Lactic Acid Fermentation of Vegetables and Fruits

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Lactic Acid Fermentation of Vegetables and Fruits

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1 Introduction

Lactic acid (LA) fermentation is considered a simple and useful form of biotechnology to keep and/or enhance the safety, nutritional, sensory and shelf life properties of vegetables and fruits (Demir et al. 2006). As shown in the data from literature of the last decade, 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, Di Cagno et al. 2013). Lactic acid bacteria (LAB) convert the carbohydrate contents of the vegetables and fruits into LA, which decreases the pH of the fermented products to around 4.0 ensuring stability. Lower pH value restricts the growth of spoilage flora and pathogenic bacteria. These bacteria improve the human intestinal microbial balance and enhance health by inhibiting the growth of pathogens such as *Escherichia coli*, *Salmonella* and *Staphylococcus* (Ohmomo et al. 2000, Ross et al. 2002). They are often considered as probiotic, beneficial for human health and active in lowering the serum cholesterol level (Kaur et al. 2002). They also stimulate immune responses and prevent tumour formation by inhibiting

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carcinogenic compounds in the gastro-intestinal tract through reducing fecal bacteria enzyme activity (Nakphaichit et al. 2011) or breaking down certain enterotoxins (Bernardeau et al. 2006).

Fruits are commonly processed for alcoholic fermentation of wine and beer as they are rich in sugars, vitamins, minerals. As juices are slightly acidic, they are therefore a suitable medium for the growth of yeasts, and fruit sugars are rapidly converted into ethanol. Vegetables on the other hand, have low sugar content but are rich in minerals, vitamins, have neutral pH and thus provide a natural medium for fermentation by LAB. Fermentation of fruits and vegetables can occur 'spontaneously' by the natural lactic acid bacterial surface microflora, i.e., *Lactobacillus*, *Leuconostoc*, *Pediococcus*, etc.; however, the use of starter culture such as *Lactobacter plantarum*, *Lb. rhamnosus*, *Lb. gasseri* and *Lb. acidophilus* (all probiotic strains) provides consistency and reliability of performance (Di Cagno et al. 2013). Pasteurizing or adding preservatives after fermentation, which are commonly done during the industrial production of lactic acid fermented vegetables (e.g., sauerkraut), destroy most of the LAB present, thus cancelling any possible probiotic effects (Montet et al. 2006).

This chapter describes the exploitation of vegetables and fruits through LA fermentation. The LAB microbiota, the occurring spontaneous fermentation, the main features of commercial/allochthonous and autochthonous starters, and the emerging and traditional fermented vegetable and fruit products are reviewed. The botanical names of vegetables and fruits mentioned in this chapter are given in Table 1.

2 Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a group of organisms that ferment sugar (i.e., glucose) predominantly to LA. They are gram positive, non-sporulating rods and cocci having low guanine-cytosine content. Most of the LAB grow an-aerobically but they are also aero-tolerant. This group of bacteria is divided into two sub-groups.

(i) Homo-fermentative

This sub-group of bacteria produces a single fermentation product, i.e., LA *via* the glycolytic (Embden- Meyerhof) pathway (Steinkraus 2002). Members of the genera are *Pediococcus*, *Streptococcus* and *Lactococcus*. The fermentation of one mole of glucose yields two moles of LA.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{Lactic acid}}{2CH_3CHOHCOOH}$$

Table 1. Common and botanical names of the fruit and vegetables mentioned in the text.

Common Name	Botanical Name
"Almagro" eggplants	*Solanum melongena* L. var. *esculentum depressum*
Artichoke	*Cynara cardunculus* var. *scolymus*
Apple	*Malus sylvestris* L.
Bamboo	*Bambusa glaucescens* L.
Beet root	*Beta vulgaris* L.
Black berry	*Rubus fruticosus*
Black pepper	*Piper nigrum* L.
Brinjal (syn. Aubergine/Eggplant)	*Solanum melongena* L.
Cabbage	*Brassica oleracea* var. *capitata* L.
Capsicum (Yellow or green pepper)	*Capsicum annum* L.
Carrot	*Daucus carota* L.
Cassava	*Manihot esculenta* Crantz
Casper berry	*Capparis spinosa* L.
Cauliflower	*Brassica oleracea* var. *botrytis* L.
Celeriac	*Apium graveolens* var. *rapaceum* L.
Chinese cabbage	*Brasicca chinensis* L.
Cucumber	*Cucumis sativus* L.
False banana	*Ensete ventricosum*
Fennels	*Phaseolus vulgaris* L.
French beans	*Foeniculum vulgare* L.
Garlic	*Allium sativum* L.
Ginger	*Zingiber officinale* L.
Green pea	*Pisum sativum* L.
Horseradish	*Lactuca sativa* L.
Immature palm	*Phoenix dactylifera* L.
Lemon	*Citrus lemon* L.
Lime	*Citrus aurantifolia* L.
Kiwi fruits	*Actinidia deliciosa* L.
Mango	*Mangifera indica* L.
Mustard	*Brassica juncea* L., *B. campestris* L.
Okra	*Abelmoschus esculentus* L.
Olives	*Olea europea* L.
Onion	*Allium cepa* L.
Pak-sian	*Gynadropsis pentaphylla*
Papaya	*Carica papaya* L.
Parsley	*Petroselinum crispum* L.
Pear	*Pyrus communis* L.
Peas	*Pisum sativum* L.
Pepper	*Piper nigrum* L.
Pineapple	*Ananas comosus* L.
Pomegranate	*Punica granatum* L.
Plums	*Prunus domestica* L.

Table 1. contd....

Table 1. contd.

Common Name	Botanical Name
Radish	*Raphanus sativus* L.
Red chilli	*Capsicum frutescens* L.
Sesame	*Sesamum indicum* L.
Spinach	*Spinacea oleracea*
Sweet cherry	*Prunus avium* L.
Sweet orange	*Citrus sinensis* L.
Sweet potato	*Ipomoea batatas* L.
Taro	*Colocasia esculenta* var. *antiquorum* L.
Tomato	*Lycopersicum esculentum* L.
Turnip	*Brassica rapa* L.

(ii) Hetero-fermentative

This sub-group of bacteria produces LA plus appreciable amount of ethanol, acetate and CO_2 *via* the 6-phosphogluconate/phosphoketose pathway (Steinkraus 2002). Bacteria involved in this group belong to genera *Leuconostoc* and *Lactobacillus*. The biochemical pathway is as follows.

$$C_6H_{12}O_6 \longrightarrow 2CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Glucose Lactic acid Ethanol Carbon dioxide

2.1 Information on D (–) Lactic Acid

Lactic acid is a three carbon carboxylic acid with the chemical formula $C_3H_6O_3$. In water solution, LA can lose a proton from the acidic group, producing the lactate ion CH_3CH (OH) COO; its pKa is 3.86. The higher acidity is the consequence of the intra-molecular hydrogen bridge between the α-hydroxyl and the carboxylate group, making the latter less capable of strongly attracting its proton. Two different isomers of LA may be produced during fermentation. They are classified depending on whether the polarized light rotates to the right L (+) or to the left D (–). The L (+) LA isomer is absorbed by the intestinal mucus and is used as energy substrate during the metabolic activity. On the other hand, the D (–) form is not assimilated and is eliminated by the kidneys in salt forms, leading to a loss of calcium and magnesium. Both of the LA isomers are usually present in the homemade or small-scale fermented vegetable preparations. However, the D (–) LA isomer concentrations in LA fermented vegetables are reported to be generally low.

2.2 Bacteriocin Production

LAB produce bacteriocins, which are peptides or small proteins that are frequently inhibitory towards many undesirable bacteria, including food-borne pathogens (e.g., *Listeria monocytogenes*, *Salmonella*, *Staphylococcus*, *Escherichia coli* and *Clostridium botulinum*) (Leroy et al. 2002). They can be subdivided into four groups:

- Class I of bacteriocins consists of lanthibiotics. These are small and heat stable peptides that contain thio-ether amino acids such as lanthionine (Hernandez et al. 2005)
- Class II is divided into three sub-groups of which Class IIa is the most common. This group is composed of pediocins like bacteriocins with anti-listerial activity. Pediocins are produced by *Pediococcus* spp. and while they are not very effective against spores, they are more effective than nisin in some food systems (O'Sullivan et al. 2002)
- Class III comprises large heat labile proteins (Eijsink et al. 2002), and
- Class IV is a complex of bacteriocins with glyco-and/or lipid moieties (Rodriguez et al. 2003)

An advantage of bacteriocins over classical antibiotics is that digestive enzymes destroy them. Bacteriocin-producing strains can be used as part of or adjuncts to starter cultures for fermented foods in order to improve safety and quality.

3 Principles of Lactic Acid Vegetable Fermentation

Lactic acid fermentations are carried out under three basic types of conditions: dry-salted, brined and non-salted. Salting provides a suitable environment for the growth of LAB, which imparts acidic flavour.

3.1 Dry-Salted Fermented Vegetables

In this process, dry salt is voluntary added to vegetables. For 100 kg of vegetables, approximately 3 kg of salt is needed. Salt extracts the juice from the vegetables and creates the brine. The vegetable is sliced, washed in potable water and drained. Then they are placed in a layer of about 2.5 cm depth in the fermenting container (a barrel or keg). Salt is sprinkled over the vegetables. Another layer of vegetables is added and more salt is added. This is repeated until the container is three quarters full. Usually, weight (stones) is placed to compress the vegetables and assists the formation of brine, which takes about 24 hr. As soon as brine is formed, fermentation starts and bubbles of CO_2 begin to appear. Fermentation takes place between

1 to 4 wk depending on the ambient temperature. Fermentation is complete when no more bubbles appear, then the pickle can be packaged in a variety of mixtures, i.e., vinegar and spices or oil and spices (Liu et al. 2011).

3.2 Brine-Salted Fermented Vegetables

In this process, a brine solution is prepared by dissolving salt in water (15 to 20% salt solution). Brine is used for vegetables that inherently contain lower water content. Best fermentation takes place in brine of about 12.5 to 20° Salometer (Liu et al. 2011). The strong brine solution draws sugar and water out of the vegetables, which decreases the inner salt concentration. It is crucial that the salt concentration does not fall below 10%; otherwise, conditions will not allow fermentation (Panda et al. 2009). To achieve this level, extra salt is added periodically to the brine mixture.

Once the vegetables have been brined and the container sealed, a rapid development of microorganisms is observed in the brine. The natural parameters that affect the microbial populations of the fermenting vegetables include the concentration of salt and temperature of the brine, the availability of fermentable materials and the numbers and types of microorganisms present at the start of fermentation. The rapidity of the fermentation is correlated with the concentration of salt in the brine and its temperature (Ray and Panda 2007).

Most vegetables can be fermented at 12.5 to 20º Salometer. If so, the sequence of LAB generally follows the classical sauerkraut fermentation. At higher salt levels of about 40º Salometer, the sequence is skewed towards the development of a homo-fermentation, dominated by *Lactobacillus plantarum*. At highest salt concentrations as 60º Salometer, lactic fermentation stops and if any acid is detected during brine storage, it is acetic acid, presumably produced by acid-forming yeasts which are still active at this salt concentration (Montet et al. 2006).

3.3 Non-Salted Lactic Acid Fermented Vegetables

Some vegetables can be fermented by LAB, without prior addition of salt or brine. Examples of non-salted products include gundruk (consumed in Nepal), sinki and other wilted fermented leaves (Dahal et al. 2005, Tamang et al. 2005). The detoxification of cassava through fermentation includes an acid fermentation, during which the cyanogenic glycosides are hydrolyzed to liberate the toxic cyanide gas (Onabolu et al. 2002a,b). The fermentation process relies on the rapid colonization of the food by LA-producing bacteria, which lower the pH and make the environment unsuitable for the growth of spoilage organisms. Oxygen is also excluded as the lactobacilli favour an anaerobic atmosphere. Restriction of oxygen ensures that yeasts do not grow.

4 Different Processes of Lactic Acid Fermentation

Lactic acid fermentations are of the following types:

- Spontaneous Fermentation
- Controlled Fermentation

4.1 Spontaneous Fermentation

Spontaneous fermentation leads to variations in the sensory properties of the products which differ according to the quality of raw material, temperature and harvesting conditions (Paramithiotis et al. 2010, Wouters et al. 2013). Fresh vegetables and fruits may present high microbial loads (around 10^5 to 10^7 microorganisms/g) after harvesting, most of which are Gram (–) ve bacteria and Gram (+) ve bacilli, yeasts and moulds. Lactic acid bacteria are least prevalent, accounting for less than 0.1% of the autochthonous microbial population as these bacteria require high nutrients such as amino acids, fatty acids, vitamins and certain minerals for their growth and metabolism. Therefore, the plant environment is not suitable for their development. But the plant medium can be enriched by salting or by the addition of certain protein ingredients (whey, bran, etc.) for their growth (Rao et al. 2004). During the fermentation process, lactic and acetic acids are formed and pH decreases thus inhibiting the Gram (–) ve and sporulating bacteria. In these conditions, only LAB are able to grow.

Some of the LAB isolated from naturally fermented vegetables are *Lactobacillus plantarum*, *Lb. brevis*, *Lb. lactis*, *Lb. paraplantarum*, *Lb. hilgardii*, *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, and *Lactococcus lactis*, etc. (Table 2) (Dahal et al. 2005, Tamang et al. 2005, Montet et al. 2006, Ponce et al. 2008, Paramithiotis et al. 2010, Di Cagno et al. 2013). Lactobacilli do not only produce LA but also H_2O_2 and bacteriocins, which inhibit the growth of pathogenic bacteria (Ray and Joshi, chapter 1 in this volume). Along with organic acids, the hetero-fermentative lactobacilli produce CO_2 which also has a preservative effect on foods.

Recently, metagenomic and metabolomic approaches were used to characterize the microbial community during spontaneous fermentation (Jung et al. 2011). The microbiota responsible for the spontaneous fermentation of kimchi was one of the largely investigated (Lee et al. 2002, 2005, Kim and Chun 2005, Park et al. 2010, Jung et al. 2011). In particular, *Lc. mesenteroides* and *Pediococcus pentosaceus* started the first stage of fermentation, and the combination of *Lb. plantarum* and *Lb. brevis*

Table 2. Microorganisms isolated from indigenously lactic acid fermented vegetables and fruits.

Product name	**Country**	**Main Ingredients**	**Microorganisms**
Burong mustala	Philippines	Mustard	*Lactobacillus brevis* *Pediococcus cerevisiae*
Cucumbers	Asia, USA	Cucumbers, vinegar, salt	*Lactobacillus plantarum*, *Pediococcus pentosaceus*
Dakguadong	Thailand	Mustard leaf, salt	*Lactobacillus plantarum*
Dhamuoi	Vietnam	Cabbage, various vegetables	*Leuconostoc mesenteroides* *Lactobacillus plantarum*
Gundruk	Nepal	Cabbage, radish, leafy vegetables	*Lb. plantarum*, *Lb. casei* subsp. *casei*, *Lc. pseudoplantarum*, *Lb. fermentum*, *P. pentosaceus*
Hardaliye	Turkey	Vegetables	*Lb. paracasei* subsp. *paracasei*, *Lb. casei Lb. pontis*, *Lb. brevis*, *Lb. acetotolerans*, *Lb. sanfranciscoensis*
Jiang-gua	Rep. of China	Cucumbers, salt, sugar, vinegar, soy sauce	*Enterococcus casseliflavus*, *Leuconostoc lactis*, *Lc. mesenteroides*, *Lb. pentosus*, *Lb. plantarum*, *Lb. paraplantarum*, *Lactococcus lactis* subsp. *lactis*, *Weissella hellenica*, *Weissella cibaria*
Kanji	India and Pakistan	Carrots	*Lactobacillus plantarum* *Lactobacillus brevis*
Khalpi	Nepal	Cucumber	*Lactobacillus plantarum*, *Lb. brevis*, *Leuconostoc fallax*, *Pediococcus pentosaceus*
Kimchi	Korea	Cabbage, radish, various vegetables and spices (ginger, pepper, garlic, onion)	*Leuconostoc mesenteroides*, *Leuconostoc kimchii*, *Leuconostoc citreum*, *Leuconostoc gasicomitatum*, *Lc. pseudomesenteroides*, *Lactobacillus plantarum*, *L. brevis*, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactobacillus maltaromicus*, *Lactobacillus bavaricus*, *P. pentosaceus*, *Weissella confusa*, *Weissella kimchii*, *Weissella koreensis*

Table 2. contd....

Table 2. contd.

Product name	Country	Main Ingredients	Microorganisms
Olive	Spain, Italy	Olive	*Lactobacillus plantarum*, *Lb. paracasei*, *Lb. pentosus*, *Lb. casei*, *Lb. vaccinostercus*, *Lb. suebicus*, *Lb. paracollinoides* *Lactobacillus brevis* *Pediococcus cerevisiae* *Leuconostoc mesenteroides*, *Lc. lactis*
Pak-sian-dong	Thailand	Leaves of Pak-sian	*Lactobacillus brevis* *Pediococcus cerevisiae* *Lactobacillus plantarum*
Salgam	Turkey	Black/Violet carrots, turnip, bulgur flour, sourdough, salt and water	*Lb. plantarum*, *Lb . paracasei* subsp. *paracasei*, *Lb. fermentum*, *Lb. brevis*
Sauerkraut	International	Cabbage	*Leuconostoc mesenteroides* *Lactobacillus plantarum* *Lactobacillus brevis*
Sinki	India, Nepal and Bhutan	Radish	*Lactobacillus plantarum* *Lactobacillus brevis* *Lactobacillus fermentum* *Leuconostoc fallax* *Pediococcus pentosaceus*
Sunki	Japan	Leaves of otaki- turnip	*Lactobacillus plantarum* *Lactobacillus brevis* *Pediococcus pentosaceus* *Bacillus coagulans*
Suan-tsai	Taiwan	Mustard leaves	*Pediococcus pentosaceus* *Tetragenococcus halophilus*
Tursu	*Turkey*	Cucumbers, cabbage, green tomatoes, green peppers and other vegetables	*Lb. plantarum*, *Lc. mesenteroides*, *Lb. brevis*, *P. pentosaceus*, *Enterococcus fecalis*

Source: Montet et al. 2006, Ray and Panda 2007, Hurtado et al. 2012, Di Cagno et al. 2013
Lb. = *Lactobacillus*, Lc. = *Leuconostoc*, P = *Pediococccus*

or *Lb. maltaromicus* and *Lb. bavaricus* further dominated depending on the temperature of incubation (20–30ºC or 5–7ºC, respectively). Distinct kinetics of growth characterized the three genera, *Leuconostoc, Lactobacillus* and *Weissella*, which dominated the fermentation. Similarly, phylogenetic analysis based on partial 16S-rRNA gene sequences exhibited that spontaneous cauliflower fermentation was characterized by an initial hetero-fermentative stage driven by strains belonging to *Lc. mesenteroides*-group that was followed by a homo-fermentative one with strains of *Lb. plantarum*-group dominating. Strains belonging to *Enterococcus faecium*-group and *Enterococcus faecalis*-group were also isolated but only at the early stages of fermentation (Paramithiotis et al. 2010).

4.2 Controlled Fermentation

Quality control is essential for the industrialization of fermentation process (Ray and Sivakumar 2009). For controlled LA fermentation, conditions must be created which favour the growth of commensal and/or inoculated LAB while excluding other microorganisms (Gardner et al. 2001, Di Cagno et al. 2008a,b, 2011a). 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 starters for fermentation of raw vegetables and fruits.

Two main options may be pursued for the controlled LA fermentation of vegetables and fruits: the use of autochthonous or allochthonous starters (Di Cagno et al. 2008a,b, 2009, 2010, 2011b). Autochthonous starters mean 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 (Di Cagno et al. 2013).

4.2.1 Commercial/allochthonous starters

Majority of the reports show the use of commercial/allochthonous starters in LA fermentation of vegetables and fruits (Gardner et al. 2001, Plengvidhya et al. 2004, Demir et al. 2006, Johanningsmeier et al. 2007). Few examples are cited here. Peeled and blanched garlic was fermented with commercial *Lb. plantarum* (de Castro et al. 1998). The allochthonous starter grew well in blanched garlic after two days of fermentation and LA was the main fermentation end-product. Allochthonous starters (e.g., *Lb. plantarum* RSKK

1062) 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 *Lb. plantarum* NK-312, *Pediococcus pentosaceus* AFERM 772 and *Lc. mesenteroides* BLAC to ferment a mixture of cabbages, carrots, beets and onions (Gardner et al. 2001). However, Di Cagno et al. (2013) have outlined some limitations in using allochthonous culture such as: (i) the selection time (days) did not consider other features except 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.

4.2.2 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. 2013). Autochthonous *Lb. 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, these allochthonous strains showed longer latency phases of growth and acidification. Tomato juices fermented with autochthonous strains maintained the highest values of ascorbic acid, glutathione and total antioxidant activity during storage. In another study, when fermented with selected autochthonous starters (*Lb. plantarum* M1, *Lc. 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 (Di Cagno et al. 2013). 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 (*Lb. plantarum* PE21, *Lb. curvatus* PE4 and *Weissella confusa* PE36) was preferable to the spontaneous fermentation during processing of red and yellow peppers (Di Cagno et al. 2009). Sweet cherry (*Prunus avium* L.) puree added with stem infusion was fermented with selected autochthonous *P. pentosaceus* SWE5 and *Lb. plantarum* FP3 (Di Cagno et al. 2011b). Although the environment was hostile (pH 3.9 and 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). Autochthonous *Lactobacillus pentosus* and *Lb. plantarum*, and *Candida diddensiae* were used as starters for the traditional fermentation of Arbequina naturally green olives (Hurtado et al. 2012, Aponte et al. 2012). Compared to the spontaneous fermentation, the survival of *Enterobacteriaceae* was inhibited. *Lb. 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.

5 Factors Affecting Lactic Acid Fermentation

There are seven factors that influence the growth and activity of LAB in fermenting fruits and vegetables. Those are pH, moisture and water activity, O_2 concentration, temperature, nutrients, selected starter culture and inoculum concentration (Lee and Salminen 1995, Ballesteros et al. 1999).

5.1 pH

The pH is a critical factor in preservation and developing aroma and flavour of many fermented fruits and vegetables like cabbage, olives, etc. (Muyanja et al. 2003, Rao et al. 2004). Most LAB favour conditions with a near neutral pH (Battcock and Azam-Ali 2001). Certain bacteria are acid tolerant (i.e., *Lactobacillus* and *Streptococcus*) and can survive at reduced pH levels (3.0–4.0) (Ray and Panda 2007).

5.2 O_2 Availability

The O_2 requirements vary from species to species. Unlike many anaerobes, however most LAB are not sensitive to O_2 and can grow in its presence as well as in absence. They are aero-tolerant anaerobes (Molenaar et al. 2005).

5.3 Temperature

Temperature is a critical factor for vegetable fermentation. Most LAB have a temperature optimum between 20ºC to 30ºC, there are some (thermophiles) which prefer high temperatures (50–55ºC) and those with colder temperatures optima (15–20ºC) (Ross et al. 2002). Most LAB work best at temperatures of 18–22ºC (Ray and Panda 2007).

5.4 Salt Concentration

Salting is an important step in vegetable fermentation. Sodium chloride concentration can range from 20 to 80 g/l during fermentation. LAB can tolerate high salt concentrations. This salt tolerance gives them an advantage over less tolerant species and allows LA fermentation that inhibits growth of non-desirable organisms (Rao et al. 2004). Salt induces plasmolysis in plant cells which releases mineral salts and nutrients from the vacuole and creates anaerobic conditions for proper growth of LAB around the submerged product (Gardner et al. 2001, Rakin et al. 2004, Wouters et al. 2013).

5.5 Water Activity

In general, LAB require a fairly high water activity (0.9 or higher) to survive. There are a few species, which can tolerate water activities lower than this, but usually, the yeasts and fungi will predominate on foods with a lower activity (Ray and Panda 2007).

5.6 Nutrients

All bacteria require a source of nutrients for metabolism. The fermentative bacteria require carbohydrates, either simple sugars such as glucose and fructose or complex carbohydrates such as starch or cellulose (Ray and Panda 2007, Wouters et al. 2013).

5.7 Selected Starter Cultures

The selection of starter cultures (either allochthonous or autochthonous) is based principally on the competitiveness between the starter and the natural flora, as well as on the sensory properties of the resulting products (McFeeters 2004). They are selected on the following criteria for fermentation of fruits and vegetables:

- Lack of production of toxic chemicals
- Ability to produce only (L+) lactic acid
- Low or nil production of biogenic amines
- Genetic stability of the species
- Rapid brine acidification
- Production reproducibility between different batch cultures
- Total depletion of fermentable sugars
- Resistance to bacteriocins and bacteriophages from natural strains
- Potential of strain preservation by drying, freezing or freeze-drying

6 Lactic Acid Fermentation of Vegetables and Fruits

Vegetables are rich in nutrients, vitamins and minerals and some of them contain coloured pigments such as flavonoids, lycopene, anthocyanin, β-carotene and glucosinolates, which act as antioxidant in the body by scavenging harmful free radicals implicated in degenerative diseases like cancer, arthritis and ageing (Kaur and Kapoor 2001).

Lactic acid fermentation of vegetables and fruits can be divided into four main categories:

A. Vegetable fruits such as cucumbers, tomatoes, peppers, okra and green-peas (Dahal et al. 2005).
B. Fruits such as olives, apples, pears, immature mangoes, immature palms, lemons and fruit pulps (Zhang et al. 2000).
C. Root and tubers such as carrots, turnips, beetroot, radishes, celeriac, cassava and sweet potato (Ray and Sivakumar 2009).
D. Innovative juices and smoothies from fruits and vegetables (Yoon et al. 2006, Di Cagno et al. 2013).

6.1 Lactic Acid-Fermented Vegetables

There are several traditional and non-traditional fermented vegetables available around the globe. Few important ones are discussed.

6.1.1 Sauerkraut

This is an example of dry salted fermented vegetables. It results from the natural LA fermentation of salted and shredded cabbage. It is a common way of preserving fresh vegetable in the Western world, in China and Korea (Liu et al. 2011). The high nutritive value of sauerkraut is mainly due to the increased digestibility in comparison to raw cabbage and relatively low vitamin C losses. For at least 150 yr, sauerkraut has been made in the home for saving fresh cabbage before spoilage (Steinkraus 2002, Viander et al. 2003). Other vegetables which are fermented by sauerkraut process are carrots, onions, garlic and beets (Gardner et al. 2001). Presently, the production of sauerkraut has become an important food industry in Korea and Vietnam.

Sauerkraut processing at industrial scale consists of trimming the mature and sound heads of cabbage to remove the outer green, broken or dirty leaves (Karovicova and Kohajdova 2002). The head of cabbage is then sliced by rotary knives into long and fine shreds. Salt is sprinkled on the shreds which are conveyed to the fermentation tanks. Spices could also be added. A salt concentration in the range of 2.25–2.5% is used for facilitating

LA fermentation. Forks are used to uniformly distribute the shreds which are dumped and squeezed into the vat. Once the tank has been filled to the proper level, it is closed. The shredded cabbage should be completely immersed to favour anaerobic conditions and prevent undesirable darkening and flavour changes. Within a few hours the brine is formed and the fermentation is started, the latter is initiated by *Lc. mesenteroides*, which produces lactic and acetic acids and CO_2. The pH is quickly lowered, thus limiting the activity of undesirable microorganisms and enzymes that might soften the cabbage shreds. In the next phase, homo-fermentative bacteria such as *Lactobacillus* and *Pediococcus* continue the fermentation to a final pH of 3.5 to 3.8 (Yoon et al. 2006, Xiong et al. 2012). The CO_2 replaces air and creates an anaerobic atmosphere which prevents the oxidation of ascorbic acid and the darkening of the natural cabbage colour. The optimal temperature for sauerkraut fermentation is 18ºC (Viander et al. 2003). At this temperature fermentation is completely performed in three weeks.

6.1.2 Kimchi

Kimchi is the name given to a group of traditional fermented vegetables in Korea (Cheigh and Park 1994, Cho et al. 2009). It is a popular side dish that is served at every meal with rice. Kimchi production in Korea is estimated at over 1.5 million tonnes, mainly at household level and daily consumption is estimated at 150 to 250 g (Cho et al. 2009). The main ingredient of kimchi is either Chinese cabbage to which radish and cucumber may be eventually added. Cabbages are cut and brined in salt (5–10%) solution for 12 hr or in 15% brine for 3–7 hr. Salting is followed by rinsing and draining the water. Minor ingredients such as garlic, onions, black pepper, ginger, mustard, parsley, sesame grains and fermented anchovies or shrimps are then added at 10% w/w of the main ingredient (Jung et al. 2011). The mixture is finally left to ferment in jars. Due to its nutritional properties, kimchi was recently included in the list of the top five World's Healthiest Foods (http://eating.health.com/2012/02/01/worlds-healthiest-foods-kimchi-korea/). These beneficial effects are attributed either to functional components (vitamins, minerals, fibre and phytochemicals) or to fermentation by LAB (Lee et al. 2011). The main kimchi are tongbaechu-kimchi, tongkimchi and bossam-kimchi (Di Cagno et al. 2013).

The LAB profile during kimchi fermentation varies with pH and acidity. In a multiplex PCR assay, *Lc. mesenteroides* was observed during early fermentation (pH, 5.64–4.27 and acidity, 0.48–0.89%), and *Lb. sakei* become dominant later in fermentation (pH, 4.15 and acidity, 0.98%) (Cho et al. 20). The other organisms found in kimchi are *Lb. plantarum*, *Lb. brevis*, *Lc. mesenteroides*, *Streptococcus faecalis* and *Pediococcus cerevisiae*, and aerobic bacteria such as *Achromobacter*, *Flavobacterium* and *Pseudomonas* spp. (Lee et al. 2005, Kim and Chun 2005). The main microorganism responsible for kimchi fermentation is *Lc. mesenteroides* and the main acidifying microorganism is *Lb. plantarum* (Lee et al. 2011).

6.1.3 Kocho

Kocho is a fermented product from false banana (*Ensete ventricosum*) pseudostem. It is produced in Ethiopia (Steinkraus 2002). False bananas are peeled before placing in the pit and left to ferment for three to six weeks, after which it becomes soft, has a strong odour and a paste-like consistency. During fermentation, CO_2 builds up in the pit creating an anaerobic atmosphere. As a result of bacterial activity, the temperature rises much higher than the ambient temperature. The pH of the fruit within the pit decreases from 6.7 to 3.7 within about four weeks. Inoculation of the fruit in the pit with LAB greatly speeds up the process. The pit therefore provides a good, reliable, cheap means of storage (Ray and Panda 2007).

6.1.4 Cucumbers

Pickled cucumbers are made in Africa, Asia and Latin America. Cucumbers undergo lactic acid fermentation and change from a pale product to a darker green and more transparent product. Khalpi is a popular cucumber pickle in Nepal during summer months (McGee 2004, Dahal et al. 2005). The gherkin (Fig. 1), popularly known as pickling small cucumber, has emerged as a potential export-oriented delicacy, fetching foreign exchange of US$ 33.3 million from 50,000 tonnes every year from India (Kapur and Singh 2003).

Fully ripe undamaged cucumbers are washed in potable cold water and drained. One kg of salt is added to the cucumbers. As soon as the brine is formed, fermentation starts and bubbles of CO_2 appear. Fermentation takes in between one to four weeks depending on the ambient temperature. Proper salt concentration exerts a selective effect on natural flora, resulting in the growth of lactic acid bacteria. When the pH is about 4.7, the brine is inoculated with either *Lb. plantarum* or *P. pentosaceus* or a combination of these organisms (Steinkraus 2002). At the end of fermentation, salt (16% w/w of brine) is traditionally added to the LA fermented cucumbers in order to stop any undesirable bacterial growth during storage (Kapur and Singh 2003). Cucumber pickle is usually stored in clean capped jars. They keep well if stored in a cool place. Due to high acid level (3.1–3.5) of the final product, the risk of food poisoning is low (Tamang et al. 2005).

Fig. 1. Fermented gherkin (Ray and Panda 2007).

Color image of this figure appears in the color plate section at the end of the book.

6.1.5 Gundruk

Gundruk is popular pickle consumed in Nepal (Dahal et al. 2005, Tamang et al. 2005). It is obtained by LA fermentation of leafy vegetables such as cabbage and radish. It is an important source of minerals, particularly when the diet consists of mostly starchy roots, tubers and maize which are low in minerals. This is mainly served as side dish with the main meal and also used as an appetizer.

Shredded leaves are tightly packed in an earthen pot and warm water is added to cover all the leaves. After 5 to 7 days, a mild acidic taste indicates the end of fermentation. The ambient temperature at the time of fermentation is 18ºC (Steinkraus 2002). *Pediococcus* and *Lactobacillus* species are the predominant microorganisms during fermentation (Tamang et al. 2005).

6.1.6 Ethnic Pickled Vegetables

'Almagro' eggplants (*Solanum melongena* L. var. *esculentum depressum*) is a pickle, virtually exclusive to Spain and more specifically to the town of Almagro and surrounding areas in the province of Ciudad Real (Sanchez et al. 2000b). The effect of a commercial *Lactobacillus* starter and sodium chloride concentration on the fermentation of "Almagro" eggplants was studied. The results of fermentation using added starter and varying salt concentrations (4, 6, and 10% w/v) in brine were compared with the results of spontaneous fermentation taking place in brine with a salt concentration of 4%. Fresh fruits, medium in size (34–44 g), were used in all cases; all fruits were blanched under identical conditions. Temperature in the fermenters was 32ºC. The results indicated that addition of a suitable starter shortened the fermentation process, provided the salt concentration in the brine did not exceed 6%. In the conditions tested, the eggplants obtained after fermentation were found to be of good quality though somewhat bitter which may explained by the starter employed (Ballesteros et al. 1999). Other ethnic pickled vegetables are:

- Brovoda: Pickled turnips (*Brassica rapa*) (Maifreni et al. 2004)
- Naw-mai-dong: pickled bamboo shoots (*Bambusa glaucescens*) from Thailand (Tanasupawat and Komagata 1994)
- Hom-dong: pickled red onions from Thailand (Tanasupawat and Komagata 1994)
- Jeruk: pickled vegetables including ginger and papaya from Malaysia
- Pickled carrots and turnips are produced in Asia and Africa. They are known as hua-chai po in Thailand and tai tan tsoi in China (Liu et al. 2011)
- Nukamiso-zuke: vegetables fermented in rice bran, salt and water in Japan
- Dak-dua-dong: fermented mustard leaves from Thailand (Tanasupawat and Komagata 1994)
- Dhamuoi, fermented cabbage and other vegetables in Vietnam
- Torshi felfel: Fermented sweet peppers (capsicum) are produced in West Asia and Africa
- Cauliflower stalks are fermented to produce achar tandal in India
- Torshi bentigen: Aubergines (brinjal) are pickled in West Asia
- Pak-sian-dong: Pak Sian (*Gynadropsis pentaphylla*) vegetables fermented in Thailand
- Sayur asin: fermented wilted mustard and cabbage (*Brassica juncea*) from Indonesia
- Kaktugi: fermented radish produced in Korea
- Tursu: fermented vegetables and fruits such as cucumbers, cabbages, green tomatoes, green peppers, carrots, red beets, eggplants or melon, in Turkey (Kabak and Dobson 2011)
- Suan-tsai and fu-tsai: Fermented mustard products of Taiwan (Chao et al. 2009)
- Poi: Fermented taro tubers in Papua New Guinea and other Pacific islands (Ray and Ward 2006)

6.2 Lactic Acid-Fermented Fruits

Most of the research works have concentrated on olives. Nevertheless, there are some other fruits which have been LA fermented.

6.2.1 Olives

Green olives are lactic acid fermented following sodium hydroxide (lye) treatment to remove bitterness (Sanchez et al. 2000a). The bacteria involved are *Lb. plantarum, Lb. brevis, P. cerevisiae* and *Lc. mesenteroides* (Borcakli et al. 1993, Duran-Quintana et al. 1999). The pH varied between 4.0–4.5. The optimum fermentation temperature is 24ºC. The fermentation period usually takes between 2–3 mon. Once the fermentation is complete, the olives are packed in air-tight jars and sterilized, which produces a good quality product with a long storage life. Salting is normally done at 1–10% brine solution (Tsapatsaris and Kotzekidou 2004). With the aim of formulating a probiotic food, the green olive was used as a vehicle for incorporating probiotic bacterial species such as *Lactobacillus rhamnosus*, *Lb. paracasei*, *Bifidobacterium bifidum* and *B. longum*. All these strains showed a good survival rate with a recovery of about 10^6 cfu (colony forming units)/g in olives after 30 d of storage (Lavermicocca et al. 2005).

Naturally black fermented olives are of equal importance in the international market as green olives (Nychas et al. 2002). After harvesting, black olives are transported to the factory, sorted to separate damaged fruits, washed to remove superficial dirt and finally brined in a 6–10% salt solution (Ozay and Borcakli 1996). In a recent study, the effect of controlled fermentation processes on the microbial association and biochemical profile of cv. *Conservolea* naturally black olives processed by the traditional anaerobic method was studied. The different treatments included (a) inoculation with a commercial starter culture of *Lactobacillus pentosus*, (b) inoculation with a strain of *Lb. plantarum* isolated from a fermented cassava product and (c) un-inoculated spontaneous process. Microbial growth, pH, titratable acidity, organic acids and volatile compounds were monitored throughout the fermentation. The initial microbiota consisted of Gram (–) ve bacteria, LAB and yeasts. Inhibition of Gram (–) ve bacteria was evident in all processes. Both starter cultures were effective in establishing an accelerated fermentation process and reduced the survival period of Gram-negative bacteria by 5 days compared with the spontaneous process, minimizing thus the likelihood of spoilage. Higher acidification of the brines was observed in inoculated processes without any significant difference between the two selected starter cultures (113.5 and 117.6 mM for *Lb. plantarum* and *Lb. pentosus*, respectively). *Lb. pentosus* was also determined as the major species present during the whole process of spontaneous olive fermentation. It is characteristic that LA fermentation was also initiated rapidly in the spontaneous process, as the conditions of fermentation, mainly the low salt level (6%, w/v), favoured the dominance of LAB over yeasts. Lactic, acetic and propionic were the organic acids detected by HPLC in considerable amounts, whereas citric and malic acids were also present at low levels and degraded completely during the processes. Ethanol, methanol, acetaldehyde, ethyl acetate were the major volatile compounds identified by gas chromatography (Panagou et al. 2008).

6.2.2 Sweet Cherry

Sweet cherry fruits have a very short life since they are subject to rapid microbial spoilage. Fermentation by LAB is a simple technological option for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of these fruits. Di Cagno et al. (2011b) isolated several strains of LAB such as *Pediococcus acidilactici, P. pentosaceus, Lb. plantarum,* and *Lc. mesenteroides* subsp. *esenteroides* from spontaneous fermentation of eight cultivars of these fruits by partial 16S rRNA gene sequence and subjected to typing by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis. Lactic fermentation has increased the anti-oxidant activity, anthocyanin content and sensory quality of these fermented fruits.

6.2.3 Caper berries

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

6.3 Lactic Acid-Fermented Roots and Tubers

There are several traditional products such as sunki, kanji, etc. obtained by lactic acid fermentation of roots and tubers.

6.3.1 Sunki

Sunki is a non-salted fermented vegetable prepared from the leaves of the otaki-turnip in the Kiso District of Japan. Sunki is eaten with rice or in miso soup. The otaki turnip is boiled, mixed with zumi (a wild small apple) and dried sunki from the previous yr and allowed to ferment for one to two months. Sunki is produced under low temperature (in winter season).

Microorganisms involved include *Lb. plantarum*, *Lb. brevis*, *Bacillus coagulans* and *Pediococcus pentosaceus* (Battcock and Azam-Ali 2001).

6.3.2 Sinki

Sinki is a sour pickle prepared from radish tap roots. It is consumed traditionally in India, Nepal and parts of Bhutan (Dahal et al. 2005). Fresh radish roots are harvested, washed and wilted by sun-drying for one to two days. They are then shredded, re-washed and packed tightly in glass jars, which are sealed and left to ferment. The optimum fermentation time is 12 days at 3ºC. Sinki fermentation is initiated by *Lactobacillus fermentum* and *Lb. brevis*, followed by *Lb. plantarum*. The pH drops from 6.7 to 3.3. After fermentation, the radish substrate is sun-dried to a moisture level of about 21%.

6.3.3 Kanji

In Northern India and Pakistan, carrots, especially a variety that is deep purple in colour, are fermented to make a traditional ready-to-serve drink known as kanji. Kanji is very popular and considered to have cooling and soothing properties and to be of high nutritional value. After thorough washing, the carrots are finely grated. Each kg of grated carrot is mixed with 7 L of water, 200 g of salt, 40 g of crushed mustard seed and 8 g of hot chilli powder. The mixture is then placed in a glazed earthenware vessel, which is almost entirely sealed, leaving only a tiny hole for gases released during fermentation to escape. The mixture is then allowed to ferment naturally for seven to ten days. The final product is slightly acidic in taste and has an attractive purple-red colour. After fermentation, the drink is strained through fine muslin and has to be consumed within three to four days after which it goes bad. Each kg of grated carrot yields just over 7 L of kanji (Ray and Panda 2007).

6.3.4 Onion and garlic

Lactic acid fermentation was conducted on sweet, white, and yellow storage onions to produce sour onion. The onions were sliced to 0.3 cm thick, salt was added at 1.5, 2.0, and 2.5 g/100 g without or with sugar at 1.0 and 2.0 g/100 g, and the fermentation temperature was 18ºC. Since onions did not have the necessary LAB for anaerobic fermentation, they were inoculated using either brine from sauerkraut or slices of cabbage. The fermentation produced sour onion with pH between 3.25–3.35 and 1.2–1.5 g LA/100 ml, which is in the range as that of sauerkraut. Sensory evaluation showed that the yellow storage sour onion was a favourable product with respect to colour, texture and flavour. The sour onion had a tartaric acidic taste, characteristic of sauerkraut, with the onion flavour but without the pungency of raw onions (Roberts and Kidd 2005).

The controlled fermentation of peeled, blanched garlic, using a starter culture of *Lb. plantarum*, was studied and compared with that of un-blanched garlic. Blanching was carried out in hot water (90ºC) for 15 min. The starter grew abundantly in the case of blanched garlic, producing mainly LA and reaching a pH of 3.8 after 7days, but its growth was inhibited in un-blanched garlic. Ethanol and fructose, coming from the enzymatic activities of garlic, and a green pigment were formed during the fermentation of un-blanched garlic, but not of blanched garlic. The blanched garlic fermented by *Lb. plantarum*, even without a preservation treatment (such as pasteurization), was microbiologically stable during storage at 30ºC in an acidified brine (approximately 3% (w/w) NaCl and pH 3.5 at equilibrium), but the fructans were hydrolyzed (De Castro et al. 1998).

6.3.5 Sweet potato

Bio-fortified sweet potato roots, rich in colour pigments such as anthocyanin or β-carotene, were pickled by LA fermentation by brining both cut and blanched roots in 2–10% NaCl solution and subsequently inoculated with a probiotic strain of *Lb. plantarum* MTCC 1407 for 28 days (Fig. 2). Treatment with 8–10% brine solution was found to be organoleptically most acceptable (based on texture, taste, aroma, flavour and after tasting). The pickle prepared from anthocyanin-rich sweet potato had a pH (2.5–2.8), titratable acidity (1.5–1.7 g/kg), LA (1.0–1.3 g/kg), starch (56–58 g/kg) and anthocyanin content (390 mg/kg) on fresh weight basis (Panda et al. 2009). Similarly, β-carotene-rich pickle had a pH (2.9–3.0), titratable acidity (2.9–3.7 g/kg), lactic acid (2.6 3.2 g/kg), starch (58–68 g/kg) and β-carotene (163–165 mg/kg) (Panda et al. 2007).

Figure 2 can not be paste on the this page.

Sweet potato roots (non-boiled/fully-boiled) rich in β-carotene pigments were fermented with *Lb. plantarum* MTCC 1407 at 28± 2ºC for 48 hr to make lacto-juice. There were no significant variations in biochemical constituents (pH, 2.2–3.3; LA, 1.19–1.27 g/kg root and titratable acidity, 1.23 1.46 g/kg root) of lacto-juices prepared from non-boiled and fully-boiled sweet potato roots except β-carotene concentration [130 ± 7.5 mg/kg (fully-boiled roots) and 165± 8.1 mg/kg (non-boiled roots)] (Panda and Ray 2007).

Fig. 2. Lacto-pickle from orange fleshed sweet potato (Panda et al. 2007).

Color image of this figure appears in the color plate section at the end of the book.

6.3.6 Cassava

Cassava is another tropical crop in which roots are consumed as food in Africa and Latin America. Several fermented foods (gari, fufu, lafun, dawa dawa, chickwanghe, agbelima, attieke, kivunde and peujeum in Africa, gaplek and putto in Indonesia and cheese bread and sour starch in Latin America) are prepared from cassava roots based on LAB and yeast fermentations (Ray and Ward 2006). Fermentation reduces the cyanogenic toxicity and enhances flavour, taste and aroma of the fermented products (Onabolu et al. 2002a,b).

6.4 Innovative Juices and Smoothies from Fruits and Vegetables

Innovation in food technology has an important role to develop new LA fermented products to improve the nutritional features; few of them are described below.

6.4.1 Lactic acid-fermented vegetable juices

Lactic acid bacteria have been added to a variety of dairy-based products such as fermented milks and yoghurts for their probiotic human health benefits. In recent yrs, consumers' demand for nondairy-based probiotic products particularly lacto-juice prepared from vegetables has gained importance (Karovicova et al. 1999, 2002b). Lacto-juices have been prepared from vegetables such as carrot, turnip, tomato pulp, onion, sweet potato, beet and horseradish (Karovicova et al. 1999, 2002b, Klewicka et al. 2004). For fermentation of juices of highest quality, it is imperative to use commercially supplied probiotic starter cultures such as *Lactobacillus acidophilus*, *Lb. plantarum*, *Lb. bavaricus*, *Lb. xylosus*, *Lb. bifidus*, and *Lb. brevis* (McFeeters 2004).

Lu et al. (2001) carried out the fermentation of cucumber juices inoculated by *Lb. plantarum*. The juices were fortified with glucose, fructose or a mixture of glucose and fructose. When the cucumber juice was supplemented with fructose and/or glucose, the starter culture continued to ferment fructose, but not glucose, resulting in an increase in lactic acid production and decrease in terminal pH. Karovicova et al. (2002a) performed spontaneous fermentation of cabbage juices, one fermentation by *Lb. plantarum* 92H and another by a mixed culture of *Lb. plantarum* and *Saccharomyces cerevisiae* C11-3. It was found that the highest amount of lactic acid was produced in the juice inoculated by *Lb. plantarum* 92H and the highest decrease in pH was observed in juice inoculated by the mixed culture. The spontaneously fermented juice had the highest intensity of harmonic taste, acceptance of taste, odour and flavour. Yoon et al. (2004) studied the probiotic strain of tomato juice by *Lb. acidophilus* LA39, *Lb. plantarum* C3, *Lb. casei* A4 and *Lb. delbrueckii* D7. Tomato juice was inoculated with a 24 hr-old culture and incubated at 30ºC. The LA fermentation reduced the pH to 4.1 and increased the acidity to 0.65% and the viable cell count reached nearly 1.0–9.0 x 10^9 cfu/ml, after 72 hr fermentation. Similar studies were carried out by Yoon et al. (2006) for production of probiotic cabbage juice.

Red beets were evaluated as a potential substrate for the production of probiotic beet juice by four species of LAB (*Lb. acidophilus*, *Lb. casei*, *Lb. delbrueckii*, and *Lb. plantarum*). All the lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and LA production. However, *Lb. acidophilus* and *Lb. plantarum* produced a greater amount of LA than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 hr of fermentation at 30ºC (Yoon et al. 2005). In another study, Bergqvist et al. (2005) reported that LA fermentation by *Lb. pentosus* FSC1 and *Lc. mesenteroides* FSC2 strongly improved iron solubility in carrot juice. 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. 10^8 c/ml (Rivera-Espinoza and Gallardo-Navarro 2010).

6.4.2 Lactic acid-fermented fruit juices

Lactobacillus and *Bifidobacterium* strains survived for a longer time in orange and pineapple juices than in cranberry juice. *Lactobacillus casei*, *Lb. rhamnosus*, and *Lb. paracasei* remained viable in orange juice at a cell number higher than 10^7 during 12 weeks of storage (Sheehan et al. 2007). *Lactobacillus plantarum* and *Lb. delbrueckii* were able to survive at 10^8 cfu (colony forming units)/ml for 2 weeks in pomegranate juice, while *Lb. paracasei* and *Lb. acidophilus* showed a marked decrease of the viability (Mousavi et al. 2010). In a more recent study, Filannino et al. (2013) reported enhanced concentration of polyphenolics (ellagic acid), anti-oxidant activity and anti-microbial activity of LA fermented probiotic pomegranate juices that were fermented with *Lb. plantarum* (POM 1 and POM 2) strains isolated previously from tomatoes and carrots.

6.4.3 Smoothies

The manufacture of smoothies is based on the use of a mixture of fruits and vegetables which are processed into pulp or puree, after removing seeds and peel (Qian 2006). In most 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 *Lb. plantarum, Lb. pentosus* and *Weissella cibaria* strains. Lactic acid fermentation by selected starters positively affected the content of antioxidant compounds and enhanced the sensory attributes.

7 Health and Nutritional Benefits of the Fermented Vegetables and Fruits

Lactic acid fermented vegetables and fruits have several health and nutritional attributes beside the scope of food preservation. These are discussed below in brief.

7.1 Food Preservation

The main motive of LA fermentation is to preserve food, increase its shelf-life, and improve food quality and palatability (Ray and Sivakumar 2009).

There are several options for preserving fresh fruits and vegetables including drying, freezing, canning and pickling (Karovicova et al. 1999). But, many of these techniques are not suitable for small-scale or household use in developing countries (Steinkraus 2002). For instance, the small-scale canning of vegetables has food safety problems and contamination by foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* might occur (Reina et al. 2005). Lactic acid fermentation requires very little sophisticated equipment for carrying out the fermentation process (Steinkraus 2002). Further, the pathogenic microflora is inhibited by LAB (Reina et al. 2005).

7.2 Removal of Anti-Nutritional Factors

Many vegetables and fruits contain naturally-occurring toxins and anti-nutritional compounds (Drewnowski and Gomez-Carneros 2000). For example, cassava roots contain two cyanogenic glucosides, linamarin and lotaustralin (Ray and Ward 2006). When the roots are naturally fermented by a mixed population of yeasts (*Saccharomyces cereviseae* and *Candida* spp.) and LAB (*Lactobacillus*, *Leuconostoc* and *Pediococcus*), the cyanogen level is reduced drastically (Kostinek et al. 2005). Likewise, LAB reduce the toxic elements in African locust beans and in leaves of *Cassava obtusifolia* during preparation of kawal, a Sudanese food (Dirar 1993).

7.3 Mineral and Vitamin Preservation

The micronutrient availability is enhanced in LA fermented vegetables because of significant reduction in the phytase enzyme. Iron bioavailability was also reported to be higher in carrots (Rakin et al. 2004), beet (Yoon et al. 2005) and sweet potato (Panda et al. 2007) after LA fermentation.

7.4 Improvement of Food Digestibility

Lactic acid bacteria contain various intracellular and extracellular food digestive enzymes, i.e., α-amylase (Guyot et al. 2000, Rao et al. 2004), pectinase (Kunji et al. 1996) and proteinase (Shurkhno et al. 2005). These enzymes aid in improving the digestibility of fermented fruits and vegetables. Proteases digest vegetable proteins during fermentation and some indigestible compounds such as sulfur compounds (aliin, allicin, ajoene, allylpropl, trisulfide, sallylcysteine, vinyldithiine and S-allylmercaptocystein) in garlic or onion improving the food digestibility (Di Cagno et al. 2013). Single culture lactic fermentation (*Lb. casei*, *Lb. plantarum* and sequential culture fermentation (*Saccharomyces boulardii* + *Lb. casei*, *S. boulardii* + *Lb. plantarum*) drastically reduced the content of phytic acid, polyphenols and trypsin inhibitors of a food mixture containing rice flour, green paste and tomato pulp while significantly improved the *in vitro* digestibility of starch and protein (Sindhu and Khetarpaul 2002).

7.5 Some Disadvantages of Lactic Acid Fermentation

Microbial de-carboxylation of amino acids results in the formation of biogenic amines that can be found in fermented foods. These amino-compounds can confer an unpleasant flavour to the product or can be toxic (Arena and Manca de Nadra 2001, Garcia- Ruiz et al. 2011). Ingestion of certain amines by human can cause headaches, fever and vomiting, symptoms similar to those of microbial food poisoning. Histidine, ornithine, tyrosine and lysine are the main amino acids that can be de-carboxylated into biogenic amines such as histamine, putrescine, tyramine, and cadaverine, respectively. A minimum histamine level of 1 g/kg in a foodstuff or the consumption of 0.07 to 1 g of histamine per meal is supposed to elicit histamine toxicity. The European regulation 2073/2005 stated that the average histamine content must be 100 mg/kg or less in food.

Some bacteria commonly involved in LA fermentation, such as *Lc. mesenteroides*, have been found to contain the amino acid decarboxylase, which induces the biogenic amine formation (Arena et al. 1999). Conversely, other bacteria such as *Lb. plantarum* and *Pediococcus* can limit biogenic amines through the production of amine oxidase enzymes (Garcia-Ruiz et al. 2011).

8 Perspectives and Conclusion

The daily intake of fruits and vegetables is estimated to be lower than the recommended dietary intake of 450 and 500 g of fruits and vegetables, respectively, as advised by the Food and Agricultural Organization (Rome). Fruits and vegetables are an essential part of the human nutrition. In particular, they are rich in water-soluble vitamins (vitamin C and group B vitamins), pro-vitamin A, phytosterols, and show a high variety of minerals and phytochemicals depending on the plant species. The major part of fruits and vegetables are consumed fresh or as industrially processed such as canned, dried, juice, paste, salad, sauce and soup preparations. Nevertheless, LAB perform an essential role in the preservation and production of wholesome foods ranging from fermented fresh vegetables such as cabbage, cucumber to fermented fruits (olive). Due to LA fermentation, foods become resistant to microbial spoilage and to development of toxins, and enriched with vitamins, minerals, dietary fibres and antioxidants. Regular consumption of LA fermented food products enriched with natural pigments such as anthocyanin, lutein and β-carotene would be helpful in combating several chronical diseases such as night blindness, liver injury, aging, and related ailments. This is in addition to health benefits from probiotics.

Keywords: Food preservation, Lactic acid bacteria, Lactic fermented vegetables, Food fermentation

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