Chapter 7

The Use of Lactic Acid Bacteria in the Fermentation of Fruits and Vegetables — Technological and Functional Properties

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Additional information is available at the end of the chapter

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1. Introduction

The relationship between food and health has been investigated for many years, and therefore, the development of foods that promote health and well-being is a key research priority of the food industry [1]. Fruits and vegetables are an essential part of human nutrition. Unfortunately, the daily intake of fruits and vegetables is estimated to be lower than the recommendation of the World Health Organization (WHO) [2], who suggest a dietary intake of 450 and 500 g of fruits and vegetables, respectively. Vegetables are strongly recommended in the human diet because they are rich in antioxidants, vitamins, dietary fibres and minerals. The majority of vegetables consumed in the human diet are fresh, minimally processed, pasteurised or cooked by boiling in water or microwaving, and vegetables can be canned, dried, or juiced or made into pastes, salads, sauces, or soups. Fresh vegetables or those that have been minimally processed have a particularly short shelf-life because they are subjected to rapid microbial spoilage. In addition, the above cooking processes can cause a number of potentially undesirable changes in physical characteristics and chemical composition [3,4].

Therefore, these drawbacks could be reduced by novel technologies, such as new packaging systems, high-hydrostatic pressure processing, ionisation radiation and pulsed electric fields [5-7]. The use of natural antimicrobial preservatives is considered to be the simplest and most valuable biological technique to keep and/or enhance the safety, nutrition, palatability and shelf-life of fruits and vegetables [5]. 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. Three technology options are usually considered for lactic acid fermentation of vegetable: spontaneous fermentation by autochthonous lactic acid bacteria, fermentation by starter cultures that are added into raw vegetables, and fermentation of mild heat-treated vegetables by starter cultures [18]. For thousands of years, microorganisms have been used to produce and preserve foods through the process of fermentation. Fermented foods have been adopted in various ways depending on the properties of the available raw materials and the desired features of the final products [8-10]. Food produced by traditional methods has become popular among consumers who know that their food is manufactured from high quality raw materials, without preservatives and other synthetic additives that are characterised by unique flavour values [11].

2. Fermentation from a biochemical point of view

Bourdichon et al. [12] describe the fermentation process as "a metabolic process of deriving energy from organic compounds without the involvement of an exogenous oxidising agent". Fermented foods are subjected to the actions of microorganisms or enzymes. Fermentation plays different roles in food processing, such that desirable biochemical changes have occurred [13]. The fermentation process is very important in the improvement of technological properties of preservation, such as a relative cost-effectiveness and low energy requirements, which are essential for ensuring the shelf-life and microbiological safety of the product [8]. The major roles of fermentation are considered to be the following:

1. preservation of food: the formation of inhibitory metabolites, such as organic acid (lactic acid, acetic acid, formic acid, propionic acid), ethanol, bacteriocins, etc., often in combination with a decrease in water activity (by drying or the use of salt) [14-15].
2. improving food safety through the inhibition of pathogens [16,17] or the removal of toxic compounds [18].
3. improving nutritional value: biological enrichment of food substrates with proteins, essential amino acids, essential fatty acids and vitamins [19,20].
4. organoleptic food quality: enrichment of the diet through the development of a diversity of flavours, aromas, and textures in food substrates [21-24].
5. decrease in cooking times and fuel requirements [25].

Interest in the biopreservation of food has created a demand for more natural and minimally processed food, with particular interest in naturally produced antimicrobial agents [26].

3. Lactic acid bacteria (LAB) in food fermentation and new natural antimicrobial compounds

LAB have traditionally been associated with food fermentation. LAB are generally considered beneficial microorganisms, with some strains even considered to promote good health

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(probiotic), and their extensive historical use contributes to their acceptance as being GRAS (generally recognised as safe) for human consumption [27]. LAB are used as natural or selected starters in food fermentation and exert health benefits through the antimicrobial effect produced from different metabolic processes (lactose metabolism, proteolytic enzymes, citrate uptake, bacteriophage resistance, bacteriocin production, polysaccharide biosynthesis, metal-ion resistance and antibiotic resistance) [28,29,9]. Spontaneous fermentation typically results from the competitive activity of a variety of autochthonous and contaminating microorganisms, which may lead to a high risk for failure. Both from a hygiene and safety perspective, the use of starter cultures is recommended, as it leads to rapid inhibition of spoilage and pathogenic bacteria while yielding processed fruit with consistent sensory and nutritional quality [30].

Interest in the biopreservation of food has prompted the quest for novel antimicrobial compounds from different natural origins. The LAB of genera such as *lactobacilli* and *lactococcus* are amongst the most important known members that have probiotic activity [31]; these bacteria produce antimicrobial peptides most frequently referred to as bacteriocins [32,33]. Bacteriocins ensure the stability of fermented plant products, reduce microbial contamination during fermentation, inhibit the growth of moulds and delay microbiological spoilage of baked goods [34].

LAB have strong inhibitory effects on the growth and toxin production of other bacteria. This activity can occur due to the following factors: competition for available nutrients; decrease in redox potential; production of lactic acid and acetic acid and the resulting decrease in pH; production of other inhibitory primary metabolites, such as hydrogen peroxide, carbon dioxide or diacetyl; and production of special antimicrobial compounds, such as bacteriocins and antibiotics [35].

Each of these properties, particularly when combined, can be used to extend the shelf-life and safety of food products [36].

Amongst the various technologies, lactic acid fermentation may be thought of as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of fruits and vegetables [37,38]. Overall, LAB are a small part (2–4 $\log_{10}$ CFU g^{-1}) of the autochthonous microbiota of raw vegetables, and their cell density is mainly influenced by the vegetable species, temperature and harvesting conditions [37].

Interest in the use of LAB fermentation of vegetable products stems largely from the nutritional, physiological and hygienic aspects of the process and their corresponding implementation and production costs [39].

LAB fermentation represents the easiest and most suitable way to increase the daily consumption of nearly fresh fruits and vegetables.

4. Unique features of fermented fruits and vegetables

Buckenhuskes and colleagues [40] generally agreed that fermented plant products are the "food of the future". The following factors support this idea: products can be marked as

"natural" or "biological"; desirable flavour compounds are enhanced while negative flavour compounds (for example, glucosinolates) are destroyed; handling and storage (without cooling) is simple; easy methods exist for the pre-handling of raw material before further processing; desired metabolites (lactic acid, amino acids) are enriched; and the process results in the detoxification of pathogens [41]. Fruits and vegetables preserved using LAB with antimicrobial properties are perceived as suitable products for the human diet [42].

Dieticians and physicians recommend fermented fruits and vegetables due to the health-promoting properties of these foods. Fermented fruits and vegetables are low-calories foods because they contain considerably lower quantities of sugars compared to their raw counterparts. Fermented vegetables are a source of dietary fibre, which impedes the assimilation of fats and regulates peristalsis in the intestines; they are also a valuable source of vitamin C, B-group vitamins, phenolics and many other nutrients present in the raw material. Lactic acid may also lower gut pH, thereby inhibiting the development of putrefactive bacteria [42].

Many types of fermented fruit and vegetable products exist in the world: sauerkraut, cucumber pickles, and olives in the Western world; Egyptian pickled vegetables in the Middle East; and Indian pickled vegetables, Korean kim-chi, Thai pak-sian-don, Chinese hum-choy, Malaysian pickled vegetables and Malaysian tempoyak. Lactic acid-fermented cereals and tubers (cassava) include Mexican pozol, Ghanaian kenkey, Nigerian gari; boiled rice/raw shrimp/raw fish mixtures such as Philippine balao-balao and burong dalag; lactic-fermented/leavened breads such as sourdough breads in the Western world; Indian idli, dhokla, khaman and Sri Lankan hoppers; Ethiopian enjera, Sudanese kisra and Philippine puto; and Chinese sufu/tofu-ru [9,43].

Commercial distribution of these fermented products lags far behind that of fermented meat and dairy products due to a lack of standardised manufacturing protocols; in addition, their ingredients are subject to limiting and unpredictable weather and geographic conditions [44]. The lactic acid fermentation of vegetables currently has industrial significance only for cucumbers, cabbages and olives [45]. Several other varieties of vegetables cultivated mainly in Southern Italy or, more generally, in the Mediterranean area, such as carrots, French beans, marrows, artichokes, capers and eggplants, may benefit from increased safety, nutritional, sensory and shelf-life properties through standardised industrial lactic acid fermentation [46].

5. The use of microorganisms in our diet opens new opportunities

Either as traditional fermented foods or as novel approaches, the rationalised use of microorganisms in our diet could reveal new opportunities. Low dietary quality is an important factor that limits adequate nutrition in many resource-poor settings. Bioavailability is a key aspect of dietary quality with respect to the adequacy of micronutrient intake [47]. Prebiotic food ingredients encourage the growth of probiotic bacteria. The appropriate combination of prebiotics and probiotics manifest in a higher potential for synergistic effects 48]. Probiotic foods are fermented products that contain a sufficient number of a certain live microorganism to favourably modify the intestinal microbiota of the host [49]. Recently developed probiotics

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tend to be milk-based, although in recent years other substrates have been explored for 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 protection against bile resistance [50].

According to Kim et al. [51], cabbage (including the Chinese cabbage), pH-adjusted tomato (pH 7.2), carrot and spinach media give relatively higher fermentability than other vegetables because they have more fermentable saccharides. The 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 [52-54]. Approximately 90 % of the lycopene in dietary sources is found in the linear, all-*trans* conformation, while human tissues mainly contain *cis*-isomers. It has been suggested that the *cis*-isomers of lycopene are better absorbed than the all-*trans* form because they are shorter, have greater solubility in mixed micelles, and have a lower tendency to aggregate [55]. Studies have shown that lycopene levels in plasma increase only after the consumption of red tomato paste and purified lycopene [54]. It has also been revealed that the absorption of lycopene is greater from processed tomatoes than from fresh tomatoes because processing breaks down the tomato cell matrix and makes the lycopene more available [56,57].

The red colour of tomatoes is a result of the degradation of chlorophylls and the increased biosynthesis of carotenoids [58]; thus, a tomato's colour is related to its maturity and post-harvest treatment. Colour is therefore an important attribute indicating the quality of tomato fruit, and it is used in the food industry to predict the colour of finished products. Additionally, the application of instrumental colour measurement to objectively define the colour of tomatoes is an important research topic [59,60]. It was reported that the colour coordinates of a product could relate to its concentration of lycopene and other carotenoids [61,62].

There is an increasing consumer demand for high quality meat products that taste good and are both nutritious and easy to prepare. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage microorganisms and common food-borne pathogens. It is therefore essential to apply adequate preservation techniques to maintain its safety and quality [63]. The processes used in meat preservation are principally concerned with inhibiting microbial spoilage, although other methods of preservation seek to minimise additional deteriorative changes in colour and oxidation [64]. The most investigated new preservation technologies for fresh meat involve non-thermal inactivation, such as high hydrostatic pressure (HHP), novel packaging systems, including modified atmosphere packaging (MAP) and active packaging (AP), natural antimicrobial compounds and biopreservation. Storage life is extended and safety is increased by using natural or controlled microflora, including the extensively studied LAB and their antimicrobial products, such as lactic acid and bacteriocins. Bacteriocins are a heterogeneous group of antibacterial proteins that vary in their spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties [65].

The destruction of the total BLIS (bacteriocin-like inhibitory substances) activity after treatment with proteinase K, trypsin, pepsin and chymotrypsin indicates that antimicrobial

substances produced by the tested LAB possess a proteinaceous nature. They might be bacteriocins because protease sensitivity is a key criterion in the classification of antimicrobial substances as BLIS [66]. In our previous studies, BLIS produced by *Lactobacillus sakei* KTU05-6 and *P. pentosaceus* KTU05-9 were designated as sakacin 05-6 and pediocin 05-9 [67]. We proposed that due to their broad inhibition spectrum, the presence of BLIS and organic acids in tested LAB is an indication that these bacteria can be used widely in the food industry as bio-preservatives.

Consumer interest for diverse fermented foods has increased in recent years because of the positive perception of their beneficial impact on health. Hence, there is an evident need to find novel methods and new food preservation agents from natural origins. Biopreservation refers to extending the shelf-life and enhancing the safety of foods using microorganisms or their metabolites [68]. In this aspect, LAB are very good candidates [69].

The food matrices in vegetables offer promising potential as sources and carriers of probiotic strains [70]. Vegetables are fundamental sources of water-soluble vitamins (vitamin C and group B vitamins), provitamin A, phytosterols, dietary fibres, minerals and phytochemicals [71] in the human diet. LAB are a small part (2.0–4.0 $\log_{10}$ CFU g^{-1}) of the autochthonous microbiota of raw vegetables [37]. Under favourable conditions of anaerobiosis, water activity, salt concentration and temperature, raw fruits and vegetables may be subject to spontaneous lactic acid fermentation. In some cases, alcoholic fermentation takes place concomitantly [72].

Tomatoes are a rich source of a variety of nutritional compounds, especially key antioxidant components, such as the carotenoid lycopene, vitamin C, and a range of polyphenols. The possible protective characteristics of these antioxidants are of great interest, and consumers have already become aware of their potential importance. A survey of the literature revealed that a great deal of research has been conducted on the biochemical composition of tomatoes and their products [73]. Lycopene, a natural carotenoid found in tomatoes, has been reported to possess various health benefits, such as preventive properties against cardiovascular disease and cancer [74].

6. Lactic acid fermentation of tomatoes: effects on *cis*/*trans* lycopene isomers, β-carotene concentration and the formation of L(+) and D(-)-lactic acid

The production of L-lactic acid and D-lactic acid isomers during the fermentation of different tomato varieties (var. Ronaldo and var. Cunero) by the bacteriocin-producing LAB *Lactobacillus* and *Pediococcus* spp. have been investigated. The influence of lacto fermentation on the lycopene and β-carotene contents and their relation to the colour characteristics of fermented tomato products were also investigated [75]. Tomato var. Cunero and Ronaldo, the LAB strains were used in this investigation. Tomato var. Cunero and Ronaldo were obtained from the Lithuanian Institute of Horticulture (Babtai, Lithuania) harvested in 2011. Pure cultures of *Lactobaccilus sakei KTU05-6*, *Pediococcus acidilactici KTU05-7* and *Pediococcus pentosaceus KTU05-8*, characterized as a bacteriocin producing strains [76] are from collection of Kaunas University of Technology (Kaunas, Lithuania) [75].

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The LAB strains were propagated in nutrition media (moisture content 72 %), prepared by mixing extruded rice flour (100 g) and tap water. After addition of pure LAB cell suspension (5 g, 10.2 $\log_{10}$ colony-forming units (CFU) g^{-1}) 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 purpose 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 MRS agar at 30 °C for 48 hours. Products obtained after propagation of individual LAB in rice media were used for fermentation of tomato pulp [75].

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. [77] in this investigation. Extraction of carotenoids and carotenoid analysis by Reverse Phase Liquid Chromatography (RP-HPLC) were used [75] and the colour characteristics of fermented and untreated tomato pulp were evaluated of the surface using CIEL*a*b* [78].

6.1. The effect of selected fermentation media on LAB viability

As reported in the literature, the behaviour of different LAB depends on substrate composition, where bacteria in different substrates are able to produce different metabolites or increased biomass [79]. For maximum health benefits, it is important to have a significant number of viable LAB present in the probiotic product [80].

Extruded rice flour, a current product of the cereal processing industry, was found to show good fermentability. Counts of viable bacteria cells were measured between 6.62 and 8.50 $\log_{10}$ CFU g^{-1} after 48 h of analysed LAB cultivation in selected media (Table 1) [75]. The lowest biomass of bacteria was found in the spontaneously fermented rice media (5.57 $\log_{10}$ CFU g^{-1}). According to the obtained results, rice flour is a suitable medium for LAB cultivation to produce a functional food while most likely maintaining the other functional properties of rice. These results are in agreement with Trachoo et al. [81], who showed a biomass increase of lactobacilli over 2.5 $\log_{10}$ CFU mL^{-1} during 24 h in a germinated rice broth [75].

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

The numbers are means followed by standard deviations (n = 3).

Means 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 – spontaneous fermented.

Table 1. The influence of fermentation media on LAB cell counts ($\log_{10}$ CFU g^{-1}), pH and TTA values.

L. sakei, *P. acidilactici* and *P. pentosaceus* were found to be capable of sufficient rapid utilisation of tomato pulp for cell synthesis and organic acid production. They reduced the pH to 3.5–3.7 and increased the TTA to as high as 6.4. The viable cell counts reached 6.61 $\log_{10}$ CFU g^{-1} after 48 h of fermentation. In either case, tomato products treated with spontaneous fermentation had pH values that were higher by 7.2% and TTA values that were lower by 17.3 % than products treated with lactofermentation (Table 1) [75].

Acid production depends on the concentration of viable bacteria able to utilise the available carbohydrate sources in the substrate [82]. The viable LAB cells in the fermented tomato products were found to be lower on average by 30% (lactofermentation) or 49.2% (spontaneous fermentation) compared to the rice media (Table 1); however, three LAB counts measured after 48 h of fermentation varied between 4.54 and 6.61 $\log_{10}$ CFU g^{-1}. To achieve health benefits, probiotic bacteria must be viable and available at a high concentration, typically approximately 6 $\log_{10}$ CFU g^{-1} of product [80]. According to Sindhu and Khetarpaul [83], probiotic fermentation of indigenous food mixtures containing tomato pulp increases the acidity and improves the digestibility of starch and protein. Our results support the hypothesis that rice media contain the essential nutrients to support the growth of lactobacilli and can be directly used as a fermentation substrate of LAB. The obtained biomass levels are above the minimum required for a probiotic formulation.

Classic lactic acid vegetable fermentation is a microbial process that involves heterofermentative and homofermentative LAB, generally *Lactobacillus* and *Pediococcus* [82]. At a pH between 3.5 and 3.8, vegetables will be preserved for a long period of time [83]. Tomatoes treated by lactofermentation could 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 [75].

6.2. The production of L- and D-lactic acid during lactofermentation of tomato pulp

Our results showed that all the analysed LAB produced a mixture of L- and D-lactic acid (Figure 1), and the highest amounts of each form were determined in tomato products treated by spontaneous fermentation (7.18±0.03 and 7.67±0.11 mg/100 g, respectively). As reported by Hartman [85] and Li and Cui [86], *Lactobacilli amylophilus, L. bavaricus L. casei*, *L. maltaromicus*, and *L. salivarius* predominantly yield the L-isomer. Strains such as *L. delbrueckii*, *L. jensenii*, and *L. acidophilus* yield D-lactic acid or mixtures of both forms. LAB such as *L. pentosus*, *L. brevis* and *L. lactis* can ferment glucose into lactic acid through homolactic fermentation. The fermentation of rice with two strains of *L. delbrueckii* yielded 3.23 and 5.04 mg/100 g of D-lactic acid [87].

The concentration of D-lactic acid in fermented tomato products was measured between 4.05±0.05 and 6.34±0.04 mg/100 g, and the concentration of L-lactic acid ranged from 4.26±0.04 to 7.19±0.08 mg/100 g (Figure 1). The results of our study indicate that compared to spontaneous fermentation, the use of *P. pentosaceus* allowed a reduction in the content of D-lactic acid in tomato products by 11.8% (Figure 1). Fermentation with *P. acidilactici* and *L. sakei* reduced the content of the latter isomer at a higher level (on average by 40.6%).

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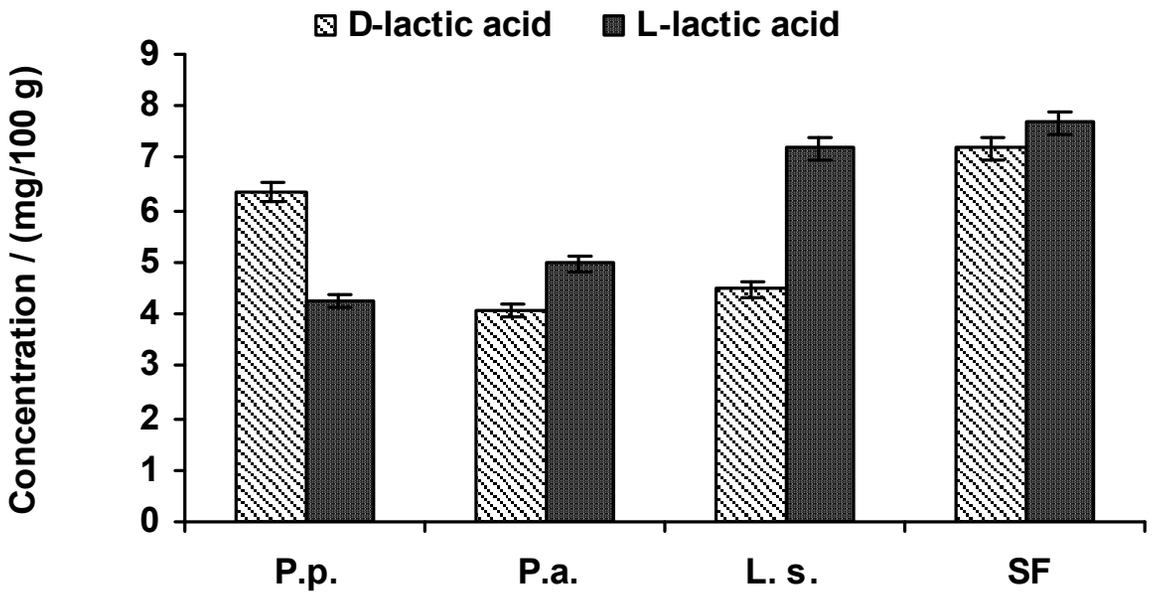

Figure 1. Concentrations of L- and D-lactic acid in fermented tomato products. Samples: fermented with LAB: P.p. – *P. pentosaceus*, P.a. – *P. acidilactici*, L.s. – *L. sakei*; SF – spontaneous fermented

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

By evaluating our knowledge of the potential toxicity of D-lactic acid in terms of nutrition, we can report that tomato products prepared using a pure culture of LAB were found in all cases to be safer than those treated with spontaneous fermentation. The level of D-lactic acid in pure LAB-fermented tomato products was significantly lower ($p<0.05$) than that in those spontaneously fermented (Figure 1). Based on these results, *L. sakei KTU05-6* could be selected as the L-lactic acid bacteria and is recommended for the fermentation of tomatoes [75].

6.3. Trans/cis lycopene and β-carotene contents in fermented tomato products

The results from our analysis of lycopene and β-carotene contents in fermented tomato products are presented in Figure 2. The highest concentration of total carotenoids (on average 6.83 mg/100 g) were measured in a var. Cunero sample fermented with *P. pentosaceus* and in a var. Ronaldo sample fermented with *L. sakei*. However, fermentation with the latter bacteria increased the total level of carotenoids by 41.1 and 33.6%, respectively, compared to untreated samples. Compared to untreated tomatoes, fermentation with *P. acidilactici* reduced the concentration of total carotenoids by 3.6% in the samples of var. Cunero and var. Ronaldo (3.96 and 4.61 mg/100 g, respectively), which was accompanied by a reduction in β-carotene content (Figure 2) [75].

On average, the fermented tomato samples of var. Cunero had 24.7 % lower β-carotene and 11.5% higher lycopene content compared to untreated tomatoes. In contrast, the β-carotene

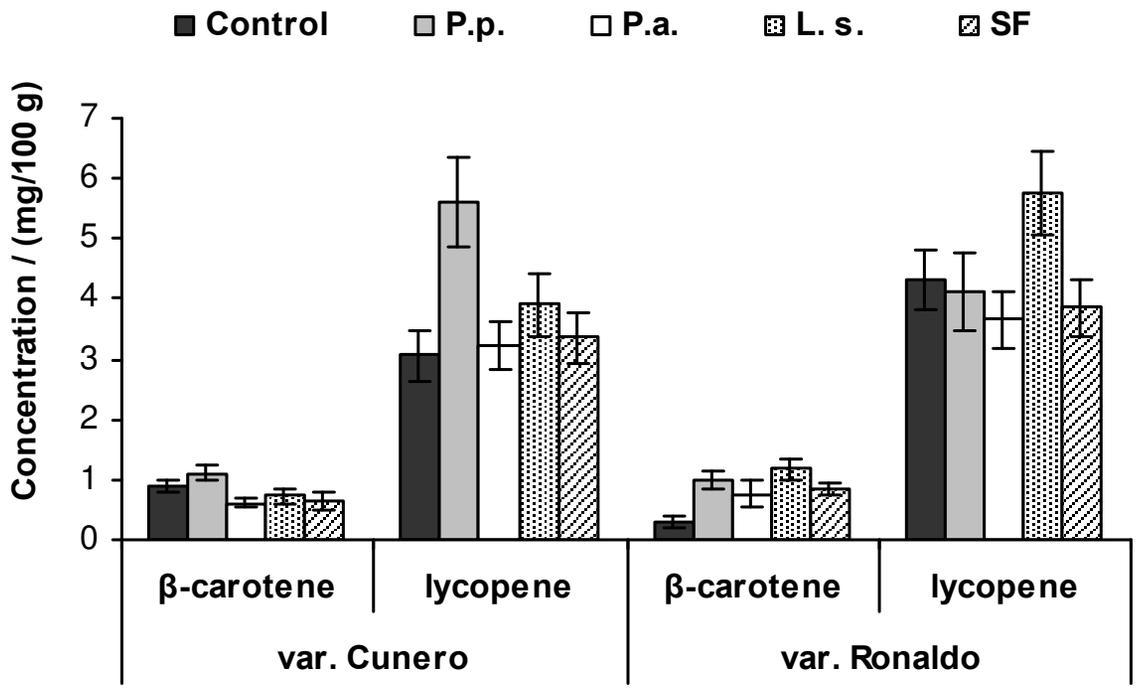

Figure 2. Carotenoid contents in untreated and fermented with different LAB tomato products. Samples: Control – untreated tomato pulp; tomato pulp fermented with: P.p. – *P. pentosaceus*; P.a. – *P. acidilactici* MI807; L.s. – *L. sakei,* SF – spontaneous fermented.

concentrations in all the fermented tomato products of var. Ronaldo were generally higher, with an average increase of 69.4% compared to untreated tomatoes (Figure 2) [75].

A 24.8% increase in lycopene content was reached in the var. Ronaldo samples after fermentation with *L. sakei.* Spontaneous fermentation or treatment by *P. pentosaceus* reduced the concentration of lycopene by 11.0 and 4.4%, respectively, compared to the control sample (Figure 2).

According to these results, lactic acid fermentation generally had a positive effect on the lycopene and total carotenoid contents of the fermented tomato products. The β-carotene contents were influenced not only by which LAB was used but also by the variety of tomato. As reported in the literature, compositional variation of lycopene in tomatoes occurs as a consequence of varietal differences, climate conditions, agricultural variables, stage of maturity, harvesting and post-harvest handling and conditions during storage [75]. Other researchers reported lycopene values within the range of 3.1–7.7 mg/100 g for different tomato cultivars [88]. However, Camara et al. [89] reported a lycopene concentration of 6–15 mg/100 g for whole fresh tomato fruit [89], which is higher than the results of this investigation. Lycopene content may be directly affected by the pH of the fruit, as the low pH of red tomatoes accumulates more lycopene [90].

Our analysis of all-*trans* and *cis*-lycopene showed that the amounts of both isomers depended significantly on the tomato variety and were slightly affected by the LAB strain used for fermentation (Figure 3). The fermented tomato products of var. Ronaldo had all-*trans*- and *cis*-

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lycopene contents that were higher on average by 25.9 and 62.6%, respectively, compared to the tomato products of var. Cunero [75].

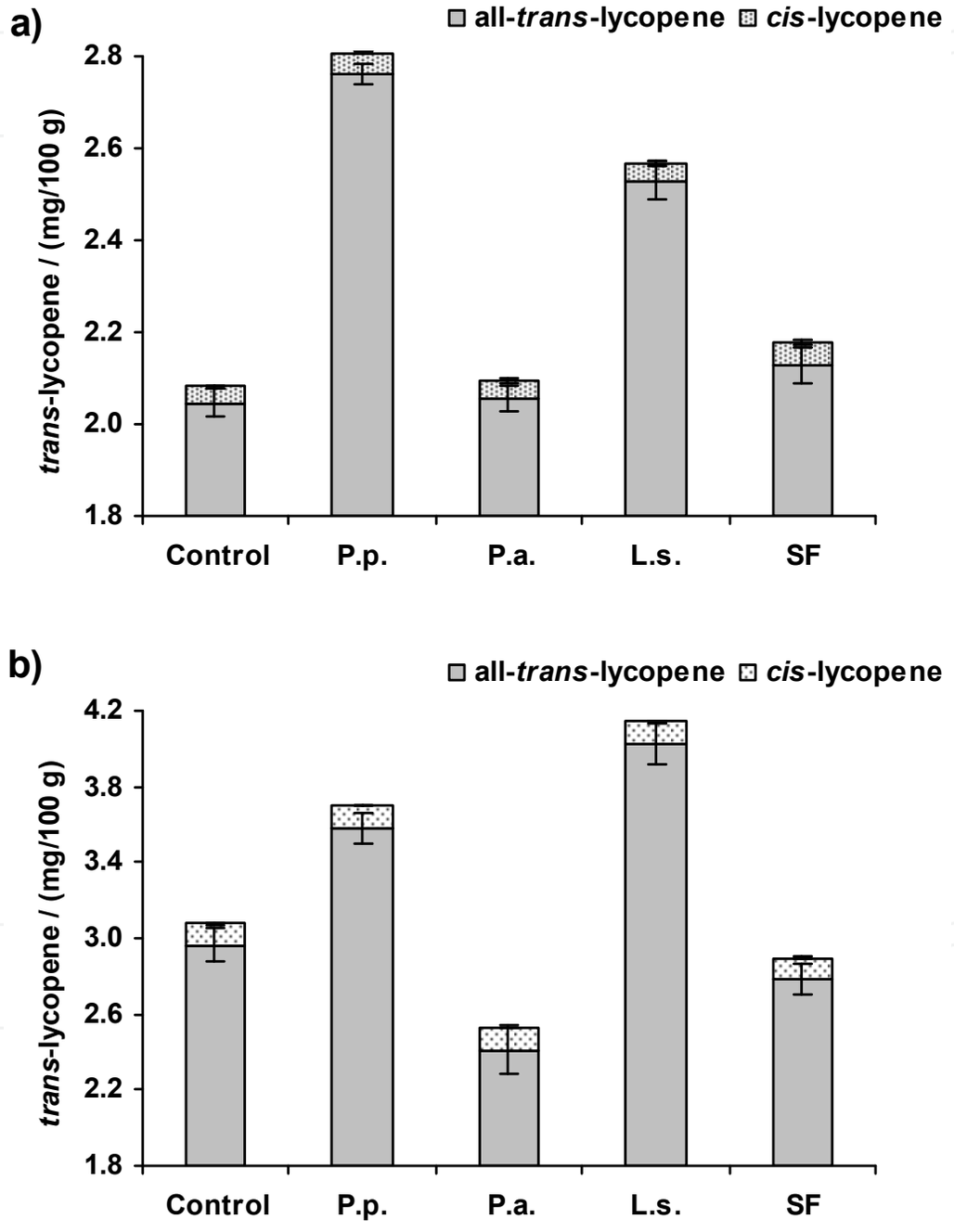

Figure 3. Content of all-*trans*- and *cis*-lycopene in fermented tomato of var. Cunero (a) and var. Ronaldo (b) products. Samples: Control – untreated tomato pulp; tomato products fermented with: P.p. – *P. pentosaceus*, P.a. – *P. acidilactici*, L. s. – *L. sakei*; SF – spontaneous fermented.

The control samples of var. Ronaldo had 3.3-fold higher *cis*-lycopene (3.4 mg/kg) compared to var. Cunero (11.3 mg/kg) (Fig. 3). Fermentation by *P. pentosaceus* or *L. sakei* increased the *cis*-lycopene contents on average by 30.6 and 8.5%, respectively in the products of var. Cunero and var. Ronaldo. A lower increase in *cis*-lycopene was noticed during fermentation of the var. Cunero tomatoes with *P. acidilactici* as well as during spontaneous fermentation (an average increase of 9%). Similarly, lactofermentation using *P. acidilactici* and *L. sakei* increased the *cis*-lycopene contents by 5.8% on average in the tomato products [75].

The fermentation of var. Cunero and var. Ronaldo tomatoes by *P. pentosaceus* and *L. sakei* produced an average of 22.2% more all-*trans*-lycopene compared to the controls (204.6 and 296.0 mg/kg, respectively) (Figure 3) [75].

The *cis*/*trans* ratio of var. Cunero and var. Ronaldo tomatoes were 1.67 and 3.81, respectively. The highest *cis*/*trans* ratio was found in the var. Cunero samples fermented by *L. sakei* (2.08), following that of var. Ronaldo samples fermented by *P. acidilactici* (4.90) and spontaneous fermentation (4.09) [75].

It is known from the literature that in human subjects, lycopene from *cis*-isomer-rich tomato sauce is more bioavailable than that from all-*trans*-rich tomato sauce [91]. Because of the positive effect of lactofermentation on the *cis*/*trans* lycopene ratio, fermented products of the var. Ronaldo tomato, fermented with *P. acidilactici* or *L. sakei*, could be recommended as more biologically accessible products with greater functional value.

6.4. Colour characteristics of fermented tomato products

The results from our analysis of the red (a*) and yellow (b*) colour coordinates of fermented tomato products are presented in Table 2. No relation was found in the var. Cunero samples between the yellow colour coordinate (b*) and total carotenoid, lycopene or β-carotene contents ($p > 0.05$) (Table 2). However, the red colour coordinate (a*) slightly correlated ($R^2 = 0.672$) with β-carotene content.

In contrast, a weak relation was noticed between colour coordinate b* of var. Ronaldo and total carotenoid or β-carotene contents ($R^2 = 0.581$ or $R^2 = 0.596$, respectively) (Table 2). In addition, samples of this variety showed a strong relation between colour coordinate b* and lycopene content ($R^2 = 0.825$, $p = 0.03$). No significant relations were observed between a* and β-carotene or lycopene contents ($p > 0.05$) (Table 2) or between total carotenoids and the colour tone (h^o) or colour purity (C) values of the var. Cunero and var. Ronaldo samples (Table 3) [75].

The best estimation for β-carotene content was obtained using the b* chromaticity value from the whole fruit measurements or the transformed a^{*2} value from the pure measurements [91]. Neither model, however, could explain more than 55% of the variation in β-carotene levels, suggesting that chromaticity values may not be appropriate for estimating tomato β-carotene content. It has been stated that the inspection of different chromaticity values and regression models suggest that colorimeter readings may not be highly useful for estimating β-carotene content in the tomato fruit [92].

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Samples	var. Cunero			var. Ronaldo		
	a*	b*	a*/b*	a*	b*	a*/b*
K	13.97±1.3c	15.47±0.8a	0.903	13.84±0.9c	16.71±1.1b	0.828
P.p.	14.57±1.1d	16.46±1.3b	0.885	15.15±0.8e	18.28±0.9c	0.829
P.a.	11.41±0.9a	17.29±1.3bc	0.660	13.38±1.1b	19.37±1.3d	0.691
L. s.	13.03±1.1b	15.09±1.3a	0.864	13.85±0.5c	15.54±0.7a	0.891
SP	13.44±1.3b	19.13±1.4d	0.703	14.26±1.2d	19.54±1.3d	0.730
a; b and a/b correlation with total carotenoid content						
R	0.3905	0.04763	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	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 means followed by standard deviations (n = 3). Means within a column with different superscript letters are significantly different ($p < 0.05$).

Samples: control – untreated tomato pulp; tomato products fermented with: P.p. – *P. pentosaceus,* P.a. – *P. acidilactici L.,* s. – *L. sakei;* SF – spontaneous fermented; R^2 – correlation coeficient.

Table 2. Colour coordinates (a*, b*) of tomato var. Cunero and var. Ronaldo samples and their correlations between total carotenoids, lycopene and β-carotene contents

Samples	var. Cunero		var. Ronaldo	
	C	h°	C	h°
Control	22.01±2.3b	47.03±3.1a	21.97±2.4b	46.85±2.4a
P.p.	21.93±1.5b	48.34±3.2bc	23.75±1.9c	50.11±1.9c
P.a.	20.77±1.7a	56.60±2.7de	23.44±1.8c	55.30±2.9d
L.s.	20.04±1.3a	49.46±2.4c	20.99±1.6ab	48.52±1.4b
SP	23.24±2.1c	55.20±1.7d	24.35±1.3cd	53.66±2.3d
Correlation with total caratenoid content				
R	0.00000565	0.2332	0.4974	0.2043
p	0.9970	0.4099	0.1834	0.4448

The numbers are means followed by standard deviations (n = 3). Means within a column with different superscript letters are significantly different ($p < 0.05$).

Samples: control – untreated tomato pulp; tomato products fermented with: P.p. – *P. pentosaceus,* P.a. – *P. acidilactici L.,* s. – *L. sakei;* SF – spontaneous fermented; R^2 – correlation coeficient.

Table 3. Colour tone (h°) and purity (C) of tomato var. Cunero and var. Ronaldo samples and their correlation with total carotenoid contents

The overall results indicate that lycopene content could be measured simply and quite accurately across a wide range of tomato genotypes using chromaticity values taken from fruit puree [91]. In contrast, Liu et al. [93] reported that treating tomatoes with a daily light treatment enhances exocarp lycopene accumulation with minimal effect on the colour. Arias et al. [59] also observed that the b* characteristic was not appropriate for predicting the lycopene content of tomatoes.

According to the obtained results, colour tone (h^o) and purity (C) are not suitable indicators of the total carotenoid content in the evaluation of tomato products. We postulate that measuring the yellow coordinate (b*) could be a simple and non-destructive method for predicting lycopene concentration in tomato products [94].

7. The use of tomato additives fermented with *Pediococcus pentosaceus* KTU05-9 and *Lactobacillus sakei* KTU05-6 to improve the quality of ready-to-cook minced meat products

The influence of lactic acid fermentation with BLIS-producing lactobacilli (*Pediococcus pentosaceus* KTU05-9, *Lactobacillus sakei* KTU05-6) on the parameters of tomato powder and the impact of fermented tomato products on the acceptability, colour characteristics and carotenoid content of ready-to-cook minced pork meat products (RCMP) have been investigated [95]. In this experiment used tomato powder was obtained from "Obipectin AG" (Bischofszell, Switzerland). The lactic acid bacteria (LAB) P. pentosaceus KTU05-9 and Lactobacillus sakei KTU05-6, previously isolated from spontaneous rye sourdoughs [96] revealed antimicrobial activity against undesirable microorganisms in the food industry by producing organic acids and BLIS [97,98,67] were used for tomato powder fermentation. The LAB were stored at –70 °C and cultured at temperatures of 35 °C (KTU05-9) or 30 °C (KTU05-6) for 48 h in MRS broth (CM0359, Oxoid Ltd, Hampshire, UK) supplemented with 40 mmol L^{-1} fructose and 20 mmol L^{-1} maltose. Solid state fermentation of tomato powder was used [95].

The cell growth results observed at 48 h of fermentation in tomato media are presented in Figure 4. We found the highest amount of LAB in samples treated with *L. sakei* (8.15 $\log_{10}$ CFU g^{-1}). The spontaneously fermented samples yielded 6.69 $\log_{10}$ CFU g^{-1} of LAB. The lowest amount of LAB was found in samples fermented with *P. pentosaceus* (4.58 $\log_{10}$ CFU g^{-1}).

Different substrates may affect microorganism growth and metabolism [99]. High viable counts are necessary to obtain the desired acid production and pH reduction, which affects the organoleptic properties and shelf-life of the products while preventing contamination. However, the success of fermented products does not rely solely on the ability to provide enough LAB cells; in addition, the consumer must find these organoleptic properties acceptable, which is related in many cases to the organic acid content. We found the lowest pH after 48 h of fermentation in samples fermented with *P. pentosaceus* (pH = 4.1) (Figure 5). Samples fermented with *L. sakei* or through spontaneous fermentation had a pH of 4.16 [95].

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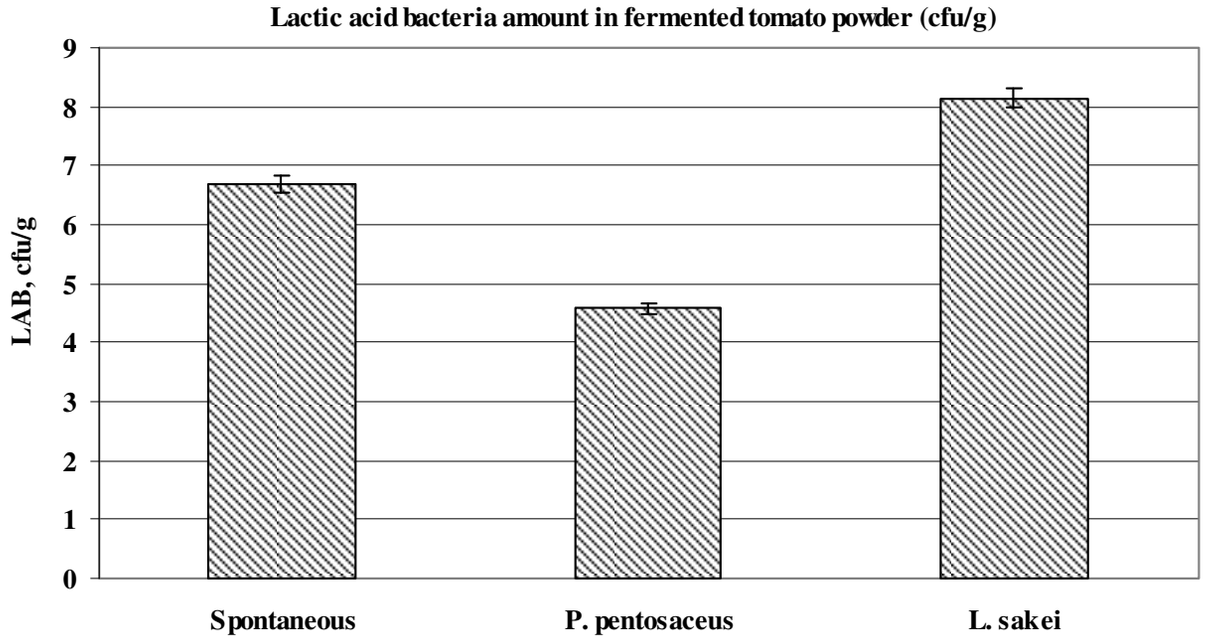

Figure 4. Lactic acid bacteria (LAB) amount (cfu/g) in fermented tomato powder (Samples: Spontaneous – tomato powder fermented spontaneous; P. pentosaceus - tomato powder fermented with *P. pentosaceus;* L. sakei – tomato powder fermented with *L. sakei*; $p < 0.05$).

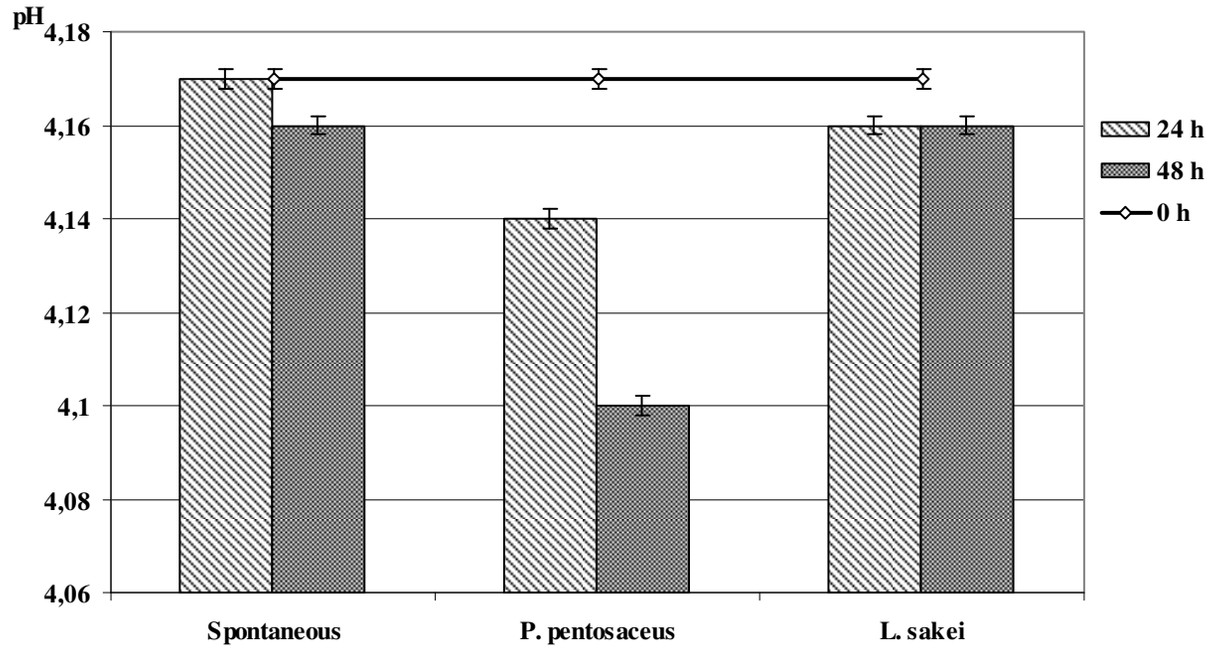

Figure 5. pH of fermented tomato powder (Samples: Spontaneous – tomato powder fermented spontaneous; P. pentosaceus - tomato powder fermented with *P. pentosaceus;* L. sakei – tomato powder fermented with *L. sakei*).

7.1. Colour parameter relation with carotenoid content in fermented tomato products

By influencing consumer choice and preferences, colour is an important quality attribute in the food and bioprocessing industries. Food colour is governed by the chemical, biochemical, microbial and physical changes that occur during growth, maturation, post-harvest handling and processing. Measuring the colour of food products has been used as an indirect measure of other quality attributes, such as flavour and pigment contents, because it is simple, fast and correlates well with other physicochemical properties [100]. We found that fermentation influenced the colour characteristics and carotenoid content of tomato products (Figure 6) [95]. The highest concentration of carotenoids was found in samples fermented with LAB starters (*P. pentosaceus, L. sakei*). Spontaneous fermentation also increased the content of carotenoids in the tomato samples, but not as effectively (the total carotenoid content in the spontaneously treated samples was 54.78 mg/100 g). A strong and significant relation was found between colour tone (ho) and lycopene content and between colour tone (h°) and total carotenoid content ($R^2 = 0.9045$; $p = 0.0489$ and $R^2 = 0.9035$; $p = 0.0495$, respectively). We found correlations ranging from 0.8922 to 0.5091 between others colour characteristics and β-carotene, lycopene and total carotenoid content, but they were not significant [101].

The beneficial effects of lycopene on health have been reviewed [102-104]. According to our results, fermentation with *L. sakei* and *P. pentosaceus* increases the carotenoid concentration in tomato products by two-fold. We did not study the effects on tomato product fermentation using different LAB starters, and more research is needed to explain the mechanism of increasing carotenoids in fermented tomato products [95].

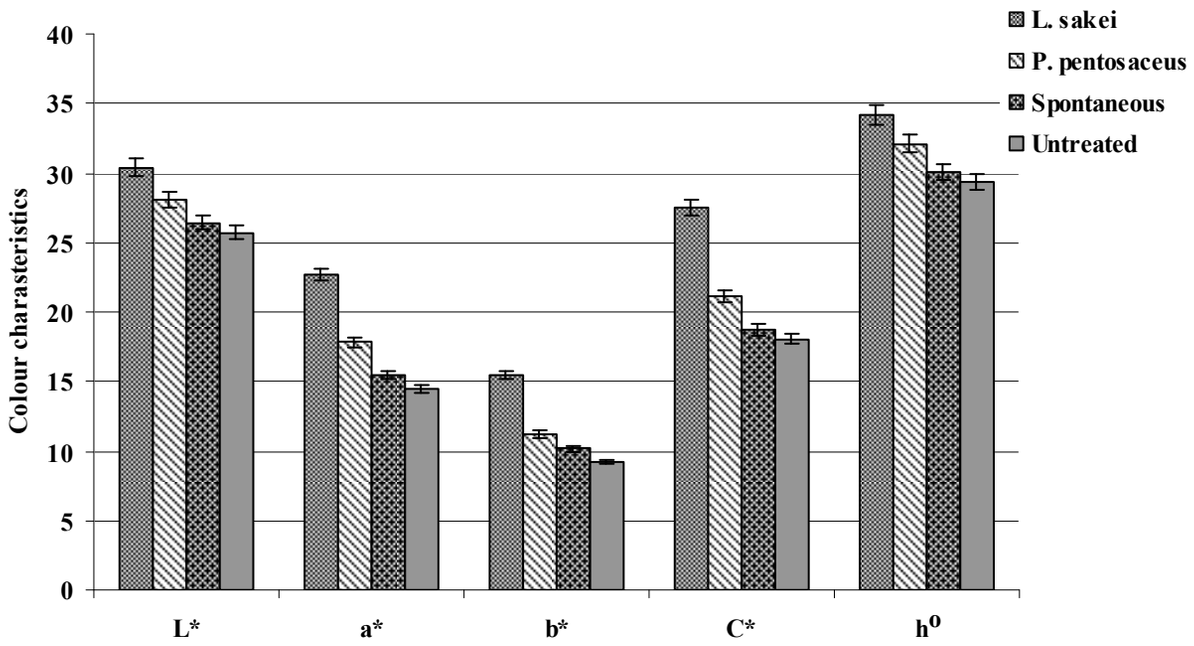

Figure 6. Colour characteristics and carotenoids content (mg/100 g) of fermented and untreated tomato products (Samples: Untreated – untreated tomato powder; Spontaneous – tomato powder fermented spontaneous; P. pentosaceus - tomato powder fermented with *P. pentosaceus*; L. sakei – tomato powder fermented with *L. sakei*; $p < 0.05$).

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7.2. The influence of fermented tomato additives on the acceptability of Ready-to-cook Minced Meat Products (RCMP)

We found significant differences in the acceptability of RCMP with and without 10 or 30 % tomato powder treated with different LAB (*L. sakei* KTU05-06, *P. pentosaceus* KTU05-09) or spontaneous fermentation (Figure 7) [95]. The highest RCMP acceptability was found with 10 % L. sakei fermented tomato powder (an average score of 9.38). Control samples (without additives) were found to be less acceptable (an average score of 5.86) compared to samples with 10 % fermented tomato additives. Ready-to-cook minced pork meat products with 30 % additive were found to be less acceptable than samples with 10 % additive. Compared to samples with 30 % additive, the most acceptable samples were those without fermented tomato products (an average score of 7.72) [95].

7.3. The influence of fermented tomato additives on the colour characteristics of Ready-to-cook Minced Meat Products (RCMP), and the influence of carotenoid content on thermal-treated and untreated RCMP

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) previously endorsed the use of lycopene (both natural and synthetic) as a food colour at its eighth, eighteenth, and twenty-first meetings [106-108] but was not able to establish an Acceptable Daily Intake (ADI) due to the limited information available. At its sixty-seventh meeting, JECFA agreed that both synthetic lycopene and lycopene extracted from *Blakeslea trispora* are acceptable as food colours and established a group ADI of 0-0.5 mg/kg bw/day for both preparations [109]. Adding tomato, tomato products or lycopene to meat could lead to products with health benefits. Few studies have been reported regarding the use of tomato products or lycopene in meat products. Candogan [110] reported on the use of tomato paste in beef patties, while Deda, Bloukas, and Fista [111] investigated its use in frankfurters. Calvo et al. [112] reported on the use of lycopene from tomato peel in dry fermented sausages. However, we could not find data on tomato product fermentation with different LAB starters or how fermented tomato products influence RCMP quality parameters [95].

We found that the addition of tomato products significantly affected ($p < 0.05$) all colour parameters (Table 4) of the final product (thermal-treated and untreated). The controls had the highest ($p < 0.05$) lightness and the lowest ($p < 0.05$) redness and yellowness as a consequence of lower hue angle and saturation index. These tendencies were found for both thermal-treated and untreated products [95].

High variation in the colour parameters of fermented meat products has been reported [113-115]. These variations could be due to the calibration plate used in the determinations, the composition of the meat products, the size of the meat particles and the ripening time.

Furthermore, the addition of tomato products affects the carotenoid content of RCMP (Table 5) [95]. We found that thermal treatment decreases the carotenoid concentration in RCMP. After thermal treatment, we found 23.71 and 52.03 % less ß-carotene (in samples with 10 % spontaneously treated products and in samples with 30 % L. sakei-fermented tomato products, respectively). Additionally, 10.78 and 50.00 % less lycopene was found in samples with 30 %

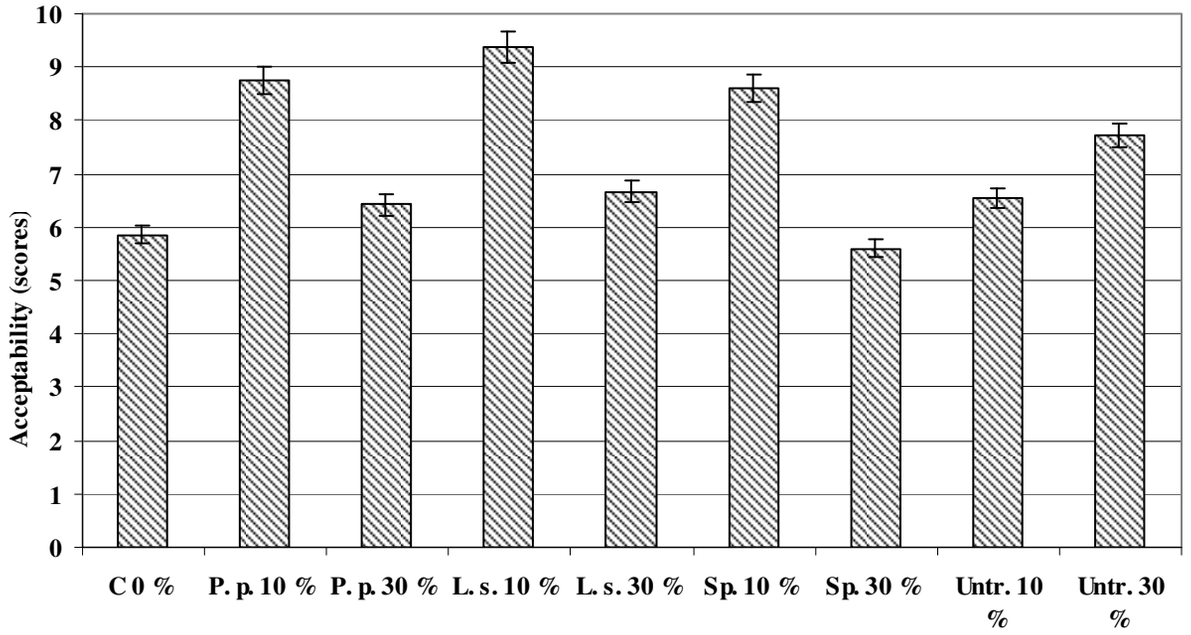

Figure 7. Acceptability of ready-to- cook minced meat products (RCMP) (Samples: C0 % - RCMP without tomato products; P.p. 10 % - RCMP with 10 % with P. pentosaceus fermented tomato products; P.p. 30 % - RCMP with 30 % with P. pentosaceus fermented tomato products; L.s. 10 % - RCMP with 10 % with L. sakei fermented tomato products; L.s. 30 % - RCMP with 30 % with L. sakei fermented tomato products; Sp. 10 % - RCMP with 10 % spontaneous fermented tomato products; Sp. 30 % - RCMP with 30 % spontaneous fermented tomato products; Untr. 10 % - RCMP with 10 % untreated tomato powder; Untr. 30 % - RCMP with 30 % untreated tomato powder; $p > 0.05$)

spontaneously treated products and in samples with 30 % untreated tomato products, respectively, and as a consequence, the highest loss of total carotenoid content was found in samples with 30 % untreated tomato product (49.25 %) [95].

RCMP samples	L*	a*	b*	C	h°
Thermal treated					
P. p. 30 %	45.64±0.11^{a}	19.09±0.21^{a}	28.16±0.69^{a}	34.02±0.71^{a}	55.87±0.96^{a}
P. p. 10 %	52.76±0.18^{c}	12.8±0.81^{b}	23.05±0.52^{b}	29.02±0.32^{b}	63.83±0.78^{b}
L. s. 30 %	47.96±0.20^{c}	19.27±0.93^{a}	29.93±0.71^{a}	35.60±0.23^{a}	57.22±0.63^{a}
L. s. 10 %	48.45±0.19^{c}	9.31±0.74^{c}	21.89±0.64^{b}	23.79±0.18^{c}	66.96±0.88^{b}
Sp. 30 %	44.54±0.23^{a}	12.01±0.63^{b}	20.33±0.55^{b}	19.02±0.13^{c}	54.32±0.49^{a}
Sp. 10 %	43.51±0.11^{a}	8.21±0.32^{b}	17.75±0.39^{c}	16.57±0.42^{d}	52.65±0.55^{a}
Untr. 30 %	42.10±0.25^{a}	7.56±0.41^{c}	14.24±0.44^{c}	14.99±0.54^{d}	53.66±0.86^{a}
Untr. 10 %	42.14±0.17^{b}	4.32±0.37^{d}	12.32±0.60^{d}	15.01±0.30^{d}	52.75±0.97^{a}
C 0%	60.07±0.43^{d}	2.41±0.30^{d}	11.84±0.22^{d}	12.99±0.21^{e}	82.71±0.60^{d}
Thermal untreated					
P. p. 30 %	46.03±0.25^{a}	17.27±0.51^{b}	26.90±0.74^{a}	31.97±0.65^{a}	57.30±0.40^{d}
P. p. 10 %	50.64±0.19^{b}	12.90±0.72^{a}	28.96±0.83^{a}	31.70±0.82^{a}	65.99±0.93^{c}

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RCMP samples	L*	a*	b*	C	h°
L. s. 30 %	45.63±0.63[a]	18.08±0.61[d]	27.60±0.52[a]	32.99±0.74[b]	56.77±0.68[b]
L. s. 10 %	50.9±0.79[a]	12.14±0.21[a]	28,50±0.48[a]	30.98±0.65[a]	66.93±0.88[c]
Sp. 30 %	47.23±0.28[a]	15.23±0.34 [a]	25.36±0.39[b]	29.59±0.54[c]	62.39±0.45[a]
Sp. 10 %	45.21±0.41[a]	14.02±0.19 [a]	21.45±0.41[d]	28.96±0.91[c]	61.33±0.46[a]
Untr. 30 %	46.27±0.52[a]	13.25±0.16[a]	21.55±0.57[d]	28.69±0.58[c]	61.45±0.41[a]
Untr. 10 %	44.25±0.39[a]	11.03±0.11[c]	19.56±0.59[d]	26.98±0.62[d]	60.84±0.93[a]
C 0%	58.38±0.48[c]	3.93±0.18[e]	15.33±0.78[e]	25.63±0.61[d]	81.18±0.54[e]

Samples: C 0 % - RCMP without tomato products; P.p. 10 % - RCMP with 10 % with *P. pentosaceus* fermented tomato products; P.p. 30 % - RCMP with 30 % with *P. pentosaceus* fermented tomato products; L.s. 10 % - RCMP with 10 % with *L. sakei* fermented tomato products; *L.s.* 30 % - RCMP with 30 % with *L. sakei* fermented tomato products; Sp. 10 % - RCMP with 10 % spontaneous fermented tomato products; Sp. 30 % - RCMP with 30 % spontaneous fermented tomato products; Untr. 10 % - RCMP with 10 % untreated tomato powder; Untr. 30 % - RCMP with 30 % untreated tomato powder.

Means in column with common letter are not different (p> 0.05).

Table 4. Colour coordinates (a*, b*), L* - lightness, colour tone (h°) and purity (C) of thermal treated (10 min in 100 °C temperature water) and untreated ready- to-cook minced meat products (RCMP)

Samples	ß–carotene	Lycopene	Total carotenoids content
	mg/100 g		
Thermal untreated			
Untr. 10%	0.19±0.02[a]	0.48±0.06[a]	0.67 [a]
Untr. 30%	0.25±0.01[b]	1.46±0.10[b]	1.71[b]
Sp. 10%	0.69±0.02[c]	1.02±0.09[b]	1.71[b]
Sp. 30%	0.97±0.02[c]	2.13±0.15[c]	3.1[c]
L. s. 10%	1.23±0.09[d]	3.59±0.21[d]	4.82[c]
L. s. 30%	1.95±0.08[e]	9.66±0.17 [e]	11.61[e]
P. p. 10%	1.01±0.07[d]	3.67±0.21[d]	4.68[d]
P. p. 30%	1.76±0.03[e]	10.32±0.11[e]	12.08[e]
Thermal treated			
Untr. 10%	0.10±0.02[a]	0.24±0.07[a]	0.34[a]
Untr. 30%	0.15±0.04[a]	0.93±0.05[b]	1.08[b]
Sp. 10%	0.49±0.07[b]	0.91±0.06[b]	1.40[b]
Sp. 30%	0.74±0.04[c]	1.75±0.09[c]	2.49[c]
L. s. 10%	0.59±0.06[b]	2.15±0.13[c]	2.74[c]
L. s. 30%	1.47±0.08[d]	7.32±0.14[d]	8.79[d]

Samples	ß–carotene	Lycopene	Total carotenoids content
	mg/100 g		
P. p. 10%	0.76±0.06[c]	2.62±0.20[c]	3.38[c]
P. p. 30%	1.17±0.10[d]	6.31±0.26[d]	7.48[d]

Samples: C0 % - RCMP without tomato products; P.p. 10 % - RCMP with 10 % with *P. pentosaceus* fermented tomato products; P.p. 30 % - RCMP with 30 % with *P. pentosaceus* fermented tomato products; L.s. 10 % - RCMP with 10 % with *L. sakei* fermented tomato products; *L.s.* 30 % - RCMP with 30 % with *L. sakei* fermented tomato products; Sp. 10 % - RCMP with 10 % spontaneous fermented tomato products; Sp. 30 % - RCMP with 30 % spontaneous fermented tomato products; Untr. 10 % - RCMP with 10 % untreated tomato powder; Untr. 30 % - RCMP with 30 % untreated tomato powder.

Means in column with common letter are not different (p> 0.05).

Table 5. ß–carotene, lycopene and total carotenoids content in thermal treated and untreated *ready- to-cook minced meat products (RCMP)*

8. Conclusions

Lactic acid fermentation represents the easiest and most suitable way to increase daily consumption of nearly fresh fruits and vegetables. The health and safety of the products can be aided by the development of starter cultures. Progress in the field of antimicrobial LAB strains with multi-functional properties, including the degradation of mycotoxins, can be engineered to significantly improve the quality, safety and acceptability of plant foods.

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

The results of our tomato product colour analysis offered the possibility of evaluating the level of lycopene using the yellow colour characteristic of tomato products; this method was reproducible and accurate enough to substitute for the chemical extraction determinations and may be a useful tool for the tomato industry.

The direct use of *Pediococcus pentosaceus* KTU05-9 and *Lactobacillus sakei* KTU05-6 for tomato product fermentation increases the carotenoid content in tomato products, which is a beneficial additive that improves the colour, functional value and acceptability of ready-to-cook minced meat products. Ready-to-cook minced meat products that have been enriched with carotenoids, which lend good sensory quality and are produced to contain a high level of lycopene and ß-carotene, can increase the intake of carotenoids in the diet. This is the first time that selected lactobacilli-fermented tomato products have been used as source of lycopene and ß-carotene for food, and more research is needed to explain the mechanism of carotenoid increase in fermented tomato products.

All the tested LAB produced a mixture of L- and D-lactic acid, with the latter isomer at a lower level. Because of the potential toxicity of D-lactic acid in food, we report that the tomato products prepared using pure cultures of tested LAB were found in all cases to be safer than those treated by spontaneous fermentation.

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References

[1] Klaenhammer T.R., Kullen M.J. Selection and design of probiotics. International Journal of Food Microbiology 1999;50 45-57.

[2] The World Health Organization (WHO) (www.who.int) (accessed 3 September 2014).

[3] Zia-ur-Rehman Z., Islam M., Shah W.H. Effect of microwave and conventional cooking on insoluble dietary fibre components of vegetables. Food Chemistry 2003;80 237-240.

[4] Zhang D.L., Hamauzu Y. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry 2004;88 503- 509.

[5] Devlieghere F., Vermeiren L., Debevere J. New preservation technologies: possibilities and limitations. International Dairy Journal 2004;14 273-285.

[6] Gómez-López V.M., Devlieghere F., Bonduelle V., Debevere J. Intense light pulses decontamination of minimally processed vegetables and their shelf-life. International Journal of Food Microbiology 2005;103 79-89.

[7] Elmnasser N., Guillou S., Leroi F., Orange N., Bakhrouf A., Federighi M. Pulsed-light system as a novel food decontamination technology: a review. Canadian Journal of Microbiology 2007;58 813-821.

[8] Liu S., Han Y., Zhou Z. Lactic acid bacteria in traditional fermented Chinese foods. Food Research International 2011;44 643-651.

[9] Juodeikiene G., Bartkiene E., Viskelis P., Urbonaviciene D., Eidukonyte D., Bobinas C. Advances in Applied Biotechnology. In: Petre M. (ed.) Fermentation Processes Using Bacteriocins Producing Lactic Acid Bacteria for Biopreservation and Improving Functional Properties of Food Products. Rijeka: InTech; 2011. p63-100.

[10] Sanchez B., Ruiz L., Gueimonde M., Ruas-Madiedo P., Margolles A. Toward improving technological and functional properties of probiotics in foods. Trends in Food Science and Technology 2012;26 56-63.

[11] Clark J.P. Fermentation - new products from an old process. Food Technology 2004;58 75-76.

[12] Bourdichon F., Casaregola S., Farrokh C., Frisvad J.C., Gerds M.L., Hammes W.P., Harnett J., Huys G., Laulund S., Ouwehand A., Powell I.B., Prajapati J.B., Seto Y., Ter Schure E., Van Boven A., Vankerckhoven V., Zgoda A., Tuijtelaars S., Hansen E.B. Food fermentations: microorganisms with technological beneficial use. International Journal of Food Microbiology 2012;154 87-97.

[13] Caplice E., Fizgerald G. F. Food fermentations: Role of microorganisms in food production and preservation. International Journal of Food Microbiology 1999;50(1-2) 131-149.

[14] Ross R. P., Preservation and fermentation: past, present and future. International Journal of Food Microbiology 2002;79 3-16.

[15] Gaggia F., Di Gioia D., Baffoni L., Biavati B. The role of protective and probiotic cultures in food and feed and their impact on food safety. Trends in Food Science and Technology 2011;22 58-66.

[16] Adams M., Mitchell R. Fermentation and pathogen control: a risk assessment approach. International Journal of Food Microbiology 2002;79 75-83

[17] Adams M.R., Nicolaides L. Review of the sensitivity of different foodborne pathogens to fermentation. Food Control 2008;8 227-239.

[18] Hammes W.P., Tichaczek P.S. The potential of lactic acid bacteria for the production of safe and wholesome food. Zeitschrift für Lebensmittel-Untersuchung und -Forschung 1994;198 193-201.

[19] van Boekel M., Fogliano V., Pellegrini N., Stanton C., Scholz G., Lalljie S., Somoza V., Knorr D., Jasti P.R., Eisenbrand G. A review on the beneficial aspects of food processing. Molecular Nutrition and Food Research 2010;54 1215-1247.

[20] Poutanen K., Flander L., Katina K. Sourdough and cereal fermentation in an nutritional perspective. Food Microbiology 2009;7 693-699.

[21] Marilley L., Casey M.G. Flavors of cheese products: metabolic pathways, analytical tools and identification of producing strains. International Journal of Food Microbiology 2004;90 139-159.

[22] Smit G., Smit B.A., Engels W.J. Flavor formation by lactic acid bacteria and biochemical flavor profiling of cheese products. FEMS Microbiology Reviews 2005;29 591-610.

[23] Lacroix N., St Gelais D., Champagne C.P., Fortin J., Vuillemard J.C. Characterization of aromatic properties of old-style cheese starters. Journal of Dairy Science 2010;93 3427-3441.

[24] Sicard D., Legras J.L. Bread, beer and wine: yeast domestication in the Saccharomyces sensu stricto complex. Contes rendus biologies 2011;334(3) 229-236.

[25] Steinkraus K.H. Introduction to indigenous fermented foods. In: Steinkraus K. H. (ed.) Handbook of indigenous fermented foods. New York: Marcel Dekker; 1996. p1-6.

[26] Cleveland J., Thomas J.M., Ingolf F.N., Michael L.C. Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology 2001;71 1-20.

[27] Silva J., Carvalho A. S., Teixeira P., Gibbs P. A. Bacteriocin production by spray-dried lactic acid bacteria. Letters in Applied Microbiology 2002;34(2) 77-81.

[28] Zotta T., Parente E., Ricciardi A. Viability staining and detection of metabolic activity of sourdough lactic acid bacteria under stress conditions. World Journal of Microbiology and Biotechnology 2009;25(6) 1119-1124.

[29] Corsetti A., Settanni L., Van Sinderen D. Characterization of bacteriocin-like inhibitory substances (BLIS) from sourdough lactic acid bacteria and evaluation of their in vitro and in situ activity. Journal of Applied Microbiology 2004;96(3) 521-534.

[30] Rodriguez S.J., Alexopoulos L.G., Epperlein J., Samaga R., Lauffenburger D.A., Klamt S., Sorger P.K. Discrete logic modelling as a means to link protein signalling networks with functional analysis of mammalian signal transduction. Molecular System Biology 2009;5 1-19.

[31] Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology 1989;66 365-378.

[32] Vaughan A., Eijsink V.G.H., O'Sullivan T.F., O'Hanlon K., van Sinderen D. An analysis of bacteriocins produced by lactic acid bacteria isolated from malted barley. Journal of Applied Microbiology 2001;91 131-138.

[33] Altuntas E.G., Cosansu S., Ayhan K. Some growth parameters and antimicrobial activity of a bacteriocin-producing strain Pediococcus acidilactici 13. International Journal of Food Microbiology 2010;141 28-31.

[34] Juodeikiene G., Salomskiene J., Basinskiene L., Vidmantiene D., Narbutaite V., Kasnauskyte N. The influence of novel fermented products on wheat bread spoilage and staling. Food Chemistry and Technology 2009;43 36-46.

[35] Karovicova J., Kohajdova Z. Lactic acid fermented vegetable juices. Horticultural Science (Prague). 2003;30(4) 152-158.

[36] Kalantzopoulos G. Fermented products with probiotic qualities. Anaerobe. 1997;3 185-190.

[37] Buckenhüskes H. J. Fermented vegetables. In: Doyle P.D., Beuchat L.R., Montville T.J. (ed.) Food Microbiology: Fundamentals and Frontiers. Washington: ASM Press; 1997. p595-609.

[38] Bartkiene E., Juodeikiene G., Vidmantiene D., Viskelis P., Urbonaviciene D. Nutritional and quality aspects of wheat sourdough bread using L. luteusand L. angustifolius flours fermented by Pedioccocus acidilactici. International Journal of Food Science and Technology 2011;46 1724-1733.

[39] Karocicova J., Drdak M., Greif G., Hybenova E. The choice of strains of Lactobacillusspecies for the lactic acid fermentation of vegetable juices. European Food Research and Technology. 1999;210 53-56.

[40] Buckenhuskes H.J. Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. FEMS Microbiological Reviews 1993;12 253-272.

[41] Maki M. Lactic acid bacteria in vegetables fermentation. In: Salminen S., Von Wright A., Ouwehand A. (ed.) Lactic acid bacteria microbiological and functional aspects. New York: Marcel Dekker; 2004. p419-430.

[42] Jazwiak K.P., Rozmierska J., Chablowska B., Stecka K.M., Skapska S., Kliszcz M., Rzeszowiak E.S. Starter cultures for lactic acid fermentation of sweet pepper, pattypan squash and tomatoes. Polish Journal of Food and Nutrition Science. 2013; 63(2) 95-102.

[43] Peres C. M., Peres C., Hernandez-Mendoza A., Malcata F. X. Review on fermented plant materials as carriers and sources of potentially probiotic lactic acid bacteria – With an emphasis on table olives. Trends in Food Science and Technology 2012;26 31-42.

[44] Cetin B. Production of probiotic mixed pickles (Tursu) and microbiological properties. African Journal of Biotechnology 2011;10 14926-14931.

[45] Montet D., Loisea, G., Kakhia-Rozis N. Microbial technology of fermented vegetables. In: Ray R.C., Ward O.P. (ed.) Microbial Biotechnology in Horticulture. New Hampshire: Science Publishers; 2006; p309-343.

[46] Di Cagno R., Surico R.F., Paradiso A., De Angelis M., Salmon J.-C., Buchin S., De Gara L., Gobbetti M. Effect of autochthonous lactic acid bacteria starters on health-pro-

moting and sensory properties of tomato juices. International Journal of Food Microbiology 2008;128 473-483.

[47] Hotz C., Gibson R.S. Traditional food-processing and preparation pactices to enhance the bioavailability of micronutrients in plant-based diets. Journal of Nutrition 2007;137 1097-1100.

[48] Ranadheera R.D.C.S., Baines S.K., Adams M.C. Importance of food in probiotic efficacy. Food Research International 2010;43 1-7.

[49] Yoon K.Y., Woodams E.E., Hang Y.D. Probiotication of tomato juice by lactic acid bacteria. Journal of Microbiology 2004;42 315-318.

[50] Parrado J., Miramontes E., Jover M., Gutierrez J.F., Collantes de T.L., Bautista J. Preparation of a rice bran enzymatic extract with potential use as functional food. Food Chemistry 2006;98 742-748.

[51] Kim H.Y., Min J.H., Lee J.H., Ji G.E. Growth of lactic acid bacteria and Bifidobacteria in natural media using vegetables, seaweeds, grains and potatoes. Food Science Biotechnology, 2000;9: 322-324.

[52] Rao A.V., Agarwal S. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. Nutritional Research 1999;99 305-323.

[53] Giovannucci E. Tomato products, lycopene and prostate cancer: a review of the epidemiological literature. The Journal of Nutrition 2005;135 2030-2315.

[54] Talvas J., Caris-Veyrat C., Guy L., Rambeau M., Bernard L., Minet-Quinard R., Lobaccaro J.-M.A., Vasson M.-P., George S., Mazur A., Rock E. Differential effects of lycopene consumed in tomato paste and lycopene in the form of a purified extract on target genes of cancer prostatic cells. The American Journal of Clinical Nutrition 2010;91 1716-1724.

[55] Van Breemen R.B., Sharifi R., Viana M., Pajkovic N., Zhu D., Yuan L., Yang Y., Bowen P.E., Stacewicz-Sapuntzakis M. Antioxidant effects of lycopene in African American men with prostate cancer or benign prostate hyperplasia: a randomized, controlled trial. Cancer Prevention Research (Phila.) 2011;4 711-418.

[56] Gardner N.J., Savard T., Obermeier P., Caldwell G., Champagne C.P. Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. International Journal of Food Microbiology 2001;64 261-275.

[57] Richelle M., Bortlik K., Liardet S., Hager C., Lambelet P., Baur M., Applegate L.A., Offord E.A. A food-based formulation provides lycopene with the same bioavailability to humans as that from tomato paste. Journal of Nutrition 2002;132 404-408.

[58] Harris W.M., Spurr A.R. Chromoplasts of tomato fruits. II. The red tomato, American Journal of Botanic 1969;56 380-389.

[59] Arias R., Lee T.C., Logendra L., Janes H. Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. Journal of Agriculture and Food Chemistry 2000;48 1697-1702.

[60] Raffo A., Leonardi C., Fogliano V., Ambrosino P., Salucci M., Gennaro L. Nutritional value of cherry tomatoes (Lycopersicon esculentum Cv. Naomi F1) harvested at different ripening stages. Journal of Agriculture and Food Chemistry 2002;50 6550-6556.

[61] Heredia A., Peinado I., Barrera C., Grau A.A. Influence of process variables on colour changes, carotenoids retention and cellular tissue alteration of cherry tomato during osmotic dehydration. Journal of Food Composition and Analysis 2009;22 285-294.

[62] Melendez-Martinez A.J., Escudero-Gilete M.L., Vicario I.M., Heredia F.J. Study of the influence of carotenoid structure and individual carotenoids in the qualitative and quantitative attributes of orange juice colour. Food Research International 2010;43 1289-1296.

[63] Aymerich T., Picouet P. A., Monfort J. M. Decontamination technologies for meat products, Meat Science 2008;7 114-129.

[64] Zhou G. H., Xu X. L., Liu Y. Preservation technologies for fresh meat – A review. Meat Science 2010;86 119-128.

[65] Stiles M. E., Hastings J. W. Bacteriocin production by lactic acid bacteria: potential for use in meat preservation Trends in Food Science and Technology 1991;2 247-251.

[66] Klaenhammer T. R. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiology Reviews 1993;12 39-86.

[67] Cizeikiene D., Juodeikiene G., Paskevicius A., Bartkiene E. Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. Food Control 2013;31 539-545.

[68] Ross R. P., Morgan S., Hill C. Preservation and fermentation: past, present and future. International Journal of Food Microbiology 2002;79 3-16.

[69] El-Ghaish S., Ahmadova A., Hadji-Sfaxi I., El Mecherfi K.E., Bazukyan I., Choiseta Y., Rabesona H., Sitohy M., Popov Y.G., Kuliev A.A., Mozzig F., Chobert J.-M., Haertle T. Potential use of lactic acid bacteria for reduction of allergenicity and for longer conservation of fermented foods. Trends Food Sciences Technology 2011(22) 509-516.

[70] Peres C.M., Peres C., Hernández-Mendoza A., Malcata F.X. Show more Review on fermented plant materials as carriers and sources of potentially probiotic lactic acid bacteria – With an emphasis on table olives. Trends in Food Science and Technology 2012;26 31-42.

[71] Gebbers J. O. Atherosclerosis, cholesterol, nutrition, and statins – a critical review. German Medical Science 2007;5 1-11.

[72] Di Cagno R., Coda R., De Angelis M., Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology 2013;33 1-10.

[73] Capanoglu E., Beekwilder J., Boyacioglu D., De Vos R.C., Hall R.D. The Effect of Industrial Food Processing on Potentially Health Beneficial Tomato Antioxidants. Critical Reviews in Food Science and Nutrition 2010;50 919-931.

[74] Palozza P., Parrone N., Catalano A., Simone R. Tomato Lycopene and Inflammatory Cascade: Basic Interactions and Clinical Implications. Current Medicinal Chemistry 2010;17 2547-2563.

[75] Bartkiene E., Vidmantiene D., Juodeikiene G., Viskelis P., Urbonaviciene D. Lactic acid fermentation of tomato: effects on cis/trans lycopene isomer and β-carotene concentration and formation of L(+) and D(-)-lactic acid. Food Technology and Biotechnology 2013;51(4) 471-478.

[76] Digaitiene A., Hansen A.S., Juodeikiene G., Eidukonyte D., Josephsen J. Lactic acid bacteria isolated from rye sourdouhs produce bacteriocin-like inhibitory substances active against Bacillus subtilis and fungy. Journal of Applied Microbiology. 2012;112 732-742.

[77] De Lima C.J.B., Coelho L.F., Blanco K.C., Contiero J. Response surface optimization of D(-)-lactic acid production by Lactobacillus SMI8 using corn steep liquor and yeast autolysate as an alternative nitrogen source. African Journal of Biotechnology 2009;8 5842-5846.

[78] McGuire R.G. Reporting of objective color measurements. Horticulture Science 1992;27 1254-1255.

[79] Kedia G., Vázquez J.A., Pandiella S.S. Fermentability of whole oat flour, PeriTec flour and bran by Lactobacillus plantarum. Journal of Food Enginiering 2008;89 246-249.

[80] Shah N.P. Functional Foods from probiotics and prebiotics. Food Technology 2001;55 46-53.

[81] Trachoo N., Boudreaux C., Moongngarm A., Samappito S., Gaensakoo R. Effect of germinated rough rice media on growth of selected probiotic bacteria. Pakistan Journal of Biological Science 2006;9 2657-2661.

[82] Steinkraus K.H. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control 1997;8 311-317.

[83] Sindhu S.C., Khetarpaul N. Probiotic fermentation of indigenous food mixture: effect on antinutrients and digestibility of starch and protein. Journal of Food Composition and Analysis 2001;14 601-609.

[84] Gartner C., Stahl W., Sies H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes American Journal of Clinical Nutrition 1997;66 116-22.

[85] Hartmann M.H. Biopolymers from Renewable Resources. In: Kaplan D.L. (ed.) Berlin: Springer. 1998; p367-411.

[86] Li Y.B., Cui F. Sustainable Biotechnology: Sources of Renewable Energy. In: Singh O., Harvey S. (ed.) Dordrecht: Springer. 2010; p211-228.

[87] Lee C.W. Production of D-lactic acid by bacterial fermentation of rice. Fiber and Polymers 2007;8 571-578.

[88] Nguyen M.L., Schwartz S.J. Lycopene: chemical and biological properties. Food Technology 1999;53(2) 38-45.

[89] Camara M., Matallana M.C., Sánchez-Marta M.C., Liko Ayne R., Labra E. Lycopene and hydroxymethylfurfural (HMF) evaluation in tomato products. Acta Horticulturale 2003;613 365-371.

[90] Gould W.A. Quality evaluation of processed tomato juice. Journal of Agricultural and Food Chemistry 1978;26 1006-1011.

[91] Unlu N.Z., Bohn T, Francis D.M., Nagaraja H.N., Clinton S.K., Schwartz S.J. Lycopene from heat-induced cis-isomer-rich tomato sauce is more bioavailable than from all-trans-rich tomato sauce in human subjects. British Journal of Nutrition 2007;98 140-146.

[92] Hyman R.J., Gaus J., Foolad R.M. A rapid and accurate method for estimating tomato lycopene content by measuring chromaticity values of fruit puree. Journal of the American Society for Horticultural Science 2004;129(5) 717-723.

[93] Liu L.H., Zabaras D., Bennett L.E., Aguas P., Woonton B.W. Effects of UV-C, red light and sun light on the carotenoid content and physical qualities of tomatoes during post-harvest storage. Food Chemistry 2009;115 495-500.

[94] Fernandez-Ruiz V., Torrecilla J.S., Camara M., Mata M.C., Shoemaker C. Radial basis network analysis of color parameters to estimate lycopene content on tomato fruits. Talanta 2010;83 (2010) 9-

[95] In press: Bartkiene E., Juodeikiene G., Vidmantiene D., Viskelis P., Urbonaviciene D. The use of fermented with Pediococcus pentosaceus KTU05-9 and Lactobacillus sakei KTU05-6 tomato additives for ready-to- cook minced meat products quality improving. 2015.

[96] Parrado J., Miramontes E., Jover M., Gutierrez J.F., Collantes de Teran L., Bautista J. Preparation of a rice bran enzymatic extract with potential use as functional food. Food Chemistry 2006;98 742-748.

[97] Yun J.S., Wee Y.J., Kim J.N., Ryu H.W. Fermentative production of dl-lactic acid from amylase-treated rice and wheat brans hydrolyzate by a novel lactic acid bacterium Lactobacillus spp., Biotechnol. Lett. 2004;26 1613-1616.

[98] Digaitiene A., Hansen A.S., Juodeikiene G., Eidukonyte D., Josephsen J. Lactic acid bacteria isolated from rye sourdoughs produce bacteriocin-like inhibitory substances

active against Bacillus subtilis and fungi. Journal of Applied Microbiology 2012;112 732-742.

[99] Rathore S., Rathore S., Salmeron I., Pandiella S.S. Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. Food Microbiology 2012;30 239-244.

[100] Pathare P. B., Opara U. L., Al-Julanda Al-Said F. Colour Measurement and Analysis in Fresh and Processed Foods: A Review. Food and Bioprocess Technology 2013;6 36-60.

[101] Urbonaviciene D., Viskelis P., Viskelis J., Jankauskiene J., Bobinas C. Lycopene and β-carotene in non-blanched and blanched tomatoes. Journal of Food, Agriculture and Environment 2012;10 (2): 142-146.

[102] Choski P. M., Joshi V. Y. A review on lycopene – Extraction, purification, stability and applications. International Journal of Food Properties 2007;10 289-298.

[103] Kavanaugh C. J., Trumbo P. R., Ellwood K. C. The US food and drug administration's evidence-based review for qualified health claims: Tomatoes, lycopene and cancer. Journal of the National Cancer Institute 2007;99 1074-1085.

[104] Khachik F. Carvalho L., Bernstein P.S., Muir G.J., Zhao D.Y., Katz N.B. Chemistry, distribution and metabolism of tomato carotenoids and their impact in human health. Experimental Biological and Medicine 2002;227 845-851.

[105] Agarwal S., Rao A.V. Tomatoes, lycopene and human health and chronic disease. CMAJ 2000;163(6) 739-44.

[106] FAO/WHO, 1965. Specification for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants (Eighth report on the Joint FAO/WHO Expert Committee on Food Additives). Geneva, World Health Organization (WHO Technical Report Series, No. 309).

[107] FAO/WHO, 1975. Evaluation of certain food additives (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva, World Health Organization (WHO Technical Report Series, No. 557).

[108] FAO/WHO, 1978. Evaluation of certain food additives (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva, World Health Organization (WHO Technical Report Series, No. 617).

[109] FAO/WHO, 2006. Evaluation of certain food additives (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). Geneva, World Health Organization (WHO Technical Report Series, No. 940).

[110] Candogan K. The effect of tomato paste on some quality characteristics of beef patties during refrigerated storage. European Food Research and Technology 2002;215 305-309.

[111] Deda M. S., Bloukas J. G., Fista G. A. Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters. Meat Science 2007;76 501-508.

[112] Calvo M. M., Garcia M. L., Selgas M. D. M. Dry fermented sausages enriched with lycopene from tomato peel. Meat Science 2007;80(2) 167-172.

[113] Gimeno O., Astiasaran I., Bello J., Calcium ascorbate as a potential partial substitute for NaCl in dry fermented sausage: Effect on colour, texture and hygienic quality at different concentration. Meat Science 2001;57 23-29.

[114] Jakobsen M.,. Bertelsen G. Colour in food. Modelling colour stability. London: Woodhead Publishing Limited. 2002. p233–247.

[115] Perez-Alvarez J. A. Physicochemical characteristics of spanishtype dry-cured sausage. Food Research International 1999;32 599-607.