Investigation on sweaty off-flavors in small mill sesame oil and its formation mechanism via odor-screening and secondary verification strategy

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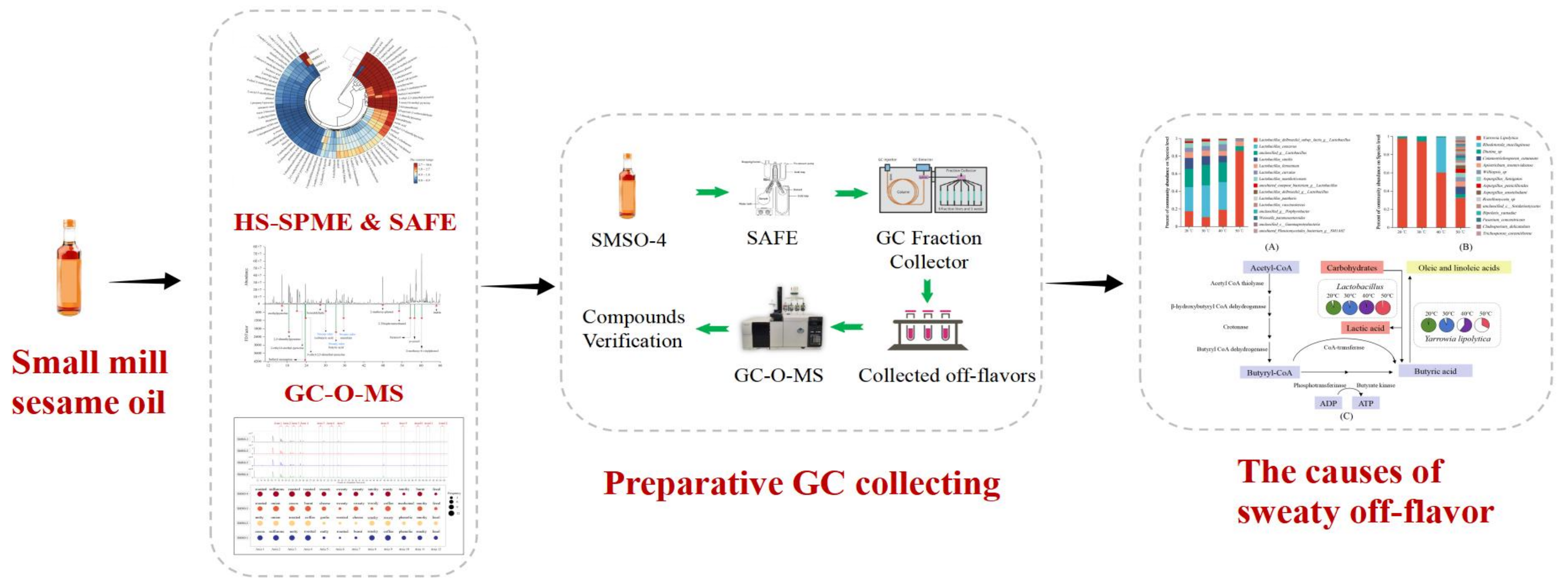
Introduction

Sesame oil, a globally valued product with an annual production nearing 6.8 million tonnes, is celebrated for its health benefits and unique aroma. The traditional aqueous extraction method yields small mill sesame oil (SMSO) with a superior flavor. However, suboptimal production, particularly in summer, can lead to an undesirable sweaty off-flavor, likely due to microbial activity during the prolonged sedimentation process. By combining sensory analysis (GC-O) with advanced chemical isolation (prep-GC), we identified key sweaty odor compounds. Furthermore, we analyzed the microbial communities in sesame residue at different temperatures to understand their role in producing these off-flavors. Our findings clarify the potential mechanisms behind the formation of sweaty off-flavors, offering a scientific basis for quality improvement in SMSO production.

Methods

- Aroma Extraction: Volatile flavors were extracted using HS-SPME and SAFE.
- Odor-Screening: Compounds were screened and identified using GC-O-MS.
- Off-Flavor Verification: Sweaty off-flavors were isolated and verified by preparative-GC for secondary verification.
- Microbial Analysis: Microbial DNA from sesame residue was sequenced for biodiversity and community analysis.



Small mill sesame oil

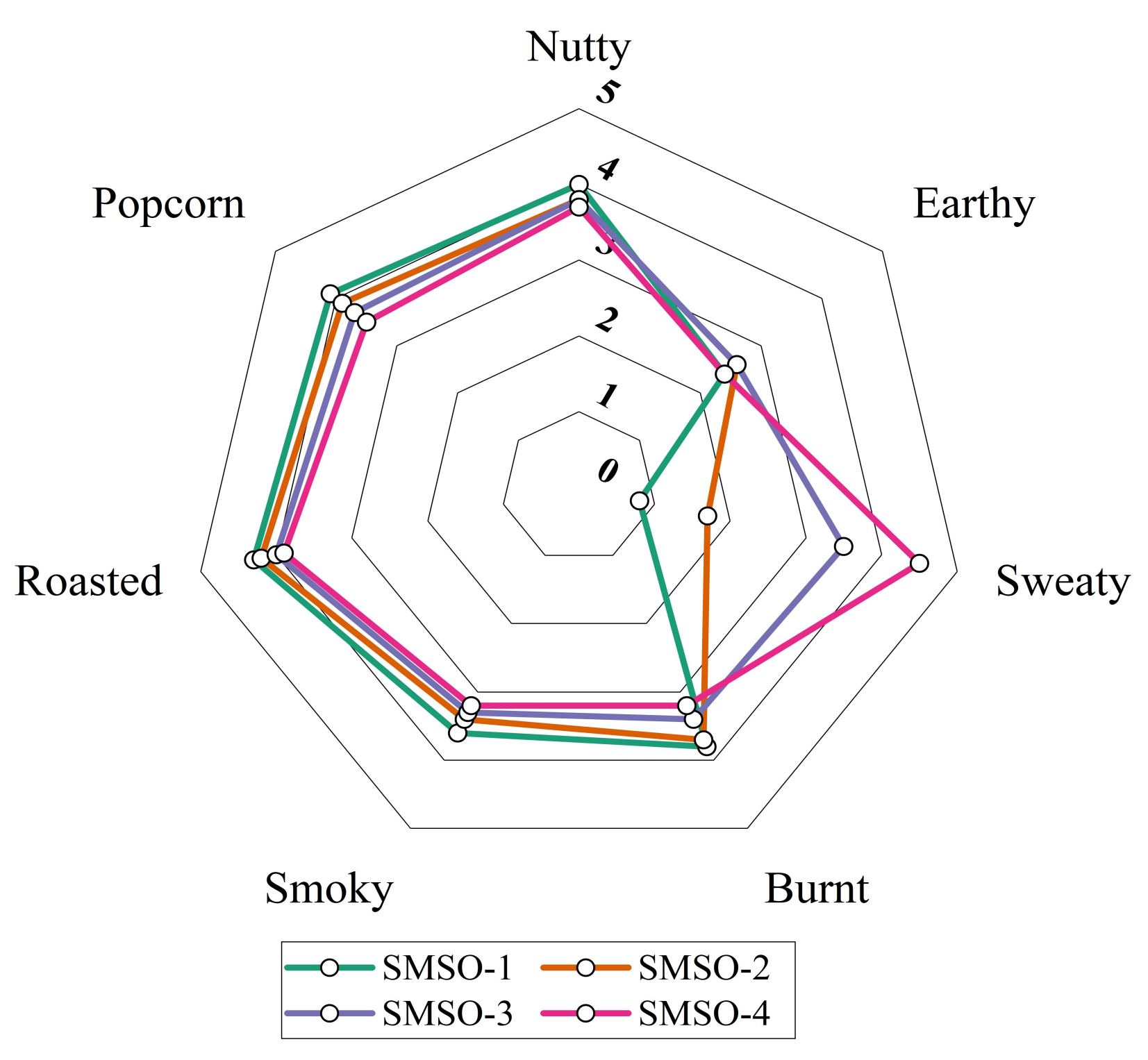
HS-SPME & SAFE

GC-O-MS

Preparative GC collecting

The causes of sweaty off-flavor

Results



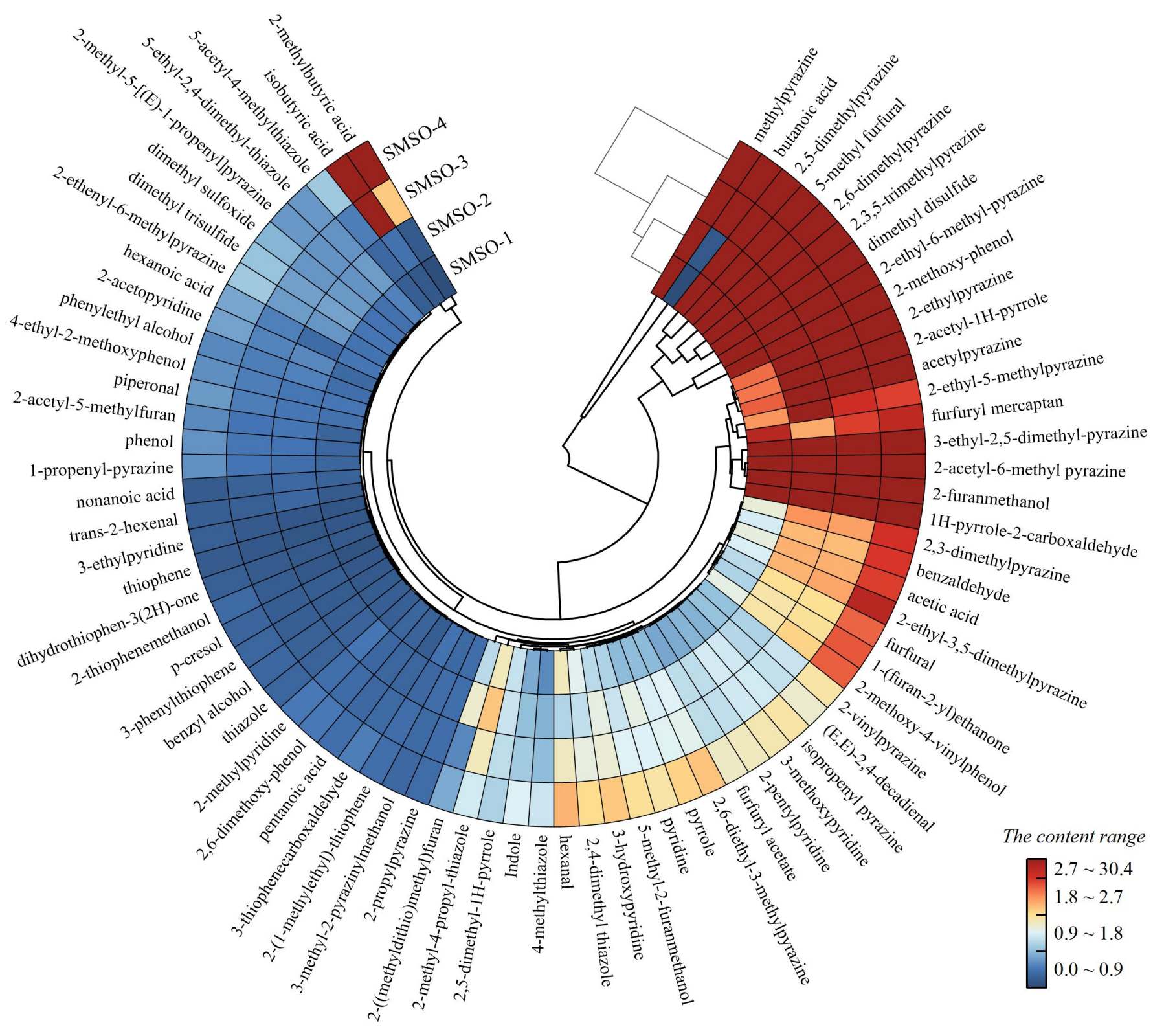
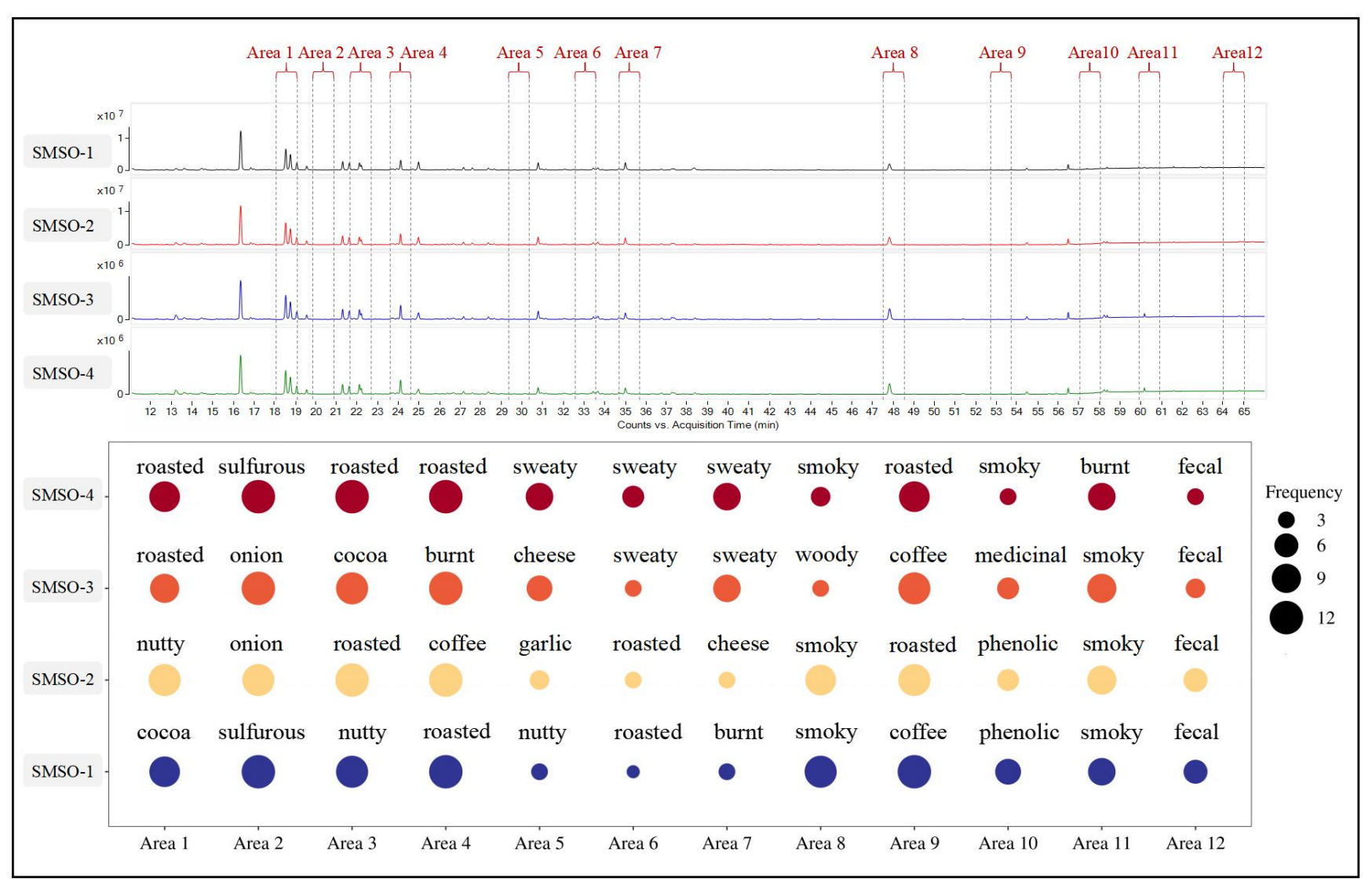


Figure 1 : *Sensory evaluation and ratings on different kinds of SMSO.*

Figure 2 *: The comparison of volatile flavor compounds in different kinds of SMSO identified by HS-SPME.*

The sensory evaluation was undertaken to better compare the flavor attributes of SMSO obtained at different sedimentation temperatures (20 ℃, 30 ℃, 40 ℃, 50 ℃). The odor attributes and scores of SMSO-1, SMSO-2, SMSO-3, and SMSO-4 were presented in **Figure 1**, exhibiting different degrees of nutty, earthy, sweaty, burnt, smoked, and roasted odors. Among them, SMSO-4 had the highest score for sweat odor, with a rating of 4.5. Significantly, as the temperature increased during sedimentation, the intensity of the sweaty odor increased progressively, which was in line with our previous observation that sweaty off-flavors were presented in SMSO when the external ambient sedimentation temperature was elevated.

As shown in **Figure 2**, a total of 76 aroma compounds were identified, including 19 pyrazines, 17 volatile sulfur-containing compounds, 8 aldehydes, 7 acids, 7 pyridines, 6 phenols, 4 alcohols, 3 pyrroles, and 5 other compounds. It is essential to point out that isobutyric acid, butanoic acid, and 2-methyl butyric acid found in this identification However, their contributions to the whole flavor still need further confirmation. Hence, GC-O analysis for different kinds of SMSO is required.



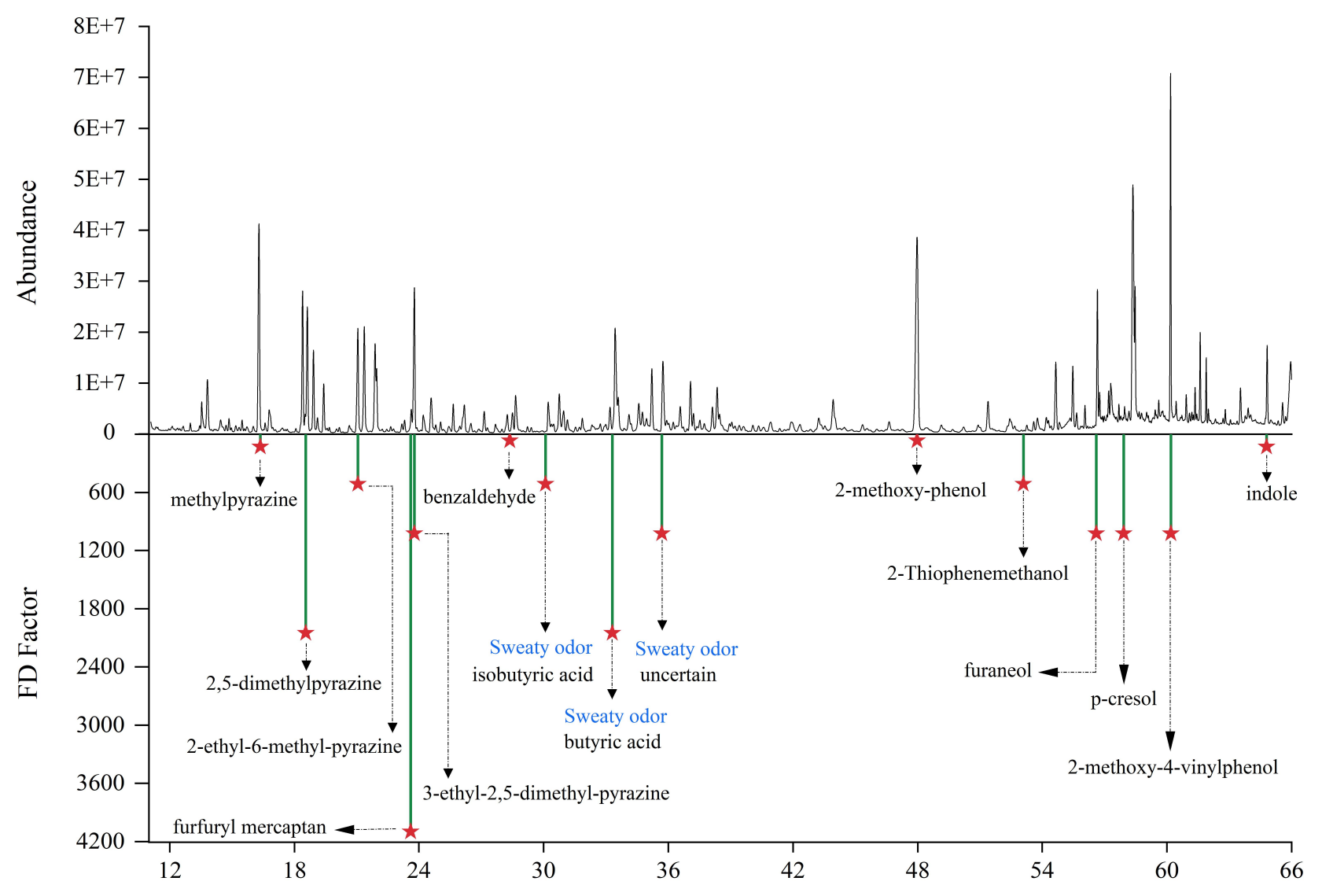
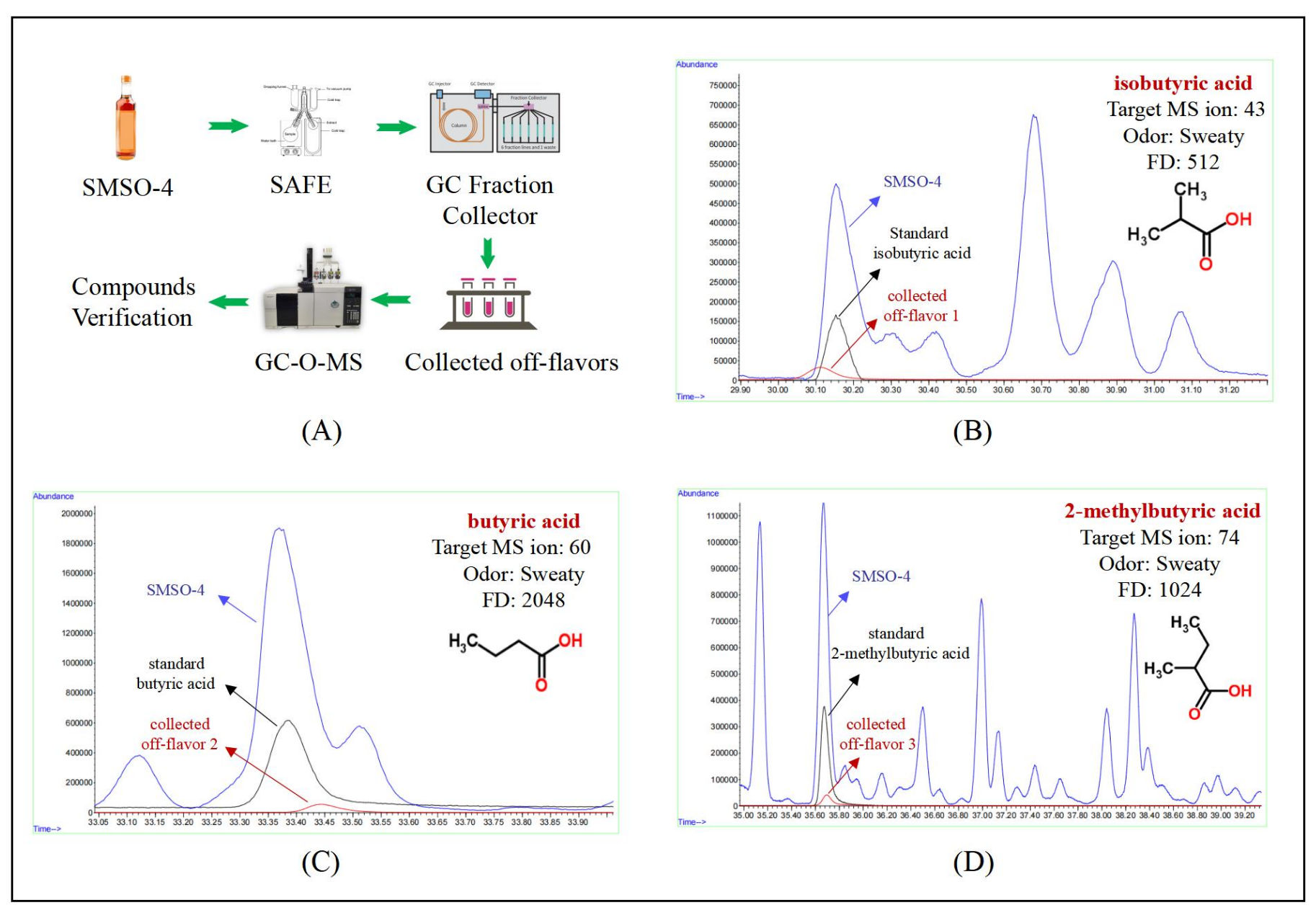


Figure 3 *: The key odor areas detected in different kinds of SMSO via HS-SPME combined with GC-O (A) and comparison of olfactory descriptions and frequencies in different areas (B). The odor description for each area is determined by the highest frequency recorded by all the sensory group.*

Figure 4 : *Comparison of volatile flavor compounds with FD greater than 64 in SMSO-4 by SAFE extraction combined with GC-O.*

Based on **Figure 3**, there were a total of 12 distinct areas where individuals in olfactory tests can detect the odor. It is worth noting that areas 5, 6, and 7 exhibit notable variations in the frequency and description of odors in 4 kinds of SMSO. These areas, where a sweaty off-flavor can be perceived via the two different extraction methods, remain consistent, both appearing in areas 5, 6, and 7 in **Figure 4**. Then, additional validation tests specifically targeting the compounds in these areas were required.



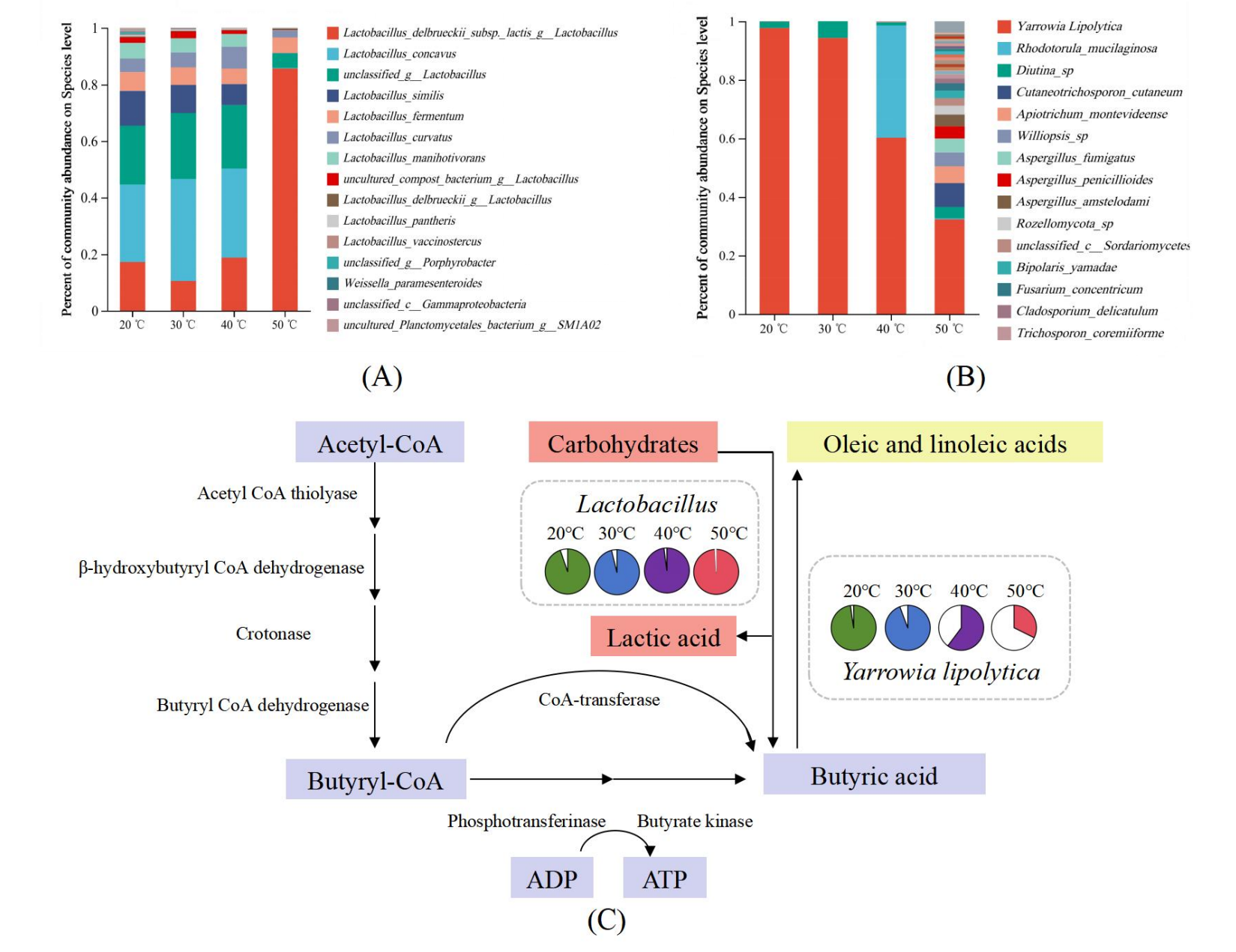


Figure 5: *Comparison of total ion chromatograms, odor descriptions, and structures of sweaty off-flavors, standards, and SMSO-4 sample in three key areas.*

Figure 6 : *Comparison of relative abundance of bacteria (A) and fungi (B) at the species level of four different temperature-settled sesame residues and possible mechanisms for the generation of sweaty off-flavors (C).*

Figure 5 displayed a comparison of the retention times, target MS ions, and odor description of the three collected off-flavors and standards. The secondary verification confirms that the compounds in areas 5, 6, and 7 were isobutyric acid, butyric acid, and 2-methylbutyric acid, which was significant source of compounds that led to the sweaty odor of SMSO.

As shown in **Figure 6**, when the sedimentation temperature varied between 20°C and 50°C, the proportion of *Lactobacillus* species producing butyrate in sesame residue gradually increased. Simultaneously, butanoate 1-phosphotransferase activity decreased during the sedimentation process, inhibiting the conversion of butyrate to butyryl phosphate. Furthermore, the decrease in the abundance of *Yarrowia lipolytica* during the temperature increase promoted butyrate accumulation.

Conclusion

This study utilized molecular sensory science, prep-GC, and microbiomics to investigate the sweaty off-flavor in SMSO. Among 76 volatile compounds identified, sensory analysis pinpointed three key areas with a sweaty odor. Secondary validation using prep-GC confirmed the specific culprits as butyric acid, 2-methylbutyric acid, and isobutyric acid. Furthermore, microbiological analysis of sesame residue revealed that these off-flavors likely originate from the collaborative interaction of Lactobacillus, Yarrowia lipolytica, and butanoate 1-phosphotransferase. Based on the odor screening and verification strategy, the combination of prep-GC and GC-O olfactory analysis provides a novel insight into the detection of off-flavors in vegetable oil. Simultaneously, this study provides a theoretical foundation for the quality control and flavor improvement of SMSO.

Acknowledgement

Thanks for the (financial) support from Arawana Charity Foundation.