Chapter 9

Review of Vegetable Fermentations With Particular Emphasis on Processing Modifications, Microbial Ecology, and Spoilage

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9.1 BRIEF HISTORY OF FOOD FERMENTATIONS

Fermentation is a primitive preservation method primarily used to enable the long-term storage of foods. The elaboration of bread, cheese, or wine is a tradition introduced thousands of years ago and developed all around the world from rural areas to villages. It is well documented that fermented drinks were consumed in Babylon 5000 years ago (Dirar, 1993) and that bread was consumed in Egypt around the 1500 BC (Suhigara, 1985). Anthropologists even suggest that the production of alcoholic beverages was the cause of settling down in primitive inhabitants, which indirectly allowed the introduction of agriculture. The origin of the fermentation of mixed vegetables is situated in China and associated with the diet of the workers who built the Great Wall in the 3rd century BC (Anderson et al., 1988). Furthermore, the use of *chu*, a fermented grain product, is described in the book *Shu-Ching* (written about 1121−256 BC) (Yokotsouka, 1985). Sauerkraut production was described as early as the 1st century by Plinius the elder (Buckenhüskes et al., 1990). The diverse preparation forms of table olives are also described by Columela in his book *De Re Rustica* in the 1st century (Columela, 54).

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Although extremely beneficial to humankind, the fermentation process remained largely uncharacterized for centuries. Deprived of an understanding of the microbiology behind successful and desirable fermentations, antique generations use the cover brines or doughs from fermentations with desirable attributes to initiate fresh ones, a technique known as back slopping. Processors frequently passed the "high-quality" inocula or "yeast paste" to subsequent generations in an attempt to perpetuate specific organoleptic attributes in the desired fermented foods. The possibility to further control the outcome of a fermentation emerged with the discovery of yeasts as living cells by Anton van Leeuwenhoek in 1680 and Cagnard-Latour in 1838 (Nanniga, 2010). The early perception of yeasts as an inanimate paste was transformed into living cells that convert extracted malt sugars to ethanol and CO_2 in 1838 (Nanniga, 2010). Subsequent studies by Louis Pasteur of a faulty alcoholic fermentation in Lille, France catalyzed the discovery of the role of lactic acid bacteria (LAB) in food fermentations (Brock, 1961). A few years later in 1873, Joseph Lister, a leading surgeon in antiseptic surgery, prepared the very first pure starter culture of a LAB species, by diluting fermented milk (Brock, 1961). Defined fermentative cultures were introduced commercially in New Zealand in 1934 (Cogan and Hill, 1993), beginning the era of "controlled" fermentations.

9.2 PRODUCTION OF FERMENTED VEGETABLES

Under the denomination of fermented vegetables are usually included both fruits, considered as the edible part of a plant or tree, consisting of the seed and its envelope or pulp, and vegetables defined as the plant or root cultivated for food. Fruits are mainly considered acidic foods while vegetables have a lower acidity, which makes them more prone to spoilage by microorganisms. However, most of the vegetable fermentation processes imply acidification by microorganisms, regardless of the type of substrate.

In spite of the fact that fermentation of vegetables is an old tradition everywhere, the system still retains increasing popularity due to a series of favorable factors. Stamer (1988) defines such factors as: (1) the minimal requirement of technical training or knowledge, enabling its use from households to industrial production in any country, (2) the low cost of the process, with a reduced energy consumption, able to preserve in bulk very different commodities for a gradual commercialization, (3) the long-lasting safety record making fermented products very reliable for consumers, and (4) also produces a series of metabolites which contribute to the flavor, texture, and appearance as well as the nutritional (e.g., detoxification or increase in absorption of components) characteristics of the products. In addition, the consideration of vegetable fermented products is in agreement with the current trend for more natural products with potential beneficial effects on health, such as decrease of cholesterol, risk of certain types of cancer,

modulation of the immunologic system, or probiotic effect. Thus, the prospects for the future of fermented vegetables are promising.

The consumption of vegetables is widespread in the world and represents an important component of the human diet. China is the largest producer and the probable origin of pickles (Fleitag, 2012). In the European Union (EU) the canned pickles, olives, and sauerkraut have been valued at approximately €1746, €1123, and €67.0 million, respectively (CBI, 2009). The largest producer in the EU is Germany with values reaching 800 million euros for pickles (mainly cucumbers). Kimchi (cabbage fermented in garlic, chillies, and vinegar) is also very popular in Asia, being South Korea's national dish (per capita consumption around 24 kg); however, production still relies on household or small-scale users (56%). There are also many other fermented vegetable products all around the world, for which production statistics are not detailed in this report.

9.3 GENERAL DESCRIPTION OF THE MICROBIOLOGY OF FERMENTED VEGETABLES

The commonality of fermented vegetables is their dependence on the growth of LAB, which results in the production of acidity and a decrease in pH. These changes, together with the presence of salt, are the essence of the production of stable and safe fermented vegetables. Total aerobic microbial counts in fresh cucumbers and cabbage may reach 5 $\log_{10}$ CFU/mL (Pérez-Díaz et al., 2015a). It is well known that the community of Enterobacteriaceae, LAB, and yeasts dominate the natural microbial population of vegetable fermentations. The natural microbial population in olives is a fraction of that found in cucumbers and cabbage, presumably due its antimicrobial phenolic content. Most microbes colonize the outermost interior of vegetables (Mattos et al., 2005; Samish et al., 1963). Microbial counts for fresh produce vary with seasons, maturity stage, environmental humidity, temperature, and the use of pesticides among other factors. The diversity of the microbial population of vegetables is drastically reduced as the result of fermentation, which supports the safety record of such preserves. The various Gram-positive and Gram-negative bacteria and yeasts and molds naturally present in fresh vegetables compete for dominance. Enterobacteriaceae, aerobic spore-formers, LAB, and other groups of bacteria and yeasts may be active for several days or weeks depending on factors such as temperature, dissolved oxygen, and the salt concentration used in the cover brines. Very often LAB gain predominance due to their ability to produce lactic acid inducing a drop in pH tolerable by them. Six species of lactic acid-producing bacteria may be naturally and actively present in many vegetable fermentations: *Leuconostoc mesenteroides*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Lactobacillus pentosus*. Recent studies suggest that *Weissella* spp. are also present during the early stages of sauerkraut fermentation (Plengvidhya et al., 2007). Generally, *L. mesenteroides* survives

best in vegetable fermentations with less than 2% NaCl, while *L. plantarum* and *L. brevis* are more resistant to the acidic pH (McDonald et al., 1990). The genome sequences for the main LAB present in vegetable fermentations, *L. plantarum* and *L. pentosus*, are available. The first complete genome sequence for a *L. plantarum* isolate became available in 2003 (Kleerebezem et al., 2003). The human saliva isolate was found to contain putative genes encoding the necessary components of a facultative heterofermentative LAB, and several regulatory and transport-related systems. A study of the genetic diversity among 185 *L. plantarum* isolates obtained from various niches identified 2000 genes as the core genome for this species, including 121 unique *L. plantarum*-marker genes absent in other LAB. More than 50 genes found in the reference *L. plantarum* genome published in 2003 were unique to the saliva isolate (Siezen et al., 2009). Further analysis of multiple *L. plantarum* genome sequences showed the existence of a genomic lifestyle islands consisting of numerous functional gene cassettes related to carbohydrate utilization that may be acquired, shuffled, substituted, or deleted in response to extrinsic environmental factors (Siezen and van Hylckama Vlieg, 2011). The genome sequence for *L. pentosus* MP-10 isolated from naturally fermented Aloreña green table olives with potential as probiotic was published in 2011 (Abriouel et al., 2011). The *L. pentosus* genome sequence has significantly more similarity with the *L. plantarum* than the *L. fermentum* counterparts. It was determined that the *L. pentosus* genome is shaped by horizontal gene transfer mediated by mobile genetic elements. Later it was learned that *L. pentosus* IG1 possess one of the biggest lactobacilli genomes (Maldonado-Barragán et al., 2011). This strain was found to encode for 16 two-component regulatory systems suspected to aid in adapting to changing extrinsic conditions such as those characteristics of active fermentations. The genes encoding for the two-component class IIb plantaricin were found to be identical to open reading frames found in the *L. pentosus* IG1 genome sequence. A comparison of the stress response systems among *L. pentosus*, *L. plantarum*, and *L. paraplantarum* revealed that there is a generalized response in stationary phase to increases in oxidative, heat, and starvation stress (Ricciardi et al., 2012).

A limited number of fermented vegetable processors choose to use starter cultures of *P. pentosaceus/acidilactici* or *L. plantarum* to enhance product consistency and the efficiency of the fermentation as recommended by Etchells et al. (1964, 1973) for cucumber fermentations and Vega Leal-Sánchez et al. (2003) for the olive counterpart. Utilization of *L. mesenteroides* as a starter culture for low salt sauerkraut fermentations has also been evaluated (Johanningsmeier et al., 2007). A method for the preparation of *L. plantarum* and other LAB starter cultures that meets kosher requirements for vegetable fermentations was developed by Pérez-Díaz and McFeeters (2011). The performance of specific lactobacilli as starter cultures in selected vegetable fermentation has been evaluated and is described below. Various species of fermentative yeasts are also typically present in selected vegetable fermentations. Yeasts were implicated in cucumber

fermentations in 1941. Salt concentrations in the cover brine were found to be responsible for the extent of the yeasts growth lag and logarithmic phases (Etchells, 1941). Yeast cells are primarily found on the skin of the cucumber fruits, exposed mesocarps, and the fermentation cover brines. The size of yeast cells prevents their penetration into the mesocarp through the fruits skin (Daeschel et al., 1985). Predominating yeasts genera in cucumber fermentations include *Torulopsis*, *Brettanomyces*, *Zygosaccharomyces*, *Hansenula*, *Torulaspora*, and *Kloeckera* (Etchells and Bell, 1950). Oxidative yeasts of the genera *Debaryomyces*, *Pichia*, and *Mycoderma* have also been associated with the formation of a film on the surface of cucumber fermentation cover brines, popularly known as a scum by film-forming yeasts (Etchells and Bell, 1950; Mkar and Bonar, 1939). The fermentation of table olives is known to support the proliferation of *Candida*, *Pichia*, and *Saccharomyces* (a review by Botta and Cocolin, 2012). Such yeasts are known to contribute to the definition of the final organoleptic profile of fermented table olives due to their ability to produce volatile compounds. The utilization of selected yeast cultures as adjunct cultures for vegetable fermentations is been currently evaluated.

9.4 DESCRIPTION OF THE PROCESSING AND MICROBIAL ECOLOGY OF THE MAIN TYPES OF FERMENTED VEGETABLES

A concise description of the processing peculiarities and microbiology of the most relevant fermented vegetables are included below.

1. *Cucumbers (Cucumis sativus)*. This vegetable is one of the most important fermented vegetables in the United States, with an average production of around 480,000 tons in 2012−14 and an economic value of between 145 and 175 million dollars (USDA, 2015a). Usually, the fermentation is carried out in large open-top fiberglass tanks to prevent the growth of surface yeasts and molds by the ultraviolet sunlight radiation. The cucumbers are covered with fresh (or recycled brine) containing NaCl and $CaCl_2$ in the adequate proportions to reach 6% and 0.1−0.4%, respectively, at equilibrium. The presence of calcium is recommended for the retention of texture and crispness of the fruits. The main carbohydrates used during the fermentation are fructose and glucose (about 1%) and malic acid. After brining, it is imperative to achieve the removal of the CO_2 produced by both the fruit's respiration and, the gradual malolactic metabolism by LAB, to avoid the formation of bloaters. Although air purging is widely utilized at the commercial-scale production to reduce the incidence of bloating, it also introduces oxygen to the system, which leads to the growth of undesired aerobic organisms such as yeasts and molds, among others. Thus, the incorporation of sorbic acid or benzoic acid in the fermentation cover brines is recommended. The

fermentation is usually spontaneous despite the development of specific malic acid decarboxylase deficient starter culture, recommended for the reduction of the incidence of bloaters (Daeschel et al., 1984). After fermentation, the lactic acid concentration may be about 2% and the pH between 3.1 and 3.5. These conditions, together with the salt and other metabolites produced during fermentation, stabilize the fermented fruits for long-term bulk storage for up to 1 year, which is processed into finished products in line with the market demand. A review of the biochemistry of vegetable fermentation and the genomic research on the LAB involved in them, especially for cucumbers, was presented by Breidt et al. (2013b). As deduced from the description of the cucumber fermentation process described above, a large proportion of waste-waters are generated from the fermentation cover brines and the desalting steps are necessary in order to decrease the sodium chloride concentration from 6% in bulk storage to less than 2% in the finished products to achieve the edibility threshold. Modifications to the traditional cucumber fermentation process have been suggested recently and are being currently optimized for commercial-scale production. One of the most promising modifications is the total substitution of the NaCl by $CaCl_2$. The fermentation biochemistry observed in the modified system brined with calcium chloride is similar to that observed in the traditional fermentation described above (McFeeters and Pérez-Díaz, 2010; Pérez-Díaz et al., 2015b). Due to the high amount of calcium Ca^{++}, the texture is also maintained (McFeeters and Pérez-Díaz, 2010). Further studies on the consumer acceptability of cucumber pickles brined with $CaCl_2$ showed that there was no difference if bulk storage happens for 2−8 months (Wilson et al., 2015). However, a significant preference for cucumber pickles brined with NaCl was observed if a 10-month storage period was considered. It was further concluded that the presence of Ca ions was not the cause of such rejection after 10 months of bulk storage, given that the amount of the calcium salt present in the finished product was well below the taste threshold for $CaCl_2$ at 62 mM. Cucumber fermentations brined with $CaCl_2$ instead of NaCl are a viable process at the commercial scale with a single desalting step and reduced environmental impact from waste waters (Wilson et al., 2015).

2. *Sauerkraut (Brassica oleracea)*. The name is a compound German word derived from *sauer* meaning sour and *kraut* meaning cabbage. In the United States, its production during 2012−14 was about 140,000 tons with an economic value of approximately 10 million dollars (USDA, 2015b). In Germany, production of sauerkraut reached around 100 million euros in 2011 (Fleitag, 2012). It is produced from the sweet and white cabbage varieties, specifically developed for the commercial process, due to their large and dense heads with a minimum of green outer leaves. The sugar content of cabbage is especially high (about 5%), and

mainly composed of fructose and glucose. Also, the raw material is characterized by the presence of a series of inhibitory compounds such as glucosinolates that by hydrolysis produce isothiocyanates, nitriles, and thiocyanates. Processing is relatively simple. After removing the outer leaves, the head is sliced into shreds <0.2 cm, depending on available machinery or tradition, although long, fine shreds are preferred. Then, the shreds are transported into the fermentation tanks (usually concrete tanks, covered by fiberglass, with diverse capacities of up to 180 tons), and are then mixed with the appropriate solid salt so that the final equilibrium reached is around 2.5%. The brine is formed with the liquid extracted by the salt. Finally, the tanks are covered with plastic to prevent the presence of oxygen and to keep the shreds submerged. Fermentation by LAB is initiated immediately after the cabbage is brined. The lactic acid producing microbiota is initially composed of *L. mesenteroides*, *L. citreum*, and *Weissella* spp. which decrease the pH to approximately 6.5 (Plengvidhya et al., 2007). These species are progressively substituted with predominantly *L. plantarum*, *L. curvatus*, and other lactobacilli, which continue to drop the pH to about 4.5 (Plengvidhya et al., 2007). *Lactobacillus brevis* ends the production of acids spearheading the final decrease in pH to $\sim$4.0. *Leuconostoc* and *Weissella* spp. convert the cabbage-derived fructose and glucose heterofermentatively to lactic and acetic acids, and CO_2. In general, adequate progression of cabbage primary and secondary fermentations by the various LAB species is sufficient to stabilize and preserve it preventing microbial spoilage. Although the fermentation may be completed in $\sim$20 days, bulk storage usually occurs in the same tanks until the sauerkraut is processed into finished products according to consumer demand (Fleitag, 2012). The use of selected starter culture of *L. plantarum* and *L. mesenteroides* to control the process improved ascorbigen content (x12), antioxidant (x2.0), and nitric oxide production (2.6). The product obtained with the supplementation of fermentations with starter cultures are known to enjoy higher acceptability by consumers and improved nutritional characteristics (Martinez-Villaluenga et al., 2012). The reduction of the nitrite content in Chinese cabbage using selected starter cultures of *Lactobacillus delbrueckii* and *Lactobacillus paracasei* has also been recently documented (Han et al., 2014). Fermentation of cabbage causes a dramatic degradation of glucosinolates between the second and fifth day of the process, while increasing the concentration of potential bioactive components (ascorbigen and indol-3-carbinol) (Palani et al., 2016).

3. *Table Olives (Olea europaea).* World production of table olives was around 2,600,000 tons in the 2013/14 season, with Spain as the main producer with 25% of the total production (IOC, 2015). There are different types of table olives (green, turning color, and naturally black) which can

be debittered using an alkaline solution (treated olives) or by dilution of the bitter compound into the brine (natural). All of them are subjected to fermentation processes after brining. However, the so-called ripe olives (Californian style) are stored in brine or acid solutions just while waiting for the final oxidation process, which is the most important processing step. The most popular style is the green Spanish-style table olives (which account for about 60% of the market) followed by natural black olives (Greek-style). For the Spanish-style, olives are harvested at the green maturation stage, usually by hand (although research into the introduction of mechanical harvesting is in progress). After transportation in bulk 1000 kg bins, the fruits are treated with a NaOH solution (~2.5%, w/v) until the alkali reaches 2/3 of the flesh. Then, the lye is removed and the olives are washed with tap water for 18–24 hours to eliminate the excess alkali. After washing, the olives are submerged in a NaCl solution (10–12% w/v) for a few hours and finally are sent to the fermentation fiberglass tanks with a total capacity of about 16,000 L. Acids such as HCl, lactic acid, and acetic acid are occasionally added to the fermentation cover brines. The fermentation begins just after brining. The first microorganisms to grow are Enterobacteriaceae, followed by LAB, specifically and predominantly *L. plantarum* and *L. pentosus* (De Angelis et al., 2015). Yeasts and LAB may coexist in brined olives (Garrido Fernández et al., 1997). It is relevant to mention that the use of bulk tanks for table olive fermentations have indirectly modified the predominant microbiota, inducing the replacement of lactic acid producing cocci such as *Pediococcus* and *Leuconostoc* to the rods *L. plantarum* or *L. pentosus* (Botta and Cocolin, 2012). The use of *L. plantarum* LPCO10 as a starter culture and of *Enterococcus casseliflavus* cc45 and *L. pentosus* 5138A in sequential inoculations has proven effective in accelerating acid production, and the die-off of pathogenic microbes as compared to spontaneous fermentations (De Castro et al., 2002; Leal-Sánchez et al., 2002; Vega Leal-Sánchez et al., 2003). Application of recently-developed molecular methods have shown that the biodiversity of the microbiota in green Spanish-style fermented olives is higher (Lucena-Padrós et al., 2014) than previously estimated. The fermentation of green Spanish-style olives is mainly spontaneous; however, the formation of biofilms on the olive surface (Dominguez-Manzano et al., 2012) may favor the selection and use of appropriate starter cultures for the making of probiotic olives (Rodríguez-Gómez et al., 2014). For preparing natural black olives, the fruits should be completely ripe but not overripe. Traditionally, the olives are placed in cement open tanks protected with polyester resins. However, they are being progressively substituted with fiberglass tanks with 10-ton capacity, similar to those used for green olives. The brine used has 8–10% (w/v) NaCl concentration or lower (~6% NaCl) as is the case in cold areas. As the black olives are not lye treated, the leakage of nutrients into the brine and the debittering of these olives is slow and, therefore,

fermentation is rather long. In fact, the presence of residual fermentable material in the brine of these olives may be observed during the entire fermentation. Fermentation of naturally black olives is mainly achieved by yeasts with a weak lactic acid production, although the growth of LAB is also possible in the presence of low salt levels in cultivars with reduced content of polyphenol. In Spain, the traditional anaerobic process has been substituted with an aerobic fermentation to prevent the formation of gas-pockets in some cultivars (Hojiblanca and Manzanilla) prone to such defects. The modification aims at an increase in facultative yeasts (Garrido-Fernández et al., 1997). Starter cultures are also being developed for this type of olive fermentation (Bleve et al., 2015). The adequate selection of mixed cultures of LAB and yeast for starter cultures could lead to the development of probiotic natural black olives, due to the development of biofilms on the fruits (Rodríguez-Gómez et al., 2014). Combinations of native yeasts and *L. plantarum* have been tested as starter cultures for Bella di Cerignola table olives and have been shown to significantly reduce the biodiversity of the microbiota during the active fermentation period, to modify the concentration of free amino acids and phenolic and volatile compounds, and to generate finished products with increased consumer acceptability (De Angelis et al., 2015).

4. *Other vegetable products*. There are numerous fermented vegetable products that are traditional in specific countries and popular in some other areas around the world. Among them are kimchi, sour onion, mixed vegetables, capers (from *Capparis* sp.) and caper berries. *Capers* and *caper berries* are very common in the counties around the Mediterranean basin. The traditional process for the manufacturing of capers consists of a pretreatment in highly concentrated cover brines, followed by packaging in a 6% NaCl (w/v) brine containing 1% acetic acid (Alvarruiz et al., 1990). The initial step for the making of fermented caper berries consists of storage in water for 4 to 7 days. The water is then substituted with a 10−12% cover brine, the concentration of which is gradually increased to 15% to assure product stability until commercialization (Sánchez et al., 1992). The fermentation of these products consists of lactic acid production by LAB such as *L. plantarum*, which predominates, *L. paraplantarum*, *L. pentosus*, *L. brevis*, *L. fermentum*, *P. pentosaceus*, *P. acidilactici*, and *Enterococcus faecium* (Pérez Pulido et al., 2005). The use of a *L. plantarum* starter culture for caper berry fermentation induces a consistent process and faster sugar catabolism (Palomino et al., 2015). The antioxidant compounds quercetin, kaempferol, rutin, kaempferol-3-O-β-rutinoside and N(1),N(5),N(10)-triphenylpropenoyl spermidine amides have been identified as the most relevant in caper buds extract (Wiese et al., 2013). Although the actual origin of *kimchi* is still disputable, it is the traditional fermented Korean vegetable food, in particular when fermented with red paper (Jang et al., 2015). The name is not related to an individual vegetable, but to a range of fermented products. The two main

raw materials used in the making of kimchi are selected cultivars of Chinese cabbage and radish. In contrast to the sauerkraut preparation, the making of kimchi requires half-cuts or quartered brined cabbage. The fermentation of kimchi proceeds at 18°C for a few days, followed by a longer incubation period at refrigerated temperatures to promote microbial stability and the development of excess sourness. Such temperature control provides the advantageous proliferation of heterofermentative *Leuconostoc* spp. at the outset, followed by the growth of homofermentative lactobacilli and *Weissella* spp. (Jung et al., 2012). The use of *L. citreum* as a starter culture for kimchi fermentation has proven to prevent over-ripening and growth of yeasts during refrigerated storage (Chang and Chang, 2010). The health benefits derived from the LAB isolated from kimchi fermentations, in particular with respect to the immunomodulatory effects, have been recently described and mimic those identified for bacteria isolated from dairy products (Choi et al., 2015). The application of metabolomic and transcriptomic tools to study the dynamics of kimchi fermentation suggests that the main changes occur in relation to amino acids, organic acids, and sugars (Jung et al., 2013; Park et al., 2016). Other vegetable fermentations, for which popularity is currently increasing, is that of carrots, onions, and garlic. Carrot lactic acid fermentation is achieved when a lye treatment is applied to a system containing 2.5% NaCl and a mixed starter culture of *L. plantarum* and *Saccharomyces oleaginosus* (Montaño et al., 1997). A mixed LAB starter culture is known to lead a more complete sugar consumption with a concurrent higher acidity as compared to spontaneous fermentation of mixed vegetables including carrots, cabbage, beet, and onions (Gardner et al., 2001). Lactic acid garlic fermentation (3% NaCl) is only possible after blanching the vegetables and yields products with a similar flavor to those packed prior to processing (De Castro et al., 1998). Inhibition of LAB in nonblanched garlic may be associated with the antimicrobial activity of thiosulfinates (Yoshida et al., 1999). However, the same thiosulfinates are ineffective against the abundant population of Gram-negative rods heterofermentative LAB found on prepeeled garlic (Shim and Kyung, 1999). Fermented aged black garlic has shown high favorable bioactivity against induced obesity, hyperlipidemia nephropathy, and hepatopathy, apparently potentiated by yeast fermentation (Jung et al., 2011). The garlic fructan has also been identified as an effective prebiotic component, suggesting the beneficial use of garlic to prevent selected gastrointestinal diseases (Zhang et al., 2013). The anaerobic lactic acid fermentation of onions requires supplementation with sauerkraut cover brine, or slices of cabbage as a source of inocula (Roberts and Kidd, 2005). An insufficient or ineffective natural population of LAB in onions seems to preclude spontaneous fermentation. The fermented product known as sour-onion has a tart acidic taste similar to that which is characteristic of sauerkraut, with the basic onion flavor but without the pungency of raw onion (Roberts and Kidd, 2005).

9.5 SPOILAGE OF FERMENTED VEGETABLES

As mentioned previously, microorganisms play an important role in vegetable fermentations, affecting the quality and safety of the final products. The main alterations suffered by fermented vegetables are described in Table 9.1.

TABLE 9.1 Main Microbial Alterations in Fermented Vegetables and Causative Agents

Type of Alteration	Product Affected	Microorganisms Involved	References
Gas pocket or "alambrado"	Table olives	*Enterobacter* sp.	Garrido-Fernández et al. (1997)
		Citrobacter sp.	
		Escherichia sp.	
		Klebsiella sp.	
		Aeromonas sp.	
		Saccharomyces kluyveri	Duran-Quintana et al. (1979)
		S. cerevisiae	
		W. anomalus	
	Cucumbers	*L. brevis*	Fleming et al. (1975)
Putrid fermentation	Table olives	*Desulfovibrio aestuarii*	Levin and Vaughn (1966)
		Clostridium sp.	Gililland and Vaughn (1943)
Butyric fermentation	Table olives	*C. butyricum*	
		Clostridium beijerinckii	
		Clostridium fallax	
	Cucumbers	*C. bifermentans*	Franco et al. (2012)
Zapatería	Table olives	*Propionibacterium* sp.	Kawatomari and Vaughn (1956)
		Clostridium sporogenes	
		C. bifermentans	

(*Continued*)

TABLE 9.1 (Continued)

Type of Alteration	Product Affected	Microorganisms Involved	References
Lactic acid depletion	Cucumbers	*L. buchneri*	Johanningsmeier and McFeeters (2013)
		Acetobacter pasteurianus	Medina et al. (2016)
		Acetobacter sp.	
		Pectinatus sp.	Breidt et al. (2013a)
		Propioni bacterium sp.	
		E. cloacae	Franco et al. (2012)
		P. manshurica	Franco et al. (2012)
		I. occidentalis	
	Table olives	Oxidative yeasts	Ruiz-Cruz and Gonzalez-Cancho (1969)
Softening	Table olives	*R. minuta*	Hernandez et al. (2007)
		W. anomalus	Arroyo-López et al. (2012)
		D. hansenii	
	Table olives and cucumbers	*Aerobacter*	King and Vaughn (1961)
		Aeromonas	
		Achoromobacter	
		Escherichia	
		Paracolobactrum	
	Cucumbers	*Alternaria*	Costilow et al. (1980)
		Fusarium	
		Mucor	Etchells et al. (1958)
		Filamentous fungi	
	Kimchi	*P. kudriavzevii*	Moon et al. (2014)
White spots	Table olives	LAB species	Vaughn et al. (1953)
Pink/red pigmentation	Sauerkraut	Yeast species	Fred and Peterson (1922)
	Cucumbers	LAB species	Díaz-Muñiz et al. (2007)

Besides LAB and yeast, typically found in vegetable fermentation, Gram-negative bacteria of the *Enterobacter*, *Citrobacter*, and *Escherichia* genera are usually present in the first stage of vegetable fermentations. Such microbial communities decrease during the first few days of fermentation due to the production of lactic acid with a concomitant drop in pH (Breidt and Caldwell, 2011; Garrido-Fernández et al., 1997). If the decline in pH is protracted, Gram-negative bacteria such as Enterobacteriaceae may derive energy for growth from the metabolism of sugars, producing CO_2 and compromising the quality and safety of the preserve (Garrido-Fernández et al., 1997; West et al., 1941). Formation of CO_2 in vegetable fermentations leads to defects associated with significant economic losses. CO_2 can accumulate below the epidermis of the fruits, forming superficial or internal gas-pockets (*alambrado*) in table olives (Garrido-Fernandez et al., 1997) (Fig. 9.1) and cucumbers (Fleming et al., 1975). Formation of gas-pockets is

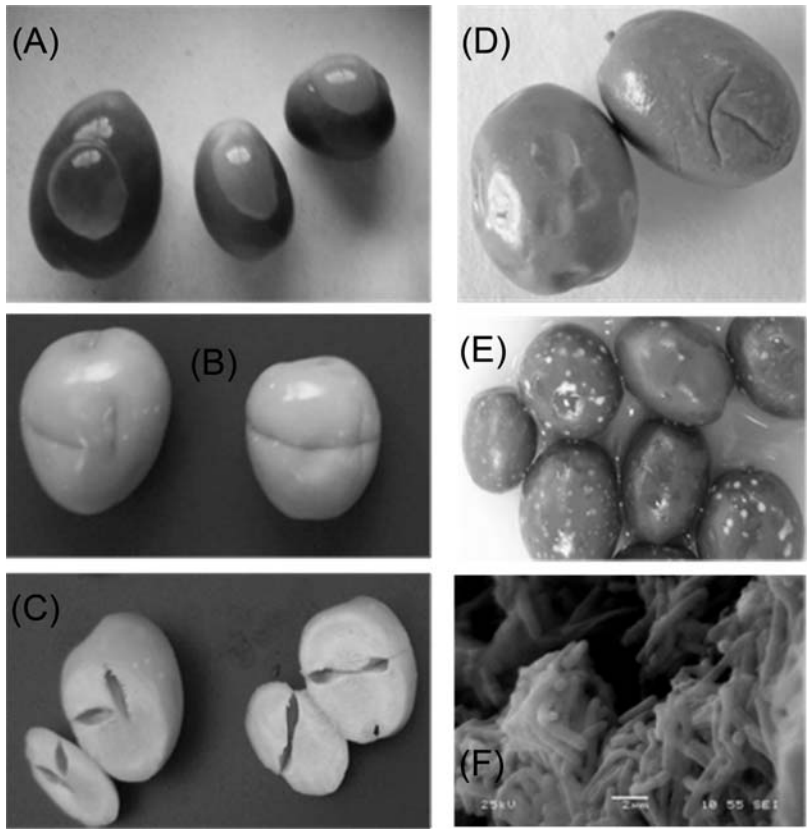

FIGURE 9.1 Olive fruits showing diverse types of spoilage: cuticular (A) and intramesocarpic gas-pockets (B and C), softening (D), presence of white spots on surface (E) and amplified SEM image of the stomatal openings (F). *Pictures courtesy of Dr. Antonio Castro, Dr. Pedro Garcıá and Dr. Francisco Rodriguez from Instituto de la Grasa (CSIC).*

the most frequent defect in the production of table olives and cucumber pickles. It may be also induced by the production of CO_2 by yeasts, heterofermentative LAB and homofermentative LAB able to catabolize malic acid, by the respiration of the fresh fruits prior to brining during storage without refrigeration, and by the fruits growing conditions in the field (Etchells et al., 1968, 1975; Duran-Quintana et al., 1979; McFeeters et al., 1984). The use of a nitrogen purging system, a correction of the pH at the beginning of the fermentation process, and the use of preservatives against yeasts would be an effective means with which to control this type of defect (Asehraou et al., 2002; Fleming et al., 1975; Garcia-Garcia et al., 1982).

The richness of nutrients and close to neutral pH existing at the beginning of lactic acid vegetable fermentations may contribute to the development of putrid and butyric fermentations (Gililland and Vaughn, 1943). The main characteristic of putrid fermentation is reminiscent of the smell of decomposing organic matter by the metabolisms of *Clostridium* spp. They are spore-forming anaerobic bacteria able to colonize the bottom of tall fermentation vessels, where anaerobic conditions can develop in the absence of purging. *Clostridium butyricum* in particular has been associated the development of butyric fermentations with a rancid butter smell (Gililland and Vaughn, 1943). *Desulfovibrio* spp. have also been isolated from putrid fermentations characterized by the production of sulfhydric compounds (Levin and Vaughn, 1966). Lack of sanitation practices for fermentation vessels, or the use of contaminated water, are the main causes of this type of spoilage.

Upon completion of a lactic acid vegetable fermentation, physicochemical conditions, high acidity, and low pH ensure the preservation of the product. However, sometimes, an undesired secondary fermentation or spoilage may be initiated by propionic acid bacteria, in particular *Propionibacterium* spp. This group metabolizes sugars or the lactic acid form during the primary fermentation, to produce propionic acid, acetic acid, and CO_2, inducing an increase in pH and volatile acidity (Gonzalez-Cancho et al., 1980). These conditions also encourage the development of *Clostridium* species, which together with *Propionibacterium* can promote *zapatería* spoilage, giving abnormal odors and tastes in table olives (Kawatomari and Vaughn, 1956; Plastourgos and Vaughn, 1957). The resulting higher pH enables the growth of other spoiling microbes such as the spore-forming *Clostridium botulinum*, responsible for the production of the lethal botulinum toxin (Medina-Pradas and Arroyo-López, 2015). Control of pH and salt concentration in brine would prevent the growth of these spoilage microorganisms and their off-odor fermentations, especially when temperatures are warmer during the summer months (Gonzalez-Cancho et al., 1970). In spite of lactic acid depletion, the propionic acid generated by propionic acid bacteria results in desirable attributes in other fermented foods, in particular sauerkraut (Babuchowski et al., 1999). Propionic acid is a potent mold inhibitor, preventing the growth of pathogenic fungi able to produce off-flavors and hazardous substances.

Undesired secondary fermentation or spoilage is also common in cucumber fermentations. The presence of acetic acid bacteria (AAB) and *Lactobacillus* spp. seems to play a relevant role in the initiation of fermented cucumber spoilage (Johanningsmeier and McFeeters, 2013; Medina et al., 2016). AAB can convert lactic acid to acetic acid. *Lactobacillus buchneri* are able to produce acetic acid and 1,2-propanediol from lactic acid during the first stage of the undesired secondary fermentation (Johanningsmeier and McFeeters, 2015). Should the undesired secondary fermentation enable the increase in pH above 4.2, *Propionibacterium* and *Pectinatus* species, and *Clostridium bifermentans* and *Enterobacter cloacae* are able to convert lactic acid to propionic acid (Breidt et al., 2013a) and produce butyric and propionic acids, respectively (Franco and Perez-Diaz, 2013). Production of such organic acids would explain the cheesy and manure-like aroma characteristic of the spoilage of fermented cucumbers.

Yeast populations coexist with the presence of LAB during vegetable fermentation. In fact, yeasts predominate in certain types of elaboration such as directly brined (natural) olives. The roles of yeasts in the processing of fermented vegetables are associated with the development of taste and aroma (Arroyo-López et al., 2012) However, yeasts may be the culprit of certain types of spoilage. *Pichia kudriavzevii* is considered as the main yeast responsible for kimchi spoilage due to superficial film formation, off-odor production, and bad-tastes (Moon et al., 2014). An excessive growth of fermentative yeast, such as *Saccharomyces cerevisiae* and *Wicherhamomyces anomalus*, could lead to a vigorous production of CO_2 that may damage the fruits, due to the formation of gas pockets or *alambrado* (Duran-Quintana et al., 1979; Garrido-Fernandez et al., 1997; Vaughn et al., 1972). Moreover, food spoilage can result by oxidative yeasts which can consume the lactic acids produced during olive fermentation under aerobic conditions (Ruiz-Cruz and Gonzalez-Cancho, 1969). *Pichia manshurica* and *Issatchenkia occidentalis* have the ability to use lactic and acetic acids during aerobic metabolism with the concomitant increases in pH, and have been associated with the initiation of spoilage of fermented cucumbers (Franco et al., 2012).

Another unfavorable property of some yeast strains in vegetable fermentation is the ability of enzyme productions that could cause the softening of fruits, such as proteases, xylanases, and pectinases (Fig. 9.1). Some yeast strains of *Rhodotorula minuta*, *W. anomalus*, and *Debaryomyces hansenii* have been described in green table olive fermentation with these capacities (Hernandez et al., 2007), and *P. kudriavzevii* can lead to softening in kimchi deterioration (Moon et al., 2014). Also, pectinolytic molds belonging to the genera *Alternaria*, *Fusarium*, and *Mucor* are responsible for the softening of brined cucumbers (Costilow et al., 1980). The metabolism of pectinolytic and cellulolytic yeast acts on pectic substances, cellulose, hemicelluloses, and polysaccharides, which damage the cell walls associated with the softening of the fruit (Arroyo-Lopez

et al., 2012; Golomb et al., 2013). Also, certain pectinolytic Gram-negative bacteria such as *Aerobacter*, *Aeromonas*, *Achoromobacter*, *Escherichia*, and *Paracolobactrum* produce the softening deterioration and the skin sloughing of the fruit in black olive oxidation process when the temperature is high (Garrido-Fernandez et al., 1997; King and Vaughn, 1961; Vaughn et al., 1969).

Regardless of the habitual low pH and high salt concentration obtained in the final product, packed fermented vegetables can suffer spoilage by microorganisms if residual nutrient concentration is present in the packaging (Garrido-Fernandez et al., 1997). Thermal treatments are an effective method for increasing the microbiological stability of final products. In some products, such as cracked green olives, thermal treatments are not appropriate and result in loss of organoleptic characteristics (Abriouel et al., 2014). Also, residual sugar content can be found in partial lactic acid fermentation, with the subsequent risk of postfermentation. Spoilage microorganisms in packaging are the same as those found in a typical fermentation, LAB and yeasts, and their growth can reach high population levels producing clouding of brines (Arroyo-Lopez et al., 2012). Moreover, yeasts in table olives can also consume the lactic and acetic acids produced during fermentation, under aerobic conditions (Ruiz-Cruz and Gonzalez-Cancho, 1969), suggesting that the development of yeast is stabilized under anaerobic conditions.

Other common alterations in table olive processing is the presence of white spots (see Fig. 9.1). This abnormality is very common in Spanish-style green olives where a vigorous lactic fermentation is carried out by LAB. Vaughn et al. (1953) noticed that the small white spots are colonies of LAB species established in stomatal openings.

Pink sauerkraut is one of the most common defects in the production of that commodity. Although pink sauerkraut has been reported to be edible and is often sold at a lower price, it has been related to changes in texture, flavor, and odor (Fred and Peterson, 1922). Pink sauerkraut is caused by the yeasts naturally present in the cabbage and favored by the concentration and type of sugars present in the cabbage, sodium chloride concentrations above 3%, high acidity, and extrinsic factors such as temperature and the supply of oxygen (Fred and Peterson, 1922). *Lactobacillus brevis* has also been associated with the formation of a water-soluble red pigment in sterile cabbage juice favored at pH values between 5.2 and 6.3 and suppressed by anaerobic conditions (Stamer et al., 1973). Sporadic red-colored fermented cucumber spoilage cases have also been documented within the US cucumber pickling industry. *Lactobacillus casei* and *L. paracasei* have been implicated in such types of spoilage due to their ability to degrade the azo dye tartrazine (FD&C yellow nr 5) used as a yellow coloring in cover brines (Díaz-Muñiz et al., 2007). This type of spoilage can be prevented by the addition of 0.1% sodium benzoate.

9.6 CONTROL MEASURES TO REDUCE ALTERATION OF FERMENTED VEGETABLES

In many industries, vegetable fermentation still occurs spontaneously. Thus, the process is not fully predictable and sometimes can lead to spoilage. Alteration of vegetable packaging can also occur if residual sugar content and microorganisms are present in the finished product. To prevent these problems, the elaboration processes can be controlled through physicochemical or microbiological approaches. Below, we detail the most important factors with easy implementation and control in the industry.

1. *Salting* and *acidification* are two common factors in many vegetable -fermentations, which convert the system into an adverse habitat for spoilage and pathogenic microbes. Salt, mainly sodium chloride, determines the flavor of the final products, reduces the water activity and consequently influences the type and extent of microbial metabolism, indirectly helping to prevent the softening of vegetable tissue, and facilitating the diffusion of components from the fruit tissues into the cover brines. As an example, levels of salt higher than 8% (wt/vol.) are necessary in table olives for long-term bulk storage in order to inhibit spoilage by *Propionibacteria* species (Garrido-Fernández et al., 1997). The pH and/or acidity of a food are generally used to determine processing requirements and applicability of specific regulations. Reduction of pH as the result of fermentation or acidification with organic acids is also a critical preservation factor. Improper acidification to pH above 4.6 may lead to spoilage with consequences of public health significance due to the potential proliferation of toxin-producing *Clostridium* species (Garrido-Fernández et al., 1997; Pérez-Díaz et al., 2015a). The recommended pH and salt concentration varies widely among vegetables (Pérez-Díaz et al., 2015a).
2. A *starter culture* is defined as a preparation of living microorganisms, which are deliberately used to assist the beginning of fermentation, producing specific changes in the chemical composition and the sensorial properties of the substrate to obtain a more homogeneous product. For many years, the search for starter cultures with application in vegetable fermentations was practically focused on the technological activity of LAB. The traditional selection of LAB starter cultures was based on diverse technological criteria, including fast consumption of sugars and acidification rate, homo-fermentative metabolism, pH, and salt tolerance, resistance to bacteriophages, production of bacteriocins, flavor development, wide temperature range for growth and minimum nutritional requirement, among many other characteristics. The starter culture must also dominate the indigenous microbiota by its fast and predominant growth under fermentation conditions. *Lactobacillus pentosus* and *L. plantarum* are among the LAB species with major applications as starter cultures in such

fermented vegetables as cucumber, capers or tables olives, albeit *L. mesenteroides* is also habitually used in low salt fermentations such as sauerkraut, as mentioned previously (Corsetti et al., 2012; Perez-Díaz et al., 2015a). Recently, several publications have emphasized the importance of the role played by yeasts as bioprotective agents when used as starter cultures during vegetable processing. Apart from their technological characteristics (lipase, esterase, β-glucosidase or catalase activities, improvement of LAB growth and the organoleptic profile of raw material), the competitive traits against undesirable microorganisms have attracted increasing attention from scientists, who proposed their application as biocontrol agents in the food industry (Arroyo-López et al., 2012; Muccilli and Restuccia, 2015). These antagonistic activities rely on the production and tolerance of high concentrations of ethanol, competition for nutrients, as well as the synthesis of a large class of antimicrobial compounds (killer toxins, ethyl acetate, ethanol, etc.) which showed clearly a large spectrum of activity against foodborne pathogen and spoilage microorganisms. Thus, latest trends for the development of starters in vegetables are focused on the production of mixed-multifunctional (LAB-yeasts) starters in order to improve and expand the mode of action by the use of two complementary microorganisms with different properties and with the ability to inhibit spoilage microorganisms.

3. *Hazard Analysis and Critical Control Point (HACCP) system*. Fermented vegetables must be prepared and handled in accordance with the appropriate sections of the *General Principles of Food Hygiene* (CAC/RCP 1-1969, Codex Alimentarius Commission, 2003), the *Code of Hygienic Practice for Low-Acid and Acidified Low-Acid Canned Foods* (CAC/RCP 23-1979, Codex Alimentarius Commission, 1993), and the *Code of Hygienic Practice for Canned Fruit and Vegetable Products* (CAC/RCP 2-1969). The product should comply with any microbiological criteria established in accordance with the *Principles for the Establishment and Application of Microbiological Criteria for Foods* (CAC/GL 21-1997, Codex Alimentarius Commission, 1997). HACCP is a valuable tool for complying with all these hygiene criteria and good manufacturing practices in preparation of fermented vegetables. The HACCP is a systematic and preventive procedure, internationally recognized, to tackle biological, physical, and chemical risks by means of applying prevision and prevention principles, instead of final product testing. The preventative approach is based on remarking all essential hygiene controls in each processing step. The industry of fermented vegetables must comply with the requirement for elaborating, applying, and maintaining a permanent procedure based on HACCP principles. Therefore, the HACCP concept permits a systematic approach to the identification of hazards, and an assessment of the likelihood of their occurrence during the manufacture, distribution, and use of the food product, and defines measures for their control in order to ensure food

safety and quality. When being applied, food handlers can acquire knowledge and awareness about food safety and quality, thus improving their handling practices and preventing food alteration. Despite the advantages of HACCP system, food industries have to deal with its implementation across a large variety of food commodities produced. Additionally, for small-scale industries (<20 workers) HACCP implementation is often more difficult given the associated cost and effort. Therefore, HACCP should be adapted for different situations and adjusted to changes in equipment, processing methods or technological achievements.

4. *Preservatives* and *thermal treatment*. To increase shelf life and avoid spoilage of fermented vegetables packaging, the finished products may be pasteurized or sterilized, depending on their characteristics. Thermal treatment considerably reduces the microbial load of finished products, increasing shelf life and stability during storage. Contrarily, it can reduce the texture and produce losses associated with the fresh appearance of the preserved fruits (Garrido-Fernández et al., 1997). Additionally, processed vegetables which undergo thermal treatment may maintain stability at less acidic pH values and reduced salt concentration. Preservatives may substitute thermal treatments if specific processing constraints exist, such as the use of plastic bags or 5-gallon plastic pales for packaging, or the use of heat sensitive seasonings. Such prerogative is applicable in the United States to the stabilization of the products for transportation purposes only. Preservatives such as sorbic and benzoic acids, or their respective salts, are commonly used in finished fermented vegetable products and as processing aids. Potassium sorbate is effectively used to inhibit the growth of yeasts and molds in fermentation cover brines and some processed products. Sodium benzoate is an important preservative used in certain fermented and fresh-pack products to inhibit bacterial growth, especially LAB (Pérez-Díaz et al., 2015a). The effectiveness of these preservatives is dependent on the pH of the medium. The disadvantages in applying such preservatives include their ability to preferably accumulate in the fatty components of vegetables such as olives, limiting the antimicrobial effect, generation of undesirable sensorial notes for consumers, induction of browning of fruits, and degradation by selected microbes compromising the stability of the products. As a result, the table olive sector is demanding research for obtaining more appropriate preservatives (sodium metabisulphite, zinc chloride, or natamicin) as well as other technologies to increase shelf life (e.g., modified atmosphere or high hydrostatic pressure).

9.7 CONCLUDING REMARKS

Vegetable fermentations will continue to be central to the production, availability and supply of foods for a growing population. The use of traditional approaches for spontaneous fermentations is in line with the demand for

natural, healthier food products. Safe fermented vegetables with improved quality can be produced provided certain hygienic rules are followed and sufficient knowledge of the underlying microbiology and chemistry is developed (Demarigny, 2012). Consumer trends will likely encourage the emergence of innovative fermented food products made with vegetable materials from tropical and warm countries, using environmentally friendly technologies and applying new scientific knowledge. With the development of DNA sequencing technology and bioinformatics it is currently possible to obtain and translate genomic DNA sequencing information into functional predictions. Microbial genome sequences may be compared in order to improve the selection of starter cultures species that can positively influence the organoleptic attributes of finished products instead of inducing spoilage. The possibility of identifying missing growth factors in vegetable matrixes that could enhance and accelerate the fermentation process and prevent spoilage is around the corner. Identification of specific strains dominating in vegetable fermentations at variable stages is imminent and has been achieved for other environments. Increased knowledge of how to manipulate fermentation systems to increase their bioactivity and retain that which is naturally present awaits.

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