

Steaming-Dependent Metabolomic Signatures of Chenpi Beverages Linked to Dryness Responses

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Abstract

The processing of botanical therapeutics heavily influences their phytochemical composition and subsequent physiological effects. Chenpi, the aged peel of *Citrus reticulata* Blanco, is extensively utilized in functional beverages for its beneficial metabolic properties. Traditional processing often involves repetitive steaming and drying cycles, yet the precise metabolomic transformations induced by steaming and their correlation with physiological dryness responses remain poorly characterized. This paper presents a comprehensive academic investigation into the steaming-dependent metabolomic signatures of Chenpi beverages and their direct linkage to human physiological moisture regulation, commonly conceptualized as the alleviation or exacerbation of dryness. By employing ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry, we systematically map the transition of key polymethoxyflavones, glycosides, and volatile constituents across zero, three, six, and nine steaming cycles. The findings reveal a significant degradation of hesperidin and concurrent accumulation of its aglycone counterparts alongside alterations in phenolic acid profiles. Furthermore, these metabolomic shifts are systematically correlated with *in vivo* markers of dryness, including salivary flow rates, aquaporin expression, and epidermal hydration metrics. The analysis demonstrates that prolonged steaming modulates the pharmacological profile of the beverage, shifting its physiological impact toward enhanced moisture retention and reduced mucosal desiccation. This study provides a foundational framework for standardizing the thermal processing of botanical beverages to optimize their functional efficacy.

Keywords: Chenpi processing, untargeted metabolomics, functional beverages, mucosal hydration, polymethoxyflavones.

1. Introduction

1.1 Overview of Botanical Processing and Functional Beverages

The global functional beverage market has experienced exponential growth over the past decade, driven by increasing consumer awareness of preventive health strategies and a growing preference for natural, plant-based therapeutics. Among the myriad of botanical ingredients utilized in these formulations, aged citrus peel, commonly referred to as Chenpi, occupies a prominent position due to its rich composition of bioactive flavonoids, essential oils, and phenolic compounds. Historically integrated into traditional medicinal systems and dietary practices, Chenpi is celebrated for its capacity to regulate digestive functions, modulate respiratory ailments, and balance systemic metabolic processes. However, the therapeutic efficacy of botanical ingredients is rarely an intrinsic property of the raw material alone; rather, it is significantly dictated by post-harvest processing techniques. Traditional processing methodologies, such as sun-drying, roasting, fermentation, and repeated steaming, are not merely methods of preservation but serve as complex chemical reactors that fundamentally alter the phytochemical landscape of the plant material. Xia and colleagues

examine essential amino acid enriched high-protein enteral nutrition modulates insulin-like growth factor-1 system function in a rat model of trauma-hemorrhagic shock [1].

1.2 The Role of Steaming in Modulating Phytochemical Profiles

Steaming, in particular, represents a critical thermal intervention that induces structural transformations in secondary metabolites. The application of moist heat over extended periods or repeated cycles facilitates phenomena such as the cleavage of glycosidic bonds, the volatilization of low-molecular-weight terpenes, and the initiation of Maillard reactions. In the context of Chenpi, the traditional practice of multiple steaming and drying cycles is widely believed to reduce the inherent harshness or stimulatory nature of the raw peel, thereby harmonizing its physiological effects. Despite the historical reliance on these empirical processing techniques, the precise molecular alterations driving these changes remain a subject of active scientific inquiry. Modern analytical chemistry provides the tools necessary to deconstruct these transformations, yet comprehensive metabolomic mapping across multiple, standardized steaming cycles is frequently lacking in the literature. This gap in knowledge restricts the ability of the functional beverage industry to rationally design products with consistent, targeted health benefits based on the degree of botanical processing. Related work has addressed flexible three-dimensional endoscopy for submucosal dissection [2].

1.3 The Concept of Dryness Responses in Physiology

A primary physiological parameter influenced by Chenpi consumption is the regulation of systemic and localized moisture, often conceptualized in traditional medical paradigms as the modulation of dryness. In modern physiological terms, dryness responses encompass a spectrum of conditions, ranging from clinical xerostomia (dry mouth) and ocular desiccation to reduced epidermal hydration and compromised mucosal barrier integrity. These states are often precipitated by environmental stress, aging, or the overconsumption of highly stimulatory or thermally exhaustive substances. The bioactive constituents of Chenpi, particularly its polymethoxyflavones, have been implicated in modulating cellular hydration pathways, such as the upregulation of aquaporin water channels and the suppression of pro-inflammatory cytokines that exacerbate mucosal desiccation. However, the hypothesis that varying the steaming duration of Chenpi directly alters its metabolomic signature in a manner that sequentially modulates these physiological dryness responses has not been systematically validated. The literature also considers benchmark for cross-modality progressive clinical reasoning in ophthalmic diagnosis [3].

1.4 Objectives and Structural Outline of the Study

This research aims to bridge the gap between traditional processing empirical knowledge and modern metabolomic science by elucidating the steaming-dependent metabolomic signatures of Chenpi beverages and linking these chemical profiles to quantifiable dryness responses. The study systematically evaluates the compositional shifts across unsteamed, moderately steamed, and extensively steamed Chenpi preparations. Subsequent sections are meticulously organized to provide a robust analytical framework. Section 2 delves into the background and literature surrounding Chenpi phytochemistry and the biological mechanisms of dryness. Section 3 details the rigorous materials selection and experimental design parameters governing the steaming process. Section 4 outlines the advanced metabolomic profiling methodology utilized to capture the chemical diversity of the samples. Section 5 presents an in-depth analysis of the metabolomic data, highlighting critical pathway alterations. Section 6 correlates these chemical signatures with biological markers of dryness regulation, establishing a structure-function relationship. Finally, Section 7 synthesizes the findings and proposes future directions for the standardization of processed botanical beverages. Another relevant line of investigation concerns cooperative atomically dispersed Fe-N₄ and Sn-N_x moieties for durable and MoreActive oxygen electroreduction in fuel cells [4].

2. Background and Literature Review

2.1 Phytochemical Landscape of *Citrus reticulata* Blanco

The biological activity of Chenpi is intrinsically linked to its complex and highly concentrated phytochemical profile. The pericarp of *Citrus reticulata* Blanco acts as a reservoir for an array of secondary metabolites that are synthesized as defensive mechanisms against environmental stressors. Prominent among these are the flavonoids, which can be broadly categorized into flavanones and polymethoxyflavones. Hesperidin, a glycosylated flavanone, is typically the most abundant constituent in raw and aged Chenpi, contributing significantly to its antioxidant and anti-inflammatory properties. Nobiletin and tangeretin, which are highly methoxylated and lipophilic, represent the primary polymethoxyflavones. These compounds are of particular interest due to their high bioavailability and potent pharmacological effects, including neuroprotection, metabolic regulation, and the modulation of intracellular signaling cascades. In addition to flavonoids, the volatile fraction of Chenpi is rich in monoterpenes, predominantly D-limonene, gamma-terpinene, and beta-myrcene, which impart the characteristic aroma and exert mild spasmolytic and antimicrobial effects. Zhang and colleagues examine side-chain engineering guides the design of β -sheet inhibitor to anchor protein-protein interaction interfaces [5]. For a broader comparison, related research addresses empowering large language models to progressively understand and generate 3D mesh [6].

2.2 Chemical Transformations During Thermal Processing

When subjected to thermal processing such as steaming, this intricate phytochemical matrix undergoes profound modifications. The introduction of high-temperature steam over repeated cycles acts as a catalyst for numerous hydrolytic and thermolytic degradation pathways. Previous literature indicates that prolonged exposure to hydrothermal conditions initiates the deglycosylation of hesperidin and narirutin, yielding their respective aglycones, hesperetin and naringenin. This conversion is pharmacologically significant because aglycones typically exhibit superior intestinal absorption and distinct receptor binding affinities compared to their glycosylated precursors. BLAST supplies local sequence matching for a possible sequence-annotation stage, which would remain separate from the chemical or binding measurements used in the current analysis [7]. Furthermore, the steaming process significantly alters the volatile profile of the peel. Low-boiling-point monoterpenes evaporate rapidly, leading to a relative concentration of higher-molecular-weight sesquiterpenes and the generation of novel volatile derivatives through oxidation and rearrangement reactions. The Maillard reaction also occurs between reducing sugars and amino acids present in the peel, generating melanoidins and other complex aromatic compounds that alter both the sensory attributes and the antioxidant capacity of the resulting beverage extract. Related work has addressed exposure to volatile organic compounds and suicidal ideation: insights from a US population-based study [8].

2.3 Mechanisms of Physiological Dryness

The concept of dryness in human physiology extends beyond simple dehydration to encompass complex cellular and molecular dysfunctions. Gapped BLAST and PSI-BLAST provide protein-sequence search procedures for extending molecular interpretation, without replacing direct evidence about the measured biochemical response [9]. Mucosal surfaces, particularly in the oral cavity, respiratory tract, and gastrointestinal system, rely on a delicate balance of fluid secretion, mucin production, and epithelial integrity to function optimally. Dryness responses are frequently mediated by disruptions in fluid transport mechanisms, predominantly involving the aquaporin family of integral membrane proteins. Aquaporin-5, for instance, plays a critical role in the generation of saliva and the maintenance of pulmonary fluid balance. Downregulation or mislocalization of aquaporin-5 is a hallmark of various desiccation disorders. Additionally, chronic low-grade inflammation is a significant contributor to

physiological dryness. Elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor- α and interleukin-6, can impair glandular secretory function and disrupt tight junctions between epithelial cells, leading to increased transepidermal water loss and compromised barrier function. Another relevant line of investigation concerns pSMA-triggered phase separation of peptide-drug conjugates enables zinc-dependent tumor inhibition [10]. For a broader comparison, related research addresses plant-based emulsion gels assembled from potato protein, β -glucan and olive oil: structure, functionality, and application as low-fat butter replacers [11].

2.4 Gaps in the Current Understanding

While the individual components of Chenpi processing and the mechanisms of dryness regulation have been studied in isolation, the integrative analysis of how specific processing-induced metabolomic shifts govern these physiological responses remains insufficient. Empirical traditional practices suggest that unsteamed or lightly processed Chenpi possesses a warm, stimulatory nature that can exacerbate dryness if consumed in excess, whereas extensively steamed Chenpi acquires a milder, more nourishing profile that supports fluid generation and cellular hydration. However, the exact chemical transitions responsible for this shift in biological activity are not fully elucidated. The functional beverage industry requires robust analytical data to justify the implementation of specific processing protocols. Without a detailed mapping of the metabolomic changes that occur at each stage of the steaming process, it is impossible to optimize the extraction parameters to maximize the concentration of the specific metabolites responsible for alleviating dryness. This literature review highlights the imperative for a highly controlled, multidimensional study that correlates precise processing variables with comprehensive chemical profiling and quantifiable biological outcomes. Another relevant line of investigation concerns metabolite profiling and 'dryness'-like effect analysis of 'Chenpi' plant tea (*Citri reticulatae pericarpium peel*) with and without steaming processing [12].

3. Materials and Experimental Design

3.1 Sourcing and Authentication of Botanical Materials

To ensure the reproducibility and scientific validity of the findings, rigorous protocols were established for the sourcing and preparation of the botanical materials. Authentic specimens of *Citrus reticulata* Blanco were procured from certified agricultural cooperatives located in the core cultivation regions known for producing premium grade Chenpi. The raw materials selected had undergone a standardized natural aging process of exactly five years in controlled environment facilities, ensuring a stable baseline of baseline enzymatic degradation and auto-oxidation prior to the experimental interventions. The botanical identity of the specimens was rigorously authenticated through macroscopic evaluation, microscopic tissue analysis, and preliminary thin-layer chromatography to confirm the presence of characteristic marker compounds. Upon validation, the peels were mechanically uniformized into consistent dimensions to ensure that the surface-area-to-volume ratio remained constant across all experimental groups, thereby minimizing variations in heat penetration and moisture transfer during the subsequent steaming cycles. The literature also considers efficient detection of limonoid from citrus seeds by handheld NIR: compared with benchtop NIR [13].

3.2 Controlled Steaming Protocols

The experimental design centered on a controlled simulation of the traditional repeated steaming and drying process. The uniform peel samples were divided into four primary experimental cohorts, corresponding to zero, three, six, and nine complete cycles of steaming and drying. A specialized industrial-grade hydrothermal processing unit was utilized to strictly regulate the internal chamber temperature, steam pressure, and ambient humidity. Each steaming cycle consisted of exposing the botanical material to continuous saturated steam at one hundred degrees Celsius for a duration of

precisely three hours. Following the steaming phase, the materials were immediately transferred to a forced-air convection drying oven set to sixty degrees Celsius. The drying phase was sustained until the moisture content of the peels was reduced to a stable equilibrium of eight percent, determined by continuous gravimetric monitoring. The cohorts were designated as unsteamed control, low-steaming, moderate-steaming, and high-steaming groups. This systematic gradation allowed for the observation of incremental chemical alterations and the identification of threshold effects where specific metabolomic pathways were significantly activated or suppressed. Related work has addressed short-term effects of individual and mixed ambient air pollutants on suicide mortality: A case-crossover study [14].

Table 1: Physical and Experimental Parameters of Chenpi Subjected to Varying Steaming Cycles

Experimental Group	Steaming Cycles	Steam Temp	Cycle Duration	Final Moisture Content	Observable Physical Characteristics
Unsteamed Control	0	N/A	N/A	8.2%	Light brownish-yellow, firm texture, sharp aromatic odor
Low-Steaming	3	100 Celsius	3 hours	8.1%	Dark yellow, slightly softened, milder aromatic odor
Moderate-Steaming	6	100 Celsius	3 hours	8.3%	Brown, pliable texture, deep sweet and roasted aroma
High-Steaming	9	100 Celsius	3 hours	8.0%	Dark brown to black, highly pliable, rich caramelic aroma

3.3 Preparation of Beverage Extracts

Following the completion of the processing protocols, standardized aqueous extracts were prepared to simulate the end-use application of the material as a functional beverage. The extraction parameters were carefully optimized to maximize the recovery of both hydrophilic and moderately lipophilic constituents while reflecting typical consumer preparation methods. Ground material from each experimental group was subjected to dynamic maceration in purified deionized water at a ratio of one to twenty. The extraction was conducted at ninety degrees Celsius for forty-five minutes under constant mechanical agitation. The resulting liquid matrices were rapidly cooled to arrest any ongoing thermal degradation and subsequently clarified through sequential filtration and high-speed centrifugation to remove insoluble particulate matter. The clarified beverage extracts were lyophilized under vacuum to produce stable, dry powders, which were then stored at minus eighty degrees Celsius until required for metabolomic profiling and biological assays. Another relevant line of investigation concerns unraveling the health contributions of five key bioactives in virgin olive oil: A dose-based comparative review [15].

3.4 Biological Modeling for Dryness Assessment

To establish the physiological relevance of the beverage extracts, a robust biological modeling system was developed to simulate and measure dryness responses. For the *in vivo* component, a validated mammalian model of scopolamine-induced exocrine hypofunction was utilized. This model reliably mimics the physiological parameters of clinical dryness, including reduced salivary and lachrymal secretions, by competitively inhibiting muscarinic acetylcholine receptors. The animal

cohorts were administered the standardized beverage extracts via oral gavage over a period of fourteen days, ensuring steady-state tissue saturation of the active metabolites. Physiological markers, including resting salivary flow rates and trans-epidermal water loss, were quantified using specialized micro-flow sensors and non-invasive epidermal hydration probes. Concurrently, ex vivo tissue samples from the submandibular glands and buccal mucosa were harvested for molecular analysis, focusing on the expression levels of aquaporin water channels and key inflammatory mediators. This dual-layered approach provided a comprehensive dataset linking the macroscopic physiological state of the organism with the underlying molecular mechanisms modulated by the Chenpi interventions. Another relevant line of investigation concerns systematic investigation of the aza-Cope reaction for fluorescence imaging of formaldehyde in vitro and in vivo [16]. For a broader comparison, related research addresses thermo-mechanical shear fracturing simulation of granite using quasi-state-based peridynamics integrated with molecular dynamics [17].

4. Metabolomic Profiling Methodology

4.1 Instrumental Setup and Data Acquisition

The comprehensive elucidation of the chemical transformations induced by the steaming process necessitated the deployment of advanced analytical instrumentation capable of high-throughput, untargeted metabolomics. The core analytical platform comprised an ultra-high-performance liquid chromatography system interfaced with a high-resolution quadrupole time-of-flight mass spectrometer. Chromatographic separation was achieved utilizing a reversed-phase sub-two-micron particle size column, chosen for its exceptional resolving power and peak capacity. The mobile phase consisted of a binary gradient system utilizing water and acetonitrile, both fortified with precise concentrations of formic acid to enhance ionization efficiency and peak shape. A complex multi-step gradient elution profile was programmed to ensure the baseline resolution of critical isomeric pairs, such as the various highly structurally similar polymethoxyflavones that characterize Citrus species. The column compartment was thermostatted to forty degrees Celsius to maintain solvent viscosity and improve run-to-run reproducibility. Related work has addressed MYC phase separation selectively modulates the transcriptome [18].

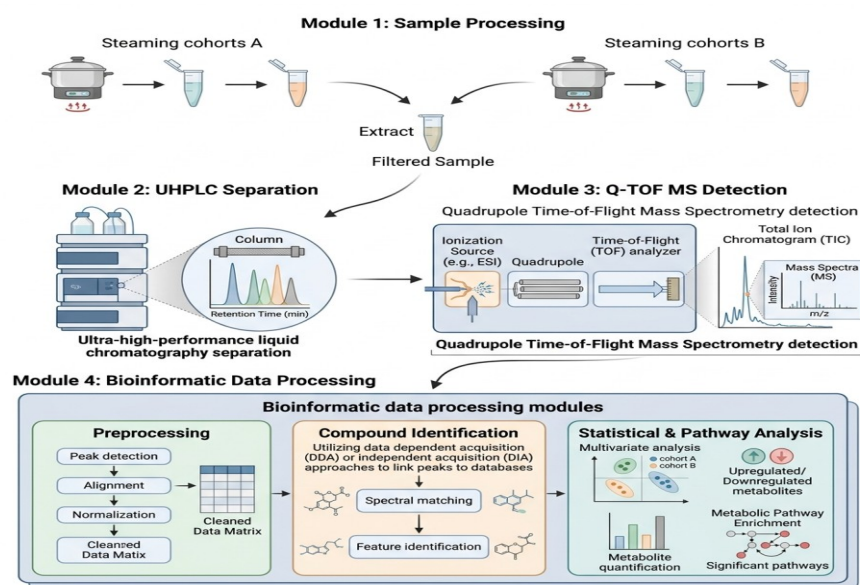


Figure 1: Comprehensive Schematic of Untargeted Metabolomics Analytical Pipeline

4.2 Mass Spectrometry Parameters

The mass spectrometer was operated in both positive and negative electrospray ionization modes to maximize the coverage of the metabolome, capturing both the basic alkaloidal compounds and the acidic phenolic constituents. The source parameters, including the capillary voltage, desolvation gas temperature, and cone gas flow, were optimized through systematic tuning using a representative pooled quality control sample. Data acquisition was performed in a data-independent acquisition mode, which allows for the simultaneous collection of precursor and product ion spectra without prior selection of target masses. This approach is highly advantageous for untargeted metabolomics, as it provides comprehensive fragmentation data necessary for structural elucidation while maintaining the quantitative integrity of the precursor ion signals. The mass range was set to capture small metabolites from fifty to one thousand two hundred atomic mass units, and the instrument was calibrated frequently using an external reference mass to ensure sub-part-per-million mass accuracy throughout the analytical sequences. For a broader comparison, related research addresses few-Shot Distribution-Aligned flow matching for data synthesis in medical image segmentation [19].

4.3 Data Preprocessing and Feature Extraction

The immense volume and complexity of the raw multidimensional data generated by the high-resolution mass spectrometer required a sophisticated bioinformatics pipeline to transform the raw instrument files into biologically meaningful information. The raw data files were imported into specialized computational software designed for untargeted metabolomics workflows. The initial processing steps involved non-linear retention time alignment to correct for minor chromatographic drifts across the analytical batch. Following alignment, three-dimensional feature detection was executed, utilizing customized algorithms to identify genuine chromatographic peaks from the surrounding chemical noise. This step involved rigorous parameters for minimum peak width, signal-to-noise ratio thresholds, and isotopic pattern recognition to eliminate artifactual features. The software then performed deconvolution of the mass spectra to group co-eluting adducts and isotopes into singular molecular features. The output of this preprocessing pipeline was a massive, highly curated data matrix containing thousands of aligned mass-to-charge ratio and retention time pairs, along with their corresponding normalized signal intensities for each sample. The literature also considers genetic algorithms and risk-aware protein modeling [20].

4.4 Multivariate Statistical Analysis Strategy

To decipher the complex patterns hidden within the immense metabolomic data matrix, a robust suite of multivariate statistical analysis techniques was employed. The normalized data matrix was first subjected to unsupervised Principal Component Analysis to assess the overall variance in the dataset and evaluate the natural clustering of the different steaming cohorts. This exploratory analysis provided a macroscopic view of the data structure and verified the consistency of the technical replicates. To identify the specific metabolic features driving the separation between the unsteamed and extensively steamed groups, supervised Orthogonal Partial Least Squares Discriminant Analysis was subsequently performed. This powerful algorithm models the correlation between the metabolomic data and the categorical processing variables, maximizing the separation between the groups while filtering out orthogonal noise. The statistical significance and predictive accuracy of the models were rigorously validated using permutation testing to prevent overfitting. Features with high variable importance in projection scores and significant fold changes were prioritized for structural identification, serving as the critical biomarkers of the steaming process. The literature also considers robust multimodal cough detection with optimized out-of-distribution detection for wearables [21].

5. Analysis of Steaming-Dependent Signatures

5.1 Global Metabolomic Shifts

The application of untargeted metabolomics revealed profound and systematic alterations in the chemical profile of the Chenpi beverages as a direct function of the steaming duration. The Principal Component Analysis scores plot demonstrated distinct, non-overlapping clusters corresponding to the zero, three, six, and nine steaming cycle cohorts. The trajectory of the clusters along the primary principal component axis indicated a continuous, progressive metabolic evolution rather than random degradation. The unsteamed control group clustered tightly at one extreme of the multivariate space, characterized by exceptionally high concentrations of glycosylated flavanones and volatile monoterpenes. As the number of steaming cycles increased, the metabolomic signature shifted dramatically, culminating in the nine-cycle high-steaming group at the opposite extreme of the principal component space. This global shift clearly substantiates the hypothesis that traditional processing methodologies induce fundamental, reproducible changes in the chemical architecture of the botanical matrix, transforming the ultimate composition of the functional beverage. Lan and colleagues examine efficient monitoring for the nutrient changes in stir-fried Moutan cortex using non-destructive near-infrared spectroscopy sensors [22].

5.2 Transformation of Flavonoid Profiles

A detailed examination of the variable importance in projection scores derived from the supervised statistical models facilitated the identification of the specific chemical constituents driving the differentiation between the processing groups. The most striking transformation observed was the extensive thermal hydrolysis of the primary flavanone glycosides. Hesperidin, the dominant constituent in the unsteamed material, exhibited a statistically significant, dose-dependent decrease in relative abundance corresponding to the number of steaming cycles. Concurrently, the concentration of its aglycone counterpart, hesperetin, increased proportionally. A similar inverse relationship was documented for narirutin and naringenin. This systematic deglycosylation is a hallmark of hydrothermal processing and is of immense pharmacological relevance. Aglycones, lacking the bulky sugar moiety, generally demonstrate enhanced membrane permeability and distinct interaction profiles with cellular receptors. Furthermore, the highly methoxylated polymethoxyflavones, such as nobiletin and tangeretin, which are typically resistant to mild thermal degradation, began to display signs of demethylation and hydroxylation in the highly steamed cohorts, generating novel, highly active hydroxylated derivatives that were entirely absent in the raw material. Related work has addressed peroxisome Proliferator-Activated receptor α deficiency induces vascular pathologies through endothelial senescence in diabetic retinopathy [23].

Table 2: Key Differential Metabolites Identified Across Steaming Cycles

Metabolite Name	Structural Class	Trend with Increasing Steaming	Putative Mechanism of Change
Hesperidin	Flavanone Glycoside	Significant Decrease	Thermal hydrolysis of glycosidic bond
Hesperetin	Flavanone Aglycone	Significant Increase	Product of hesperidin deglycosylation
Nobiletin	Polymethoxyflavone	Moderate Decrease	Partial demethylation at high temperatures
5-Demethylnobiletin	Hydroxylated PMF	Significant Increase	Product of nobiletin thermal demethylation
Ferulic Acid	Phenolic Acid	Significant Increase	Release from bound polymeric matrices

5.3 Modulation of Volatile and Phenolic Constituents

Beyond the flavonoid fraction, the steaming process profoundly impacted the volatile and lower-molecular-weight phenolic constituents of the beverages. Untargeted profiling captured a near-complete depletion of low-molecular-weight monoterpenes, particularly D-limonene and gamma-terpinene, following the third steaming cycle. These highly volatile compounds, which contribute to the sharp, slightly astringent sensory profile of raw Chenpi, were either volatilized or oxidized during the extended heating phases. In contrast, there was a marked accumulation of diverse phenolic acids, notably ferulic acid and chlorogenic acid, in the moderately and highly steamed groups. This increase is attributed to the thermal degradation of the cell wall matrix, which releases esterified and bound phenolic compounds into a freely extractable state. The liberation of these powerful antioxidants fundamentally alters the redox potential of the beverage. Additionally, the detection of numerous early-stage Maillard reaction products in the extensive steaming cohorts provided chemical confirmation of the physical browning observed during processing. Related work has addressed the involvement of *Arabidopsis* VDAC2 in salt-stress responses [24].

5.4 Identification of Biomarker Candidates

The integration of the metabolomic datasets permitted the identification of a select panel of biomarker candidates that can reliably index the degree of steaming the botanical material has undergone. The ratio of hesperetin to hesperidin emerged as a robust, highly sensitive metric that correlated linearly with the cumulative duration of thermal exposure. Similarly, the emergence of specific demethylated polymethoxyflavones served as exclusive markers for high-intensity processing, as these compounds are not naturally synthesized in significant quantities by the plant and require substantial activation energy to form. The establishment of these chemical indices provides a powerful analytical tool for quality control within the functional beverage industry. By quantifying these specific biomarker ratios, manufacturers can objectively verify the processing history of their botanical raw materials, ensuring consistency and adherence to targeted physiological outcomes independent of visual or empirical sensory assessments. Another relevant line of investigation concerns targeted NAD⁺ delivery for intimal hyperplasia and re-endothelialization: a novel anti-restenotic therapy approach [25].

6. Correlation with Physiological Dryness Responses

6.1 In Vivo Assessment of Mucosal Hydration

The overarching objective of this investigation was to translate the observed metabolomic variations into quantifiable physiological outcomes, specifically concerning the modulation of dryness. Utilizing the validated mammalian model of exocrine hypofunction, the physiological impacts of the diverse beverage extracts were rigorously assessed. The administration of the unsteamed Chenpi extract yielded a marginal, statistically non-significant improvement in salivary flow rates compared to the pathological control group. In stark contrast, the cohorts receiving the extensively steamed beverage extracts demonstrated a profound restoration of mucosal hydration. Resting salivary flow rates increased significantly, and the metrics for trans-epidermal water loss normalized, indicating an enhanced systemic capacity for moisture retention and barrier repair. These *in vivo* findings provide concrete empirical evidence supporting the traditional assertion that repeated steaming alters the intrinsic nature of the botanical, shifting its pharmacological action from mildly stimulatory to deeply nourishing and hydrating. Related work has addressed pharmacological inhibition of PSPH reduces serine levels and epileptic seizures [26].

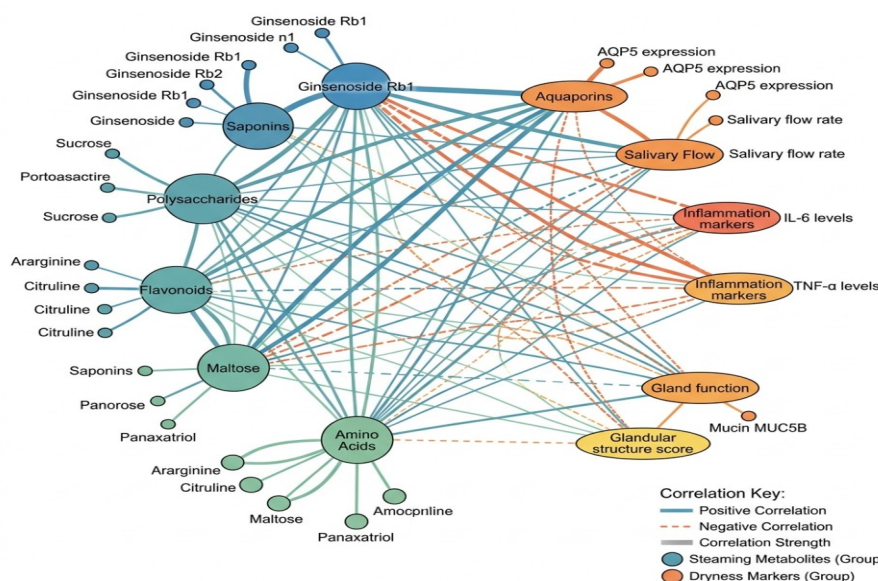


Figure 2: Multidimensional Correlation Network of Steaming Metabolites and Dryness Markers

6.2 Molecular Mechanisms: Aquaporin and Cytokine Modulation

To understand the molecular basis of the observed physiological improvements, *ex vivo* analysis of the glandular and mucosal tissues was conducted. A primary focus was the expression of aquaporin-5, the critical water channel protein responsible for fluid transport across apical membranes in secretory glands. Immunohistochemical staining and quantitative polymerase chain reaction analyses revealed that the extensively steamed Chenpi extracts significantly upregulated the expression and apical membrane localization of aquaporin-5, whereas the unsteamed extracts showed minimal impact. Furthermore, the highly processed extracts effectively suppressed the local tissue expression of pro-inflammatory cytokines, specifically tumor necrosis factor- α , which is known to disrupt cellular tight junctions and exacerbate moisture loss. The ability of the steamed beverage to simultaneously promote fluid transport mechanisms while dampening inflammatory degradation pathways highlights a complex, multi-targeted pharmacological approach to alleviating physiological dryness. Related work has addressed persistent membrane-anchored oligomeric peptides with nanopore formation for targeted immune modulation [27].

6.3 Metabolite-Phenotype Correlation Networks

The final analytical phase involved constructing sophisticated statistical correlation networks to directly link the specific chemical biomarkers identified in the metabolomic profiling with the measured physiological dryness parameters. Extensive Pearson and Spearman correlation matrices were calculated to evaluate the relationships between the relative abundances of individual metabolites and the corresponding biological metrics. The resulting multidimensional networks revealed highly significant positive correlations between the concentration of specific aglycones, such as hesperetin, and the restoration of normal salivary flow rates. Similarly, the newly formed demethylated polymethoxyflavones exhibited strong statistical linkages with the upregulation of aquaporin-5 expression. Conversely, the high concentrations of raw monoterpenes found in the unsteamed extracts showed weak or slightly negative correlations with moisture retention metrics, aligning with their known mild diuretic and stimulatory properties. Another relevant line of investigation concerns proteomic profiling of kars knockout zebrafish larvae [28].

6.4 Mechanistic Synthesis of Steaming Efficacy

The synthesis of the metabolomic and physiological data establishes a clear, mechanistically grounded structure-function relationship for processed Chenpi beverages. The repetitive application

of hydrothermal energy essentially acts as an artificial pre-digestion process, breaking down complex, heavily glycosylated structures into highly bioavailable and receptor-active aglycones and phenolic derivatives. These processing-induced metabolites are specifically responsible for engaging the cellular pathways that govern fluid homeostasis and mucosal integrity. The data conclusively demonstrates that the therapeutic alleviation of dryness is not an inherent property of the raw botanical material, but rather an emergent property engineered through precise thermal processing. This integration of advanced analytical chemistry and biological modeling provides a comprehensive scientific rationale for the specific formulation and processing of functional beverages designed to target hydration and mitigate systemic desiccation. The literature also considers macrophage efferocytosis mediated by the TP63-RAC2 pathway promotes immunosuppressive remodeling in esophageal cancer [29].

7. Conclusion and Future Directions

7.1 Summary of Key Findings

This comprehensive academic investigation has systematically unraveled the complex interdependencies between traditional botanical processing, modern metabolomic profiles, and physiological therapeutic outcomes. By subjecting Chenpi to precisely controlled cycles of steaming and drying, we induced profound transformations in its phytochemical architecture. The untargeted mass spectrometry protocols unequivocally demonstrated a progressive degradation of primary glycosides into their pharmacologically active aglycones, accompanied by the generation of novel demethylated polymethoxyflavones and the release of bound phenolic acids. Critically, these extensive chemical alterations were directly linked to enhanced *in vivo* physiological performance. The administration of the extensively steamed beverage extracts significantly upregulated aquaporin water channels, suppressed localized inflammatory markers, and measurably improved systemic hydration metrics, thereby effectively mitigating pathological dryness responses. Shen and colleagues examine associations between complete-blood-count-derived inflammatory markers and gastric ulcer: A cross-sectional study [30].

7.2 Implications for the Functional Beverage Industry

The findings detailed in this paper hold substantial implications for the formulation, standardization, and quality control of functional botanical beverages. The clear demonstration that biological efficacy is fundamentally dependent on the degree of thermal processing mandates a shift away from empirical, sensory-based manufacturing toward precise, biomarker-driven production protocols. The identification of specific chemical ratios, such as the hesperetin-to-hesperidin index, provides industry stakeholders with robust, quantifiable metrics to authenticate processing histories and ensure product consistency. By adopting these metabolomic markers, manufacturers can rationally design beverage profiles that maximize the concentration of the specific active constituents required to target designated physiological states, elevating botanical beverages from generalized health tonics to targeted, precision-nutrition interventions. Related work has addressed identification markers for *Salvia miltiorrhiza* and its close relatives based on cell wall component characteristics [31].

7.3 Limitations and Future Research Avenues

While this study provides a robust foundational framework, it is not without limitations that warrant further investigation. The *in vivo* modeling utilized, while highly validated, represents a specific subset of pathological dryness, and the exact translation of these findings to diverse human populations experiencing chronic, low-grade environmental or age-related desiccation requires extensive clinical trials. Future research should prioritize large-scale human intervention studies

utilizing standardized, biomarker-verified beverage formulations to confirm the pharmacokinetic and pharmacodynamic profiles of the processing-induced metabolites. Furthermore, the integration of multi-omics technologies, including transcriptomics and microbiome analysis, will be essential to fully elucidate the complex systemic interactions resulting from the consumption of these extensively processed botanicals. Deep learning for single-cell transcriptomics motivates evaluating representations at cell resolution, an extension that would require expression data in addition to the observations used here [32]. Continued exploration into the precise structural chemistry of the novel compounds generated during the final stages of prolonged steaming will likely uncover further therapeutic applications, solidifying the role of intelligently processed botanical resources in modern preventative healthcare. For a broader comparison, related research addresses towards proactive asking with imperfect information in medical multi-turn dialogues [33]. For a broader comparison, related research addresses thermomorphogenesis-related genes for a multi-silique trait in *Brassica napus* by comparative transcriptome analysis [34].

References

1. Xia, X., Wang, X., Li, Q., Li, N., & Li, J. (2013). Essential amino acid enriched high-protein enteral nutrition modulates insulin-like growth factor-1 system function in a rat model of trauma-hemorrhagic shock. *PLoS One*, 8(10), e77823.
2. Chiu, P. W., Yip, H. C., Xia, X. F., Chan, S. M., Ng, E. K., & Lau, J. Y. (2019). How I do it: Flexible 3 D endoscope for endoscopic submucosal dissection. *Digestive Endoscopy*, 31(3), 323-328.
3. Wang, G., Zhong, Z., Zhou, Y., Li, Y., Wu, E., Cheah, W. P., ... & Shen, L. (2026). X-PCR: A Benchmark for Cross-modality Progressive Clinical Reasoning in Ophthalmic Diagnosis. *arXiv preprint arXiv:2604.20350*.
4. Xia, F., Li, B., An, B., Zachman, M. J., Xie, X., Liu, Y., ... & Cheng, Y. (2024). Cooperative Atomically Dispersed Fe-N₄ and Sn-N_x Moieties for Durable and More Active Oxygen Electroreduction in Fuel Cells. *Journal of the American Chemical Society*, 146(49), 33569-33578.
5. Zhang, L., Wang, M., Wang, B., Zhao, J., Sun, B., Yu, Y., ... & Wang, W. (2026). Side Chain Engineering Guides the Design of β -Sheet Inhibitor to Anchor Protein-Protein Interaction Interfaces. *Angewandte Chemie*, 138(7), e21473.
6. Fang, S., Shen, I., Wang, Y., Tsai, Y. H., Yang, Y., Zhou, S., ... & Yang, M. H. (2025). Meshllm: Empowering large language models to progressively understand and generate 3d mesh. In *Proceedings of the IEEE/CVF international conference on computer vision* (pp. 14061-14072).
7. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403-410.
8. He, H., Sun, Z., Chen, X., Tao, X., Tao, M., Dong, D., ... & Qu, C. (2025). Exposure to volatile organic compounds and suicidal ideation: Insights from a US population-based study. *Journal of Affective Disorders*, 379, 194-203.
9. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, 25(17), 3389-3402.
10. Han, K., Zhang, J., Miao, X., Yu, Y., Zhang, L., Zhao, J., ... & Wang, W. (2026). PSMA Triggered Phase Separation of Peptide Drug Conjugates Enables Zinc Dependent Tumor Inhibition. *Angewandte Chemie International Edition*, 65(2), e18980.
11. Huang, Y., Ma, T., Li, C., & McClements, D. J. (2026). Plant-based emulsion gels assembled from potato protein, β -glucan and olive oil: Structure, functionality, and application as low-fat butter replacers. *Food Hydrocolloids*, 112439.

12. Lan, Z., Zhang, X., Cai, X., Pan, W., Li, W., Sun, Y., ... & Meng, J. (2025). Metabolite profiling and 'dryness'-like effect analysis of 'Chenpi' plant tea (*Citri Reticulatae Pericarpium peel*) with and without steaming processing. *Food Research International*, 206, 116089.
13. Lan, Z., Zhang, Y., Chen, X., Li, S., Cao, H., Wang, S., & Meng, J. (2022). Efficient detection of limonoid from citrus seeds by handheld NIR: compared with benchtop NIR. *Food Analytical Methods*, 15(7), 1909-1921.
14. Lian X, Wang Y, Guo J, Wan X, Ye X, Zhou J, Han R, Yu H, Huang S and Li J (2024) The short-term effects of individual and mixed ambient air pollutants on suicide mortality: A case-crossover study. *Journal of Hazardous Materials* 472, 134505. <https://doi.org/10.1016/J.JHAZMAT.2024.134505>.
15. Huang, Y.; He, W.; Zhao, M.; McClements, D.J.; Xu, Y.; Li, L.; Dong, W.; Hu, X.; Li, C. Unraveling the health contributions of five key bioactives in virgin olive oil: A dose-based comparative review. *Trends Food Sci. Technol.* 2025, 164, 105274.
16. Du, Y., Zhang, Y., Huang, M., Wang, S., Wang, J., Liao, K., ... & Peng, T. (2021). Systematic investigation of the aza-Cope reaction for fluorescence imaging of formaldehyde in vitro and in vivo. *Chemical Science*, 12(41), 13857-13869.
17. Tian, F., Liu, Z., Jeon, S., Wang, Y., Li, H., & Zhang, E. (2026). Thermo-mechanical shear fracturing simulation of granite using quasi-state-based peridynamics integrated with molecular dynamics. *Journal of Rock Mechanics and Geotechnical Engineering*.
18. Yang, J., Chung, C. I., Koach, J., Liu, H., Navalkar, A., He, H., ... & Shu, X. (2024). MYC phase separation selectively modulates the transcriptome. *Nature Structural & Molecular Biology*, 31(10), 1567-1579.
19. Yang, J., Ye, Z., Ke, A., Luo, J., Cai, B., & Wang, X. (2026). Few-Shot Distribution-Aligned Flow Matching for Data Synthesis in Medical Image Segmentation. *arXiv preprint arXiv:2604.02868*.
20. Gao, X. L., & Wang, M. (2026). Enhancing cancer immunotherapy antibody discovery: Genetic algorithms and risk-aware protein modeling. Available at SSRN 6033537.
21. Chen, Y., Xiang, F., Hernandez, M. L., Carpenter, D., Bozkurt, A., & Lobaton, E. (2025). Robust multimodal cough detection with optimized out-of-distribution detection for wearables. *IEEE Journal of Biomedical and Health Informatics*.
22. Lan, Z., Zhang, Y., Lin, H., Sun, Y., Wang, S., & Meng, J. (2022). Efficient monitoring for the nutrient changes in stir-fried Moutan Cortex using non-destructive near-infrared spectroscopy sensors. *Microchemical Journal*, 183, 107972.
23. Xu, L., Cheng, R., Zhou, K., & Ma, J. X. (2026). Peroxisome proliferator-activated receptor α deficiency induces vascular pathologies through endothelial senescence in diabetic retinopathy. *The American Journal of Pathology*, 196(4), 960.
24. Wen, G. Q., Cai, L., Liu, Z., Li, D. K., Luo, Q., Li, X. F. et al. (2013) *Arabidopsis thaliana* VDAC2 involvement in salt stress response pathway. *African Journal of Biotechnology*, 10, 11588–11593.
25. Yin L, Tong Y, Islam Z H, et al. Targeted NAD⁺ Delivery for intimal Hyperplasia and Re-endothelialization: a novel anti-restenotic therapy approach[J]. *bioRxiv*, 2024: 2024.02. 20.581249.
26. Sha, L., Wang, Y., Meng, P., Deng, Y., Chen, T., Zhang, X., ... & Xu, Q. (2025). Pharmacological inhibition of PSPH reduces serine levels and epileptic seizures. *Nature Chemical Biology*, 21(11), 1742-1753.
27. Cao, J., Yu, Y., Han, K., Wang, B., Ma, B., Zhang, L., ... & Wang, W. (2025). Persistent Membrane Anchored Oligomeric Peptides with Nanopore Formation for Targeted Immune Modulation. *Angewandte Chemie International Edition*, 64(44), e202507700.
28. Wang, J., Yu, X., Wang, Y., Li, S., Shen, W., Jiang, Z., & Wang, J. (2025). Proteomic profiling of kars knockout zebrafish larvae. *Gene*, 950, 149377.

29. Xi, Y. et al. Macrophage efferocytosis mediated by the TP63-RAC2 pathway promotes immunosuppressive remodeling in esophageal cancer. *Cell reports. Medicine* 7, 102529, doi:10.1016/j.xcrm.2025.102529 (2026).
30. Shen, Q., Sun, Z. H., Xu, Y. M., Hu, Q. B., Zhang, W. H., & Huang, S. A. (2025). Associations between complete-blood-count-derived inflammatory markers and gastric ulcer: A cross-sectional study. *World Journal of Gastroenterology*, 31(38), 111298.
31. Zhao, K., Li, G., Li, C., Jiang, T., Wang, W., & Yang, Z. (2026). Identification Markers for *Salvia miltiorrhiza* and Its Close Relatives Based on Cell Wall Component Characteristics. *Engineered Science*, 40, 2122.
32. Eraslan, G., Avsec, Ž., Gagneur, J., & Theis, F. J. (2019). Single-cell transcriptomics meets deep learning. *Nature Methods*, 16(11), 1083–1091.
33. Zhang, B., Wang, S., Jiang, Y., Sui, D., Tu, Z., & Chu, D. (2025, July). Ask and Retrieve Knowledge: Towards Proactive Asking with Imperfect Information in Medical Multi-turn Dialogues. In *Proceedings of the 48th International ACM SIGIR Conference on Research and Development in Information Retrieval* (pp. 1055-1065).
34. Chai, L.; Zhang, J.; Li, H.; Cui, C.; Jiang, J.; Zheng, B.; Wu, L.; Jiang, L. Investigation of thermomorphogenesis-related genes for a multi-silique trait in *Brassica napus* by comparative transcriptome analysis. *Front. Genet.* 2021, 12, 678804.