METATAXONOMIC ANALYSIS OF THE BACTERIAL DIVERSITY IN TABLE OLIVE DRESSING COMPONENTS

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Running title: Bacterial diversity in olive dressings

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Abstract

In this work, we have used a metataxonomic analysis to study the bacterial biodiversity in three types of commercial dressing and ingredients habitually used in table olive packaging. The bioinformatics analysis showed that salt samples (marine and spring) had the highest biodiversity indexes, followed by the aromatic herbs (thyme, oregano, and fennel) and finally by fermented dressing samples (garlic, pepper, and pepper paste). In salt samples, a significant high presence of *Cytophagia*, *Sphingobacteria*, *Deltaproteobacteria* and *Halobacteria* classes was observed. On the contrary, the presence of *Bacilli* class in fermented dressing samples was significantly higher compared to the other samples. Finally, the abundance of *Gammaproteobacteria* was statistically higher in the samples of herbs. At genus or family taxonomic level, *Pseudomonas* and diverse non-assigned genus of the *Enterobacteriaceae* family predominated in many samples of herbs. In fermented dressing samples, diverse genus belonging to the *Enterobacteriaceae* family appeared in a high relative abundance in pepper paste and garlic, and presence of *Vibrio* sp. was detected in pepper. However, *Lactobacillus*, *Weisella*, and *Pediococcus* were the predominant genera found in many of these fermented samples. *Salinibacter* was the most representative genus in both types of salt samples. Multivariate analysis (PCA) of the metataxonomic data sets discriminated well the samples belonging to the three different groups, showing a high proportion of sequences shared by samples-group. This survey reports for the first time the presence of new bacterial species not previously related with table olive processing, as well as the presence of others which could compromise the stability of olive packaging.

Keywords: Table olives; Packaging; Metabarcoding; Seasoning ingredients; Salt

1. INTRODUCTION

The olive tree is an iconic species in the Mediterranean cultural history and diet. Its multiple uses in the food industry (olive oil and table olives) and its omnipresence in many traditional agrosystems have made this species an economic pillar and cornerstone of Mediterranean agriculture. Specifically, table olives are one of the most important fermented vegetable in Europe and especially in the Mediterranean basin, with an average annual worldwide production of 2.7 million tonnes in the 2013-2018 season (IOC, 2018). The fermentation of fruits is mainly carried by lactic acid bacteria (LAB) and yeasts in an acid and saline environment, consuming sugars and producing diverse volatile compounds and organic acids (mainly lactic acid) with a considerable influence on the flavour, quality and safety of the final products (Arroyo-López et al., 2008; Garrido-Fernández et al., 1997). Then, fruits are packaged in diverse types of containers (glass, tin, bags, etc.) using preservatives, pasteurization, modified atmospheres or other stabilization systems to increase shelf life.

Herbs, spices and other seasoning material are used in many cases as essential condiments in table olive packaging as they help to enhance their flavor. Many of them, such as garlic or pepper, are also previously fermented independently, and used as stuffed material in pitted olives. Numerous studies have indicated high microbial loads in spices and herbs which could pose a problem for food manufacturers (Baumgartner et al., 2009; Beuchat et al., 2013; Vij et al., 2006). These studies showed the presence of *Salmonella* sp., considered the principal foodborne pathogen associated with dried herbs and spices, *Cronobacter* species, verotoxigenic *Escherichia coli*, *Staphylococcus* sp., spore forming microorganisms (*Bacillus* and *Clostridium* genera), and *Listeria*

monocytogenes. All of them were isolated from low-moisture foods including spices and dry herbs.

On the other hand, sodium chloride has been reported to suppress the growth of undesirable bacteria and other spoilage microorganisms by decreasing water activity in foods. During olive processing, salt can also give fruits a crunchier texture, since the salt hardens the pectin in vegetables. Nevertheless, there is scarce information on the microbial load provided by salt in olive elaboration, and how it could affect the stability of the final product. Recently, Benítez-Cabello et al. (2016) reported the presence of resistant bacteria in salt through culture-dependent methods. Species belonging to the *Bacillus*, *Staphylococcus* and *Enterococcus* genera, not detected during the fermentation process, were found in the solid marine salt used by the industry for preparation of brines. Also, some halophilic bacteria such as *Halolactibacillus halophilus* or species from *Marinilactibacillus* genus have been previously described during table olive processing (Lucena-Padrós et al., 2015; Lucena-Padrós and Ruiz-Barba, 2016; Rodríguez-Gómez et al., 2017).

So far, most of the studies conducted on microbial biodiversity in foods or condiments have been approached through classical and culture-dependent techniques. However, the use of new molecular techniques in food microbiology, such as culture-independent methods, complement the studies carried out and allow to overcome the limitations of these traditional methods. In this sense, Next Generation Sequencing techniques represent a change in the way that researchers address microbial ecology in foods. Indeed, the application of metataxonomic analysis allows a detailed understanding of microbial diversity. Specifically, in table olives, diverse metataxonomic studies (Arroyo-López et al., 2016; Cocolin et al., 2013; De Angelis et

al., 2015; Medina et al., 2016, 2018; Rodríguez-Gómez et al., 2017) have been carried out to determine the bacterial and fungal evolution during olive fermentation, but this approach was never applied to the study of olive dressings or salt used to prepare brines in table olive processing.

The goal of this study is to provide a more precise knowledge of the bacterial biodiversity in some of the main ingredients and dressing material used in table olive packaging through metataxonomic approaches. The possible role of these microorganisms in the final product quality is discussed. Data obtained could be used by industry to design new strategies to improve the quality, safety and stability of the final products.

2. MATERIALS AND METHODS

2.1. Types of samples

Samples were provided from different table olive producer companies from the regions of Seville and Málaga (Spain) during the 2015/2016 season and transported to the laboratory for analysis in the same day. A total of 19 samples divided in three groups were processed. Five samples were obtained from diverse aromatic herbs (thymus, fennel, and oregano), seven samples from fermented dressings material (sliced red pepper, pepper paste, and garlic) and seven from salts (marine and spring salt). All these samples are ready-to-use by industry and they are directly added to olives as ingredient or stuffed material during the packaging step.

2.2. DNA extraction

Five grams of every herb sample was homogenized with 50 mL of sterile saline solution (0.9 % NaCl) in a Stomacher® homogenizer (Seward Laboratory Systems, Inc.

Bohemia, NY, USA) for 2 min, and the aqueous phase was centrifuged at 9,000 $\times$ g for 15 min. For fermented dressing samples, 25 g were processed in 50 mL of sterile saline solution in the same conditions described above. Finally, for salt samples, 25 g were diluted in 100 ml of distilled water and centrifuged as described above. In all cases, the supernatant was withdrawn and pellets were washed twice with sterile saline solution before stored at -80°C until use.

Microbial genomic DNA was isolated and purified from pellets using the PowerFood® Microbial DNA Isolation Kit (MoBio, Carlsbad, Calif) according to the manufacturer instructions, and was stored at -20 °C until use. Prior to sequencing, the amount of purified DNA, that ranged from 5.6 to 42.2 ng/ μ l, was measured using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, USA).

2.3. Sequencing

DNA samples were sent for sequencing to FISABIO (Valencia, Spain). 16S rDNA gene amplicons were amplified following the 16S rDNA gene Metagenomic Sequencing Library Preparation Illumina protocol (Part # 15044223 Rev. A). The gene-specific sequences used in this protocol target the 16S rDNA gene, both V3 and V4 regions (Klindworth et al., 2013). Libraries were sequenced using a 2x300 bp paired-end run (MiSeq Reagent kit v3 (MS-102-3001) on a MiSeq Sequencer, according to manufacturer's instructions (Illumina). Quality assessment was performed by the use of prinseq-lite program (Schmieder and Edwards, 2011) by applying the following parameters: minimum sequence length of 50 bp, trim_qual_right of 30, trim_qual_type of mean and trim_qual_window of 20 bp. R1 and R2 from Illumina sequencing were joined using FLASH program (Magoč and Salzberg, 2011) by applying default parameters.

2.4. Metataxonomic analysis

For the metataxonomic data analysis, the *.fna format files were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline (version 1.9.1, <http://qiime.sourceforge.net/>) in a server running Ubuntu v16.04. Sequences were first screened for quality control, being retained those with a length between 220-300 bp and mean sequence Phred quality score >35. Any sequence not meeting these parameters was excluded from downstream analyses. Then sequences were sorted by barcode into their respective samples. In order to calculate downstream diversity measures (alpha diversity indices), 16S rRNA Operational Taxonomic Units (OTUs) were defined at the level of $\geq 97\%$ sequence homology. Chimeric sequences were removed using ChimeraSlayer, and SILVA108 was used as reference sequence database (McDonald et al., 2012). OTUs were assigned using uclust (Edgar, 2010) by using the script pick_de_novo_otus.py. This script produced an OTU mapping file, a representative set of sequences, a sequence alignment file, taxonomy assignment file, a filtered sequence alignment, a phylogenetic tree, and a biom-formatted OTU table. The hierarchical clustering based on population profiles of most common and abundant taxa was performed using UPGMA clustering (Unweighted Pair Group Method with Arithmetic mean). *Cyanobacteria* and mitochondrial OTUs were removed from the resulting OTU table. Taxonomic abundances within each identified Phylum to genus level were visualized using Krona hierarchical data browser (Ondov et al., 2011)

Alpha diversity indexes were calculated through the alpha_diversity.py script using different metrics (Chao, Observed Species, Shannon, Simpson, Margalef, Good's coverage, PD whole tree) after performing a rarefaction analysis. Rarefied OTU tables

to 11.400 sequences (lowest number of reads obtained) were used to obtain these alpha diversity metrics.

The OTU tables were collapsed at five taxonomic levels (Phylum, Class, Order, Family, and Genus), exported in tab-delimited text format and analyzed using STAMP v2.0.1 (Parks and Beiko, 2010). A non-supervised principal component analysis (PCA) was conducted after grouping the variables in three categories; fermented dressings (Garlic, pepper and paste of pepper), herbs (thymus, oregano and fennel), and salts (marine and spring salt). In order to determine the unique and shared OTUs between groups, a Venn diagram was constructed on the basis to relative abundance of bacteria on the level of class. An ANOVA/Tukey Kramer (*post hoc*) test was running to elucidate the taxa association in the different grouping variables. The false discovery rate correction [FDR, (Benjamini and Hochberg, 1995)] was finally applied in all cases and significant differences in taxa were only considered below a *p*-value of 0.05 and a *q*-value below 0.3.

3. RESULTS

3.1 Phylogenetic analysis

This study generated a total of 3,478,486 raw sequences for the 19 samples, from which 3,435,826 sequences with high quality were recovered after the quality control (98.77%). From those, we obtained 2,660,444 (83.79%) sequences that could be assigned into OTUs. Finally, after removing chloroplast and mitochondria, a total of 1,011,230 sequences (29.43%) were used for metataxonomic analysis with a mean of 126,403 sequences per sample.

Table 1 shows the total OTUs at class level found in every group and their alpha diversity indexes. The maximum number of OTUs were assigned to the salt samples (6,925), followed by herb group (6,744), and much further the food fermented group with less of the half of OTUs (3,072). This high number of OTUs found in the salt samples was also translated into greater biodiversity, as shown either by Chao1, Simpson, Shannon, or Margalef indexes. Despite the rarefaction curves analysis, around 97% of Good's coverage values were obtained for all the samples, which indicated a satisfactory coverage of bacterial diversity.

Krona plots show the relative abundance of taxa at class level for the different group of samples (Figure 1). *Bacilli* was the dominant bacteria class in the fermented dressings with an average proportion of 64.29% of the sequences, followed by *Gammaproteobacteria* (22.84%) (Figure 1 upper panel). The rest of sequences in this group belonged to minority classes. In the case of herbs, the 61.49% of the total sequences was covered by the *Gammaproteobacteria* class, followed by *Alphaproteobacteria* (20.62%). *Bacilli* class was only present in the 3.90% of the sequences in this type of sample, similar to the *Actinobacteria* class (5.86%) (Figure 1 middle panel). Referring to the salt samples, *Cytophagia* and *Halobacteria* classes predominated with the 30.77 and 21.77 % of the sequences, respectively. Other classes appeared to a lesser extent, such as *Alphaproteobacteria* (12.50%), *Deltaproteobacteria* (7.60%) or *Gammaproteobacteria* (8.61%) (Figure 1, bottom panel).

The Venn diagram (Figure 2a) shows that a total of 55 OTUs (36.4%) were shared among the three groups of samples at class level. Salt group shared the same number of OTUs with both, fermented and herb groups (16 OTUs; 10.6%). Meanwhile, fermented dressing and herb groups shared the lowest number of OTUs (5 OTUs;

3.3%). On the other hand, whereas only a single OTU (non-shared by any other group) appeared in fermented and herbs groups, 57 OTUs (37.7%) showed to be unique in the salt group. Figure 2b shows the unique and shared OTUs between samples after selecting those with more than 1% representation from all OTUs in at least one of the groups. None of the groups showed unique OTUs with more than 1% representation. *Bacilli*, *Halobacteria*, *Flavobacteria*, *Cytophagia*, *Gammaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, and *Clostridia* classes were shared by all groups. Meanwhile, *Alphaproteobacteria*, *Actinobacteria*, and *Sphingobacteria* classes were shared exclusively between fermented dressing and salt groups. Lastly, no OTUs with high representation were shared between salt and fermented groups or salt and herbs groups. Table S1 (supplementary material) shows the total OTUs assigned at class level, shared and unique among the three groups of samples. Specifically, *Cytophagia*, *Bacilli*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Sphingobacteria*, and *Halobacteria* classes were found to be over-represented in these communities (Figure 3 and Figure S1 in supplementary material) being the only classes with high representation that showed significant differences among the three groups. Box-and-whisker plot showing the difference in mean proportions among the different groups is shown in Figure 4. Significant differences were found between salt samples compared to the other groups. This was due to a higher presence of *Cytophagia*, *Sphingobacteria*, *Deltaproteobacteria*, and *Halobacteria* classes. No significant differences in the presence of these classes were found between fermented dressing and herb groups. However, the presence of *Bacilli* class in fermented group was highly significant in this group respecting to salt and herbs group. The difference in the proportion of sequences of the class of *Bacilli* between salt and herbs was not statistically significant. Finally, the proportion of *Gammaproteobacteria* was statistically higher in herbs group

respecting fermented and salt groups. No significant differences in the proportion of *Gammaproteobacteria* were found between fermented and salt groups.

Figure 5 shows the relative abundance of sequences (%) to genus or family level by specific samples, after filtering OTUs with less of 1% of representation in at least one of the samples. *Pseudomonas* genus and *Enterobacteriaceae* family predominated in most samples of herbs. The first one showed 40.04% of sequences in thyme, 22.29% in oregano, and 20.58% in fennel. The non-assigned genus was majority in the fennel samples with 50.62% of the sequences. In thyme and oregano the representation of this genus was 13.44 and 19.35% of sequences, respectively. *Pseudomonas* was also found in fermented dressing samples in less proportion. However the genus of *Enterobacteriaceae* family appeared in a relative high proportion in pepper paste (12.49%) and garlic (9.52%). *Sphingomonas* genus was also found exclusively in thyme (8.91%) and oregano (14.77%) samples. The presence of 5.55% of *Vibrio* genre sequences in pepper is noteworthy. *Lactobacillus*, *Weisella* and *Pediococcus* were the predominant genera in the fermented dressing samples, except in the sample of pepper paste, in which *Pediococcus* genus was only found in 0.38% of sequences. The proportion of sequences of *Lactobacillus*, *Weisella* and *Pediococcus* genera in pepper was 27.06, 25.89 and 21.33% respectively, and 22.88, 26.27 and 18.86% in garlic. The lack of presence of *Pediococcus* sp. in pepper paste was complemented by other OTUs with less of 1% of the total sequences and a higher proportion of the non-assigned genus of *Enterobacteriaceae*. A higher biodiversity of OTUs with less of 1% of representation was found in salt samples. *Salinibacter* was the most representative genus, with 54.87 and 34.49% of sequences in spring and marine salt samples, respectively.

3.2. Multivariate analysis

Principal component analysis (PCA) showing the clustering of metagenomes of the diverse samples, suggests a right selection of data for each group. PCA generated mainly two components. The first and second principal components (PC1 and PC2) explained 80% of the total variability of the data set. PC1, which explained 45.3% of the variance had the highest positive loading of herbs samples, and PC2 (34.7% of the variance), had the highest positive loading of fermented dressing and salt sample. Similar positive loading of fermented dressing samples was shared by the two components. A clear cluster of communities of fermented, herb and salt samples was observed. However, two of the herbs group samples (Oregano, Thymus) were found to be closer to salt samples.

4. DISCUSSION

New generation sequencing techniques, based on independent culture techniques, have meant a revolution for the knowledge of the microbiota present in foods. Until now, the classical methodology only allowed us to know the presence of some cultivable microbial groups, what has kept us blind to the real biodiversity understanding of a given sample. The current challenge is not only to determine the presence or absence of the microorganisms in a sample, but to clarify the role that they could develop in relation to quality and safety aspects. The initial data reveal the great difference in the bacterial population observed among the three types of samples analyzed (fermented dressings, aromatic herbs, and salts). We must bear in mind that the methodology used allows us to determine the presence of DNA of the species in the samples, but this is not a guarantee of the metabolic activity of the microorganisms. With the NGS procedure analyzed, we do not have the certainty that the microorganisms are alive or dead. However, high proportions of DNA sequences

suggest that at some point microorganisms have been present in high concentrations, indicating a possible important role in terms of activity that may represent a risk for the product in the case of the presence of genera with pathogenic species or strains, also due to the presence of possible toxins produced by them.

Pseudomonas genus and *Enterobacteriaceae* family represent the two major groups of saprophytic bacteria found on the surface of freshly harvested plants (Blackburn et al., 2006). The high presence of both groups of microorganisms in the aromatic herbs analyzed in this work is not surprising. *Pseudomonas* genus is considered as ubiquitous due to its metabolic diversity and genome plasticity (Madigan et al., 1997). In general, species belonging to this genus are innocuous to humans but there are also opportunistic pathogens for humans and plants, such as *P. aeruginosa* and *P. syringae*. Several species of this group can cause food spoilage. (Raposo et al., 2016). The presence of *Pseudomonas* genus during fermentation of *Aloreña de Málaga* table olives has been previously described (Abriouel et al., 2011; Medina et al., 2016). However, no information about the origin was given. Our data could explain the presence of this genus in table olives, since these condiments are common ingredients in the elaboration of *Aloreña de Málaga* packaging. On the other hand, the *Enterobacteriaceae* family, although its presence does not necessarily imply a risk, it contains a large number of pathogenic species, such as *E. coli*, *Salmonella* sp. or *Erwinia* sp., among others. The abundance of *Enterobacteriaceae* sequences in the group of herbs was high, especially in fennel, which has been previously reported as a carrier of these microorganisms (Sospedra et al., 2010).

Weissella, *Pediococcus* and *Lactobacillus* are some of the typical genera commonly found in fermented foods (Erten et al., 2017). Our results support the

presence of these microorganisms as the most representative genera in samples of fermented dressing. All of them belong to the group of bacteria generally known as lactic acid bacteria. Apart from a perceived technical role of certain *Weissella* species involved in such traditional fermentations, specific strains are also receiving attention as potential probiotics (Fusco et al., 2015). *Weissella* genus has been reported commonly in fermented food, such as *W. koreensis*, the dominant specie in the fermentation of Kimchi, a fermented food made with red pepper powder and garlic, among other ingredients (Kang et al., 2015). These researchers also reported that red pepper powder had a strong influence in the abundance of these species. On the other hand, while some *Lactobacillus* species have been described as spoilage microorganisms in some fermented food (Andreevskaya et al., 2018; Gram et al., 2002). It is known that most species of *Lactobacillus* and *Pediococcus* play an important role in the processes of conservation and fermentation of food by lowering the pH and producing bacteriocins, which may prevent the growth of pathogenic and spoilage microorganisms (Fontana et al., 2005; Papagianni and Anastasiadou, 2009; Sivakumar et al., 2010)

Vibrio sp. was found in a high abundance in one of the samples of pepper. Several species of this genus have been described as pathogenic, causing diseases of the digestive tract (Jones et al., 2017). Recently, Benítez-Cabello et al. (2016) reported the presence of *Vibrio vulnificus* and *Vibrio* sp., through RT-PCR-DGGE technique in both, brines and fruits during table olive processing. Isolates from *Vibrio furnissii* and *Vibrio fluvialis* were also found by Lucena-Padrós et al. (2014) during the initial stage of the table olive fermentation; they were reported as human pathogens, causing acute gastroenteritis (Ballal et al., 2017; Ramamurthy et al., 2014).

Despite the intrinsic restrictive conditions of salt, our results revealed a high bacterial diversity in this group of samples. The genus *Salinibacter* was the most representative in salt samples and was described for the first time by Antón et al. (2002), being *S. ruber* the only specie of this genus. Recently, Medina et al. (2016) described the presence of the genus during the *Aloreña de Málaga* table olive processing in brine and fruit samples. Lucena-Padrós and Ruiz-Barba (2016) studied the diversity of halophilic bacteria in Spanish-style green table-olive fermentations, revealing the presence of *Amphibacillus*, *Natronobacillus*, *Catenococcus* and *Streptohalobacillus* genera among others, but, no evidence of the presence of *Salinibacter* was reported. This work elucidated that the salt used for preparing the cover brines could be the possible origin of *Salinibacter* in table olives.

5. Conclusions

The use of seasoning and salt material may involve the entry of a high microbial load directly into the packaging of the olive, including spoilage microorganisms that may alter the stability of the table olive packaging.

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Figure legends

Figure 1. Krona plots showing the relative abundance of taxa (%) at class level for fermented dressing (upper panel), herbs (middle panel) and salts (bottom panel) groups.

Figure 2. Venn diagrams showing the total number of unique and shared bacterial OTUs at class level **(a)** and unique and shared bacterial class with percentage >1 representation in at least one of the groups **(b)**, between fermented dressing samples (F), salt (S), and herb (H) groups.

Figure 3. ANOVA Tukey-Kramer post hoc comparison test executed with STAMP v2.0.1 to elucidate significant differences for *Cytophagia* class in the diverse group of samples. Significant differences were only considered for $p \leq 0.05$ and a q -value below 0.3. Meaning of symbols: Cross, star and horizontal line inside box denoted for outlier, mean, and median of the percentage of sequences obtained. F, fermented dressing group; H, herbs group; S, salt group of seasoning.

Figure 4. Comparison of bacterial community profiles at the levels of class between OTUs (defined at >97%) which showed significant differences and only present in appreciable numbers within communities sampled from seasoning groups. Analysis was performed using STAMP and significant differences in taxa were only considered below a p -value of 0.05 and a q -value below 0.3. Percentage of mean proportion of taxa by groups (left) and difference in the mean proportion (right) are illustrated.

500 Figure 5. Relative abundance of sequences (%) to genus or family levels obtained by
501 metataxonomic analysis from thymus (T), oregano (O), fennel (Fn), pepper (P),
502 pasta pepper (PP), garlic (G), marine salt (SM) and salt spring (SS) samples.

503 Figure 6. PCA plot comparing class-level taxonomic profiles of the different group of
504 samples (F, fermented dressing group; H, herbs group; S, salt group).

Table 1. Number of assigned sequences and OTUs at class level (after chloroplast and mitochondria removal), alpha diversity indices and estimated sample coverage for 16S amplicons (bacteria) according to the group of samples¹ and single samples²

Sample reference	chao1	PD_whole_tree	shannon	simpson	margalef	dominance	Counts/sample	observed_otus	goods_coverage
S (n=7)	3892.07	109.55	6.32	0.92	137.92	0.08	150034.21	6925.42	0.97
F (n=7)	3444.37	51.63	4.40	0.80	81.29	0.20	71647.11	3072.89	0.97
H (n=5)	3695.20	54.79	5.45	0.89	110.05	0.11	142579.17	6744.83	0.96

¹S-Salt, F-Fermented dressing, H-Herbs.

Type of sample	chao1	PD_whole_tree	shannon	simpson	margalef	dominance	Counts/sample	observed_otus	goods_coverage
SM (n=4)	4920.65	116.21	7.31	0.97	166.54	0.03	150772.75	8818.50	0.96
SS (n=3)	2863.49	102.89	5.34	0.88	109.30	0.12	149295.67	5032.33	0.98
G (n=3)	2877.99	50.40	4.76	0.83	79.29	0.17	54753.33	2427.67	0.97
P (n=2)	3725.88	56.81	3.74	0.74	87.57	0.26	96103.50	4160.00	0.97
PP (n=2)	3729.25	47.69	4.70	0.83	77.02	0.17	64084.50	2631.00	0.97
O (n=2)	2907.21	68.01	6.01	0.94	115.61	0.06	94946.50	4083.50	0.97
T (n=2)	4703.38	56.30	5.74	0.90	118.83	0.10	212358.00	10462.00	0.96
Fn (n=1)	3475.01	40.05	4.62	0.82	95.70	0.18	120433.00	5689.00	0.96

²SM-marine salt, SS-spring salt, G-garlic, P-pepper, PP-pepper paste, O-oregano, T-thymus, Fn-Fennel.

^aAlpha diversity metrics were obtained after rarefaction of the samples to 11400 sequences.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6