Original Research

Study of Microbial Diversity in Fresh Ingredients and Their Wastes

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Abstract

With economic development, the food industry and people's nutrition have experienced new growth. Food ingredients, as core elements of the diet, play an essential role in the food industry, but their microbial diversity and associated waste remain understudied. This study selected vegetables, fruits, seafood, meat, and their associated waste, commonly found at farmers' markets, for analysis using high-throughput techniques. It was found that *Pseudomonas*, *Pantoea*, *Psychrobacter*, and *Acinetobacter* were common bacteria in the samples. Meanwhile, methanogens and nitrogen-cycling archaea, such as *Methanobrevibacter*, *Methanomicrobium*, and *Candidatus Nitrocosmicus,* dominated the samples. However, the archaea showed no significant similarity between the samples. Further analysis revealed no significant differences between the microbial structures of fresh ingredients and their wastes, but more pronounced differences were observed between the microbial structures of fruits and vegetables and their respective wastes. Nitrogen-cycling archaea dominated fruit and vegetable wastes, whereas methanogens predominated in seafood and meat wastes. The co-fermentation process of fruit, vegetable, and meat wastes may facilitate C and N removal in the future.

Keywords: fresh ingredients, food waste, bacterial diversity, archaeal diversity, waste resource utilization

Introduction

With the development of the economy, food safety is becoming an essential basis for safeguarding the health of citizens [1]. Ingredients are generally classified as vegetables and meat, while fruits and seafood, as complementary categories, are often analyzed and studied separately. Fresh ingredients provide rich nutrients for the human body but also create opportunities for microbial growth, including therapeutic and opportunistic pathogenic bacteria that threaten human health [2]. Therefore, the freshness of

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ingredients is an important factor in food quality and safety, protecting human health from microorganisms.

Presently, research on the microbial diversity of food ingredients mainly focuses on pathogenic and opportunistic pathogens [2], particularly on detecting microbial indicators at different stages of industrial production [3]. However, studies on the microbial diversity of food ingredients and their wastes remain insufficient. The microbiological structure of different ingredients varies due to their diverse sources, leading to differences in the pathogenic microorganisms they contain. Meanwhile, food waste from everyday life and industrial production is often improperly disposed of [4]. Direct landfilling of food waste increases the supply of N and P to the soil [5], reducing soil oxygen content, which provides a favorable environment for pathogenic bacteria to multiply on a large scale, potentially leading to epidemics. Even harmless treatment increases costs and energy consumption, hindering the sustainable and green disposal of ingredient waste. Therefore, studying the microbial diversity of ingredients and their wastes provides essential data to support the elimination of pathogenic bacteria and the safe treatment of wastes.

The main purpose of food waste treatment is to reduce the content of N, P, and organic matter and to eliminate harmful microorganisms [5, 6]. At present, microbial treatment is the most promising method for utilizing food waste resources [6], but industrialization has been slow due to the different microorganisms required for the variety of ingredients [7]. Native microorganisms associated with the biodegradation of food waste have better environmental adaptability [8], reducing the potential impact of microorganisms on environmental safety. The residues after waste degradation can be used for fertilization or environmental modification to achieve green and sustainable elemental recycling and energy flow [9].

In this study, common meats, seafood, fruits, vegetables, and their corresponding wastes from farmers' markets were selected as research objects. The diversity of bacteria and archaea in the samples was detected using high-throughput analysis and analyzed with PCoA and Venn to clarify the distribution characteristics of strains in different ingredients and the factors influencing them. The results of the study will provide data to support the resource treatment of ingredient waste and the removal of pathogenic microorganisms.

Material and Methods

Food Waste Collection

Food waste samples were collected in 1.5 mL sterile centrifuge tubes and labeled on the outside with information. The categories included Fresh Seafood (FS), Fresh Fruit (FF), Fresh Vegetables (FV), Fresh Livestock Meat (FLM), Fruit Waste (FW), Vegetable Waste (VW), Seafood Waste (SW), and Livestock Meat Waste (LMW), which were collected from daily meals on 4 January 2023. All tubes were placed in a -20°C incubator and quickly transported to the Qing University of Science and Technology lab, where they were frozen and stored in -80°C freezers for microbiological testing.

DNA Extraction and Sample Delivery

High-Throughput Microbial Screening: according to the standard protocol, sample DNA was extracted using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, CA). PCR was performed using archaeal primers AR109F/AR915R and bacterial primers BA27F/BA907R, targeting the V4-V5 region of 16S rRNA. The reaction parameters were as follows: pre-denaturation at 94°C for 2 min; 94°C denaturation for 30 s; annealing at 55°C for 30 s. The reaction lasted for 25 cycles at 72 °C for 1 min. PCR products were detected via gel electrophoresis, then excised and purified for high-throughput sequencing. The library was prepared using the TruSeq Nano DNA LT Library Prep Kit from Illumina. The End Repair Mix2 kit was used to excise the base protruding at the 5′ end of DNA, complete the 3′ end, and add a phosphate group to the 5′ end. Sequencing was performed on the NGS platform Illumina using the NovaSeq instrument.

The Quantitative Insights Into Microbial Ecology (QIIME) version 1.7.0 pipeline (http://www.qiime.org) was used to process raw sequencing data with the default parameters. Briefly, representative sequences from each OTU were defined by a 97% identity threshold, after which chimeric and low-quality reads were removed. Using the Ribosomal Database Project (RDP) classifier, the taxonomic classification of each OTU was assigned. The average relative abundance (%) of the predominant genus-level taxonomy in each sample was assessed by comparing the assigned sequence number of a particular taxon to the total obtained sequence number. To clarify microbial community differences, PCoA and Venn were computed using R.

Results and Discussion

The Bacterial Community in Food Waste

In the analysis presented in Fig. 1, the microbial composition on the surfaces of fresh vegetables was predominantly characterized by *Pseudomonas* (45.42%), *Staphylococcus* (16.52%), *Acinetobacter* (14.45%), and *Erwinia* (12.39%). In contrast, the microbial communities on vegetable waste were primarily composed of *Pectobacterium* (27.25%), *Pseudomonas* (25.27%), and *Pantoea* (9.42%). The surfaces of fresh fruits were predominantly colonized by *Rosenbergiella* (67.10%) and *Pantoea* (17.59%), whereas fruit waste was dominated by *Kocuria* (58.44%) and *Massilia* (16.19%). Fresh seafood samples were overwhelmingly colonized by *Psychrobacter* (79.50%), while in seafood waste,
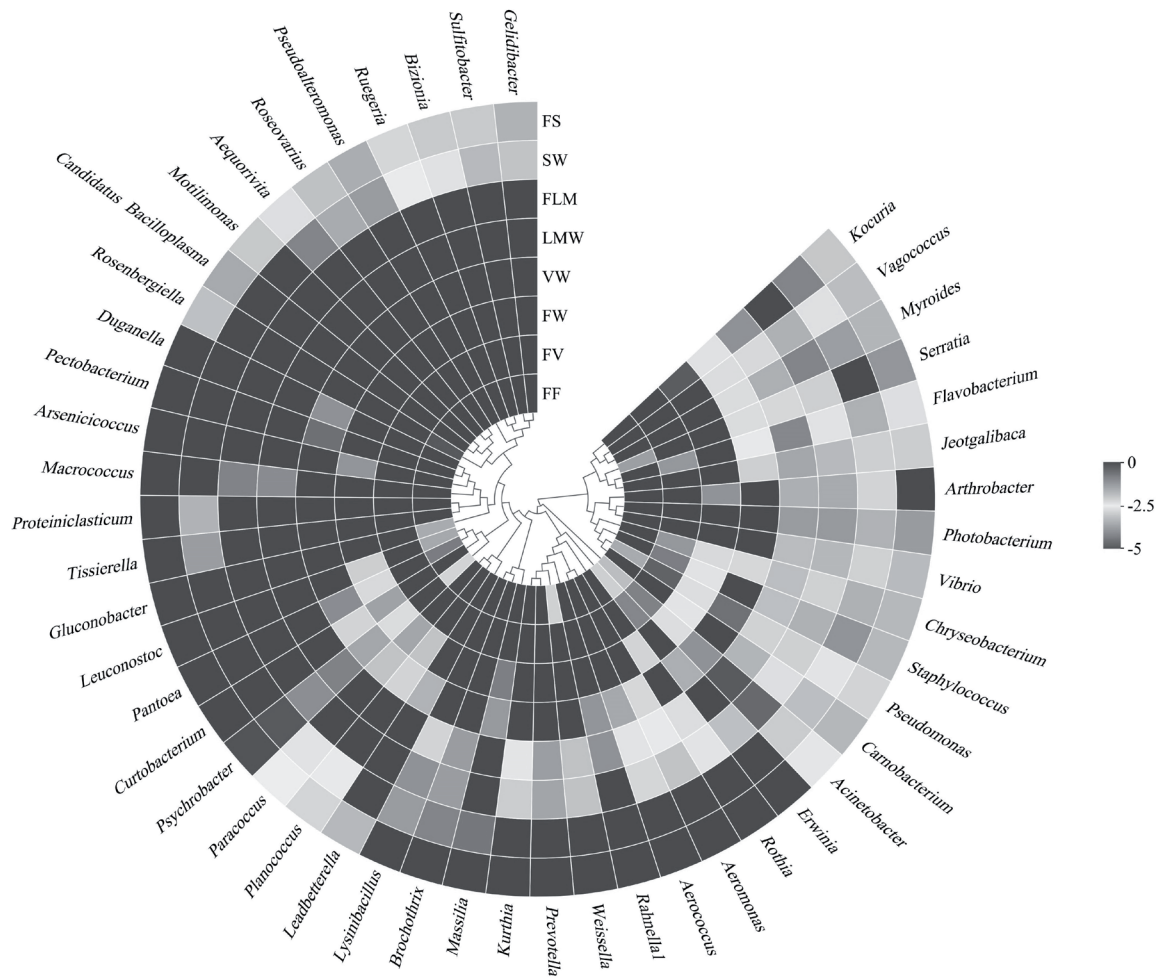
Fig. 1. Relative abundance of bacteria.
Note: The raw data is plotted by taking the logarithm of lg.

Psychrobacter (64.64%) and *Aequorivita* (13.21%) were the dominant species. Livestock meat was primarily inhabited by *Acinetobacter* (36.68%), *Macrococcus* (14.06%), and *Myroides* (13.18%), and the waste was similarly dominated by *Acinetobacter* (62.43%), *Macrococcus* (9.56%), and *Psychrobacter* (14.56%). The results showed a clear continuity of bacteria between fresh seafood, meat, and their wastes, which were close in species and relative abundance. Fresh vegetables and their wastes had similar bacterial communities, except for *Pectobacterium* (27.25%). In contrast, the bacterial communities between fresh fruits and their wastes were very different. This suggests that fruit wastes, rich in sugars and nutrients, became an excellent medium, allowing microorganisms to multiply rapidly in the trash.

The high-throughput analyses showed that the microbial species in the vegetable, fruit, meat, and seafood samples were not uniform, and there was some continuity in microbial species between the ingredients and their wastes. Among the bacteria, *Pseudomonas* was present in both vegetables and their wastes and is a common microorganism associated with lung infections [10]. Additionally, *Acinetobacter* was detected in

both and is known as a common drug-resistant gram-negative bacterium [11]. Of these, *Staphylococcus* and *Erwinia* were found in fresh vegetables with high relative abundance (>10%) as human and plant pathogens, respectively [12, 13]. *Pectobacterium* was widely present in waste as a plant pathogen [14] but was below the detection limit in fresh vegetables. As a result, the prolonged placement of fresh vegetables and the high intensity of human traffic led to an increase in pathogenic microorganisms on the surface layer of the vegetables [15], as well as some phytopathogenic organisms due to the growing environment. In contrast, the waste placed in the bins was isolated from human traffic, thereby removing the source of contact and providing survival material, which created conditions conducive to the growth of vegetative pathogens. This resulted in a decrease in human pathogens and an increase in vegetative pathogens. Unlike vegetables and their waste samples, which were very similar, fruits and their waste were more distinct. *Rosenbergiella*, a genus of gram-negative bacteria that can be isolated from the nectar of fresh fruit [16], suggests that bees may play a role in the pollination process of this fruit. *Pantoea*, on the other hand, is a widespread and
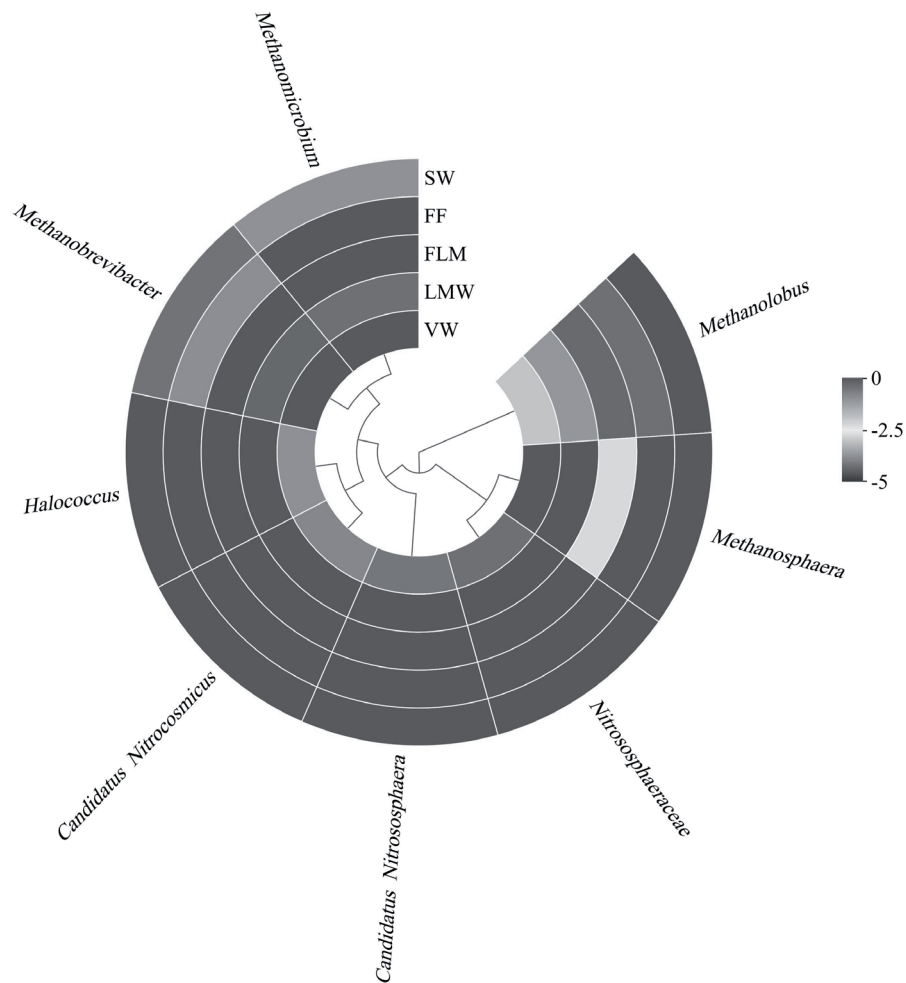
Fig. 2. Relative abundance of archaea
Note: The raw data is plotted by taking the logarithm of lg.

diverse genus of bacteria that plays a beneficial role in medicine [17], bioremediation, and pest control, although it is primarily responsible for conditions such as plant wilting. *Kocuria*, a genus of gram-positive cocci found in fruit wastes [18], is widespread in various environments and is partially pathogenic. *Massilia*, on the other hand, is a functional and widespread gram-negative bacterium that shows significant potential for ecological remediation [19]. In contrast, fruits and their wastes contain fewer pathogenic bacteria and are mostly composed of widespread genera, highlighting notable differences between vegetables and fruits in their growing and trading environments. The differences between fruits and their waste were primarily due to the release of nutrients, such as fructose and vitamins, from damaged fruit. These nutrients provided a food source for microorganisms in the waste bin [20], causing the microbial community on the surface of the fruit skin to no longer be dominant.

The main microorganisms in seafood and its waste were *Psychrobacter*, gram-negative bacteria widespread in various environments [21], including fish, animal skin, and intestines, with some genera exhibiting pathogenic potential. *Aequorivita*, a common gram-

negative genus with some salt tolerance [22], was found in higher relative abundance in waste than in fresh samples, suggesting it may have potential degradative capacity. *Acinetobacter* was present at a high relative abundance in meat and its waste. As a common human pathogen [23], its presence may be linked to higher foot traffic at farmers' markets, although its high abundance also suggests it may originate during animal husbandry. *Macrococcus* is a rare gram-positive bacterium found in mammals, and the pathogenic effects of this genus on humans have not been reported [24]. *Myroides* is a genus of low pathogenicity and environmental origin that primarily affects immunocompromised individuals and is resistant to antibiotics [25].

Seafood and meat, as protein sources, have a higher zoonotic potential for pathogenic bacteria, with livestock being the most likely source. Seafood shows a higher degree of generic concordance with meat and their wastes compared to vegetables and fruit. This may be because meat consists primarily of proteins and fats, with the epidermis mainly composed of phospholipids and proteins. In contrast, the inner skin of vegetables and fruit is rich in nutrients [26], and compression
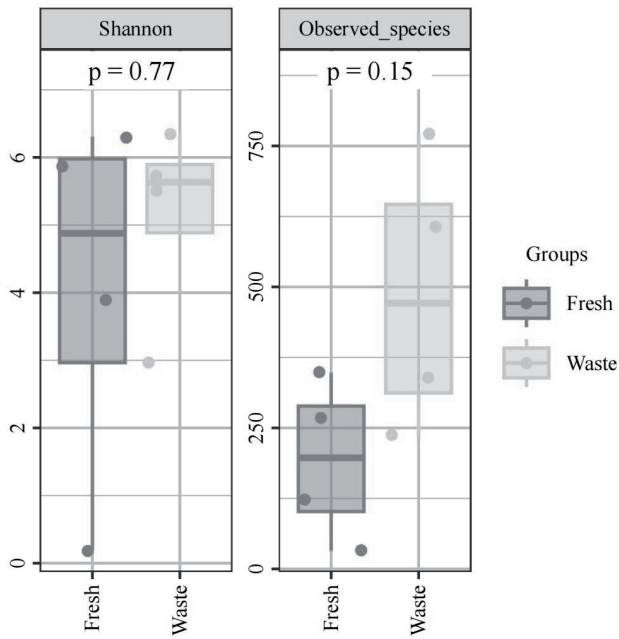
Fig. 3. Boxplot of bacteria classified by fresh and waste.

after disposal leads to the exudation of these nutrients, providing energy for microorganisms in the waste bins.

The Archaeal Community in Food Waste

As can be seen in Fig. 2, the levels of archaea in fresh seafood and vegetables and fruit waste were below the detection limit, with *Methanobrevibacter* (99.37%) dominating fresh meat and *Methanobrevibacter* (58.78%) and *Methanomicrobium* (41.20%) dominating waste. Fresh fruit was dominated by *Candidatus Nitrocosmicus* (99.98%). Seafood waste was dominated by *Methanolobus* (99.95%), while vegetable waste was dominated by *Nitrososphaeraceae* (41.42%). *Candidatus Nitrososphaera* (30.02%), *Candidatus Nitrocosmicus* (16.37%), and *Halococcus* (10.95%) were also dominant. These results indicate that the species and abundance of archaea were low in fresh ingredients, especially seafood and vegetables. Archaea in fruit waste were also below the detection limit, suggesting that archaea may be less adapted to this environment. This finding aligns with the observation that there were fewer archaeal species than bacteria. Meanwhile, the higher number of archaeal species in vegetable waste may be attributed to its rich nutrients, which provide an opportunity for archaeal growth. Different types of methanogens accounted for
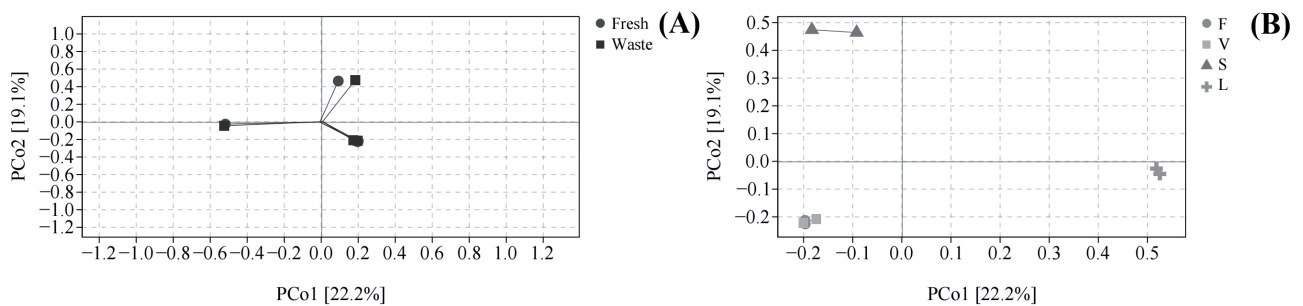

Fig. 4 . PCoA analysis of bacterial community in food waste classified by freshness (A) and types (B).
Note: Fresh: Fresh Seafood, Fresh Fruit, Fresh Vegetables, Fresh Livestock Meat; Waste: Fruit Waste, Vegetable Waste, Seafood Waste, Livestock Meat Waste. V: Fresh Vegetables, Vegetable Waste; S: Fresh Seafood, Seafood Waste; F: Fresh Fruit, Fruit Waste; L: Livestock Meat, Livestock Meat Waste.
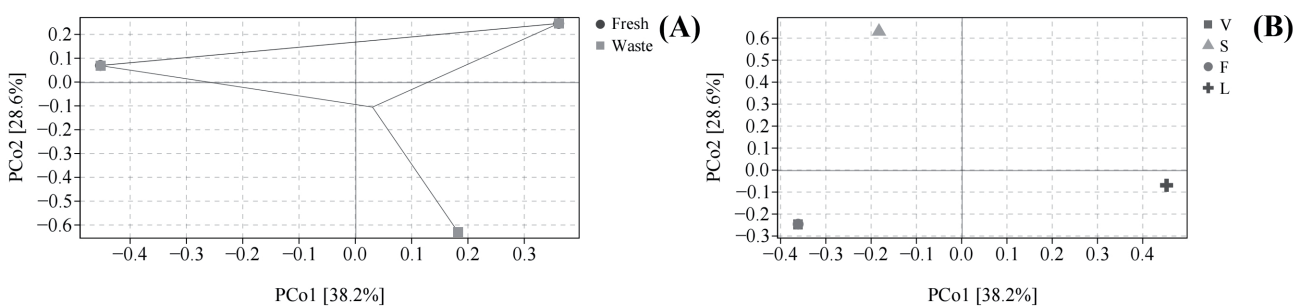

Fig. 5. PCoA analysis of archaeal community in food waste classified by freshness (A) and types (B).
Note: Fresh: Fresh Seafood, Fresh Fruit, Fresh Vegetables, Fresh Livestock Meat; Waste: Fruit Waste, Vegetable Waste, Seafood Waste, Livestock Meat Waste. V: Fresh Vegetables, Vegetable Waste; S: Fresh Seafood, Seafood Waste; F: Fresh Fruit, Fruit Waste; L: Livestock Meat, Livestock Meat Waste.

Fig. 6. Venn analysis of bacterial community in food waste classified by samples (A), freshness (B) and types (C).
Note: Fresh Fruit (FF); Fresh Livestock Meat (FLM); Vegetable Waste (VW); Seafood Waste (SW); Livestock Meat Waste (LMW). Fresh: Fresh Seafood, Fresh Fruit, Fresh Vegetables, Fresh Livestock Meat; Waste: Fruit Waste, Vegetable Waste, Seafood Waste, Livestock Meat Waste. V: Fresh Vegetables, Vegetable Waste; S: Fresh Seafood, Seafood Waste; F: Fresh Fruit, Fruit Waste; L: Livestock Meat, Livestock Meat Waste.
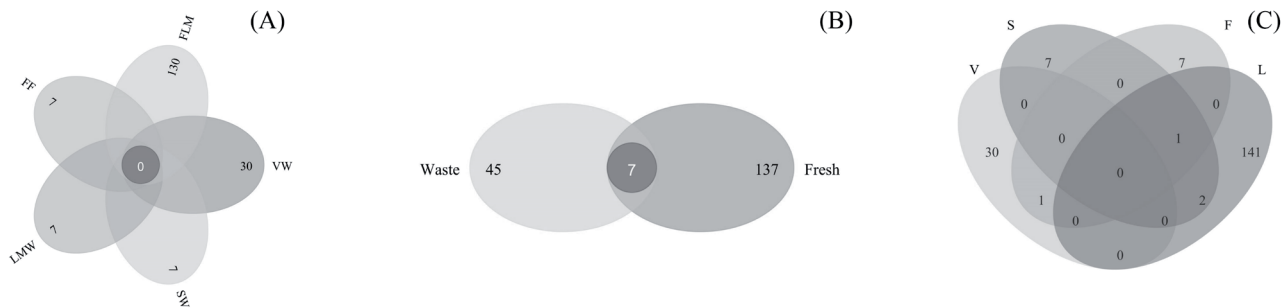

Fig. 7. Venn analysis of archaeal community in food waste classified by samples (A), freshness (B), and types (C).
Note: Fresh Fruit (FF); Fresh Livestock Meat (FLM); Vegetable Waste (VW); Seafood Waste (SW); Livestock Meat Waste (LMW). Fresh: Fresh Seafood, Fresh Fruit, Fresh Vegetables, Fresh Livestock Meat; Waste: Fruit Waste, Vegetable Waste, Seafood Waste, Livestock Meat Waste. V: Fresh Vegetables, Vegetable Waste; S: Fresh Seafood, Seafood Waste; F: Fresh Fruit, Fruit Waste; L: Livestock Meat, Livestock Meat Waste.

a greater proportion of seafood, livestock, and meat waste, suggesting that these wastes have high potential for methanogenesis.

In terms of archaea, vegetable waste was dominated by the nitrogen cycle-related archaea *Nitrososphaeraceae* [27], *Candidatus Nitrososphaera* [28], *Candidatus Nitrocosmicus*, and the salt-tolerant archaea *Halococcus* [28, 29]. In contrast, the surface of fresh fruit was dominated by the nitrogen cycle-related archaea *Candidatus Nitrocosmicus* [28]. This phenomenon is probably due to nutrient leaching from the waste, which is inhibited by the relative cleanliness of the fresh fruit surface [26]. Meat and its wastes were dominated by the hydrotrophic *Methanobrevibacter* [30], with the relative abundance of the hydrotrophic *Methanomicrobium* also being higher in the wastes [31], whereas seafood wastes were dominated by the methylotrophic *Methanolobus* [32].

The above results indicate that the degradation process of vegetable and fruit wastes is mainly based on the nitrogen cycle [33], while the degradation of meat wastes primarily relies on anaerobic processes [34]. Therefore, the resource utilization process for fruit and vegetable wastes should focus on nitrogen (N)

removal, while the resource utilization of meat wastes should focus on carbon (C) removal. This suggests that the resource utilization of fruit, vegetable, and meat wastes may be complementary, with the carbon source from meat providing energy to facilitate nitrogen removal from fruit and vegetable wastes. However, the final electron acceptor or oxygen supply in this process deserves further investigation.

The Analysis of Microbial Community in Food Waste

As shown in Fig. 3, the differences in Shannon and observed species of bacteria between fresh ingredients and waste were insignificant. For archaea (Table 1), boxplot analysis was not possible because some samples were not detected. Among the detected samples, meat had the highest observed species count at 136, while the Shannon was lower than that of vegetable waste (3.54), at 3.25. The above results indicate that the microbial content of food ingredients in the market is not high, and even in the wastes, the number of bacterial colonies remains low due to the short time period [35].

Table 1 Observed species and Shannon of archaea.

Sample	Observed species	Shannon
VW	30.90	3.54
SW	9.20	0.03
LMW	13.80	2.25
FF	8.70	0.14
FLM	136.00	3.25

Note: Fresh Fruit (FF); Fresh Livestock Meat (FLM); Vegetable Waste (VW); Seafood Waste (SW); Livestock Meat Waste (LMW).

The PCoA analysis shows that the explained amount of variation in bacterial differences between fresh ingredients and waste is 41.3%, but the source of the difference is not freshness. Instead, the explained variation of inter-bacterial differences was 41.3% according to the nature of the ingredients themselves, with differences in the sample sources reflected in the second principal axis (19.1%). However, the differences between fruits and vegetables could not be distinguished. For archaea, the explained variation between samples was 66.8%, and the source of variation was not freshness but primarily the nature of the ingredients themselves. Except for vegetables and fruit, the differences caused by the ingredients were reflected in the first and second principal axes. The above results suggest that the microbiological diversity of ingredients in the market is primarily due to the characteristics of the ingredients rather than the degree of freshness [36].

The figure shows no common OTUs for bacteria across all samples, and the number of bacterial OTUs in waste is higher than in fresh ingredients of the same type. The number of shared OTUs between fresh ingredients and waste was 181, with fewer OTUs in fresh ingredients and more in waste. Among the ingredients, seafood had the highest number of OTUs (796), while fruit and its waste had the lowest number (353). For archaea, there were no common OTUs between samples. The number of shared OTUs between fresh ingredients and waste was 7, with fewer OTUs in waste. Among the ingredients, meat had the highest number of OTUs (144), while seafood and fruit had the lowest number (8). These results suggest that the type of ingredient has a significant influence on bacterial OTUs. Furthermore, the higher number of bacterial OTUs in waste compared to fresh ingredients may be attributed to the microorganisms present in the bin and the stuffy environment [37].

Based on the boxplot analysis, it was found that the difference between fresh ingredients and their waste was not significant in terms of bacterial diversity and types. According to the PCoA analysis, freshness is not the core factor affecting the structure of bacteria and archaea; instead, the type of ingredients themselves is the primary determinant [38, 39]. This result may be due to the timely cleaning of the waste bins. Regarding diversity analysis, different genera of bacteria were identified in the fresh ingredients and their waste, which may be attributed to the lack of waste removal. According to the Venn analysis, the number of common OTUs between the ingredients was low, and the bacterial OTUs in the waste were higher than those in the fresh ingredients, but the difference was insignificant. However, the waste provided rich nutrients for the bacteria and inhibited the growth of the archaea [7].

Conclusions

In conclusion, *Pseudomonas*, *Pantoea*, *Psychrobacter*, and *Acinetobacter* were the common bacteria found in the samples. Meanwhile, methanogens and nitrogen cycle archaea, such as *Methanobrevibacter*, *Methanomicrobium*, and *Candidatus Nitrocosmicus*, dominated the samples. However, archaea showed no significant similarity between the samples. There were no significant differences in the microbial structures of fresh ingredients and their wastes, although greater differences were observed in the microbial structures of fruits and vegetables and their wastes.

In the future, more emphasis will be placed on detecting and analyzing detailed information on microbial communities in food waste, including the macro-genome, transcriptome, and proteome. At the same time, more advanced methods of colony isolation will be developed based on microbial information to isolate functional strains for food waste resource utilization.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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