

The Differences in *Lactobacillus spp.* Between Traditional and Industrial Vegetable Pickles

Geleneksel ve Endüstriyel Sebze Turşuları Arasındaki Lactobacillus Spp. Farklılıkları

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ABSTRACT

Aim: This study investigates the content of Lactobacillus acidophilus, Levilactobacillus brevis and Lactiplantibacillus plantarum in traditional and industrially prepared cabbage and cucumber pickles.

Material and Method: Pickle samples were categorized according to the industrial (n=20) and traditional (n=38) production methods. The chemical compositions, including salt contents and pH of the pickles, were evaluated. The salt content of the pickles produced by the traditional method was recorded based on the manufacturer's declaration. Label information was assessed in industrially produced pickles. pH measurements were made using a desktop pH meter. Microbial load, including Lactobacillus acidophilus, Levilactobacillus brevis and Lactiplantibacillus plantarum counts of the pickles was carried out in a Real-time PCR device (RotorGene-Q, Germany) using the Diagen Real-time PCR Kit. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) program version 22.0.

Results: The pH values of the pickles produced by the traditional method had higher pH values than the industrial products (p <0.05). Conventional and industrial pickles had similar salt content except for the industrial cabbage pickles (3.16 g/100 g, p=0.002). Lactobacillus acidophilus and Levilactobacillus brevis contents of traditional cucumber pickles (4.25±0.88 and 5.55±1.37 log₁₀ cfu/g, respectively) were found to be significantly higher than those of industrial cabbage pickles (3.33±0.55 and 1.53±2.11 log₁₀ cfu/g, respectively, p <0.05).

Conclusion: This study's results, which found higher Lactobacillus spp. content in traditionally produced pickles than industrial ones, provide preliminary data for future studies investigating the bacterial community patterns and the proportions of predominant bacteria in pickles, which are fermented products consumed frequently in Türkiye.

ÖZET

Amaç: Bu çalışmanın amacı geleneksel ve endüstriyel olarak hazırlanan lahana ve salatalık turşularında Lactobacillus acidophilus, Levilactobacillus brevis ve Lactiplantibacillus plantarum içeriğini araştırmaktır.

Materyal ve Metot: Turşu örnekleri endüstriyel (n=20) ve geleneksel (n=38) üretim yöntemine göre sınıflandırılmıştır. Turşuların tuz içerikleri ve pH derecesi dâhil olmak üzere kimyasal bileşimleri değerlendirilmiştir. Geleneksel yöntemle üretilen turşuların tuz içerikleri üretici beyanı esas alınarak kayıt altına alınmış endüstriyel olarak üretilen turşularda ise etiket bilgileri değerlendirilmiştir. PH ölçümleri masaüstü pH metre kullanılarak yapılmıştır. Turşuların Lactobacillus acidophilus, Levilactobacillus brevis ve Lactiplantibacillus plantarum içeriği, Diagen Real-time PCR Kiti kullanılarak bir Realtime PCR cihazında (RotorGene-Q, Almanya) gerçekleştirilmiştir. İstatistiksel analiz IBM Sosyal Bilimlerde İstatistik Paket Programı (SPSS) sürüm 22.0 kullanılarak yapılmıştır.

Bulgular: Geleneksel yöntemle üretilen turşular, endüstriyel ürünlere göre daha yüksek pH değerlerine sahiptir (p<0,05). Geleneksel ve endüstriyel turşular, endüstriyel lahana turşuları dışında benzer tuz içeriğine sahipti (3,16 g/100 g, p=0,002). Geleneksel salatalık turşularının Lactobacillus acidophilus ve Levilactobacillus brevis içerikleri (sırasıyla 4,25±0,88 ve 5,55±1,37 log10 kob/g) endüstriyel lahana turşularına göre (sırasıyla 3,33±0,55 ve 1,53±2,11 log10 kob/g) önemli ölçüde yüksek bulunmuştur (p <0,05).

Sonuç: Geleneksel olarak üretilen turşularda Lactobacillus spp içeriğinin endüstriyel olanlara göre daha yüksek olduğu tespit edilmiştir. Bu çalışma Türkiye'de sıklıkla tüketilen fermente ürünler arasında yer alan turşularda bulunan bakteri türlerinin, baskın bakteri oranlarının araştırıldığı ilerideki çalışmalara ön veri sağlayacaktır.

Anahtar kelimeler: *endüstriyel turşu; geleneksel turşu; salatalık; lahana*

Key words: *industrial pickles; traditional pickles; cucumber; cabbage*

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Pickling is one of the oldest historical methods of preserving various foods, including fruits, vegetables, meat, and fish. Pickling imparts unique and desirable changes in texture, color and flavor that take place over time in fermented pickles. In pickling food products, microorganisms (primarily Micrococcaceae, lactic acid bacteria, yeasts, Bacilli, and filamentous fungi) significantly impact the end product's quality and safety¹. It has been revealed that especially *Lactobacillus* species are effective in the pickling process, which is widely applied to various fruits and vegetables today, and that these bacteria convert hexoses such as glucose and lactose to lactic acid and subsequently to acetic acid and also produce different metabolites including bacteriocins and exopolysaccharides, the positive effects of pickles on health are also investigated^{$1-3$}.

Pickle consumption is widespread in Türkiye, both as a method of preserving vegetables and traditionally in food consumption. Fermented pickles have often been homemade products manufactured by spontaneous fermentation in the traditional way, but they are still changing to solve difficulties with quality, safety, and mass production. Almost all investigations revealed that the dominant genus was Lactobacillus or Leuconostoc, regardless of the pickling duration, even though the microbiota in each variety of pickle is not the same⁴.

In the studies carried out on most preferred pickles, cucumber and cabbage, the lactic acid bacteria found in the highest count as a result of the fermentation process5–6 *Leuconostoc mesenteroides, Lactobacillus brevis, Enterococcus faecalis, Pediococcus pentosaceus, Lactobacillus pentosus, and Lactobacillus plantarum* are Lactic acid bacteria (LAB) that predominate in the medium during pickle fermentation. *L. plantarum* is the bacterium that completes fruit and vegetable fermentation because it has a higher acid tolerance than other LABs. The starter cultures of *L. plantarum and L. mesenteroides*⁷ impact the quality of sauerkraut in low-salt conditions.

The fermentation stage is essential to pickle-making, with its health effects and unique characteristic flavor properties. However, there may be differences in the other steps applied in the pickling process. When traditionally produced, pickles are fermented by adding salt at 2–5% concentrations and sometimes acids such as vinegar or lemon juice; when commercially produced various preservatives such as potassium sorbate (E202) and sodium benzoate (E211) or acidity regulators such as acetic acid (E260) and citric acid (E330) can be added or pasteurization can be applied after fermentation to extend the storage period¹. It has been shown that these additional processes, mainly used in some commercially produced pickles, affect the lactic acid bacteria content and that lactic acid bacteria are more dominant in traditionally produced pickles⁸. Certain species of lactic acid bacteria, such as *Lactiplantibacillus plantarum* and *Levilactobacillus brevis*, are mainly reported to be dominant in traditionally produced pickles, according to their tolerance levels of salt and acid $concentrations⁸⁻⁹$.

Fermentation-produced lac improves the product's storage capacity by lowering the brine's pH, inhibiting the growth of acid-sensitive microorganisms. Highsalinity brine is also used to enhance storability in the preparation of salt stock. Few halotolerant microorganisms can survive in brines with salinities up to 20%; therefore, salt stock preparation preserves vegetables rather than aiming for fermentation1. Pickling procedures remain crucial and are of great interest to scholars worldwide.

This study aimed to clarify the changes in microbial composition and concentrations of *Lactiplantibacillus plantarum*, *Levilactobacillus brevis* and *Lactobacillus acidophilus* by focusing on the processes method and salt stock preparation that have previously been overlooked. The results obtained from this study will help explain the effects of salinity and other pickling preservatives or natural additives like vinegar or lemon juice on the microbiota by comparing the results from the different processing conditions. This may provide valuable knowledge for improving pickle production.

Materials and Methods

Sample Collection

This study randomly obtained samples from significant retailers that produce industrially and traditionally produce pickles sold in Ankara, the capital of Türkiye. Within the scope of the study, 58 pickle samples (22 traditional cucumber pickles, 16 traditional cabbage pickles, 10 industrial cucumber pickles, and 10 industrial cabbage pickles) were collected by the researchers themselves (Fig. 1). While canned samples were presented in original containers without cooling, fresh samples were taken in their purchase containers

Figure 1. The distinctions between industrial and traditionally produced pickles in the study.

and transported to a laboratory in an insulated ice box $(4\pm2^{\circ}C)$ within two hours of collection. Upon arrival, all samples underwent quick biochemical and microbiological analysis. The categorization of traditional and industrially produced pickles was made as in Fig. 1. The study did not include pickles that have been pasteurized in industrial pickles. In the study, pickles evaluated within the scope of industrial pickles that contain preservatives and acidity regulators, while pickles produced by the traditional method, which do not contain preservatives and contain only lemon juice and/or vinegar in addition to salt, were evaluated.

DNA Extraction in Samples

The QuickGene (DNA extraction kit from tissue) extraction equipment was utilized for the extraction technique. Firstly, $250 \mu l$ of MDT (Tissue Lysis) solution and 50 mg of pickle samples were added to the homogenization tube. To homogenize, 15 mg of 0.1 mmø glass beads or 10 1.0 mmø zirconia beads were added to the tube. 2×120 seconds of application was made at 5000 rpm in the homogenizer. Twenty-five µl EDT (Proteinase K) solution was added after the sample had been homogenized, and it was incubated

at 56°C for 60 minutes. Then, it was centrifuged at 5000 g for 5 minutes at room temperature. After centrifugation, 200 µl of supernatant was transferred to a 1.5 mL microtube. The microtube was filled with 180 µl of LDT (Cell Lysis) solution and vortexed for 15 seconds before being incubated at 70°C for 10 minutes. The following step involved adding 240 µl of 99% cold ethanol and vortexing it for 15 seconds. The QuickGene (Kurabo)¹⁰ filtered cassette was filled with the complete contents of the microtube, and the instrument protocol was followed for performing washes and elutions. Three washes were performed using 750 µl of WDT (wash buffer) solution. As a result of the extraction process, genomic DNA diluted with 50 µl CDT (elution buffer) was obtained.

Determination of Lactiplantibacillus plantarum, Levilactobacillus brevis and Lactobacillus acidophilus counts by real-time PCR

Diagen Real-time PCR Kit was used for the analysis of *Lactobacillus acidophilus, Lactiplantibacillus plantarum* and *Levilactobacillus brevis* (catalog numbers were 3010–100, 3019–100, and 3017–100, respectively). The manufacturer's procedure carried out test

steps. The analyses were completed by performing the polymerase activation, denaturing and bonding/ extension stages, respectively, and the changes in the time and temperatures to be applied depending on the Lactobacillus species to be analyzed. Studies were performed in a Real-time PCR device (RotorGene-Q, Germany) using 0.2 microcentrifuge tubes. As a result of the real-time PCR process, the device determined how many copies were in the samples by correlating the samples used as the standard according to the obtained ct values. The PCR reactions were conducted using the following program: 5 min of polymerase activation at 95°C, 10 s for denaturation at 95°C, 40 cycles of 30 s at 95°C, 30 s for elongation at 50°C and 60°C. PCR reactions were performed in 20 μL mixture containing five μL of *L. acidophilus* specific oligonucleotide mix, *L. plantarum* specific oligonucleotide mix, *L. brevis* specific oligonucleotide mix, ten μL of qPCR master mix (2×), five μL of Sample/Positive Control/Negative Control.

Chemical Composition

The pH was measured using a WTW Inolab pH 720 Desktop pH meter. The salt contents of traditional pickle samples were obtained by questioning the producers. Label information was used to record the salt content of industrial pickle samples.

Statistical Analysis

The microbial content of the pickles was expressed as cfu/g, and subsequently, log_{10} transformed before analysis. Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS) program version 22.0 (IBM Inc., Chicago, IL, USA). Data were expressed as the means \pm standard deviation. The parameters, including Lactobacillus counts, pH values, and salt contents, were not normally distributed, so the Kruskal-Wallis tests were conducted to compare these parameters. The significance of pairwise differences was examined using the Mann-Whitney U test with the Bonferroni correction to account for multiple comparisons. The level of type-I error used to determine statistical significance was 5% overall¹¹.

Results

The microbiological and physicochemical properties of the pickles are shown in Table 1. The pH values of the pickles produced by the traditional method had higher pH values than the industrially produced alternatives ($p \le 0.05$). In addition, the mean pH value of conventional cucumber pickles was statistically significantly higher than that of traditional cabbage pickles $(3.66$ and 3.49 , respectively; $p \le 0.05$), and a similar result was also obtained in industrial pickles, but the result in industrial pickles was not statistically significant. When the salt content of pickles was examined, traditional and industrial pickles had similar salt content except for industrial cabbage pickles. Industrial cabbage pickles had the lowest salt concentration, with a salt content of 3.16 g/100 g ($p=0.002$).

In traditional and industrial cucumber and cabbage pickles, *Lactiplantibacillus plantarum* was the most abundant species analyzed. The preparation techniques of the pickles (traditional or industrial) and types of the pickles (cucumber and cabbage) affected the microbial load pH characteristic of the pickles. The highest *Lactobacillus acidophilus* and *Levilactobacillus brevis* counts were observed in traditional cucumber $(4.25\pm0.8 \text{ and } 5.55\pm1.37 \text{ log}_{10}$ cfu/g, respectively) and traditional cabbage pickles $(4.78\pm0.50$ and 6.16 ± 1.37 \log_{10} cfu/g, respectively; p <0.05). Although there is no statistically significant difference between industrial production and traditional production in the counts of *Lactiplantibacillus plantarum* in both cucumber and cabbage, the mean *Lactiplantibacillus plantarum* counts of pickles made with conventional production are higher ($p > 0.05$). *Lactobacillus acidophilus* and *Levilactobacillus brevis*

Table 1. Microbial load and physicochemical properties of pickles canned traditional and industrial

Preparation technique	Types of pickles	N	рH	Salt (q/100 q)	Lactobacillus acidophilus $(log_{10} c f u/g)$	Levilactobacillus brevis $(log_{10} c f u/g)$	Lactiplantibacillus plantarum $(log_{10} c f u/g)$
Traditional	Cucumber	22	$3.66 \pm 0.20a$	$3.77 \pm 0.16a$	4.25 ± 0.88 a.b	$5.55 \pm 1.37a$.b	$7.91 \pm 0.86^{a,b}$
	Cabbage	16	3.49 ± 0.15 b	$3.77 \pm 0.15a$	$4.78 \pm 0.50a$	$6.16 \pm 1.37a$	7.98 ± 0.61 ^a
Industrial	Cucumber	10	3.34 ± 0.28 b.c	$3.74 \pm 0.09a$	3.43 ± 0.43 b.c	1.23 ± 2.56 b.c	$5.95 \pm 0.65^{\circ}$
	Cabbage	10	$3.17 \pm 0.18c$	$3.16 \pm 0.34b$	$3.33 \pm 0.55c$	$1.53 \pm 2.11c$	$7.10 \pm 1.31^{a,b}$
			$\bm{p} = 0.000$	$p = 0.002$	$p = 0.002$	$p = 0.000$	$p = 0.002$

Values are the mean (±standard deviation) of pickle samples. a-c means sharing different superscripts in each column significantly differs at $p < 0.05$. cfu means colony-forming unit. N: number of samples

Figure 2. Quantitation data for Cycling A. Green by quantitative PCR of pickle samples. Color lines represent the same samples indicated in Fig. 1, from the left to the right.

contents of traditional cucumber pickles (4.25±0.88 and 5.55 \pm 1.37 log₁₀ cfu/g, respectively) were found to be significantly higher than those of industrial cabbage pickles $(3.33\pm0.55 \text{ and } 1.53\pm2.11 \text{ log}_{10} \text{ cftu/g},$ respectively, $p \leq 0.05$). All three strains of bacteria analyzed in traditional cabbage were statistically significantly higher than in industrial cucumbers and cabbage ($p < 0.05$).

The results of real-time PCR demonstrated that the samples reached the amplification threshold

beginning after cycle 13 for *Lactobacillus acidophilus*, cycle 8 for *Levilactobacillus brevis*, and cycle 16 for *Lactiplantibacillus plantarum* (Fig. 2). This finding could be due to the high quantity of specific DNA in pickle samples.

In Fig. 3, the varying ratios of *Lactobacillus acidophilus*, *Levilactobacillus brevis* and *Lactiplantibacillus plantarum* counts, respectively, from the inside to the outside in the rings, were presented according to the pickle and preparation techniques expressed in different colors.

As shown in Fig. 3, Lactiplantibacillus plantarum, expressed by the outermost ring, had the highest relative abundance in traditional cucumber pickles. In contrast, *Levilactobacillus brevis* (middle ring) and *Lactobacillus acidophilus* (innermost ring) had the highest relative abundance in traditional cabbage pickles. It is schematized that industrial cucumber pickles (purple color) contained all three bacterial species in minor relative abundance compared to the other pickle types.

Discussion

This study aimed to investigate and compare the bacterial communities of traditional and industrial production of cucumber and cabbage pickles. Pickles are an integral part of the diet, and various pickles are produced using different methods from different vegetables worldwide. Cucumber and cabbage pickles are most popular and frequently consumed in Türkiye and many countries around the world $12-13$. This study determined that traditional pickles had a higher microbial content than industrial pickles, and *Lactiplantibacillus plantarum* was the most abundant species analyzed.

Traditional fermented foods like cucumber and cabbage pickles are stored at room temperature and are abundant sources of LAB strains with unprecedented antimicrobial activity and probiotic characteristics¹⁴. Lactic acid bacteria are known to control serum cholesterol levels and also exhibit antiviral, antimutagenic, and antiplatelet aggregation attributes. The pickling process also helps effectively preserve and restore the natural bioactive compounds and antioxidant capacities of fruits and vegetables, which contain pigments such as anthocyanins, flavonoids, carotenoids, etc.¹⁵

With the changing pH, while the amount of some microorganisms decreases, other ones become more dominant¹⁶. Most *L. plantarum* and *L. brevis* strains showed more robust resistance to low pH17 than other LAB strains. Within the scope of the study*, L. brevis, L. blantarum and Lactobacillus acidophilus* species were evaluated in traditional and industrial pickles of cabbage and cucumber, and it was determined that all species were dominant, especially *L. plantarum* species that were resistant to low pH. In a study to identify the LABs of vegetable pickles, *Leu. mesenteroides, P. pentosaceus, L. brevis and L. plantarum* have been determined as the most frequently isolated species¹⁸. The study conducted by Boricha19 revealed that Lactobacillus strains isolated from pickles showed growth at pH three at the level of 6.2–7.7 log cfu/

mL. In the study by Tokatlı et al. 17, the isolates obtained from pickle samples produced from different vegetables were examined regarding probiotic properties. As a result, it was stated that *P. ethanolidurans, L. plantarum,* and *L. brevis* remained viable at pH 2.5, and especially *L. brevis and L. plantarum* species could maintain their viability better at pH 2.5¹⁷. The average pH values of the pickles analyzed within the scope of this study were determined as 3.66 and 3.34 in cucumber pickles and 3.49 and 3.17 in cabbage. Similarly*, L. plantarum and L. previs* were dominant at low pH levels (Table 1).

It has been observed that the appropriate salt concentration for LAB growth is $2.5-10\%$ NaCl²⁰. The number of LAB cultures decreases with high salt concentration. LAB counts increase within 24 hours during fermentation when the salt concentration is below 5%21, with salt's effect at the later fermentation stage, *Leu. mesenteroides, P. pentosaceus and L. plantarum* species become dominant¹⁷. In our study, salt content was determined to be 3.77 (g/100) in traditional cabbage and cucumber pickles, 3.16 (g/100) in industrial cabbage, and 3.74 (g/100) in cucumber pickles. The low salt concentration in pickles provides an opportunity for microbial diversity and load of strain.

The fermentation process of cabbage pickles is dominated by *L. mesenteroides*, which is salt tolerant. The acidic environment created by the growth of this microorganism not only inhibits the non-lactic competitive flora but also favors the development of other LABs. Thus, it is reported that with the decrease in the *Leuconostoc* population, other LABs, such as L. brevis and L. plantarum, become dominant²². Similarly, in our study, *L. brevis* and *L. plantarum* strains were the dominant species in all types of pickles. Ragul et al.²³ followed the development of LAB isolated from traditional brine pickles in the presence of 0.5% and 1% bile salt. According to this research, at 0.5% bile salt, none of the isolates' survival rates changed, and the isolates showed much greater tolerance to 1% bile salt than the reference strain. In a study conducted by Rao et al. 24 in China, *L. plantarum* strains were isolated from the traditionally produced pickle, and it was determined that the isolates survived in the presence of different bile salts.

It is stated that *L. plantarum* can be used as a probiotic starter for industrial pickle fermentation, and the pickled product may be utilized as a potential anti-Candida probiotic 24 . Considering the possible effects

of *L. plantarum*, which was determined as the dominant strain in our study, the beneficial effects of traditional pickles can be mentioned. Pickle manufacturers typically use non-iodized salt for fermenting pickles. It is hypothesized that iodine prevents fermentation by preventing the growth of LAB²⁵. Traditional pickles produced in Türkiye mainly utilize non-iodized table salt to ferment cucumbers and cabbage; therefore, traditional pickles may have higher LAB content. In addition, the amount of salt in pickles has been reduced with the Sodium/Salt Reduction Guide, prepared by the Ministry of Health to minimize salt consumption²⁶.

Although fungus and bacteria contribute to the microbial variety in pickles, most studies on the microbial community of pickles have concentrated on bacteria, mainly lactic acid bacteria $(LAB)^{27}$. The conditions for processing and storage differ in some ways between commercial and traditional pickle production. Traditional pickles are susceptible to various contaminants during manufacturing and storage, such as pathogens, pests, or cross-contamination, whereas industrial pickles are standardized with stringent control. Therefore, the traditional pickles made with household production methods also have richer microbial diversity²⁸. Similarly, a richer microbial diversity was observed in our study, and *L. acidophilus*, *L. brevis*, and *L. plantarum* levels in traditional pickles were higher in both cucumber and cabbage pickles than in industrial pickles. The type of dominant species in naturally fermented vegetable pickles is slightly higher than in inoculation fermentation. However, the microbial diversity in naturally fermented vegetable pickles was more enriched than inoculated fermentation²⁹.

Food safety is increasing as a significant public health problem. Antibiotics are commonly used to reduce the harm caused by microbial contamination. As a result of excessive use of antibiotics, multidrug-resistant pathogens occurred. Therefore, finding favorable biological preservatives that are promising alternative antibiotics30 is essential. Fermentation by LAB is the best way of preserving and retaining natural ingredients while improving taste, aroma, and quality 31 . In addition to their probiotic functions, LAB can enhance food flavor and nutritional value by generating aromatic compounds 32. A study conducted by Çetin⁵ revealed that adding *L. plantarum* as a starter culture into the pickles improved the taste and beneficial properties of the product. According to another study conducted by Al-Shaw33, adding *L. acidophilus* as a starter culture

into the pickles improved taste, flavor and odor. In this study, *L. plantarum* and *L. acidophilus* species in cucumber and cabbage pickles were higher in traditional pickles than in industrial pickles. Traditional homemade production is preferred instead of industrial output to obtain pickles' desired taste and flavor. The fact that conventional pickles are still produced in large quantities and stand out with their unique ingredients, production methods, and unique taste and flavor may be related to LAB contents.

A significant strength of the research is the analysis of a large sample of 58 different pickles. However, there are several significant limitations in this study. First, the process of making pickles could not be questioned in detail. Salt content, pH value, and fungal diversity in fermented pickles were necessary; no research has been done on these. This needs to be measured in future studies.

Conclusion

Pickle quality is affected by microbial diversity and the number of core microorganisms. Although a wide variety of microflora is involved in traditional and industrial vegetable pickles, traditional pickles have higher microbial content than industrial ones. This study result provides preliminary data for future studies investigating the microbiota of pickles. There are several health benefits thanks to the microorganisms contained in the pickled products. LABs found in pickled foods have been significantly associated with various probiotic properties, such as enhanced natural resistance to infectious disease in the gastrointestinal tract, prevention of urogenital infections, suppression of cancer, and improved digestion. It will be helpful in the food industry to develop pickles products by seriously following the process of traditional pickles, such as the raw material, production, storage and fermentation. This would help select specific microorganisms and create a standardized fermentation process to provide better microbial diversity and produce the characteristic flavor of industrial pickles. Further research is needed to understand the traditional community succession process of the core strains and how to develop the microbial diversity and flavor.

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Compliance with ethics requirements

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