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Production of Pickles by Mixed Culture Fermentation

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Abstract

The potential of mixed culture fermentation has gained importance in researches and food industry. In fact, fermented food preferences and enriching of human dietary habits are growing interest in less salt intake, chemical additives, better nutritional properties and safety. However, traditional pickle production is performed by spontaneous fermentation, which is used natural microflora instead of defined starter culture in fermentation process. Controlled fermentation could be divided into two groups such as single culture and mixed culture fermentations. Developments in mixed culture fermentation provide significant features on pickles. In particular, high amount of acid production in a short time, longer shelf life, rich in organoleptic characteristics including various texture, aroma, flavor components and better nutritive effects are presented in the current review. Nevertheless, some obstacles are existing in processing of fermented foods. This review demonstrates the recent advances in the use of mixed culture and focus on its mechanism and impacts on fermentation process.

Keywords

Lactic Acid Bacteria, Yeasts, Fermentation Biotechnology, Pickle, Mixed Culture, Food Quality

1. Introduction

Pickle is a traditional lactic acid-fermented food made from various vegetables or fruits with aromatic herbs and seeds, which are considered as nutritious foods rich in omega values (omega 3;6;9), dietary fibers, vitamins, minerals and lactic acid [1, 2].

Fermentation is a crucial process for vegetables to improve textural quality and upgrade nutritive value by enrichment with LAB [3]. Other benefits are longer shelf life, better functional product, providing easily digestible substance by lactic acid fermentation, enhancement of sensory properties and increase the vitamins such as thiamine, riboflavin, niacin and folic acid that can directly influence on health of the consumers of such product [4].

Fermentation performed either homofermentative or heterofermentative pathways and hexoses are metabolized by two types of LAB. It is known that the homofermentative LAB use Embden-Meyerhof-Parnas (EMP) pathway (Figure 1) to produce mainly lactic acid (LA) via aldolase enzyme. Heterofermentative LAB produce acetic acid, ethanol, CO_2 as well as LA with phosphoketolase enzyme via pentose phosphate pathway (hexose monophosphate shunt) (Figure 2) [2]. Spontaneous fermentation and single culture fermentation are materialized by heterofermentative and homofermentative pathways, respectively.

Starter culture have an importance for consistency which are selected according to their effectiveness, predictable and controllable fermentation process. The use of starter culture must be one of the prerequisites for the production of fermented vegetables at the industrial scale [5, 6].

Recently, mixed culture (MC) has been a better option as starter culture which theoretically relies on a sequential microbial process including heterofermentative and homofermentative LAB. In high level inoculation as MC, fermentation could be controlled and accelerated to prevent spoilage and attained consistently high quality and reliable products.

Currently, numerous studies focused on the classification of various traditional or novel fermented product, screening microbial community of LAB, phenotypic and genotypic identification of LAB, characterization of pure and co-culture or MC in lactic acid fermentation to improve production, gain probiotic attribute, minimize risks, enhance organoleptic properties, increase the bacteriocin level etc. [7-10].

The purpose of this review is to present recent literature and advances on production of pickles by MC, single culture and spontaneous fermentations. Additionally, we presented the ways of such production by single and MC techniques that recently shown as new approach in pickle process.

2. The General Characteristics and Problems During Fermentation of Pickle

Within the vegetable pickles; cucumber, olive and cabbage are most known products. Other vegetables are beet, radish, carrot, cauliflower, garlic, pepper, turnip, green tomato, green bean, artichoke, ginger roots and bamboo shoot [11], [12]. Moreover, various Asian traditional-fermented products have innovative combinations ranging from mild to spicy with some unique ingredients and techniques [12]. For instance, one of the most famous Korean fermented product is kimchi usually made from Chinese cabbage, radish, red pepper powder, garlic, ginger, onion, glutinous rice paste, fruits and optionally fishery products by direct-salting [13]. Next, Sichuan pickle produced in China which is mildly salted and fermented vegetable [14]. The vegetables such as Chinese leaf mustard, cabbage, radish, bamboo shoot, tender ginger and chili are used in Sichuan fermentation by brine-salted [15].

A number of natural and synthetic additives are often used to improve the quality, shelf life, nutritional status and enhance of the product profiles in fermentation of vegetables [16]:

1) Acetic acid-acetate: Preservative and bacteriostatic effect; antimicrobial activity,
2) Citric acid: Synergistic effect with antioxidants; antimicrobial effect; taste-promoting; colour stabilization,
3) Benzoic acid-benzoate: Preservative effect; inhibition of specific enzyme systems; antimicrobial activity; synergistic effect with sodium chloride, boric acid, sugar and sorbic acid,
4) Sorbic acid-sorbate: Inactivation of enzyme systems of microorganisms; antimicrobial and antibacterial effect; synergistic effect with acids (i.e acetic acid), sugar, temperature; extend shell-life;
5) Calcium chloride: Increase product endurance; providing texture firmness; prevention of pectolitic activity,
6) Riboflavin (Lactoflavin, B2 vitamin): Fortification with vitamin; colouring agent and stabilization,
7) Aromatic plant seed, leaf and root such as herbs, spices, oils: Antimicrobial and anti-oxidative effect, increasing palatability, providing aromatic pleasant odour, enrichment of nutritive value.

Various seeds (dill, coriander, mustard, poppy, black peppercorn) and leaves (of bay, olive, rosemary, celery, cherry) are used in pickle production.

Traditionally, there are two brine technics in the pickling process including direct salting to withdraw juice from the vegetable (dry-salting) and brine-salted [17], [18]. Salt concentration is the most important factor for progression of fermentation and end-product quality. Fermentation performs slowly or falls through in 10% and above. Meanwhile, high salt concentration (10-20%) causes the growth of fermentative (*Torulopsis, Hansenula, Saccharomyces*) and oxidative (*Debaryomyces, Pichia, Candida*) yeasts [16]. In spontaneous fermentation, oxidative yeasts decrease of acidity consuming LA which have deterioration activity.

Undesirable conditions in table olive especially off-odours could be formed during processing because of its low sugar content. A red ring is considered as a series defect and causes buttery flavour due to low acid production. Prone to shot berries, thin skin, sensitive to lye, white skin spots, easily bruised and possibility of split stones could be observed. Other defects are darkening, skin hardness, and rancid, mouldy, metallic taste. Moreover, gas pockets (fish-eyes) originated from gram-negative bacteria and some of yeast in spontaneous fermentation of olives. Gas collected in pockets under the skin and causes soft-wrinkled olives. Processing or storing of brines with a high salt concentration shows some problems in skin and texture [2].

According to pickle industry, encountered problem is softening especially in cucumber and olive fermentation or their storage. Problem overcome by controlling pH and salt levels in fermentation. Using food grade acid (lactic acid, citric acid and acetic acid) is other solution apart from starter cultures. Moreover, good hygienic conditions, clean water, process equipment, good quality ingredients and good manufacturing practice should consider for corrective and preventive outputs.

In vegetables fermentations, there are control parameters (pH, temperature and salt level). The brine pH is characteristically lowered around 4.5 or below. The growth of Enterobacteriaceae and undesirable microorganisms are suppressed initially at lower pH [19]. Lower temperatures decrease the productivity of fermentation and cause high process time. Furthermore, the optimum temperature is in the range 15-32°C, salt level is 5-8% NaCI, and fermentation takes 21-30 days [16]. In controlled conditions, parameters are revised using starter culture.

In the last few years, the potential of starter cultures have been emphasized as controlling and safety of fermentation process. The selection of starter cultures comprises suitability of their phenotypic characteristics and technological features. Moreover, improving nutritional value, health effects and organoleptic properties should be considered in selection.

Starter cultures convert fermentable sugars to lactic acid, organic acids and alcohol depending on metabolic pathway as well as flavour components enhanced savouriness. The most important microbial populations are lactic acid bacteria in pickle technology. Based on sugar fermentation patterns, there are two wide metabolic categories of LAB: homo fermentative and hetero fermentative. First of all, homo fermentative LAB include some *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus* species which catabolize one mole of glucose to yield two moles of pyruvate via Embden-Meyerhof pathway (EMP) (Figure 1). Secondly, hetero fermentative LAB utilize the the phosphoketolase pathway (pentose phosphate pathway). Representative hetero fermentative LAB genera involve some *Lactobacillus, Leuconostoc* and *Weissella* species (Figure 2).

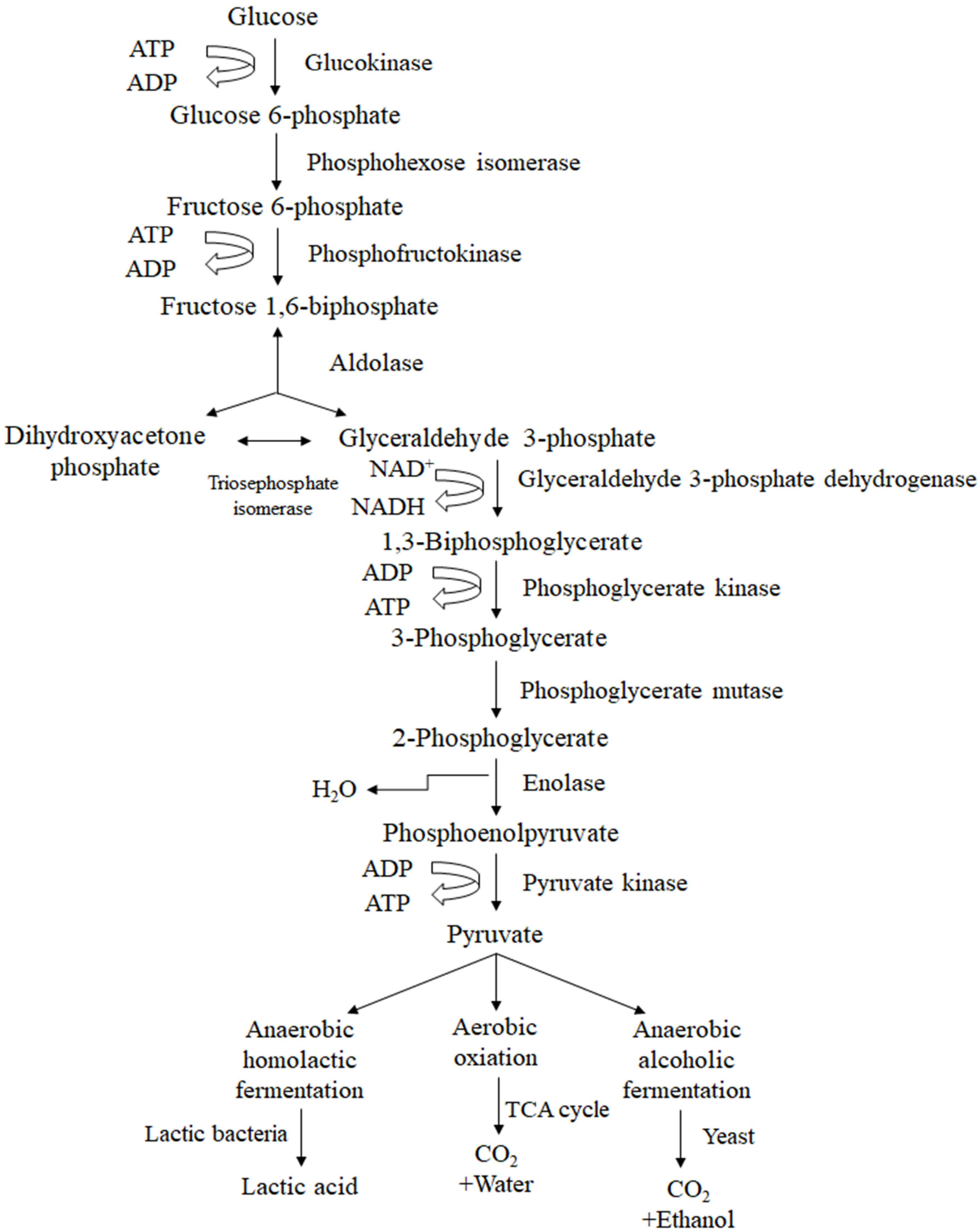

Figure 1. *Homolactic fermentation pathway of glucose (Embden-Meyerhof Pathway)-EMP-Glycolysis.*

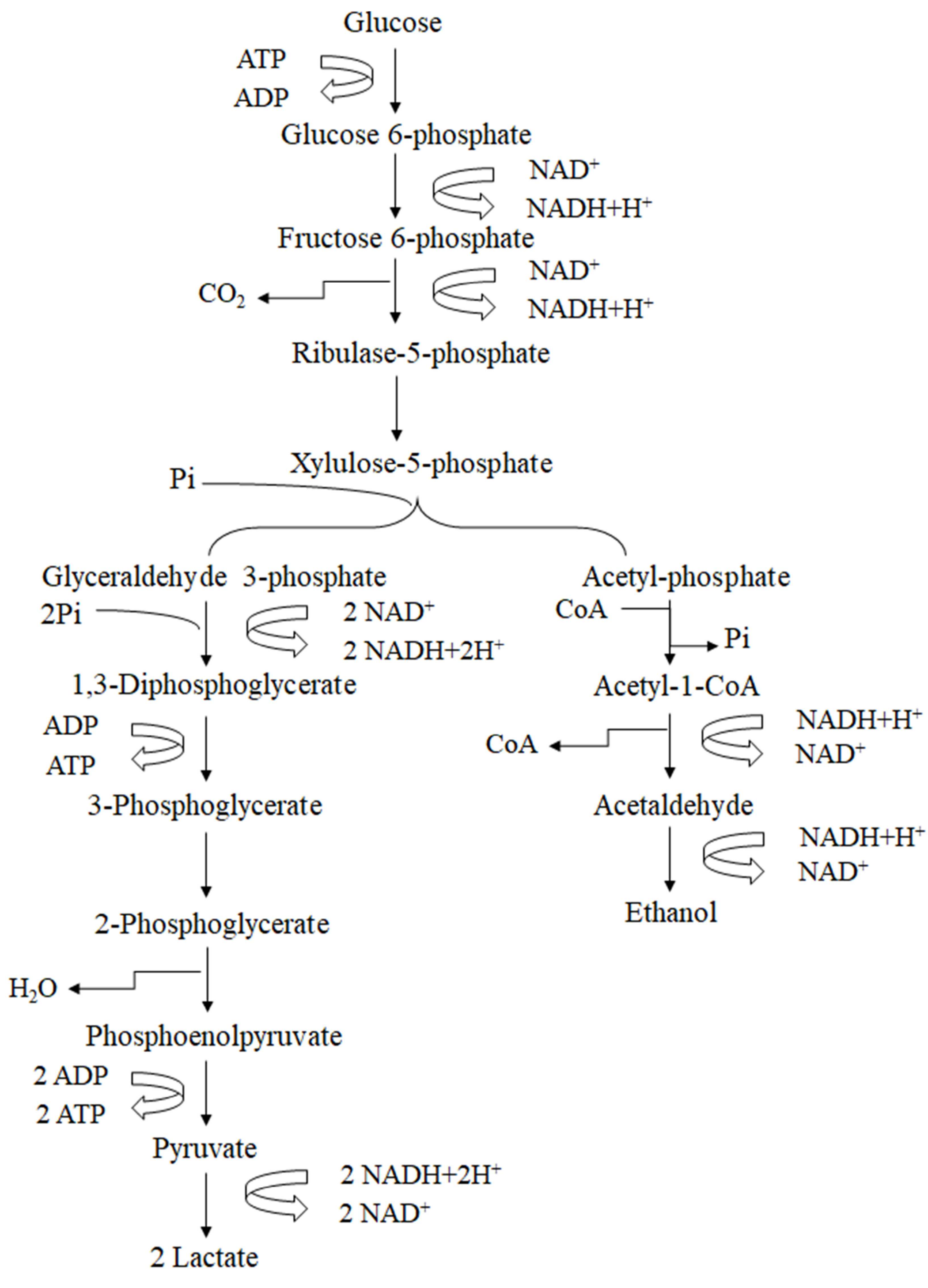

Figure 2. *Heterolactic fermentation pathway of glucose (6-phosphogluconate/ phosphoketolase pathway).*

As can be seen in the Table 1; homo fermentative and hetero fermentative LAB species related with pickle. For instance; it was indicated that *Pediococcus* are commonly associated with various vegetables and their products such as cabbage, sauerkraut, cucumbers and olive [20]. Starter cultures can be basically classified as shown in Table 2. The use of these defined cultures provides better fermentation process. Nowadays changing demands of customers has refused of using chemical additives struggling with undesired bacteria growth in fermented foods. In the selection of starter cultures, technological characteristics are survival of LAB in brine, production of large amount of LA, salt tolerance and adaptation of pH values. It was shown at proceeded topics in the light of the committed scientific studies within the scope of the review.

Table 1. *Classification of lactic acid bacteria and Prevalent LAB species related with pickles.*

Morphology	Family	Genus	L. A. isomer form	Carbohydrate fermentation	Species
Rods	Lactobacillacea	*Lactobacillus*	D, L, DL	Homo- or hetero fermentative, facultative hetero-	*L. acidophilus, L. delbrueckii, L. helveticus, L. lactis, L. leichmanii, L. salivarius, L. casei, L. curvatus, L. plantarum, L. bavaricus, L. sakei, L. pentosus, L. paracasei, L. fermentum, L. buchneri, L. brevis, L. hilgardii, L. reuteri, L. malefermentans, L. diolivorans, L. parabrevis, L. namurensis, L. rhamnosus, L. coryniformis*
		*Pediococcus**	DL, L	Homo-or facultative hetero-	*P. damnosus, P. dextrinicus, P. parvulus, P. cerevisiae, P. pentosaceus, P. acidilactici, P. ethanolidurans*
Cocci	Carnobacteriaceae	*Carnobacterium*	L	Homo-	*C. maltaromaticum*
	Enterococcaceae	*Enterococcus*	L	Homo-	*E. faecalis, E. faecium, E. casseliflavus*
		Tetragenococcus	L	Homo-	*T. halophilus V. fluvialis*
		Vagococcus	L	Homo-	
	Leuconostocaceae	*Leuconostoc*	D	Hetero-	*L. mesenteroides, L. cremoris,*
		Oenococcus	D	Hetero-	*L. paramesenteroides, L. citreum,*
		Weissella	D, DL	Hetero-	*L. pseudomesenteroides, L. gelidum, L. dextranicum, L. inhae, L. gasicomitatum, O. oeni, W. carnosum, W. cibaria, W. confusa, W. hellenica, W. kimchii, W. koreensis, W. paramesenteroides, W. viridescens*
	Streptococcaceae	*Streptococcus*	L	Homo-	*S. bovis, S. faecalis, S. lactis, S. thermophilus,*
		Lactococcus	L	Homo-	*Lactococcus lactis,*

*Pediococcus also has a tetrad morphology
Adapted from [20], [31], [44], [45], [46]

Table 2. *Classification of starter cultures.*

Name	Definitions
Single strain starter culture	Include a single strain of species
Multiple strain starter cultures	Include more than one strain of a single species
Mixed strain starter cultures	Include different strains from different species

3. Traditional Pickle Production

In spontaneous fermentation; vegetables are fermented based on autochthonous microbiota consisting mainly gram-negative bacteria, LAB and yeasts [21]. The autochthonous flora of raw vegetables and their cell density are closely associated with agronomic practices consisting of irrigation water, fertilizers, harvesting conditions, soil type of field, temperature as well as raw material quality [1, 22].

The natural microflora of fresh vegetables occurs from a great variety of microorganisms (Table 3). Some LAB and microorganisms lead to spoilage within these populations. However, LAB remain considerably a small part (2-4 log CFU g-1) in total microflora [1, 16].

Table 3. *LAB and some yeasts occuring spontaneously in fermented vegetable.*

Pickle types	Growing microorganisms	References
Cucumber	*Leuconostoc* spp., *L. brevis*, *L. pentosus*, *L. plantarum, Enterobacter cloacae, L. buchneri* and some yeasts (*Pichia manshurica, Issatchenkia occidentalis)*	[19], [47], [48]
Eggplant	*L. brevis*, *L. plantarum*, *L. pentosus* and *L. fermentum*	[38]
Olive	*Enterobacteriaceae, L. mesenteroides, L. citreum, P. cerevisiae, L. plantarum, L. brevis, L. pentosus and yeasts (Zygotorulaspora mrakii, Rhodotorula mucilaginosa)*	[39], [40]
Cauliflower	*Weissella kimchii, L. plantarum, L. sakei/curvatus, W. viridescens, L. mesenteroides, Enterococcus spp. P. pentosaceus, L. brevis*	[49]
Turnip	*L. plantarum, L. hilgardii, L. corynigormis, P. parvulus, L. maltaromicus, L. viridescens* and some yeasts (*Candida* spp., *Issatchenchia* spp., *Hasenula* spp.)	[50]
Sauerkraut	*Enterococcus faecelis, L. mesenteroides, L. brevis, P. pentosaceus, L. plantarum* and some yeasts (*C. lambica*)	[43], [51]
Kimchi	*L. mesenteroides, L. citreum, L. pseudomesenteroides*, *L. sakei, Lactococcus lactis, L. brevis, L. plantarum*	[25], [52]
Takanazuke	*Pseudomonas*, *L. curvatus*, *L. plantarum*, *L. sakei*, *Weissella*, and *L. alimentarius*	[53]

Spontaneous fermentation is separated into four stages, each characterized by different groups of microorganisms. First of all, Gram-(+) and Gram-(-) bacteria naturally present in fresh product growing at the initiation of fermentation. During this stage, LAB allowed decreasing of pH. Consequently, Gram-(-) bacteria and spore-forming bacteria are inhibited. On the other hand, some fermentative yeasts are situated in medium, which lead to deterioration by gas formation. Growth of microorganisms proceeded until completion of sugar in medium. Secondary fermentation is phase of growing fermentative yeast. Yeasts ferment residual sugar in medium after regressing growth of lactic acid bacteria due to low pH. Finally, in post-fermentation stage any microbial growth can not be monitored under anaerobic conditions owing to running out of carbohydrate source in medium. However, if brine surface is in contact with air, oxidative yeasts, molds and bacteria made spoilage (Figure 3).

During spontaneous fermentation, the LAB populations are dominated by a succession of hetero- and homo-fermentative LAB, circumstantially with yeast or without yeast, which are responsible for multi-step fermentation processes. *Streptococcus faecalis* or *Enterococcus faecalis*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Pediococcus pentocaceus* and *Lactobacillus plantarum* dominated in medium [16]. Fermentation of vegetables spontaneously performed by distinctive sequential microorganisms.

Spontaneous fermentation has a few disadvantageous compared to fermentations with pure culture, co-culture or MC. In fact, different starter culture systems have been proposed for fermented food production to minimize the impact of undesirable sources of variation.

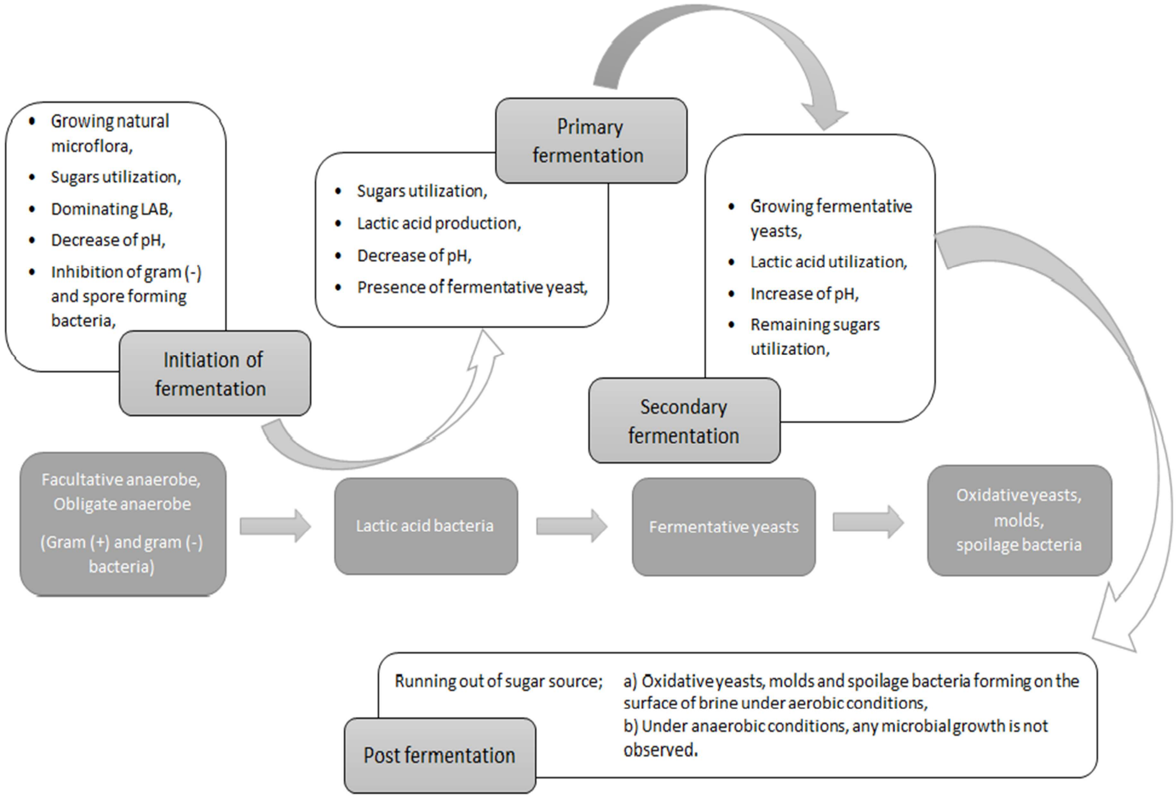

Figure 3. *Classic Fermentation Stages Occurring Spontaneously.*

4. Pickles Production by Single Culture

Starter culture inoculation control the fermentation process with low salt, less chemical addition and rich in desirable microflora, which provides technologic, nutritional and healthy advantages [2], [23].

L. curvatus isolated from Nozawana-zuke was used for production of low-salt pickle made from leafy turnip plant. *L. curvatus* strain showed antibacterial activity against some food-spoiling bacteria such as *S. marcescens* JCM20012, *Listeria monocytogenes* JCM7671 and *S. aureus* JCM20624 due to their bacteriogenic properties in pickle preservation [24].

Some LAB such as *L. mesenteroides*, *L. citreum* and *L. plantarum* are suggested as suitable starter culture for improvement of quality in vegetable fermentation [25]. It was stated that the use of one starter culture help standardize the fermentation by controlling the microbial flora [18]. Eom et al. (2007) collected nine hundred colonies of *Leuconostoc* from kimchi, sauerkraut and fermented cucumber. Meanwhile, sequencing of 16S rRNA genes of the strains, *L. mesenteroides* (HJ-S7 and HJ-S13) and *L. citreum* (HJ-P4 and HJ-P5) were revealed. Results showed that *L. citreum* could be a suitable starter culture for kimchi fermentation to maintain of its predominance and good organoleptic quality. This strain was detected as more tolerant to acid (pH 3.9) than other *Leuconostoc* spp. which maintains low pH and extends preservation of kimchi inhibiting growth of other microflora.

Anthocyanin-rich sweet potato pickles inoculating *L. plantarum* MTCC 1407 would be an alternative product similar to fermented cucumber, cabbage, garlic, [26]. Nutritional changes during fermentation revealed better organoleptic features (aroma, taste, flavor, texture) and useful probiotic properties of LAB. Contents of starch (56-58 g kg-1), sugar, anthocyanin pigment (390 mg kg-1) and LA (1.0-1.3 g kg-1) were acceptable for production. Regular consumption of these pickles rich in anthocyanin would be helpful for gastrointestinal disorders, aging and related ailment thanks to antioxidant principles.

In pepper fermentation, it was investigated the inhibition of spoilage bacteria by *Bacillus coagulans* B179 as pure cultures. Positive effects of results were attributed to the several advantages of Bacillus strains with simple nutrition requirements [23].

Since nitrite produced during fermentation adversely affects health due to formation of methemoglobin and conversion to carcinogenic nitrosamine (N-nitrosamines). Lactobacillus species contribute to reduction of the nitrite in fermented food. According to Xia et al. (2017), *L. brevis* AR123 exhibited efficient nitrite degredation and had good fermentation performance which was screened in different Chinese pickle fermentation. In the same study, *L. brevis* AR123 inoculated pickle as pure culture showed better sensory characteristics than spontaneous fermentation. Besides, the mixed starter (*L. brevis* and commercial starter) exhibited highest efficiency in nitrite degredation (final nitrite level; 0.88 mg/kg) in pickle juice. Moreover, it was recommended that *L. brevis* and the mixed starter could be effective for pickle fermentation [27]. Furthermore, pure starter cultures are beneficial to accelerate ripening time, inhibit pathogenic and spoilage bacteria and diminish problems in variations of organoleptic quality and microbiological stability in indigenous fermented foods. Hence, quality of products could be improved by use of pure starter culture which is more effective in lowering nitrite concentration compared to spontaneous fermentation [17, 28].

In some cases, single LAB strain could trigger acidification seriously throughout fermentation, consequently affect food flavor. Various LAB or yeasts could be utilized for improving the quality and minimize the risks in the production of fermented food which can be a good example of positive interaction. [6], [29]. However, using of single cultures are associated with basic properties of pickles, but using of MC could promise more complex pickle characters.

5. Pickles Production by Mixed Culture

Most of food fermentation processes involve mixtures of microorganisms such as yeast, LAB and fungi and depend on these microorganisms which take joint action to produce the desired product characteristics [30]. MC is suggested as a tool to maintain the benefits of spontaneous fermentation, while preventing the difficulty and deficiency of inoculated fermentation by single culture. Advances of MC application have been attributed to the selection and usage of new combinations of microorganisms originating from the native microflora and recently indicated as better approach to improve pickle quality [1].

MC fermentations in controlled inoculation of different strains provide a feasible opportunity towards improving the complexity. Particular and specific characteristics of fermented vegetable could be enhanced using different microorganisms [31]. In addition, possible synergistic interactions between different LAB and/or yeasts could be tool for improvement of fermentation process as a new biotechnological approach. Complexity in fermentation medium can provide thriving of microorganisms and increasing bioactive substances in end-product. Besides, some bioactive components such as exopolysaccharides produced food fermentations are related to interactions of the microorganism with food matrix and fermentation conditions [32]. In addition, mixed fermentation consisting of LAB and yeast has been specified to promote higher levels of folates, sterols, lignans, free ferulic acids and alkylresorcinols during fermentation [10]. The inoculation of MC compared to single culture appears to be more advantageous options to improve the specific characteristics of fermented foods [31]. The combination of different strains may improve aromatic profile and sensory properties of pickles by metabolic interactions in lactic acid fermentations [33]. The reason is that enzymes derived from vegetables or fruits and its microbiota promotes to the modification of raw material composition, hence bioactive compounds (total phenols, flavonoids, carotenoids) release and nutritional value of fermented foods can increase [10]. In the meantime, the composition of mixed or complex starter cultures changes, so the relative abundance of particular aroma-forming strains are influenced in several stages of fermentation process [33].

For successful understanding of traditional fermented product's microbial communities (mainly LAB, *Leuconostoc, Lactobacillus, Weissella* and *Lactococcus* species) and its metabolites with transcriptomic and metabolomics analysis have been performed by culture-dependent and culture-independent approaches [10, 12, 30, 34, 35]. Metabolites including organic acids (lactic acid, acetic acid, etc.) and other flavoring components are crucial compounds for organoleptic features of fermented product. Metabolite profiles also contribute to phenotypic view of fermented product community [34]. In a study done by Jeong et. al. (2013), metabolite analysis of kimchi showed organic acids (lactate, acetate and succinate) and bioactive compounds such as mannitol and gamma-aminobutyric acid (GABA) produced during fermentation. In addition, *Leuconostoc* strains and *L. sakei* were detected as the producers of mannitol and GABA, respectively. Mannitol and amino acids have been shown flavoring compounds [34]. Furthermore, the mixed culture of *Leuconostoc* strains and *L. sakei* produce both mannitol and GABA during fermentation. It was reported that culture-dependent fermentation could enhance the quality of fermented vegetables and develop the production yield [36]. In terms of a broad range of traditional fermented products, mixed starter cultures technique could be better option to maintain quality.

Overall, complex microbiota perform more complex activities from multi-properties (versatility) and tolerate more variation in the environmental conditions in terms of robustness compared to pure cultures [30]. Versatility and robustness could be explained with two factors according literature. By considering of mechanism in MC system, first factor is that members of the microbiota interact reciprocatively by dealing with metabolites or by exchanging molecular signals [30]. Mechanism mediates bacterial cell interaction by signal transduction and identification of inductive molecules, additionally contributes to the culture in following up population density [37]. Moreover, each microorganism in the mixture responds to the presence of others in the microbiota. Second factor is that members of the microbiota participate of different task in fermentation process and members carry out individual transaction in biotransformation by combining task performed by constituent individuals or sub-populations. This is important for the production of traditional fermented products containing functional strains such as probiotic ability [30, 37].

What are the main differences between application of spontaneous, single and mixed culture?

Characterization of viable strains were determined by RAPD-PCR (Randomly Amplified Polymorphic DNA) which is useful method for monitoring inoculated strains isolated from spontaneous fermentation. The most appropriate mixed starter cultures consisting of *L. plantarum* 3G33 (having the highest acidifying capacity), *L. brevis* 3G22 and *L. fermentum* 6G14 (produced high amount of hydrogen peroxide) were selected by the sensorial evaluation [38]. Hydrogen peroxide showed an inhibitory effect on undesirable microflora which produced by several species of LAB.

At present, there are limited information about LAB and yeasts and their fermentation characteristics which have a major role in fermented food production due to enhancement efficiency and quality improvement [39-40]. Hence, technological properties of yeasts could be considered in their selection as starters. Besides that, the ability of *Saccharomyces* was reported to dominate a mixed-culture fermentation and produce sufficient ethanol for inhibition of undesirable microbes [10]. Co-culture system comprising *L. plantarum* Shanghai brewing 1.08 and *Zygosaccharomyces rouxii* CGMCC 3791 used in sauerkraut production was compared to spontaneous fermentation. Accordingly, increasing formation of various volatile compounds such as esters (one of the most important flavor components), alcohols (give pleasant aromas and sweet flavors), acids (contribute to sensory profiles), phenols provide stronger flavor for pickled radish and cabbage [6]. The addition of *L. plantarum* and *Z. rouxii* may inhibit the growth of gram-negative bacteria led to the rapid growth of nitrate reducing bacteria in sauerkraut. Nitrite content was detected at the end of the MC fermentation (2.9 mg/kg) and spontaneous fermentation (12.2 mg/kg). Thus, co-culture of LAB and yeast are capable of reducing the accumulation of biogenic amines (putrescine, cadaverine, tyramine) and nitrite concentration. Otherwise, nitrate and nitrite can be converted into hazardous substance for human health. In another study regarding MC of LAB (*L. plantarum* UFLA CH3, *P. acidilactici* UFLA BFFCX 27.1) and yeast (*Torulaspora delbrueckii* UFLA FFT2.4) as starter cultures to ferment *Cucumeropsis manni* cotyledons, combination of these cultures improved safety of final-products by lowering pH, controlling of *Enterobacteriaceae* population and producing high concentrations of organic acids and esters [41]. To improve the characteristics and safety of traditional fermented products, the combinations of mixed starter cultures have a great importance. Chen et al. (2014) investigated isolation and idendification of essential LAB species and yeasts from spontaneous fermentaion of mustard leaves. Then, using of selected LAB (*Enterococcus faecalis, L. plantarum, L. pentosus, L. buchneri*) and yeast (*Issatchenka orientalis, I. occidentalis*) species as starter cultures was evaluated and compared with spontaneous fermentation of mustard leaves. According to results indicated in Table 4, *L. plantarum* and *I. orientalis* strains exhibited optimum MC combination [36].

So far, L. plantarum and L. mesenteroides have been widely used as starter culture combination in fermentation of vegetables (Table 4). In addition, LAB (the most used ones) are applied to fermentation process as concurrently or sequential manner in MC system [12], [32]. Kimchi fermentation performed by mixed starter cultures indicated better acceptance in terms of taste preference and higher radical scavenging activity as well as anticancer effects than spontaneously fermented kimchi [32]. In white cabbage fermentation, *Leuconostoc mesenteroides* CECT 219 (LM) and *Lactobacillus plantarum* CECT 748 (LP) used as MC (1:1 ratio inoculations from bot strains) showed 12-fold higher ascorbigen (phytochemical) content and up to 2-fold higher oxygen radical absorbance capacity (ORAC) values due to the presence of myrosinase activity of starter cultures (enzymes role in ABG formation) compared to spontaneous fermentation [42].

Controlling of microbial community during fermentation is important to produce high-quality and commercial fermented products. Therefore, LAB as starter culture have been tested and used in fermentations. In addition, LAB have been isolates and developed for fermentation trials to enhance fermentation characteristics and probiotic activities due to improvement of quality and bioactivity of fermented foods [32].

While LAB produce lactic acid, some LAB strains are known to preserve foods restraining putrefactive bacteria via producing inhibitory agents (hydrogen peroxide, lactoperoxidase, diacetyl and bacteriocins). In this regard, bacteriocin-like substances production, fermentation performance and antibacterial activity against *E. coli* CICC 21525, *S. aureus* ATCC 6538 and *Listeria innocua* CICC 21633 were evaluated in MC of *L. plantarum* E11 and *L. mesenteroides* CICC 21859 [15]. Inhibition activity of paired starter cultures against pathogenic microorganisms was the highest in the fifth day of fermentation compared to Sichuan spontaneous fermentation. It is necessary to develop bacteriogenic starter culture combinations to enhance the organoleptic qualities and nutritional properties of fermented food without addition of chemical preservatives and heat treatment. In sauerkraut production, *L. sakei* and *L. mesenteroides* used as starter culture have the highest inhibitory effects on *E. coli*, *L. monocytogenes* in cabbage brine [43]. In a study related with carrot juices was reported by Farnworth (2008), LA fermentations performed by *L.*

pentosus FSC1 and *L. mesenteroides* FSC2 enhanced iron solubility in carrot juices.

Mixed starters of bacteriocinogenic *L. plantarum* L4 (most acid tolerant species) and *L. mesenteroides* LMG 7954 (produces a mild, pleasantly aromatic flavor) were applied to fermentation of sauerkraut for production of probiotic pickles with low salt concentration (4%-2.5%) [8]. Cultivation medium had antibacterial activity against *S. aureus* (3048; K-144), *E. coli* 3014, *Salmonella* enterica serovar Typhimurium FP1, *B. subtilis* ATCC 6633 and *B. cereus* TM2. Many varied factors such as shorter fermentation time (14 days), lower salt concentration (2.5%), probiotic properties of final product (≥106 CFU of probiotic strains per gram) positively influenced on product quality. Rapid acid production was also observed from beginning of fermentation. Whereas LA concentration was 8.03 g/L (2.5% NaCI) and 8.23 g/L (4% NaCI) in the end of the controlled fermentation, 4.50 g/L was observed after completion of the spontaneous fermentation. Moreover, PFGE (Pulsed Field Gel Electrophoresis) was indicated as effective method confirmation of presence of probiotic culture. Even that PFGE could be used for identification of LAB. There is a new method, genotyping could be used for identification especially probiotic properties of LAB [8].

More recently, advances in molecular technology and genetic engineering have enabled researchers to improve fermentation process. Thus, enhancement of starter culture systems has been substantially depending on screening natural isolates for character of interest.

Table 4. *Pickles made from different vegetables related with mixed culture combinations, improved features during fermentation process..*

Fermented foods	Starter Cultures Combinations	Enhanced beneficial attributes	References
Sauerkraut -Brassica oleracea-	*L. mesenteroides* NCU1426 – *L. plantarum* NCU1121	Higher viable LAB and acidification profiles; rapid lactic acid production	[51]
	L. mesenteroides – P. dextrinicus	Improvement of organoleptic properties; lower of nitrite and nitrate concentrations; rapidly increasing LAB population	[54]
	L. delbrueckii IWQ – *L. paracasei* J21	Better texture and sensory qualities; higher nitrite degradation ability; fast fermentation; high and rapid LA production	[17]
	L. pentosus – L. plantarum	Highly efficient nitrite degradation; improve the quality and flavor; shorten fermentation period; applicable for industrial production of sauerkraut	[55]
Paocai -Brassica pekinensis-	*L. plantarum* 8m-9-*L. mesenteroides* 3m-1	Suitable for industrial production; significantly lower nitrite content; effectively prevention of over-acidity and over-ripening; improving quality; rich in volatile flavor components	[56]
Kimchi	*L. curvatus* ML17 – *Saccharomyces servazzi* MY7	Increasing acidity; higher LA content; improvement of texture, flavor, sourness, carbonated flavor, savory taste, and overall acceptability; better appearance	[57]
Takanazuke -Brassica juncea-	*L. plantarum* B17-4 –*P. parvulus* C120-3	Improvements of shelf-life; lowering of salt level; highly producing LA	[58]
Fermentation of carrots, French beans and marrows	*L. plantarum* M1 – *P. pentosaceus* F4 – *L. mesenteroides* C1	Enhancement of texture, firmness, color, sensory properties; higher vitamin C concentration; producing acid rapidly; better preservation, inhibition of *Enterobacteriaceae* and yeasts	[1]
Fermentation of cabbage, carrot, beet and onion	*L. plantarum* NK-312 – *P. acidilactici* AFERM 772 – *L. mesenteroides* BLAC	Reducing production of ethanol and gas; effectively acidifying; enhancer shelf-life; accelerating fermentation; prevention of deterioration; improving organic acid composition; stabilization of end-product; prevention of spoilage up to 90 days	[22]
Sweet potato -Ipomoea batatas-	*L. plantarum* FNCC 0123 – *L. mesenteroides* FNCC 0023	Reducing of non-LAB contamination; improvement of organoleptic properties; prevention of off-flavor; rapidly increasing LA content; better sensory score for taste and aroma	[3]
Eggplant -Solanum melongena-	*L. plantarum* $3G_3$ – *L. brevis* $3G_2$ – *L. fermentum* $6G1_4$	Higher LAB population; maintenance of typical organoleptic characteristics; decreasing pectin esterase, cellulose, polygalacturonase activities; reduction of biogenic amines forming	[38]
Mustard leaves -Brassica juncea-	*L. plantarum* G-1 - *I. orientalis* J-1	Fast acidification; higher growth ability; low-pH tolerance; high concentrations of volatile aroma flavors (esters, alcohols, sulfides); identification of new alcohols and esters; lower nitrite content; provide industrial-scale production	[36]

6. Conclusion

In the current review, spontaneous fermentation, single and MC fermentation were examined demonstrating the priorities of MC applications. However, it was highlighted importance of accelerated production and improved quality and safety from technological and microbiological approaches in pickling process. Meanwhile, using of starter cultures isolated from traditional products during fermentation of vegetables has been suggested as a prevalent strategy in manufacturing pickles [36].

Mixed culture fermentation provides faster acid production from beginning of the fermentation compared to single culture and spontaneous fermentations. Other consequences are:

1) Offering low salt concentration,
2) Suppressing non-LAB contaminants,
3) Helping stabilization of end-product,
4) Providing benefits from economic aspects.

Mixed culture fermentation by LAB and yeast is a complex microbial process in which the composition of dominant microflora changes at sequential process. In the meantime, symbiotic cultivation of cultures could emphasize production of some required metabolites compared to single culture inoculation [16]. Thus, the interactions among different microorganisms' influence on product quality and food safety whereas MC provides richness for organoleptic characteristics compared to pure culture process. Furthermore, complex microbiota tolerates more variation in food fermentation system and interactions among microorganism provide substantially product functionality in the presence of using mixed starter cultures [33, 37].

Importantly, the present review demonstrates improvement of organoleptic features with the formation of various esters, acids, phenolic, alcohols and some bioactive substances by addition of starter cultures during fermentation. For all these results, MC applications seem as promising approach in industrial application and functional fermented production.

7. Future Prospects

Further research regarding mixed culture needs to be considered nutritional and microbial quality of end-product under different fermentation conditions. Hence, it could be found a remedy for many diseases such as neurological defects, anti-cancer, anti-aging, cardiovascular diseases. Further studies are required selection of the best combination of different strains for specific fermented product. There is a significant scope of further investigation of these strains to determine their mutual interaction and choose more effective ones for each pickle. Symbiosis and/or synergist effects of different cultures should be investigated in detail for further research. Moreover, the characteristics of strains regarding probiotic attributes should improve with molecular genetics. By considering MC system, combination of LAB and/or yeasts, future researches should be focus on different key aspects of pickle technology in detail.

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Conflict of Interest

No conflict of interest declared.

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