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Microbial communities and main features of labneh Ambaris, a traditional Lebanese fermented goat milk product

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ABSTRACT

Labneh Ambaris is a traditional Lebanese dairy product typically made using goat milk in special earthenware jars. Its production is characterized by the regular additions of milk and coarse salt, all while draining the whey throughout a process that lasts for a minimum of 2 mo. In this study, 20 samples of labneh Ambaris, all produced by spontaneous fermentation, were studied. They were collected at the end of fermentation from different regions in Lebanon. Physicochemical and sensory properties were studied and microbial diversity was analyzed using culture-dependent and independent techniques. The V3–V4 region of the 16S rRNA gene and the ITS2 region were sequenced by DNA metabarcoding analyses for the identification of bacteria and yeast communities, respectively. Out of 160 bacterial and 36 fungal taxa, 117 different bacterial species and 24 fungal species were identified among all labneh Ambaris samples studied. The remaining ones were multi-affiliated and could not be identified at the species level. *Lactobacillus* was the dominant bacterial genus, followed by *Lentilactobacillus*, *Lactiplantibacillus*, *Lacticaseibacillus*, and *Lactococcus* genera, whereas *Geotrichum* and *Pichia* were the dominant fungal genera. The 20 samples tested had varying levels of salt, protein, and fat contents, but they were all highly acidic (mostly having a pH < 4). According to the sensory scores generated by classical descriptive analysis, all samples were described as having basic similar characteristics such as goat smell and flavor, but they could be differentiated based on various intensities within the same descriptors like salty and acidic. This work could be considered as a base toward obtaining a quality label for labneh Ambaris.

Key words: spontaneous fermentation, raw goat milk, microbial diversity, metabarcoding, sensory analyses

INTRODUCTION

Labneh Ambaris is a traditional Lebanese dairy product that has been made in rural areas for hundreds of years. From a socio-economic point of view, this product is important in its production regions where it represents cultural heritage and allows a significant income to farmers. In Lebanon, goats have always been associated with rural areas, the local "Baladi" breed being the most common (Serhan and Mattar, 2018). The particularity of goat flocks is that they are managed mainly under transhumance and nomadic systems (Srour et al., 2006). Local goat milk and coarse salt are the only ingredients used for the production of labneh Ambaris, where they are placed in special earthenware jars and left to ferment for several days. After coagulation, whey is drained, then milk and coarse salt are again added to the jar and left to ferment. These steps are repeated until the jar is full of coagulum, and the product can be consumed at this point. Labneh Ambaris can be regarded as a fermented milk rather than cheese, as per the definition of fermented milks according to Codex Alimentarius Standard (2018; CXS 243–2003), because of its manufacturing process involving reduction in pH due to fermentation of milk by microorganisms. The repeated additions of milk and salt throughout the fermentation process characterize this product. During this process, the salt quantities added throughout production vary largely between producers, which will affect salt's final concentration and the product's taste

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and physicochemical characteristics, such as texture and moisture content (Møller et al., 2013). Aside from the salting aspect, high acidity characterizes the product as it was shown in other studies where the pH of labneh Ambaris varied between 3.50 and 3.80 (Serhan and Mattar, 2013; Tabet et al., 2019). Salt content and high acidity are known to influence the growth and activities of microorganisms and to participate in the safety of a product by contributing, along with other factors, to the inhibition of pathogenic microorganisms (Arboatti et al., 2014; Bourdichon et al., 2021).

Today, labneh Ambaris is being slowly abandoned by consumers. This is mainly the result of urbanization waves and several consumer lifestyle expectations, such as the consumption of safe food products (Arja et al., 2001; Licitra, 2010). In addition, changes to the traditional production process were applied by some industries and households, either for economical purposes, by the use of nontraditional production containers such as plastic jars, or for safety concerns by the use of pasteurized goat milk (Tabet et al., 2019). From a sustainability point of view, it is essential to characterize labneh Ambaris and contribute to its preservation as a Lebanese cultural heritage because it can be presented as a potential food product to obtain a national quality label, as it was the case recently with Cyprus' Halloumi, recognized as a national dairy product and classified in 2021 as a Protected Designation of Origin cheese by the European Commission (European Commission, 2021).

To our knowledge, limited studies were published concerning labneh Ambaris. Tabet et al. (2019) demonstrated that, beside differences in moisture contents, the physicochemical characteristics (pH, ash, protein, and fat contents) of labneh Ambaris produced in earthenware and plastic jars were not significantly different from each other, whereas Dimassi et al. (2020) showed significant differences in fat, moisture, pH, and protein contents between samples collected from different Lebanese regions. However, none of these studies explored the sensory properties of labneh Ambaris, nor used culture-independent methods for the microbial analyses.

In a more global perspective and to preserve this sustainable food product, the present study aims to provide the first overall characterization of the Lebanese labneh Ambaris, produced by spontaneous fermentations (no starter cultures added). Therefore, different samples, each from a distinct production jar, were collected at the end of fermentation when the product can already be consumed, and the following distinct aspects were studied for the same samples: (1) physicochemical, (2) microbiological by using culture-dependent (viable counts) and culture-independent (DNA metabarcoding for the identification of bacteria and yeasts) approaches, and (3) sensory by using an original approach which combines the Pivot Profile (**PP**) method with classical descriptive analysis.

MATERIALS AND METHODS

Sampling

Twenty labneh Ambaris samples (**LA-1** through **LA-20**) were collected from 17 producers located in 3 different regions in Lebanon. The producers were selected according to their respect for animal welfare. The details of the collected samples as well as the production flowchart are presented in Supplemental Table S1 (https://figshare.com/articles/figure/TableS1/19582057; Abi Khalil, 2022d). All these samples were produced by spontaneous fermentations where no starter cultures were added. Samples were taken directly from the production jars (earthenware, plastic, or stainless steel). Each sample's quantity was divided in halves, the first one was refrigerated at 4°C for sensory and culture-dependent microbiological analyses, whereas the remaining half was frozen at −20°C for DNA metabarcoding and physicochemical analyses.

Physicochemical Analyses

The 20 samples were analyzed by Agrolabs for DM content (%; NF EN ISO 5534–10), fat content (%; NF V04–287), protein content (%; NF EN ISO 8968–3), and salt content (%; NF EN ISO 5943). The percentage of moisture content on a fat-free basis was calculated as mentioned in the General Standard for Cheese (Codex Alimentarius Standard, 2021). Salt-to-moisture ratio was calculated as mentioned by D'Amico et al. (2010). The pH values were recorded in triplicates at room temperature using a calibrated pH meter (HQ11 HD, HACH). Descriptive statistical analyses (mean values, standard deviations, quartiles, and minimum and maximum values) were applied to the collected data using XLSTAT (Addinsoft, 2022).

Microbiological Analyses: Microbial Enumeration and Pathogens Detection

Microbial enumeration was performed on the 20 collected samples. A 10-fold dilution was prepared by homogenizing 10 g of each sample in 90-mL sterile buffered peptone water (Scharlau). Successive 10-fold dilutions were then prepared and plated on nutritive and selective media and incubated with respect to necessary conditions (Table 1). Two values for de Man, Rogosa, and Sharpe agar, and M17 lactose agar were identified

Table 1. List of media used for enumeration along with their respective incubation conditions

Medium	Reference or composition	Targeted microorganism	Incubation condition	Oxygen incubation condition
de Man, Rogosa, and Sharpe agar	HIMEDIA	Mesophilic lactobacilli	30°C for 72 h	Anaerobiosis
M17 with 0.5% lactose	Fluka	Mesophilic lactococci and streptococci	30°C for 72 h	Aerobiosis
Plate count agar	Liofilchem	Mesophilic aerobic total flora	30°C for 72 h	Aerobiosis
Yeast peptone dextrose	1% yeast extract powder (HIMEDIA), 2% glucose (Scharlau), 2% peptone (Scharlau), 2% agar (Liofilchem)	Yeast and molds	25°C for 5 d	Aerobiosis

as outliers by Grubbs statistical test (Velasco et al., 2000) and removed from the analysis. The results were expressed as log cfu/g of sample. In addition, samples were tested in an external laboratory (Microbiological Quality Control Laboratory, Saint Joseph University USJ, Beyrouth) for the presence of the following food pathogens: *Brucella melitensis* (NF U47–105 ISO), *Escherichia coli* (RAPID' E.coli 2 Agar – NF validation according to ISO 16140, AOAC-RI approved N° 050601), *Staphylococcus aureus* (ISO 6888_1–1999) followed by coagulase and DNase confirmation tests, *Listeria monocytogenes* (ISO 11290–1/A1–2004), and *Salmonella* spp. (ISO 6579–2002). In addition, coliform bacteria at 37°C and thermotolerant coliforms at 44°C (ISO 4832–1991) were enumerated.

Culture-Independent Analysis: DNA Metabarcoding

DNA Extraction. Five grams of the sample was placed in 45 mL of sterile peptone water and homogenized in a stomacher (Mayo International Srl) for 3 × 1 min at maximum speed. Five milliliters of the dilution was placed in a sterile tube and centrifuged for 3 min at 13,000 × *g* at 4°C. The supernatant was then discarded and the pellet was washed with sterile peptone water and centrifuged several times while removing the fat layer present on the sides of the tube after each centrifugation. DNA was extracted using the DNeasy PowerFood Microbial Kit (Qiagen), which uses combined chemical and mechanical lysis conditions. DNA concentrations were determined using a nanodrop spectrophotometer (NanoDrop 2000/2000c, Thermo Fisher Scientific) and were adjusted to 10 ng/μL.

Mock Communities. Positive controls of mock communities (a mix of DNA of multiple known species) were used for the following 2 main reasons: to make sure that the primers used for 16S rDNA and ITS2 region did not over-amplify certain species, and to detect any false positives in the case of tag-jumping between samples (Francioli et al., 2021). They were subjected to the same analysis as the samples and as described in the Illumina MiSeq Sequencing and Bioinformatics Analysis sections. Previously identified bacterial and fungal species isolated from labneh Ambaris samples were used to constitute theses mock communities. The bacterial mock community was composed of equimolar DNA concentrations (10 ng/μL) of the following 6 species: *Lentilactobacillus diolivorans*, *Lentilactobacillus parabuchneri*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Lactobacillus delbrueckii*, and *Lactococcus lactis*. The fungal mock community was composed of equimolar DNA concentrations (10 ng/μL) of the following 10 species: *Kazachstania exigua*, *Kluyveromyces marxianus*, *Pichia kudriavzevii*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Rhodotorula mucilaginosa*, *Pichia terricola*, *Debaryomyces hansenii*, *Torulaspora delbrueckii*, and *Candida parapsilosis*.

Illumina MiSeq Sequencing. The V3–V4 region of the 16S rRNA gene for bacteria and ITS2 region for fungi were amplified. The primers used for the amplification of the 16S region were 16SV3 and 16SV4, and the primers used for the amplification of the ITS2 region were ITS3 tagmix1 and ITS4ngs (Tedersoo et al., 2015). To constitute forward and reverse primers that should be added to the PCR mix for 16S and ITS2 amplifications, an equal mix of the 3 primers, each containing a different frameshift, was made (Supplemental Table S2; https://doi.org/10.6084/m9.figshare.19582585.v4; Abi Khalil, 2022e). To prepare the multiplexed Illumina libraries, 2 amplifications are needed; particularly, the first PCR (Supplemental Table S3; https://doi.org/10.6084/m9.figshare.19582642.v2; Abi Khalil, 2022f) was used to add Illumina adapters and frameshifts to the target genes. The second PCR (indexing PCR, 8 cycles) was done by the sequencing platform to tag each sample with a unique nucleotide sequence (barcodes). The amplified DNA of all the samples were then pooled together to form the library. The mix was then sequenced with an Illumina MiSeq protocol, generating 2 × 300 bp paired-end reads.

Bioinformatics Analysis. Bacterial and fungal populations were assessed using 2 pipelines: DADA2

package version 1.14.1 in R program version 3.6.1 and FROGS version 3.2.2 (Escudié et al., 2018).

Pre-processing Using DADA2. The first demultiplexing step was done directly by the sequencing platform. The files received were the R1 and R2, constituting the forward and reverse sequences, respectively, for each sample. The bioinformatics pipeline applied consists mainly of the following steps: (1) removing primers using cutadapt (Martin, 2011), (2) filtering and trimming reads as per their quality, (3) dereplicating identical reads, (4) sample inference, (5) merging paired reads, (6) constructing amplicon sequence variants (**ASV**) table, and (7) removing chimeras.

Taxonomic Affiliation Using FROGS. Affiliation was performed using DAIRYdb database version 1.1.2 (Meola et al., 2019) and UNITE database version 8.2 (Nilsson et al., 2019) for 16S rRNA gene and ITS2 sequences, respectively. Multi-affiliated ASV were manually inspected using affiliation Explorer (Mariadassou, 2021) to choose between conflicting affiliations. The final ".biom" table was then uploaded into FROGS (Galaxy Version 3.2.2) for further processing and visualization using phyloseq R package (McMurdie and Holmes, 2013). For visual simplification, ASV tables were sorted and the most abundant (top 15) genera or species were presented for 16S and ITS2. The microbial compositions of samples were visualized using the Phyloseq Composition Visualization tool in FROGS (Galaxy Version 3.2.2). Amplicon sequence variants tables were then normalized by rarefaction before assessing the diversity indices. Species diversity within each sample was estimated using α-diversity indices (richness, Chao1, Shannon, and inverse Simpson). Species diversity between communities (β-diversity) was estimated using Bray-Curtis dissimilarity index and visualized by applying the metric multidimensional scaling ordination method.

Sensory Evaluation

All sensory tests were approved by INP-EI Purpan and the Lebanese University Institutions. The sensory tests took place during autumn season of 2020. The sensory panel was divided into 2 groups. The first one took the tests in a dedicated room at the Lebanese University–Faculty of Agriculture, and the second group was gathered in a dedicated room close to the panelists' houses or work places.

Sample Preparation. Five out of the 20 samples were discarded from the sensory analyses because 4 contained pathogens (LA-1, LA-4, LA-17, and LA-18) and 1 (LA-7) had insufficient quantity for testing. Refrigerated samples were placed at room temperature before being served to the panelists. Approximately 5 g of each sample was used for each tasting. To prevent saturation of the taste buds of the panelists, a maximum of 5 samples were tested per session because labneh Ambaris is strongly acidic and salty. Samples were presented in random order and the panelists were asked to cleanse their palates with a small piece of Lebanese bread (pita), then with room temperature water between samples.

Pivot Profile Method. Pivot Profile is a relatively new sensory method where panelists compare samples to a pre-identified reference, the pivot, using their own words (Thuillier et al., 2015). Samples should be described using terms less "*X*" than the pivot and more "*Y*" than the pivot (e.g., less salty, more acidic). Twenty panelists, 12 women and 8 men, aged between 24 and 65 yr, were selected. Panelists were informed about the nature of the products and provided written consent to participate in the analysis. Throughout the sampling campaign, most of the samples were tested by several persons, independently from the analysis, and one labneh Ambaris sample was chosen as the pivot for being the most appreciated and having balanced sensory characteristics. Fourteen samples were compared with the pivot, each placed on an opaque white plate on one side and the pivot on the other side. Samples were each coded with a random 3-digit number, whereas the pivot had always the same code. At the end of each session, panelists were asked for the exact meaning of their written descriptors, and words having the same meaning were grouped to form the final dictionary noting that only affirmative, 1-word descriptors were accepted.

Descriptors were entered into TASTEL software version 2015.2 along with the dictionary created to obtain contingency tables. The pivot was reintegrated into the analysis according to the method described previously (Fonseca et al., 2016). Statistical analysis was applied to the contingency tables generated using XLSTAT (Addinsoft, 2022).

As done by Fonseca et al. (2016), global chi-square was first used to assess the existence of a relationship between descriptors and samples in the contingency table. Then, it was possible to apply the chi-squared per cell statistical test to analyze, within each cell, the source of the variation obtained with a 95% confidence interval.

Classical Descriptive Analysis. The PP sensory sessions were considered as the "descriptive lexicon" generating sessions. Four men and 11 women were selected from the previous panel according to their motivation, commitment, and availability, and received 6 h of additional training. Eleven descriptors were retained from the PP results to constitute the "descriptive lexicon" of labneh Ambaris. Fifteen samples were

described, each presented individually to the panelists on an opaque white plate. The samples were each coded with a random 3-digit number and were scored on a 7-unit scale with a minimum of 1 (the descriptor exists in low intensity) and a maximum of 7 (the descriptor exists in very high intensity).

Statistical tests were applied using XLSTAT (Addinsoft, 2022). Analysis of variance test was applied to the sensory scores to detect significant differences between samples regarding the chosen attributes with a 95% confidence interval, followed by a principal component analysis on the 10 discriminatory descriptors. A hierarchical ascendant classification on the principal components based on Ward's linkage was then used to group labneh Ambaris samples having similar sensory characteristics.

Similarity Between the Sensory Methods. Multiple factors analysis was applied to the contingency table and the means table derived from the 2 methods. The regression vector coefficient (**RV coefficient**) was used to compare the similarities between the 2 sensory methods. The RV coefficient is an index that ranges from 0 (no correlation) to 1 (perfect correlation; Fonseca et al., 2016).

Correlation Between Descriptive Sensory Analysis and Physicochemical Characteristics. A correlation matrix between descriptors and physicochemical characteristics was generated with a 95% confidence interval.

Results

Physicochemical Analyses.

The pH values of the 20 samples varied between 2.92 and 4.14, with a mean value of 3.42 ± 0.28 (Figure 1A), which shows that labneh Ambaris is a highly acidic food product. For salt contents, high variability was recorded between the samples with a minimum of 0.52% and a maximum of 10.99%, and a mean value of 2.76 ± 2.47%, noting that 15 out of the 20 samples had values equal to or below 2.98% (Figure 1B). Salt-to-moisture ratio values ranged from 0.69 to 17.95% with a mean value of 4.39 ± 4.11% (Figure 1B). As for the fat, protein, and moisture contents, important variations existed between the samples. Values ranged from 9.5 to 30.5% for fat contents with a mean value of 18.07 ± 5.34, from 7.02 to 14.92% for protein contents with a mean value of 12.17 ± 2.09 and from 52.83 to 74.59% for moisture contents with a mean value of 65.08 ± 6.21 (Figures 1C and 1D). Details of all physicochemical characteristics of each sample is available in Supplemental Table S4 (https://doi.org/10.6084/m9.figshare.20306520.v3; Abi Khalil, 2022g).

Microbiological Analyses: Microbial Enumeration and Pathogens Detection

Total mesophilic aerobic flora levels varied between 4.80 and 8.81 log cfu/g, with a mean value of 7.10 ± 1.16 log cfu/g. For presumptive mesophilic lactococci and streptococci, levels varied between 5.22 and 7.85 log cfu/g with a mean value of 6.81 ± 0.82 log cfu/g. Presumptive mesophilic lactobacilli counts ranged between 5.87 and 8.76 log cfu/g with a mean value of 7.57 ± 0.78 log cfu/g. Yeasts were found in all the samples with levels varying between 4.30 and 8.39 log cfu/g and a mean value of 6.65 ± 1.25 log cfu/g. The details of the microbial enumerations are presented in Figure 2.

Out of the 20 samples tested, only 2 showed the presence of coliforms (with one of them also positive for *E. coli*); specifically, sample LA-1 had 4 log cfu/g coliforms at 37°C, 3.90 log cfu/g thermotolerant coliforms at 44°C and 3.90 log cfu/g *E. coli*. Sample LA-4 had 3.30 log cfu/g coliforms at 37°C and 2.47 log cfu/g thermotolerant coliforms at 44°C. Two samples collected from the same producer (LA-17 and LA-18) were positive for *L. monocytogenes*. None of the samples contained *Salmonella* sp., *B. melitensis*, or *Staph. aureus*.

Identification of Microbial Communities by DNA Metabarcoding Analyses

16S Sequencing for Bacterial Identification. After demultiplexing, 16S rRNA gene sequencing produced 1,909,754 paired-end reads for all samples, mock communities, and negative controls. This means that on average, 68,205 reads were obtained per sample, ranging from 37,385 reads to 95,126. These raw paired-end reads were then filtered according to their quality, dereplicated, and the background noises and chimeras removed, which leaves a total of 929,771 merged reads that were classified into 419 ASV. Within the bacterial composition, *Bacillota* was the dominant phylum in the 20 samples of labneh Ambaris with 95.31% of total sequences, followed by *Pseudomonadota* with 4.32%. The remaining 0.37% were composed of *Actinomycetota*, *Bacteroidota*, and *Mycoplasmatota*. The dominant phyla were translated as *Bacilli* and *Gammaproteobacteria* classes (95.30 and 4.31%, respectively). The most dominant bacterial family was *Lactobacillaceae* with a very high percentage (88.08% of the total sequences), followed by *Streptococcaceae* (6.11%) and *Enterobacteriaceae* (3.14%). At the genus level, *Lactobacillus* was the dominant one (63.14%), followed by *Lentilactobacillus* (11.98%), *Lactiplantibacillus* (7.17%), *Lacticaseibacillus* (5.02%), *Lactococcus* (4.62%), *Streptococcus* (1.49%), and *Aeromonas* (0.71%). Several ASV (constituting 1.29% of total sequences) could not be identified at

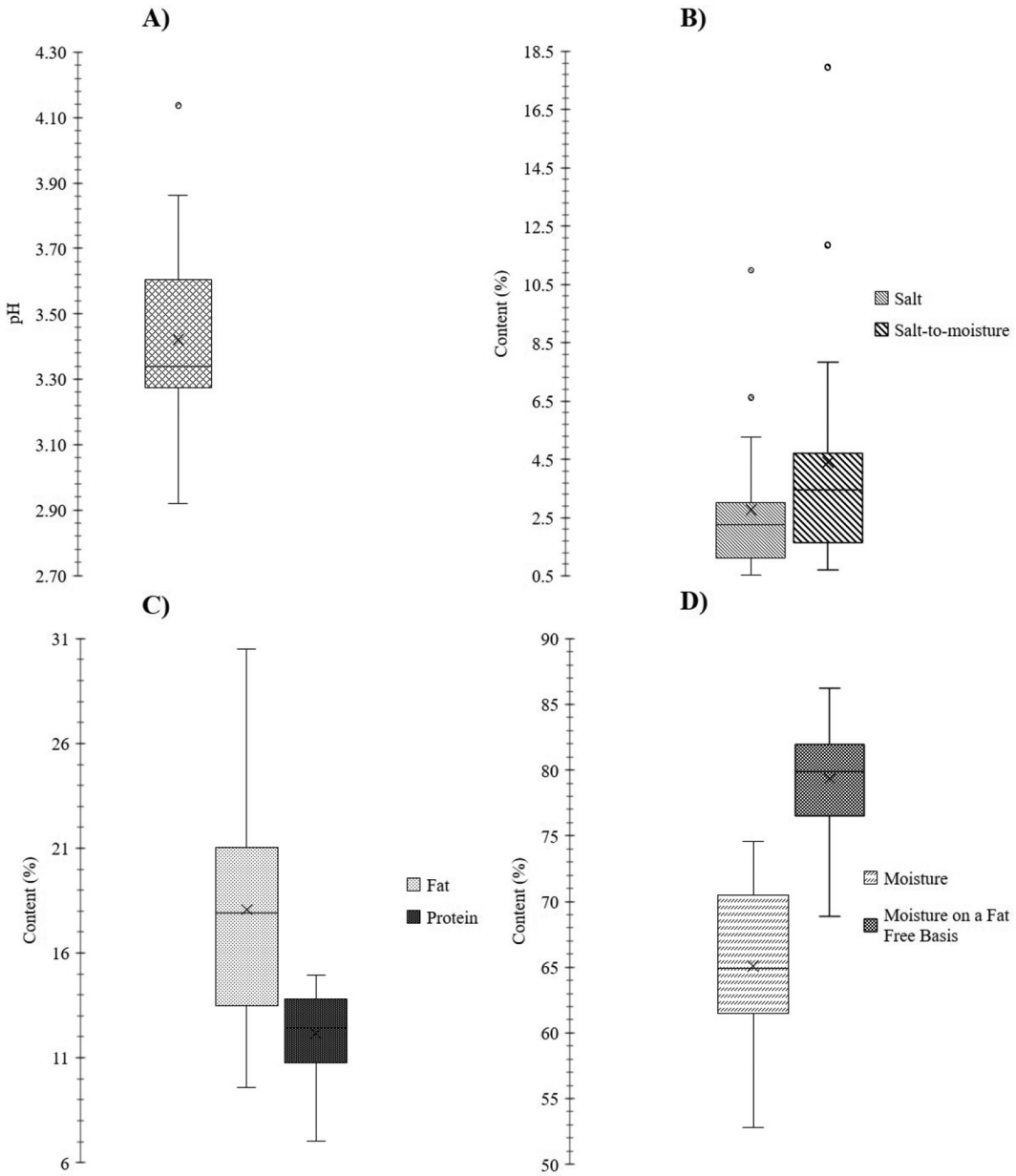


Figure 1. Physicochemical characteristics of 20 labneh Ambaris samples: (A) pH values, (B) salt and salt-to-moisture contents (%), (C) fat and protein contents (%), and (D) moisture and moisture on fat-free basis contents (%). Values are scattered according to the following: top of box = maximum, bottom of box = minimum, horizontal line = median, x = mean, lower whisker = quartile 1, upper whisker = quartile 4.

the genus level, but they all belonged to the family *Enterobacteriaceae* and were presented as "unidentified" genus. Out of the 20 samples tested, 13 had *Lactobacillus* as the dominant genus (Supplemental Figure S1; https://doi.org/10.6084/m9.figshare.19582657.v4; Abi Khalil, 2022a), whereas the remaining ones had *Lactiplantibacillus* (LA-3 and LA-6), *Lactococcus* (LA-15), *Lentilactobacillus* (LA-17), *Streptococcus* (LA-18), *Lacticaseibacillus* (LA-20), and *Enterococcus* (LA-7) as the dominant genus. It is important to note that 2 samples (LA-10 and LA-11) were strictly dominated by *Lactobacillus* genus (with over 99% within each sample), whereas 7 out of 20 samples showed more than 10 different bacterial genera. A significant amount of ASV could not be identified at the species level; particularly, when blasted into the currently available databases, the sequences gave several options having the same identity percentages and query coverage. *Lactiplantibacillus* sp. could either be the species *plantarum*, *paraplantarum*, or *pentosus*. *Lentilactobacillus* sp. could either be one

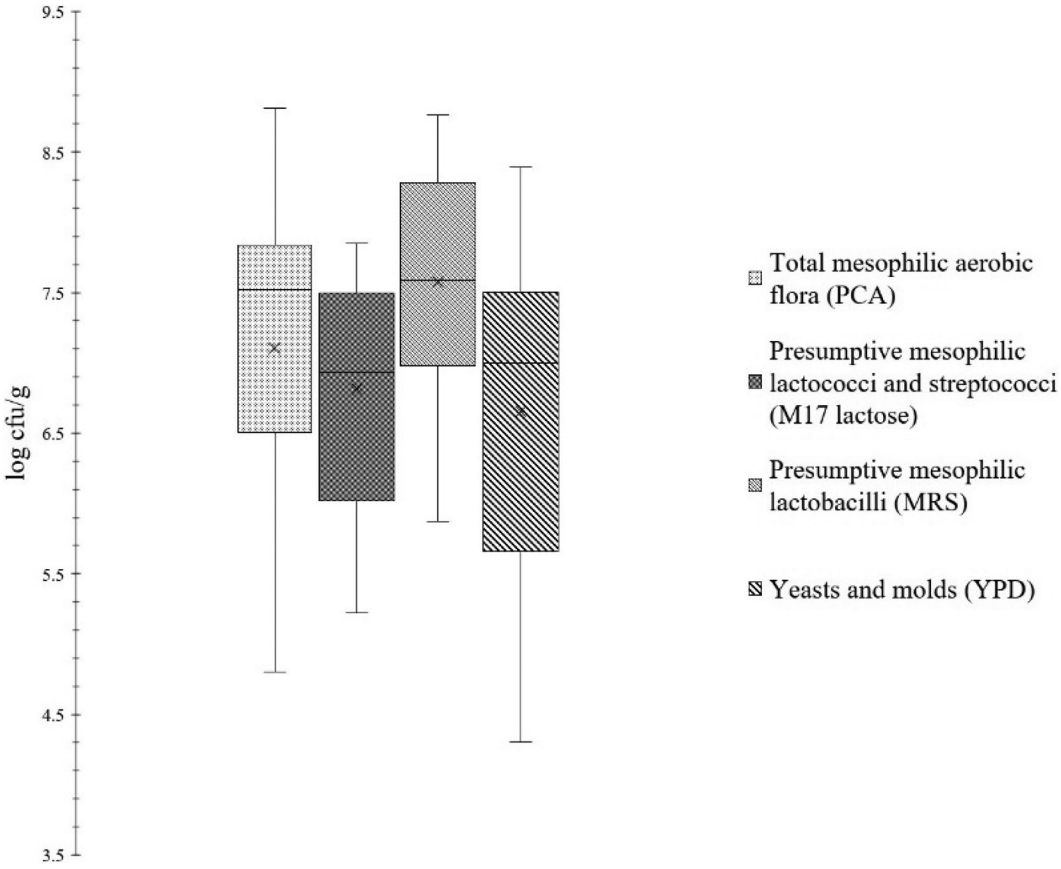


Figure 2. Microbial counts of 20 labneh Ambaris samples plated on selective and nutritive media. The results are expressed in log cfu/g. Values are scattered according to the following: top of box = maximum, bottom of box = minimum, horizontal line = median, x = mean, lower whisker = quartile 1, upper whisker = quartile 4.

of the species *hilgardii* or *diolivorans*, or the species *kefiri*, *sunkii*, or *otakiensis*. *Lactobacillus* sp. could be *helveticus*, *gallinarum*, or *crispatus* species. The options for *Enterococcus* sp. were the species *durans*, *faecium*, *hirae*, or *ratti*. A total of 160 bacterial species were detected within the samples. As shown in Figure 3A, within the identified species, *Lactobacillus kefiranofaciens* was the most dominant among samples with 40% of the total sequences obtained. Out of the 20 samples, this species was present in 16, in varying abundances. It constituted more than 90% of the relative abundance in 5 samples. The species *Lactobacillus helveticus* was the next most dominant with 12% of the total sequences, but it was only found in 4 samples, and it constituted more than 99% of the bacteria in sample LA-10 that was produced in a stainless-steel container. *Lactobacillus delbrueckii* (with 7% of total sequences) was present in 11 samples, but in low abundance, except for the sample LA-11 (produced in a plastic jar) where it was the only species present. *Lacticaseibacillus rhamnosus* with 4.7% of total sequences was detected in 4 samples and was present in a very high percentage (77.73%) in sample LA-20. As for *Lactococcus lactis*, it constituted 4.5% of total sequences and was detected in 18 samples but at varying relative abundances (Figure 3A and 3B). It is important to note that the unidentified species *Lentilactobacillus sp.* constituted around 12% of the total sequences and was present in 17 samples in varying abundances. Other species were identified among the samples but in lower percentages (<4.5% of total sequences). The species *Lc. lactis*, *Lentilactobacillus* sp., and *Lb. kefiranofaciens* were present in 80% and more of the samples and can be consequently considered as main keystone bacterial species (Figure 3B).

Species richness for each sample was assessed using several α-diversity indices presented in Supplemental Table S5 (https://doi.org/10.6084/m9.figshare.19582738.v2; Abi Khalil, 2022h). A comparison between the richness and the Chao1 indices, which is the estimated richness, revealed that our study captured almost all the bacterial diversity in the studied samples (more than 94% of the estimated richness for all the samples except for LA-10 with 83%). Based on the number of different ASV, the most diverse sample

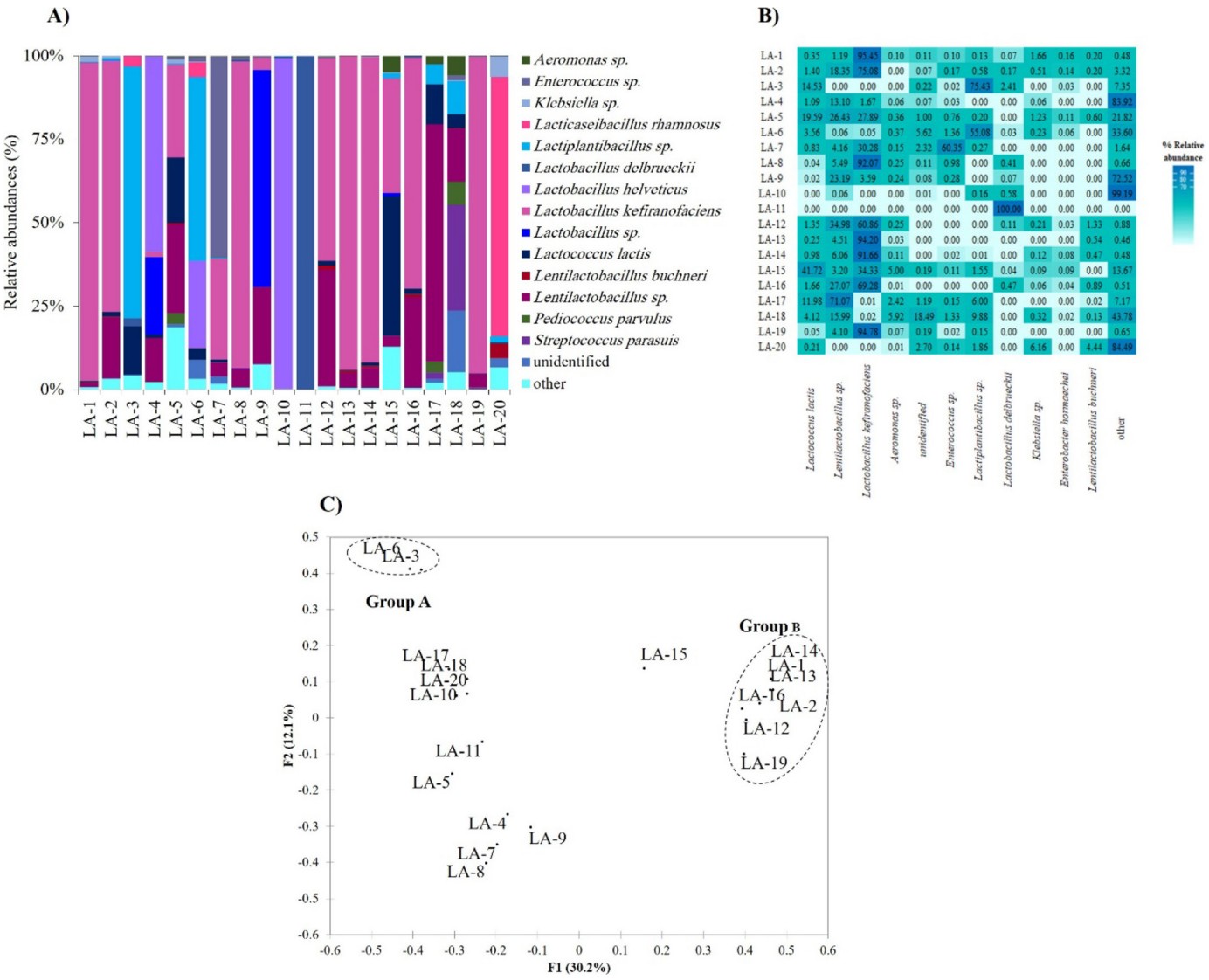


Figure 3. Analysis of bacterial communities in 20 labneh Ambaris samples (LA-1 to LA-20) by sequencing of the V3–V4 region of the 16S rRNA gene. (A) Distribution of the major 15 bacterial species, (B) heat map showing the keystone bacterial species present in 50% and more of the samples, and (C) metric multidimensional scaling plot based on Bray-Curtis dissimilarity matrix.

was LA-5 (richness index 72 and 56 different species present), followed by LA-18 (richness index 50 and 38 different species present). The least diverse sample was LA-11 (richness index 5 and only 1 species present). The richness within each sample was validated by the Shannon index, which showed the highest number for sample LA-5 (value 2.49) and the lowest number for LA-11 (value 0.12). As for species evenness, the inverse Simpson index showed that the sample LA-18 (value 7.93) had the highest value, whereas the sample LA-11 had the lowest (value 1.04). Samples dissimilarities based on the identified ASV were visualized using the metric multidimensional scaling ordination method as per Bray-Curtis indices (Figure 3C). Samples LA-3 and LA-6 (group A) that were dominated by *Lactiplantibacillus* sp. were grouped together. Samples mainly dominated by the species *Lb. kefiranofaciens* and *Lentilactobacillus* sp., where these 2 combined species constitute more than 95% of relative abundance, were clustered (group B) and separated from the others.

ITS2 Sequencing for Yeast and Mold Identification. After demultiplexing, ITS2 spacer sequencing produced 5,042,238 paired-end reads for all samples, mock communities, and negative controls. This means that on average, 180,080 reads were obtained per sample, ranging from 114,408 reads (LA-20) to 343,771 (LA-14). These raw paired-end reads were then filtered according to their quality, dereplicated, and the back-

ground noises and chimeras removed, which leaves a total of 1,580,496 merged reads that were classified into 111 ASV.

Ascomycota was the dominant fungal phylum in the 20 samples of labneh Ambaris representing 96.40% of the sequences, whereas the remaining 3.60% were for the *Basidiomycota* phylum. These ASV were classified into *Saccharomycetes* (96.24%), *Moniliellomycetes* (3.56%), *Eurotiomycetes* (0.16%), and *Tremellomycetes* (0.04%) classes. The most dominant fungal family was *Dipodascaceae* with 60.72% of the total sequences, followed by *Pichiaceae* with 33.05%, *Moniliellaceae* with 3.56%, and *Saccharomycetaceae* with 1.94%. Important differences in terms of relative abundances were recorded between samples at the family level. The Dipodascaceae family ranged from 0.042 to 92.77%, Pichiaceae from 0.64 to 99.45%, and Moniliellaceae from nonexistent in 13 out of 20 samples to 45.09% in the sample LA-14. At the genus level, *Geotrichum* was the most dominant with 60.56% of the total sequences, followed by *Pichia* with 33.04% and *Kazachstania* with 1.04%. The majority of the samples were either dominated by *Geotrichum* genus (more than 80% of the sample abundance in 6 samples) or by *Pichia* genus (more than 80% of sample abundance in 5 samples). The samples LA-13 and LA-14 (collected from the same producer) were co-dominated by *Geotrichum*, *Pichia*, and *Moniliella*. The genera *Kazachstania*, *Torulaspora*, *Kluyveromyces*, and *Starmerella* were among the top 15 identified, but they were found in lower abundances compared with *Geotrichum* and *Pichia*. The detailed composition of the top 15 fungal genera is available in Supplemental Figure S2 (https://doi.org/10.6084/m9.figshare.19582753.v4; Abi Khalil, 2022b). A total of 36 fungal species were detected among samples. *Geotrichum candidum* was identified as the most abundant species among the samples with 60.56% of the total sequences. It was detected in all 20 samples with varying relative abundances ranging from 3.54 to 92.73%, except for 2 samples LA-6 and LA-20 where it was found in traces (<1%; Figure 4A). *Pichia kudriavzevii* was the next most abundant species and was found in all 20 samples with varying percentages. *Moniliella* sp. was found in high percentages in 3 samples collected from the same producer (LA-12, LA-13, and LA-14) compared with the other samples where it was either absent or present in very low percentages (<2.5%). *Kazachstania unispora*, *K. marxianus*, *Starmerella sp.*, and *T. delbrueckii* were also identified in the samples with different relative abundances ranging from inexistent to 3.2% in sample LA-8. It is noticeable that the species *Yarrowia galli* and *Yamadazyma triangularis* were only found in the sample LA-11. The species *G. candidum*, *P. kudriavzevii*, *Geotrichum* sp., *Pichia* sp., and *K. marxianus* were present in 50% and more of the samples, and can be considered as the main keystone fungal species of labneh Ambaris samples (Figure 4B).

To normalize the data, samples LA-7 and LA-11 were removed due to their very low number of sequences in the ASV table (124 and 514, respectively). Species richness for each sample was assessed using several α diversity indices presented in Supplemental Table S6 (https://doi.org/10.6084/m9.figshare.19582765.v3; Abi Khalil, 2022i). By comparing the richness and the Chao1 indices, we conclude that our study captured almost all the fungal richness in most of the samples (100% for all samples and 95% for sample LA-12). Based on the number of different ASV, the richest samples were LA-14 and LA-4 (richness index 22 with 14 and 12 different species detected, respectively). The least rich samples were LA-17 and LA-6 (richness index 7 with 6 and 5 different species detected, respectively). These values were emphasized by the Shannon index, which showed the highest number for sample LA-14 (value 1.47) and the lowest number for LA-6 (value 0.08). As for species evenness, the inverse Simpson index showed that sample LA-14 (value 3.41) had the highest value whereas the sample LA-6 had the lowest (value 1.03). The grouping of samples based on identified ASV was visualized using the multidimensional scaling ordination method as per Bray-Curtis distance indices between samples (Figure 4C). Samples could be divided into 2 groups according to the dominant fungal species. The first (group A) was mainly dominated by the species *G. candidum* with more than 75% of relative abundance within each sample (8 out of 18 samples). The second one (group B) was dominated by *P. kudriavzevii* (6 out of 18 samples) with more than 50% of relative abundance. The sample LA-8 was co-dominated by the species *P. kudriavzevii* (41%) and *G. candidum* (52%) and was therefore placed in between the 2 groups A and B. As for the samples LA-12, LA-13, and LA-14, collected from the same producer, they were ordinated at a distance from all the other samples because they had a high percentage of the species *Moniliella* sp. compared with the other samples.

Sensory Evaluation

Pivot Profile Method. Overall, the PP method generated descriptors specific for labneh Ambaris. A first sensory description was given using this method, which showed that all products share similar attributes, but with different intensities. After the dictionary was created, attributes along with their synonyms were entered into TASTEL software. To create the contingency table where the frequencies of each attribute were written per sample, the 15 terms having the highest oc-

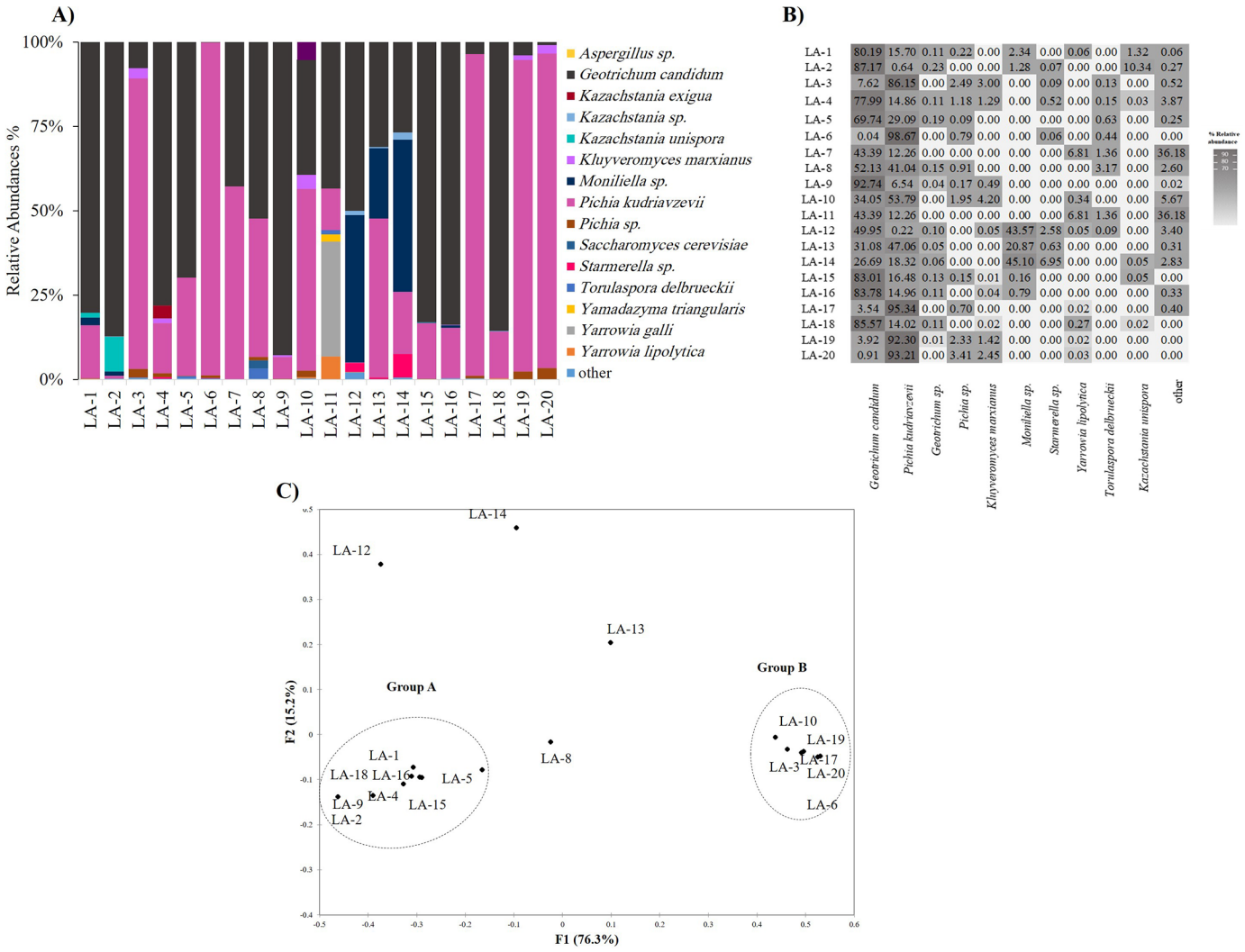


Figure 4. Analysis of fungal communities in labneh Ambaris samples (LA-1 to LA-20) by sequencing of the ITS2 region. (A) Distribution of the major 15 fungal species, (B) heat map showing the keystone fungal species present in 30% and more of the samples, and (C) metric multidimensional scaling plot based on Bray-Curtis dissimilarity matrix.

currences were retained, which are as follows: "acidic," "granulated texture," "white," "salty', "goat smell," "beige," "melting," "goat flavor," "bitter," "fatty," "humid," "granulated visually," "shiny," "acidic smell," and "fresh smell." According to Supplemental Table S7 (https://doi.org/10.6084/m9.figshare.19582771.v5; Abi Khalil, 2022j), the taste attribute "acidic" was the most used to describe labneh Ambaris samples with 424 total citations, followed by texture attribute "granulated texture" and color attribute "white" with 412 and 324 citations, respectively. The attributes "bitter," "fatty," and "fresh smell" were cited less than 100 times with 90, 68, and 48 citations, respectively. As for the attributes "beige" and "white," they were cited oppositely of one another concerning a given sample, such as the sample LA-2, where it was described as whiter than the pivot (20 positive citations for "white" and 18 negative citations for "beige"). Same for the attributes "granulated texture" and "melting," where they were cited in opposition to one another. When compared with the pivot, the descriptors "granulated visually" and "granulated texture" both varied in the same direction, either positively or negatively.

The global chi-squared test applied to the contingency table, after reintegration of the pivot and translation, was highly significant ($P < 0.0001$). This means that some labneh Ambaris samples were descriptors-dependent. Consequently, chi-squared per cell tests were applied to identify the source of statistical differences. According to the P-values, no particular sample was linked to the descriptors "bitter," "fatty," and "fresh smell." As for the following descriptors, they were sig-

nificantly correlated with only 1 or 2 samples: "goat flavor" with LA-9, "goat smell" with LA-19, "shiny" with LA-8, and "acidic smell" with LA-6 and LA-19.

Classical Descriptive Analysis. From the 15 descriptors generated by the PP method, 11 were kept for the descriptive analysis. "Acidic smell" and "fresh smell" were discarded for the difficulty in training panelists to distinguish them. For the descriptors "granulated visually" and "granulated texture," only the second one was kept because, as shown previously, a high correlation was obtained between the 2 attributes. The descriptors "beige" and "white" were grouped into 1 "color intensity." According to ANOVA statistical analysis, the following 8 descriptors had a high discriminating potential ($P < 0.05$): "humid," "melting," "shiny," "acidic," "fatty," "salty," "color intensity," and "granulated texture." As for the descriptors "goat flavor" and "goat smell," only 2 samples for the first (LA-8 and LA-12) and 1 sample for the second (LA-11) were found significantly different from the other samples. For the descriptor "bitter," no significant differences were recorded between the samples. A principal component analysis was performed using only the 10 discriminatory descriptors. According to Figure 5, the first principal component analysis plan explains 58.86% of the variability. As per the cosines square values, the descriptors "melting," "humid," "shiny," "acidic," and "granulated texture" were explained predominantly by the first axis F1 (34.25%), whereas the descriptors "goat smell" and "color intensity" were explained by the second axis F2 (24.59% of the variability). The descriptors "salty" and "goat flavor" were explained by the third axis F3 (18.39% of the variability). According to the dendrograms obtained by Ward linkage method based on the principal components (Supplemental Figure S3; https://doi.org/10.6084/m9.figshare.19582777.v3; Abi Khalil, 2022c), the 15 labneh Ambaris samples could mainly be classified into 3 groups according to their characteristics. The first group A was situated on the left side of the axis F1 and was characterized by having a "granulated texture," and was neither "acidic," "humid," "melting," nor "shiny." Sample LA-5 was classified within group A, though it was explained by both axes F1 and F2. Group B was explained by axis F2 and was described as having higher "color intensity," higher "goat smell," and being "fatty." The final group C was dispersed between the other 2 groups and was composed of several diverse samples. Some of them (LA-3, LA-9, and LA-15) were "humid," "melting," "shiny," and "acidic," but did not have "granulated texture," whereas others (LA-11, LA-2) had a whitish color and were not "fatty" compared with the other samples. It was clear that the terms used describe all labneh Ambaris samples well, whereas the latter are differentiated based on the ranges that exist within each descriptor.

Both sensory methods were compared using the RV coefficient used to calculate the degree of correlation between the 2 sets of values, each generated by a method. The RV coefficient obtained was 0.706, indicating a relatively good similarity between the PP and the classical descriptive analysis, noting that only 20 untrained panelists participated in the PP method.

Correlation Between Classical Descriptive Analysis and Physicochemical Characteristics. Important correlations were recorded between sensory descriptors and physicochemical characteristics (Supplemental Table S8; https://doi.org/10.6084/m9.figshare.19634595.v4; Abi Khalil, 2022k). "Humid" was positively correlated with "shiny," and negatively correlated with "granulated texture" and "salt content." The descriptor "fatty" was positively correlated with "goat smell," whereas "color intensity" was positively correlated with fat and protein contents.

DISCUSSION

Labneh Ambaris is a traditional and sustainable fermented dairy product made in Lebanese rural areas, typically through spontaneous lactic fermentation. Its unique sensory characteristics linked to the environment and a special expertise make it well appreciated by consumers. For the first time, our study described labneh Ambaris using different combined approaches. Twenty samples were collected from 3 regions in Lebanon, covering the range of all available product variants that are manufactured without added starter cultures. Results showed that labneh Ambaris is a high acid food product with varying salt contents and a soft cheese-like texture because the minimum value recorded for the moisture content on a fat-free basis was 68.85%. Labneh Ambaris appears to be generally free of pathogens, while the main microbial group present in it is lactic acid bacteria (**LAB**) with *Lactobacillus* being the dominant genus, in addition to the presence of various yeasts. It could be mainly described as salty, acidic, having different textures, and distinctive goat smell and flavor, all with varying intensities.

The pH values obtained within our study (ranging from 2.92 to 4.13) were in accordance with those from other authors (Serhan and Mattar, 2013; Dimassi et al., 2020). The dominance of LAB present in labneh Ambaris, their high-count numbers (around 10^7 cfu/g), and their diversity could explain the low pH values obtained. Indeed, within our samples, the bacterial species found in labneh Ambaris such as *Lc. lactis*, *Lb. kefiranofaciens*, *Lentilactobacillus* sp. (mainly *kefiri*),

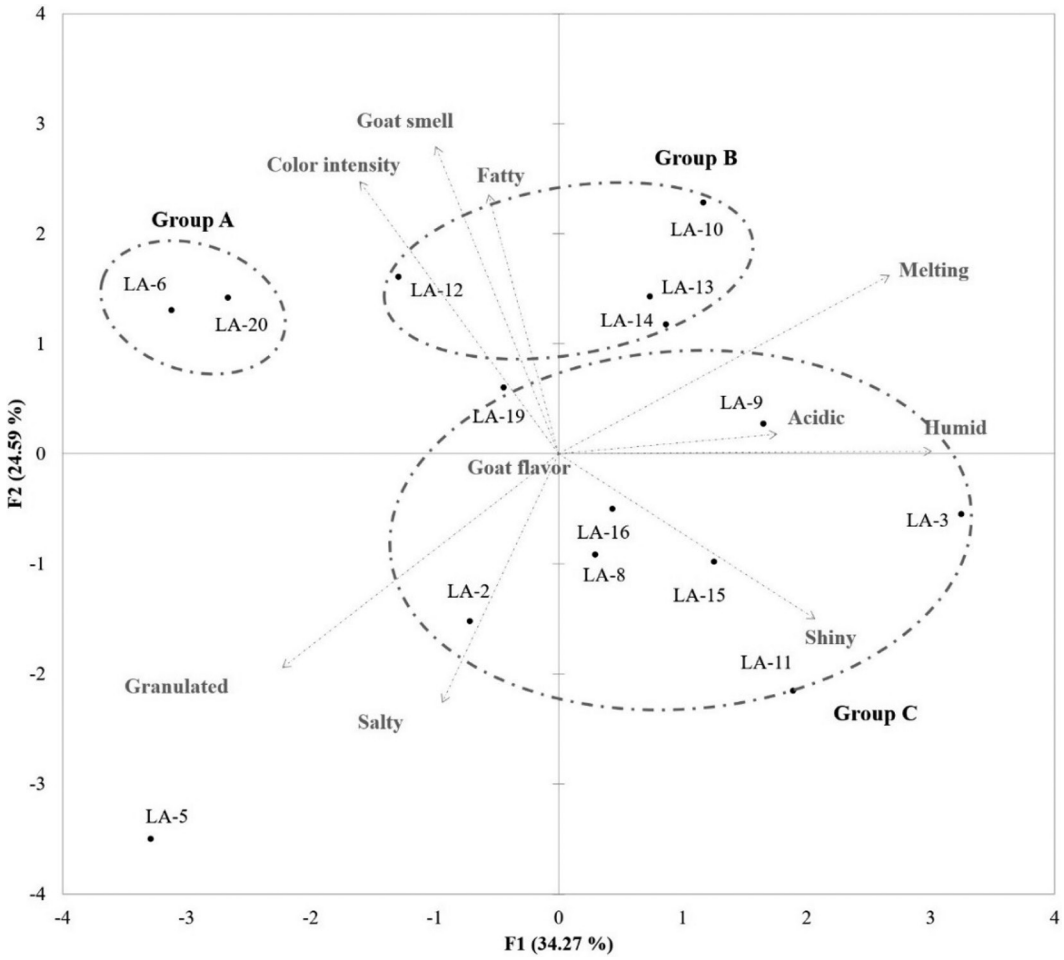


Figure 5. Principal component analysis showing the sensory profiles of 15 labneh Ambaris samples (LA-1 to LA-20) based on the sensory notes given by panelists during classical descriptive analysis sessions. Only discriminating descriptors are shown.

Lb. helveticus, *Lentil. diolivorans*, *Lactic. rhamnosus*, and *Lactip. plantarum* are similar to those mentioned in literature for milk kefir (Bengoa et al., 2019; Yilmaz et al., 2022). A study showed a drastic pH decrease for milk fermented with whole kefir grains compared with milks fermented with individual bacterial species mainly found in kefir grains (*Lb. kefiranofaciens*, *Lentilactobacillus kefiri*, and *Lentilactobacillus parakefiri*; Duran et al., 2022). Interestingly, herein among the consortia of LAB, 2 species, *Lb. kefiranofaciens* and *Lc. lactis*, could play a significant role in the low pH values measured. Indeed, Wang et al. (2021) demonstrated that a coculture of *Lb. kefiranofaciens* and *Lc. lactis* leads to lower pH values compared with pH obtained with their own individual fermentations. In our study, we have also shown the presence of *G. candidum*, *P. kudriavzevii*, *K. marxianus*, and *T. delbrueckii*, fungal species well known to grow in acidic matrices like milk kefir and contribute to acidity development (Witthuhn et al., 2005; Diosma et al., 2014).

In addition to acidity, the presence and distribution of microorganisms in milk products are also greatly affected by other abiotic factors such as the salt content and water activity of products (Zheng et al., 2021). Salt is usually added to milk products for several purposes such as to control the microflora, inhibit the growth of undesirable microorganisms (spoilage and pathogenic), and enhance consumer appreciation and mouthfeel (Møller et al., 2013; Dugat-Bony et al., 2016). High variability existed between our samples regarding salt content. This was not surprising because labneh Ambaris is a homemade traditional dairy product and salting is done arbitrarily, according to the product's needs, the producer's taste, and inherited expertise. It is safe to say that 60% of the samples had values ranging between 1 and 3% salt content, which are in accordance with the results found by Dimassi et al. (2020).

Fecal coliforms were detected in 2 samples (with one containing *E. coli*). This result was in agreement with

previous studies (Serhan and Mattar, 2013; Tabet et al., 2019). These bacteria are commonly found in artisanal milk products as shown by Samelis and Kakouri (2007) who compared industrial and artisanal Galotyri cheese (an acid-curd type Greek Protected Designation of Origin cheese). No *Staph. aureus*, *Salmonella* sp., or *Brucella* sp. were detected in our study. It is commonly known that salt and organic acids play a crucial role in inhibiting pathogenic microorganisms, although studies have shown that these 2 factors are not enough on their own to inhibit certain food pathogens, and other factors take part in this. Kim et al. (2016) demonstrated that organic acids solutions with 3.5 pH (lactic acid solution and acetic acid solution) and ethyl alcohol solution (2% wt/wt) inoculated with *Staph. aureus*, *L. monocytogenes*, *Enterococcus faecalis*, *E. coli*, *Salmonella enteritidis*, and *Pseudomonas aeruginosa* failed to inhibit the growth of these pathogens. Interestingly, antibacterial activity was observed when these pathogens were inoculated in different milk kefirs during fermentation process, noting that the longer fermentation time reinforced the antibacterial effect (Kim et al., 2016). Lactic acid bacteria have an inhibitory effect toward pathogenic bacteria due to their abilities to lower the pH, in addition to the secretion of bacteriocins and other antibacterial substances such as peroxides, alcohols, and carbon dioxide (Vardjan et al., 2013; Ołdak et al., 2020). Further, short-chain fatty acids produced by LAB in acidic environments are mainly responsible for the antimicrobial effect of these bacteria against pathogens (Rhoades et al., 2021). Herein, *L. monocytogenes* was detected in 2 samples collected from the same producer in which high salt concentrations were recorded. Even if the optimal growth of this species is under 5% of salt (Zarei et al., 2012), these bacteria can survive in media with salt concentrations up to 10% (Gérard et al., 2018). Beside this physicochemical aspect, *L. monocytogenes* is mainly found in products processed under low sanitary conditions (Ulusoy and Chirkena, 2019), as observed in this production facility.

It is well known that raw milk microbiota is an important factor that shapes the microbial composition of traditional milk products. The milk microbiota comes from many sources such as dairy equipment, the surrounding environment, animal feed, or seasonality (Montel et al., 2014; O'Sullivan and Cotter, 2017). Pasteurization is also an important factor that affects the diversity of milk microbiota. In our study, samples made from pasteurized milk apparently had the same diversity levels and species composition as raw milk samples. However, the low number of pasteurized milk samples (3 among 20) was not sufficient to make definitive conclusions about the effects of milk's heat treatment on the microbial composition of labneh Ambaris. The variability that exists between the microbial compositions of our samples could also be influenced by other factors such as a house microbiota in the production facility, the processes, or the production materials (earthenware, plastic, and stainless steel). Indeed, in our study, 2 out of 6 samples produced in non-earthenware materials showed lower bacterial diversity than all the other samples, with practically only 1 dominant species in each, these species being minor in the other samples. Here also, we cannot be conclusive from our limited data set about the influence of the container material. Taken together, these results illustrate possible various factors (including production material and milk pasteurization) that could influence the microbial diversity present in the final product. Additional investigations will be needed to confirm any possible role these factors might play in shaping the microbial communities of labneh Ambaris.

Through an original approach combining both PP and classical descriptive sensory analysis, our work generated descriptors that are well suited to the specific description of labneh Ambaris. The sensory analysis showed different intensities between the tested samples within the same descriptor. These differences are the result of the diversified combined microbial compositions and physicochemical characteristics observed. The majority of labneh Ambaris samples had distinctive "goat smell" that could mainly be explained by the presence of fatty acids originating from goat milk (Watkins et al., 2021), because a positive correlation was shown between "fatty" and "goat smell" descriptors. Salt content is described to affect texture and moisture content of the product (Møller et al., 2013). In our study, salt content was positively correlated with "granulated texture" and negatively correlated with "humid" and "melting" descriptors. Fat and protein contents are important factors that affect color intensity, and our study showed a positive correlation between these 2 physicochemical characteristics. They are initially linked to the milk composition which varies according to season, stage of lactation, and feed (Kljajevic et al., 2018). The activities of microbial communities could also influence labneh Ambaris sensory features, although no clear correlation was found between microbial compositions and sensory scores. For example, *G. candidum*, present in the majority of our samples, produces enzymes responsible for fat and protein degradation (Boutrou et al., 2006). Furthermore, other yeasts such as *P. kudriavzevii* also found majorly in our samples can degrade fat molecules (Costas et al., 2004). The texture and mouthfeel of fermented milk products can be influenced by the presence of exopolysaccharides, which are particularly and extensively produced by LAB species, such as *Lb. kefiranofaciens*, *Lactip. plantarum*, and *Lacticaseibacil-*

lus paracasei (Hamet et al., 2015) and some yeasts such as *K. marxianus* and *P. kudriavzevii* (Rahbar Saadat et al., 2020), and all of these species were present in our labneh Ambaris samples. Exopolysaccharides not only affect the rheological properties of dairy products but can also have health-promoting beneficial effects. For example, exopolysaccharides reduce blood cholesterol levels and protect the intestinal epithelial barrier against food pathogens (Hamet et al., 2015). In addition, it is commonly agreed that fermented milk products, especially milk kefir, which microbial composition can be closely compared with labneh Ambaris, provide various health benefits to humans upon consumption, either directly through the living probiotics that they harbor or indirectly through the functional metabolites present in the product (Rosa et al., 2017; Aguilar-Toalá et al., 2018; Kim et al., 2019; Ganatsios et al., 2021). Our study showed, via DNA metabarcoding analysis approach, that labneh Ambaris harbors a wide diversity of potential beneficial dead or alive microorganisms. However, future studies could be done to elucidate the probiotic or postbiotic healthy effect, or both, of labneh Ambaris.

CONCLUSIONS

Labneh Ambaris is a fermented milk product with high acidity, important salt contents, and a soft cheese-like texture. Our study showed, via DNA metabarcoding analysis approach, that it harbors a wide diversity of microorganisms closely related to kefir. The keystone microbial species were shown to be *Lb. kefiranofaciens*, *Lentilactobacillus sp.*, and *Lc. lactis* for bacteria and *G. candidum*, *P. kudriavzevii*, and *K. marxianus* for yeasts. Despite these common features, high variabilities were recorded regarding the microbiological compositions, physicochemical, and sensory aspects. This could be explained by the different raw materials used, the producer-dependent production processes adopted, and the processing environments and materials involved. These factors could be investigated in further studies. This study can be considered as a base toward initializing the process of obtaining a quality label for labneh Ambaris as a sustainable cultural food product to be preserved.

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