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Lactic Acid Fermentation of Tomato: Effects on *cis/trans* Lycopene Isomer Ratio, β-Carotene Mass Fraction and Formation of L(+)- and D(–)-Lactic Acid

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Summary

Fermentation of tomato pulp by the bacteriocin-producing lactic acid bacteria (*Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8) was applied as a preservation method for the production of tomato products. The changes in L- and D-lactic acid contents during fermentation of different tomato varieties (Ronaldo and Cunero) were analysed. Additionally, the effects of lacto-fermentation on the *cis/trans* lycopene ratio, β-carotene content, and their relation to colour characteristics of fermented tomato products were investigated. Mass fractions of L- and D-lactic acid in the fermented tomato products varied from (4.25±0.04) to (7.19±0.08) mg per 100 g, and from (4.05±0.05) to (6.34±0.04) mg per 100 g, respectively. Fermentation with *P. acidilactici* or *L. sakei* culture resulted in the the decrease of D-lactic acid content by 43.6 and 37.7 %, respectively, compared to spontaneous fermentation. The fermentation with *P. pentosaceus* or *L. sakei* increased the content of lycopene on average from 3.70 to 5.68 mg per 100 g, and β-carotene from 0.89 mg per 100 g (in Cunero var.) and from 0.28 mg per 100 g (in Ronaldo var.) to 1.14 mg per 100 g. Fermentation of tomato with selected lactic acid bacteria resulted in a greater lycopene bioavailability accompanied by an increase in *cis*-lycopene isomer content.

Key words: tomato, lactic acid fermentation, lycopene, β-carotene, L(+)/D(–)-lactic acid

Introduction

The lactic acid fermentation of vegetable products, applied as a preservation method, is considered as an important technology and it is further investigated because of the growing amount of raw materials processed in this way in the food industry. Tomato (*Lycopersicon esculentum* L.) is one of the most popular and extensively consumed vegetable crops worldwide. The nutritional significance of lycopene, a carotenoid with potent antioxidant activity, has been reported and accumulating evidence has shown an inverse correlation between the consumption of tomato products rich in lycopene and the risk of several types of cancer and cardiovascular disease (*1–3*). About 90 % of the lycopene in dietary sources is found in the linear, the all-*trans* conformation,

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while human tissues contain mainly *cis*-isomers. It has been suggested that *cis*-isomers of lycopene are better absorbed than the all-*trans* form because of their shorter length, greater solubility in mixed micelles, or as a result of their lower tendency to agregate (*4*). Studies have shown that lycopene level in plasma increased only after the consumption of red tomato paste and purified lycopene (*3*). It has also been indicated that the absorption of lycopene is greater from the processed tomatoes than from fresh tomatoes, since processing breaks down the tomato cell matrix and makes lycopene more available (*5,6*).

Fermentation used with a purpose of the extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds is an approach to the problem of food preservation that has gained increasing attention in recent years. Consequently, certain lactic acid bacteria (LAB) have demonstrated antimicrobial properties which derive from the production of one or more antimicrobially active metabolites such as organic acids (lactic and acetic), hydrogen peroxide, and antimicrobial peptides (bacteriocins) (*7–9*).

On the other hand, microbially produced lactic acid is usually a mixture of the L(+)- and D(–)-forms. As the latter cannot be metabolized by humans, excessive intake can result in acidosis, which is a disturbance in the acid-alkali balance in the blood. The potential toxicity of D-lactic acid is of particular concern for malnourished and sick people (*10*).

Dietary quality is an important limiting factor to adequate nutrition in many resource-poor settings. One aspect of dietary quality with respect to adequacy of micronutrient intake is bioavailability (*11*). Prebiotic food ingredients encourage the growth of probiotic bacteria. The appropriate combination of prebiotics and probiotics has a higher potential for a synergistic effect (*12*). Probiotic foods are fermented products containing sufficient number of a certain live microorganism that favourably modifies the intestinal microbiota of the host (*13*). The recently developed probiotics tend to be milk-based, although in recent years other substrates have been explored in new probiotic formulations. Amongst these substrates, cereals are becoming one of the most promising alternatives to milk due to their ability to support the growth of probiotic bacteria and their protective bile resistance effect (*14*). Rice and rice bran from processing wastes have previously been used to grow lactic acid bacteria for the production of lactic acid (*15*). Rice broths have also been used to support the growth of probiotic cultures (*16*).

The red colour of tomatoes is a result of the degradation of chlorophylls and the increased biosynthesis of carotenoids (*17*), thus it is related to the degree of maturity and postharvest life. Colour is therefore an important quality attribute of tomato fruit and it is used in the food industry to presume the colour of finished products. Also, the application of instrumental colour measurements to objectively define the colour of tomatoes is an important research topic (*18,19*). There are reports that the colour coordinates of a product could be in relation with the concentration of lycopene and other carotenoids (*20–22*).

The aim of the study is to evaluate the production of L- and D-lactic acid isomers during fermentation of different tomato varieties (Ronaldo and Cunero) by the bacteriocin-producing LAB of *Lactobacillus* and *Pediococcus* spp. The influence of lacto-fermentation on lycopene and β-carotene contents and their relation to the colour characteristics of fermented tomato products are also investigated.

Materials and Methods

Tomato samples and microorganisms

Tomato var. Cunero and Ronaldo, harvested in 2011, were obtained from the Lithuanian Institute of Horticulture (Babtai, Lithuania). Raw tomato fruit reference samples were refrigerated for later analysis. After defrosting at 4 °C, tomato samples were homogenized in a blender (Bosch, Stuttgart, Germany) and then analyzed. A sample was ground to a fine pulp using mortar and pestle. Extruded rice flour (moisture content of 8.4 %) produced by a single-screw extruder (Ustukiu malunas Ltd, Pasvalys, Lithuania) was tested as the target medium. Pure cultures of *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7 and *Pediococcus pentosaceus* KTU05-8, characterized as bacteriocin-producing strains (*23*), were from the collection of Kaunas University of Technology (Kaunas, Lithuania). Strains were stored at –70 °C in a Microbank system (Pro-Lab Diagnostics, Bromborough, UK).

The LAB strains were propagated in nutrition media (moisture content of 72 %), prepared by mixing the extruded rice flour (100 g) and tap water. After the addition of pure LAB cell suspension (5 g, 10.2 log colony-forming units (CFU) per g), the mixture was incubated at optimal temperatures (30 °C for *L. sakei*, 32 °C for *P. acidilactici* and 35 °C for *P. pentosaceus*) for 24 h. For comparison, a control product was prepared using spontaneous fermentation of rice flour without bacterial inoculum at 30 °C for 48 h. Enumeration of LAB was carried out by plating the diluted samples onto De Man-Rogosa-Sharpe (MRS) agar at 30 °C for 48 h. Products obtained after the propagation of individual LAB in rice media were used for fermentation of tomato pulp.

Microbiological analysis

Ten grams of sample were homogenized with 90 mL of saline (0.9 %). The suspension was diluted, and 100 μL of each 10^{-4}–10^{-8} solution was grown in MRS agar. The plates were incubated under anaerobic conditions at 30 °C (for *L. sakei* and spontaneous fermentation), 32 °C (for *P. acidilactici*) and 35 °C (for *P. pentosaceus*) for 72 h. The LAB cell number was calculated and expressed as log CFU per g.

Lactic acid fermentation of tomato pulp

The homogenized tomato pulp (140 g) and fermented rice product prepared using the individual LAB (20 g) or treated by spontaneous fermentation were mixed, and the mixture was incubated at appropriate temperatures (30–35 °C) for 48 h. The pH values of the fermented products were measured and recorded by pH electrode.

The total titratable acidity (TTA) was determined according to Sadler and Murphy (*24*) and expressed as g per L of citric acid.

Simultaneous determination of L- and D-lactic acid

A rapid and specific Megazyme assay kit for simultaneous determination of L- and D-lactic acid (Megazyme Int., Bray, Ireland) in foods was used as reported by De Lima *et al.* (*25*).

Extraction of carotenoids

The carotenoids were extracted from the samples (5 g) in hexane-acetone (1:1 by volume) using Celite® Filter Cel (Fluka Chemical Corp., Ronkonkoma, NY, USA) in a mechanical Waring blender according to Gama *et al.* (*26*). The mixture was centrifuged and the supernatant was collected. The residue was further extracted and centrifuged until all colour was removed, and the successive supernatants were pooled. The pigments in this organic extract were then transferred to petroleum ether, washed with distilled water and concentrated in a rotary evaporator at a temperature not exceeding 35 °C (*26*). Extracted samples were stored at –20 °C and equilibrated to room temperature before analysis by HPLC.

Carotenoid analysis by reversed-phase liquid chromatography

Solvents used for liquid chromatography were of analytical grade. All solvents for use as the mobile phase in HPLC were filtered through a 0.45-μm regenerated cellulose membrane filter and degassed using an ultrasonic bath. The β-carotene and lycopene standards were purchased from Sigma-Aldrich (Taufkirchen, Germany). Working solutions of 1 mg per mL of the standards were prepared daily.

The β-carotene and lycopene were analysed using reversed-phase high-performance liquid chromatography (RP-HPLC) with isocratic elution. A Waters 2695 liquid chromatograph (Waters Corp., Milford, MA, USA) connected to a Waters 2489 UV-VIS detector was used. A carotenoid C_{30} reversed-phase column (250×4.6 i.d.; 3 μm) from YMC corporation (Waters, Zellik, Belgium) was used. The column temperature was 28 °C. The samples were filtered through a 0.45-mm syringe filter (polypropylene, PVDF; Millipore) before they were injected. The injection volume was 10 μL. For analysis, samples were dissolved in a known amount of injection solvent (40 % acetonitrile, 20 % methanol, 20 % dichloromethane, 20 % hexane, 0.1 % diisopropylethylamine (DIPEA)). The pump solvent modules: solution A (acetonitrile 10 % and methanol 90 %), and solution B (hexane 45 %, dichloromethane 45 %, methanol 10 %, and DIPEA 0.1 %) were used at a flow rate of 1.5 mL/min. Carotenoids were identified based on the elution times in comparison with standard reference samples and concurrence with wavelengths for standard compounds.

Colour measurement

The colour characteristics of fermented and untreated tomato pulp were evaluated at three different positions of the surface using CIELab system (ChromaMeter CR-400, Konica Minolta, Japan). L^* is a measure of lightness, from completely opaque (0) to completely white (100), a^* is a measure of redness (or $-a^*$ of greenness), and b^* of yellowness (or $-b^*$ of blueness). The C^* value, colour purity, indicates the intensity of brightness:

$$C^*=(a^{*2}+b^{*2})^{1/2} \qquad /1/$$

and $h°$ value indicates the colour tone (*27*):

$$h°=\arctan\,(b^*/a^*) \qquad /2/$$

Statistical analysis

All experiments were replicated three times and the results were expressed as the mean values±standard deviations. Statistical analysis was performed using SPSS v. 16.0 software (SPSS, IBM, Chicago, IL, USA). Data were analysed using one-way ANOVA followed by Duncan's test. The confidence interval was 95 % ($p<0.05$).

Results and Discussion

Effect of selected fermentation media on the LAB viability

As reported in the literature, the behaviour of different LAB depends on the substrate composition, while in different substrates bacteria are able to produce different metabolites or increase the biomass (*28*). It is important to have a significant number of viable LAB present in the probiotic products for maximum health benefits (*29*).

Extruded rice flour, currently a product of the cereal processing industry, was found to show good fermentability. The counts of viable cells of LAB in selected media were between 6.62 and 8.50 log CFU per g after 48 h of cultivation (Table 1). The lowest biomass of bacteria was found in the spontaneously fermented rice media (5.57 log CFU per g). According to the obtained results, rice flour could serve as a suitable medium for LAB cultivation to produce functional foods, while probably still maintaining other functional properties of the rice. The results are in agrement with Trachoo *et al.* (*16*), who observed the increase of the biomass of lactobacilli over 2.5 log CFU per mL during 24 h using a germinated rice broth.

The analysed *L. sakei*, *P. acidilactici* and *P. pentosaceus* were found to be capable of sufficiently rapid utilization of tomato pulp for cell synthesis and organic acid production. They reduced the pH up to 3.5–3.7 and increased the TTA to as high as 6.4. The viable cell counts reached 6.61 log CFU per g after 48 h of fermentation. Spontaneously fermented tomato products had pH values higher by 7.2 % and TTA values lower by 17.3 % than the lacto-fermented products (Table 1).

Acid production is dependent on the number of viable bacteria that are able to utilize carbohydrate sources available in the substrate (*30*). The cell count of LAB in fermented tomato products was lower on average by 30 % (lacto-fermentation) and by 49.2 % (spontaneous fermentation) compared to rice media (Table 1). The viable cell counts of three LAB measured after 48 h of fermentation varied between 4.54 and 6.61 log CFU per g. For health benefits, probiotic bacteria must be viable and

Table 1. The influence of fermentation media on LAB cell counts, pH and total titratable acidity (TTA)

Samples	Extruded rice			Tomato products		
	N(LAB) / log CFU/g	pH	TTA	*N*(LAB) / log CFU/g	pH	TTA
P.p.	(8.51±0.05)^{d}	(3.37±0.01)^{a}	(8.2±0.2)^{b}	(6.61±0.03)^{c}	(3.50±0.01)^{a}	(6.4±0.3)^{b}
P.a.	(6.62±0.03)^{b}	(3.40±0.01)^{a}	(8.2±0.2)^{b}	(4.54±0.04)^{b}	(3.71±0.01)^{b}	(6.8±0.2)^{c}
L.s.	(7.75±0.03)^{c}	(3.42±0.01)^{a}	(8.3±0.2)^{b}	(4.83±0.03)^{b}	(3.70±0.01)^{b}	(7.1±0.2)^{d}
SF	(5.57±0.02)^{a}	(3.73±0.01)^{b}	(7.2±0.2)^{a}	(2.83±0.02)^{a}	(3.92±0.01)^{c}	(5.6±0.2)^{a}

The numbers are mean values followed by standard deviation (*N*=3)
Mean values within a column with different superscript letters are significantly different ($p<0.05$)
Samples: tomato products fermented with: P.p.=*P. pentosaceus*, P.a.=*P. acidilactici*, L.s.=*L. sakei*; SF=spontaneously fermented

available at a high content, typically about 6 log CFU per g of product (*29*). According to Sindhu and Khetarpaul (*31*), probiotic fermentation of indigenous food mixtures containing tomato pulp increased the acidity and improved the digestibility of starch and protein. The obtained results support the hypothesis that rice media contain the essential nutrients to support the growth of lactobacilli and can directly be used as fermentation substrates for probiotic lactic acid bacteria. The reached biomass levels are above the minimum required in a probiotic formulation.

The traditional lactic acid fermentation of vegetables is the microbial process that involves heterofermentative and homofermentative lactic acid bacteria, generally *Lactobacillus* and *Pediococcus* (*30*). At the pH between 3.5 and 3.8 the vegetables can be preserved for a long period of time (*30,32*). Tomatoes treated with lacto-fermentation can be recommended as useful and safe products for human nutrition. Furthermore, fermented tomatoes could serve as a healthy product for vegetarians and consumers who are allergic to dairy products.

The production of L- *and* D-*lactic acid during lacto-fermentation of tomato pulp*

Results showed that all analysed LAB produced the mixture of L- and D-lactic acid (Fig. 1). The highest levels of both forms were determined in spontaneously fermented tomato products ((7.18±0.03) and (7.67±0.11) mg per 100 g, respectively). As reported by Hartmann (*33*) and Li and Cui (*34*), *Lactobacillus amylophilus*, *L. bavaricus*, *L. casei*, *L. maltaromicus* and *L. salivarius* predominantly yield the L-isomer. Strains such as *L. delbrueckii*, *L. jensenii* or *L. acidophilus* yield the D-lactic acid or mixtures of both forms. Lactic acid bacteria such as *L. pentosus*, *L. brevis* and *L. lactis* can ferment glucose to lactic acid by homolactic fermentation. Fermentation of rice with two strains of *L. delbrueckii* yielded 3.23 and 5.04 mg of D-lactic acid per 100 g (*35*).

Mass fractions of D-lactic acid in fermented tomato products were determined to be between (4.05±0.05) and (6.34±0.04) mg per 100 g, and mass fractions of L-lactic acid ranged from (4.26±0.04) to (7.19±0.08) mg per 100 g (Fig. 1). Results of our study indicated that the use of *P. pentosaceus* allowed to reduce the content of D-lactic acid in tomato products by 11.8 %, compared to the spontaneously fermented ones (Fig. 1). Fermentation with *P. acidilactici* and *L. sakei* reduced the content of the latter isomer at a higher level (on average by 40.6 %).

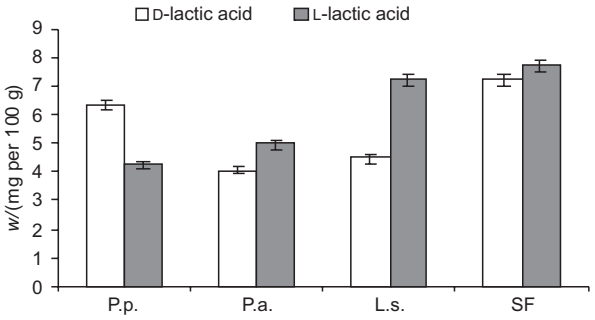

Fig. 1. Mass fractions of L- and D-lactic acid in fermented tomato products. Samples: P.p.=*P. pentosaceus*, P.a.=*P. acidilactici*, L.s.=*L. sakei*; SF=spontaneously fermented

In summary, *P. pentosaceus* produces mainly D-lactic acid (L/D ratio of 0.64), while *L. sakei* produces mainly L-lactic acid (L/D ratio of 1.61). Fermentation with *P. acidilactici* as well as the spontaneous fermentation gave almost equal amounts of both lactic acid isomers (L/D ratio of 1.17 and 1.07, respectively).

Regarding the potential toxicity of D-lactic acid, in all cases tomato products prepared using pure culture of LAB were found safer than the spontaneously fermented ones. The levels of D-lactic acid in the tomato products fermented with pure LAB were significantly lower ($p<0.05$) than those of spontaneously fermented ones (Fig. 1). The obtained results indicate that *L. sakei* KTU5-06 can be recommended for fermentation of tomato since it produces mainly L-lactic acid.

Cis/trans lycopene ratio and β-carotene content in fermented tomato products

Results of the analysis of total carotenoids, lycopene and β-carotene contents in fermented tomato products are presented in Fig. 2. The highest mass fractions of total carotenoids (on average of 6.83 mg per 100 g) were measured in the Cunero variety fermented with *P. pentosaceus* and in Ronaldo variety fermented with *L. sakei*. However, the fermentation with the latter bacteria increased the levels of total carotenoids by 41.1 and 33.6 % respectively, compared to the untreated samples. Fermentation with *P. acidilactici* reduced the mass fraction of total carotenoids in the samples of Cunero and Ro-

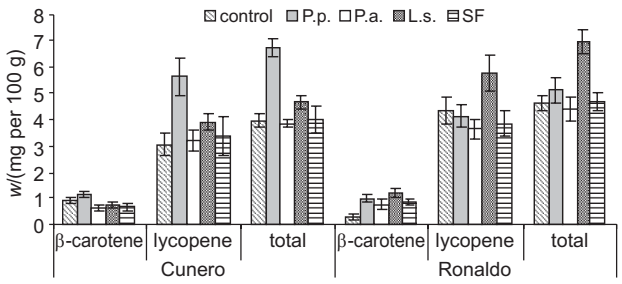

Fig. 2. β-Carotene, lycopene and total carotenoid mass fractions in the untreated and fermented with different LAB tomato products. Samples: control=untreated tomato pulp; tomato pulp fermented with: P.p.=*P. pentosaceus*, P.a.=*P. acidilactici*, L.s.=*L. sakei*; SF=spontaneously fermented

naldo varieties by 3.6 %, compared to the untreated tomatoes (3.96 and 4.61 mg per 100 g, respectively) accompanied by reduced β-carotene content (Fig. 2).

The fermented tomato samples of Cunero variety had lower content of β-carotene on average by 24.7 %, and higher content of lycopene by 11.5 % compared to the untreated tomatoes. On the contrary, β-carotene mass fractions in all fermented tomato products of Ronaldo variety were generally higher; the increase was on average 69.4 % compared to the untreated tomatoes (Fig. 2).

The increase of 24.8 % in lycopene content in the samples of Ronaldo variety was reached after fermentation with *L. sakei.* Spontaneous fermentation as well as the treatment with *P. pentosaceus* reduced lycopene mass fractions by 11.0 and 4.4 %, respectively, compared to the control sample (Fig. 2).

It can be concluded that lactic acid fermentation generally had a positive effect on lycopene and total carotenoid contents of the fermented tomato products. β-Carotene content was influenced not only by the used LAB, but also by the tomato variety. As it was reported in the literature (*36*,*37*), compositional variation of lycopene in tomato occurs as a consequence of varietal differences, climatic conditions, agricultural variables, stage of maturity, harvesting, postharvest handling and conditions during storage. Lycopene values within the range of 3.1–7.7 mg per 100 g were previously reported for different tomato cultivars (*38*). The lycopene mass fraction of 6–15 mg per 100 g of whole fresh tomato fruit reported by Camara *et al.* (*39*) was higher than the results of this investigation. Lycopene content may be affected directly by the pH of the fruit as low pH of red tomatoes accumulates more lycopene (*40*).

Analysis of the all-*trans*- and *cis*-lycopene showed that the amounts of both isomers significantly depended on the tomato variety and were slightly affected by LAB strain used for fermentation (Fig. 3). The fermented tomato products of Ronaldo variety had higher content of all-*trans*- and *cis*-lycopene on average by 25.9 and 62.6 %, respectively, than tomato products of Cunero variety.

The amount of *cis*-lycopene was found higher by 3.3-fold in the control samples of Ronaldo variety (0.034 mg per 100 g) than that of Cunero variety (0.115 mg per 100 g) (Fig. 3). Fermentation by *P. pentosaceus* as well as *L. sakei* increased the *cis*-lycopene content on average by 30.6 and 8.5 %, respectively, in products of Cunero and

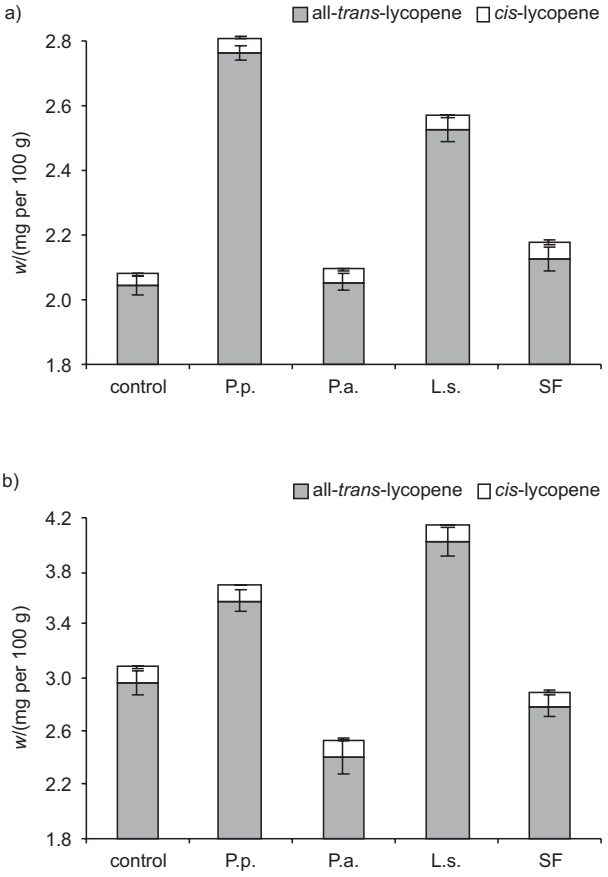

Fig. 3. Mass fractions of all-*trans*- and *cis*-lycopene in fermented tomato varieties: a) Cunero, and b) Ronaldo. Samples: control=untreated tomato pulp; tomato pulp fermented with: P.p.=*P. pentosaceus*, P.a.=*P. acidilactici*, L.s.=*L. sakei*; SF=spontaneously fermented

Ronaldo varieties. Lower increase in *cis*-lycopene was noticed during the fermentation of Cunero variety with *P. acidilactici* as well as during spontaneous fermentation (increase on average by 9 %). Similarly, the lacto-fermentation using *P. acidilactici* and *L. sakei* incresed the *cis*-lycopene content in tomato products on average by 5.8 %.

The fermentation of different tomato varieties with *P. pentosaceus* and *L. sakei* gave on average 22.2 % higher contents of all-*trans*-lycopene compared to controls (2.05 mg per 100 g of Cunero and 2.96 mg per 100 g of Ronaldo variety) (Fig. 3).

The *cis/trans* lycopene ratio of Cunero and Ronaldo tomatoes was 1.67 % and 3.81 %, respectively. The highest *cis/trans* ratio was calculated for Cunero samples fermented by *L. sakei* (2.08 %), followed by Ronaldo samples fermented by *P. acidilactici* (4.90 %) and by spontaneous fermentation (4.09 %).

The findings in the literature show that lycopene from *cis*-isomer-rich tomato sauce is more bioavailable in the human body than from all-*trans*-rich tomato sauce (*41*). Because of the positive effect of lacto-fermentation on *cis/trans* lycopene ratio, products of Ronaldo variety fermented with *P. acidilactici* or *L. sakei* could be recommended as more biologically accessible products with functional value.

Colour characteristics of fermented tomato products

The results of the analysis of red (a^*) and yellow (b^*) colour coordinates of fermented tomato products are presented in Table 2. The relation between the yellow colour coordinate (b^*) of Cunero variety and total carotenoid, lycopene and β-carotene contents was not found ($p>0.05$) (Table 2). However, a red colour coordinate (a^*) slightly correlated ($R^2=0.672$) with β-carotene content.

On the contrary, weak relation was noticed between colour coordinate b^* of Ronaldo variety and total carotenoid and β-carotene contents ($R^2=0.581$) (Table 2). Also, a strong relation between colour coordinate b^* and lycopene content of the samples of this variety were found ($R^2=0.825$, $p=0.03$). Significant relations between a^* and β-carotene and lycopene contents ($p>0.05$) (Table 2) or between total carotenoids and colour tone ($h°$) and colour purity (C^*) values of Cunero and Ronaldo varieties were not found (Table 3).

The best estimation of β-carotene content was obtained by using the b^* chromaticity value from whole fruit measurements or the transformed a^{*2} value from pure measurements (*41*). Neither model, however, could explain more than 55 % of the variation in β-carotene content, suggesting that chromaticity values may not be appropriate for estimating tomato β-carotene content. In another research the inspection of different chromaticity

Table 2. Colour coordinates (a^* and b^*) of tomato varieties Cunero and Ronaldo and the correlations between total carotenoid, lycopene and β-carotene contents

Samples	Cunero			Ronaldo		
	a^*	b^*	a^*/b^*	a^*	b^*	a^*/b^*
control	$(14.0\pm1.3)^c$	$(15.5\pm0.8)^a$	0.903	$(13.8\pm0.9)^c$	$(16.7\pm1.1)^b$	0.828
P.p.	$(14.6\pm1.1)^d$	$(16.5\pm1.3)^b$	0.885	$(15.2\pm0.8)^e$	$(18.3\pm0.9)^c$	0.829
P.a.	$(11.4\pm0.9)^a$	$(17.3\pm1.3)^{bc}$	0.660	$(13.4\pm1.1)^b$	$(19.4\pm1.3)^d$	0.691
L.s.	$(13.0\pm1.1)^b$	$(15.1\pm1.3)^a$	0.864	$(13.9\pm0.5)^c$	$(15.5\pm0.7)^a$	0.891
SP	$(13.4\pm1.3)^b$	$(19.1\pm1.4)^d$	0.703	$(14.3\pm1.2)^d$	$(19.5\pm1.3)^d$	0.730
a, b and a/b correlation with total carotenoid content						
R^2	0.3905	0.0476	0.2673	0.001697	0.5808	0.5985
p	0.2597	0.7243	0.3723	0.9476	0.1342	0.1248
a, b and a/b correlation with lycopene content						
R^2	0.3186	0.02779	0.1973	0.004398	0.8248	0.7373
p	0.3215	0.7887	0.4537	0.9156	0.0329	0.0624
a, b and a/b correlation with β-carotene content						
R^2	0.6718	0.1955	0.6326	0.001697	0.5808	0.5985
p	0.0894	0.4560	0.1077	0.9476	0.1342	0.1248

The numbers are mean values followed by standard deviation ($N=3$)
Mean values within a column with different superscript letters are significantly different ($p<0.05$)
Samples: tomato products fermented with: P.p.=*P. pentosaceus*, P.a.=*P. acidilactici*, L.s.=*L. sakei*; SF=spontaneously fermented; R^2=correlation coeficient

Table 3. Colour tone (h^o) and purity (C^*) of Cunero and Ronaldo tomato variety samples and their relation with total carotenoid content

Samples	Cunero		Ronaldo	
	C^*	$h°$	C^*	$h°$
Control	$(22.0\pm2.3)^b$	$(47.0\pm3.1)^a$	$(22.0\pm2.4)^b$	$(46.9\pm2.4)^a$
P.p.	$(21.9\pm1.5)^b$	$(48.3\pm3.2)^{bc}$	$(23.8\pm1.9)^c$	$(50.1\pm1.9)^c$
P.a.	$(20.8\pm1.7)^a$	$(56.6\pm2.7)^{de}$	$(23.4\pm1.8)^c$	$(55.3\pm2.9)^d$
L.s.	$(20.0\pm1.3)^a$	$(49.5\pm2.4)^c$	$(21.0\pm1.6)^{ab}$	$(48.5\pm1.4)^b$
SP	$(23.2\pm2.1)^c$	$(55.2\pm1.7)^d$	$(24.4\pm1.3)^{cd}$	$(53.7\pm2.3)^d$
Correlation with total carotenoid content				
R^2	0.00000565	0.2332	0.4974	0.2043
p	0.9970	0.4099	0.1834	0.4448

The numbers are mean values followed by standard deviation ($N=3$)
Mean values within a column with different superscript letters are significantly different ($p<0.05$)
Samples: tomato products fermented with: P.p.=*P. pentosaceus*, P.a.=*P. acidilactici*, L.s.=*L. sakei*; SF=spontaneously fermented; R^2=correlation coeficient

values and regression models suggested that colorimeter readings may not be highly useful for estimating β-carotene content in tomato fruit (*42*). The overal results indicate that lycopene content could be measured simply and rather accurately across a wide range of tomato genotypes using chromaticity values of fruit purée (*41*). On the contrary, Liu *et al.* (*43*) reported that daily light treatment of tomato enhances lycopene accumulation in the exocarp with minimal effect on the colour. Arias *et al.* (*18*) also observed that colour characteristic *b** was not appropriate for predicting the lycopene content of tomato.

According to the obtained results, colour tone (*h*°) and purity (*C**) are not suitable as indicators for the evaluation of total carotenoid content in tomato products. As was reported by Fernández-Ruiz *et al.* (*44*), the measurement of the yellow coordinate (*b**) can be used for predicting lycopene content in tomato products.

Conclusions

All tested lactic acid bacteria produced the mixture of L- and D-lactic acid, the latter isomer being at a lower level. Because of the potential toxicity of D-lactic acid, tomato products prepared using pure culture of the tested LAB in all cases were found safer than spontaneously fermented ones.

Processing of tomatoes resulted in several important changes in carotenoid concentration and lycopene isomer profile. Treatment with LAB breaks down the tomato cell matrix and makes the carotenoids more available, which resulted in higher level of total carotenoids. Moreover, lactic acid fermentation of tomato resulted in a large lycopene bioavailability accompanied by increased *cis*-lycopene content. According to our results, *P. pentosaceus* and *L. sakei* may be useful for preservation of tomatoes, which could be recommended as a way of obtaining more biologically accessible products with functional value.

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