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1.INTRODUCTION

1.1 Exploitation of vegetables and fruits through lactic acid fermentation

Fruits and vegetables are essential components of the human diet, being fundamental sources of vitamins, phytosterols, dietary fibers, minerals, and phytochemicals. The scientific community strongly suggests consumption of five daily portions of fruits and vegetables to prevent hypertension, coronary heart disease, and risk of stroke. Vegetables and fruits are usually consumed fresh, minimally processed or, alternatively, cooked and pasteurized. Traditional technology options for processing of these food products may cause undesirable changes in either their physical characteristics or chemical compositions. To overcome these drawbacks, in the last decades, non-thermal novel technologies have been developed and applied to vegetables, including high-hydrostatic pressure, pulsed-electric fields, new packaging systems, and use of natural microbial compounds. Besides these technologically valid options, lactic acid fermentation still represents a simple and valuable method for obtaining safe products, with enhanced nutritional and sensory properties.

1.1.1 The lactic acid bacteria microbiota and spontaneous fermentation

Vegetables and fruits represent a suitable habitat for microorganisms, which colonize their surfaces and/or tissues. In vegetables, loads of epiphytic microflora commonly range between 5.0 and 7.0 log cfu g^{-1} (Lindow and Brandi, 2003). Each plant is associated with a specific microbiota, whose composition largely depends not only on microbial growth factors but also on farming, harvesting, and storage conditions (Di Cagno et al., 2012). A recent study has suggested that differences in the farming practices (i.e. organic vs conventional farming) can influence the abundance of specific microbial taxa on plants surface area (Leff and Fierer, 2013). Autochthonous microflora of raw vegetables is mainly represented by aerobic bacteria such as

pseudomonas, enterobacteria, corynebacteria, yeasts, and moulds. Lactic acid bacteria - being fastidious microorganisms- represent a minor fraction of the total microbiota of fruits and vegetables, accounting for less than 0.1%. *Lactobacillus plantarum* is undoubtedly the most common species isolated from foods of vegetable origin; it is a ubiquitous species with a very versatile metabolism. Members of the species *Lactobacillus plantarum* possess several technologically relevant characteristics, such as the degradation of oleuropein, the production of bacteriocins and flavouring compounds, the tolerance to salt and acidic environments or even the resistance to gastrointestinal conditions (Di Cagno et al., 2012). Under suitable conditions, raw vegetables and fruits are spontaneously fermented by lactic acid bacteria. Spontaneous fermentation typically results from the competitive activity of a variety of autochthonous and contaminating microorganisms. During fermentation, lactic and acetic acids are produced, thus leading to a drop in pH, which in turn inhibits Gram negative and sporulating bacteria. Under these conditions, lactic acid bacteria can easily dominate. To date, several lactic acid bacteria species have been isolated from naturally fermented vegetables, including *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus lactis, Lactobacillus paraplantarum, Lactobacillus hilgardii, Pediococcus cerevisiae, Leuconostoc mesenteroides*, and *Lactococcus lactis.* Besides to organic acids, lactic acid bacteria produce also H_2O_2 and bacteriocins; these compounds have an inhibitory activity towards pathogenic bacteria. Hetero-fermentative lactobacilli produce CO_2 as well; this metabolite exerts a preservative effect on foods. Regarding fermented vegetables, to date numerous available studies have been focused on the investigation of the microbiota responsible for the spontaneous fermentation of kimchi (Lee et al., 1997; Lee et al., 2002; Lee et al., 2005; Kim and Chun, 2005; Park et al., 2010; Jung et al., 2011). Other examples of spontaneously fermented vegetables, which have attracted the attention of the scientific community, are *gari* from West Africa (Oguntoyinbo et al., 2010), cauliflower from Greece (Paramithiotis et al., 2010), and eggplants from Spain (Sanchez et al., 2000).

A technique that exploits the spontaneous fermentation is back slopping. Back slopping is a common practice in household operations or in the preparation of some

traditional fermented foods (e.g., bread and fermented milks). With this technique the microbiota naturally contaminating the initial raw materials ("mother cultures") are transferred – after fermentation - to new unfermented materials. Hence, back slopping results in the dominance of the best-adapted strains. Spontaneous fermentation and back slopping undoubtedly represent cheap approaches to produce fermented foods, especially in less-developed countries (Leroy and De Vuyst, 2004). In addition, indigenous fermented foods are strongly linked to the culture and tradition of their country of origin (Guo et al., 2015). A common problem of spontaneous fermentation is that fermentation conditions are difficult to standardize and variations in product stability and quality attributes may occur. For this reason, the industrial manufacturing of fermented vegetables generally relies on the use of selected starter cultures, which ensure less variations in the dominant microorganisms (Di Cagno et al., 2009).

1.1.2 The use of starter cultures

Starter cultures can be defined as "preparations containing living microorganisms, which are deliberately added with the intention of making use of microbial metabolism" (Piasecka-Jóźwiak et al., 2013). The direct addition of selected starter cultures to raw materials in the processing of fermented foods allows a high control over the fermentation process and a full standardization of the product. The inoculation of starter cultures not only accelerates and controls the fermentation process, but it also contributes to the safety of the final products as well as to the enhancement of sensory, nutritional, and healthy traits (Wouters et al., 2013a). Starter cultures are massively exploited for the manufacturing of dairy products and in vinification. As regards vegetable products, numerous available studies investigated lactic acid fermentation of cucumbers, beans, carrots, cabbage, beets, onions, cauliflowers, and others (Piasecka-Jóźwiak et al., 2013). Concerning the use of selected starters, two alternative approaches are currently applied: autochthonous starters, being microorganisms isolated and reused on the same food matrix or allochthonous starters, being

microorganisms isolated from different raw matrices in respect with those inoculated for fermentation (Di Cagno et al., 2012).

1.1.2.1 Allochthonous starters and commercial starters

Commercial starters used to ferment fruits and vegetables are mainly represented by allochthonous starters (Di Cagno et al., 2012). In literature, there are many reports on the use of these starters. An example is the exploitation of isolates of *Lactobacillus alimentarius* (collected from a commercial preparation) and *Pediococcus pentosaceous* (collected from sour carrot strips) in conjunction with *Candida sake* for fermentation of sour cabbage (Petaja et al., 2000). All the starters assayed in this study grew well and efficaciously lowered the pH; in addition, almost all the experimental batches were dominated by pediococci. *Lactobacillus plantarum* undoubtedly represents a further promising candidate species for formulation of starter cultures destined for vegetables and fruit fermentation. In a recent investigation, the strain *Lactobacillus plantarum* IMDO 788 isolated from spontaneously fermented leek was used as a starter culture for fermentation of different vegetables (Wouters et al., 2013a). The microbial inoculation led to a rapid prevalence of the starter and to a rapid pH drop, which contrasted the growth of pathogenic and spoilage bacteria. Commercial starters were successfully used to produce sauerkraut and sauerkraut juice starting from white cabbage both in laboratory and large-scale fermentation. The results showed that combination of *Leuconostoc mesenteroides* and *Lactobacillus plantarum* was the best in term of sensory qualities (Wiander et al., 2005).

1.1.2.2 The autochthonous starters

Selection of autochthonous starter cultures is recognized as the best option for fermentation of fruits and vegetables since these starters may ensure better performance compared to allochthonous strains (Di Cagno et al., 2008a). Autochthonous bacteria are well-adapted to the food matrices used for fermentation and hence are more competitive than microorganisms collected from different sources (Ponce et al., 2008). In a past study, autochthonous mixed starters including the strains *Lactobacillus plantarum* M1, *Leuconostoc mesenteroides* C1, and *Pediococcus pentosaceous* F4 were used for fermentation of carrots, French beans, and marrows in comparison with mixed allochthonous starter strains belonging to the same species but previously isolated from fermented green olives in brine (Di Cagno et al., 2008b). Overall vegetables fermented with allochthonous starters were characterized by longer latency phases of growth and acidification in respect with autochthonous ones. Products fermented by the autochthonous starters showed better colour preservation, firmness, and other sensory properties than those produced with the allochthonous cultures (Di Cagno et al., 2008b). In a further study, tomato juices fermented with autochthonous strains maintained elevated higher values of ascorbic acid, glutathione and total antioxidant activity (TAA) than juices either inoculated with allochthonous starters or spontaneously fermented (Di Cagno et al., 2009). The aroma profiles of the final products were different too: allochthonous bacteria synthesized aldehydes (e.g. butanal, pentanal and 2,4- hexadienal), which can be associated with off-flavours while autochthonous bacteria mainly produced 3-methyl-3-butan-1-ol, 2-3- butanedione, 3-hydroxy-2-butanone, and other compounds (Di Cagno et al., 2009). Good overall quality were obtained with autochthonous starters on red and yellow peppers (Di Cagno et al., 2009), pineapple (Di Cagno et al., 2010), sweet cherry (Di Cagno et al., 2011b) and sauerkraut (Tolonen et al., 2002).

1.1.2.3 Criteria for selection of autochthonous lactic acid bacteria

The criteria for selection of candidate lactic acid bacteria starters can be divided into the following main categories:

- pro-technological criteria: growth rate, acidification rate, salt tolerance, tolerance to low pH values, tolerance to phenols, etc.;
- sensory criteria: synthesis of volatile compounds, etc.;
- nutritional criteria: increase of antioxidant activity, synthesis of exo-polysaccharides, etc.;
- safety criteria: synthesis of biogenic amines, occurrence of transferable resistance determinants, etc.

(Di Cagno et al., 2012)

Various factors are known to affect the growth of these microorganisms in food, being temperature, degree of exposure to air, presence of fermentable sugars, buffer capacity, pH, acidity, occurrence of inhibitory compounds, etc. An important metabolic trait is the ability to tolerate phenols. Studies on *Lactobacillus plantarum* demonstrated its ability to degrade gallic acid, m-coumaric acid, ferulic acid, caffeic acid, and p-coumaric acid (Rodriguez et al., 2008). Another important feature of autochthonous lactic acid bacteria in vegetables is that almost all the fermentable sugars occurring in this food matrix are depleted, thus preventing the alcoholic fermentation carried out by yeasts. Some microorganisms possess the ability to metabolize the toxic compounds of vegetables. As an example, strains of *Lactobacillus plantarum*, *Streptococcus lactic*, *Leuconostoc mesenteroides*, and *Pediococcus pentosaceus* were able to breakdown linamarin, a cyanoglycoside with a high level of toxicity, found in cassava (Giraud et al., 1993). As far as the production of hydrogen peroxide (H_2O_2) is concerned, this molecule is highly reactive and it is known to cause the degradation of antioxidant components and the loss of the product colour (Di Cagno et al., 2012).

1.1.2.4 Functional properties of autochthonous lactic acid bacteria

Today's consumers are more aware and concerned about their lifestyle than ever before. The demand for foods that promote health such as products containing probiotic cultures is tremendously increasing. Dairy products are well known sources of probiotics. However, in the last decades, nondairy probiotic products have attracted the attention of both food business operators and researches, as a consequence of the increased incidence of lactose intolerance and new lifestyles (veganism and vegetarism). Fermented vegetables and fruit can be sources and carriers of probiotic microorganisms. Probiotics are viable microorganisms, including bacteria (lactobacilli, bifidobacteria, lactococci and others) and yeasts, usually isolated from gastrointestinal tracts of humans or other animals, with a beneficial effect on the health of the host, including: (i) stimulation of the resident microbiota; (ii) alleviation of certain intolerances such as lactose intolerance; (iii) enhancement of nutrient bioavailability; (iv) and reduction of some allergies in susceptible individuals (Isolauri et al., 2001; Chiang and Pan, 2012). Probiotic strains should resist to gastric acid and bile, have the capacity to adhere to the gut epithelium and metabolize substances that are not digested by the host (Lee et al., 2011). In a recent study, the probiotic potential of autochthonous lactic acid bacteria isolated from carrots, French beans, cauliflower, celery, tomatoes, and pineapples has been investigated (Vitali et al., 2012). As a result, almost 35% of the isolates showed promising characteristics as probiotic cultures, including: (i) in vitro adhesion to Caco-2 cells; (ii) ability to use fructooligosaccharides as carbon source; (iii) in vitro inhibition of human pathogenic strains such as *Escherichia coli* K12 and *Bacillus megaterium* F6; (iv) stimulation of immune-mediators including cytokines with pro and anti-inflammatory activities.

1.1.3 Investigation into the microbial composition and dynamics of fermented vegetables

A fermentation process greatly depends on both the microbial species but also on their load (Giraffa 2004). Microbial communities are not static, but they change over time and space. These changes depend on numerous variables, including the intrinsic characteristics of the food matrix, the activities of microorganisms and the environmental conditions. Reliable identification and/or quantification of the microorganisms occurring in fermented foods is of crucial importance to control safety and quality of the final products (Temmerman et al., 2004). In the last decades, several techniques have been developed for the detection and enumeration of microorganisms. These techniques are mainly grouped into: (i) culture-dependent methods and (ii) culture-independent methods.

1.1.3.1 Culture-dependent approaches

Culture-dependent methods mainly rely on the isolation and cultivation of microorganisms. With such an approach, enumeration of microorganisms can be performed by using two alternatives being pour plate method and spread plate method. Classic plating techniques generally give an estimate of microbial group or *genera* thus for identification at species level and typing are carried out phenotypic and genotypic techniques (Giraffa 2004).

1.1.3.2 Culture-independent approaches

Monitoring the microbial population and their dynamics it is also possible without the need to subculture the microorganisms. Culture-independent techniques have been developed to overcome all the limitations and constraints of culture-dependent

approaches. If only culture-dependent techniques are applied, underestimation of the true number of strains capable of biological activity would occur. Culture-independent approaches give the possibility to detect the population in its entirety including the not culturable (VBNC), the stressed and the injured microorganisms (Botta and Cocolin 2012). These techniques allow the quantification of viable cells by: (i) evaluating their metabolic activity; (ii) measuring the presence of intact membrane; or (iii) detecting the presence of their nucleic acids (DNA, mRNA, or rRNA). *In vitro* amplification of DNA molecules by Polymerase Chain Reaction (PCR) is commonly applied to the enumeration of live bacteria, based on the assumption that DNA is degraded more rapidly after cell death than other cell components. The main disadvantage of this approach is that only "expected" microorganisms can be detected (Temmerman et al., 2004). Other molecular techniques for the enumeration of microorganisms are reverse transcriptase PCR (rt-PCR) and quantitative PCR (q-PCR), both targeting the nucleic acids.

Next generation sequencing

The new frontier in food microbiology is undoubtedly represented by high-throughput sequencing (HTS) approaches. As literally reported by Ercolini (2013) "*Culture-independent HTS analysis of microbiota is considered quantitative because the number of sequences obtained is ultimately proportional to the abundance of the microorganism in question*". To date, culture-independent HTS has been applied in a number of studies investigating the microbial ecology of fermented foods (Nam et al., 2012; Sakamoto et al., 2011; Kim et al., 2011). HTS includes 454 pyrosequencing, Illumina and SOLiD sequencing methods, which are commonly referred to as Next Generation Sequencing (NGS) methods. Main advantages and disadvantages of these new sequencing techniques are listed in table 1.

Advantages	Disadvantages
High-throughput screening of many food samples at the same time	Need for bioinformatic skills
Reliable identification of most microbes in raw materials	Overall cost
Safer bench work compared to other molecular approaches	

Table 1. Advantages and disadvantages of the use of high-throughput sequencing (HTS) to study food associated microbial ecology (Ercolini 2013).

1.2 Microbial interactions in fermented vegetables

In fermented foods as well as in other natural ecosystems, microbes are interconnected. In fermented vegetables, commensalism is the most common interaction, followed by antibiosis. An example of antibiosis in these food products is the production of organic acids and bacteriocins by lactic acid bacteria; these microbial metabolites have a detrimental effect on co-existing microbial species. A further microbial interaction, which can occasionally occur in fermented vegetables, is parasitism. An example of parasitism in vegetable fermentations is the infection of starter cultures by bacteriophages. To date, very little is known about bacteriophages, though some authors have evidenced that they can be closely correlated with bacterial successions occurring during fermentation processes (Lu et al., 2003b).

1.3 Fermented vegetables

Actually, sauerkraut, kimchi, fermented cucumbers, and table olives are the most consumed fermented vegetables worldwide, with a considerable market share in the agri-food sector (Di Cagno et al., 2016).

Sauerkraut

Sauerkraut, which is very popular in Europe and United States, is obtained from the natural lactic acid fermentation of salted and shredded cabbage. The fermentation process is initiated by *Leuconostoc mesenteroides*, which lowers the pH at such an extent that spoilage microorganisms and enzymes are inhibited (Montet et al., 2014). Homo-fermentative lactic acid bacteria such as *Lactobacillus* spp. and *Pediococcus* spp. dominate in the last stage of the fermentation process up until a final pH comprised between 3.5 and 3.8 is reached.

Pickled cucumbers

Pickled cucumbers are obtained from fully ripened cucumbers, previously washed in potable cold water and drained. Brine (usually containing 5-7 % of NaCl) exerts a selective effect on the microbiota.

Table olives

Table olives are the most important fermented vegetables on the international food market, with the European Union accounting for 70% of the total production. The

presence of oleureopein, a bitter component, and the low concentration of sugars prevent the consumption of olive fruits prior to their processing. At industrial scale, Spanish-style processing is the most diffused; it combines the treatment of olive fruits with NaOH and a fermentation step. During the spontaneous fermentation, yeasts and lactic acid bacteria compete with lactobacilli, which generally dominate the process (Botta and Cocolin, 2012).

Traditional fermented vegetables

Fermentation of vegetables is an ancient practice, which is carried out worldwide. In table 2, traditional products of fermented vegetables from different geographical areas are listed.

Name of the product	Vegetable	Country or region	References
"Almagro" eggplant	Eggplant	Spain	Sanchez et al., 2004
Brovoda	pickled turnips	Italy	Maifreni et al., 2004
Tursu	different vegetables	Turkey	Kabak and Dobson, 2011
Şalgam	black /violet carrots	Turkey	Di Cagno et al., 2012
Naw-mai-dong	pickled bamboo shoots (*Bambusa glaucescens*)	Thailand	Tanasupawat and K., 1994
Hom-dong	pickled red onions	Thailand	Tanasupawat and K.,1994
Pak-sian-dong	pak Sian (*Gynadropsis pentaphylla*)	Thailand	Montet et al., 2014
Dak-dua-dong	fermented mustard leaves	Thailand	Tanasupawat and K.,1994
Jeruk	pickled vegetables including ginger and papaya	Malaysia	Montet et al., 2014
Tempoyak	durian fruit (*Durio zibethinus*)	Malaysia	Di Cagno et al., 2016
Burong mangga	pickled green mango	Philippines	Di Cagno et al., 2016
Sinki	radish taproots	Nepal	Di Cagno et al., 2016

Gundruk	leafy vegetables such as cabbage and radish	Nepal	Di Cagno et al., 2016
Khalpi	cucumbers	Himalayana region	Di Cagno et al., 2016
Jiang-gua	cucumbers	China	Di Cagno et al., 2012
Nukamiso-zuke	vegetables fermented in rice bran, salt and water	Japan	Montet et al., 2014
Kaktugi	fermented radish	Korea	Montet et al.,2014
Dhamuoi	fermented cabbages and other vegetables	Vietnam	Montet et al., 2014
Suan-tsai and Fu-tsai	mustard	Taiwan	Chao et al., 2009

Table 2. Typical fermented vegetables manufactured worldwide.

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Innovative fermented vegetables

In the last decades, the increasing consumers' demand of healthy products has led to a dramatic increase of marketed innovative vegetable- and fruit-based fermented products (Di Cagno et al., 2016). In addition, the ever growing incidence of vegetarianism and the demand for lactose-free and low cholesterol products has led the research towards non-dairy products. In this scenario, the exploitation of lactic acid fermentation for the development of novel products obtained from vegetables and fruits has attracted both food operators and researchers. These new products include fermented fruits and vegetable juices, smoothies, minimally processed vegetables and yogurt-like products.

Fruit and vegetable juices

To date, watermelon, carrot, beetroot, tomato, pomegranate, lemon, cabbage, spinach, orange, grapes, sweet potatoes, prickly pear, orange, and grape have been exploited for manufacturing of lactic acid-fermented juices also known as "lacto-juices" (Di Cagno et al., 2016). Fermentation may be spontaneous if the process is carried out by the resident lactic acid bacteria or controlled, if a starter culture is used. In a recent study, pomegranate juices fermented with selected *Lactobacillus* strains showed enhanced functional properties, including antioxidant activity and immunomodulation (Di Cagno et al., 2016). In a further study exploiting selected *Lactobacillus* strains, fermented carrot juices showed improved sensorial properties and acidity, as well as high viscosity. Moreover, lactic acid fermentation strongly improved solubility of minerals, including iron (Bergqvist et al., 2005). Probiotication of carrot juice with *Bifidobacterium* strains was also investigated (Kun et al., 2008).

Smoothies

Smoothies consist of mixtures of fruits and vegetables processed to pulp or puree. A study carried out on white grape juice, *Aloe vera* extract, and other vegetables fermented with autochthonous starters revealed that the final products were characterised by improved antioxidant activity, texture, color, browning index, and sensory properties (Di Cagno et al., 2011a).

Minimally processed products

Minimally processed vegetables and fruits are packed soon after trimming, peeling or cutting. Examples of these novel products are fermented slices of pineapple, peppers or artichokes. Recently, Rößle and colleagues (2010) have developed an innovative

functional product from fresh-cut apple wedges inoculated with the probiotic strain *Lactobacillus rhamnosus* GG.

Fruit- and vegetable-based yogurt-like products

Fruit- and vegetable-based yogurt-like products are manufactured from "milks" mainly obtained from soy, cereals, or nuts. Grape must, cereals, soy, and two strains of *Lactobacillus plantarum* have recently been exploited for the manufacturing of vegetable yogurt-like beverages (Coda et al., 2012), while in a further study, hazelnut milk was successfully fermented with *Lactobacillus rhamnosus* and *Streptococcus thermophilus* (Di Cagno et al., 2012).

1.4 Kimchi

Kimchi is a traditional Korean food manufactured by fermenting vegetables with lactic acid bacteria. Kimchi is a generic term used to indicate salted and fermented vegetables; it includes more than one hundred preparations that differ depending on the main ingredients, geographical region, and processing conditions. In Korea, kimchi has been prepared and consumed since the 3rd or 4th century A.D. In 2013, the UNESCO recognized the cultural importance of kimchi and inscribed it in the "Representative List of The Intangible Cultural Heritage of Humanity". This product is traditionally made at home and served as side dish, eaten

alone, with rice or used to prepare several dishes such as *kimchi jjigae* (kimchi stew) or *kimchi guk* (kimchi soup).

1.4.1 Recipe

The raw materials used for the different kimchi recipes can be divided into four classes:
-(i) major raw materials: Chinese cabbage (*Brassica pekinensis*) and radish;
-(ii) spices: red and black pepper, cinnamon, garlic, ginger, onion, and mustard;
-(iii) seasonings: salt, salt-pickled seafood, corn syrup, sesame seed, and soybean sauce;
- (iv) other additional ingredients: mushrooms, carrot, leek, water cress, seafood like oyster and shrimp, cereals like barley and rice, fruits like apple and pear, meats like pork and beef etc (Patra et al., 2016).

1.4.2 Manufacturing process

Kimchi prepared with *baechu* (Chinese cabbage) is mostly appreciated and consumed in Korea. The main ingredients of *baechu* kimchi are *baechu* cabbage (Chinese cabbage), *gochu* (Korean red pepper) and many other seasoning ingredients such as green onion, garlic, ginger and fermented seafoods *jeotgal* (Park et al., 2014). The traditional preparation is quite simple. Chinese cabbage is trimmed to small pieces, thoroughly washed, and overnight brined. After brining, water in excess is drained and cabbage is mixed with the other ingredients, which should have previously been washed, graded, and cut. Hence, the mixture is placed in fermentation vessels where it is covered (pressed) with stones under anaerobic conditions. The fermentation process is strictly dependent on the temperature. Ripening takes approximately 1 week at 15°C or 3 days at 25° C; however, a better overall quality and a lower acidity are guaranteed by incubating at refrigeration temperatures (2-6 °C) (Di Cagno et al., 2016).

1.4.3 Industrial-scale production

Standardization of kimchi production process is extremely challenging. Besides the quality of raw materials and the application of optimal fermentation conditions, the choice of the starter culture is fundamental (Park et al., 2014). The shelf life of kimchi represents a serious concern because the fermentation process carried out by lactic acid bacteria continues during storage and distribution. At this regard, over ripened kimchi has no market value, being characterized by a lower nutritional quality, an extremely high acidity, and an excessive softening of vegetable tissues. The latter trait is mainly due to microorganisms, which dominate in the last stage of the fermentation process and produce polygalacturonases, such as *Bacillus subtilis, Saccharomyces fragilis, Candida pseudotropicalis, Oospora lactis, Aspergillus* sp., and *Penicillium* sp. (Cheig and Park, 1994).

1.4.4 Kimchi fermentation and its microorganisms

Many studies reported that the lactic acid bacteria involved in kimchi fermentation include *Leuconosoc mesenteroides, Leuconostoc citreum, Leuconostoc gasicomitatum, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus plantarum, Lactobacillus sakei, Lactococcus lactis, Pediococcus pentosaceus, Weisella confusa,* and *Weisella Koreensis* (Park et al., 2012). The early fermentation stage is generally dominated by *Leuconostoc mesenteroides*, while the successive stages by *Lactobacillus plantarum*. However, there may be exceptions in this microbial succession due to the absence of kimchi-associated lactic acid bacteria in the raw materials or to differences in kimchi recipes, raw materials, ingredients, and fermentation conditions (Jung et al., 2011). In some processes, *Weisella cibaria* and

Weisella confusa are predominant even during the early fermentation stage, whereas *Leuconostoc gelidum* and *Leuconostoc gasicomitatum* are dominant during the late fermentation stage (Jeong et al., 2013). Denaturing Gradient Gel Electrophoresis (DGGE) profiles of different kimchi varieties evidenced the presence of yeasts ascribed to *Lodderomyces* spp., *Trichosporon* spp., *Candida* spp., *Saccharomyces* spp., *Pichia* spp., *Sporisorium* spp., and *Kluyveromyces* spp. (Chang et al., 2008). *Saccharomyces* is responsible for consumption of sugars and production of ethanol, occurring approximately 30 days since the beginning of the fermentation process, whereas *Pichia* is often found during the late fermentation stage. Moreover, in a recent metagenomic analysis carried out on kimchi (Jung et al., 2011) large amounts of phage DNA sequences have been found, thus possibly indicating that bacteria were infected by bacteriophages during fermentation. A few other studies reported that bacteriophages can affect the microbial consortia and the overall quality of kimchi but how to eradicate this problem is still a challenge (Lu et al., 2003a; Lu et al., 2003b).

1.4.5 Health benefits of kimchi

Baechu kimchi is a probiotic food with a high nutritional value, due to its high levels of vitamins, minerals, dietary fibers, and other functional components such as capsaicin, allyl compounds, gingerol, and chlorophyll. Based on the available literature, kimchi and kimchi lactic acid bacteria functionality includes antimutagenic and anticancer, antioxidative and antiaging effects, antiobesity, and anti-atherosclerotic, as well as immunostimulatory functions. In some studies kimchi was responsible of lowering the blood glucose concentration, showing antidiabetic effects (Islam and Choi 2009; Lee et al., 2012). Consumption of 300 g/day kimchi dramatically increases *Lactobacillus* spp. and *Leuconostoc* spp. in human feces, thus demonstrating that these microorganisms can pass and colonize the human gastro intestinal tract exerting probiotic functions (Park et al., 2014). In a study carried out in 2007, some isolates from kimchi, ascribed to *Lactobacillus buchneri*, produced considerable amounts of γ-aminobutyric acid (GABA), a non-protein amino acid

known for its neuroprotective effect on neuronal cells (Cho et al., 2007). In a further investigation, autochthonous *Lactobacillus sakei* starter cultures produced high amounts of GABA in kimchi (Seok et al., 2008). Moreover, kimchi contains very low level of NO_3, NO_2, and carcinogenic nitrosamines originating from the reaction of secondary amines and NO_2. Lactic acid bacteria can metabolize almost all NO_2 decreasing the mutagenicity of toxic nitrosamines during fermentation (Park et al., 2014).

2.AIM OF THE RESEARCH WORK

The aim of this Master thesis was to conduct a preliminary investigation into the microbiota of kimchi as a potential source of autochthonous starters.

3. MATERIALS AND METHODS

3.1 Kimchi manufacturing and sampling

Two production batches of kimchi, labelled as KB1 and KB2, were prepared in May 2019 at a small artisanal producer located in Santa Maria Nuova (AN, Italy) using the ingredients listed in table 3.

Ingredients	Percentage % (g /100 g)
Chinese cabbage	60,34
Turnip	12,93
Water	11,49
Onion	3,74
chili powder	2,37
red pepper	2,01
garlic leaves	2,01
spring onion	1,44
Carrot	1,44
Ginger	0,43
Sucrose	0,72
Salt	0,65

Table 3. Raw ingredients used for the preparation of kimchi.

Steps and process parameters for the manufacturing of kimchi are reported in the flowchart shown in figure 1. Briefly, Chinese cabbage was removed from outer leaves, trimmed, and then steeped in salt [10% (w/v) NaCl] for approximately 16-18 h at room temperature. The sauce was obtained by mixing chopped vegetables (turnip, onion,

carrot, ginger, spring onion, red pepper and garlic leaves) with chili powder, water, sugar, and salt. Hence, the sauce was stored under refrigeration conditions (5°C) for approximately 16-18 h. The cabbage was rinsed, and water in excess drained. Hence, all the cabbage leaves were spread with the sauce one after another. Kimchi was fermented at approximately 5 ± 1 °C for 57 days. Kimchi was sampled immediately after preparation and after 2, 5, 15, 36, 43, 50 and 57 days of fermentation. The samples were transported to the laboratory under refrigerated conditions and processed immediately after arrival.

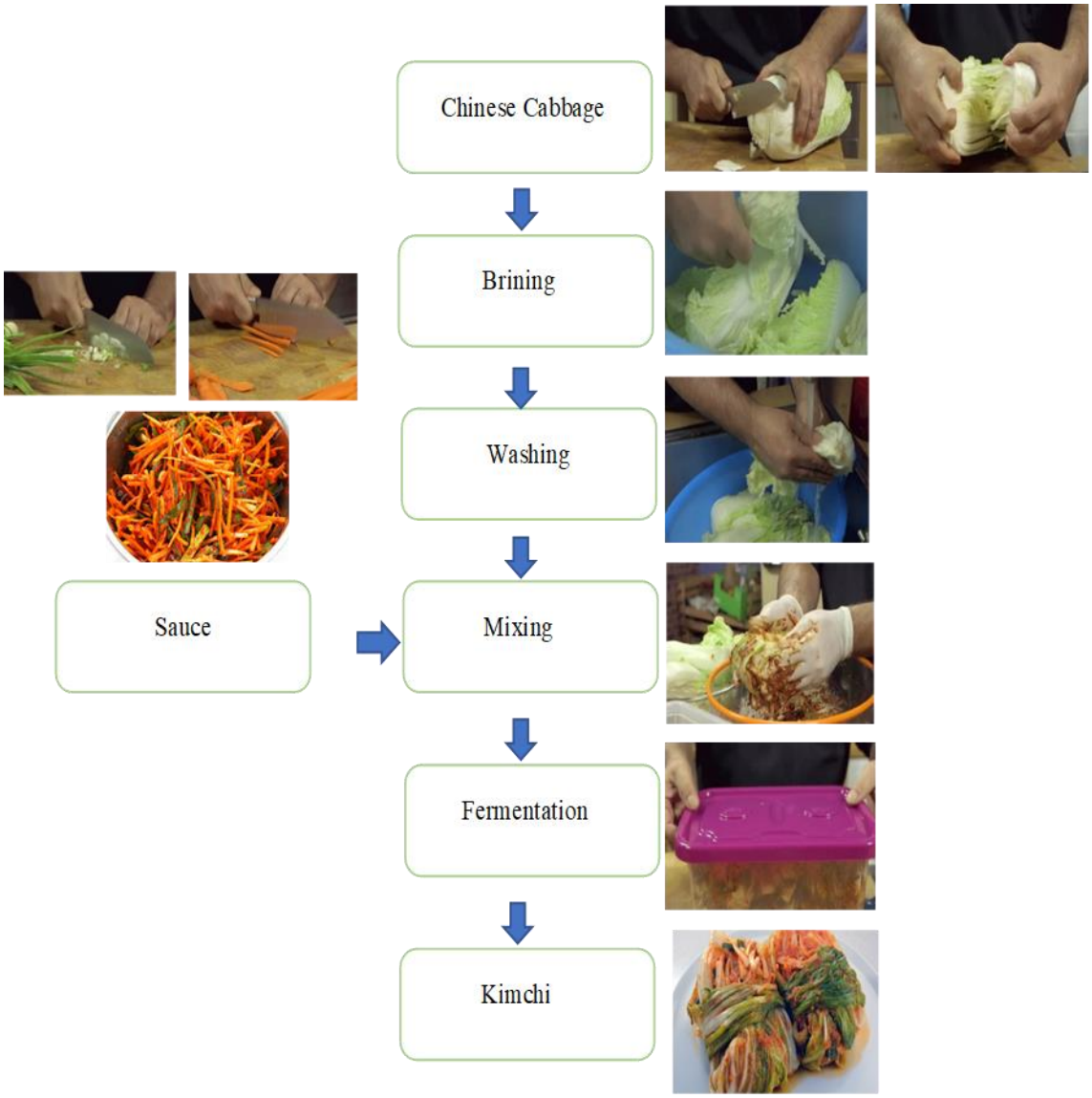

Figure 1. Kimchi manufacturing process (Park et al., 2014).

3.2 Enumeration of microorganisms

The load of different microbial groups in kimchi was determined by standard plate count procedures. An aliquot (100 g) of each kimchi sample was put in a Stomacher bag and processed with a Stomacher apparatus (400 Circulator, International PBI, Milan, Italy) for 15 min at 260 rpm to extract the juice. One mL of juice was added to 9 mL of a sterile peptone water solution (1 g/L of bacteriological peptone) and homogenized by vortexing. The obtained homogenates were further ten-fold diluted and subjected to viable counts. Pour plate method was used for the enumeration of: (i) total mesophilic aerobes on Plate Count Agar (PCA) (VWR, Radnor, Pennsylvania, USA) incubated at 30 °C for 48 h, (ii) lactobacilli on De Man Rogosa Sharpe (De Man et al., 1960) agar (VWR) incubated at 30 °C for 48h, (iii) lactococci on M17 agar (Merck KGaA, Darmstadt, Germany) incubated at 22 °C for 96 h and (iv) Enterobacteriaceae on Violet Rose Bile agar (VWR) incubated at 37 °C for 24h. MRS and M17 were supplemented with cycloheximide (VWR) (100 mg/L) to inhibit the growth of eumycetes. For the enumeration of yeasts, aliquots (0,1 mL) of ten-fold serial dilutions were inoculated by surface spreading on Rose Bengal agar (Liofilchem srl, Roseto degli Abruzzi, Italy) added with 200 mg/L of chloramphenicol (PanReac AppliChem, Barcelona, Spain) and incubated at 25 °C for 96 h. Each growth medium except Violet Rose Bile Agar was also prepared with 3% (w/v) NaCl for the enumeration of the halophilic fraction. Enumeration of *Pseudomonas* spp., *Salmonella* spp. and *Listeria monocytogenes* were carried out by Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche (Perugia, Italy). All the analyses were performed in duplicate and the results of viable counts were expressed as means of the log of colony forming units (CFU) per g of sample ± standard deviations.

3.3 pH analysis

The pH potentiometric measurements on kimchi samples were carried out with a model 300 pH meter equipped with an HI2031 solid electrode (Hanna Instruments, Padova, Italy). The analysis were performed in duplicate and mean values ± standard deviations were calculated for each sample.

3.4 Organic acids

Kimchi homogenates (10-1) were clarified and decoloured prior to the analysis of organic acids. Three solutions were prepared for clarification:

(i)Carrez I solution, containing 3.60 g of potassium hexacyanoferrate (II) {$K_4[Fe(CN)_6] \times 3H_2O$} (Sigma Aldrich, Milan, Italy) dissolved in 100 mL of distilled water; (ii) Carrez II solution containing 7.20 g of zinc sulphate ($ZnSO_4.7H_2O$) (Sigma) dissolved in 100 mL of distilled water; and (iii) sodium hydroxide solution (NaOH, 100 mM) containing 4 g of NaOH dissolved in 1 L of distilled water. Ten mL of each sample of kimchi juice was weighted in a 100 mL-graduated flask and subsequently added with 60 mL of distilled water, 5 mL of Carrez I solution, 5 mL of Carrez II solution, and 10 mL of NaOH solution (100 mM). Hence, the mixture was adjusted to 100 mL, mixed, and filtered. Clarified kimchi juice still possessed strong colour, thus it was treated with polyvinylpolypyrrolidone (VWR) (0.2 g/10 mL of sample), shooked vigorously for 5 min, and then filtered through Whatman No. 1 filter paper. Quantification of acetic acid in decoloured and clarified samples was carried out with K-ACETRM 06/18 kit (Megazyme, USA) while D-lactic acid and L-lactic acid were assayed with K-DLATE 08/18 kit (Megazyme, USA).

3.5 RNA extraction

An aliquot (2 mL) of kimchi juice used for RNA extraction was centrifuged at 14000 rpm for 5 minutes (Eppendorf Centrifuge 5415 C, Hamburg, Germany); the supernatant was discarded and RNAlater ™ Solution (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) was added to cover the pellet. The pellets were stored at -80° C until use.

Total RNA was extracted from the pellet using *Quick*-RNA™ MiniPrep kit (Zymo Research, USA) following the manufacturer's protocol with slight modifications.

1. The tubes were centrifuged for 2 min at 14000 rpm and the RNAlater ™ Solution (Thermo Fisher Scientific) was removed from cell pellets by pipetting.
2. 400 µL of RNA lysis buffer were added to lyse the sample and centrifuged for 30 sec at 14000 rpm.
3. The clear supernatant was transferred into a Spin-Away™ Filter (yellow) in a collection tube and centrifuged for 30 sec at 14000 rpm. The flow-through was saved.
4. 1 volume ethanol (95-100%) was added to the flow-through and mixed well.
5. The mixture was transferred to a Zymo-Spin™ IIICG Column (green) in a collection tube and centrifuged for 30 sec at 14000 rpm. The flow-through was discarded.

DNase I treatment:

(1) 400 µL of the RNA suspension wash buffer were added to the column and centrifuged for 30 sec at 14000 rpm.
(2) 5 µL of DNase I and 75 µL of DNA digestion buffer was mixed by inversion in an RNase free tube. The mix was added directly to the column matrix.
(3) The column was incubated at room temperature (20-30 °C) for 15 minutes. Proceeded to step 6.

6. 400 µL of RNA preparation buffer were added to the column and centrifuged for 30 sec at 14000 rpm. The flow-through was discarded.
7. 700 µL of wash buffer were added to the column and centrifuged for 30 sec at 14000 rpm. The flow-through was discarded.
8. 400 µL of RNA wash buffer were added to the column and centrifuged for 2 min at 14000 rpm to ensure complete removal of the wash buffer. The column was transferred carefully into a RNase-free tube.
9. To elute RNA, 100 µL of DNase/RNase-free water were added directly to the column and centrifuged for 30 sec at 14000 rpm.

The extracted RNA was stored at – 80 °C until use.

3.6 cDNA synthesis

Starting from the RNA extracts, cDNA was synthesized using the SensiFAST™ cDNA Synthesis Kit (Bioline, London, United Kingdom) according to the following protocol:

1. Prepare the mastermix on ice (table 4).
2. Vortex solutions and centrifuge briefly before use.

Component	Volume
Total RNA	10 µL
5x TransAmp Buffer	4 µL
Reverse Transcriptase	1 µL
DNase/RNase free-water	5 µL

Table 4.Mastermix components for the rt-PCR amplification of the V3 region of the 16S rRNAs.

3. Mix gently by pipetting.
4. Set up following program (figure 2) in thermal cycler MyCycler (Bio-Rad Laboratories, Hercules, California, USA).

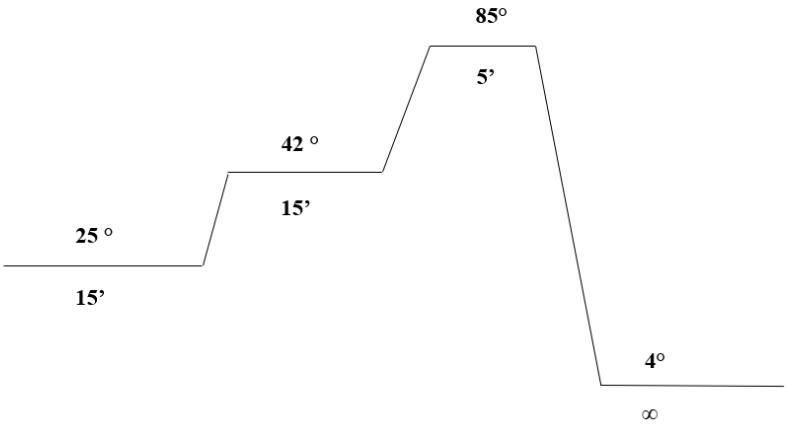

Figure 2. Program for the rt-PCR amplification of the V3 region of the 16S rRNAs.

cDNA was then stored at -20°C.

3.7 PCR

The amplification reaction was performed using 2 µL of cDNA in a total volume of 25 µL containing the mix components listed in table 5. The primer set 338f (5′-ACT CCT ACG GGA GGC AGC AGC AG-3′) and 518r (5′-ATT ACC GCG GCT GCT GG-3′) and MyFi™ mix (BioLine, London, UK) were used to amplify the V3 region of the 16S rRNA gene (Osimani et al., 2015).

Reagent	**Ci**	**Cf**	**Vi** (µL for each sample)
MyFi™ mix	10 X	1X	12,5 µL
338f	10 µM	0,2 µM	0,5 µL
518r	10 µM	0,2 µM	0,5 µL
H_2O	up to volume		

Table 5. PCR mixture composition for amplification of V3 region of the 16S rRNA gene with primer set 338f-518r.

Ci initial concentration; Cf final concentration; Vi initial volume.

The amplification reactions were performed in the thermal cycler My Cycler, Bio-Rad Laboratories, using the cycling program shown in figure 3.

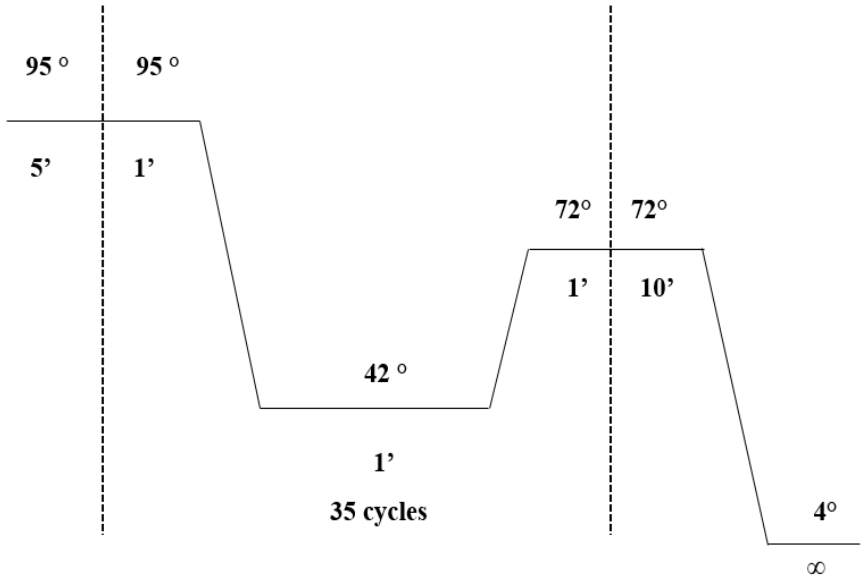

Figure 3. PCR cycling program of V3 region of the 16S rRNA gene with primer set 338f-518r.

3.2 Electrophoresis

Five microlitres of each PCR product were analysed by electrophoresis in 1.5% (w/v) agarose gel 0,5 X Tris Borate-EDTA (TBE) containing 0.5 μg/mL ethidium bromide at 100 V for 45 min, using HyperLadder™ 100bp Mix (BioLine, London, United Kingdom) as a molecular weight standard. Gels were visualised under UV light and photographed with the Complete Photo XT101 system (Explera, Jesi, Italy).

4. RESULTS AND DISCUSSION

4.1 Microbiological analyses

Viable counts of total mesophilic aerobes enumerated on Plate Count Agar (PCA) with no addition of NaCl or added with 3% (w/v) NaCl are shown in figure 4 and 5, respectively. Overall, no differences in the viable counts of this microbial group were seen between the two growth media used (with and without salt). In addition, a similar trend was seen between the two kimchi production batches analysed (KB1 and KB2), with almost stable loads during the whole fermentation period, attesting at 5 log CFU/g. In a past study carried out by Hong and colleagues (2013), notable higher loads of total mesophilic aerobes were found in kimchi, with viable counts > 8 log CFU/g after 9 days of fermentation.

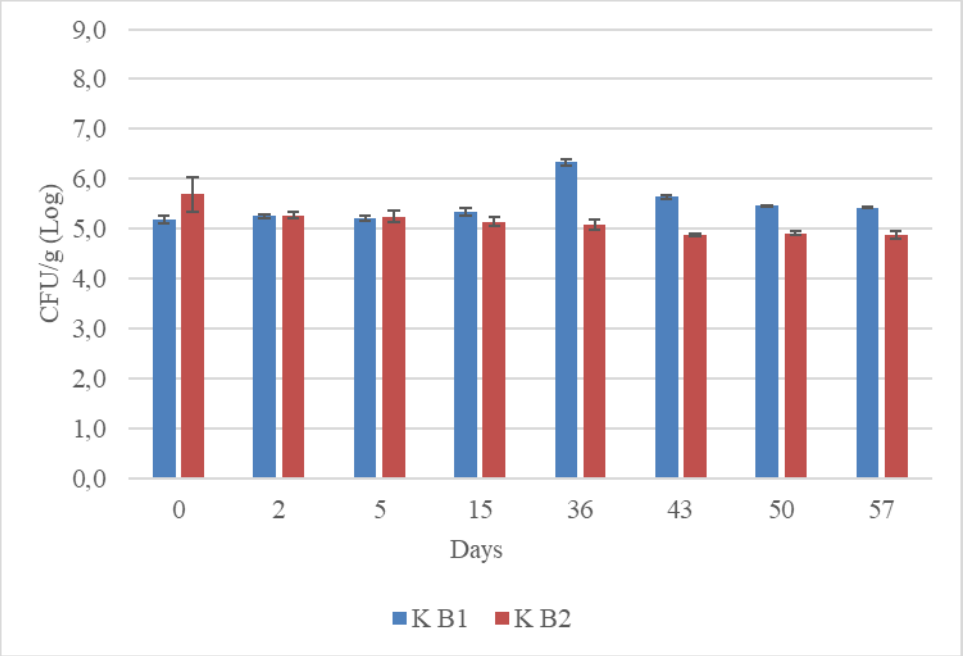

Figure 4. Viable counts of total mesophilic aerobes on Plate Count Agar (PCA). KB1 kimchi batch 1; KB2 kimchi batch 2.

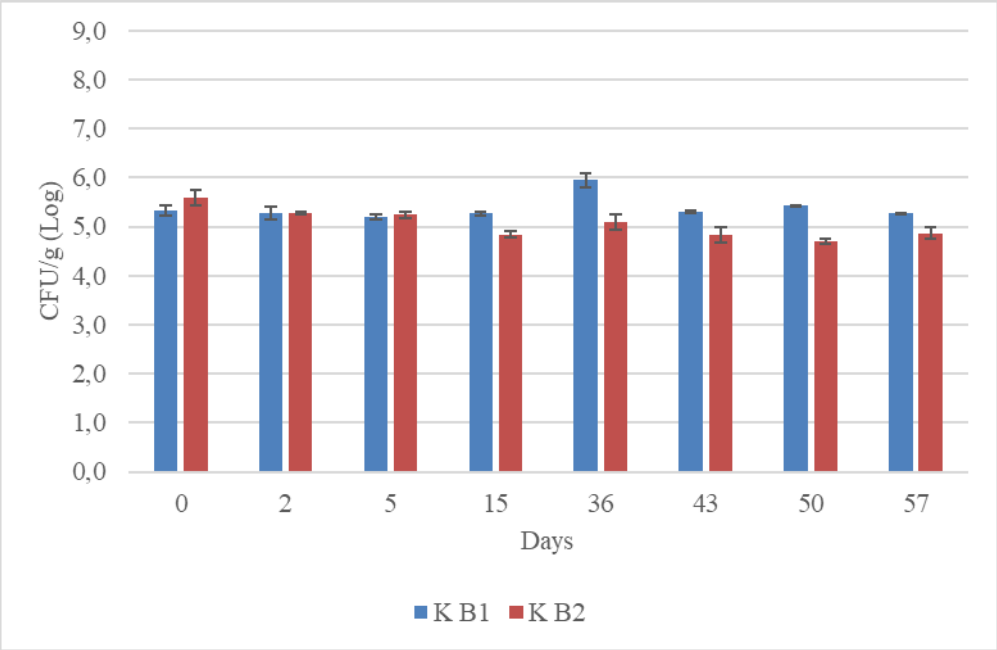

Figure 5. Viable counts of total mesophilic aerobes on Plate Count Agar added with 3% (w/v) NaCl.

KB1 kimchi batch 1; KB2 kimchi batch 2

As regards viable counts (figure 6 and figure 7), two different trends were seen in the two production batches of kimchi. This evidence might be due to differences in the microbiota composition of vegetables used as ingredients as well as to the microbial interactions established during fermentation. In KB1, the load of lactobacilli progressively increases up until day 36, when they reached a maximum mean value of 6 log CFU/g. In KB2, the load of lactobacilli remained almost stable during the early stage of fermentation (first 15 days), which was followed by a rapid increase. Markedly higher viable cell counts of lactobacilli, attesting around 9 log CFU/g were reported by Hong et al. (2013) in a previous research work dealing with the microbial dynamics of kimchi during its fermentation.

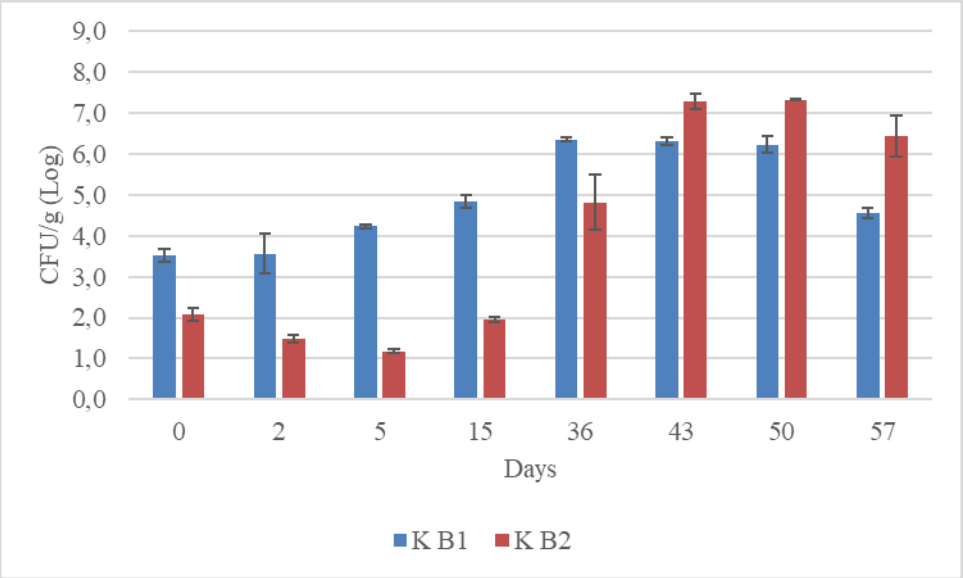

Figure 6. Viable counts of lactobacilli on De Man Rogosa Sharpe (MRS) agar.

KB1 kimchi batch 1; KB2 kimchi batch 2.

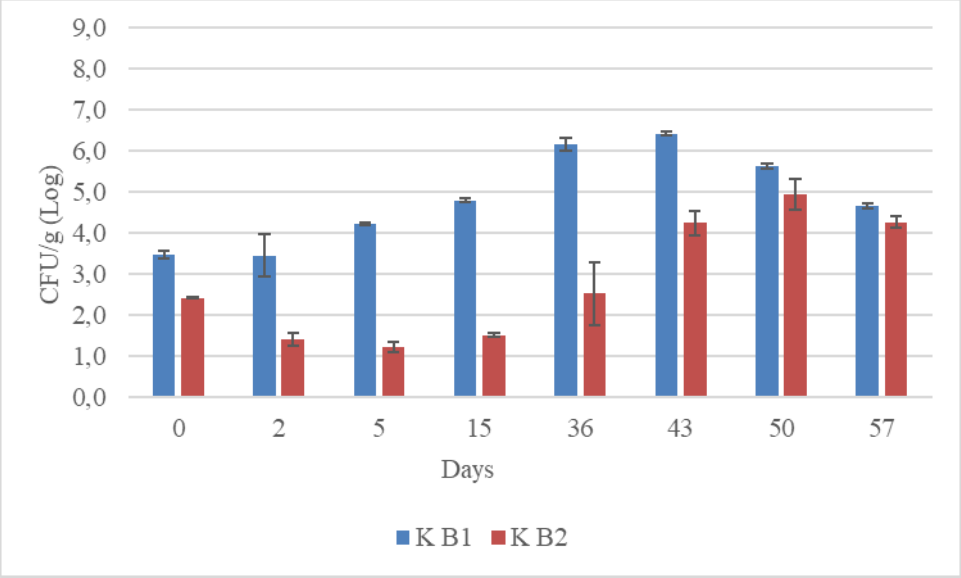

Figure 7. Viable counts of lactobacilli on De Man Rogosa Sharpe (MRS) agar added with 3% (w/v) NaCl.

KB1 kimchi batch 1; KB2 kimchi batch 2.

Viable counts of lactococci on M17 without and with 3% (w/v) NaCl addition are shown in figure 8 and 9, respectively. Overall, no differences were seen among the two production batches.

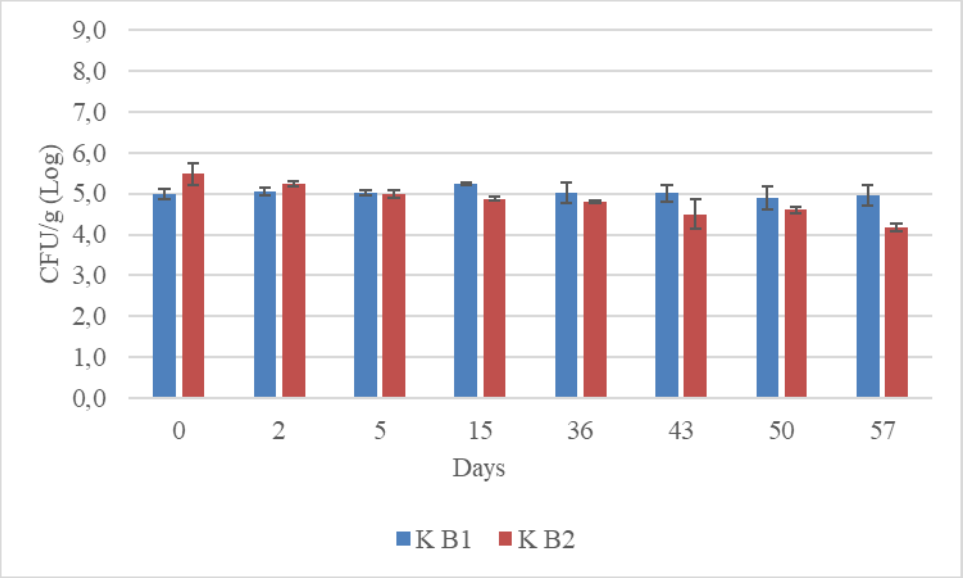

Figure 8. Viable counts of lactococci on M17.

KB1 kimchi batch 1; KB2 kimchi batch 2.

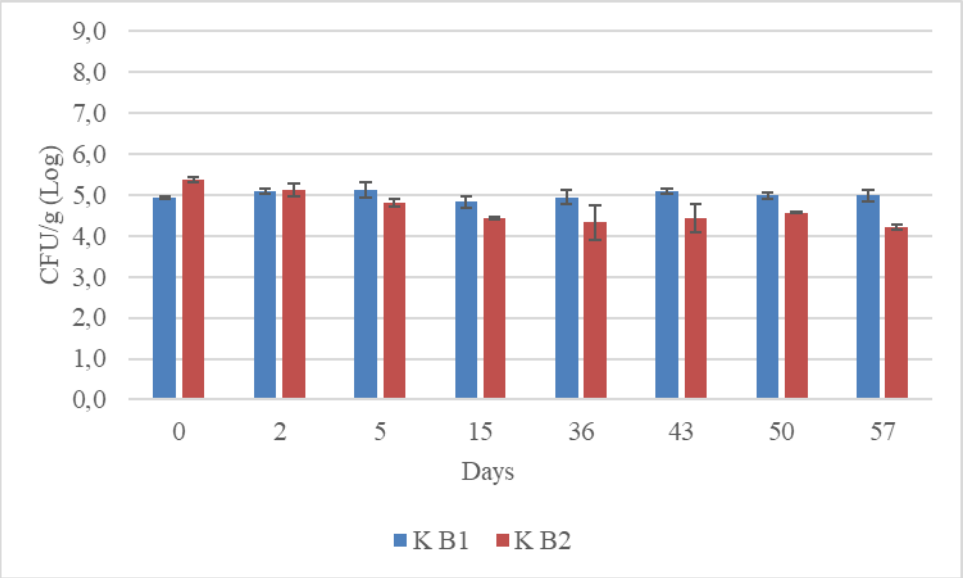

Figure 9. Viable counts of lactococci on M17 added with 3% (w/v) NaCl. KB1 kimchi batch 1; KB2 kimchi batch 2.

Microbial counts of total Enterobacteriaceae are shown in figure 10. As expected, in both production batches KB1 and KB2 a progressive decrease of this microbial group was seen, with a complete disappearance at day 36 and 50, respectively. As previously found in numerous fermented vegetables, the acidity due to accumulation of organic acids produced by lactic acid bacteria is mainly responsible for the inhibition and death of Enterobacteriaceae (Yamani et al., 1993; Tamang B. and Tamang J. P., 2010; Wouters et al., 2013; Hurtado et al., 2008). Members of the family *Enterobacteriaceae* are acknowledged as indicators of process hygiene hence, their load in foods should be carefully assessed (Osimani et al., 2018).

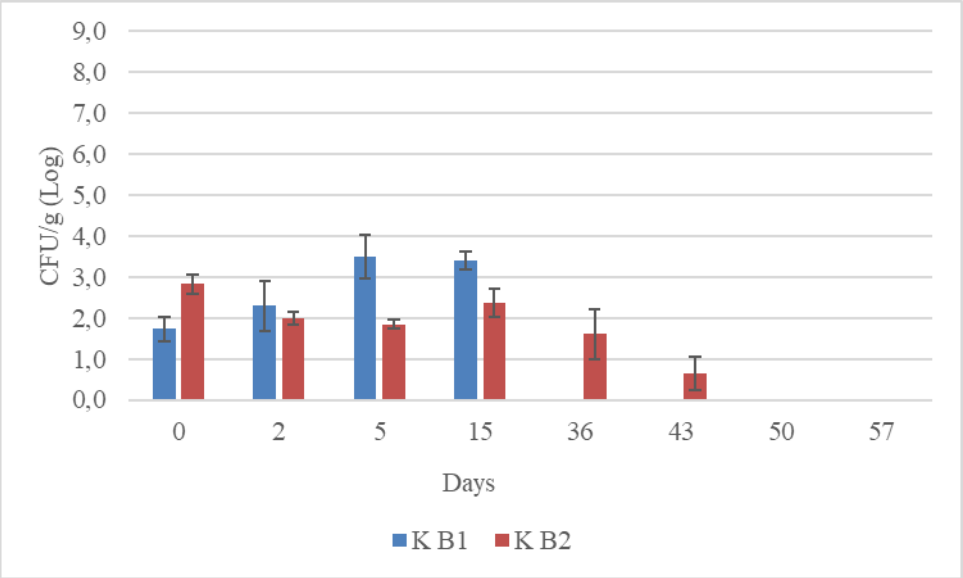

Figure 10. Viable counts of Enterobacteriaceae on Violet Rose Bile Agar (VRBGA). KB1 kimchi batch 1; KB2 kimchi batch 2.

The microbiological analyses of *Listeria monocytogenes* and *Salmonella* spp. revealed that these pathogens were absent in 25 g of each analysed sample. Viable counts of Pseudmonaceae are shown in figure 11. Members of this microbial group were detected at early stages of fermentation (from day 0 to 15). This finding agrees well with what reported by Park and colleagues (2012) about the possible dominance of pseudomonads during the first stages of kimchi fermentation.

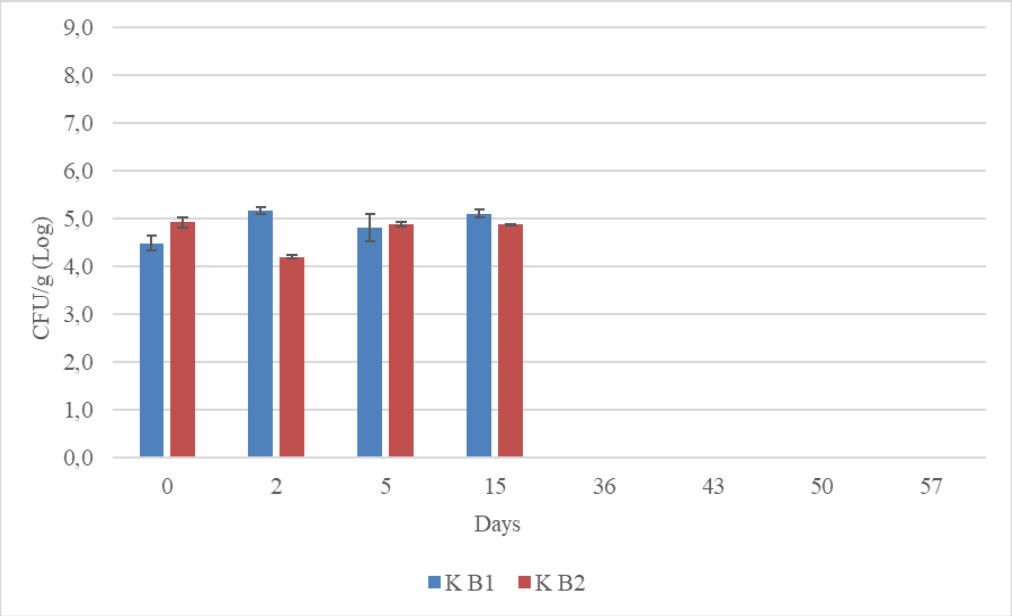

Figure 11. Pseudomonoaceae viable counts.

KB1 kimchi batch 1; KB2 kimchi batch 2.

Finally, viable counts of yeasts in Rose Bengal agar without and with 3% (w/v) NaCl. NaCl and are shown in figure 12 and 13, respectively. Again, a similar trend was shown by both production batches, with a slow but progressive decrease of these microorganisms during the fermentation process up until the end of the monitoring period (57 days). A quite different picture was drawn by Jeong and colleagues (2013), who reported an increase in the load of *Saccharomyces* spp. during fermentation of kimchi, from day 19 day up until day 45.

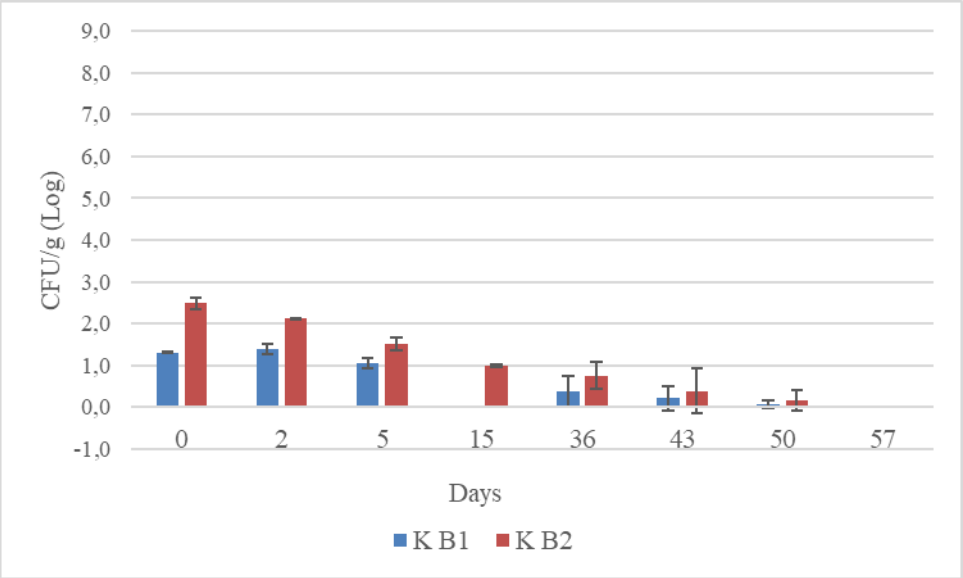

Figure 12. Viable counts of yeasts on Rose Bengal agar.

KB1 kimchi batch 1; KB2 kimchi batch 2.

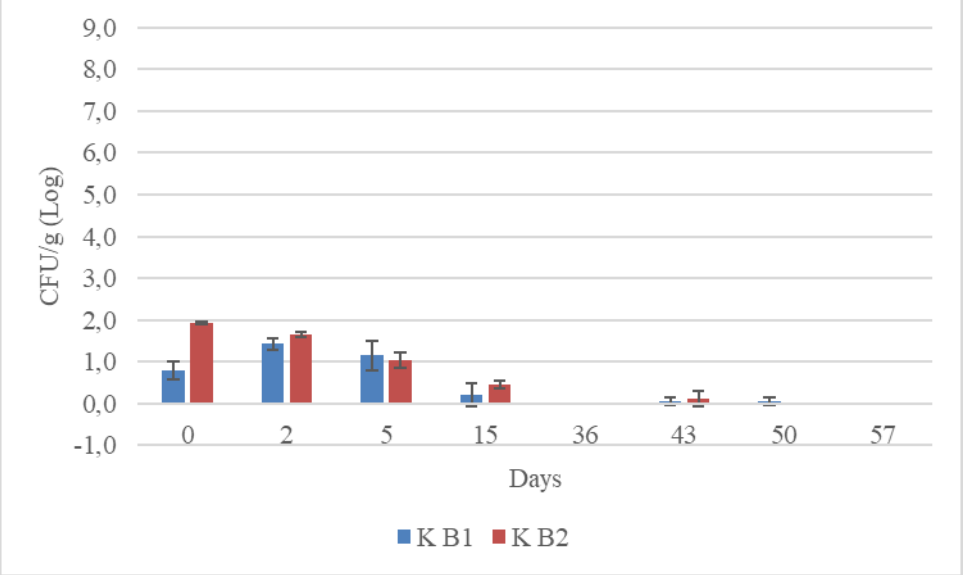

Figure 13. Viable counts of yeasts on Rose Bengal added with 3% (w/v) NaCl.

KB1 kimchi batch 1; KB2 kimchi batch 2.

3.2 pH analysis

Data of pH measurements are shown in table 6. Through the two production batches showed a similar trend, KB1 was characterized by a faster pH reduction than KB2, which agrees well with the faster increase of lactic acid bacteria load in the same batch. Overall, pH values measured in this study were in the range of those previously reported by Hong and colleagues (2016). It has previously been reported that the optimum overall quality of kimchi is achieved when the pH is around 4.2 (Mheen and Kwon, 1984; Hong et al., 2013). For KB1 and KB2, pH values around this threshold were reached at day 50 and 57, respectively. In a further study carried out by Hong and colleagues (2013) on kimchi fermented at 4°C, pH values around 4.2 were reached between 18 and 27 days of fermentation.

Time	KB1	KB2
0	5,44 ± 0,01	5,22 ± 0,06
2	5,62 ± 0,09	5,53 ± 0,18
5	5,69 ± 0,01	5,58 ± 0,02
15	5,48 ± 0,08	5,53 ± 0,01
30	5,18 ± 0,25	5,67 ± 0,04
36	4,69 ± 0,07	5,67 ± 0,04
43	4,46 ± 0,08	4,97 ± 0,19
50	4,15 ± 0,01	4,34 ± 0,17
57	3,99 ± 0,01	4,17 ± 0,01

Table 6. pH of kimchi sampled at different fermentation time.

KB1 kimchi batch 1; KB2 kimchi batch 2.

3.3 Organic acids

The results of acetic acid quantification in samples of kimchi collected at day 0, 15 and 57 are shown in table 7. As it emerges from this table, at the end of the monitoring period KB1 is characterized by a higher content of this organic acid; this finding agrees well with the low pH recorded in KB1 in respect with KB2 at the end of the fermentation period. As far as lactic acid is concerned, the results obtained are shown in table 8,9,10. A similar trend was seen for the two kimchi production batches, with a progressive increase of lactic acid during fermentation. If compared with the available literature, the content of both acetic and lactic acid found in the two production batches of kimchi were in the range of those reported by Cho and colleagues (2006).

Time	KB1	KB2
0	u.d.*	u.d.
15	u.d.	0,0002 ± 0,0001
57	0,0765 ± 0,0042	0,0495 ± 0,0011

Table 7. Acetic acid content (g/100g) of kimchi samples collected at different fermentation times.

KB1 kimchi batch 1 KB2; kimchi batch 2.; u.d. under detection limit; detection limit= $7,62 * 10^{-6}$ g/100g

Time	KB1	KB2
0	0,0002 ± 0,0001	0,0002 ± 0,0000
15	0,0002 ± 0,0001	0,0001 ± 0,0000
57	0,0585 ± 0,0014	0,0626 ± 0,0017

Table 8. D-lactic acid content (g/100g) of kimchi samples collected at different fermentation times.

KB1 kimchi batch 1; KB2 kimchi batch 2.

Time	KB1	KB2
0	0,0009 ± 0,0002	0,0011 ± 0,0001
15	0,0007 ± 0,0000	0,0009 ± 0,0001
57	0,0166 ± 0,0014	0,0145 ± 0,0006

Table 9. L- lactic acid content (g/100g) of kimchi samples collected at different fermentation times.

KB1 kimchi batch 1; KB2 kimchi batch 2.

Time	KB1	KB2
0	0,0011 ± 0,0002	0,0013 ± 0,0001
15	0,0009 ± 0,0001	0,0011 ± 0,0001
57	0,0751 ± 0,0023	0,0771 ± 0,0023

Table 10. Total lactic acid content (g/100g) of kimchi samples collected at different fermentation time.

KB1 kimchi batch 1; KB2 kimchi batch 2.

3.4 cDNA detection

The results of the rt-PCR amplification with 338f and 518r of the DNA extracts from kimchi sampled during its fermentation are shown in figure 14. Amplification of the V3 region of the 16S rRNA gene confirmed the presence of cDNA; the latter has been sent to DISAFA (University of Turin) for Illumina sequencing aimed at the disclosure of the bacterial microbiota.

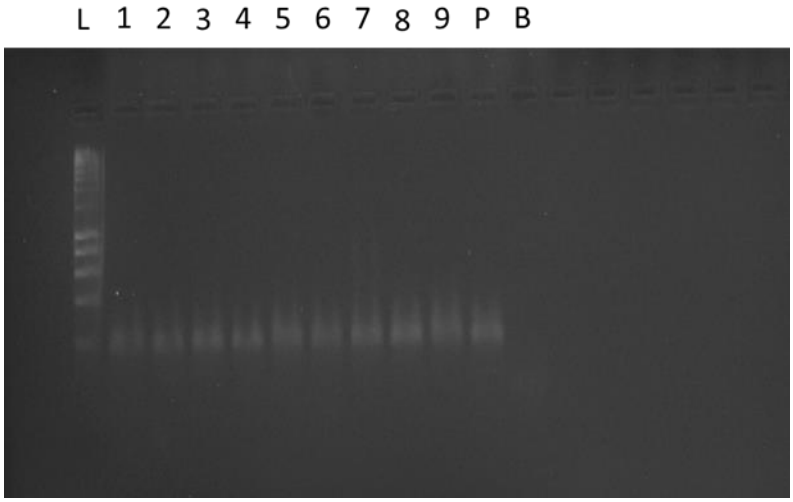

Figure 14. Amplification of V3 region of the 16S rRNA with 338f and 518r.

(L): ladder, (1): KB1 day 0, (2): KB1 day 15, (3): KB1 day 36, (4): KB1 day 57, (5): KB2 day 0, (6): KB2 day 5, (7): KB2 day 36, (8): KB2 day 57, (9) KB2 day 15, (P): positive, (B): blank.

5. CONCLUSIONS

Since kimchi is fermented by naturally occurring lactic acid bacteria mainly originating from the raw materials, the quality of the final products varies between different recipes and production batches. For this reason, the use of starter culture has been suggested as a possible solution to standardize kimchi production. However, it has been demonstrated that the starter cultures, and especially commercial selected strains, cannot be enough competitive against the natural microbiota associated to raw vegetables or they are not always well adapted to kimchi fermentation conditions such as low temperature, salts, and low pH. For these reasons it is very important to accurately investigate kimchi fermentation microbial dynamics. The aim of this Master thesis was to conduct a preliminary investigation into the microbiota of kimchi as a potential source of autochthonous starters.

The study showed that cultivation methods and chemical analyses can give a significant insight into the microbial populations and their dynamics during kimchi fermentation. Results obtained evidenced that the increases in the total number of viable cells of lactic acid bacteria were positively associated with the increase in the levels of fermentation products such as lactic acid and acetic acid. Moreover, results obtained in this study support the consideration that predominant species in kimchi can be cultured on MRS medium as stated by Cho et al. (2006). Monitoring the growth kinetics during the fermentation process gave also information on the hygienic quality of the product. Concluding, the data presented in this study may be combined with culture-independent techniques to draw a clear overview of the microbial population starting and guiding the fermentation process of kimchi produced at a local semi-industrial plant. The information overall collected could be exploited in the next future for the design of autochthonous starter cultures.

6. REFERENCES

- Bergqvist S.W., Sandberg A.S., Carlsson N.G., Andlid T., (2005). Improved iron solubility in carrot juice fermented by homo- and hetero-fermentative lactic acid bacteria. Food Microbiol ;22:53–61.

- Botta C., Cocolin L., (2012). Microbial dynamics and biodiversity in table olive fermentation: culture-dependent and -independent approaches. Front Microbiol 3: 245.

- Chang H.W., Kim K.H., Nam Y.D., Roh S.W., Kim M.S., Jeon C.O., Oh H.M. and Bae J.-W., (2008). Analysis of yeast and archaeal population dynamics in kimchi using denaturing gradient gel electrophoresis. Int. J. Food Microbiol., 126: 159–166.

- Chao S. H., Wu R. J., Watanabe K. and Tsai Y. C., (2009). Diversity of lactic acid bacteria in *suan-tsai* and *fu-tsai*, traditional fermented mustard products of Taiwan. Int. J. Food Microbiol. 135, 203–210.

- Cheigh, H.S., Park, K.Y., (1994). Biochemical, microbiological and nutritional aspect of kimchi. Critical Reviews in Food Scienceand Nutrition 34, 175–203.

- Chiang S.S., Pan T.M., (2012). Beneficial effects of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products. Appl. Microbiol. Biotechnol. 93, 903-916.

- Cho J., Lee D., Yang C., Jeon J., Kim J., Han H. (2006). Microbial population dynamics of kimchi, a fermented cabbage product. FEMS Microbiol Lett 257:262–267.

- Cho Y.R., Chang J.Y., Chang H.C., (2007). Production of c-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from kimchi and its neuroprotective effect on neuronal cells. J Microbiol Biotechnol; 17:104–109.

- Coda R., Lanera A., Trani A., Gobbetti M., Di Cagno R., (2012). Yogurt-like beverage made of a mixture of cereals, soy and grape must: microbiology, texture, nutritional and sensory properties. International Journal of Food Microbiology 155, 120-127.

- De Man J.C., Rogosa M., and Sharpe M.E., (1960). A medium for the cultivation of lactobacilli. J. Appl. Bact. 23, 130–135.

- Di Cagno R., Cardinali G., Minervini G., Antonielli L., Rizzello C.G., Ricciuti P., Gobbetti M., (2010). Taxonomic structure of the yeasts and lactic acid bacteria

microbiota of pineapple (*Ananas comosus* L. Merr.) and use of autochthonous starters for minimally processing. Food Microbiology 27, 381-389.

- Di Cagno R., Minervini G., Rizzello C.G., De Angelis M., Gobbetti M., (2011a). Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. Food Microbiology 28, 1062-1071.

- Di Cagno R., Minervini G., Rizzello C.G., Lovino R., Servili M., Taticchi A., Urbani S., Gobbetti M., (2011b). Exploitation of sweet cherry (*Prunus avium* L.) puree added of stem infusion through fermentation by selected autochthonous lactic acid bacteria. Food Microbiology 28, 900-909.

- Di Cagno R., Surico R.F., Minervini G., De Angelis M., Rizzello C.G., Gobbetti M., (2009). Use of autochthonous starters to ferment red and yellow peppers (*Capsicum annum* L.) to be stored at room temperature. International Journal of Food Microbiology 130, 108-116.

- Di Cagno R., Surico R.F., Paradiso A., De Angelis M., Salmon J.-C., Buchin S., De Gara L., Gobbetti M., (2008a). Effect of autochthonous lactic acid bacteria starters on health-promoting and sensory properties of tomato juices. International Journal of Food Microbiology 128, 473-483.

- Di Cagno R., Surico R.F., Siragusa S., De Angelis M., Paradiso A., Minervini F., De Gara L., Gobbetti M., (2008b). Selection and use of autochthonous mixed starter for lactic acid fermentation of carrots, French beans or marrows. International Journal of Food Microbiology 127, 220-228.

- Di Cagno, Coda R., De Angelis M., Gobbetti M., (2012). Exploitation of vegetables and fruits through lactic acid fermentation, Food Microbiology. Fresh Fruits and Vegetables.

- Di Cagno, Filannino P., and Gobbetti M., (2016). Fermented Foods: Fermented Vegetables and Other Products. Volume 2: Che-Foo Elsevier 668-674.

- Ercolini D., (2013). High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. Appl Environ Microbiol 79: 3148–3155.

- Giraffa G., (2004). Studying the dynamics of microbial populations during food fermentation – a review. FEMS Microbiol Rev 28: 251–260.

- Giraud E., Gosselin L., Raimbault M., (1993). Production of a *Lactobacillus plantarum* starter with linamarase and amylase activities for cassava fermentation. J. Sci. Food Agric. 62, 77–82.

- Guo C.F., Zhang S, Yuan Y.H. et al., (2015). Comparison of lactobacilli isolated from Chinese *suan-tsai* and *koumiss* for their probiotic and functional properties. J Funct Foods 12:294–302.

- Hong S.P., Lee E.J., Kim Y.H., Ahn D.U., (2016) Effect of fermentation temperature on the volatile composition of Kimchi. J Food Sci.;81: 2623–2629.

- Hong, Y., Yang H. S., Chang, H. C., Kim, H. Y., (2013). Comparison of bacterial community changes in fermenting kimchi at two different temperatures using a denaturing gradient gel electrophoresis analysis. J. Microbiol. Biotechnol. 23, 76–84.

- Hurtado A., Reguant C., Esteve-Zarzoso B., Bordons A., and Rozès N., (2008). Microbial population dynamics during the processing of *Arbequina* table olives. Food Res. Int. 41, 738–744.

- Islam M.S., Choi H., (2009). Antidiabetic effect of Korean traditional *baechu* (Chinese cabbage) kimchi in a type 2 diabetes model of rats. J Med Food; 12:292–297.

- Isolauri E., (2001). Probiotics in the prevention and treatment of allergic disease. Pediatr. Allergy. 12, 56-59.

- Jeong SH, Lee SH, Jung JY, Choi EJ, Jeon CO.(2013) Microbial succession and metabolite changes during long-term storage of Kimchi. J Food Sci. ,78 (5): M763-769.

- Jung J.Y., Lee S.H., Kim J.M., Park M.S., Bae J., Hahn Y., Madsen E.L., Jeon C.O., (2011). Metagenomic analysis of kimchi, a traditional Korean fermented food. Appl Environ Microbiol 77:2264–2274.

- Kabak B., Dobson A.D.W., (2011). An introduction to the traditional fermented foods and beverages of Turkey. Critical Reviews in Food Science and Nutrition 51, 248-260.

- Kim M., Chun J., (2005). Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. International Journal of Food Microbiology 103, 91-96.

- Kim Y.S., Kim M.C., Kwon S.W., Kim S.J., Park I.C., Ka J.O., Weon H.Y., (2011). Analyses of bacterial communities in *meju*, a Korean traditional fermented soybean bricks, by cultivation-based and pyrosequencing methods. J. Microbiol. 49-340.

- Kun S., Rezessy-Szabo J.M., Nguyen Q.D., Hoschke A., (2008). Changes of microbial population and some components in carrot juice during fermentation with selected *Bifidobacterium* strains. Process Biochem 43: 816–821.

- Lee H., Yoon H., Ji Y., Kim H., Park H., Lee J., (2011). Functional properties of *Lactobacillus* strains from kimchi. International Journal of Food Microbiology 145, 155-161.

- Lee J., Lee K.C., Ahn J.-S., Mheen T.-I., Pyun Y.-R., Park Y.-H., (2002). *Weissella koreensis* sp. *nov*., isolated from kimchi. International Journal of Systematic and Evolutionary Microbiology 52, 1257-1261.

- Lee J.S., Heo G.Y., Lee J.W. et al., (2005). Analysis of kimchi microflora using denaturing gradient gel electrophoresis. Int J Food Microbiol 102: 143–150.

- Lee J.S., Lee J.S., Chang O.C., Min-Chul J., Woo-Sik K., Hong-Joong K., Martin H., Sam-Bong K., Chan-Sun P., Jong-Seog A., Yong-Ha P., Tae-Ick M., (1997). Classification of isolates originating from kimchi using carbon-source utilization patterns. Journal of Microbiology and Biotechnology 7, 68-74.

- Lee S.Y., Song Y.O., Han E.S., Han J.S., (2012). Comparative study on dietary habits, food intakes, and serum lipid levels according to kimchi consumption in college students. J Korean Soc Food Sci Nutr; 41:351–361.

- Leff J.W., Fierer N., (2013). Bacterial communities associated with the surfaces of fresh fruits and vegetables. PLoS ONE

- Leroy F., De Vuyst L., (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends in Food Science & Technology 15, 67-78.

- Lindow S.E., Brandi M.T., (2003). Mini review: microbiology of the phyllosphere, Enviromental Microbiology 69,35- 37 of cassava linamarin by lactic acid bacteria Biotechnol. Lett. 14, 593-598.

- Lu Z., Breidt F., Plengvidhya V., Fleming H.P., (2003b). Bacteriophage ecology in commercial sauerkraut fermentations. Appl. Environ. Microbiol. 69:3192–202.

- Lu Z., Breidt F.,Fleming H.P., Altermann P., and Klaenhammer T.R., (2003a). Isolation and characterization of a *Lactobacillus plantarum* bacteriophage, FJL-1, from a cucumber fermentation. Int. J. Food Microbiol. 84:225-235.

- Maifreni M., Marino M. and Conte L., (2004). Lactic acid fermentation of *Brassica rapa*: chemical and microbial evaluation of a typical Italian product(*brovada*). Eur Food Res Technol 218:469–473.

- Montet, Ray R. C., Zakhia-Rozis N., (2014). Lactic acid fermentation of vegetables and fruits. Microorganisms and Fermentation of Traditional Foods, R. C. Ray and D. Montet, Eds.,pp.108–140.

- Nam Y.D., Yi S.H., Lim S.I., (2012). Bacterial diversity of *cheonggukjang*, a traditional Korean fermented food, analyzed by barcoded pyrosequencing.Food Control 28:135–142.

- Oguntoyinbo F. A., Dodd C. E. R., (2010). Bacterial dynamics during the spontaneous fermentation of cassava dough in *gari* production. Food Control 21, 306–312.

- Osimani A., Garofalo C., Aquilanti L., Milanoviæ V., and Clementi F., (2015). Unpasteurised commercial *boza* as a source of microbial diversity. Int. J. Food Microbiol. 194C, 62–70.

- Osimani A., Milanović V., Cardinali F., Garofalo C., Clementi F., Pasquini M., et al., (2018) The bacterial biota of laboratory-reared edible mealworms (*Tenebrio molitor L.*): from feed to frass. Int J Food Microbiol. ; 272: 49–60.

- Paramithiotis S., Hondrodimou O.L., Drosinos E.H., (2010). Development of the microbial community during spontaneous cauliflower fermentation. Food Res Int 45:1098–1103.

- Park E., Chun J., Cha C., Park W., Ok C., Bae J., (2012). Bacterial community analysis during fermentation of ten representative kinds of kimchi with barcoded pyrosequencing. Food Microbiol. 30, 197–204.

- Park J.M., Shin J.H., Lee D.W., Song, J.C., Suh H. Joo, Chang Un-Jae, Kim J.M., (2010). Identification of the lactic acid bacteria in kimchi according to initial and over-ripened fermentation using PCR and 16S rRNA gene sequence analysis. Food Science and Biotechnology 19, 541-546.

- Park K. Y., Jeong J. K., Lee Y. E., and Daily J. W., (2014). Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. J. Med. Foods 17, 6–20.

- Patra J.K., Das G., Paramithiotis S., et al., (2016). Kimchi and other widely consumed traditional fermented foods of Korea: a review. Front Microbiol 7, 1493.

- Petäjä E. and Myllyniemi P., (2000). Use of inoculated lactic acid bacteria in fermenting sour cabbage. Agricultural and Food Science in Finland 9, 37–48.

- Piasecka-Jóźwiak K., Rozmierska J., Chablowska B., Stecka K.M., Skapska S., Kliszcz M., Szkudzińska-Rzeszowiak S., (2013). Starter cultures for lactic acid fermentation of sweet pepper, pattypan squash and tomatoes. Pol J Food Nutr Sci 63:95–102.

- Ponce A.G., Moreira M.R., del Valle C.E., Roura S.I., (2008). Preliminary characterization of bacteriocin-like substances from lactic acid bacteria isolated from organic leafy vegetables. LWT-Food Sci Technol 41:432–441.

- Rodríguez H., Landete J.M., de las Rivas B., Muñoz R., (2008). Metabolism of food phenolic acids by *Lactobacillus plantarum* CECT 748T. Food Chemistry 107, 1393-1398.

- Rößle C., Brunton N., Gormley R. T., Ross P. R., Butler, F., (2010). Development of potentially symbiotic fresh-cut apple slices. Journal of Functional Foods, 2, 245–254.

- Sakamoto N., Tanaka S., Sonomoto K., Nakayama J., (2011). 16S rRNA pyrosequencing-based investigation of the bacterial community in *nukadoko*, a pickling bed of fermented rice bran. Int. J. Food Microbiol., 144, 352-359.

- Sánchez I., Palop L., Ballesteros C., (2000). Biochemical characterization of lactic acid bacteria isolated from spontaneous fermentation of "*Almagro*" eggplants. International Journal of Food Microbiology 59, 9-17.

- Sanchez, Sesena S. and Palop L. L.,(2004). “Polyphasic study of the genetic diversity of lactobacilli associated with ’*Almagro*’ eggplants spontaneous fermentation, based on combined numerical analysis of randomly amplified polymorphic DNA and pulsed-field gel electrophoresis pattern” Journal of Applied Microbiology,vol.97,no.2,pp.446–458.

- Seok J.H., Park K.B., Kim Y.H., Bae M.O., Lee M.K., Oh S.H., (2008). Production and characterization of kimchi with enhanced levels of c-aminobutyric acid. Food Sci Biotechnol; 17:940–946.

- Tamang, B. and Tamang, J. P., (2010). In situ fermentation dynamics during production of *gundruk* and *khalpi*, ethnic fermented vegetables products of the Himalayas. Indian J. Microbiol. 50, 93–98.

- Tanasupawat, S.,Komagata. K., (1994). Lactic acid bacteria in fermented foods in Thailand. World Journal of Microbiology and Biotechnology 11: 253–256.

- Temmerman R., Huys G., Swings J., (2004). Identification of lactic acid bacteria: culture-dependent and culture-independent methods. Trends Food Sci. Technol.15, 348–359.

- Tolonen M., Taipale M., Viander B., Pihlava J.-M., Korhonen H., Ryhänen E-L., (2002). Plant-derived biomolecules in fermented cabbage. Journal of Agricultural and Food Chemistry 50, 6798–6803 Washington, DC, pp. 595–609.

- Vitali B., Minervini G., Rizzello C.G., Spisni E., Maccaferri S., Brigidi P., Gobbetti M., Di Cagno R., (2012). Novel probiotic candidates for humans isolated from raw fruits and vegetables. Food Microbiology 31, 116-125.

- Wiander B., Ryhänan, E.L., (2005). Laboratory and large-scale fermentation of white cabbage into sauerkraut and sauerkraut juice by using starters in combination with mineral salt with low NaCl content. European Food Research and Technology 220, 191-195.

- Wouters D., Grosu-Tudor S., Zamfir M. and De Vuyst, L., (2013b). Bacterial community dynamics, lactic acid bacteria species divesity and metabolite kinetics of traditional Romanian vegetable fermentations. J. Sci. Food Agric. 93, 749–760.

- Wouters D., Grosu-Tudor S., Zamfir M., De Vuyst L., (2013a). Applicability of *Lactobacillus plantarum* IMDO 788 as a starter culture to control vegetable fermentations. J. Sci. Food Agric., 93: 3352-3361.

- Yamani Mi., (1993). Fermentation of brined tunrnip roots using *Lactobacillus plantarum* and *Leuconostoc mesenteroides* starter cultures. World Journal of Microbiology and Biotechnology. 9:176–179.