^{-1}) of tomato juices fermented for 17 h at 25 °C (▭) by autochthonous or allochthonous strains of lactic acid bacteria, and further stored until 40 days at 4 °C (▨). Data are the means of three independent experiments ± standard deviations ($n = 3$) analyzed in duplicate. Strains are indicated by codes, the name of the species is reported in materials and methods. Tomato juice not inoculated, subjected to the same protocol was used as the control (unstarted).

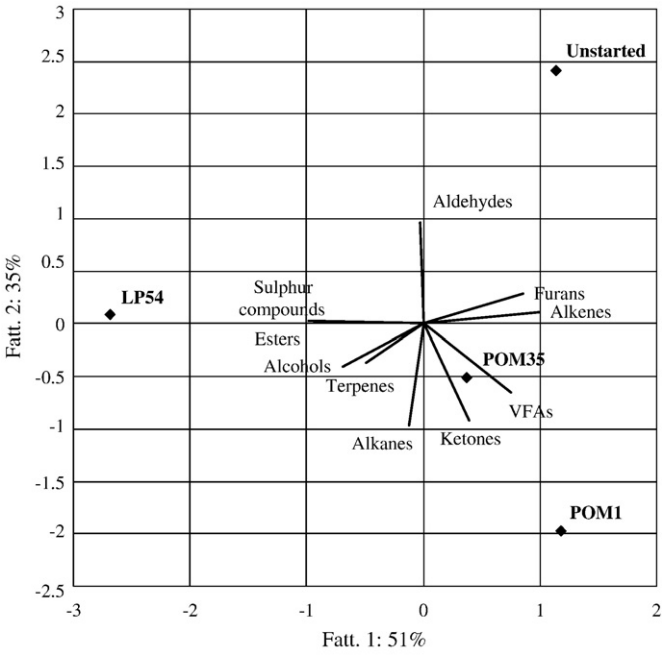

Fig. 7. Score plot of the first and second principal components (PC) after PC analysis based on volatile compounds (Table 2) of tomato juices fermented by autochthonous *Lactobacillus plantarum* POM1 and POM 35, and allochthonous *L. plantarum* LP54 after 40 days of storage at 4 °C. Tomato juice not inoculated, subjected to the same protocol was used as the control (unstarted). Chemical classes of volatile compounds were calculated as the sum of all compounds, taking for each data the normalized areas as the area ratio between fermented TJs and unstarted TJ.

Table 2
Volatile compounds [arbitrary unit of area of selected ion (x 10^{-2})] found by Purge and Trap or Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (PT or SPME-GC/MS) analysis in tomato juices fermented by the autochthonous *Lactobacillus plantarum* POM1 and POM35, and allochthonous *L. plantarum* LP54 after 40 days of storage at 4°C

Chemical class	Ion	Unstarted[a]	*L. plantarum* POM1	*L. plantarum* POM35	*L. plantarum* LP54
Aldehydes					
Butanal[b]	72	15070	3936	2419	14994
Pentanal	58	147518	48871	49943	313733
Hexanal	72	213563	51749	49528	430468
Heptanal	96	9101	3697	2823	4694
Octanal	84	13462	10175	8775	12147
Nonanal	57	28587	20889	20045	62609
Decanal	57	2419	1632	2467	298
2-butenal	70	44732	64228	42563	13648
2-pentenal	83	27374	8602	10302	4513
2-hexenal	83	111165	58666	57499	80542
2,4-hexadienal (E,E)	81	5918	2200	2778	6647
2-heptenal	83	41083	15725	16061	23660
2,4-heptadienal (E,E)	81	36450	7700	10683	3594
2-octenal	83	61503	19341	24790	22008
2,4-octadienal (E,E)	81	6074	1083	3135	1114
2,4-nonadienal	81	1268	314	690	103
2-methyl-propanal	72	20860	12662	13640	19754
3-methyl-butanal	58	25004	9890	14534	23483
2-methyl-butanal	58	29604	16061	16006	18282
benzaldehyde	106	42991	23041	34947	24283
benzeneacetaldehyde	91	55580	30589	44826	1770
5-methyl-2-furancarboxaldehyde	110	509	645	504	344
Alcohols					
Methanol	31	675948	667574	501735	818653
Ethanol	45	4360806	1377899	1302359	13514213
Propanol	59	44783	22678	15530	467201
1-butanol	56	105831	67975	57223	134761
1-pentanol	55	763001	656847	602943	644983
1-hexanol	56	7269075	7179582	7190260	3758127
1-heptanol	98	1971	1445	1941	456
1-octanol[c]	*56*	*3134*	*3415*	*3292*	*26770*
2-methyl-3-buten-2-ol	71	225254	251909	240651	225480
1-penten-3-ol	57	949806	781284	678230	1042715
3-methyl-3-buten-1-ol	68	1104	17672	4702	4702
2-penten1-ol	68	52418	48355	30580	22274
3-methyl-2-buten-1-ol	71	13054	15466	26161	20915
3-hexen-1-ol (E)	67	62866	61109	7926	63324
3-hexen-1-ol (Z)	67	1230140	1468911	1281077	1088948
2-hexen-1-ol	82	539862	426877	317997	5564
2-hepten-1-ol	96	16821	9978	8498	300
1-octen-3-ol	57	151922	68125	68926	17141
2-octen-1-ol	*68*	*8793*	*5207*	*3003*	*3211*
6-methyl-5-hepten-2-ol	95	593868	443272	437346	73396
2-propanol	45	1210610	1195752	986253	662026
2-butanol	59	164607	159162	109811	298131
2-pentanol	73	3805	4718	1545	687
3-pentanol	59	32265	103323	13578	32425
2-heptanol	45	49958	143053	11304	23588
3-octanol	59	6118	65514	936	3160
2-octanol	45	1779	1575	500	599
2-nonanol	45	2768	22912	9535	1070
2-methyl-1-propanol	74	14493	12034	11119	359652
3-methyl-1-butanol	70	741942	714523	634980	8121553
2-methyl-1-butanol	70	147485	147787	124192	139629
phenyl ethanol	*91*	*48956*	*38634*	*39959*	*72111*
2-methyl,5-isopropyl-phenol	135	5161	5100	4247	18597
Unknown alcohol mw 184	82	968	1092	1145	1
Unknown unsaturated Alcohol	43	467960	465472	476445	1713157
Ketones					
2-propanone	58	424957	581496	741425	49865
2-butanone	72	139265	210302	264900	278407
2-pentanone	43	52538	189739	162199	212737

Table 2 (*continued*)

Chemical class	Ion	Unstarted[a]	*L. plantarum* POM1	*L. plantarum* POM35	*L. plantarum* LP54
Ketones					
3-pentanone	86	71086	182719	133725	368884
2-heptanone	58	46981	211582	201537	107964
2-nonanone	58	2181	23571	108931	6954
2-undecanone	58	131	515	2023	20
3-methyl-2-butanone	86	2112	3010	3406	8254
2-methyl-3-pentanone	57	5128	3753	3 154	4558
4methyl-2-heptanone	58	13228	7568	8666	2124
6methyl-2-heptanone	58	7915	4692	7158	8014
4-methyl-6-hepten-3-one	108	20856	22006	27623	10309
6-methyl-5-hepten-2-one	108	1932072	1908857	1844330	1420130
6-methyl-3,5-heptadien-2-one	109	18756	18264	27759	1688
1-octen-3-one	70	19890	10059	8431	8523
2,3 butanedione	86	34724	890796	1295196	235052
3-hydroxy-2-butanone	88	1	55417	28340	8264
2,3-octanedione	99	14602	7759	7858	13104
cyclopentanone	84	80612	66062	57381	4517
2,2,6,trimethyl-cyclohexanone	82	20746	21524	21850	41070
3,5,5-trimethyl-2-cyclohexenone	82	39107	28782	34498	21257
2-(2-butenyl),3-methyl-2-cyclopenten-1-one	150	2101	2787	2160	2017
Esters					
Methyl acetate	74	274915	472369	451505	1361519
Methyl propanoate	88	561	499	578	20645
Methyl butanoate	71	158	10566	10067	11454
Methyl hexanoate	74	11368	15619	11435	26592
Methyl octanoate	74	10379	8662	5436	2466
Ethyl acetate	61	245012	72842	62712	5059505
Ethyl propanoate	102	502	195	183	110513
Ethyl butanoate	88	180	166	217	31365
Ethyl pentanoate	88	347	1	7	21706
Ethyl hexanoate	88	5 842	540	397	148491
Ethyl octanoate	88	3572	326	288	3498
Ethyl decanoate	88	1	1	1	301
Ethyl laurate	88	1	1	1	434
Ethyl 2-hydroxy-propanoate	45	13355	11967	17942	262734
Ethyl 2-methyl-butanoate	102	32	1	86	232
Ethyl 3-methyl-butanoate	88	2606	318	226	6191
Propyl acetate	61	1670	1141	832	75842
Propyl propanoate	75	381	155	261	558
Isopropyl acetate	61	24251	21604	13232	27959
Butyl acetate	43	2104	2882	2095	69726
Pentyl formate	70	2123	1538	1450	1150
Pentyl acetate	61	2555	2661	2538	102964
Hexyl formate	56	13553	15324	11468	5774
Hexyl acetate	84	10178	14177	11739	374408
Hexyl propanoate	75	1	1	1	2858
3-hexen-1-yl acetate	82	7615	9018	6817	388378
2-hexen-1-yl acetate	82	5878	4149	2650	1422
heptyl acetate	61	1	1	1	1268
1-methyl-propyl acetate	87	4080	3044	1990	3331
3-methyl-butyl acetate	70	74033	64937	47739	3163222
2-methyl-butyl acetate	70	6730	5903	4340	81956
3-methyl-2-buten-1-yl acetate	68	4944	5083	4928	16773
3-methyl-butyl propanoate	75	1	1	1	12796
x-methyl-1-pentyl acetate	61	1	1	1	9001

Table 2 (*continued*)

Chemical class	Ion	Unstarted[a]	*L. plantarum* POM1	*L. plantarum* POM35	*L. plantarum* LP54
Esters					
y-methyl-1-pentyl acetate	61	63	198	153	6904
phenyl-ethyl acetate	104	40	255	240	1945
2-furan-methyl acetate	98	53	52	64	7795
Sulfur compounds					
carbon-disulfide	76	339	729	729	3073
dimethyl-sulfide	62	42141	42539	33049	393
dimethyl-disulfide	94	46545	45562	37833	12371
thiophene	84	6093	4584	5847	11801
S-methyl-thioacetate	90	6442	6165	5337	2456
2-methyl-thiazole	99	2960	1265	1723	7762
2-sec-butyl-thiazole	99	487454	359336	516609	307152
Furans					
2-methyl-furan	82	19687	21851	18057	13650
3-methyl-furan	82	38279	54679	43155	11535
2-ethyl-furan	81	29343	26217	21355	28890
2-pentyl-furan	81	230819	169308	139182	231386
2,5-dimethyl-furan	96	10848	3369	5127	4060
2,4-dimethyl-furan	96	464	344	336	478
3-(4-methyl-3-pentenyl)-furan	150	52512	71615	53332	36747
Alkanes					
Butane	58	117	35	55	934
Pentane	72	703	257	261	231
Hexane	86	28	2129	1069	1069
Heptane	100	2356	2688	3896	5781
Octane	85	3725	3708	3018	2148
Nonane	85	1284	902	1178	1167
Decane	57	1369	1340	1049	5637
Undecane	57	1702	1171	1073	2785
Dodecane	57	957	1252	817	289
Tridecane	57	180	92	154	13
2-methylpentane	71	9818	11018	8587	1842
2-methylheptane	57	10589	7753	5226	4323
4-methylheptane	71	14023	14426	9927	5724
2,4 dimethyl heptane	85	12826	13275	8962	5167
Alkenes					
1-hexene	84	2688	2402	2239	229
2-hexene	84	11440	16566	11736	7466
2-methyl-1,3-butadiene	67	12741	20273	38937	3475
1,3-pentadiene (E)	67	3075	1691	1829	362
1,3-pentadiene (Z)	67	3219	1801	1993	577
1,4-hexadiene (E)	67	25987	18832	20985	16237
1,4-hexadiene (Z)	67	30332	26025	23305	18247
2,4-hexadiene (E)	67	2132	2377	2142	2536
2,4-hexadiene (Z)	67	2223	2494	1873	3265
1,3,5-hexatriene	79	6702	6024	4441	6067
1,3,5-hexatriene	79	6539	5271	3882	3252
1,3-octadiene (Z)	54	6097	3510	3005	744
1,3-octadiene (E)	54	5515	3311	2805	621
3,7-dimethyl-1,3,7-octatriene	93	11722	20591	20816	43126
2,7-dimethyl-2,6-octadiene	69	125626	125164	81065	59396
Terpenes					
alpha-pinene	93	12470	15325	9375	7844
beta-pinene	136	111	153	178	391
beta-myrcene	136	708	1251	1438	3158
l-phellandrene	93	14785	21330	13854	16209
delta-3-carene	93	941	874	1078	1173
alpha-terpinene	93	3349	5464	5837	5425
p-cymene	119	10255	12460	14995	9546
p-mentha-1,5,8-triene	91	30482	36890	29427	86270
limonene	93	15172	21145	135846	27879
cis-ocimene	93	19019	30189	26217	49599
gamma-terpinene	93	9195	3922	3736	4046
alpha-terpinolene	93	3919	6117	8242	7844
linalool	93	477	429	305	97
Terpenes					
beta-cyclocitral	152	4202	2739	5751	812
citral (Z)	69	576	560	666	134
5-tert-butyl-m-cymene	175	85305	85966	92587	13459
citral (E)	69	1798	1814	2114	415
geranyl-acetone	*151*	*18978*	*8903*	*8966*	*13370*
beta-ionone	*177*	*3477*	*2732*	*2967*	*6404*

[a] Tomato juice subjected to the same treatments, except for the use of the starter, was used as the control (unstarted).
[b] Normal: extraction by Purge and Trap.
[c] Italic: extraction by SPME.

levels of dimethyl sulphide, dimethyl disulphide and S-methyl thioacetate (Table 2). Among furans, TJs fermented with autochthonous strains were differentiated from both controls by lower levels of pentyl-2,4-dimethyl- and 2,5-dimethyl-furans.

Alkanes failed to discriminate clearly TJs, while pentadiene and octadiene isomers were highest in unstarted TJ and lowest in TJ started with the allochthonous strain (Table 2). Overall, terpenic hydrocarbons were the lowest in unstarted TJ, while oxygenated terpens such as citral isomers or linalool were the lowest in TJ fermented with allochthonous strain.

Although butanoic, 3-methyl-butanoic, 2-methyl-butanoic and hexanoic acids were identified as the volatile free fatty acids of TJs, acetic acid was found at a concentration more than 1,000 times higher. The highest concentration of acetic acid was found in the unstarted TJ (ca. 1362 ppm), it was intermediate in TJs fermented with autochthonous strains (1082–1162 ppm) and markedly lower (160 ppm) in TJ started with allocthonous *L. plantarum* LP54. Hexanoic acid also statistically differentiated the latter with respect to the other TJs (0.55–0.86 ppm).

4. Discussion

The high concentration of health-promoting compounds and the intrinsic features of tomato fruits may favor the manufacture of TJs with health-promoting properties and agreeable sensory characteristics (Suzuki et al., 2002). Processing of tomatoes into juices influences health-promoting and sensory properties. This paper aimed at showing that TJs fermented with autochthonous strains have better viscosity, color, health-promoting and flavor profile with respect to unstarted TJ and TJ fermented with allocthonous starter.

Raw tomatoes used in this study harbored autochthonous lactic acid bacteria at cell densities which corresponded to those usually found in the most common vegetables (Buckenhüskes, 1997) or in pressured TJs (ca. 4.2 log CFU ml^{-1}) (Hsu et al., 2008). Overall, *L. plantarum* and *W. cibaria*/*confusa* were the species dominating the microbiota of raw tomatoes. *L. plantarum* was commonly identified in many raw vegetables and, especially, in spontaneous fermented vegetable juices (Buckenhüskes, 1997; Karovičová and Kohajdová, 2003). Biodiversity of *L. plantarum* isolated from tomatoes was shown by RAPD-PCR analysis. Isolates of *L. plantarum* did not group in a unique cluster but were separated into different clusters. Occasionally, *Weissella* spp. were identified in vegetables also (Björkroth and Holzapfel, 2006; Di Cagno et al., 2008).

A protocol to carrying out TJ fermentation was set up. As usual for vegetable juices, it included an initial inoculum of lactic acid bacteria of ca. 7.0 log CFU ml^{-1} and fermentation at 25 °C for 17 h. A mild heat treatment at 70 °C for 5 min was included also (Demir et al., 2006; Karovičová and Kohajdová, 2003). All autochthonous strains grew well in TJ without nutrient supplementation and pH adjustment. After 17 h of fermentation, cell densities of autochthonous strains were

markedly higher than those found in tomato juice fermented for 48 h with probiotic *Lactobacillus acidophilus*, *L. plantarum*, *Lactobacillus casei* and *Lactobacillus delbrueckii* (8.0 log CFU ml^{-1}) (Yoon et al., 2004). After 40 days of storage at 4 °C, survival of the autochthonous strains was also markedly higher than that of the above probiotic strains which decreased of ca. 2.5 log cycles. All TJs fermented with autochthonous strains had values of pH below 3.9.

Autochthonous strains differed for several activities that influenced the characteristics of TJs. Only *W. cibaria*/*confusa* POM11 showed the capacity of in vitro synthesizing exo-polysaccharides (EPS) and TJ fermented with it had the highest viscosity. The synthesis of EPS is widespread in the bacterial population of vegetables and it is also positively correlated with keeping the green-red and blue-yellow tonalities during processing (Den Ouden and Van Vliet, 2002; Sánchez-Moreno et al., 2006). TJ started with *W. cibaria*/*confusa* POM11 had the highest values of the above indexes. Almost all started TJs had values of viscosity markedly higher than unstarted TJ and these differences were maintained throughout storage. Only TJ fermented with *L. plantarum* POM27 showed a marked decrease of the viscosity during storage, probably due to the synthesis and/or activation of pectolytic enzymes (Hsu et al., 2008).

Unstarted TJ and TJ fermented with the allochthonous strain showed marked decreases of ASC, GSH and TAA during storage. On the contrary, several TJs fermented with autochthonous strains, especially with *L. plantarum* POM1 and POM 35, maintained elevated values of ASC, GSH and TAA. ASC is considered one of the most sensitive vitamins in foods. Stability of ASC and GSH, and in turn of TAA, markedly varies depending on environmental factors such as pH, concentration of metal ions and redox state (Selman, 1994). In acid media such as tomatoes the non-enzymatic browning is mainly due to ASC degradation (Kaanane et al., 1988). In TJs where the degradation of ASC was more pronounced the lowest preservation of natural color indexes was found (Lee, 1997).

Based on the above results, TJs fermented with *L. plantarum* POM1 and POM35 were selected for characterizing the volatile profiles through PT-SPME-GC/MS analysis. Flavor of tomato is characterized by interactions between volatile and non-volatile compounds, mainly organic acids, sugars, FAA and salts (Petrò-Turza, 1987). Sourness of TJs and TTA were well correlated (Salles et al., 2003). TJs fermented with *L. plantarum* POM1 and POM35 had the highest values of TTA which were ca. twice with respect to both the controls. TJs fermented with *L. plantarum* POM1 and POM35 also had the lowest concentration of free glutamic acid, noticeably lower than both the controls. High concentration of FAA and, especially of glutamic acid, may lead to undesirable changes in the taste of TJs favoring the appearance of off-flavors (Kader et al., 1978). Some of the volatiles identified in this study have been previously reported as components of the aroma note of tomatoes (Buttery and Ling, 1993; Petrò-Turza, 1987). A large number of volatiles belonging to various chemical classes differentiated TJs fermented with autochthonous strains with respect to controls. PCA analysis clearly showed that the volatile profiles of TJs fermented with autochthonous strains differed. Usually, aldehydes are unstable compounds that in food matrices are reduced to alcohols or oxidized to acids, in particular under microbial activity. This could explain that highest levels were generally found without starter addition. High concentrations of aldehydes may cause off-flavors (Moio and Addeo, 1998). Butanal, pentanal and 2,4-hexadienal (E,E) were found at the highest levels in unstarted TJ and TJ started with the allochthonous strain, being responsible for cooked flavor (Servili et al., 2000). The low redox potential of TJs might favor the reduction of aldehydes and ketones to primary and secondary alcohols (Molimard and Spinnler, 1996). In particular, ethanol was the alcohol found at the highest level in unstarted TJ and TJ started with the allochthonous strain. Ethanol was found to suppress the perception of other volatile compounds in fresh and processed tomatoes products (Tandon, 1997). On the contrary, 3-methyl-3-butan-1-ol, mainly present in the TJ fermented with autochthonous *L. plantarum* POM1, has a positive log odor value in tomatoes (Ortiz-Serrano and Gil, 2007). Among ketones, both 2–3 butanedione (diacetyl) and its reduction derivative, 3-hydroxy-2-butanone (acetoin), were found at the highest levels in TJs fermented with autochthonous strains. Both are considered as flavor impact compounds in processed tomatoes (Buttery et al., 1990). The origin of esters in TJs might be attributed to reactions between acids and alcohols. Overall, esters have a low perception threshold concentration that is ca. 10 times lower than their alcohol precursors (Preininger and Grosch 1994). No esters are included within the volatile compounds that generally have positive log odor units and are likely to contribute to tomato flavor (Buttery and Ling, 1993). However, they are known for their fruity notes. TJ fermented with the allochthonous strain was characterized by the highest levels of most of the identified esters. This could be related to the highest amounts of the alcohols, limiting precursors of these compounds (Liu et al., 2004). The allochthonous strain seems to have a different metabolic pattern with respect to autochthonous ones regarding the balance between carbonyl compounds. The former synthesized mainly aldehydes and the corresponding alcohols, while the latter synthesized mainly acids. Among the other volatiles, sulphur derivatives are very aromatic compounds. Their balance was very different between allochthonous and autochthonous strains. Dimethyl sulphide and terpens, linalool and beta-cyclocitral, were at the highest levels in TJs fermented with the autochthonous strains. Dimethyl disulfide is probably deriving from the breakdown of the sulfur-containing amino acids by microbial enzymes and subsequent oxidation of hydrogen sulfide. It was at the highest level in TJs fermented with the autochthonous strains as were terpens such as linalool and beta-cyclocitral. All these compounds positively influence the flavor of processed tomatoes. In particular, linalool contributes to the floral and fruity flavor notes (Buttery et al., 1990; Guadagni and Miers 1969; Servili et al., 2000). Although, most of the terpens are naturally present in the plants, they might be synthesized as secondary metabolite by microorganisms (Carrau et al., 2005; Yoo and Day, 2002).

Not all autochthonous strains performed similarly when used as starters for TJs. Nevertheless, this study shows that especially *L. plantarum* strains may join several traits useful for enhancing health-promoting, sensory and shelf-life properties of tomato juice. Since the allochthonous strain of *L. plantarum* behaved differently from the autochthonous ones, selection of starters within the tomato microbiota seems to be indispensable.

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