

RESEARCH ARTICLE

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Glycosidically bound volatiles profiling reveals a new approach to effectively gauge aging years of Guangchenpi (*Citrus reticulata* 'Chachiensis')

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Abstract

Guangchenpi (GCP, *Citrus reticulata*), a well-known traditional Chinese medicine and homology food condiment, is predominantly consumed in Asia. Extending its aging process enhances its medicinal properties, flavor, and profitability. However, pinpointing the aging year of GCP remains a bottleneck of the industry. This study represents the first comprehensive characterization of 32 glycosidically bound volatiles in GCP, employing a combination of Amberlite XAD-2 resin extraction and gas chromatography-mass spectrometry (GC-MS) analysis. The identified compounds include bioactive substances such as 5-hydroxymethylfurfural and acorenone B. Fourteen glycosidically bound volatiles exhibited a significant correlation with the aging duration of GCP. Additionally, the aging process was associated with dynamic shifts in the microbial community, notably an enrichment of *Lactobacillus* and *Oceanobacillus*, which potentially influenced the volatile compound profile. Machine learning integration revealed a model based on the content and relative weight of the 14 glycosidically bound volatiles, demonstrating high accuracy in determining aging years of GCP. This model could aid in detecting adulteration and standardizing the GCP market.

Keywords Guangchenpi, Glycosidically bound volatiles, Microbiome, Aging years, Predictive model

Introduction

Pericarpium Citri Reticulatae (PCR), the dried peel of mature tangerine fruit, is a prominent traditional Chinese medicine and culinary ingredient widely consumed in Asia and other regions. Citri Reticulatae 'Chachiensis', derived from *Citrus reticulata* 'Chachiensis', is referred to as Guangchenpi (GCP), which is considered superior in quality compared to PCR from other tangerine cultivars (Liu et al. 2024). GCP is commonly used to treat persistent coughs and phlegm, stimulate appetite, and enhance immunity (Zhou et al. 2025). GCP from Xinhui County (Guangdong Province, China) is often regarded as the finest, based on geo-herbalistic analyses (Su et al. 2023). Various secondary metabolites contribute to the therapeutic properties of GCP, with numerous studies identifying key biochemicals such as volatiles and

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flavonoids that play significant roles in its health benefits (Duan et al. 2017; Wang et al. 2016; Zeng et al. 2017). For example, citrus essential oils exhibit strong antifungal activity (Calva et al. 2017), while the antioxidant and immunomodulatory properties of GCP polysaccharides have been quantified (Zhou et al. 2021). These biological activities substantiate the exceptional physiological properties of GCP. Empirically, the quality of GCP improves with aging, supporting the adage “the older, the better.” This enhancement is largely attributed to the accumulation of bioactive compounds during the aging process. For instance, polymethoxylated flavones, which are integral to GCP’s medicinal effects, increase with age (Zeng et al. 2017). Similarly, levels of α -sinensal and dimethyl anthranilate are higher in GCP aged ten years compared to those aged three or five years (Liu et al. 2024). Understanding the metabolite composition is essential for elucidating the mechanisms behind GCP aging.

Volatile compounds, key secondary metabolites in plants, contribute to flavor and exhibit various physiological functions (Fan et al. 2021). Numerous studies have focused on free volatiles in fruits, particularly citrus, with extensive research on volatiles in citrus fruits, juices, and peel products (Fan et al. 2009; Liu et al. 2024; Pan et al. 2023). In addition to free volatiles, which can be quickly released, volatile compounds can also exist as glycosidically bound volatiles. These conjugates are believed to serve as a detoxification, storage, and transport mechanism for cytotoxic metabolites in plants (Song et al. 2018). In tea plants, volatiles like benzyl alcohol, 2-phenylethanol, and monoterpene alcohols exist as glycosidically bound forms, conferring physiological defense and antimicrobial properties (Mizutani, et al. 2002; Zhang et al. 2006). Moreover, glucosylation of free volatiles enhances the antibacterial activity of the aglycones (Liang et al. 2020). These odorless sugar conjugates can be released through acid or enzymatic hydrolysis, yielding free volatiles such as terpenes, benzenic compounds, C13-norisoprenoids, hydroxy esters, and fatty alcohols. Therefore, glycosidically bound volatiles hold potential as natural aroma enhancers in food processing. Research on glycosidically bound volatiles in citrus, particularly in GCP, remains limited, presenting an opportunity to investigate how these volatiles influence flavor changes during GCP aging.

The microbial community in GCP also plays a critical role in its metabolic quality. The predominant microorganisms in GCP include fungi and bacteria, with *Bacillus* and *Pantoea* being abundant bacterial species, while *Aspergillus* is likely the primary fungus contributing to quality variations across different aging periods (Zhou

et al. 2025). The geo-herbalistic characteristics of GCP are influenced by microbial differences in its growing regions (Su et al. 2023). Given this, further investigation into the microbial composition and its dynamic changes during GCP aging is warranted.

Machine learning models have proven effective in identifying correlations among variables and have been widely applied in screening physicochemical and metabolite indicators (Jing et al. 2017; Kong et al. 2016; Monforte et al. 2021). For instance, Lyu (2022) employed machine learning techniques to authenticate lemon juice based on metabolomic analysis, while Zhang (2022) developed partial least squares and artificial neural network models to predict apple shelf life with high accuracy. These examples demonstrate the potential of machine learning to predict product quality from multidimensional biochemical data.

In this study, glycosidically bound volatiles in GCP were identified, and correlation network analysis was applied to examine the relationship between the content of these volatiles and GCP aging years. Additionally, the microbial composition of GCP was systematically identified, and its influence on glycosidically bound volatiles was assessed. Finally, machine learning models were used to predict GCP’s aging years based on the bound volatiles. This research offers a practical strategy for identifying the functional components of GCP and deepens the understanding of its quality changes during the aging process. Furthermore, the optimization and application of predictive models provide an effective means for determining the authentic aging duration of GCP, which can help stabilize the market and offer theoretical support for the standardization of the GCP industry.

Materials and methods

Materials and GCP sampling

A total of 37 GCP samples with varying aging years were collected in 2020 from five manufacturers in Xinhui District, Jiangmen City, Guangdong Province, China. Detailed information regarding the GCP samples is provided in Table S1. The samples were initially authenticated using characteristic identification, empirical analysis, and metabolite identification (Liu et al. 2024). All samples were stored at -80°C until further analysis.

Reagents and standards

A $\text{C}_7\text{-C}_{40}$ standard n-alkane solution was obtained from ANPEL Laboratory Technologies (Shanghai) Inc. (China), and methyl nonanoate, as well as dichloromethane (GC-grade), were purchased from Sigma Aldrich

(Saint Louis, MO, USA). Amberlite XAD-2 resin was sourced from Sigma Aldrich, while almond β -glucosidase (12 units·mg⁻¹) was acquired from Yuanye Ltd. (Shanghai, China). Disodium hydrogen phosphate and sodium dihydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Isolation of glycosidically bound volatiles

GCP samples were ground into powder using liquid nitrogen. A 2.00 g portion of the powder was then mixed with 36 mL of distilled water, followed by ultrasonic extraction at 40 °C for 20 min. After centrifugation at 8,000 rpm at 4 °C for 5 min, the supernatant was collected as the crude extract. Glycosidic precursors were isolated using a 20×3.5 cm i.d. activated column filled with Amberlite XAD-2 resin (5×3.5 cm), which was pre-conditioned with 24 mL of methanol and 24 mL of distilled water. The clean crude extract was poured onto the column and rinsed with 24 mL of distilled water to elute soluble sugars. Free volatiles were eluted using 24 mL of dichloromethane, while glycosidic precursors were eluted with 30 mL of methanol. The methanol eluate was concentrated under vacuum at 45 °C, and the residue of glycosidic precursors was re-dissolved in 1 mL of 0.15 M citric phosphate buffer solution (pH=5). The glycosidic precursors were then hydrolyzed by adding 0.10 mg of almond β -glucosidase (12 units·mg⁻¹) and incubated at 40 °C for 24 h to release volatile aglycones from the glycosidically bound volatiles.

GC-MS analysis of volatile compounds

The extraction and analysis of volatile aglycones were performed according to Liu et al. (Liu et al. 2024). The hydrolyzed liquid was mixed with 500 μ L of dichloromethane containing 17.5 μ g·mL⁻¹ of methyl nonanoate as the internal standard.

Volatiles were analyzed using a TRACE GC Ultra coupled with a DSQ II mass spectrometer (Thermo Scientific, Bellefonte, PA, USA) with a TR-5 MS column (30 m×0.25 mm×0.25 μ m; Thermo Scientific, Bellefonte, PA, USA). Compounds were identified by matching mass spectra and linear retention index against the NIST 17 database. Semi-quantification was conducted based on the relative peak area of the volatiles compared to that of the internal standard.

16S rRNA gene amplicon sequencing of the endophyte microbiome

The CTAB method was employed to extract total DNA from the microbial community of GCP samples. The

V3-V4 variable region of the 16S rRNA gene was amplified using primers 338F (5'-ACTCCTACGGGAGGC AGCAG-3') and 806R (5'-GGACTACHVGGGTWT CTAAT-3'), with three replicates per sample.

The amplification products were recovered and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). For library preparation, the NEXTFLEX Rapid DNA-Seq Kit was used, following these steps: (i) ligation of adapters; (ii) magnetic bead-based screening and removal of self-contiguous adapter sequences; (iii) enrichment of library templates via amplification; (iv) recovery of products using magnetic beads to obtain the final library. Sequencing was performed using Illumina's Miseq PE300/NovaSeq PE250 platform (Majorbio, Shanghai, China).

Machine learning models screening

The recorded aging years of GCP and the 32 glycosidically bound volatiles detected were used as the initial dataset. Several linear and nonlinear statistical models were employed to analyze the dataset, including Mixture of Experts (MOE), Random Forest (RF), Gradient Boosting Regressor (GBoost), CatBoost Regressor (CBoost), Decision Tree Regressor (DTR), Linear Regression (Linear R), Multi-layer Perceptron Regressor (MLP), Partial Least Squares (PLS), Bayesian Ridge Regression (BR), Logistic Regression (Logistic R), K-Nearest Neighbors Regressor (KNN), and Support Vector Regression (SVR) models. Among these, MOE is a hybrid expert system composed of four sub-models: GBoost, RF, CBoost, and DTR. The Mean Square Error (MSE) was used to evaluate the error between the predicted and actual values, while the Coefficient of Determination (R^2) measured the model's capacity to explain the data variation. These two parameters were key indicators for assessing model stability and predictive performance. To ensure the reliability and repeatability of the experiments, all models were trained with identical parameters, and random seed numbers were set to ensure result reproducibility. The dataset was split into a training set (80%) and a test set (20%), with a tenfold cross-validation scheme applied to optimize the regularization parameter.

The contribution direction (negative or positive effect) of each glycosidically bound volatile to the aging years was determined using an LR model. The MOE model was employed to estimate the importance of each metabolite. This model integrates multiple regression models to accommodate nonlinear relationships and interactions within the data, thereby improving prediction performance.

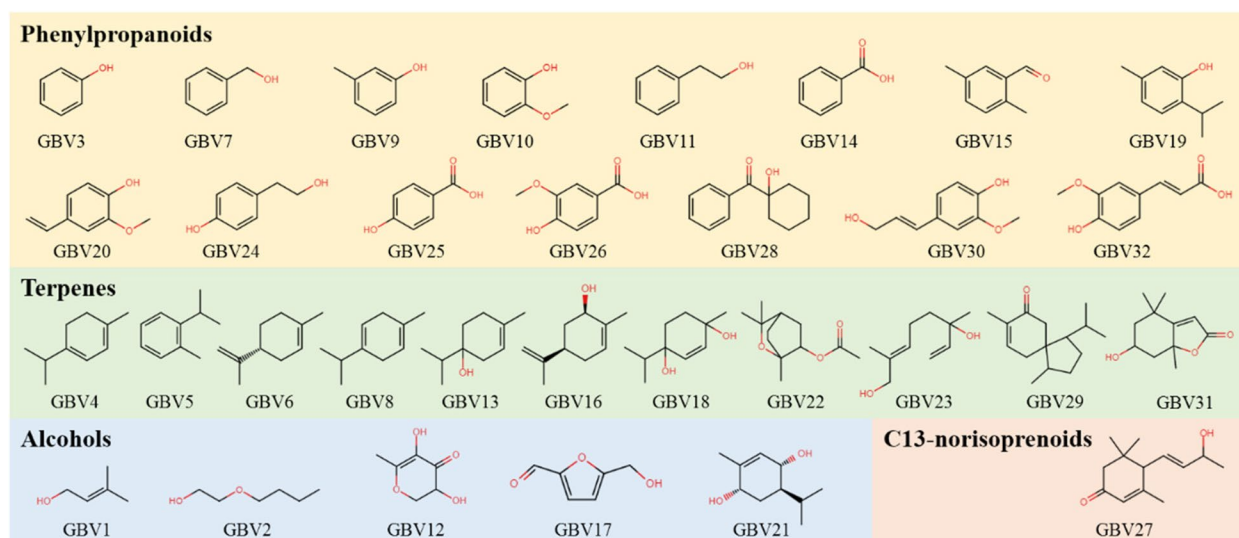


Fig. 1 Structures of the identified glycosidically bound volatiles in GCP. Sugar moieties are typically bonded to the hydroxyl groups of the volatiles. The oxygen-containing parts of the glycosidically bound volatiles in GCP, such as the hydroxyl group, are highlighted in red

To predict the authentic aging years of GCP, a machine learning model based on the composition of glycosidically bound volatiles was developed. The model's performance was evaluated using LR, which proved optimal when the feature set consisted of eleven variables. The prediction formula derived is as follows:

$$AY = 24.45 \times X_1 - 17.03 \times X_2 - 12.37 \times X_3 + 10.04 \times X_4 + 3.69 \times X_5 - 7.31 \times X_6 - 1.82 \times X_7 + 1.13 \times X_8 + 0.23 \times X_9 + 2.68 \times X_{10} - 0.83 \times X_{11} + 11.49 \quad (1)$$

where AY represents the predicted aging years of GCP, and X_1 to X_{11} correspond to the content of glycosidically bound volatiles ($\mu\text{g}\cdot\text{g}^{-1}$) in GCP, including α -terpinene (GBV4), 3-methyl-phenol (GBV9), o-cymene (GBV5), β -butoxyethanol (GBV2), 2-methoxy-phenol (GBV10), pyranone (GBV12), trans-coniferyl alcohol (GBV30), isoxylaldehyde (GBV15), d-limonene (GBV6), 4-hydroxybenzeneethanol (GBV24), and trans-ascaridol glycol (GBV18).

Statistical analysis

For glycosidically bound volatile determination, four biological replicates of each sample were performed to ensure reliable results. For microbiome sequencing, three biological replicates were conducted. All data were processed using Excel 2016 and expressed as the mean $\pm$ standard deviation. The relationship between glycosidically bound volatiles and GCP aging years was explored using Pearson's correlation analysis and visualized with Cytoscape v3.9.1. Supervised orthogonal partial least squares-discriminant analysis (OPLS-DA) was applied using SIMCA 14.1. Redundancy analysis (RDA)

was performed and visualized at <https://www.chiplot.online/>.

Results

Characterization of glycosidically bound volatiles in GCP

The composition of glycosidically bound volatiles in

GCP with varying aging years was analyzed. A total of 32 bound volatiles were identified, including 11 terpenes, 15 phenylpropanoids, five alcohols, and one C13-norisoprenoid (Fig. 1, Table S2). Terpenoids, the most abundant free volatiles in citrus fruits, play a key role in the characteristic flavor of citrus. These compounds can also serve as flavor precursors via glycosylation. In this study, terpenoids such as d-limonene, α -terpinene, γ -terpinene, terpinen-4-ol, (-)-trans-carveol, and 8-hydroxylinalool were detected among the glycosidically bound volatiles in GCP. Most of these terpenoids were previously identified as free volatiles in 'Chachiensis', with the exceptions of 8-hydroxylinalool and exo-2-hydroxycineole acetate (Liu et al. 2024). These two bound terpenoids are likely specific derivatives formed during the GCP aging process, potentially through oxidation and glycosylation of linalool and 1,8-cineole, respectively. Additionally, benzenic compounds are known as major bound volatiles in fruits with strong antioxidant properties (Gao et al. 2018; Liu et al. 2018). Many of these compounds contribute to the pungent and spicy aroma of the fruit. Notably, vanillin is a key component in various industries, including food flavoring, beverages, and perfumes (Li et al. 2008). In this

Table 1 Glycosidically bound volatiles in GCP with different aging years

No	Compound name	RI ^a	RT ^b	Average content ^c ($\mu\text{g}\cdot\text{g}^{-1}$)	Maximum content ($\mu\text{g}\cdot\text{g}^{-1}$)	Sample ID	Minimum content ($\mu\text{g}\cdot\text{g}^{-1}$)	Sample ID
GBV1	Prenol	776	5.78	1.17 ± 1.04	5.28 ± 1.24	4-18	0.04 ± 0.00	1-08
GBV2	β-Butoxyethanol	907	10.44	0.14 ± 0.02	0.19 ± 0.01	4-09	0.11 ± 0.02	2-17
GBV3	Phenol	981	14.50	0.12 ± 0.03	0.21 ± 0.01	1-10	0.07 ± 0.00	5-14
GBV4	α-Terpinene	1017	15.48	0.03 ± 0.01	0.06 ± 0.05	5-12	0.01 ± 0.00	3-18
GBV5	o-Cymene	1022	15.86	0.12 ± 0.06	0.33 ± 0.05	1-17	0.03 ± 0.00	3-18
GBV6	d-Limonene	1031	16.08	0.09 ± 0.16	1.00 ± 0.70	4-17	0.02 ± 0.00	3-18
GBV7	Benzyl alcohol	1036	16.61	2.37 ± 1.99	7.51 ± 0.82	3-17	0.09 ± 0.01	1-08
GBV8	γ-Terpinene	1060	17.66	0.03 ± 0.02	0.09 ± 0.06	1-10	- ^d	2-09
GBV9	3-Methyl-phenol	1075	17.84	0.23 ± 0.07	0.40 ± 0.04	3-17	0.06 ± 0.01	1-08
GBV10	2-Methoxy-phenol	1090	18.90	0.10 ± 0.04	0.23 ± 0.03	1-16	0.00 ± 0.00	4-17
GBV11	2-Phenylethanol	1116	20.25	0.55 ± 0.41	1.44 ± 0.15	1-18	0.07 ± 0.01	1-08
GBV12	Pyranone	1150	21.85	0.21 ± 0.07	0.37 ± 0.00	5-16	0.04 ± 0.02	1-08
GBV13	Terpinen-4-ol	1177	23.37	0.15 ± 0.13	0.71 ± 0.09	3-18	0.00 ± 0.00	1-08
GBV14	Benzoic acid	1177	24.41	1.09 ± 0.51	2.08 ± 0.54	1-13	0.01 ± 0.00	4-18
GBV15	Isoxylaldehyde	1208	24.91	0.48 ± 0.18	1.08 ± 0.55	4-11	0.19 ± 0.02	3-18
GBV16	(-)-trans-Carveol	1217	25.27	1.27 ± 1.10	4.22 ± 0.51	1-13	0.05 ± 0.01	5-14
GBV17	HMF	1232	26.00	0.64 ± 0.75	3.11 ± 0.14	1-10	-	5-14
GBV18	trans-Ascaridol glycol	1272	27.86	0.13 ± 0.12	0.65 ± 0.11	1-17	-	1-08
GBV19	Isothymol	1299	28.74	1.08 ± 1.20	5.86 ± 0.58	1-13	-	3-09
GBV20	p-Vinylguaiacol	1316	29.29	6.86 ± 4.04	20.91 ± 1.17	2-09	1.79 ± 0.53	3-18
GBV21	2-Methyl-5-(propan-2-ylidene)-cyclohexane-1,4-diol	1322	29.67	0.77 ± 0.41	1.75 ± 0.38	3-09	0.02 ± 0.01	1-08
GBV22	exo-2-Hydroxycineole acetate	1344	31.05	11.37 ± 10.40	60.79 ± 8.93	3-12	2.43 ± 0.94	3-18
GBV23	8-Hydroxylinalool	1361	31.72	0.40 ± 0.43	2.35 ± 0.26	1-08	0.04 ± 0.04	3-11
GBV24	4-Hydroxy-benzeneethanol	1442	34.71	0.93 ± 0.36	1.73 ± 0.11	1-12	0.36 ± 0.02	1-08
GBV25	p-Hydroxybenzoic	1558	40.15	1.29 ± 0.79	3.67 ± 0.68	4-11	0.01 ± 0.00	3-17
GBV26	Vanillin	1592	40.97	1.57 ± 0.86	4.15 ± 0.70	2-15	0.41 ± 0.05	3-18
GBV27	3-Oxo-α-ionol	1647	42.66	2.52 ± 0.98	4.83 ± 0.32	3-17	0.62 ± 0.17	3-09
GBV28	1-Hydroxycyclohexyl phenyl ketone	1687	44.27	0.49 ± 0.63	3.06 ± 4.35	1-16	0.04 ± 0.01	2-11
GBV29	Acorenone B	1701	44.87	0.66 ± 0.41	1.90 ± 0.25	3-12	0.15 ± 0.03	1-08
GBV30	trans-Coniferyl alcohol	1743	46.16	1.25 ± 1.12	4.00 ± 0.88	4-18	-	5-09
GBV31	HTT	1784	47.18	1.31 ± 0.48	2.39 ± 0.24	5-16	0.15 ± 0.03	1-10
GBV32	Ferulic acid	1871	50.55	2.23 ± 0.98	4.17 ± 0.92	2-10	0.61 ± 0.08	5-17
Total content				41.66 ± 13.42	98.16 ± 12.67	3-12	21.84 ± 1.32	1-10

a Linear retention index on the TR-5 MS column; **b** Retention time; **c** Average content of bound volatiles across all GCP samples; **d** - indicates that the substances were below the detection limit

study, vanillin and 2-phenylethanol, both with a sweet scent, were identified in GCP. Regarding C13-norisoprenoids, several compounds, including β-damascenone, β-ionone, and 3-oxo-α-ionol, have been identified in various fruits (Liu et al. 2018; Ubeda et al. 2012). However, 3-oxo-α-ionol is typically the only C13-norisoprenoid found in citrus cultivars (Ren et al. 2015, 2014; Zhong et al. 2014). Consistent with these reports, 3-oxo-α-ionol was identified as the unique C13-norisoprenoid in GCP in this study.

The content of bound volatiles exhibits significant variation across different GCP samples, with total bound volatile levels ranging from $21.84 \pm 1.32 \mu\text{g}\cdot\text{g}^{-1}$ (1-10) to $98.16 \pm 12.67 \mu\text{g}\cdot\text{g}^{-1}$ (Table 1). Exo-2-hydroxycineole acetate was the most prevalent bound volatile, with an average concentration of $11.37 \pm 10.40 \mu\text{g}\cdot\text{g}^{-1}$, potentially serving as a biomarker for GCP. Additionally, p-vinylguaiacol and 3-oxo-α-ionol were the second and third most abundant glycosidically bound volatiles, with mean concentrations of $6.86 \pm 4.04 \mu\text{g}\cdot\text{g}^{-1}$ and $2.52 \pm 0.98 \mu\text{g}\cdot\text{g}^{-1}$, respectively. Notably, p-vinylguaiacol is also the dominant

bound volatile in the citrus juices of Hamlin sweet orange, pink grapefruit, white grapefruit, and Owari satsuma (Ren et al. 2015). The average contents of vanillin and 2-phenylethanol were $1.57 \pm 0.86 \mu\text{g}\cdot\text{g}^{-1}$ and $0.55 \pm 0.41 \mu\text{g}\cdot\text{g}^{-1}$, respectively. However, some compounds were below the detection threshold in certain samples. For instance, terpinen-4-ol was undetectable in sample 1–08, and HMF was absent in samples 3–09 and 5–14.

Glycosidically bound volatiles exhibit different accumulation patterns during the GCP aging process

Differing accumulation levels of glycosidically bound volatiles were observed in GCP samples with varying aging durations. For example, the content of trans-coniferyl alcohol (GBV30) in GCP samples stored for three years was $3.40 \pm 0.16 \mu\text{g}\cdot\text{g}^{-1}$ (1–18), $2.58 \pm 0.11 \mu\text{g}\cdot\text{g}^{-1}$ (2–18), $2.97 \pm 0.17 \mu\text{g}\cdot\text{g}^{-1}$ (3–18), and $4.00 \pm 0.88 \mu\text{g}\cdot\text{g}^{-1}$ (4–18). However, GBV30 levels were significantly lower in GCP samples stored for longer periods; in samples aged thirteen years from two manufacturers, the contents were only $0.32 \pm 0.05 \mu\text{g}\cdot\text{g}^{-1}$ (1–08) and $0.33 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$ (4–08), respectively. These fluctuations in the bound volatile levels provide new insights into the aging process and its influence on GCP.

To uncover potential patterns in the aging process of GCP, correlation network analysis was conducted based on the glycosidically bound volatiles across all GCP samples (Fig. 2, Fig. S1). The relationship between aging years and bound volatiles reflects unspecified conversion and degradation reactions among related metabolites, facilitating the identification of aging biomarkers in GCP. The network comprised 33 nodes connected by 145 significant edges, including 96 positive correlations and 49 negative correlations (Fig. 2a). Fourteen glycosidically bound volatiles were found to be significantly correlated with the aging years of GCP. These included prenol (GBV1, $r = -0.44$), benzyl alcohol (GBV7, $r = -0.52$), 3-methylphenol (GBV9, $r = -0.62$), 2-phenylethanol (GBV11, $r = -0.48$), pyranone (GBV12, $r = -0.52$), terpinen-4-ol (GBV13, $r = -0.57$), (-)-trans-carveol (GBV16, $r = -0.42$), trans-ascaridol glycol (GBV18, $r = -0.38$), 3-oxo- α -ionol (GBV27, $r = -0.53$), and trans-coniferyl alcohol (GBV30, $r = -0.81$), all exhibiting negative correlations with aging time (Fig. 2). These compounds are known to be present as bound volatiles during the development of citrus fruits. For instance, benzyl alcohol, 2-phenylethanol, terpinen-4-ol, (-)-trans-carveol, and 3-oxo- α -ionol have been detected as bound volatiles in the peels and juices of Satsuma mandarin (Ren et al. 2015). As indicated by this study, the content of these compounds in GCP progressively decreases over the aging period, demonstrating a trend of degradation (Fig. 2b).

Conversely, four bound volatiles— α -terpinene (GBV4, $r = 0.45$), *p*-hydroxybenzoic acid (GBV25, $r = 0.33$), vanillin (GBV26, $r = 0.37$), and ferulic acid (GBV32, $r = 0.36$)—showed significant positive correlations with the aging years of GCP (Fig. 2). Among these, the three benzenic compounds, *p*-hydroxybenzoic acid, vanillin, and ferulic acid, were present at relatively high concentrations.

The physiological activity of GCP is thought to increase gradually with aging (Liu et al. 2024). In this context, the characterization of glycosidically bound volatiles offers a valuable perspective in explaining the enhanced quality of long-aged GCP. Several bound volatiles exhibited an increasing trend over time (Fig. 2b). As aging progresses, the accumulation of bound volatiles, such as vanillin and ferulic acid, likely contributes to the improved quality of GCP.

Additionally, significant correlations were observed between bound volatiles in the network. The collinearity among substances can help identify potential metabolic pathways in plants. In GCP, active physiological metabolism may have ceased, with only mild and spontaneous oxidation, browning, and transformation reactions occurring. The observed collinearity among substances in GCP could reflect transformation or degradation processes rather than active metabolic pathways. Seven positive correlations and two negative correlations were found among bound terpenoids. Bound benzenic compounds, being the most diverse group of bound volatiles in GCP, exhibited more correlations with each other than bound terpenoids. A total of 25 positive correlations and 10 negative correlations were observed among bound benzenic compounds. Clearly, the higher number of positive correlations within the same class likely results from their similar chemical structures and physicochemical properties.

Potential effects of the microbiome of GCP on its glycosidically bound volatiles

To explore the role of microorganisms in the aging process of GCP, microbial sequencing was conducted on ten samples selected from five batches, representing both the shortest and longest aging periods. The 'longer aging' group (LA group) included samples '1–08', '2–09', '3–09', '4–08', and '5–09', aged 3–4 years, while the 'shorter aging' group (SA group) contained '1–18', '2–18', '3–18', '4–18', and '5–17', aged 12–13 years. A total of 1707 OTUs were identified across all GCP samples, representing 35 bacterial phyla and 781 bacterial genera (Table S3). Among these phyla, *Proteobacteria* dominated at 37.6%, followed by *Firmicutes* (16.1%) and *Actinobacteria* (14.2%). *Delftia tsuruhatensis* was the most abundant bacterial species in all GCP samples, with an average of 9076.83 ± 4570.20 , followed by *Pseudomonas mosselii* at 763.03 ± 1558.32 .

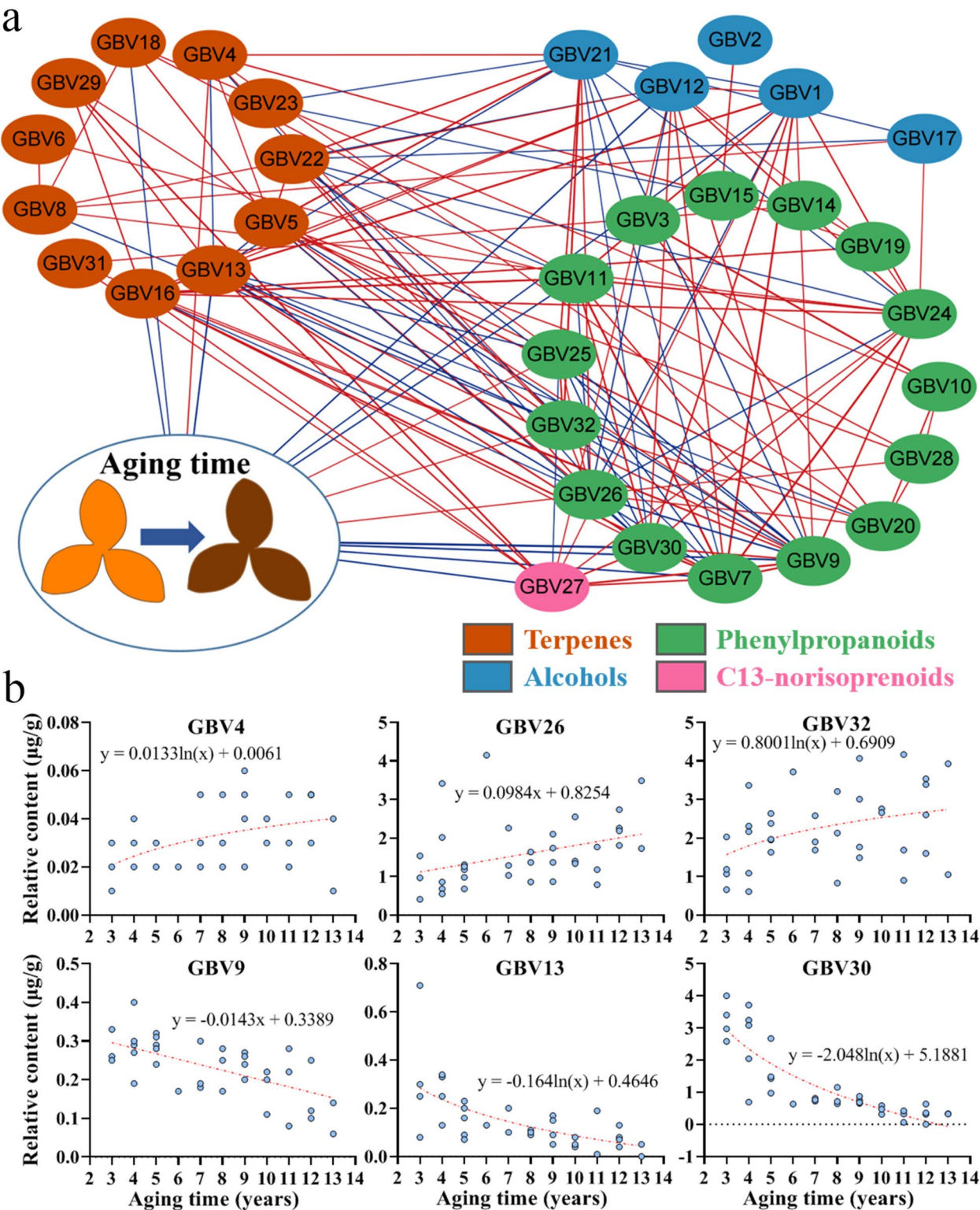


Fig. 2 Correlation analysis and glycosidically bound volatiles in GCP. **a** Correlation network analysis of GCP based on glycosidically bound volatiles. GBV1-32 represents the bound volatiles detected in GCP. The red and blue edges indicate positive and negative correlations, respectively. The thickness of the edges corresponds to the strength of the correlation coefficient. All correlations were significant at $P < 0.05$. **b** Scatter plots of selected glycosidically bound volatiles in GCP across different aging years. The red dashed line represents the fitting curve showing the relationship between bound volatile content and aging time

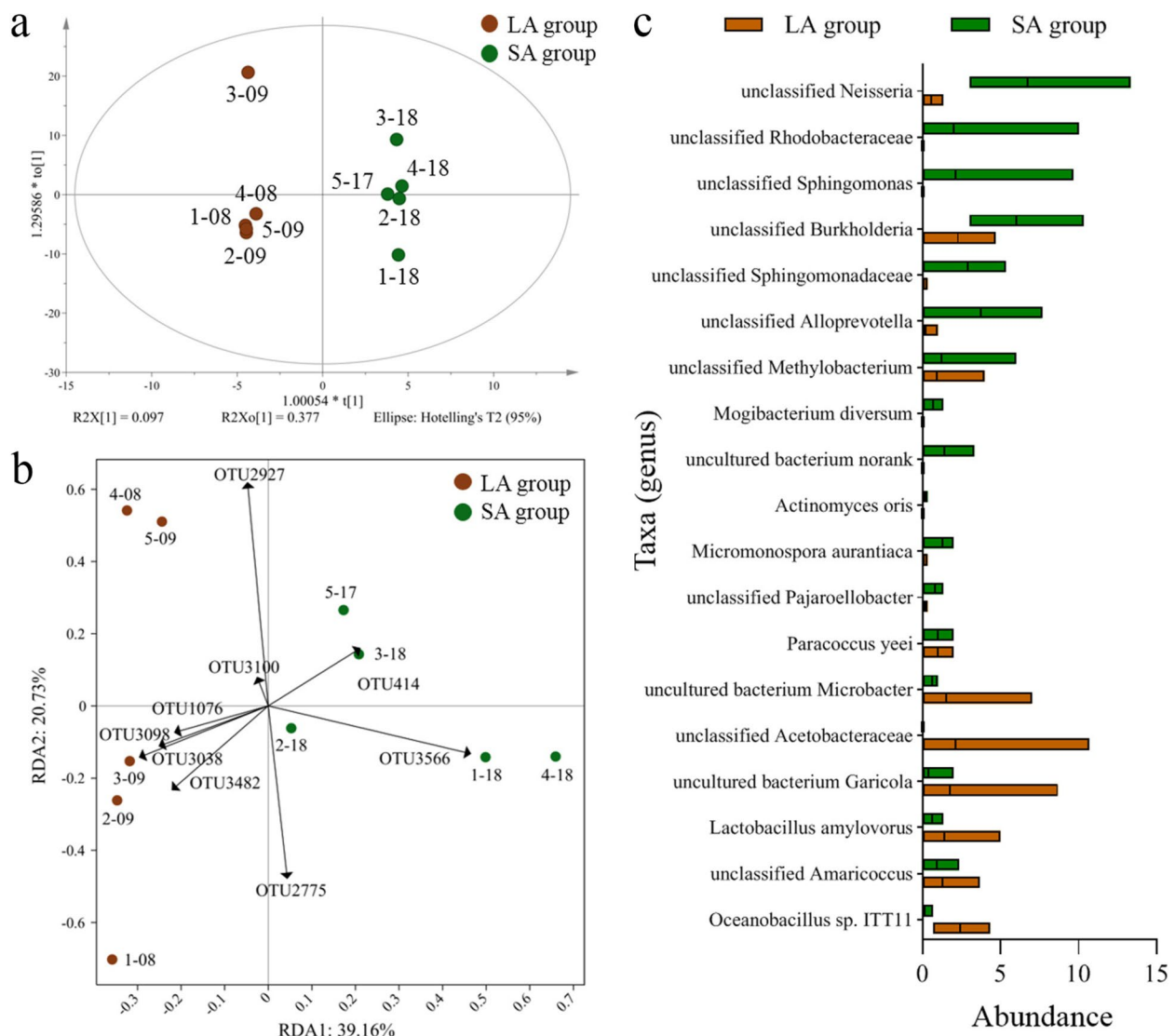


Fig. 3 Microbiome analysis of GCP with different aging years. **a** OPLS-DA score plot of the microbial community in GCP with different aging years ($R^2X = 0.907$, $R^2Y = 0.996$, $Q^2 = 0.503$). **b** The microbial community (genus level) significantly explained the observed compositional variation of glycosidically bound volatiles in GCP. **c** Differential enrichment of microbial genera between GCP with different aging years. Microbial genera identified in the OPLS-DA and RDA, as well as those differentially accumulated in GCP, are shown. LA group, the 'longer aging' group of GCP, containing '1-08', '2-09', '3-09', '4-08', and '5-09' (aged 3–4 years); SA group, the 'shorter aging' group, containing '1-18', '2-18', '3-18', '4-18', and '5-17' (aged 12–13 years)

OTUs with counts greater than 10 were considered for further analysis.

OPLS-DA analysis revealed distinct differences in microbial composition between GCP samples of different aging durations (Fig. 3a). Five bacterial genera with variable importance in projection (VIP) values greater than 2 were identified: *Oceanobacillus* (OTU1271), *Neisseria* (OTU3474), *Alloprevotella* (OTU3878), *unclassified Sphingomonadaceae* (OTU1476), and *Burkholderia-Caballeronia-Paraburkholderia* (OTU3470) (Table S4). These bacterial genera exhibited significant changes during

GCP aging. For instance, the abundance of *Neisseria* was 6.73 ± 4.28 in the SA group and 0.53 ± 0.45 in the LA group.

To investigate the impact of microbial changes on glycosidically bound volatiles in GCP, RDA was performed. The variation in GCP glycosidically bound volatiles was significantly explained by its microbiome, including *Amaricoccus* (OTU2927), *Methylobacterium-Methylorubrum* (OTU3100), *Lactobacillus* (OTU1076), *Garicola* (OTU3098), *unclassified Acetobacteraceae* (OTU3038), *Microbacter* (OTU3482), *Paracoccus* (OTU2775), *Sphingomonas* (OTU414), and *unclassified Rhodobacteraceae*

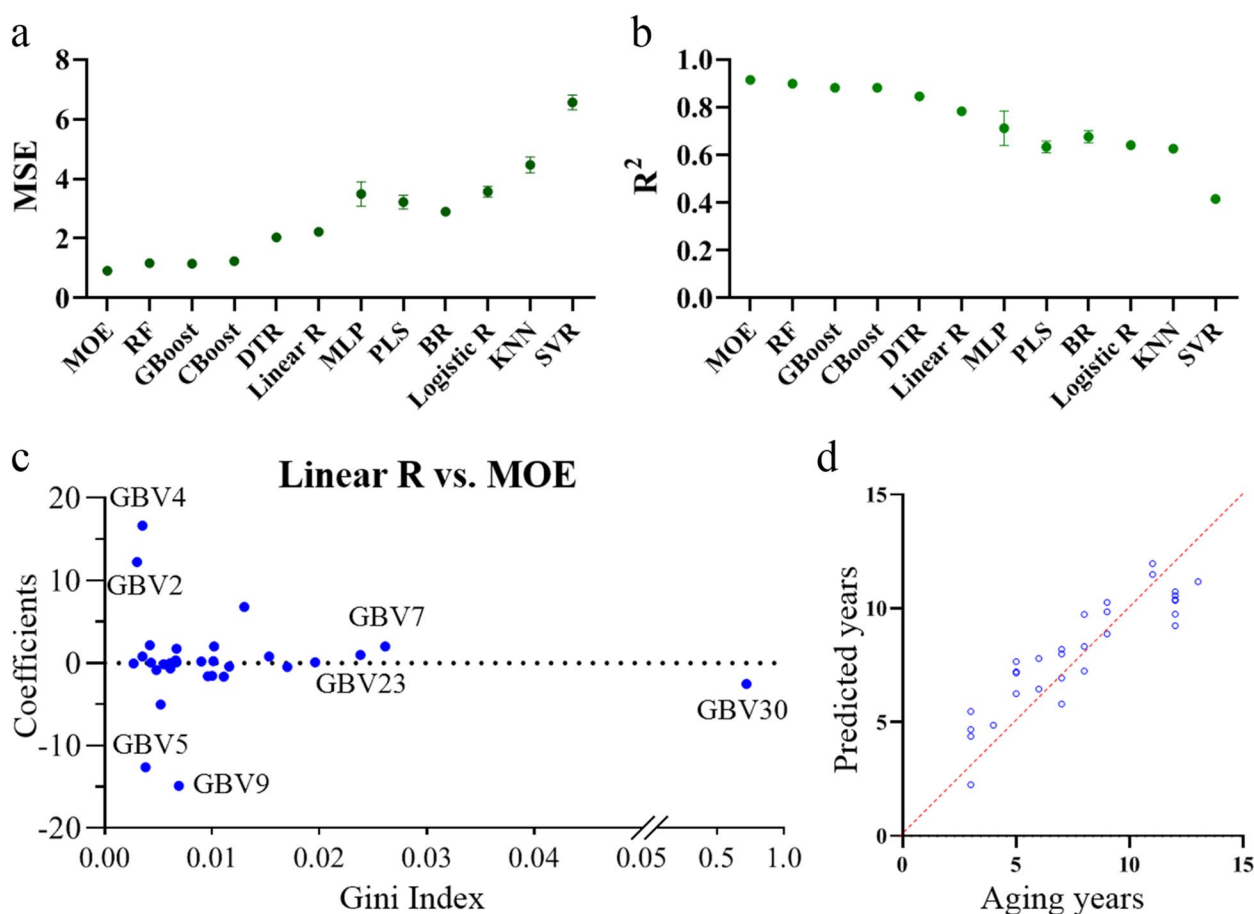


Fig. 4 Evaluation of various models incorporating glycosidically bound volatiles. **a-b** MSE and R^2 evaluation parameters of machine learning models. **c** Regression coefficients estimated using Linear Regression and variable importance determined by MOE models. **d** Relationship between authentic GCP aging years and predicted aging years based on the prediction equations

(OTU3566) (Fig. 3b). Notably, *Lactobacillus*, *Garicola*, *unclassified Acetobacteraceae*, and *Microbacter* exhibited strong positive correlations with the LA group, while *Sphingomonas* and *unclassified Rhodobacteraceae* were strongly positively correlated with the SA group. A comparative analysis revealed bacterial genera differentially enriched between the LA and SA groups (Fig. 3c). Genera such as *Oceanobacillus* sp. ITT11, *unclassified Amaricoccus*, *Lactobacillus amylovorus*, *uncultured Garicola*, *unclassified Acetobacteraceae*, and *uncultured Microbacter* were more abundant in the LA group, while other genera, including *unclassified Burkholderia*, *unclassified Sphingomonas*, *unclassified Rhodobacteraceae*, and *unclassified Neisseria*, were more abundant in the SA group.

Prediction of GCP aging years using machine learning models

To further investigate the relationship between glycosidically bound volatiles and aging years, these volatiles were used as variables in a range of machine learning

algorithms to assess prediction accuracy. Twelve models were employed for fitting and forecasting, including MOE, RF, GBoost, CBoost, DTR, Linear R, MLP, PLS, BR, Logistic R, KNN, and SVR models (Fig. 4a, 4b). The MSE for each model was calculated, with lower values indicating a better model fit. Among the tested models, the MOE model provided the best fit with an MSE of 0.918. RF and GBoost also showed strong performance, with MSE values of 1.176 and 1.156, respectively (Fig. 4a). Additionally, the explanatory degree R^2 of these models was evaluated. The MOE model explained the most variation in aging years ($R^2=0.915$), followed by RF ($R^2=0.898$) and GBoost ($R^2=0.882$) (Fig. 4b). Overall, the MOE model demonstrated the best performance and was selected for further analysis to explore the contribution of glycosidically bound volatiles in predicting aging years.

The quantification of glycosidically bound volatiles associated with GCP aging years was examined using both linear and nonlinear regression models, including

Linear R and MOE (Fig. 4c). GBV30, GBV7, and GBV23 were identified as the top three substances contributing most significantly to the prediction of aging years. Certain glycosidically bound volatiles, such as GBV30, GBV5, and GBV9, exhibited a negative contribution to aging year prediction, while GBV2 and GBV4 showed a positive contribution, consistent with earlier analyses (Fig. 2).

The regression Eq. (1) was used to predict the aging years of GCP samples (Fig. 4d). All predicted aging years were within three years of the authentic aging years. This linear regression prediction model effectively estimates the precise aging years of GCP within a reasonable error range.

Discussions

Identification of glycosidically bound volatiles in GCP offers valuable insights into the aging of GCP

This study conducted the first comprehensive characterization of 32 glycosidically bound volatiles in GCP. Notably, 5-hydroxymethylfurfural (HMF), exo-2-hydroxycyclohexyl acetate, 6-hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one (HTT), and acorenone B were identified for the first time as bound volatiles in citrus and citrus juice products (Gao et al. 2018; Ren et al. 2015; Zhong et al. 2014). During the aging process, GCP samples exhibit a color transition from orange to brown and ultimately to dark brown. Given the dry storage conditions, this browning is likely associated with the Maillard reaction (Zhou et al. 2025), and the presence of HMF supports its role as a product of the Maillard reaction (Şen and Gökmen 2022). Acorenone B and HTT, both components of plant essential oils, are known for their significant biological activities, such as anti-inflammatory effects (Dawra et al. 2020; Jayawardena et al. 2019). Ye (2011) reported that ferulic acid was the predominant extractable phenolic acid in young mandarin fruits, whereas caffeic acid and ferulic acid were the major bound phenolic acids. In 'Chachiensis' peels, the raw material for GCP, chlorogenic acid predominated, followed by ferulic acid, caffeic acid, and *p*-coumaric acid (Xi et al. 2014). However, the specific composition of bound phenolic acids in 'Chachiensis' remains unknown. In this study, bound volatiles, including benzenic compounds, were analyzed in GCP. Ferulic acid emerged as the predominant bound phenolic acid, with an average concentration of $2.23 \pm 0.98 \mu\text{g}\cdot\text{g}^{-1}$. Notably, ferulic acid, one of the few compounds positively associated with aging years, is widely recognized for its potent antioxidant activity. The characterization of these bound volatiles contributes to a clearer understanding of the functional ingredients in GCP.

Overall, the analysis of glycosidically bound volatiles in GCP offers valuable insights into the volatile composition of citrus and enhances understanding of the flavor profiles and physiological properties of GCP. Moreover, the conversion of volatiles such as terpenes and benzenic compounds into bound forms may facilitate their prolonged release over time, potentially influencing the quality changes in GCP during aging.

Microbiome plays an important role in determination of the GCP quality

Microbial composition is recognized as a key environmental factor influencing plant growth (Li et al. 2025; Lima et al. 2024; Pandey et al. 2024). In addition to its role in plant growth, microbial activity during food storage, fermentation, and aging can alter metabolite compositions, thereby affecting flavor and quality (He et al. 2019; Wu et al. 2020). In the drying process of Chachiensis peels into GCP, the dominant microorganism shifted from *Amnibacterium* to *Bacillus*, which likely influenced metabolite changes, including those in flavonoids (Zhou et al. 2025).

Additionally, a significant presence of *Bacillus* was observed in all GCP samples (Table S3), aligning with previous research (Zhou et al. 2025). *Bacillus* plays a vital role in the roasting and drying processes of *Vanilla planifolia*, contributing to vanillin production and reducing undesirable odors (Xu et al. 2020). Furthermore, *Bacillus* can degrade cell wall cellulose, promoting the release of metabolites for enhanced utilization by humans (Zhou et al. 2025). Similarly, *Lactobacillus*, a well-known probiotic, has been shown to alter the structure and physicochemical properties of plant polysaccharides, thereby enhancing their bioactivity (Teame et al. 2020; Wang et al. 2024).

In summary, this study emphasizes the potential impact of microbial activity on the transformation of glycosidically bound volatiles during the aging process of GCP. Understanding the relationship between dominant microbial strains and key active compounds could provide valuable insights for accelerating the aging process and improving the quality of GCP.

Advanced technologies facilitate the upgrading of GCP industry

The development of machine learning models based on physicochemical quality and metabolic substances to predict sample quality attributes has become a significant area of research. Wu (2022) developed a LASSO-PCA model to predict the sensory quality of matcha based on its physicochemical properties, while Ferrão (2022) demonstrated that volatiles in blueberries

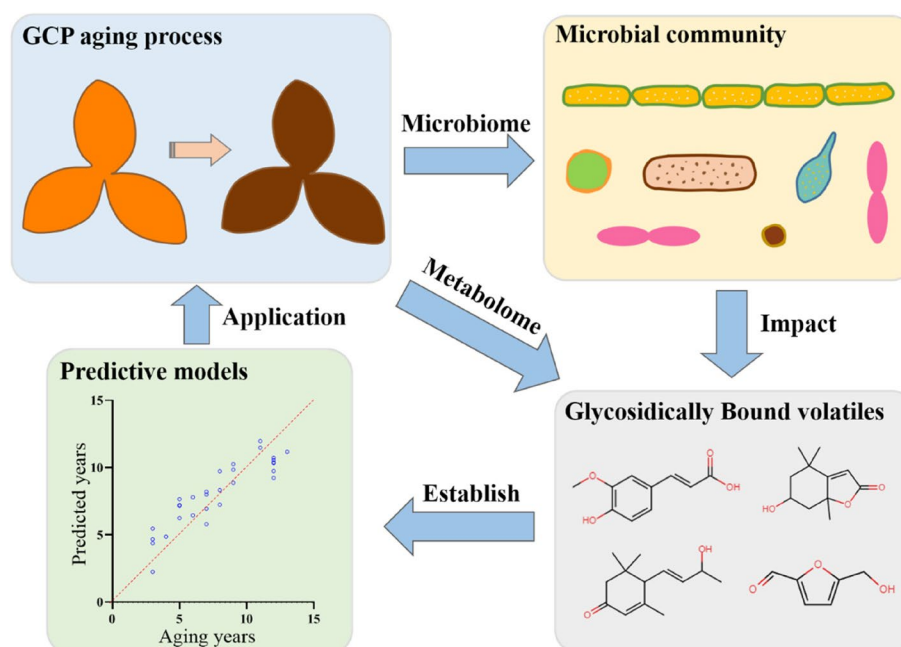


Fig. 5 A proposed model illustrating the prediction of GCP aging years based on microbial and glycosidically bound volatiles

influenced consumer preferences and perceptions of fruit quality attributes, such as sweetness. Previous research constructed a model for predicting aging years based on GCP volatiles and flavonoids, which performed well in rough aging year identification (Liu et al. 2024). However, pinpointing the exact aging year of GCP remains a bottleneck that hinders industry development. In the present study, a machine learning model was established to predict the precise aging years of GCP based on the composition of glycosidically bound volatiles.

Furthermore, compared to other metabolites like volatiles or flavonoids, there are currently no relevant commercial standards for glycosidically bound volatiles, making it challenging to manipulate sample aging years by merely adding these bound volatiles. Therefore, the profiling of glycosidically bound volatiles offers a reliable and effective method for determining the aging years of GCP. Overall, this study presents a novel approach for predicting the precise aging years of GCP, which has important implications for addressing the long-standing market confusion that has persisted for over a decade, often leading to consumer dissatisfaction.

Conclusions

In this study, glycosidically bound volatiles in GCP were characterized and utilized to estimate the aging time for the first time. A total of 32 glycosidically bound volatiles were identified in GCP samples with varying aging years, including bioactive compounds such as HME.

Throughout the aging process, the composition of these volatiles underwent notable changes, such as a gradual accumulation of vanillin and a steady dissipation of benzyl alcohol. Microbial activity also influenced the metabolism of GCP, with four bacterial genera exhibiting strong positive correlations with longer-aged GCP, and two genera correlating with shorter-aged GCP. Additionally, a machine learning model was developed to predict the authentic aging year of GCP based on the composition of glycosidically bound volatiles (Fig. 5). This predictive model provides significant practical value in addressing the current challenges faced by the GCP market. Our research offers a valuable reference for the quality assessment of GCP and the development of relevant standards, which will play a crucial role in guiding future production and development of GCP products.

Abbreviations

GCP	Guangchenpi
GC-MS	Gas chromatography-mass spectrometry
PCR	Pericarpium Citri Reticulatae
MOE	Mixture of experts
RF	Random forest
GBoost	Gradient boosting regressor
CBoost	Cat boost regressor
DTR	Decision tree regressor
Linear R	Linear regression
MLP	Multi-layer perception regressor
PLS	Partial least square
BR	Bayesian ridge regression
Logistic R	Logistic regression
KNN	Kneighbors regressor
SVR	Support vector regression

MSE	Mean square error
R ²	Coefficient of determination
OPLS-DA	Orthogonal partial least squares-discriminate analysis
RDA	Redundancy analysis
VIP	Variable importance in projection
HTT	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one
HMF	5-Hydroxymethylfurfural
OTU	Operational taxonomic unit

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44281-025-00079-2>.

Supplementary Material 1: Figure S1. Correlation analysis of GCP glycosidically bound volatiles and aging years.

Supplementary Material 2: Table S1. Source and sample information of GCP. Table S2. Glycosidically bound volatiles of GCP detected by GC-MS. Table S3. Composition of the microbial community in GCP with different aging years. Table S4. List of VIP values calculated in the OPLS-DA model.

Acknowledgements

Not applicable.

Authors' contributions

YL, methodology, writing—original draft; XW, methodology; ZY, data curation; HW, software, visualization; QC, validation; YH, sample collection; HH, field investigation; CL, sample collection; JZ, resources maintaining, field investigation; JC, project administration, supervision; JX, conceptualization, funding acquisition, writing—review & editing.

Funding

This work was supported by the National Key Research and Development Program of China (No. 2021YFD1600100) and Hubei Hongshan Laboratory (No. 2022hszd021).

Data availability

Data will be available on reasonable request.

Declarations

Ethics approval and consent to participate

No ethics approval or consent to participate was required.

Consent for publication

Not applicable.

Competing interests

JX has filed a patent application relating to the prediction of GCP aging years based on the glycosidically bound volatiles profiling. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received: 16 May 2025 Revised: 24 June 2025 Accepted: 1 July 2025

Published online: 30 August 2025

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