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Unveiling the Chemical Composition Differences in Ginseng Extract Fermentation Using LC/Q-TOF Technology and Multilevel Qualitative Analysis Strategies

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Introduction

Ginseng, a highly prized traditional Chinese medicine, owes its therapeutic benefits to ginsenosides, which feature four main structural aglycone cores: PPD, PPT, OLE, and OCO [1](Fig. 1). Fermentation presents a valuable approach to altering ginsenoside composition, potentially enhancing ginseng's bioactivity and unlocking new health benefits.

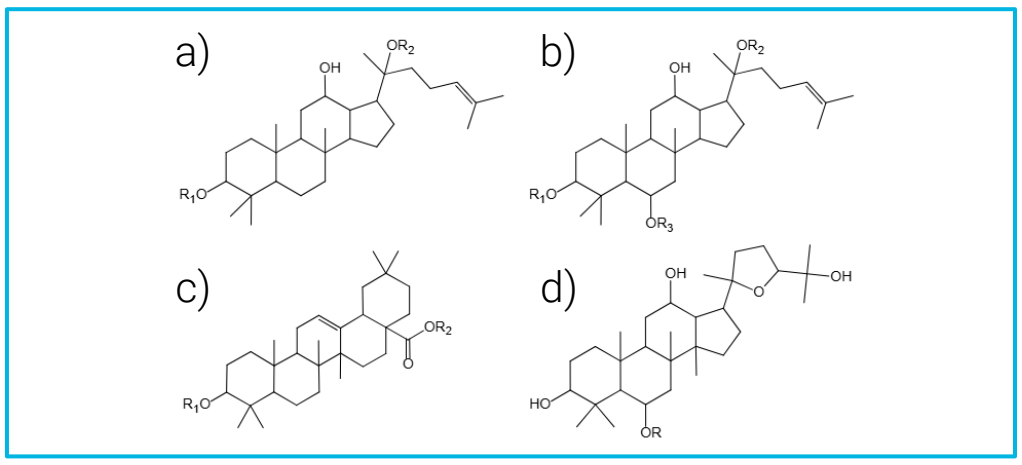

Figure 1. Ginsenosides feature four main structural aglycone cores: PPD, PPT, OLE, and OCO.

The advanced LC/Q-TOF analytical tool, when integrated with specialized software tools, MassHunter Explorer, TCM PCDL library, SIRIUS CSI:FingerID and feature based molecular networking analysis, enables a comprehensive exploration of the compositional changes in ginseng before and after fermentation (Fig. 2). Through in - depth analysis, we aim to not only identify the specific ginsenosides that are affected by the fermentation process but also trace the possible metabolic pathways involved. Understanding these changes will provide critical insights into how fermentation can enhance the bioactivity of ginseng, paving the way for the development of more potent ginseng - based products and a better understanding of the underlying mechanisms of ginseng's therapeutic effects.

Experimental

Materials & methods

This study employed two types of samples: pre- and post- fermentation ginseng samples. After vortexing for homogenization, 25 mL of each sample was transferred into a tube and mixed with 25 mL of water/methanol (1:1, v/v). The mixture was ultrasonicated and vortexed again, then centrifuged, and the supernatant was collected.

All samples were analyzed using a 1290 Infinity II LC system coupled with a 6546 LC/Q-TOF instrument. Briefly, sample separation happens in a 35 min method using an Agilent InfinityLab Poroshell EC-C18 150nm column. This gradient method for the mobile phase starts with B at 10%. It remains at 10% until 2 minutes. From 2 to 10 minutes, B increases to 35%. Between 10 and 25 minutes, it rises to 55%. From 25 to 31 minutes, B reaches 75%, and finally, from 31 to 35 minutes, it reaches 100%. The LC/Q-TOF was tuned in m/z 1700 mode and operated in MS1 mode m/z 60-1500 for each individual sample. Negative ion mode data was collected for ginsenosides. A pooled QC was made and injected throughout the MS1 worklist for data quality evaluation. Additionally, the pooled QC was used for an iterative MS/MS acquisition (n=4) for high--coverage MS/MS.

The MassHunter Explorer software was utilized to rapidly screen for compounds that vary between ginseng samples before and after fermentation. Initially, compounds were annotated using the TCM PCDL via a library - matching strategy. For those uncharacterized features, the Sirius CSI:Finger ID[2] was applied to annotate their structures. Moreover, molecular network analysis[3] was conducted to comprehensively monitor the alterations in ginsenosides based on structural similarities.

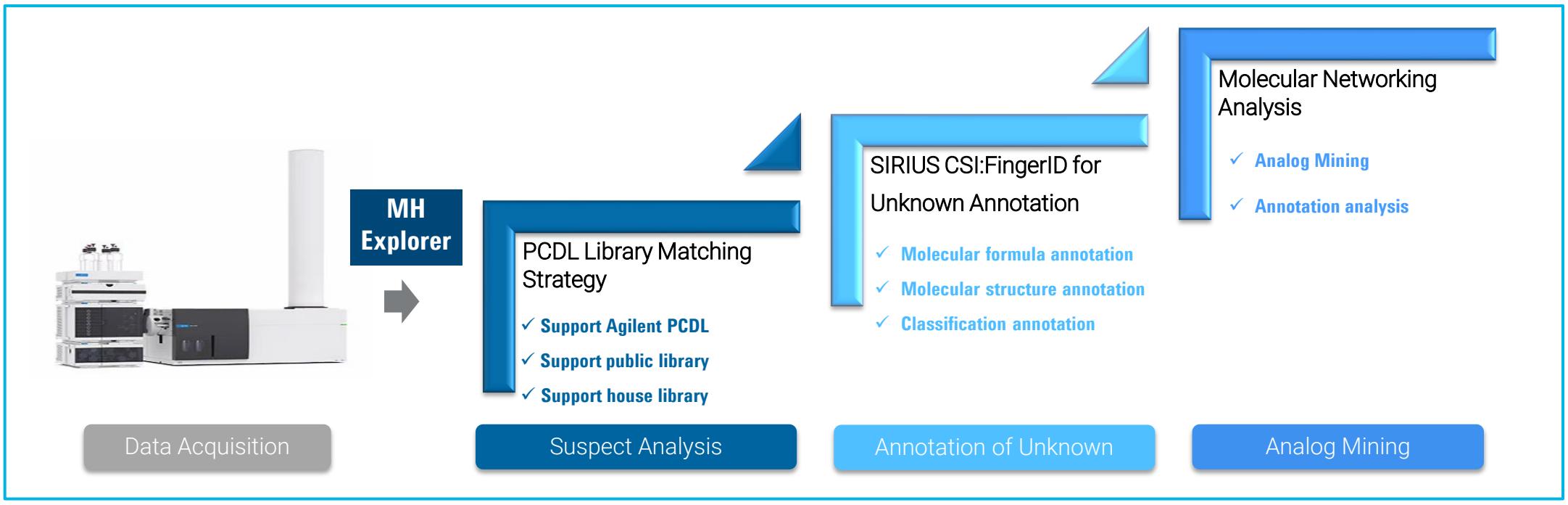

Figure 2. LC - QTOF Technology and Multilevel Qualitative Analysis Strategies enables in – depth unveiling of Chemical Compositions.

Results and Discussion

Preliminary investigation on the effect of fermentation process in ginseng

In this study, MassHunter Explorer offers comprehensive analysis and visualization, including setup conditions, data extraction, normalization, filtering, statistical analysis, and identification procedures. Here, PCA shows the 3 replicates of the 2 samples were separated into two distant groups (Fig. 3a). It indicates notable shifts in the chemical profiles of ginseng samples during fermentation. Following that, the Volcano Plot highlights significant changes in the abundance of various compounds (Fig. 3b). Compositions having |fold change| ≥ 2 at p-value < 0.01 were colored as red (significantly higher in post- fermentation ginseng) or blue (significantly higher in before- fermentation ginseng). It suggests that fermentation triggers a series of chemical reactions that may modify the original ginseng composition.

Those data demonstrate that the chemical composition can be strongly influenced by the fermentation process in ginseng.

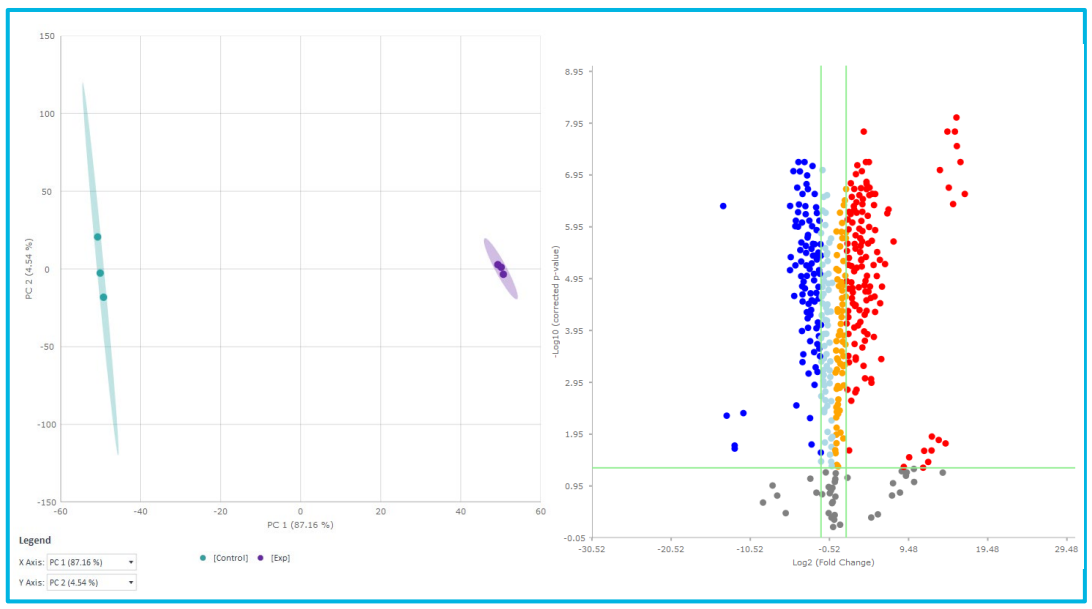

Figure 3. PCA plot for chemical composition shows distinction between before and after fermentation in ginseng is inherent (a) and the volcano plot for the compositions show up and down regulated when considering fold change of 2 and p-value of 0.05 (b).

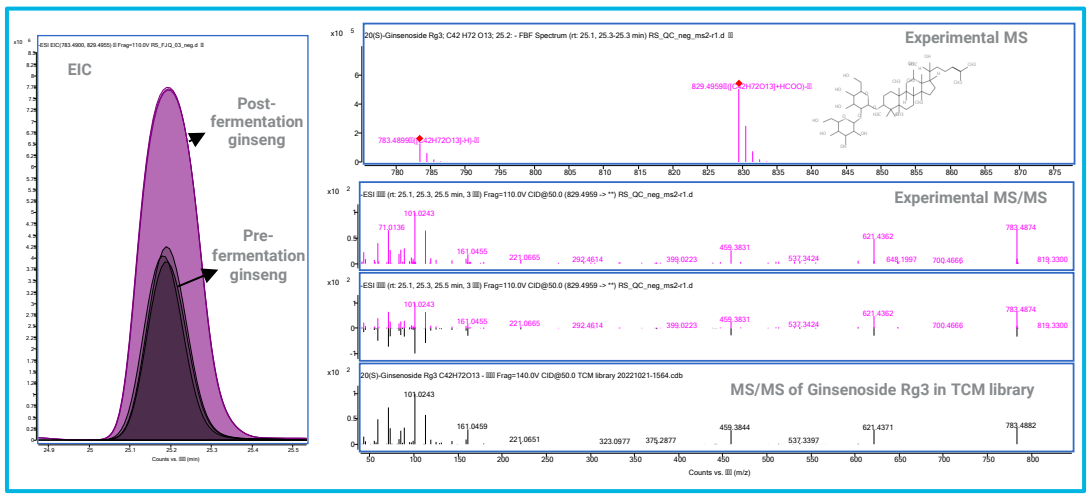

Figure 4. Experimental MS and MS/MS spectra were matched against the TCM PCDL database, indicating that the compound was ginsenoside Rg3.

Rapid structural annotation of differential components via library matching

Utilizing the TCM PCDL library enables rapid and accurate identification of components in ginseng, as well as the differential components that emerge during fermentation. In this study, 33 ginsenosides were quickly identified. The majority of these ginsenosides exhibited significant changes. Here, we take ginsenoside Rg3 as an example to demonstrate this process (Fig. 4). Through matching the experimental MS and MS/MS spectra against the TCM PCDL library, ginsenoside Rg3 could be precisely identified. Notably, the content of ginsenoside Rg3 increased significantly after fermentation. In addition to ginsenosides, other types of compounds, such as chlorogenic acid and 5 - feruloylquinic acid, were not only detected but also found to exhibit significant differences.

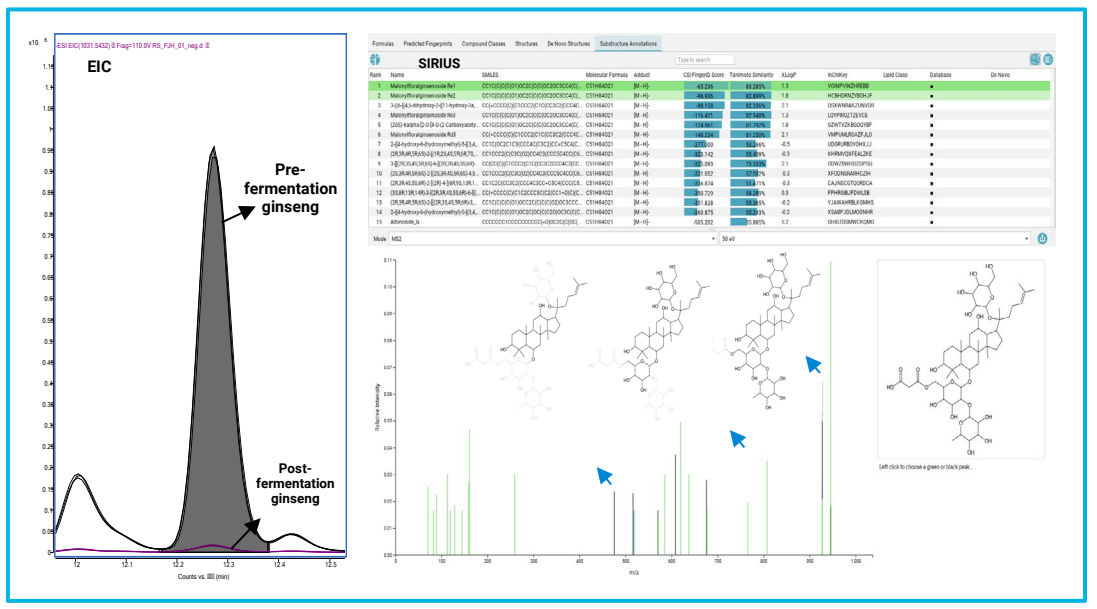

Figure 5. Structural annotation of an unknown compound using SIRIUS CSI:FingerID reveals its molecular formula and structure, identifying it may be malonyl - ginsenoside Re.

Unraveling uncharacterized ginsenosides with SIRIUS CSI:FingerID

When one component is still unidentified after applying TCM PCDL library, its MS/MS spectra would be submitted to SIRIUS CSI:FingerID for unknown structural annotation. Here, Malonyl - ginsenoside Re is presented as a representative example to illustrate the result (Fig. 5). In this study, a series of modified ginsenoside derivatives were successfully characterized. These modifications include acetylation, malonylation, hydroxylation, and others. Notably, the contents of these modified ginsenosides underwent significant changes during the fermentation process. These changes not only highlight the dynamic nature of the chemical composition of ginseng during fermentation but also provide valuable insights into the potential transformation pathways of ginsenosides, which may have implications for the enhanced bioactivity and functionality of fermented ginseng products.

Results and Discussion

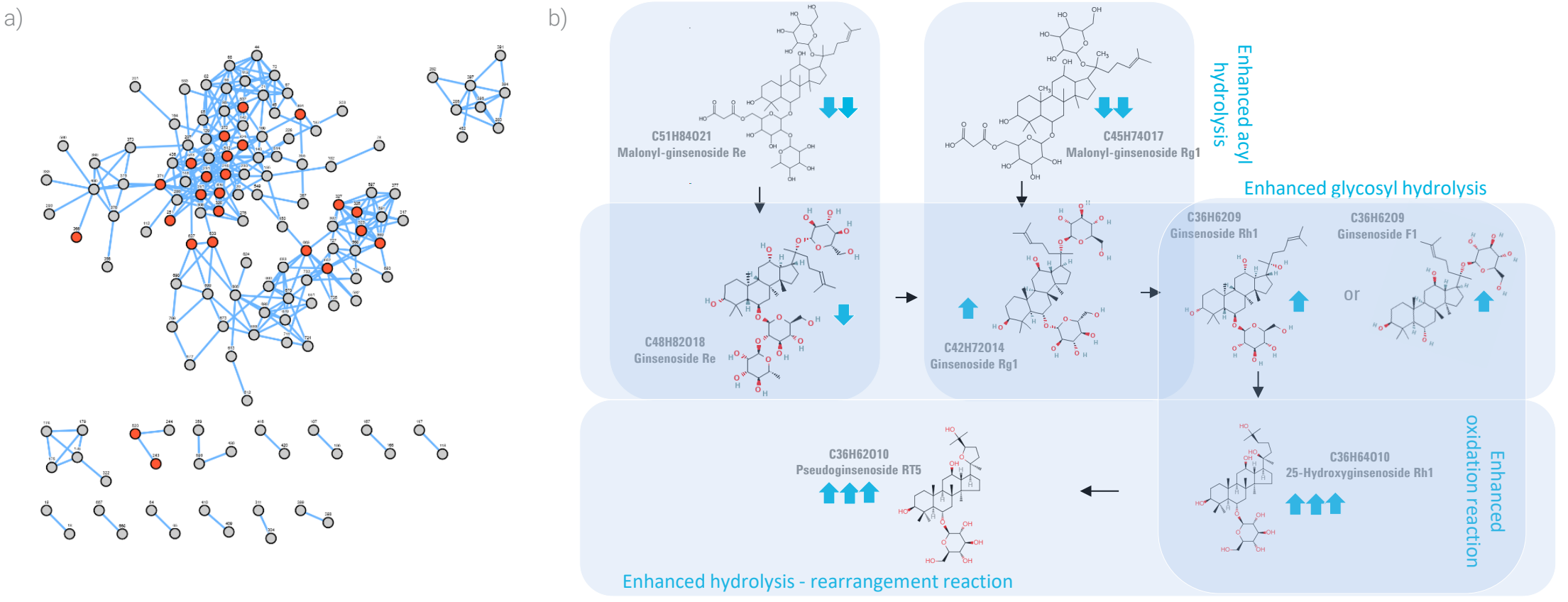

Figure 6. a)The molecular network demonstrates the structural diversity of ginsenosides. b) Structural annotation insights into fermentation - induced biotransformation

Exploring the chemical depths of ginseng: molecule networking

To clarify the changes in ginsenosides during ginseng fermentation, we conducted a molecular network analysis. By using the characteristic fragment ions of ginsenoside aglycones (m/z 459.3844 for PPD, m/z 475.3793 for PPT, m/z 455.3531 for OLE, and m/z 491.3750 for OCO) for targeted filtering, and then performing feature - based molecular network analysis, a dedicated ginsenoside molecular network was constructed (Fig. 6a). This network effectively revealed ginsenoside structural diversity and enabled precise compound annotation, facilitating the identification of rare ginsenoside forms.

Structural annotation insights into fermentation - induced biotransformation

Combining TCM PCDL, SIRIUS CSI:FingerID, molecular network analysis, and differential analysis uncovered key trends in ginseng fermentation. Fermentation resulted in: 1) a drop in acylated ginsenosides, likely from accelerated acyl - group hydrolysis; 2) a decrease in PPT/PPD - type polysaccharide - glycoside ginsenosides and an increase in di - and mono - glycoside forms, indicating enhanced glycosidic bond hydrolysis; 3) oxidation and rearrangement of 24,25 - double bonds, raising OCO - type ginsenoside content. These changes suggest fermentation promotes acyl and glycosyl hydrolysis and hydrolysis - rearrangement reactions (Fig. 6b).

Since literature indicates ginsenosides' oral bioavailability and pharmacological activity rank as mono - glycosides > di - glycosides > polysaccharides, the enhanced glycosyl hydrolysis improves ginseng's efficacy[4].
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The significant increase in OCO - type ginsenosides during fermentation, typically found in American ginseng, implies novel bioactivities, expanding fermented ginseng's therapeutic potential and highlighting fermentation's role in unlocking its functional value.

Conclusions

In conclusion, LC/Q-TOF technology and multilevel qualitative analysis effectively revealed the chemical composition differences in ginseng extract fermentation. This approach identified enhanced ginsenoside transformation, indicating potential bioactivity improvements, and offers a valuable method for developing functional ginseng - based foods.

References

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