*Food Chemistry International*

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# Effect of Three Concentration Processes on the Physicochemical Properties and Flavor Characteristics of *Anoectochilus Roxburghii* Juice

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**Received:** 10 June 2025 | **Revised:** 7 August 2025 | **Accepted:** 13 August 2025

**Funding:** This work was supported by Technology Innovation Special Fund of Fujian Agriculture and Forestry University (KFB24089).

**Keywords:** *A. roxburghii* | antioxidant capacity | bioactive compounds | freeze-concentration

## ABSTRACT

This study investigated the effects of atmospheric-pressure concentration (APC), vacuum concentration (VAC), and freeze-concentration (FC) methods on the physicochemical properties, bioactive compounds, and flavor profile of *Anoectochilus roxburghii* juice. The results indicated that after concentration, APC caused the most significant color change in *A. roxburghii* juice ($\Delta E = 3.06$), while FC was best at retaining the original color ($\Delta E = 0.45$). However, FC juice showed a lower clarification index, indicating better stability. Additionally, the pH value decreased in all groups after concentration. The pH of the normal pressure group dropped from 5.33 to 5.18, representing the most significant reduction. Meanwhile, FC juice exhibited over 85% preservation rates for both total flavonoids and total phenolics, with only a 2.37% loss of kinsenoside. This performance was significantly superior to that of the VAC and APC juices. Different concentration methods all resulted in a decrease in the antioxidant capacity of *A. roxburghii* juice, with FC juice showing the lowest degree of antioxidant capacity loss. Its total antioxidant capacity was 1.49 μmol/mL, with a retention rate of 97.98%. This retention rate was 9.97% and 14.37% higher than that of the VAC group and the APC group, respectively. In contrast, electronic tongue analysis detected elevated bitterness (response value > 8.30) and astringency (5.97) in APC juice compared to the other groups. The taste profiles of VAC and FC juices closely aligned, whereas APC processing resulted in a significant divergence from FS. Therefore, our findings provide theoretical insights for optimizing *A. roxburghii* juice processing methodologies.

## 1 | Introduction

With increasing consumer demand for healthy lifestyles, natural, additive-free foods with functional and nutrient-dense properties are gaining prominence. Fruit and vegetable juices, abundant in vitamins, sugars, minerals, antioxidants, polyphenolic compounds, and polysaccharides, serve as a critical dietary source of nutrients (Niu et al. 2024). Compared to fresh fruits and vegetables, Fruit and vegetable juice retains its flavor and nutritional components while often being hailed as "liquid fruits and vegetables" (Lan et al. 2023). However, fruits and juices comprise 75%–90% water, rendering them highly susceptible to enzymatic reactions and microbial growth during storage and transportation, which accelerates spoilage and quality degradation. To mitigate these challenges and ensure prolonged preservation, ease of handling, and efficient transportation, extracted juices are typically concentrated. Reducing water content within juices not only inhibits microbial proliferation and enzymatic activity but also substantially decreases product volume and weight, thereby

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enhancing storage and logistical efficiency (Trishitman et al. 2023).

Current industrial concentration processes primarily include atmospheric-pressure concentration (APC), vacuum concentration (VAC), and freeze-concentration (FC). APC involves heating solutions under ambient pressure to evaporate water, thereby increasing solute concentration. A study compared the effects of APC and forward osmosis concentration on watermelon juice aroma. The results indicated that APC exhibited a less desirable organoleptic profile, characterized by off-odors such as fishy and mushroom-based notes (Milczarek and Sedej 2021). VAC, which operates at low to medium temperatures under reduced pressure, mitigates the thermal degradation of nutrients, color, and flavor (Vu et al. 2024). Li et al. reported that VAC pear sauce retained higher nutrient levels and functional compounds while enhancing antioxidant capacity, freshness, and flavor compared to traditional heating methods (Y. Li et al. 2022). Similarly, Zhang et al. demonstrated that VAC enhances quality and aroma distribution in sea buckthorn juice (Z. W. Zhang, Cheng, et al. 2024b). FC is an emerging non-thermal technology for concentrating aqueous solutions. The process cools the solution below its freezing point, inducing water crystallization and forming a biphasic system: unfrozen liquid concentrate and a porous ice phase. Owing to this porosity, the concentrate is separated via gravity, centrifugation, or vacuum filtration (Pérez-Bermúdez et al. 2025). FC technology operates below 0°C, thereby facilitating the retention of thermally unstable compounds. These include volatile substances, such as flavors and aromas, as well as bioactive components such as antioxidants, phenolics, anthocyanins, and vitamin C (Vásquez-Castillo et al. 2025). Ding et al. produced apple juice at 34.50°Brix using FC, which exhibited over 90% retention of vitamin C and aromatic constituents, alongside negligible alterations in reducing sugars and color metrics (Ding et al. 2019). Yoda et al. compared FC with thermal concentration methods for apple juice processing, demonstrating that FC exhibited superior efficacy in retaining beneficial compounds present in fresh juice (Yoda et al. 2021). Pérez-Bermúdez et al. employed ultrasound-assisted block freeze concentration (US-BFC) to concentrate peppermint extract (*Mentha piperita* L.). US-BFC significantly increased solute content, bioactive components, and specific compound levels, enhancing antioxidant capacity. The technique improved concentration efficiency without compromising extract quality or nutritional properties (Pérez-Bermúdez et al. 2025). Vásquez-Castillo et al. proposed a low-temperature concentration technology, termed V-CABC, which integrates centrifugal and vacuum operations in a staged process. Experimental results demonstrated that V-CABC achieved improved concentration efficiency and product quality compared to centrifugal-assisted bulk low-temperature concentration (CABC) (Vásquez-Castillo et al. 2025). Consequently, concentration techniques distinctly influence juice quality. Systematic investigation of these effects is essential for refining processing protocols and enhancing product standards within the juice industry.

*Anoectochilus roxburghii* (Wall.) Lindl (Orchidaceae), a perennial herb, has been traditionally utilized in food and medicine for its aerial parts and fruits (Yuan et al. 2022). This species is rich in various bioactive compounds, including polysaccharides, glycosides, alkaloids, flavonoids, triterpenoids, and organic acids (Yuan et al. 2022). Previous study indicated that kinsenoside, a key constituent of *A. roxburghii*, effectively suppresses adipogenesis, thus lowering the risk of obesity-related metabolic disorders (Buacheen et al. 2023). Additionally, it has been reported kinsenoside have therapeutic potential for ischemic stroke by activating the Nrf2/HO-1 pathway and mitigating oxidative stress-induced blood-brain barrier impairment (Qiao et al. 2023). Alkaloids isolated from *A. roxburghii*, including stilbene A and aconitine, exhibited neuroprotective and analgesic properties, offering relief for neurodegenerative conditions (Han et al. 2008). As a functional food, *A. roxburghii* is consumed not only as tea, soup, or culinary ingredients but also processed into commercial health products such as snacks and juices, expanding its market applications (T. Wu et al. 2021). Current research on *A. roxburghii* predominantly emphasizes its bioactive constituents (Y. Y. Qiu et al. 2023), with limited exploration of its juice concentrate products.

To enhance the utilization of *A. roxburghii* resources and advance the development of high-quality freshly pressed juice concentrates, this study evaluates the impacts of APC, VAC, and FC on key parameters of *A. roxburghii* juice, including viscosity, stability, pH, color, kinsenoside content, reducing sugars, total polyphenol and flavonoid levels, and antioxidant properties. Additionally, electronic sensory techniques (e-nose and e-tongue) were utilized to analyze the flavor profiles and sensory characteristics of reconstituted concentrates. This study utilized fresh *A. roxburghii* as the raw material, developed a new juice extraction process, obtained the optimal processes for VAC and FC, and investigated the quality differences of *A. roxburghii* juice under different concentration methods. This laid a solid foundation for the diversified product development of *A. roxburghii*, contributed to downstream industry innovation and upgrading, and provided scientific insights for optimizing the industrial production of freshly pressed *A. roxburghii* juice concentrates.

## 2 | Materials and Methods

### 2.1 | Materials and Reagents

Anoectochilus roxburghii (*A. roxburghii*) was provided by Yong an Huangnijia Co. Ltd. Kinsenoside standard, 3,5-dinitrosalicylic acid, D-anhydrous glucose, Total Antioxidant Capacity (T-AOC) Assay Kit, and aluminum nitrate were sourced from Beijing Solepol Science and Technology Co. Ltd. 1,1-Diphenyl-2-picrylhydrazyl standard was obtained from Shanghai Yuanye Bio-Technology Co. Ltd.

### 2.2 | Sample Processing

Freshly pressed *A. roxburghii* juice (1°Brix) was uniformly concentrated to 45°Brix via three methods: atmospheric-pressure concentration (APC), vacuum concentration (VAC), and freeze-concentration (FC). For APC, samples were heated in a water bath at 100°C. VAC was performed using a rotary evaporator (vacuum: 0.95 MPa, temperature: 50°C, rotation: 100

r/min). FC involved freezing 100 mL of juice at −18°C, followed by collecting the concentrate within 10 min of thawing initiation. All concentrates were subsequently reconstituted to the original juice concentration (1°Brix) for analysis.

### 2.3 | Viscosity Measurement

Viscosity measurements were conducted using a multifunctional rotational viscometer (Brookfield viscometer DV-III + Rheometer, USA). *A. roxburghii* juice was separately concentrated using APC, VAC, and FC to the same endpoint (45°Brix). The concentrated juices were then reconstituted to the original concentration of the fresh juice (1°Brix). The viscosity of the reconstituted *A. roxburghii* juice after concentration via different methods was measured.

### 2.4 | Stability Measurements

The sedimentation rate of *A. roxburghii* juice was assessed, and color changes across experimental groups were documented. For each measurement, a 425 μL aliquot of juice was loaded into the LUMiSizer device under the following parameters: light intensity 1 × , temperature 25°C, rotational speed 4000 r/min, and a measurement duration of 1 h. Scans were performed every 30 s, yielding 120 data points.

### 2.5 | pH Measurement

pH measurements were conducted using a digital pH meter (Leizhi PHS-25, China). *A. roxburghii* juice was separately concentrated using APC, VAC, and FC to the same endpoint (45°Brix). The concentrated juices were then reconstituted to the original concentration of the fresh juice (1°Brix) solely for pH measurement.

### 2.6 | Color Measurement

*A. roxburghii* juice was separately concentrated using APC, VAC, and FC to the same endpoint (45°Brix). For color analysis, these concentrated juices were then each diluted back to the original concentration of the fresh juice (1°Brix). The color of these reconstituted juices was measured. Colorimetric analysis was performed using an automatic colorimeter to determine L* (lightness), a* (green-red axis), and b* (blue-yellow axis) values. The instrument was calibrated with a standard white plate prior to measurements. Total color difference (ΔE) was calculated using the following formula:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where ΔE represents the magnitude of color change in *A. roxburghii* juice, with smaller values indicating less perceptible variation.

### 2.7 | Determination of Kinsenoside Content

The extraction protocol followed Yang et al. with modifications: 0.1 g of *A. roxburghii* standard was ultrasonicated in 2 mL methanol at 30° C and 420 W for 45 min (B. Y. Yang et al. 2020). The supernatant was collected after centrifugation at 10,000 r/min for 10 min. Ultrasonication and centrifugation steps were repeated, and supernatants were pooled to a final volume of 2 mL, filtered through a 0.22 μm PVDF membrane, and stored at −20° C. UPLC analysis utilized a Waters Acquity UPLC C18 column (1.7 μm, 2.1 × 100 mm) with the following parameters: column temperature 30° C, injection volume 5 μL, mobile phase water-acetonitrile-methanol (90:5:5, v/v/v), and flow rate 0.2 mL/min.

### 2.8 | Determination of Reducing Sugar Content

The reducing sugar content of *A. roxburghii* juice concentrate was determined using the method of Naik et al. with minor modifications (Naik et al. 2023). Briefly, 1 mL of 100-fold diluted concentrate was mixed with 1.0 mL of DNS reagent and heated in a boiling water bath for 5 min. After cooling to room temperature, 0.5 mL of 40% potassium sodium tartrate solution was added, and the mixture was diluted to a final volume of 5 mL with deionized water. Absorbance was measured at 570 nm using a dual-beam UV–Vis spectrophotometer, with results quantified against a glucose standard curve.

### 2.9 | Determination of Total Flavonoid Content

Total flavonoid content in *A. roxburghii* juice concentrate was quantified following Chen et al. with minor adjustments (J. J. Chen et al. 2023). Briefly, 1 mL of concentrate was mixed with 0.5 mL of methanolic hydrochloric acid solution (MeOH: HCl: $H_2O$ = 80:1:19, v/v/v). Aliquots (1 mL) were treated sequentially with 0.3 mL of 5% sodium nitrite, vortexed, and incubated for 6 min; followed by 0.5 mL of 10% aluminum nitrate, vortexed, and incubated for an additional 6 min. Finally, 4 mL of 1 mmol/L NaOH was added, and the mixture was incubated for 15 min. After centrifugation at 3000 r/min for 10 min, absorbance of the supernatant was measured at 505 nm using a spectrophotometer, with rutin as the reference standard for total flavonoid quantification.

### 2.10 | Estimation of Total Polyphenol Content

Gallic acid standard solutions and a 1:10 dilution of Folin-Ciocalteu reagent were prepared according to Martins et al. (Martins et al. 2018). Aliquots of the gallic acid solution were transferred to test tubes, adjusted to a consistent volume with distilled water, and mixed with 7.5% (w/v) sodium carbonate to establish an alkaline pH. An equal volume of Folin-Ciocalteu reagent was added, and the mixture was incubated for 30 min before measuring absorbance at 760 nm. A standard curve was generated by plotting gallic acid concentration against absorbance. The total polyphenol content in diluted sample extracts

was quantified using the same protocol, with results interpolated from the standard curve.

### 2.11 | Determination of Total Antioxidant Capacity

The total antioxidant capacity (T-AOC) was determined by measuring the reduction of $Fe^{3+}$-TPTZ to blue $Fe^{2+}$-TPTZ under acidic conditions using a T-AOC assay kit.

### 2.12 | Measurement of DPPH Free Radical Scavenging Capacity

A 4 mmol/L DPPH radical solution was prepared in ethanol following the method of Wang et al. (Q. Wang, Huang, et al. 2022b). For the assay, 1 mL of DPPH solution was combined with 1 mL of 20% sample extract and 2 mL of 60% ethanol. The mixture was incubated in the dark at 25°C for 30 min, and absorbance was measured at 517 nm. Vitamin C (VC) served as the positive control. DPPH scavenging activity was calculated using the formula:

$$S_A = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \text{x } 100\%$$

Where $S_A$ is the scavenging activity of the test sample, $A_1$ is the absorbance of the reactant solution, $A_2$ is the absorbance of the sample, and $A_0$ is the absorbance of the blank.

### 2.13 | Measurement of Hydroxyl Radical Scavenging Capacity

Hydroxyl radical scavenging activity was assessed following Wang et al. (Q. Wang, Huang, et al. 2022b). A reaction mixture containing 1 mL of 20% sample extract, 2 mL of 5 mmol/L ferrous sulfate, 2 mL of 5 mmol/L hydrogen peroxide, 2 mL of distilled water, and 2 mL of 5 mmol/L ethanol-salicylic acid was vortexed and incubated at 37°C for 30 min. Absorbance was measured at 510 nm, with Vitamin C (VC) serving as the positive control. Hydroxyl radical scavenging activity was calculated using the formula:

$$S_A = \left[1 - \frac{(A_1 - A_2)}{A_0}\right] \text{x } 100\%$$

Where $S_A$ is the scavenging activity of the test sample, $A_1$ is the absorbance of the reactant solution, $A_2$ is the absorbance of the sample, and $A_0$ is the absorbance of the blank.

### 2.14 | E-Nose Assay

The E-nose analysis method was based on a previously published study with slight modifications (Du et al. 2019). Four A. roxburghii sample types (freshly squeezed juice (FS), APC, VAC, and FC) were prepared by pipetting 5 mL aliquots into headspace vials. Samples were equilibrated at 25°C for 30 min and analyzed using an electronic nose under standardized conditions: 120 s assay time, 120 s autoclave cleaning, 400 mL/min injection flow rate, and ten sensor arrays. Data acquisition utilized predefined analytical templates.

### 2.15 | Electronic Tongue Assay

Four *A. roxburghii* sample types (FS, APC, VAC, and FC) were prepared following Maguluri et al. (Maguluri et al. 2024). Aliquots (25 mL) were aspirated into a beaker and loaded into an electronic tongue autosampler. Each sample underwent 120 s analysis at room temperature, with the sensor rinsed with ultrapure water between measurements to ensure stable potential.

### 2.16 | Statistical Analysis

All experiments were performed in triplicate. Statistical analyses utilized using SPSS 19.0, and graphical data were generated using OriginPro 2023b. Distinct lowercase letters indicate significant differences ($p < 0.05$).

## 3 | Results and Discussions

### 3.1 | Effects of Different Concentration Techniques on the Color of *A. Roxburghii* Juice

Color is a key factor influencing consumers' sensory evaluation of fruit and vegetable juices, highlighting the importance of preserving their original characteristics (Cheng et al. 2020). As shown in Figure 1, APC treatment significantly increased the lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$) of *A. roxburghii* juice compared to the FS group ($p < 0.05$), which was distinct from the other treatments. The *A. roxburghii* juice reconstituted from APC exhibited pronounced yellowing, resulting in a significant color difference compared to the freshly pressed juice. In contrast, no visually detectable color variations were observed among the other experimental groups (Figure 1). The total color difference (ΔE) between concentrated and freshly squeezed juices is presented in Figure 1e, with values of 3.06 for APC, 0.71 for VAC, and 0.45 for FC. These results indicated that APC exerted the most significant impact on juice color, while FC best preserved the original hue (Figure 1). This disparity may arise from higher sugar degradation in atmospheric-pressure processing, consistent with findings by Yoda et al. (Yoda et al. 2021). Ding et al. successfully produced high-concentration apple juice (34.50°Brix) using the FC process, which showed only minimal color change while maintaining a high nutrient retention rate (Ding et al. 2019). Therefore, FC treatment could minimize the impact on the original color of *A. roxburghii* juice.

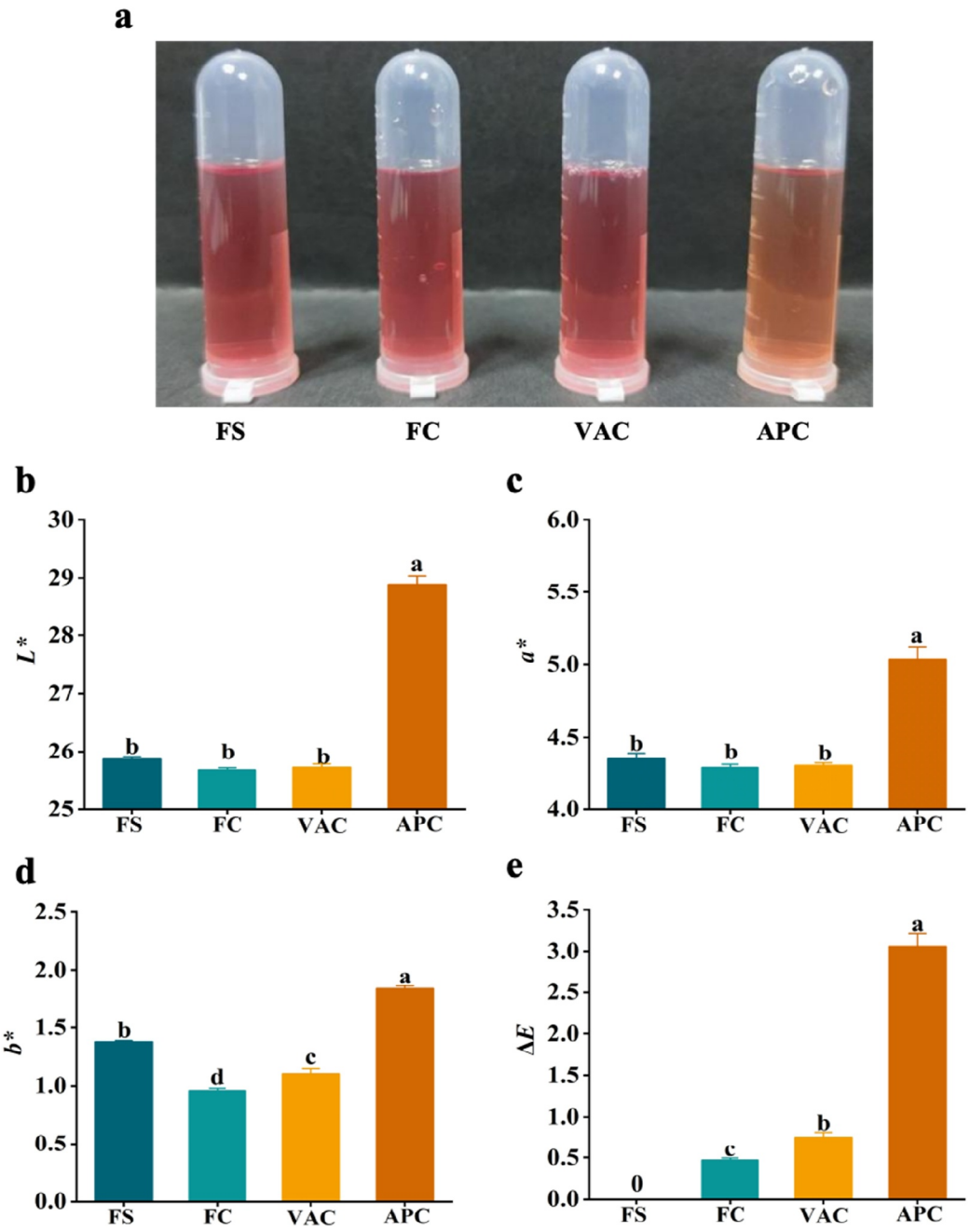



**FIGURE 1** | The effect of different concentration processes on the color of *Anoectochilus roxburghii* juice. (a) Representative images of *A. roxburghii* juice; (b) *L**; (c) *a**; (d) *b**; (e) Δ*E*.

### 3.2 | Effects of Different Concentration Techniques on the Physical Properties of *A. Roxburghii* Juice

Figure 2 illustrates the differences in viscosity, sedimentation rate, pH, and browning index of *A. roxburghii* juice concentrates processed via APC, VAC, and FC. As shown in Figure 2a, viscosity values across all groups remained consistent at 0.0030 Pa·s, with no significant differences observed ($p > 0.05$). Freeze-concentrated juice exhibited the lowest sedimentation rate (Figure 2b, $p < 0.05$), likely due to impurities being retained in ice crystals during freeze-thaw cycles, thereby enhancing clarity (Moreno et al. 2014). As illustrated in Figure 2c, all concentrates exhibited a pH decline, with APC juice showing the most pronounced decrease (5.31–5.18) compared with the FS group ($p < 0.05$). The browning index is a key indicator of juice browning. The browning index of fresh juice was 0.72, and the browning index of the juice significantly increased after atmospheric-pressure concentration (Figure 2d, $p < 0.05$). This increase is primarily attributed to the enhanced Maillard reaction caused by atmospheric-pressure concentration, which affects the non-enzymatic browning of the juice, leading to a higher browning index (Z. Xu et al. 2023). Ding et al. further confirmed this: during the evaporation of apple juice, changes in reducing sugar content significantly promoted Maillard browning, especially

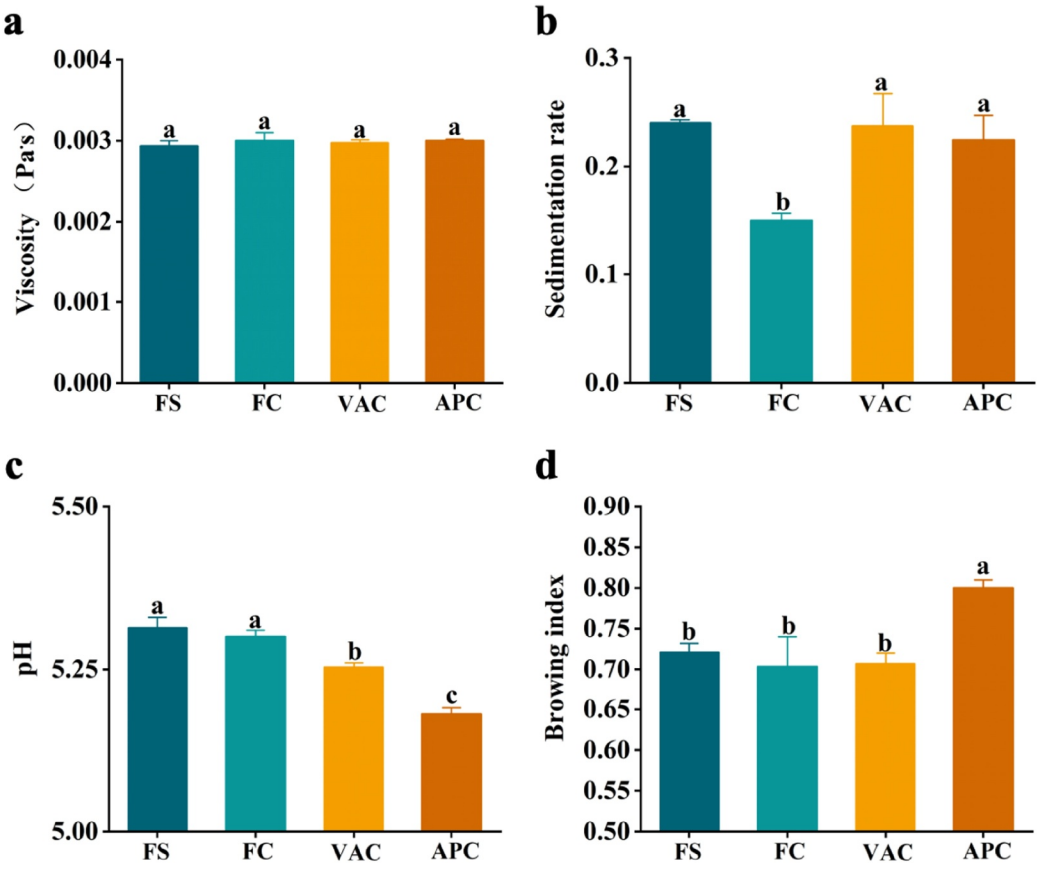



**FIGURE 2** | Effects of different concentration processes on the viscosity, sedimentation rate, pH value, and beowing index of *Anoectochilus roxburghii* juice. (a) Viscosity; (b) sedimentation rate; (c) pH; (d) Browning index.

when the temperature exceeded 60°C (Ding et al. 2019). Hence, Both VAC and FC treatments effectively maintained the physical properties of A. roxburghii juice.

## 3.3 | Effects of Different Concentration Techniques on the Bioactive Composition of *A. Roxburghii* Juice

### 3.3.1 | The Reducing Sugar Content of *A. Roxburghii* Juice

Figure 3a illustrates the effects of different concentration processes on reducing sugar content in *A. roxburghii* juice. All groups exhibited increased reducing sugar levels post-treatment: VAC juice increased from 3.27 mg/mL to 3.81 mg/mL, APC juice from 3.27 mg/mL to 3.50 mg/mL, and FC juice from 3.27 mg/mL to 3.50 mg/mL ($p < 0.05$). Thermal concentration methods significantly enhanced reducing sugar content compared to cryoconcentration, likely due to the conversion of non-reducing sugars during heating. Notably, VAC juice showed higher sugar retention than APC juice ($p < 0.05$), potentially because prolonged high-temperature exposure in atmospheric processing accelerated Maillard reactions. These reactions, involving polymerization and condensation of fructose and glucose derivatives, lead to reduction in total reducing sugars (Das et al. 2020). Ding et al. further confirmed this: during the evaporation of apple juice, changes in reducing sugar content significantly promoted Maillard browning, especially when the temperature exceeded 60°C (Ding et al. 2019).

### 3.3.2 | The Total Polyphenol Content of *A. Roxburghii* Juice

As shown in Figure 3b, the total polyphenol content in *A. roxburghii* juice decreased after concentration treatment ($p < 0.05$). The APC group exhibited the most significant reduction, with levels decreasing from 0.14 mg/mL to 0.08 mg/mL. This decline is likely due to the oxidation and degradation of phenolic compounds, whose hydroxyl groups are highly reactive under high-temperature conditions (Niveditha et al. 2023). In contrast, FC preserved polyphenol content more effectively, with a decrease from 0.14 mg/mL to 0.12 mg/mL (a 14.29% reduction) (Figure 3b, $p < 0.05$). Similar trends have been reported in studies of broccoli extract (Azhar et al. 2020), blueberry juice (Casas-Forero et al. 2020), and apple juice (Orellana-Palma et al. 2020), where low-temperature processing preserves bioactive compounds by minimizing thermal degradation.

### 3.3.3 | The Total Flavonoid Content of *A. Roxburghii* Juice

The total flavonoid content in *A. roxburghii* juice following different concentration processes is illustrated in Figure 3c. All

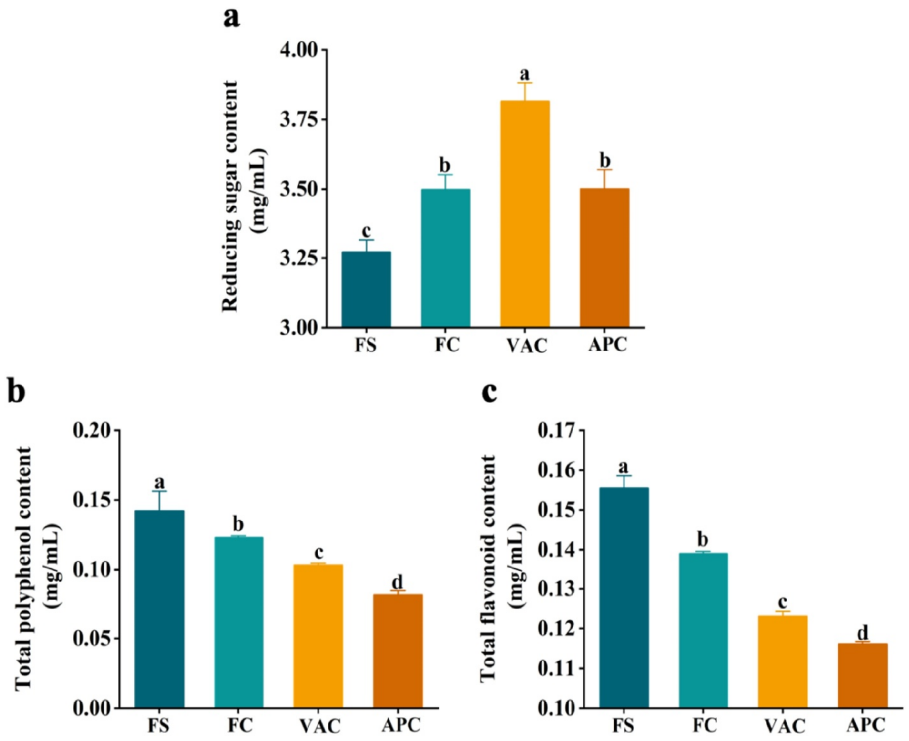



**FIGURE 3** | Effect of different concentration processes on the reducing sugar content, total polyphenol content, and total flavonoid content of *Anoectochilus roxburghii* juice.

treatments resulted in varying degrees of flavonoid degradation, with freeze-concentration exhibiting the least reduction ($p < 0.05$). The FC group retained a total flavonoid content of 0.14 mg/mL, representing 12.90% and 19.68% higher retention compared to VAC and APC juices, respectively (Figure 3c, $p < 0.05$). SU et al. demonstrated that elevated temperatures accelerate flavonoid degradation (Su et al. 2019), potentially inducing denaturation and polymerization reactions during prolonged heating. Similarly, research on broccoli extract (Azhar et al. 2020), blueberry juice (Casas-Forero et al. 2020), and apple juice (Orellana-Palma et al. 2020) converges in demonstrating that FC more effectively maintains flavonoid structural integrity by preventing extended thermal degradation.

#### 3.3.4 | The Kinsenoside Content of *A. Roxburghii* Juice

The chromatograms of *A. roxburghii* juice samples from different concentration treatments (Figure 4a–d) exhibited peaks at 10–11 min. This retention time aligned with the characteristic peak of kinsenoside in the reference standard, confirming the compound's presence in all samples. Kinsenoside, a unique bioactive compound in *A. roxburghii*, exhibits therapeutic potential against metabolic disorders and confers hepatoprotective and vascular benefits under hyperglycemic conditions (H. Z. Wang, Chen, et al. 2022a). As shown in Figure 4a–d, compared with the FS group, all treatments reduced kinsenoside levels, with freeze-concentration showing the smallest decline and atmospheric-pressure concentration the largest ($p < 0.05$). Specifically, kinsenoside concentrations were 6.64, 6.63, 6.46, and 4.73 mg/mL for FS, FC, VAC and APC juices, respectively (Figure 4 and Table 1, $p < 0.05$). These results suggest that thermal processing adversely affects kinsenoside preservation, likely accelerating its degradation. Previous research by Orellana-Palma et al. demonstrated that low-temperature concentration effectively preserves bioactive compounds, such as polyphenols and flavonoids, by mitigating thermal degradation during solute concentration (Orellana-Palma et al. 2021). These findings align with the present study, indicating that FC is recommended to preserve kinsenoside stability during *A. roxburghii* juice processing.

### 3.4 | Effects of Different Concentration Techniques on the Antioxidant Capacity of *A. Roxburghii* Juice

Total antioxidant capacity reflects the combined efficacy of antioxidant mechanisms within a sample. A high total antioxidant capacity signifies robust free radical scavenging (Q. Yang et al. 2023) and inhibition of oxidative chain reactions (Finotti et al. 2024). As shown in Figure 5a Compared to FS juice, all concentration methods could reduce antioxidant capacity ($p < 0.05$). However, FC—a low-temperature method—minimized oxidative degradation of antioxidant components (e.g., polyphenols and flavonoids), better preserving their activity (Batool et al. 2019). Consequently, freeze-concentrated *A. roxburghii* juice retained the highest total antioxidant capacity (1.47 μmol/mL, 95.74% retention), surpassing VAC and APC juices by 12.78% and 42.72%, respectively ($p < 0.05$). DPPH, a stable nitrogen-centered free radical, is widely used to assess antioxidant activity via its scavenging capacity (Alav et al. 2024). As shown in Figure 5b, FC and VAC juices exhibited comparable DPPH scavenging rates (62.40% and 62.23%, respectively), while APC juice showed a lower rate (56.70%). Hydroxyl radicals, highly reactive free radicals generated in biological systems

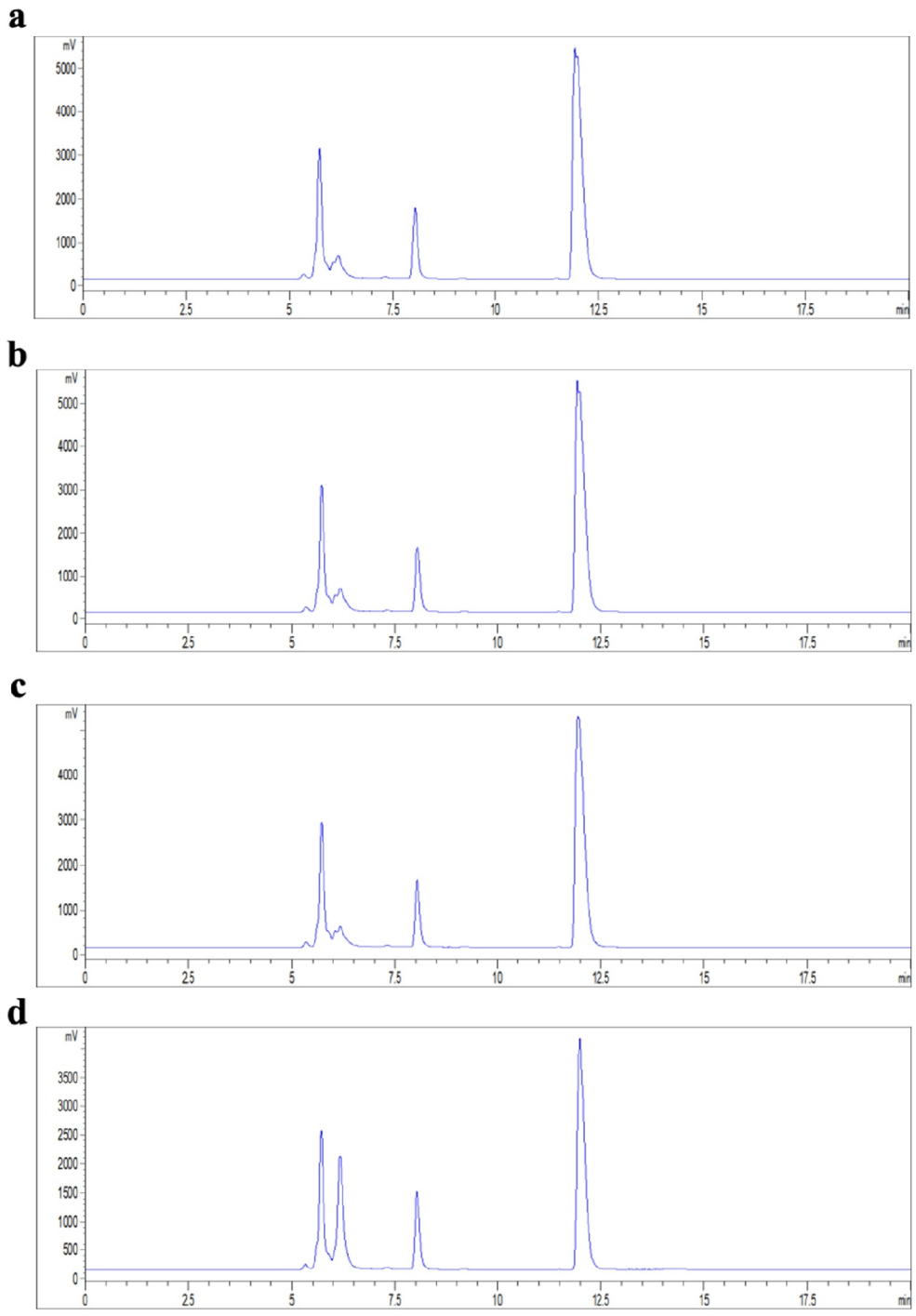



**FIGURE 4** | Chromatogram of kinsenoside of FS (a), FC (b), VAC (c), and APC (d).

**TABLE 1** | Effects of different concentration processes on the content of kinsenoside in *Anoectochilus roxburghii* juice.

| Sample | Content (mg/mL) |
|---|---|
| FS | $6.64 \pm 0.17^{a}$ |
| FC | $6.63 \pm 0.05^{a}$ |
| VAC | $6.46 \pm 0.03^{b}$ |
| APC | $4.73 \pm 0.13^{c}$ |

*Note:* Different lowercase letters indicate significant differences between the two groups.

(X. K. Qiu et al. 2024), are critical targets for evaluating antioxidant efficacy, with their scavenging rate serving as a key indicator (Y. Wang et al. 2021). As shown in Figure 5c, freeze- and vacuum-concentration processes most effectively preserved the hydroxyl radical scavenging capacity of *A. roxburghii* juice ($p < 0.05$). Both methods maintained scavenging rates at approximately 60%, demonstrating superior retention compared to the APC group. Therefore, freeze- and vacuum-concentration treatments could effectively retain the antioxidant capacity of *A. roxburghii* juice. Azhar et al. reported that in their study of blueberry juice, DPPH values in the low-temperature frozen

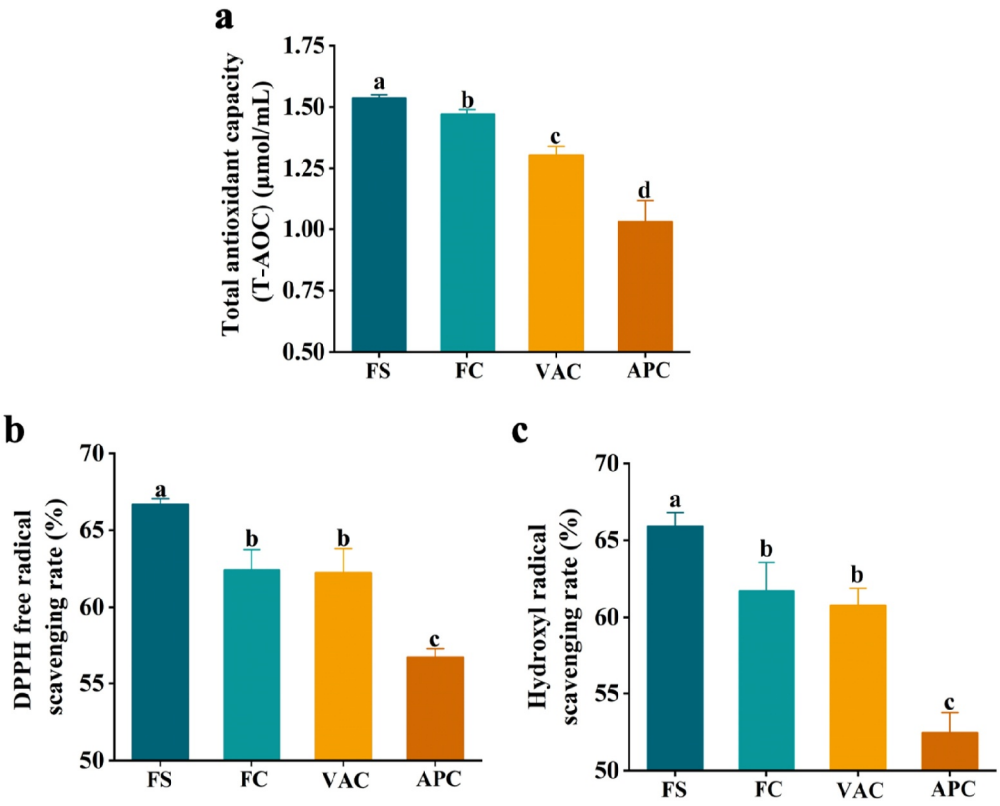



**FIGURE 5** | Effect of different concentration processes on the antioxidant capacity of *Anoectochilus roxburghii* juice. (a) Total antioxidant capacity; (b) DPPH scavenging ability; (c) Hydroxyl radical scavenging capacity.

treatment group ranged from 1916 to 5700 μmol/L, significantly higher than those in the high-temperature group (1916–4600 μmol/L) (Casas-Forero et al. 2020). This indicates robust antioxidant activity in the low-temperature group, a finding consistent with our results.

## 3.5 | Analysis of the Odor of *A. Roxburghii* Juice by Electronic Nose

### 3.5.1 | Analysis of Sensor Characteristic Response Values

Throughout electronic nose measurements, each sensor is exposed to volatile compounds within the sample, adsorbing them and inducing a characteristic change in resistivity. This yields the sensor response value, denoted as G. Subsequently, the sensor is purged with activated-carbon-purified air, and its baseline resistivity ($G_0$) is recorded. The relative resistivity is then defined as the dimensionless ratio $G/G_0$. During electronic nose (e-nose) measurements, the response curve of each sensor reflects the resistivity ratio change, which directly correlates with volatile organic compounds (VOCs) in the sample (Du et al. 2019). Figure 6 presented the electronic nose response curves for *A. roxburghii* juice. The normalized resistivity ($G/G_0$) was initially low during sample injection. As volatile compounds adsorbed onto the sensor surface over time, $G/G_0$ increased sharply, peaking at 5–10 s, before stabilizing. To ensure data reliability and reproducibility, the equilibrium stabilization phase (96–100 s) of $G/G_0$ was selected for conductivity signal analysis.

In electronic nose radargram analysis (Figure 7a), the distance of a sensor's response point from the radar center correlates with its signal intensity: farther points indicate higher responses. The FC group demonstrated superior retention of volatile compounds and flavor integrity in *A. roxburghii* juice compared to APC and VAC group. Specifically, sulfide-sensitive (W1W), alcohol-sensitive (W2S), and amine-sensitive (W2W) sensors exhibited higher responses in FC juice, closely mirroring the freshly squeezed juice profile. Similarly, Orellana-Palma et al. reported that heat-sensitive volatile compounds degraded in the untreated juice at 50°C. Compared to high-temperature treatments, freezing methods offer distinct advantages for preserving these compounds (Orellana-Palma et al. 2021).

### 3.5.2 | Loadings Analysis

Sensor distinction contribution analysis quantifies the sensitivity and relative contributions of individual sensors to sample variability (H. Wang et al. 2016). Principal component analysis (PCA) loadings plots for freshly squeezed and concentration-processed *A. roxburghii* juice are shown in Figure 7b. The first principal component (PC1) accounted for 99.21% of variance, while the second (PC2) contributed 0.46%, with a cumulative variance contribution of 99.67%. This confirms that PC1 predominantly captures the volatile compound profile of *A. roxburghii* juice.

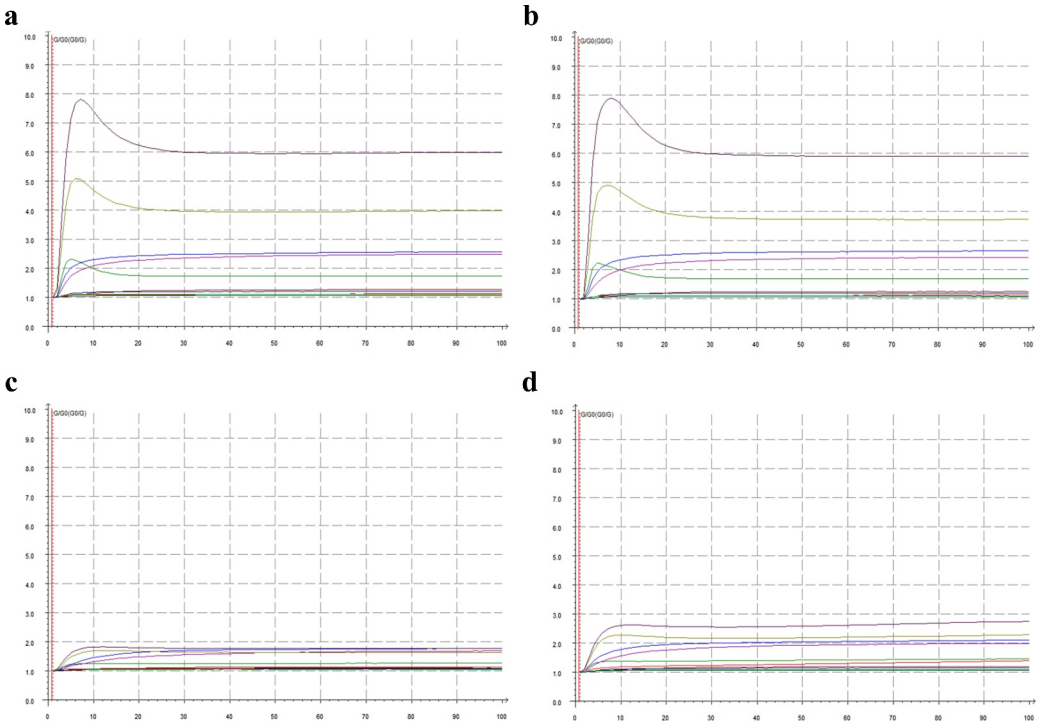



**FIGURE 6** | Electronic nose response curves of FS (a), FC (b), VAC (c), and APC (d).

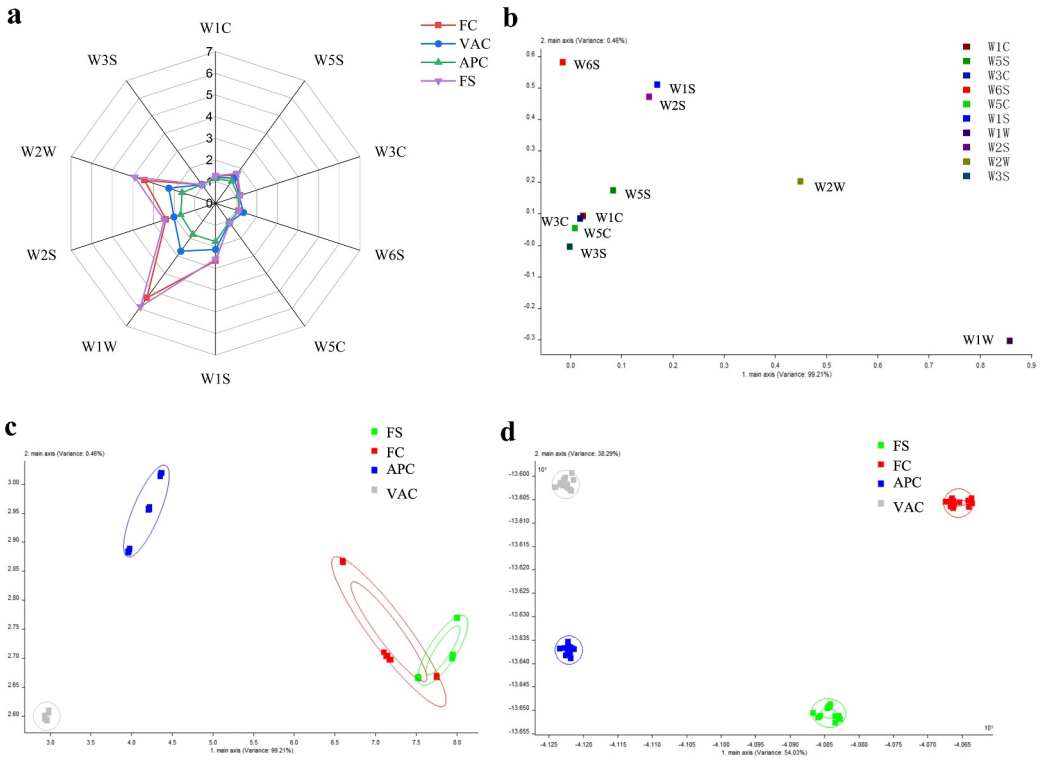



**FIGURE 7** | Electronic nose analysis of *Anoectochilus roxburghii* juice sensor under different concentration processes. (a) Electronic nose radargram; (b) Loadings analysis of aroma composition; (c) PCA analysis of aroma composition; (d) LDA analysis of aroma components.

In PCA, a sensor's proximity to the coordinate origin inversely correlates with its loading value: sensors closer to the origin contribute less to aroma discrimination, while those farther away exhibit higher discriminatory power (Y. J. Wang et al. 2023). Sensor W1W, positioned farthest from $x = 0$, contributed most to PC1. Conversely, sensor W6S, farthest from $y = 0$ and nearest to $x = 0$, contributed most to PC2 but minimally to PC1. Given PC1's significantly higher variance contribution (99.21%) compared to PC2 (0.46%), sensor contributions ranked as follows: W1W > W2W > W1S > W2S > W5S. Sensors

W3S and W5C, with response values near (0, 0), demonstrated negligible loadings and minimal impact on aroma profiling, suggesting low sensitivity to long-chain alkanes and aromatic compounds. Loadings analysis identified inorganic/organic sulfides, short-chain alkanes (e.g., methane), alcohols, ethers, aldehydes, ketones, and nitrogen oxides as key discriminants of aroma profiles in *A. roxburghii* juice. Zhang et al. employed loading analysis in their study, identifying nitrogen oxides, sulfides, terpenes, aromatic compounds, and organic sulfur compounds as key discriminant compounds for characterizing the aroma profile of red strawberry juice (J. W. Zhang et al. 2023).

#### 3.5.3 | PCA Analysis

PCA is a dimensionality reduction technique that transforms high-dimensional sensor data into simplified eigenvectors for linear analysis (Yin and Zhao 2019). In e-nose applications, larger overlaps in PCA plots reflect greater similarity in aroma profiles between samples, indicating shared volatile compounds (Zeng et al. 2023) Figure 7c presents PCA of *A. roxburghii* juice samples. PC1 and PC2 captured 99.67% of variance, effectively representing the aroma profiles of the juice. VAC and APC groups exhibited greater dispersion in the PCA plot, distinguishing them from other treatments. In contrast, FS and FC juices showed overlapping data points, indicating partial similarity in aroma composition. This overlap likely stems from freeze-concentration's lack of thermal degradation, which better preserves volatile compounds compared to heat-based methods, minimizing odor profile differences with freshly squeezed juice (Orellana-Palma et al. 2021). Yoda et al. consistently demonstrate that over half of the original flavor components are retained during freezing, whereas heat treatment fails to achieve comparable retention (Yoda et al. 2021).

#### 3.5.4 | LDA Analysis

Linear Discriminant Analysis (LDA), a widely used statistical method in e-nose studies, is a supervised pattern classification technique that projects high-dimensional sensor data into a lower-dimensional space to maximize inter-class separation (Mohammadian et al. 2024; Zhu et al. 2020). Unlike PCA, LDA employs discriminatory algorithms to orient data projection in directions that maximize between-group variance while minimizing within-group variance. This approach enhances classification accuracy by leveraging information from all sensors, thereby improving pattern recognition resolution (H. Wu et al. 2024). Figure 7d presented LDA of aroma components in *A. roxburghii* juice subjected to different concentration methods. The first and second discriminant functions accounted for 54.03% and 38.29% variance, respectively, with a cumulative variance contribution of 92.32% (exceeding the 70% threshold (Shi et al. 2020)), confirming robust classification efficacy. Data points for all four sample types clustered tightly within distinct regions of the discriminant space, showing clear separation without overlap. Compared to PCA, LDA demonstrated superior discriminatory power, effectively distinguishing FS, VAC, and APC juices from freshly squeezed juice. FC and FS juices exhibited a 72% overlap in projected coordinates, with significantly smaller spatial distances in aldehyde- and ketone-sensitive regions. APC and VAC groups showed diffuse distributions, whereas FC and FS juices clustered closely, indicating high consistency in aroma profiles between these treatments. Zhang et al. identified significant overlap in the VOC sensor response profiles for Hongyan strawberry juice stored for 12 and 48 h, suggesting remarkably similar flavor characteristics between these two storage periods (J. W. Zhang et al. 2023).

### 3.6 | Analysis of the Taste of *A. Roxburghii* Juice by Electronic Tongue

#### 3.6.1 | Effective Taste Indicators

In e-tongue analysis, sour and salty tastes are defined by detection thresholds of −13 and −6, respectively, while all other taste indicators (bitter, astringent, fresh, sweet) have a threshold of 0. Values below these thresholds are considered undetectable (N. Xu et al. 2024). Figure 8a displays taste radar plots for *A. roxburghii* juice samples, showing that only bitterness, sweetness, astringency, and freshness were detectable (sour and salty values fell below thresholds). The effective taste indicator radar plots Figure 8b reveal minimal variability among the four sample groups, with bitterness, sweetness, astringency, and freshness consistently exhibiting higher response values. Zhang et al. presented a radar chart depicting the aroma profile of strawberry juice in their study. Each curve represents the response values over time for specific sensors exposed to fresh strawberry juice. Among the ten sensors analyzed, five categories—nitrogen oxides, sulfides, terpenoids, aromatic compounds, and organic sulfur compounds—demonstrated strong correlations with key flavor characteristics in both Hongyan and Xiaobai strawberry cultivars (J. W. Zhang et al. 2023).

#### 3.6.2 | Analysis of Effective Taste Indicators

Figure 8c illustrates the astringency and bitterness profiles of *A. roxburghii* juice samples. The APC group exhibited the highest values on both axes, with astringency (~6) and bitterness (> 8.3) responses surpassing other treatments. Elevated temperatures during APC likely promote bitter compound formation via thermal degradation and Maillard reactions. For instance, high-temperature reduction of sugars and amino acids in Maillard reactions generates nitrogenous bitter-tasting compounds, which intensify perceived bitterness (Z. F. Chen et al. 2021). Figure 8d presents sweetness (Y-axis) and freshness (X-axis) scatter plots for *A. roxburghii* juice subjected to different concentration treatments. FS juice (untreated) exhibited the lowest values (freshness: 4.34–4.35; sweetness: 6.8–6.95), indicating minimal perceived sweetness and freshness. Taste profiles in *A. roxburghii* juice arise from interactions among multiple components, including reducing sugars, amino acids, and proteins. Yan et al. analyzed small peptides derived from pea protein hydrolysis and their Maillard reaction products. They found that the concentration process initiates both hydrolysis and conversion reactions, altering the content and proportions of these

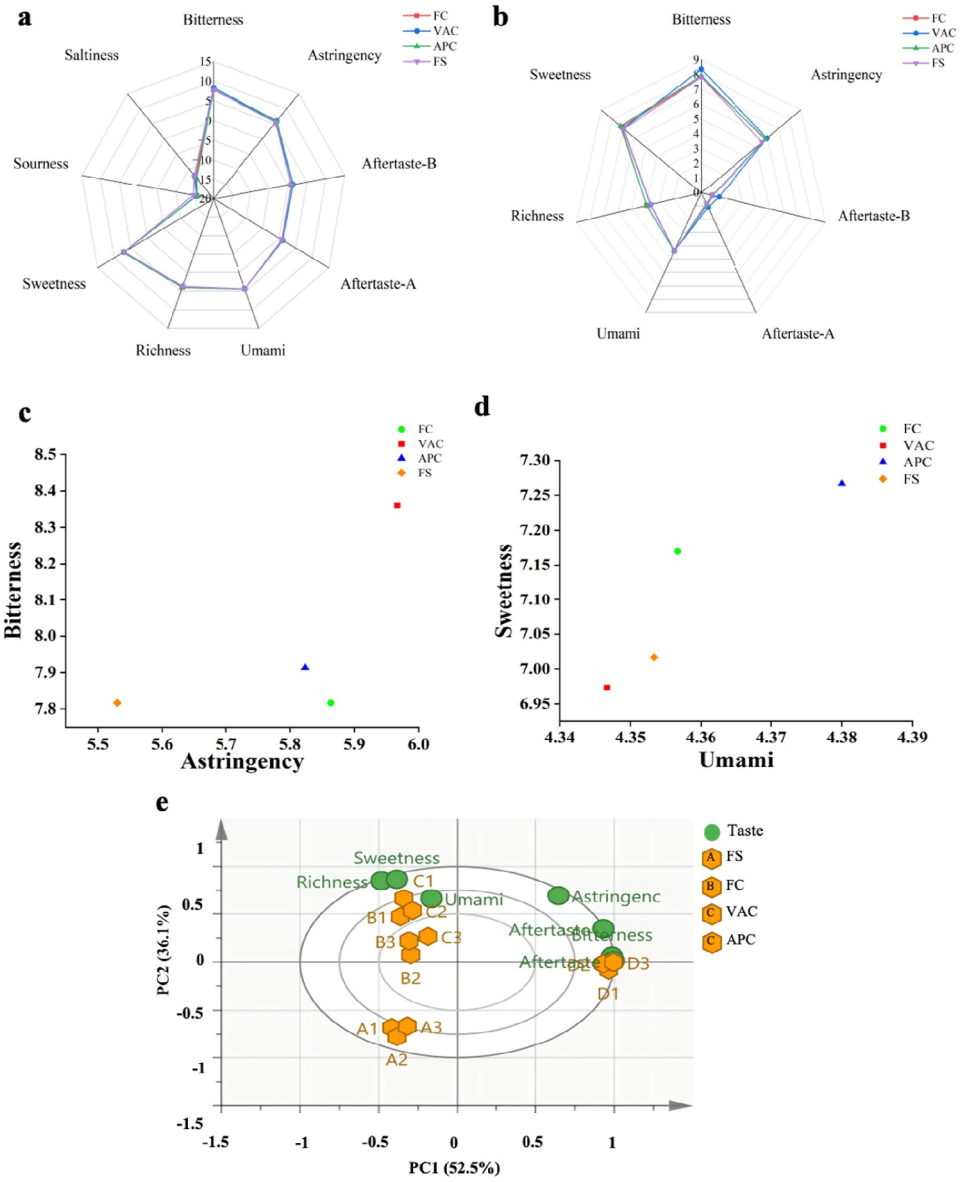



**FIGURE 8** | Electronic tongue analysis of *Anoectochilus roxburghii* juice sensor under different concentration processes. (a) Electronic tongue taste radargram; (b) Effective taste radargram; (c) Scatterplot of the taste (Astringency and Bitterness); (d) Scatterplot of the taste (Sweetness and Freshness); (e) PCA score and loading plots.

compounds and consequently modifying flavor characteristics (Yan et al. 2021).

### 3.6.3 | Principal Component and Factor Loadings Plot Analysis

A biplot integrates factor loading and PCA score plots to visualize correlations between samples and taste profiles (S. Y. Li et al. 2024; F. Zhang et al. 2024a). To investigate the relationship between *A. roxburghii* juice concentration methods and effective taste indicators, electronic tongue detection data were analyzed. A biplot was constructed using principal component analysis (PCA) in SIMCA software with unit variance (UV) scaling, representing juice samples concentrated by different methods. As shown in Figure 8e, PC1 accounted for 52.50% of variance, while PC2 contributed 36.10%, yielding a cumulative variance of 88.60%. This captures most of the variance in *A. roxburghii* juice data, effectively representing its key compositional features.

The score plot represents distinct *A. roxburghii* juice samples as discrete points. Proximity between points corresponds to greater similarity in their sensory profiles, while increased separation indicates reduced similarity. Notably, the APC sample is positioned distantly from the other three sample types, suggesting its taste profile differs considerably. The loading plot provides complementary information by illustrating influential variables. Superimposing the score plot and loading plot at a shared origin creates a biplot, where the relative positions of both samples and variables reveal their correlations. Figure 8e illustrates the e-tongue-based differentiation of *A. roxburghii* juice samples. The FS and APC groups occupied distinct quadrants, enabling complete discrimination from other treatments. In contrast, FC and VAC groups clustered closely, indicating analogous taste

profiles. Furthermore, the APC group exhibited proximity to astringency and bitterness clusters, corroborating its heightened bitter and astringent attributes, consistent with prior scatter plot analysis. Zhang et al. conducted a principal component analysis (PCA) two-coordinate graph study on wine samples from different treatment groups to gain an intuitive and comprehensive understanding of changes in wine flavor. The results clearly showed the contribution of flavor characteristics to differences in wine types (F. Zhang et al. 2024a).

## 4 | Conclusions

This study systematically evaluated the effects of APC, VAC, and FC processes on the physicochemical properties, bioactive compounds, and flavor profiles of *A. roxburghii* juice. Results showed that FC reconstituted juice most closely resembled FS juice in physicochemical properties, followed by VAC reconstituted juice. Regarding bioactive component retention, FC exhibited a significant advantage: retention rates for total flavonoids and total phenols both exceeded 85%, while the loss rate of specific glycosides was only 2.37%. Although all concentration treatments reduced the juice's antioxidant capacity (as measured by total antioxidant capacity, DPPH radical scavenging rate, and hydroxyl radical scavenging rate), the loss was minimal in FC reconstituted juice. Electronic nose and tongue analyses indicated that key volatile components include aromatic compounds, inorganic sulfides, organic sulfides, methane, short-chain alkanes, nitrogen oxides, alcohols, ethers, aldehydes, and ketones; key taste attributes were bitterness, sweetness, astringency, and umami. In summary, the FC process maximizes the preservation of *A. roxburghii* juice's natural quality, bioactive integrity, and unique flavor profile, demonstrating its feasibility as a high-fidelity industrial juice processing technology. Future applications could focus on developing high-quality nutraceutical juices using FC, a method that effectively preserves heat-stable antioxidants and nutrients in the juice without degradation. Industrially, targeting high-value functional food markets is recommended, specifically by combining this process with aseptic packaging to produce shelf-stable, additive-free concentrates.

### Author Contributions

**Xiaoyan Lu:** formal analysis, investigation, methodology, writing – original draft. **Jingwen Lv:** conceptualization, data curation, formal analysis, writing – original draft. **Yongxin Ming:** methodology, project administration, resource, software. **Sirui Leng:** data curation, formal analysis. **Shaoxiao Zeng:** resources, software, supervision, writing – review and editing. **Haoran Chen:** conceptualization, data curation, funding acquisition, writing – review and editing.


### Acknowledgments

This work was supported by Technology Innovation Special Fund of Fujian Agriculture and Forestry University (KFB24089).


### Ethics Statement

The authors have nothing to report.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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