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Starter Cultures for Lactic Acid Fermentation of Sweet Pepper, Pattypan Squash and Tomatoes

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The aim of this study was to obtain starter cultures for fermenting sweet pepper, pattypan squash and tomatoes, composed of the autochthonous lactic acid bacteria (LAB), selected from spontaneously fermented vegetables of each particular species. Antibacterial activity of the LAB strains against eight test strains of pathogenic and undesirable in food bacteria, as well as good organoleptic quality of obtained fermented product were adopted as selection criteria. Thirteen isolated LAB strains were identified as *Lactobacillus plantarum*, *Lactobacillus brevis* and *Pediococcus pentosaceus*. All strains revealed the inhibiting capability, limiting the growth of at least two test strains. Ten selected LAB strains were used as starter cultures. Sensory quality of the obtained products was at least equal to the product obtained as a result of spontaneous fermentation. Using the autochthonous LAB strains of high antibacterial activity seems to be the rational way of producing microbiologically-safe products, preserving the original, unique flavour of traditional, spontaneously fermented vegetables.

INTRODUCTION

Food produced by traditional methods acquires greater and greater interest in the whole Europe. Craftsman-made, traditional products become popular among the consumers who know that they are manufactured from high quality raw material, without preservatives and other artificial additives and are characterised by unique flavour values [Clark, 2004]. Interest in the traditional methods of food manufacturing creates an opportunity for producers to increase the diversity of their products.

Preservation of plant raw material by lactic acid fermentation is one of the oldest known methods for food storage, it is also one of the means to make the assortment of food products more attractive. Over 20 different vegetables are fermented in Europe, as whole, cut, shredded vegetables or juices [Caplice & Fitzgerald, 1999; Mäki, 2004]. The fermented vegetables are presently recommended by dietician and physicians due to their health-promoting properties, which makes them more and more popular in many countries, even in those more developed, where this kind of food was sometimes underestimated.

Fermentation process, carried out by lactic acid bacteria (LAB), provides the added value to health-promoting and organoleptic properties of vegetables [Caplice & Fitzgerald, 1999; Warmińska-Radyko *et al*., 2006]. Fermented vegetables are low-calorie foods as they contain considerably lower quantities of sugars compared to raw vegetables. The presence of lactic acid renders a pleasant, refreshing taste to the fermented products. Lactic acid may also lower the gut pH therefore inhibiting development of putrefactive bacteria [Manning *et al*., 2004]. Fermented vegetables are a source of dietary fibre which impedes assimilation of fats and regulates peristalsis of intestines. They are also a valuable source of vitamin C and B-group vitamins, phenolics and many other nutrients present in raw material. Fermented vegetables are perceived as suitable products for introducing LAB with probiotic properties into the human diet [Caplice & Fitzgerald, 1999; Yoon *et al*., 2005].

The quality of fermented vegetables, obtained as a result of natural spontaneous lactic acid fermentation, is largely dependent on microorganisms present in raw material [Akpinar-Bayizit *et al*., 2007; Desai & Sheth, 1997]. In unfavourable soil-climate conditions, the vegetables may become greatly contaminated with spore-forming bacteria, pathogens like *Salmonella*, *Shigella*, *Escherichia* or *Listeria*, mucous bacteria, yeasts and moulds which may predominate over lactic acid bacteria, deteriorating the quality of fermented products [Gomez *et al*., 2002].

It is, therefore, purposeful to initiate fermentation of vegetables or vegetable juices *via* introduction of starter cultures with the appropriately selected composition [Caplice & Fitzgerald, 1999; Desai & Sheth, 1997; Gardner *et al*., 2001; Hansen, 2002; Klewicka *et al*., 2004; Savard *et al*., 2000]. Application of starter cultures is a common practice in the dairy industry, in the production of fermented meat products, fermented olives and in bread manufacture. Some attempts were

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made to develop starter cultures for lactic acid fermentation of cucumbers, French beans, carrots, cabbage, beets, onions, cauliflowers [Desai & Sheth, 1997, Di Cagno *et al.,* 2008; Gardner *et al*., 2001; Yoon *et al*., 2005]. The experiments were also carried out on obtaining fermented vegetable products of an increased nutritive value, for example by introducing *Propionibacterium* producing vitamin B_{12} [Warmińska-Radyko *et al*., 2006]. It is, however, still difficult to find commercial starter cultures for fermentation of vegetables, such as sweet pepper, pattypan squash and tomatoes, which are not so commonly fermented as *i.e.* cucumbers or cabbage. These vegetables in Polish cuisine are usually pickled in vinegar, with the exception of tomatoes, which are popular in Russia and some Asian countries. Lactic acid fermentation is an alternative to pickling method of preservation, giving products of superior health value.

Controlled vegetable fermentation is often carried out using starters which contain well characterised strains, coming from collections, or the commercial starter cultures composed of strains originated from various plant or even dairy sources [Klewicka *et al*., 2004; Akpinar-Bayizit *et al*., 2007; Gardner *et al*., 2001; Jägerstrad *et al*., 2004]. However, many consumers believe that the fermented products, obtained in such a way, lose their traditional, characteristic taste and odour. An ideal starter culture should therefore enable obtaining fermented products with the palatability equal to good quality products, obtained as a result of spontaneous fermentation. It can be achieved when the starter culture includes LAB isolated from spontaneously fermenting vegetables. Such native, indigenous strains, called Non Starter Lactic Acid Bacteria (NSLAB), directly added to vegetables adapt easily, thus ensuring fast predomination in the environment [Gómez *et al*., 2002]. NSLAB are usually capable to synthesise compounds with antimicrobiological properties and compounds with a positive influence on organoleptic properties of the product [Akpinar-Bayizit *et al*., 2007; Gardner *et al*., 2001; Tamang *et al*., 2009].

The aim of the present study was to obtain LAB starter cultures, containing the autochthonous strains selected from spontaneously fermented pattypan squash, sweet pepper and tomatoes, characterised by antibacterial activity, which would provide the fermented product of good organoleptic quality.

MATERIALS AND METHODS

Isolation and identification of LAB strains

The vegetables: yellow pattypan squash (*Cucurbita pepo* L., var. patisoniana), red sweet pepper (*Capsicum annuum* L.) and tomatoes (*Solanum lycopersicum* L.) obtained from a local market were spontaneously fermented using traditional, artisan method (described later as control samples). After seven days, the samples of fermentation brine were diluted (1:9) in a sterile sodium chloride solution (0.9% w/v) and plated on MRS agar (Merck) with bromocresol purple 0.04% w/v and incubated at 30°C for 72 h. Bromocresol purple, a pH--indicating dye, was added to MRS to enhance the visibility of colonies forming by bacteria synthesising acids. From each type of vegetable material about 50 colonies were isolated and purified by streaking on MRS agar. Bacterial strains were preliminarily phenotypically identified on the base of their biochemical characteristics, using bioMérieux API CHL 50 tests in order to get the primary species identification [Tamminen *et al*., 2004]. The number of isolates was reduced to 23 based on the identical API test results. Further identification was carried out with the genetic method on the basis of analysis of 16S rDNA sequence.

DNA isolations were performed from 3 mL of bacterial overnight cultures with the GenElute Bacterial Genomic DNA Kit (Sigma). DNA concentration and purity was measured with Biofotometer 6131 (Eppendorf). The PCR of bacterial 16S rRNA genes was carried out with the *Lactobacillus* sp. group-specific 16S rDNA primers - 16SF (5'-TGAGAGTTTGATCCTGGCTCAG-3') and 16SR (5'-GCTACCTTGTTACGACTTCAC-3'), designed to amplify almost the entire sequence of the gene (the expected size of the amplicons in the range of 1470–1500 bp). Both DNA strands of the PCR products (previously purified with the GenElute PCR Clean-Up Kit, Sigma) were automatically sequenced with the capillary 3730 DNA Analyzer (Applied Biosystems).

The nucleotide sequences obtained after DNA sequencing were assembled with BioEdit Sequence Alignment Editor [Hall, 1999] and analysed by Basic BLAST search available in the GenBank database. If the LAB strains were assigned to the same species based on 16S rDNA sequence, additional DNA analyses were performed using RAPD-PCR and molecular typing by rep-PCR according to the procedure developed by Zawadzka-Skomiał *et al*. [2009]. Obtaining DNA profiles of individual bacteria enabled establishing 13 different strains, used for further studies. The isolates were kept in liquid nitrogen.

Growth capacity and lactic acid synthesis

The growth capacity of the examined LAB strains was evaluated after 24 h of cultivation in MRS liquid medium (Merck) at temperatures of 25°C, 30°C and 37°C. The quantity of synthesised D(-) and L(+) isomers of lactic acid was determined in samples incubated at 25°C, using Boehringer-Mannheim/R-Biopharm enzymatic tests (cat. no 11 112 821 035).

Antibacterial activity

Antibacterial activity was determined using the modified Pilet *et al*. [1995] method, as the total capacity of growth inhibition, based on the measurement of the size of inhibition zones of test bacteria strains. *Salmonella* DO serotype, *Salmonella* CO serotype, *Salmonella* Enteritidis, *Escherichia coli* 1, *Escherichia coli* 2, *Escherichia coli* 3, *Listeria monocytogenes* and *Bacillus subtilis* were used as the test strains. These strains were obtained from the collection of the National Veterinary Research Institute (PIWET PIB) in Puławy, Poland. The antibacterial activity was assessed in supernatant after 24-h incubation of LAB strains in a growth medium at 25°C. The plates with soft agar medium inoculated with test bacterial strains in log phase (10^6 cfu/mL) were spread with 10 μL of supernatant and incubated for 20 h at room temperature. The diameter of inhibition zones was then examined.

Fermentation trials

Vegetables were washed thoroughly with water. Sweet pepper was cut in four and seeds were removed, other vegetables were fermented as a whole. Vegetables were put into sterile 1 L glass jars, completely covered with the brine containing 2.5% NaCl and inoculated with single or mixed starter culture suspension to obtain the final cell density of 10^5 cfu/g. Control samples were prepared in the same way, without the starter. Vegetables were allowed to ferment for 7 days at 24–28°C. After this time, the total acidity, pH and LAB count were checked.

Sensory evaluation

Sensory quality of the fermented products was evaluated after 7 days of fermentation using a 6-score scale, in respect of their colour, aroma, taste and texture, and a 9-score hedonic scale for assessment of overall acceptability of the product, according to PN-ISO 4121:1998. Sensory evaluation was carried out by a panel consisting of seven trained assessors.

Statistical analysis

All analytical and microbiological tests were performed in two independent assays. Fermentation trials were carried out in duplicate and the results were averaged. The results of sensory evaluation were subjected to statistical analysis, using STATISTICA® 8. In order to determine the effect of the bacterial strain on the sensory properties of fermented vegetables, one-way ANOVA with RIR Tuckey's test was used.

RESULTS AND DISCUSSION

LAB strains isolated from spontaneously fermenting vegetables

Species affiliation of 23 lactic acid bacteria isolated from spontaneously fermenting vegetables, identified preliminarily with API CH 50 test, were verified using molecular biology methods. Identification was performed on the basis of 16S rRNA gene sequence with respect of at least 99% homology with related reference species reported in BLAST database. Di Cagno e*t al*. [2008] characterised bacteria isolated from vegetables analysing 16rRNA gene and using partial sequencing of the *recA*, *rpoA*, and *pheS* genes, results of that analysis were in conformity with results of 16S rDNA analysis. Taking into account that all new strains isolated in this work showed a high level of homology with reference species, the identification can be considered as reliable. On the basis of the performed characteristic, the number of examined strains was reduced to 13 (Table 1). Strains were identified as *Lactobacillus plantarum*, *Lactobacillus brevis* and *Pediococcus pentosaceus.* Most strains belonged to heterofermentative (*Lactobacillus brevis*) or "facultatively" heterofermentative (*Lactobacillus plantarum)* LAB, which is important in terms of their ability to produce sensory valuable by-products.

The results obtained are generally in line with findings of many other studies on characteristic LAB microflora in vegetables. From about 20 different vegetables, vegetable blends and vegetable juice fermented in Europe, lactic acid bacteria belonging to the genera *Lactobacillus* (*L. brevis, L. casei, L. plantarum, L. arabinosus, L. buchneri, L. fermentum*), *Leuconostoc* (mostly *L. mesenteroides*), and *Pediococcus* (*P. acidilactici, P. pentosaceus*) have been isolated. Lactococci and Enterococci also participate in the initial phase of fermentation [Mäki, 2004]. According to Sajur *et al*. [2007], *Pedioccus* and *Lactobacillus* were two of four bacterial strain groups isolated from tomato surface. It can be concluded that the diversity of bacterial microflora of fermented vegetables is not associated with many species but rather with the variety of strains of the same species.

TABLE 1. LAB strains isolated from spontaneously fermented vegetables.

Vegetable	Strain
Sweet pepper	*Lactobacillus brevis* ZF 165
	Lactobacillus brevis ZF 166
Pattypan squash	*Lactobacillus brevis* ZF 167
	Lactobacillus brevis ZF 168,
	Lactobacillus plantarum ZF 169
	Lactobacillus plantarum ZF 170
	Lactobacillus plantarum ZF 171
Tomatoes	*Lactobacillus plantarum* ZF 179
	Lactobacillus plantarum ZF 180
	Lactobacillus plantarum ZF 181
	Lactobacillus plantarum ZF 182
	Lactobacillus plantarum ZF 184
	Pediococcus pentosaceus ZF 183

Growth capacity and lactic acid production

The growth rate of starter strains is a very important attribute, since it influences their competitive behaviour during the fermentation [Ayad *et al*., 2002]. Growth of the isolated bacterial strains at three different temperatures was evaluated after 24-h incubation (Figure 1). It was found that most of the examined strains showed good growth in the whole range of tested temperatures. Only in the case of *L. plantarum* ZF 180, a weaker growth at 25°C and in the case of *P. pentosaceus* ZF 183 at 37°C, was recorded. Taking into account that vegetable fermentation is usually carried out at ambient temperature, the most significant fact was that they had grown well at 25°C.

The capability of the isolated LAB strains to biosynthesise lactic acid is presented in Figure 2. Efficiency of lactic acid

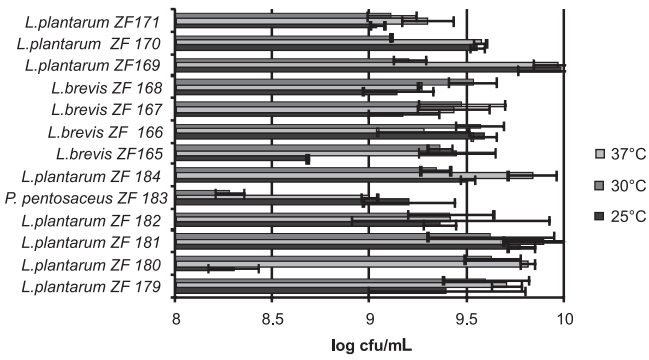

FIGURE 1. Effect of temperature on the growth of LAB strains isolated from spontaneously fermented vegetables (MRS medium, 24 h). Results are the mean of two independent experiments by duplicate ± standard deviation.

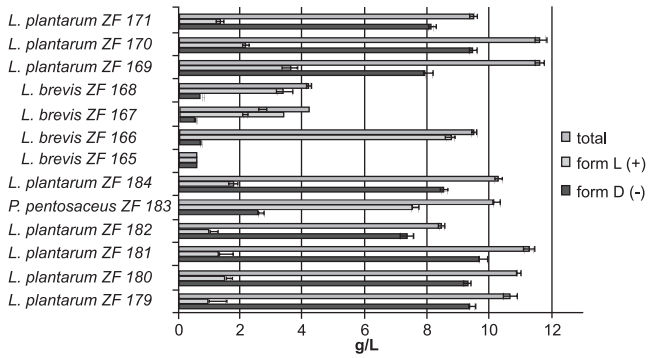

FIGURE 2. Synthesis of lactic acid by the LAB strains isolated from spontaneously fermented vegetables (MRS medium, 25°C, 24 h). Results are the mean of two independent experiments by duplicate ± standard deviation.

production has been chosen for LAB characterisation as an important preservation factor. Most of the examined strains synthesised lactic acid in the quantity of *ca*. 10 g/L during 24 h of incubation at 25°C, and in the case of 9 out of 13 strains, form D (-) of acid was prevailing. Only *L. brevis* ZF 166 and *P. pentosaceus* ZF 183 produced relatively more L(+) than D(-) isomer. The lowest amount of lactic acid was synthesised by heterofermentative strains belonging to *L. brevis* species: ZF 165, ZF 167 and ZF 168. The growth of LAB strains is not necessarily correlated with the amount of lactic acid produced by LAB, furthermore, even bacteria belonging to the same species can differ in their ability to synthesise different metabolites. Some features, such as lactic acid or bacteriocin synthesis, are strain-dependent and vary from strain to strain [Koistinen *et al*., 2007; Plumed-Ferrer *et al*., 2008].

The concentration of synthesised lactic acid, due to its higher initial sugar concentration, was higher in MRS medium than that found in fermented vegetable brine, which does not usually exceed 8 g/L, depending on raw material and bacterial strain used [Desai & Sheth, 1997; Di Cagno *et al*., 2008; Gardner *et al*., 2001; Klewicka *et al*., 2004].

Antibacterial activity

A variety of pathogenic bacteria have been found in vegetables: *Salmonella* and *Shigella* spp., enteropathogenic strains of *Escherichia coli, Aeromonas hydrophila*, *Yersinia enterocoliticia* and *Staphylococcus aureus* [Breidt & Fleming, 1997]. The presence of these bacteria can pose a health risk, and some of them may influence the fermentation process and product quality. Therefore it is important to prevent their growth due to inoculation and rapid initiation of fermentation by LAB strains having antimicrobial properties [Gomez *at al*., 2002; Klewicka *et al*., 2004].

The test strains selected for the evaluation of antibacterial activity of isolated LAB strains were bacteria pathogenic for man and animals and being undesirable in processing. From the examined LAB strains, the highest inhibiting capability was revealed by *L. brevis* ZF 166 and *L. plantarum* ZF 169 isolated from fermented sweet pepper and pattypan squash, respectively (Tables 2–4). They limited the growth of all test strains. Three following strains: *L. brevis* ZF 165 isolated from fermented sweet pepper, and *L. plantarum* ZF 170 and *L. plantarum* ZF 171 isolated from fermented pattypan squash, inhibited the growth of seven out of eight test strains. The lowest antibacterial activity was revealed by three *L. plantarum* strains isolated from fermented tomatoes, which limited the growth of only two (ZF 180) and three (ZF 179 and ZF 181) test strains.

TABLE 2. Antibacterial activity of LAB strains isolated from spontaneously fermented sweet pepper against indicatory bacteria.

Indicatory strains of bacteria	*LAB strains*	
	L. brevis ZF 165	*L. brevis* ZF 166
Salmonella DO	6.25±0.50	10.25±0.50
Salmonella CO	10.00±0.82	10.50±0.58
Salmonella Enteritidis	8.00±1.41	7.00±0.82
E .coli 1	7.75±0.50	10.00±1.41
E .coli 2	8.00±0.82	9.75±0.96
E. coli 3	nd	8.50±0.58
L. monocytogenes	8.00±0.82	8.50±0.58
B. subtilis	10.25±0.50	12.00±0.00

Results are the mean of two independent experiments by duplicate ± standard deviation. nd – non detected

The antibacterial activity shows the cumulative effect of several LAB metabolites, such as lactic acid, ethanol, diacetyl, carbon dioxide, hydrogen peroxide and bacteriocins or antibiotic-like substances. Taking into account the fermented product characteristics, it is important that bacteriocins generated by LAB strains are generally stable at acidic pH [Caplice & Fitzgerald, 1999; Ponce *et al*., 2008]. The antibacterial activity of the studied strains was not directly related to the lactic acid production determined on MRS medium, which may imply the formation of other substances of antimicrobial activity, like bacteriocins.

The antibacterial activity of LAB strains selected from vegetables has been confirmed in several previous studies, *i.e.* from: fermented cucumbers [Daeschel & Fleming 1987], fermented carrots [Uhlman *et al*., 1992], beet juice [Klewicka *et al*., 2004], organic leafy vegetables [Ponce *et al*., 2008], and fermented ethnic Himalayan vegetables [Tamang *et al*., 2009]. Also several LAB strains isolated from lettuce juice showed the antibacterial activity against, among others, *Listeria monocytogenes, Staphyllococcus aureus* and *Salmonella typhimurum* [Gómez *et al*., 2002].

Sensory quality of fermented vegetables

The examined LAB strains were used as starter cultures for the fermentation of vegetables, with the exemption of *L. plantarum* ZF 179, ZF 180 and ZF 181 of the lowest antibacterial activity. After one week of fermentation, the total acidity, pH and LAB count were checked (results not presented). Acidity (as lactic acid) of fermented sweet pepper accounted for 13.0–16.0 g/L, that of pattypan squash for 6.8–8.1 g/L and that of tomatoes for 5.0–7.5 g/L. High acid concentration in fermented sweet pepper resulted from relatively high sugars concentration in this vegetable, compared to pattypan squash and tomatoes. The pH value of fermented sweet pep-

TABLE 3. Antibacterial activity of LAB strains isolated from spontaneously fermented pattypan squash against indicatory bacteria.

Idicatory strains of bacteria	*LAB strains*				
	L. brevis ZF 167	*L. brevis* ZF 168	*L. plantarum* ZF 169	*L .plantarum* ZF 170	*L .plantarum* ZF 171
Salmonella DO	6.75±0.96	nd	9.25±0.50	9.00±0.82	8.50±0.58
Salmonella CO	nd	nd	8.00±0.82	7.75±0.50	8.00±0.82
Salmonella Enteritidis	nd	5.00±0.82	6.00±0.82	7.25±0.50	8.75±0.50
E. coli 1	7.50±0.58	nd	9.50±0.58	9.00±0.58	8.25±0.50
E. coli 2	7.25±0.50	5.00±0.00	9.75±0.95	8.00±0.82	8.25±0.50
E. coli 3	nd	6.75±0.95	10.25±0.50	8.25±0.50	8.00±1.41
L. monocytogenes	6.00±0.82	6.00±0.00	9.00±0.82	8.50±0.58	7.50±0.50
B. subtilis	9.25±0.50	nd	5.25±0.50	nd	nd

Results are the mean of two independent experiments by duplicate ± standard deviation. nd – non detected.

TABLE 4. Antibacterial activity of LAB strains isolated from spontaneously fermented tomatoes against indicatory bacteria.

Idicatory strains of bacteria	*LAB strains*					
	L. plantarum ZF 179	*L. plantarum* ZF 180	*L .plantarum* ZF 181	*L.plantarum* ZF 182	*P.pentosaceus* ZF 183	*L. plantarum* ZF 184
Salmonella DO	9.00±0.82	nd	nd	8.00±0.82	8.25±0.50	7.75±0.50
Salmonella CO	nd	nd	nd	10.25±0.50	8.00±1.41	8.00±0.82
Salmonella Enteritidis	nd	nd	nd	nd	nd	nd
E. coli 1	nd	nd	6.00±0.82	8.25±0.50	7.00±0.82	10.25±0.50
E .coli 2	9.75±0.96	9.00±0.82	7.00±0.50	9.75±0.82	8.25±0.50	10.00±0.58
E. coli 3	9.75±0.82	6.00±0.50	7.25±0.50	8.00±0.82	9.00±0.50	9.50±0.58
L. monocytogenes	nd	nd	nd	nd	nd	nd
B. subtilis	nd	nd	nd	nd	nd	nd

Results are the mean of two independent experiments by duplicate ± standard deviation. nd – non detected.

TABLE 5. Evaluation of sensory properties of sweet pepper after 7 days of fermentation.

Attribute [points]	Composition of starter culture			
	L. brevis ZF 165	*L. brevis* ZF 166	*L. brevis* ZF 165 *L. brevis* ZF 166 (1 : 1)	Control
Colour [1–6]	5.3±0.52[a]	5.2±0.75[a]	5.3±0.52[a]	5.3±0.52[a]
Odour [1–6]	5.0±0.89[a]	4.0±0.89[a]	4.6±0.49[a]	5.0±0.63[a]
Taste [1–6]	5.0±0.63[a]	4.0±0.89[a]	4.7±0.82[a]	4.5±0.55[a]
Texture [1–6]	4.5±0.55[ab]	3.7±0.52[a]	4.7±0.82[b]	3.8±0.41[ab]
Overall acceptability [1–9]	8.0±0.89[a]	5.0±0.89[b]	7.2±0.75[a]	7.2±0.75[a]

[a, b] – values in the same verse with different letters differ significantly ($p \leq 0.05$). Values are the mean of two independent experiments, assessed by 6 panellists ± standard deviation.

per and pattypan squash varied from 3.0 to 3.3; whilst fermented tomatoes showed lower pH values, from 2.7 to 2.8. All products showed LAB count of 10^7–10^8 cfu/mL, irrespective of raw material or LAB strain used.

Sensory properties were adopted as the main criterion of the quality of fermented products, being the most important attribute for the consumers.

Sweet pepper was fermented with *L. brevis* ZF 165, *L. brevis* ZF 166 and the mixture of both strains in the ratio of 1:1. The results of sensory evaluation are given in Table 5. No significant differences in colour, taste, odour nor appearance of the examined products were found. The only differences were found in texture and overall acceptability. Sweet pepper fermented using *L. brevis* ZF 165 as a starter received

TABLE 6. Sensory properties of pattypan squash after 7 days of fermentation.

Attribute [points]	Composition of starter culture					
	L. brevis ZF 167	*L. brevis ZF 168*	*L. plantarum ZF 169*	*L. plantarum ZF 170*	*L. plantarum ZF 171*	Control
Colour [1–6]	5.3±0.50[a]	5.3±0.50[a]	5.2±0.67[a]	5.0±0.87[a]	5.2±0.67[a]	5.2±0.67[a]
Odour [1–6]	5.0±0.87[a]	4.4±0.88[ab]	4.2±0.83[ab]	3.7±0.87[b]	4.6±0.73[ab]	5.0±0.71[a]
Taste [1–6]	5.0±0.71[a]	4.6±0.73[ab]	5.1±0.78[a]	4.0±0.71[b]	4.6±0.73[ab]	5.1±0.33[a]
Texture [1–6]	5.1±0.60[a]	5.2±0.67[a]	5.4±0.53[a]	5.1±0.60[a]	5.1±0.78[a]	5.0±0.87[a]
Overall acceptability [1–9]	7.4±0.88[a]	7.4±0.73[a]	7.7±0.87[a]	5.3±0.71[b]	6.8±0.97[a]	7.4±0.53[a]

[a, b] – values in the same verse with different letters differ significantly ($p \leq 0.05$). Values are the mean of two independent experiments, assessed by 6 panellists ± standard deviation.

the highest scores in the hedonic scale; on the other hand, the fermented product obtained using *L. brevis* ZF 166 acquired the lowest scores of overall acceptability (the difference was statistically significant at $p \leq 0.05$), mainly because of poor, too soft texture. Soft texture was also observed in the control sample, which may indicate that this strain did not dominate over the indigenous sweet pepper microflora. Scores of sensory attributes as well as overall acceptability value of sweet pepper fermented with the mixed culture of *L. brevis* ZF 165 and *L. brevis* 166 were placed between scores gained by products obtained with each of monocultures used. It was noteworthy that products inoculated with strains of *L. brevis* ZF 165 and ZF 167, which did not produce high amounts of lactic acid on MRS medium at 25°C, were characterised by good taste, odour and overall acceptability. It may derive from a capacity of these heterofermentative strains to biosynthesis of compounds positively influencing product flavour. The ability to synthesise significant amounts of odour-active by-products is often met among NSLAB.

Pattypan squash was fermented using all identified autochthonous LAB strains. The results of sensory evaluation of the fermented products are given in Table 6. Fermented products obtained using *L. brevis* ZF 167, *L. brevis* ZF 168, *L. plantarum* ZF 169, and *L. plantarum* ZF 171 as monoculture starters were evaluated by the sensory panel at a similarly high level as the control sample (spontaneous fermentation), according to all tested attributes and overall quality. Small differences observed in the overall acceptability in favour of the strains *L. brevis* ZF 167 and *L. brevis* ZF 169 were statistically insignificant. On the other hand, the application of *L. plantarum* ZF 170 resulted in obtaining a product which was significantly ($p \leq 0.05$) lower evaluated according to odour, taste and overall acceptability as compared to the spontaneously fermented one.

For tomatoes fermentation three strains were employed: *L. plantarum ZF 182, L. plantarum 184,* and *P. pentosaceus ZF 183.* Results are shown in Table 7. The overall acceptability of all tested samples was similar and rather low. Sensory attributes such as colour, odour and taste were evaluated at the same level for all samples, irrespective of the strain. The overall acceptability of fermented tomatoes was evaluated at a relatively low level, compared to the two other vegetables. It may result from the fact that fermented sweet pepper or pattypan squash, contrary to fermented tomatoes, are organoleptically, according to intensity of sour taste and texture, similar to other more known fermented vegetables such as cucumbers. Fermented tomatoes have a very specific, strongly acidic and even slightly astringent taste and rather soft texture, and panellists did not find them attractive.

In spontaneously fermented products, usually more differentiated ecosystem is formed than in the case when a starter culture is introduced, and predominates the environment from the very beginning. This may negatively affect product sensory attributes, which may show slightly poorer and different from traditional flavour pattern. The appropriate selection of the LAB strains for starter ensures, however, good and repeatable quality of the fermented products and the appropriate microbiological purity *via* elimination of the undesirable

TABLE 7. Sensory properties of tomatoes after 7 days of fermentation.

Attribute [points]	Composition of starter culture			
	L. plantarum ZF 182	*L. plantarum ZF 184*	*P. pentosaceus ZF 183*	Control
Colour [1–6]	4.7±0.82[a]	4.7±0.82[a]	4.2±0.45[a]	4.8±0.75[a]
Odour [1–6]	4.3±0.52[a]	3.8±1.17[a]	4.2±0.75[a]	4.5±0.55[a]
Taste [1–6]	3.0±0.63[a]	3.0±0.71[a]	4.0±0.89[a]	3.8±0.75[a]
Texture [1–6]	3.8±0.45[a]	3.8±0.45[a]	4.0±0.63[a]	3.8±0.45[a]
Overall acceptability [1–9]	4.7±1.03[b]	4.7±0.96[b]	6.0±0.82[a]	5.7±0.82[ab]

[a, b] – values in the same verse with different letters differ significantly ($p \leq 0.05$). Values are the mean of two independent experiments, assessed by 6 panellists ± standard deviation.

microflora, which is especially important when the process is conducted in the industrial scale. Some studies indicate that satisfactory results may be achieved with allochthonous LAB strains used as starter cultures. This type of starters led to better results according to process parameters (faster acidification), organoleptic quality (colour, taste) and longer shelf life in products like: fermented carrot, cabbage, beet and onion juices as well as vegetable mixes, than in the course of spontaneous fermentation [Gardner *et al*., 2001; Klewicka *et al*., 2004]. One should, however, take into consideration that using one commercial starter culture containing several chosen and accepted LAB strains of well known characteristics, for fermenting different products, may result in the unification of product properties, regardless of raw material used.

The idea of using LAB strains isolated from spontaneously fermenting vegetables of particular species as starter cultures came from the assumption that it would allow to preserve as much as possible the original, traditional organoleptic features of the craft-made product. Experiments described in this study, involving the introduction of indigenous LAB flora as starter culture for fermenting sweet pepper, pattypan squash and tomatoes, in most cases allowed obtaining a product of a good sensory quality, equal, or in some cases even higher, compared to the product obtained as a result of spontaneous fermentation. Di Cagno *et al*. [2008] also demonstrated that quality of vegetables fermented using a mixed starter consisting of autochthonous LAB strains were superior according to texture, colour and sensory properties to vegetables fermented with allochthonous LAB strains, the last being classified as "barely acceptable".

CONCLUSION

Selected autochthonous LAB strains isolated from spontaneously fermenting pattypan squash, sweet pepper and tomatoes can be applied as starter cultures for fermenting these vegetables. The starters obtained were characterised by a high overall antibacterial activity, directed towards pathogenic and undesirable-in-food bacteria and allowed to obtain fermented products of superior or at least equal sensory quality to products derived from spontaneous fermentation process. The presented approach to starter culture selection offers a means to obtain fermented vegetables which are safe and of repeatable quality and with organoleptic features similar to artisanal products, appreciated by the consumers.

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