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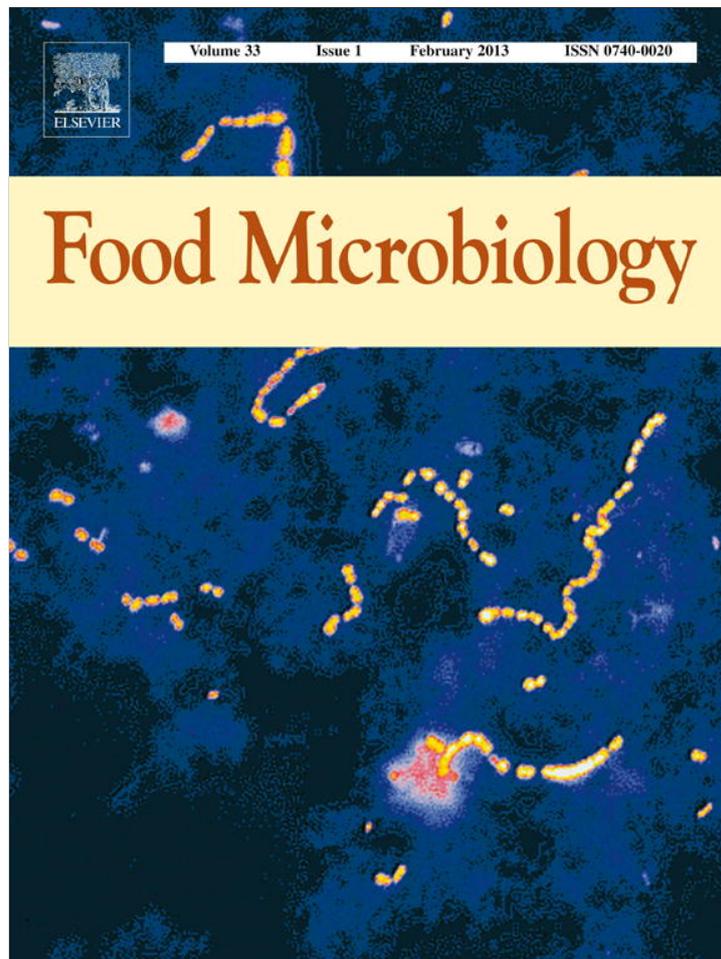
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## Review

# Exploitation of vegetables and fruits through lactic acid fermentation

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## ABSTRACT

Lactic acid fermentation represents the easiest and the most suitable way for increasing the daily consumption of fresh-like vegetables and fruits. Literature data are accumulating, and this review aims at describing the main features of the lactic acid bacteria to be used for fermentation. Lactic acid bacteria are a small part of the autochthonous microbiota of vegetables and fruits. The diversity of the microbiota markedly depends on the intrinsic and extrinsic parameters of the plant matrix. Notwithstanding the reliable value of the spontaneous fermentation to stabilize and preserve raw vegetables and fruits, a number of factors are in favour of using selected starters. Two main options may be pursued for the controlled lactic acid fermentation of vegetables and fruits: the use of commercial/allochthonous and the use of autochthonous starters. Several evidences were described in favour of the use of selected autochthonous starters, which are tailored for the specific plant matrix. Pro-technological, sensory and nutritional criteria for selecting starters were reported as well as several functional properties, which were recently ascribed to autochthonous lactic acid bacteria. The main features of the protocols used for the manufacture of traditional, emerging and innovative fermented vegetables and fruits were reviewed. Tailored lactic acid bacteria starters completely exploit the potential of vegetables and fruits, which enhances the hygiene, sensory, nutritional and shelf life properties.

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## 1. Introduction

Vegetables and fruits are fundamental sources of water-soluble vitamins (vitamin C and group B vitamins), provitamin A, phytosterols, dietary fibres, minerals and phytochemicals (Gebbers, 2007) for the human diet. Scientific evidences encouraged the consumption of vegetables and fruits to prevent chronic pathologies such as hypertension (Dauchet et al., 2007), coronary heart diseases and the risk of stroke (He et al., 2007). Unfortunately, the daily intake of vegetables and fruits is estimated to be lower than the doses (400 g, excluding potatoes and other starchy tubers) recommended by the World Health Organization (WHO), and Food and Agriculture Organization (FAO) ([www.who.int/](http://www.who.int/); [www.fao.org/](http://www.fao.org/)).

The major part of vegetables and fruits is consumed fresh or as industrially minimally processed, which include canned, dried, juice, paste, salad, sauce and soup preparations. Minimally processed and, especially, fresh vegetables and fruits have short shelf life since subjected to rapid microbial spoilage, and, in some cases, to contamination by pathogens. Cooking and pasteurization as well as the addition of chemical preservatives are the main technology

options that guarantee safe vegetables and fruits, but would bring about a number of not always desirable changes in their physical characteristics and chemical composition (Zia-ur-Rehman et al., 2003; Zhang and Hamauzu, 2004). To reduce these drawbacks, some novel technologies, like the high-hydrostatic pressure processing, ionization radiation and pulsed-electric fields (Devlieghere et al., 2004; Gómez-López et al., 2005; Elmnasser et al., 2007), new packaging systems and the use of natural antimicrobial preservatives (Devlieghere et al., 2004), are also considered.

The consumer trend towards fresh-like, highly nutritional value, health-promoting and rich flavour ready-to-eat or -to-drink foods and beverages is increasing (Endrizzi et al., 2006). Lactic acid fermentation is considered as the simple and valuable biotechnology to keep and/or enhance the safety, nutritional, sensory and shelf life properties of vegetables and fruits (Buckenhüskes, 1997; Steinkraus, 1996; Karovičová and Kohajdová, 2003; Demir et al., 2006). As shown in the last decade literature data, the combination of this ancient method of bio-preservation with the current biotechnology tools should allow controlled fermentation processes and the selection of starter cultures to increase the consumption of fresh-like vegetables and fruits (McFeeters, 2004).

This review describes the exploitation of vegetables and fruits through lactic acid fermentation. The lactic acid bacteria microbiota, the occurring spontaneous fermentation, the main features of commercial/allochthonous and autochthonous starters, and the

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emerging and traditional fermented vegetable and fruit products are reviewed.

## 2. The lactic acid bacteria microbiota and spontaneous fermentation

The epiphytic microbial population of plants is largely subjected to fluctuations of the physical and nutritional conditions (Lindow and Brandt, 2003). Each particular type of plant provides a unique niche in terms of chemical composition, buffering capacity, competitive biota and natural antagonist compounds (Buckenhüskes, 1997). Temperature and harvesting conditions also affect the microbiota. It may be concluded that each species of plant harbours a dominant and constant microbiota (Yang et al., 2000).

The microbial population of raw vegetables and fruits fluctuates between 5.0 and 7.0 log cfu g<sup>-1</sup> (Spurr, 1994). The biota of fruits is mainly dominated by yeasts and fungi, and yeasts often anticipate the colonization by fungi due to their faster growth. Yeasts range between 2.0 and 6.0 log cfu g<sup>-1</sup> (Rosini et al., 1982; Nyanga et al., 2007; Di Cagno et al., 2008a,b). Depending on fruit, species belonging to *Cryptococcus*, *Candida*, *Saccharomyces*, *Pichia*, *Hansenula*, *Debaromyces* and *Rhodotorula* genera were found to dominate the microbiota (Postmaster et al., 1997; Offonry and Achi, 1998; Nielsen et al., 2007; Chanprasartsuk et al., 2010; Di Cagno et al., 2010a). Bacteria mainly populate the biota of vegetables, especially the aerobes (pseudomonads, enterobacteria and coryneforms) (Schneider, 1988). Coagulase-positive staphylococci and other faecal bacteria may be also present in raw vegetables. Unless specific circumstances occur (use of highly contaminated water), they do not constitute a healthy risk because of the low cell density, and the inhibition by hostile environmental conditions and microbial competition (Ciafardini and Di Cagno, 2012).

Lactic acid bacteria are a small part (2.0–4.0 log cfu g<sup>-1</sup>) of the autochthonous microbiota of raw vegetables and fruits (Buckenhüskes, 1997). The main species, which were isolated from raw or spontaneously fermented vegetables and fruits, are listed in Table 1. Hetero-fermentative and homo-fermentative species belonging to *Leuconostoc*, *Lactobacillus*, *Weissella*, *Enterococcus* and *Pediococcus* genera were variously identified depending

on the species of vegetables. *Weissella cibaria*/*Weissella confusa* and, especially, *Lactobacillus plantarum* were the most frequent species.

Molecular typing of the lactic acid bacteria isolated from vegetables and fruits may be useful to group them into several clusters, which reflect their distribution in the raw material and, presumptively, their way of entry. For instance, strains of *Lactobacillus rossiae* were mainly isolated from the middle ring of pineapple fruit, indicating this layer as their optimal niche (Di Cagno et al., 2010a).

When favourable conditions of anaerobiosis, water activity, salt concentration and temperature occur, raw vegetables and fruits may be subjected to spontaneous lactic acid fermentation. In some cases, the alcoholic fermentation takes place concomitantly. Usually, Gram-negative bacteria are inhibited at the early stage of lactic acid fermentation. Back slopping (e.g., inoculation with a small amount of a previously and successfully fermented raw material) is the easiest way to induce and monitor the spontaneous fermentation. Back slopping is traditionally used for sauerkraut fermentation to favour the dominance of the best adapted strains, which shorten the fermentation and reduce the risk of failure (Leroy and De Vuyst, 2004). Overall, the spontaneous fermentation of vegetables and fruits includes the succession of hetero- and homo-fermentative lactic acid bacteria, together with or without yeasts (Plengvidhya et al., 2004). Obviously, the microbial agents responsible for the spontaneous lactic acid fermentation reflected the main composition of the microbiota of raw vegetables and fruits (Harris, 1998; Karovičová and Kohajdová, 2003; Tamminen et al., 2004; Kim and Chun, 2005). The microbiota responsible for the spontaneous fermentation of kimchi was one of the largely investigated (Lee et al., 1997; Lee et al., 2002, 2005; Kim and Chun, 2005; Park et al., 2010; Jung et al., 2011). In particular, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* started the first stage of fermentation, and the combination of *L. plantarum* and *Lactobacillus brevis* or *Lactobacillus maltaromicus* and *Lactobacillus bavaricus* further dominated depending on the temperature of incubation (20–30 °C or 5–7 °C, respectively). Recently, meta-genomic and metabolome approaches were used to characterize the microbial community and related metabolites of kimchi during spontaneous fermentation (Jung et al., 2011). The proportion of unclassified phylotypic groups was the highest (ca. 97%) during early fermentation, then it decreased as the fermentation proceeded. Distinct

**Table 1**  
Species of lactic acid bacteria, which were isolated from raw or spontaneously fermented vegetables and fruits.

Lactic acid bacteria species	Source	Reference
<i>Lactobacillus plantarum</i>	Tomatoes, marrows, carrots, cucumbers, eggplants, red-beets, capers, pineapple, plums, kiwi, papaya, fennels, cherries, cabbages	Di Cagno et al., 2008a, 2008b, 2010a, 2011a, 2011b; Pulido et al., 2012; Plengvidhya et al., 2007; Sánchez et al., 2000; Tamminen et al., 2004
<i>Lactobacillus pentosus</i>	Capers, papaya, eggplants, cucumbers	Di Cagno et al., 2011a; Pulido et al., 2012; Sánchez et al., 2000; Tamminen et al., 2004
<i>Lactobacillus rossiae</i>	Pineapple	Di Cagno et al., 2010a,b
<i>Lactobacillus fermentum</i>	French beans, red beets, capers, eggplants, melon pod	Di Cagno et al., 2008a; Seseña and Palop, 2007; Pulido et al., 2012; Sánchez et al., 2000; Offonry and Achi, 1998
<i>Lactobacillus curvatus</i>	Peppers	Di Cagno et al., 2009
<i>Lactobacillus brevis</i>	Tomatoes, capers, eggplants, cabbages, cucumbers, melon pod	Di Cagno et al., 2008b; Pulido et al., 2012; Seseña and Palop, 2007; Plengvidhya et al., 2007; Sánchez et al., 2000; Offonry and Achi, 1998
<i>Lactobacillus paraplantarum</i>	Cabbages, capers	Pulido et al., 2012; Plengvidhya et al., 2007
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	White cabbages, carrots, peppers, cucumbers, eggplants, lettuce, cherries	Lee et al., 2011; Plengvidhya et al., 2007; Sánchez et al., 2000; Di Cagno et al., 2008a; Di Cagno et al., 2009; 2011b
<i>Weissella soli</i>	Carrots	Di Cagno et al., 2008a
<i>Weissella confusa</i> , <i>Weissella cibaria</i>	Peppers, tomatoes, blackberries, papaya	Di Cagno et al., 2008b, 2009; 2011a
<i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i>	French beans, tomatoes, capers, melon pod	Di Cagno et al., 2008a, 2008b; Pulido et al., 2012; Offonry and Achi, 1998
<i>Pediococcus pentosaceus</i>	French beans, tomatoes, cucumbers, capers, cherries, cabbages	Di Cagno et al., 2008a, 2008b; 2011b; Tamminen et al., 2004; Pulido et al., 2012; Plengvidhya et al., 2007

kinetics of growth characterized the three genera, *Leuconostoc*, *Lactobacillus* and *Weissella*, which dominated the fermentation (Fig. 1). The genus *Leuconostoc* was the most abundant throughout fermentation but the abundance of *Lactobacillus* markedly increased after the first stage of fermentation.

The microbiota responsible for the spontaneous fermentation of several raw vegetables and fruits deserves a large interest as a tool to improve the microbial safety of fermented foods (Fan and Truelstrup Hansen, 2012; Paramithiotis et al., 2012). Bio-preservation is mainly due to the synthesis of a wide variety of antagonistic primary and secondary metabolites including organic acids, carbon dioxide, ethanol, hydrogen peroxide and diacetyl, antifungal compounds (e.g., fatty acids, phenyllactic acid), bacteriocins and antibiotics (reutericyclin) (Fan and Truelstrup Hansen, 2012). Among these compounds, bacteriocins have attracted the interest due to their potential use as safe and natural food preservatives. Although nisin is the only purified bacteriocin used thus far in industrially processed foods, many bacteriocins produced by various lactic acid bacteria, may have potential applications in foods. Exploitation of bacteriocinogenic lactic acid bacteria on common spoilage and poisoning of raw vegetables and fruits was largely carried out. Several examples of bio-preservation of fresh-cut salads and other vegetables (e.g., apples and lettuce) from common spoilage (yeasts and moulds) and poisoning (*Listeria innocua*, *Listeria monocytogenes* and *Escherichia coli*) microorganisms were reported (Fan and Truelstrup Hansen, 2012).

Notwithstanding the reliable value of the spontaneous fermentation to stabilize and preserve raw vegetables and fruits (e.g., cucumbers, onions, eggplants, red-beets, capers, lychee, cocoa beans, persimmon), a number of factors are in favour of the use of selected starters. The risk of fermentation failure, the inadequate inhibition of spoilage and pathogen microorganisms, and the undesirable and not predictable variations of the sensory, nutritional and rheology properties are some of these factors (Buckenhüskes, 1997). Contrarily to other fermented foods (e.g., cheeses, sausages and leavened baked goods), the use of starter cultures in vegetable and fruit fermentation is increasing only recently (Molin, 2001; Vega Leal-Sánchez et al., 2003; Montet et al., 2006).

### 3. The use of starter cultures

Two main options may be pursued for the controlled lactic acid fermentation of vegetables and fruits: the use of autochthonous or

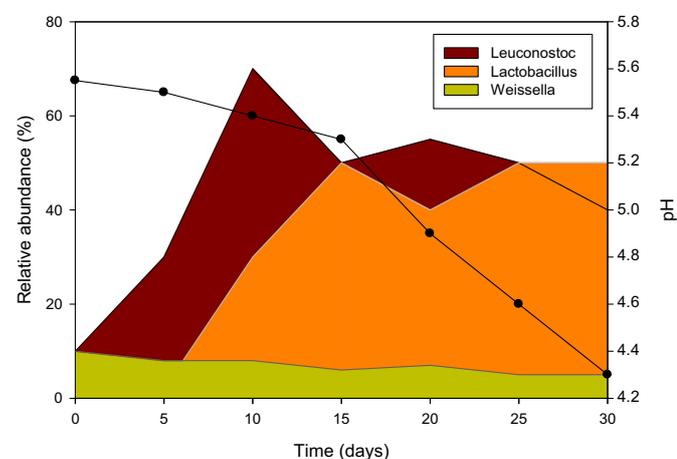


Fig. 1. Succession of lactic acid bacteria and change of pH during spontaneous fermentation of kimchi.

allochthonous starters (Di Cagno et al., 2008a, 2008b, Di Cagno et al., 2009, 2010a, 2011b). Autochthonous starters means isolated from and re-used on the same raw matrix, apart from the geographical origin. Allochthonous starters means isolated from certain raw matrices but used to ferment various products. Obviously, commercial starters, which are used to ferment a variety of vegetables and fruits, mostly coincide with the above definition of allochthonous strains. More than for other food matrices, this differentiation between starters is of fundamental importance for plant species, which represent completely different niches. Authorized lists of microorganisms with certified use in food fermentations, which cover a wide range of food matrices, including vegetables and fruits, were recently published (Bourdichon et al., 2012). These lists may represent a de facto reference of food cultures, which should be consulted to select starter for fermentation of raw vegetables and fruits.

#### 3.1. The commercial starters

The literature reports the use of several commercial/allochthonous starters (de Castro et al., 1998; Erten, 2000; Gardner et al., 2001; Plengvidhya et al., 2004; Demir et al., 2006; Johanningsmeier et al., 2007). Peeled and blanched garlic was fermented with commercial *L. plantarum* (de Castro et al., 1998). The allochthonous starter grew well in blanched garlic (ca.  $9 \log \text{cfu g}^{-1}$ , after two days of fermentation) and lactic acid was the main fermentation end-product. When blanching was omitted, the growth of the starter was inhibited, and ethanol, fructose and an undesired green pigment were found. Since non-degradable chloride ions and BOD (Biochemical Oxygen Demand) of the brine are problems for the waste management during sauerkraut fermentation, the use of commercial starters *Leuc. mesenteroides* DSM 20343, *P. pentosaceus* DSM 20336, *L. plantarum* DSM 20174, *L. brevis* DSM 20054 and *Leuc. mesenteroides* ATCC 8293 was combined with the use of low salt brine (Wiander and Ryhänan, 2005; Johanningsmeier et al., 2007). A mixture of above commercial starters, which was used to ferment cabbages and showed a fast decrease of the pH, also improved the sensory properties and gave a firm texture. As shown by RAPD-PCR and Pulse Field Gel Electrophoresis (PFGE) analyses, commercial *Leuc. mesenteroides* strains (from ATCC) dominated over the natural microbiota during the early stages of sauerkraut fermentation (18 °C for 14 days) (Plengvidhya et al., 2004). *Leuc. mesenteroides* strains NCIMB 8023 (National Collection of Industrial and Marine Bacteria, Aberdeen, UK) and NCFB 811 (National Collection of Food Bacteria, Reading, UK) preferentially used glucose with respect to fructose, and lactate and ethanol were the main fermentation end-products (Erten, 2000). Low concentrations of acetate and mannitol were also found. The activity of the starters inhibited spoilage microorganisms, prevented the softening, and stabilized ascorbic acid and the natural colours of the vegetable. Allochthonous starters (e.g., *L. plantarum* RSKK 1062, Refik Saydam Institute, Turkey) were also used for making vegetable juices, aiming at favouring the activity of pectolytic enzymes, which increases the juice yield (Wong, 1995), and at rapidly decreasing the value of pH, when the matrix was poorly acid (carrots) (Demir et al., 2006).

Usually, commercial starters are not previously selected to ferment a specific vegetable or fruit matrix. Only one report described the selection of *L. plantarum* NK-312 (Rosell Institute, Montreal, Canada), *P. pentosaceus* AFERM 772 (Quest International, Lachine, Canada) and *Leuc. mesenteroides* BLAC (Food Research and Development Centre, Canada) to ferment a mixture of cabbages, carrots, beets and onions (Gardner et al., 2001). Nevertheless, the intrinsic and extrinsic parameters markedly differentiated most of the vegetables and fruits. In some cases, commercial/allochthonous starter cultures may present several limitations: (i) the selection

did not consider other features than rapid acidification; (ii) the adaptation to the main sensory and functional properties of the matrix is poor; (iii) the metabolic flexibility is low; and (iv) the diversity did not reflect the ecosystem where they have to be used (Oberman and Libudzisz, 1998). Consequently, highly performing commercial/allochthonous starter cultures are very rare. To guarantee industrial scale fermentation of vegetables and fruits, commercial starters with high performances are needed.

### 3.2. The autochthonous starters

Selection of starter cultures within the autochthonous microbiota of vegetables and fruits should be recommended since autochthonous cultures may ensure prolonged shelf life and targeted nutritional, rheology and sensory properties (Di Cagno et al., 2008a,b; 2009, 2010a; 2011a,b).

Autochthonous *L. plantarum* starters were compared to allochthonous strains (isolated from green olives) during fermentation of tomato juice (Di Cagno et al., 2008b). Compared to selected autochthonous strains, allochthonous strains showed longer latency phases of growth and acidification (Fig. 2) After fermentation (25 °C for 17 h), all autochthonous strains grew from ca. 7.0 to ca. 9.6 log cfu mL<sup>-1</sup>. The unstarted tomato juice contained ca. 5.2 log cfu mL<sup>-1</sup> of presumptive lactic acid bacteria, while the allochthonous *L. plantarum* reached ca. 8.5 log cfu mL<sup>-1</sup>. After 40 days of storage at 4 °C, only the unstarted tomato juice and that fermented with the allochthonous strain contained yeasts. The use of autochthonous strains conferred the highest viscosity to the juice, especially, when the exo-polysaccharides (EPS) producer *W. cibaria*/*W. confusa* was used. The synthesis of EPS is widespread within the bacterial population of vegetables and it is also positively correlated with keeping the green–red and blue–yellow tonalities during processing (Sánchez-Moreno et al., 2006). Tomato juices fermented with autochthonous strains also maintained the highest values of ascorbic acid, glutathione and total antioxidant activity during storage. A large number of volatile components differentiated the tomato juices fermented with autochthonous or allochthonous strains. This latter mainly synthesized aldehydes (e.g., butanal, pentanal and 2,4-hexadienal), which may cause off-flavours (Moio and Addeo, 1998). Autochthonous strains synthesized volatile compounds that had a positive log odour value in tomato juice: 3-methyl-3-butan-1-ol (Ortiz-

Serrano and Gil, 2007), 2-3 butanedione, 3-hydroxy-2-butanone (Buttery et al., 1990), dimethyl sulphide and terpenes (Guadagni and Miers, 1969; Buttery et al., 1990; Servili et al., 2000). When fermented with selected autochthonous starters (*L. plantarum* M1, *Leuc. mesenteroides* C1 and *P. pentosaceus* F4), carrots, French beans and marrows showed a rapid decrease of pH, marked consumption of fermentable carbohydrates, and inhibition of Enterobacteriaceae and yeasts. Allochthonous starters, belonging to the same species, did not show the same performance. The differences between autochthonous and allochthonous strains were also pronounced regarding the concentration of vitamin C, colour indexes, firmness and sensory properties. The use of autochthonous strains (*L. plantarum* PE21, *Lactobacillus curvatus* PE4 and *W. confusa* PE36) was preferable to the spontaneous fermentation during processing of red and yellow peppers (Di Cagno et al., 2009). Autochthonous strains dominated the microbiota, and positively affected firmness and colour. Since fresh and minimally processed pineapples have a very short shelf life, they are canned and subjected to very intense heat treatments, which in part decrease the sensory and nutritional properties. A protocol for minimally processing, which included the use of selected autochthonous *L. plantarum* 10R12 and *L. rossiae* 2MR10, was set up (Di Cagno et al., 2010a). After 30 days of storage at 4 °C, started pineapples had a number of lactic acid bacteria up to 1,000,000 higher than the spontaneously processed pineapples, as well as the lowest number of yeasts, the highest antioxidant activity and firmness, the better preservation of the natural colours, and the preference for odour and overall acceptability. Sweet cherry (*Prunus avium* L.) puree added of stem infusion was fermented with selected autochthonous *P. pentosaceus* SWE5 and *L. plantarum* FP3 (Di Cagno et al., 2011b). Although the hostile environment (pH 3.9, high presence of phenolic compounds), the above strains grew well, showed metabolic adaptation to environment and remained viable during 60 days of storage at cell numbers, which exceeded those of potential probiotic beverages (Yoon et al., 2004). During storage, *P. pentosaceus* and *L. plantarum* markedly consumed malic acid and free amino acids. Overall, microbial adaptation to acidic conditions includes the use of non conventional carbon sources either to synthesize additional ATP moles or to favour the homeostasis of the intracellular pH (Gobbetti et al., 2005). Autochthonous *Lactobacillus pentosus* and *L. plantarum*, and *Candida diddensiae* were used as starters for the traditional fermentation of Arbequina naturally green olives (Hurtado et al., 2010; Aponte et al., 2012). Compared to the spontaneous fermentation, the survival of Enterobacteriaceae was inhibited. *L. pentosus* showed a very short latency phase of acidification and rapidly decreased the pH of the brine. Fourteen days more were needed by spontaneous fermentation to reach the same value of pH. Fig. 3 shows an example of a biotechnology protocol to ferment raw vegetables and fruits, which includes the use of single or mixed autochthonous lactic acid bacteria starters.

### 3.3. Criteria for selection of autochthonous lactic acid bacteria starters

Not all the strains that compose the lactic acid bacteria microbiota of vegetables and fruits may guarantee the same performance during processing. Therefore, their selection is indispensable. The main criteria used to select, and the corresponding metabolic traits, are usually divided into three main categories: (i) pro-technological; (ii) sensory; and (iii) nutritional (Table 2). Although heat treatments could in part change the chemical composition of the raw matrix, sterilized/pasteurized vegetable or fruit juice media are considered to be the most suitable model systems to carry out the selection (Gardner et al., 2001). Environmental adaptation of presumptive starters is the primary requisite, which affect all the

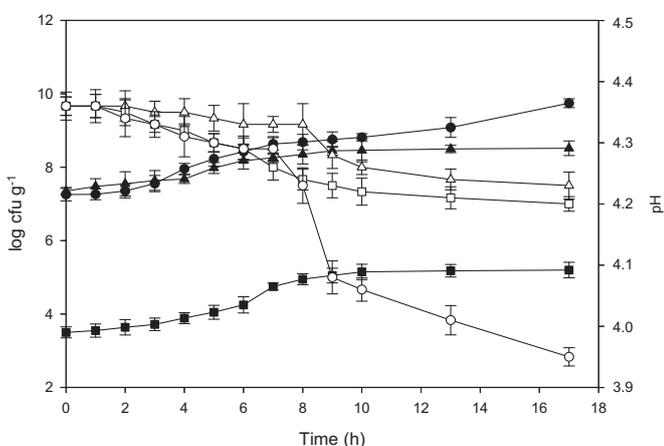


Fig. 2. Representative kinetics of growth (solid symbols) and acidification (open symbols) of unstarted tomato juice (squares), and tomato juices fermented with the autochthonous *Lactobacillus plantarum* POM35 (circles) and allochthonous *Lactobacillus plantarum* LP54 (triangles). Adapted from Di Cagno et al. (2008b).

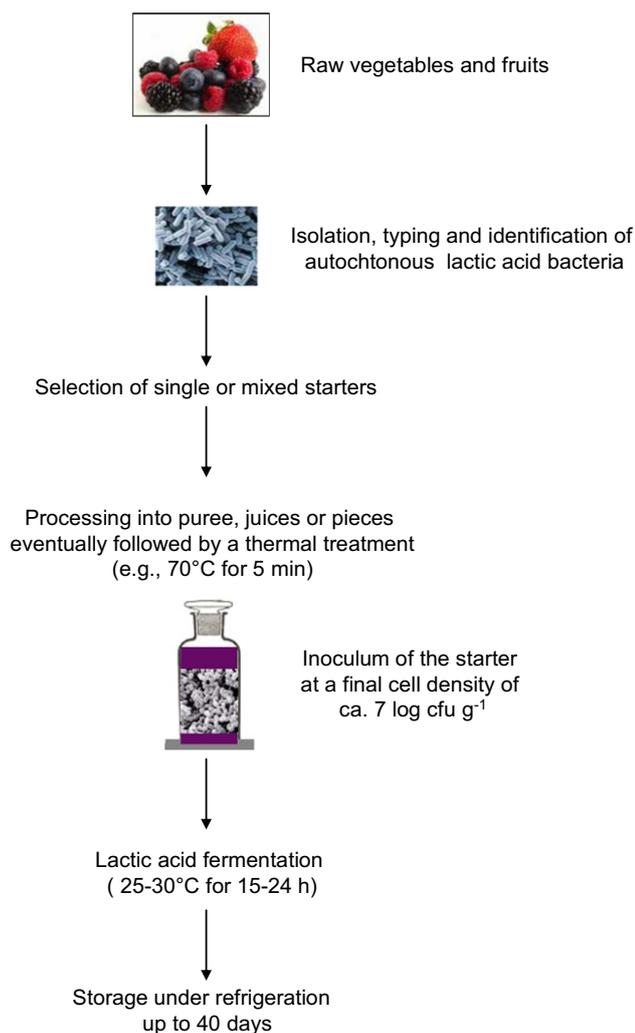


Fig. 3. Example of a biotechnology protocol to ferment raw vegetables and fruits.

other potential metabolic features (Karovicova et al., 1999; Gardner et al., 2001). Concentration of fermentable carbohydrates, buffering capacity, pH and presence of inhibitory compounds are the main environmental factors, which affect the growth and acidification of

**Table 2**  
Main criteria and corresponding metabolic traits to select starters for vegetable and fruit fermentation.

Criteria	Metabolic traits
Pro-technological	Growth rate
	Acidification rate
	Salt tolerance
	Growth at low values of pH
	Tolerance to low values of pH
	Growth at low temperature
	Completeness of fermentation
	Malolactic fermentation
	Tolerance to phenols
	Synthesis of antimicrobial compounds
	Synthesis of hydrogen peroxide
	Pectinolytic activity
	Sensory
Synthesis of aroma compounds or their precursors	
Nutritional	Synthesis of biogenic compounds
	Synthesis of exo-polysaccharides
	Increase of the antioxidant activity
	Synthesis of biogenic amine

lactic acid bacteria (Demir et al., 2006). Tolerance to high concentrations of phenols is indispensable to grow on some plant materials, where such compounds are particularly abundant (e.g., cherry, pomegranate) (Rodríguez et al., 2009; Di Cagno et al., 2011b). *L. plantarum* strains, which possess a broad portfolio of enzymes (e.g.,  $\beta$ -glucosidase, *p*-coumaric acid decarboxylase, general decarboxylase) have the capacity to degrade oleuropein (Landete et al., 2008), hydroxycinnamic acid derivatives (*p*-coumaric and ferulic acid) (Rodríguez et al., 2008), and caffeic gallic and protocatechuic acids (Rodríguez et al., 2008). Autochthonous starters have to rapidly drop the pH until values of at least 4.5. This is indispensable to get the inhibition of undesirable microorganisms from the early stage of fermentation. Several strains of *L. plantarum*, which were variously isolated from plant materials, had the capacity to ferment the main carbon sources of these ecosystems (D-fructose, gentibiose,  $\alpha$ -D-glucose, D-mannitol, D-mannose,  $\beta$ -methyl-D-glucoside and sucrose) (Di Cagno et al., 2010b). Not surprisingly, none of the above isolates had the capacity to ferment lactose. Once the lactic acid fermentation is completed, depletion of almost all fermentable carbohydrates is also required to avoid alcoholic fermentation by yeasts (Andersson et al., 1990). Survival of autochthonous starters throughout fermentation and storage processes is also expected. High cell number of starters (ca. 8.0–9.0 log cfu mL<sup>-1</sup>) guarantees the hygiene, and the eventual probiotic features. The synthesis of hydrogen peroxide by starters is another criterion to be considered for selection. Hydrogen peroxide, a strong oxidizing agent, may react with organic material, and cause, aside from degradation of antioxidant components, an undesired loss of the product colour (Zalán et al., 2012). The synthesis of EPS is another important feature, either for the viscosity of juices and purees or for the nutritional properties (Di Cagno et al., 2008b; Hernández et al., 2009; Di Cagno et al., 2011a). For a few vegetables, mild acidification is preferred (Lee et al., 2011). This is the case of kimchi, where over souring is one of the most serious defect. Autochthonous strains of *Lactobacillus sakei* were selected because of their mild acid producing properties (Rhee et al., 2011).

Despite the characterization of numerous metabolic tracts of autochthonous lactic acid bacteria, further studies on physiology, genetics and technology properties have to be encouraged to more in depth exploit their potential.

#### 4. Functional properties of autochthonous lactic acid bacteria

Nowadays, the only source where commercial probiotic strains are isolated is the gastrointestinal tract of humans. Nevertheless, other ecosystems may harbour functional strains that should possess the resistance to gastric acidity and bile toxicity, and the capacity of adhesion to the gut epithelium, to hydrolyse nutritional constituents, which cannot be metabolized by the host (e.g., fructooligosaccharides, FOS), and to synthesize antimicrobial substances (Lee et al., 2001; FAO/WHO, 2006). More than other food ecosystems, raw fruits and some vegetables possess intrinsic chemical and physical parameters that, for some traits, mimic those of the human gastrointestinal tract such as the extremely acid environment, buffering capacity, high concentration of indigestible nutrients (fibre, inulin and FOS) and anti-nutritional factors (tannins and phenols) (Buckenhüskes, 1997; Rossi et al., 2005). In most of the cases, the autochthonous microbiota of fruits and vegetables has to colonize and adhere to surfaces, and it exerts antagonistic activity towards spoilage and pathogenic microorganisms. A large number of autochthonous lactic acid bacteria, previously isolated from carrots, French beans, cauliflower, celery, tomatoes and pineapples, were assayed for functional properties (Vitali et al., 2012). In particular, strains of *L. plantarum* showed high survival under

**Table 3**  
Functional activities of autochthonous lactic acid bacteria, which were isolated from raw and fermented vegetables.

Microorganism	Food matrix	Functional activity	Reference
<i>Lactobacillus fermentum</i>	Japanese pickle	Immunoenhancing effect	Kawahara and Otani, 2006
<i>Pediococcus pentosaceus</i> MP12; <i>Lactobacillus plantarum</i> LAP6	Pickled cabbage	Adhesion on mouse epithelial cells; inhibition on pathogenic bacteria ( <i>Salmonella</i> )	Chiu et al., 2007
<i>L. plantarum</i> IB2	Ethnic fermented vegetables from Asia	Antimicrobial activity towards <i>Staphylococcus aureus</i>	Tamang et al., 2009
<i>L. plantarum</i>	Various raw fruits and vegetables	Stimulation of immune-mediators, adhesion to human intestinal Caco-2 cells, inhibition of <i>Escherichia coli</i> and <i>Bacillus megaterium</i>	Vitali et al., 2012
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	Various raw and fermented vegetables	Growth in the presence of bile salts, removal of cholesterol from the growth media	Kimoto et al., 2004
<i>L. plantarum</i> c16, c19	Olives	Growth in the presence of bile salts, adhesion to IPEC-J2 cells, inhibition of <i>E. coli</i>	Bevilacqua et al., 2010

simulated gastric and intestinal conditions, stimulated a large number of immune-mediators by peripheral blood mononuclear cells, including cytokines with pro- and anti-inflammatory activities, strongly adhered to Caco-2 cell, used FOS as the only carbon source, and inhibited pathogens from human sources. Similar results were found for autochthonous strains, which were isolated from kimchi and belonged to the species *L. plantarum* and *L. sakei* (Lee et al., 2011). For these strains, the capacity of lowering the cholesterol was also suggested. Functional properties were also attributed to lactic acid bacteria strains, which were isolated from Nozawana-zuke, a traditional Japanese pickle made with *Brassica campestris* L. var. *rapa* (Kawahara and Otani, 2006). A T helper 1-type immuno-enhancing effect was found. Strains of *L. plantarum* and *P. pentosaceus*, which were isolated from various pickled vegetables, showed an antagonistic effect towards *Salmonella* invasion (Chiu et al., 2007). Strains of lactic acid bacteria, which were isolated from ethnic fermented vegetables of the Himalayas (e.g., gundruk, sinki, khalpi, inziangsang, and fermented bamboo shoot products), had the capacity to degrade anti-nutritive factors, to inhibit *Staphylococcus aureus* and to adhere to mucus secreting HT29 MTX cells (Tamang et al., 2009).

A list of the functional properties shared by lactic acid bacteria, which were isolated from raw and fermented vegetables and fruits, is reported in Table 3.

### 5. Traditional and emerging fermented vegetables and fruits

Table 4 shows some examples of emerging and traditional fermented vegetables and fruits, which are manufactured in various worldwide regions. Nowadays, the lactic acid fermentation of vegetables has an industrial significance mainly for cabbages, cucumbers and olives (Montet et al., 2006; Rodríguez et al., 2009). An abundant literature has already described the lactic acid fermentation of cabbages and olives for making sauerkrauts and table olives (for review see Hurtado et al., 2012). The protocols used for making emerging and traditional pickles, and some novel fermented vegetables and fruits are described following.

#### 5.1. Pickles

Pickles are mainly manufactured through lactic acid fermentation of fruits and, especially, vegetables, which mainly occurs

**Table 4**  
Examples of emerging and traditional fermented vegetables and fruits, which are manufactured in various worldwide regions.

Product	Main ingredients	Main lactic acid bacteria involved	Country
Sauerkraut	Cabbage, salt	<i>Leuconostoc mesenteroides</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus plantarum</i>	Europe, USA
Cucumbers	Cucumbers, vinegar, salt	<i>Pediococcus pentosaceus</i> , <i>L. plantarum</i>	USA, Asia
Capers	Capers, water, salt	<i>L. plantarum</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus fermentum</i> , <i>L. brevis</i> , <i>Lactobacillus paraplantarum</i> , <i>Enterococcus faecium</i> , <i>P. pentosaceus</i>	Mediterranean Countries (Greece, Italy, Spain, Turkey, Morocco)
Kimchi	Cabbage, radish, salt, spices and other vegetables (ginger, pepper, garlic, onion)	<i>Leuc. mesenteroides</i> , <i>Leuconostoc kimchii</i> , <i>Leuconostoc citreum</i> , <i>Leuconostoc gasicomitatum</i> , <i>Leuc. pseudomesenteroides</i> , <i>L. plantarum</i> , <i>L. brevis</i> , <i>Lactobacillus curvatus</i> , <i>Lactobacillus sakei</i> , <i>Lactobacillus maltaromicus</i> , <i>Lactobacillus bavaricus</i> , <i>P. pentosaceus</i> , <i>Weissella confusa</i> , <i>Weissella kimchii</i> , <i>Weissella koreensis</i>	Korea
Dhamuoi	Cabbage and other vegetables	<i>Leuc. mesenteroides</i> , <i>L. plantarum</i>	Vietnam
Burong mustala	Mustard	<i>L. brevis</i> , <i>Pediococcus</i> sp.	Philippine
Dakguadong	Mustard leaf, salt	<i>L. plantarum</i>	Thailand
Gundruk	Local cabbages, mustard leaves, cauliflower leaves,	<i>L. plantarum</i> , <i>L. casei</i> subsp. <i>casei</i> , <i>Leuc. pseudoplantarum</i> , <i>L. fermentum</i> , <i>P. pentosaceus</i>	Eastern Himalaya
Sinki	Radish roots	<i>L. fermentum</i> , <i>L. plantarum</i> , <i>L. brevis</i> , <i>Leuconostoc fallax</i>	Eastern Himalaya
Khalpi	Cucumber	<i>L. plantarum</i> , <i>L. brevis</i> , <i>Leuc. fallax</i> , <i>Pediococcus</i> sp.	Eastern Himalaya
Pak-Gard-Dong	Mustard leaves	<i>L. brevis</i> , <i>L. plantarum</i>	Thailand
Turşu	Cucumbers, cabbage, green tomatoes, green peppers and other vegetables	<i>L. plantarum</i> , <i>Leuc. mesenteroides</i> , <i>L. brevis</i> , <i>P. pentosaceus</i> , <i>E. faecalis</i>	Turkey
Şalgam	Black/violet carrots, turnip, bulgur flour, sourdough, salt and water	<i>L. plantarum</i> , <i>L. paracasei</i> subsp. <i>paracasei</i> , <i>L. fermentum</i> , <i>L. brevis</i>	Turkey
Hardaliye	Red grape juice, black mustard seeds	subsp. <i>L. paracasei</i> subsp. <i>paracasei</i> , <i>L. caseipseudoplantarum</i> , <i>L. pontis</i> , <i>L. brevis</i> , <i>L. acetotolerans</i> , <i>L. sanfranciscensis</i> .	Turkey
Jiang-gua	Cucumbers, salt, sugar, vinegar, soy sauce	<i>Enterococcus casseliflavus</i> , <i>Leuconostoc lactis</i> , <i>Leuc. mesenteroides</i> , <i>L. pentosus</i> , <i>L. plantarum</i> , <i>L. paraplantarum</i> , <i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Weissella hellenica</i> , <i>Weissella cibaria</i>	Republic of China

before the pickling process. Although processes at industrial scale were developed, the manufacture of pickles mainly takes place at artisan or domestic levels.

#### 5.1.1. Kimchi

Kimchi is the name given to various traditional fermented vegetables, which are emblematic of the Korean culture. Kimchi is mainly manufactured with Chinese cabbages (*Brassica pekinensis*) and radish, but other seasonings ingredients such as garlic, green onion, ginger, red pepper, mustard, parsley, fermented seafood (jeotgal), carrot and salt may be used (Jung et al., 2011). Due to its nutritional properties, kimchi was recently included in the list of the top five “World’s Healthiest Foods” (<http://eating.health.com/2008/02/01/worlds-healthiest-foods-kimchi-korea/>). These beneficial effects are attributed either to functional components (vitamins, minerals, fibre and phytochemicals) or to fermentation by lactic acid bacteria (Lee et al., 2011). The main pickles are tongbaechu-kimchi, tongkimchi and bossam-kimchi. After soaking with water, cabbages are cut and placed into a salt solution (5–7% for 12 h), which favours an increase of the concentration of NaCl up to 2.0–4.0% of the total weight. Further, cabbages are rinsed several times with fresh water and drained. The fermentation is carried out by autochthonous lactic acid bacteria, which vary depending on the main ingredients, temperature (5–30 °C) and concentration of salt. Usually, *Leuc. mesenteroides* starts the fermentation but it is suddenly inhibited by the increasing concentration of lactic acid. Acid-tolerant species such as *L. brevis* dominate during the middle stage, being replaced by *L. plantarum* during late fermentation. Nevertheless, the best tasting kimchi is obtained before the overgrowth of *L. plantarum* and *L. brevis*, at an optimal pH of 4.5. After fermentation, kimchi is left to ripen for several weeks under refrigeration conditions (Lee et al., 2011).

#### 5.1.2. Cucumbers

Fully ripe cucumbers are washed, drained and, eventually, sliced for making fermented pickles. The manufacture consists of selection of regular shaped cucumbers, dipping into brine (5–7% of NaCl) inside plastic or glass boxes, spontaneous lactic acid fermentation and packaging. A marked reduction of the salt concentration (4% or less) is feasible when cucumbers are blanched to, in part, inhibit the autochthonous microbiota. Sometimes, calcium chloride is added into the surface to allow a crisp texture. As soon as the brine is obtained, the spontaneous lactic acid fermentation starts, lasting 2–3 weeks, which depended on the temperature (20–27 °C). The final pH ranges between 3.1 and 3.5. During fermentation, lactic acid bacteria synthesize several bacteriocins and liberated antimicrobial peptides, which are inhibitory to spoilage bacteria. Keeping the structure integrity of whole cucumbers is very important during brine fermentation. Carbon dioxide may be synthesized through cucumber respiration and malo-lactic fermentation by *L. plantarum*. Since gaseous spoilage (bloat damage) may cause serious economic losses, cucumbers may be purged with air to remove CO<sub>2</sub>. Nevertheless, this practice may increase the risk of growth of moulds and yeasts. Khalpi is the most popular cucumber pickly in the Himalayan region. Cucumbers are cut into pieces, sun dried for 2 days, put into a bamboo vessel and left to ferment at room temperature for 3–7 days (Tamang et al., 2005).

#### 5.1.3. Capers

Caper berries are the fruits of *Capparis* species (mainly *Capparis spinosa* L.), a Mediterranean shrub cultivated for its buds and fruits. Fermented capers are typical of Mediterranean countries (e.g., Greece and Italy). Fruits are harvested during June or July, immersed in tap water, and subjected to spontaneous lactic acid

fermentation for ca. 5–7 days at ambient temperature, which may markedly vary from 23 to 43 °C. Subsequently, fermented capers are placed into brine and distributed for consumption. *L. plantarum* is the main species, which is isolated from the brine of capers (Pulido et al., 2012).

#### 5.1.4. Ethnic pickles

Besides the above semi-industrial or artisanal products, a large variety of fermented pickles, which represents a culture heritage from the tradition of rural communities, is scarcely documented. Perishable and seasonal leafy vegetables, radish, cucumbers and young tender bamboo shoots are fermented into edible products using indigenous techniques of bio-preservation (e.g., pickling) (Tamang, 2012). Such fermented pickles are very popular in Asian and African countries, where they are fundamental components of the daily diet (Tamang, 2012). Under these poor technology conditions, lactic acid fermentation is the simplest, and probably the only, way to preserve fruits and vegetables.

Turşu is a traditional pickle made in Turkey with a wide variety of different vegetables and fruits such as cucumbers, cabbages, green tomatoes, green peppers, carrots, green beans, red beets, eggplants or melon (FAO, 1998; Kabak and Dobson, 2011). Vegetables or fruits are pressed into vessels and added of brine, which contains 10–15% of NaCl. Vinegar is also added, and pickles are left to ferment at ca. 20 °C for 4 weeks. Fermentation by autochthonous lactic acid bacteria dominates and yeasts (e.g., *Torulopsis* sp., *Hansenula* sp., and *Saccharomyces* sp.) occur during the late stage (Kabak and Dobson, 2011).

Sweet potato are also suitable substrates for making fermented pickles. Optimal sensory properties were achieved through the use of brine, which contained 10% of NaCl, and fermentation with selected strains of *L. plantarum*. Pickled garlic is increasing the popularity among consumers because of the specific sensory properties. Spontaneous fermentation was shown to improve some health-promoting features of pickled garlic, especially the concentration of polyphenols and the related antioxidant activity (Montaño et al., 2004).

Pak-Gard-Dong is a fermented mustard leaf (*Brassica juncea*) made in Thailand. Mustard leaves are washed, wilted, mixed with salt and left to ferment for ca. 12 h. The water is then drained and a solution, containing 3% of sugars, is added. A further fermentation is allowed for 3–5 days at room temperature. A similar pickle is Hum-choy, which is manufactured in the South of China from local leafy vegetables. After washing and draining, leaves are covered with salt, sun dried and put into pots, to allow the spontaneous fermentation, which is lasting 4 days. Pickled radish roots (Sinki) are also very common in other Asian countries such as Korea, India, Nepal and Buthan. The manufacture of these pickles resembles that described elsewhere (Tamang et al., 2005).

#### 5.2. Innovative vegetable- and fruit-based fermented products

Innovation in food biotechnology has an important role to improve the nutritional features, possibly also enhancing the hedonistic aspects.

Smoothies are an example of this trend to increase the consumption of vegetables and fruits, as an alternative and/or a complement to fresh products. Smoothies were first introduced in 1960 in United States. Further, they re-emerged worldwide in 2000 (Titus, 2008). The manufacture of smoothies is based on the use of a mixture of fruits and vegetables, after removing seeds and peel, which are processed into pulp or puree (Qian, 2006). In most of the cases, the selection of the mixtures is based on the colour, flavour, drinkable texture and, especially, to ensure high concentration of nutrients with low energy content (Watzl, 2008). Recently, a novel

protocol for the manufacture of fermented smoothies was set up (Di Cagno et al., 2011a). White grape juice and *Aloe vera* extract were mixed with red (cherries, blackberries, prunes and tomatoes) or green (fennels, spinach, papaya and kiwi) fruits and vegetables, and subjected to fermentation with mixed autochthonous starters, consisting of *L. plantarum*, *W. cibaria* and *L. pentosus* strains. Lactic acid fermentation by selected starters positively affected the content of antioxidant compounds and enhanced the sensory attributes.

The consumer demand for non-dairy beverages with high functional value is increasing also as the consequence of the ongoing trend of vegetarianism and the increasing prevalence of the lactose intolerance. Functional beverages made with a mixture of rice and barley or emmer and concentrated red grape must were fermented with selected strains of *L. plantarum*, which remained viable at ca.  $8.5 \log \text{ cfu g}^{-1}$  throughout storage at  $4^\circ\text{C}$  for 30 days (Coda et al., 2012). The use of fruit and vegetable matrices as potential non dairy vehicles for delivering probiotic strains was largely assessed (Gobbetti et al., 2010; Rivera-Espinoza and Gallardo-Navarro, 2010; do Espírito Santo et al., 2011). Probiotics have the capacity to grow and to survive, depending on the inherent characteristics of the plant species (do Espírito Santo et al., 2011). *Lactobacillus acidophilus*, *L. plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii*, *Leuc. mesenteroides*, and species of the genus *Bifidobacterium* were mainly used. Compared to cranberry juice, *Lactobacillus* and *Bifidobacterium* strains survived for a longer time in orange and pineapple juices. *L. casei*, *L. rhamnosus*, and *Lactobacillus paracasei* remained viable in orange juice at a cell number higher than  $7.0 \log \text{ cfu mL}^{-1}$  during 12 weeks of storage (Sheehan et al., 2007). *L. plantarum* and *L. delbrueckii* were able to survive at ca.  $8.0 \log \text{ cfu mL}^{-1}$  for 2 weeks in pomegranate juice, while *L. paracasei* and *L. acidophilus* showed a marked decrease of the viability (Mousavi et al., 2010). Tomato, carrot, cabbage, artichokes and reed beet juices were proven to be particularly suitable for probiotic fermentation, allowing a rapid growth of the strains and viable cell population above ca.  $8 \log \text{ cfu mL}^{-1}$  (Valerio et al., 2006; Rivera-Espinoza and Gallardo-Navarro, 2010). Nutrients essential for microbial growth and survival are also added to vegetable juices. *L. plantarum* and *L. acidophilus* strains showed optimal growth and acidification in beetroot juice when it was added of yeast extract (Rakin et al., 2007). Probiotics not only have to survive at high cell numbers but do not have to impart unsuitable modifications of the sensory properties of the fruit juice. The careful selection of the fruit matrix, probiotic strains and the addition of other ingredients are all biotechnology options to optimize the manufacture of probiotic non-dairy beverages (Mousavi et al., 2010).

## 6. Conclusion

Lactic acid fermentation undoubtedly represents the easiest and the most suitable way for increasing the daily consumption of fresh-like vegetables and fruits. Often lactic acid fermentation occurs spontaneously following protocols of manufacture, which are strongly linked to the culture and traditions of worldwide countries. New molecular approaches to study the composition of the microbiota and to select autochthonous starters targeted for different vegetables and fruits have to be encouraged to get new insights and to allow controlled fermentation processes as it was done for other fermented foods (cheeses, sausages, leavened baked goods). Lactic acid bacteria tailored to the various intrinsic and extrinsic environmental conditions completely exploit the potential of vegetables and fruits, which enhances the hygiene, sensory, nutritional and shelf life properties.

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