Original Research Article

Assessment of dried star anise (*Illicium verum Hook.f.*) for color, volatile oil, shikimic acid, and flavor during room-temperature storage affected by packaging methods

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Abstract: Consumer demands for food packaging and quality are increasing, and the preservation of food quality during storage is critical, especially for spices. In this study, four different packaging methods, which were packaging using a transparent sealing bag (TSB), a transparent plastic bag (TPB), a lightproof sealing bag (LSB), and a woven bag (WB), were applied to the storage of star anise at room temperature for one year. Changes in moisture content, volatile oil (VO), trans-anethole in VO (TA-O), shikimic acid (SA), main flavor substances, and mycotoxins were monitored during storage. The results show that the two sealed packaging methods (TSB and LSB) were better at preventing moisture absorption than unsealed packaging (WB and TPB). In addition, the color of star anise packaged using the TSB method was the best, although the LSB method effectively reduced the loss of SA, VO, and TA-O. The gas chromatography–mass spectrometry (GC-MS) and principal component analysis (PCA) results show that the flavor substance scores of the LSB sample were the highest, followed by those of the TSB, TPB, and WB samples. Finally, the quality characteristics of star anise packaged using various methods for preservation were assessed using hierarchical clustering analysis (HCA). The LSB method was found as the best storing method for dried star anise at room temperature.

Keywords: star anise; packaging methods; storage; physicochemical properties; flavor components

1. Introduction

Star anise (*Illicium verum Hook.f.*) is popularly known as Chinese star anise or Da Liao. It belongs to the Magnoliaceae (Magnolia) family and is extensively cultivated in northern Myanmar and southern China[1]. The seed of star anise is capsule-like and the aggregate is mostly eight-petaled and octagonal in shape[2], which is widely used in foods, drinks, candies, and bakeries, offering some special flavors or reducing the fishy smell[3]. The dried fruit of *Illicium verum* plants is one of the traditional spices used in the Chinese catering industry. Star anise has been reported to contain many active substances, including sesquiterpene lactone, phenylpropanoids, polysaccharides, lignans, flavonoids, shikimic acid, and trans-anethole[4,5]. Modern pharmacology studies have demonstrated that star anise possesses a series of pharmacological bioactivities, including antimicrobial, anti-inflammatory, analgetic, sedative, insecticidal, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radical scavenging activities[6–8]. Star anise is a major source of shikimic acid, which plays an important role in the synthesis of oseltamivir phosphate (Tamiflu)[9]. Trans-anethole, the main flavor substance found in the volatile oil of star anise, has been demonstrated to have strong

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anti-apoptotic, anticataract, anti-inflammatory, antioxidant, and anti-diabetic activities[10,11]. Therefore, star anise has great potential for utilization in both the food and pharmaceutical industries.

Spices are important cooking ingredients and are primarily responsible for food flavor thanks to their high levels/contents of special flavoring substances, which are easily affected by light, oxygen, water, temperature, and other factors during storage, resulting in the deterioration of flavor components, the decrease in the content of bioactive ingredients, and even microbial contamination[12–14]. Most spices, especially star anise, are not consumed immediately after drying and need to be appropriately stored in packages for some period of time[14]. Misra[15] reported that almost all spices were susceptible to fungal contamination under storage conditions of 80% humidity and 20 ℃. Korkmaz et al.[14] analyzed the changes in dried pepper quality during storage at room temperature and found that the flavor components of dried samples can deteriorate significantly. According to Mizani et al.[16], good spice packaging should be able to preserve volatile chemicals, inhibit/minimize oxidation, and prevent the loss of spice color and flavor. As a result, in recent decades, there has been a renewed emphasis on storage conditions, particularly packaging methods, in order to mitigate sample quality loss.

The commercial star anise packaging methods mainly include directly bulked storage (non-packaging), woven bag (WB) packaging, and transparent plastic bag (TPB) packaging. Of note, these packaging approaches may be associated with some food safety problems. It was emphasized in the "Code of Practice for the Prevention and Reduction of Mycotoxins in Spices"[17] that the storage environment of star anise must be dry, ventilated, and not accompanied by other foods; otherwise, increased moisture content or contamination by non-food products will result in the growth of toxigenic fungi and a change in the flavor and color of star anise. Previous studies showed that vacuum and non-light packaging are the best options for preserving the quality (color, numbness, and aroma) of dried *Zanthoxylum armatum* during room-temperature storage, followed by ventilation and non-light packaging[18]. Yang[19] found that, in comparison with sealed packaging, the pungent components of *Zanthoxylum* packaged in woven bags and plastic bags at room temperature were destroyed more. Also, according to Duman[20], airtight and vacuum storage methods are better than the traditional technique (stacked bags) for long-term red chili pepper storage to preserve the quality of red chili peppers and avoid mycotoxin formation. However, Ding et al.[21] demonstrated that atmospheric sealing packaging without vacuum helped preserve the volatile taste constituents of dried chili peppers. Giuffrida et al.[12] stored red chili powder in a dark environment for 12 months, and both carotenoid and capsaicinoid contents were found to decrease at room temperature. Therefore, proper packaging can help postpone the loss of spice taste and extend the shelf life of spices. However, previous studies on star anise mainly focused on its active chemicals (e.g., flavonoids, shikimic acid, trans-anethole, etc.) and health benefits, with little attention paid to packaging and storage. Therefore, the impacts of four commonly used packaging approaches on the quality of star anise were compared. The quality of star anise was studied while it was stored at room temperature. This study is expected to lead to a better knowledge of appropriate star anise packaging methods and storage conditions and provide greater data-driven support for successful star anise spice preservation.

2. Material and methods

2.1. Material

Star anise (*Illicium verum Hook.f.*) was hand-picked in Liuxian Town, Qinzhou, Guangxi, China (latitude N 22°28′58.83″, longitude E 109°47′20.57″). Fresh star anise of uniform size was selected, washed, and then dried using the drying method described by Shi et al.[22] The standard star anise obtained was first-class star anise from Guangxi, China.

2.2. Packaging and storage methods

A detailed flow chart of packaging and storage for the dried star anise is described in **Figure 1**. The tested star anise samples were packaged using the following methods: packaging using a transparent sealing bag (TSB), a transparent plastic bag (TPB), a lightproof sealing bag (LSB), and a woven bag (WB). After storage, the star anise samples were kept at room temperature for varied periods of time (0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 months), and the temperature and humidity of the environment were recorded using a temperature and humidity meter (VMS-3003-WS, Shandong Wemsee Technology Co., Ltd.). **Table S1** shows the results.

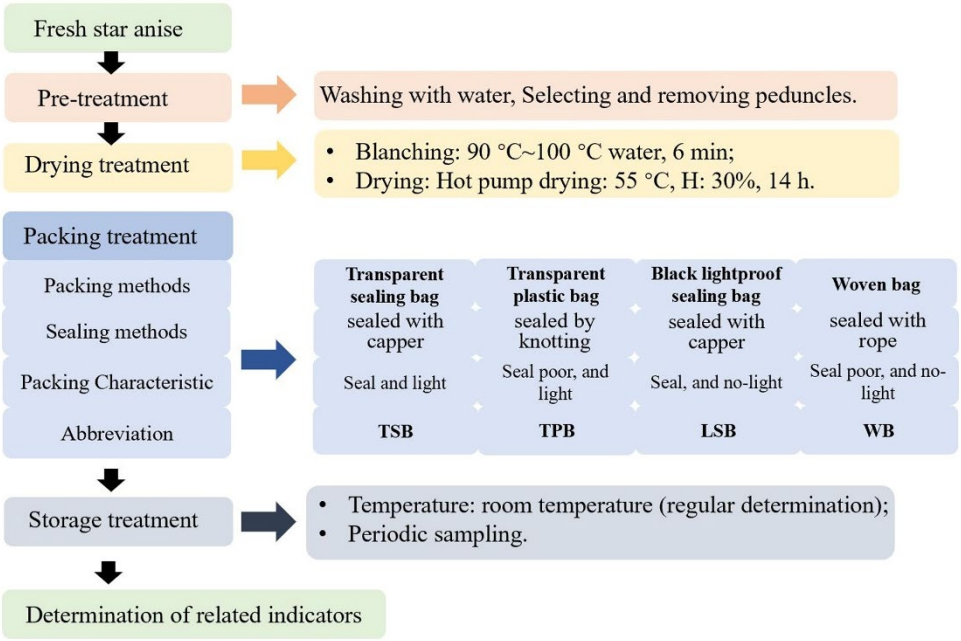

Figure 1. Flow chart of sample treatment.

2.3. Determination of physicochemical index

2.3.1. Moisture content

The moisture content of the star anise samples was measured as described previously by Liu et al.[23]

2.3.2. Color

The color of the star anise samples was determined as described previously by Wu et al.[24]

2.3.3. Volatile oil (VO)

Distillation of dried star anise powder (20.0 g) was performed in 200 mL of water (with added zeolite) for 3 h until no more oil was obtained. In a separating funnel, the distilled fluid was collected and extracted twice using 20 mL of petroleum ether and then dried with sodium sulfate. After removing the petroleum ether using rotary evaporation, the concentrated solution was weighed. The VO was obtained to determine the trans-anethole content[22].

2.3.4. Trans-anethole content in star anise volatile oil (TA-O)

The VO (1 mL) extracted from a variety of dry materials was diluted with ethanol and then subjected to gas chromatography (GC) (7890A, Agilent, USA) analysis. The temperatures of the injector and detector were 220 °C and 250 °C, respectively. The temperature program was performed as described by Shi et al.[22]. The contents of trans-anethole in the crude extract were determined in comparison with that of the standard trans-anethole.

2.3.5. Shikimic acid (SA)

Dried star anise was blended with deionized water, put aside overnight, and then boiled for 1 h in a water bath at a steady temperature of 85 ℃. The mixture was filtered, and the filtrate was immediately diluted and stored. Based on the method by Zhang[25], the content of SA was determined using high-performance liquid chromatography (HPLC) (1260, Agilent, USA) coupled with a diode array detector (DAD) with a 4.6×250mm column (ZPRBAX SB-Aq, Agilent) at 25 ℃. The mobile phase was composed of solvent A (0.1% phosphoric acid) and solvent B (acetonitrile) according to the following isocratic elution (97% A, v/v). A volume of 20 μL was injected at a flow rate of 0.7 mL/min. The content of SA is expressed as mg per g dry basis (mg/g db).

2.3.6. Flavor components

Based on the methods by Jia et al.[26] and Gholivand et al.[27], dried star anise (1.0 g) was immersed in a headspace bottle with octanol (Sigma, 100 μL, 4 mg/mL) as an internal standard. The sample vial was equilibrated at 50 ℃ for 15 min. Solid-phase microextraction (SPME) fibers (85-micron PA, polyacrylate) were then introduced to the headspace and absorbed for another 40 min at 50 ℃.

After extraction, the material was immediately injected into a gas chromatography–mass spectrometry (GC-MS) (Shimadzu, Japan) injection port with a DB-5MS column (30 m × 250 μm × 0.25 μm; Agilent) and desorbed at 250 ℃ for 2 min. The temperature schedule was as follows: the temperature was raised to 220 ℃ at 4 ℃/min after the beginning temperature of 50 ℃ (3 min and held for 10 min). The carrier was 1.0mL/min gas helium. The electron-impact ionization mode (+EI, 70 eV) was used to run the mass spectrometer. The temperature of the ion source was 200 ℃. The scan speed ranged from 35 to 500 m/z[26]. The qualitative and quantitative examinations of volatile compounds were performed by following the procedures described by Kulapichitr et al.[28] and Zhao et al.[29].

2.4. Determination of mycotoxins

2.4.1. Determination of aflatoxin (AFT: G1, G2, B1, and B2)

We extracted, purified/enriched, and separated aflatoxin from the material by following the manufacturer's instructions for the AFT immunoaffinity column (NXA-Q001, Beijing Naxun Technology Co., Ltd.).

The contents of AFT (G1, G2, B1, and B2) were measured using HPLC (1260, Agilent, USA) coupled with a fluorescence detector (FLD), with an excitation wavelength of 360 nm and emission wavelength of 440 nm. The test solution (50 μL) was injected into a Venusil MP C18 column (4.6 mm × 150 mm × 5 μm, Agela Technologies) at a flow rate of 0.8 mL/min. The column temperature was 40 ℃. The mobile phase consisted of methanol (55%) with isocratic elution (25 min)[30]. The AFT (G1, G2, B1, and B2) standards (Romer Labs, Beijing, China) were used, and each content of AFT is expressed as ng per mL. The linear equations and correlation coefficients of the AFT standard curves are shown in **Table S2**.

2.4.2. Determination of ochratoxin A (OTA)

We extracted, purified/enriched, and separated OTA from the material by following the manufacturer's instructions for the OTA immunoaffinity column (NXA-Q007, Beijing Naxun Technology Co., Ltd.).

The resulting extracts were determined as described by Giancarlo et al.[31], with slight modifications. The content of OTA was measured using HPLC with an FLD, with an excitation wavelength of 333 nm and an emission wavelength of 477 nm. The test solution (50 μL) was injected into the Venusil MP C18 column at a flow rate of 0.8 mL/min. The column temperature was 40 ℃. The mobile phase consisted of water/acetonitrile/acetic acid (99/99/2) with isocratic elution (25 min). The OTA standard (Romer Labs,

Beijing, China) was used, and the content of OTA is expressed as ng per mL. The linear equation and correlation coefficient of the OTA standard curve are shown in **Table S2**.

2.5. Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA) via SPSS Statistics 26.0 (IBM SPSS Inc., USA). Duncan's test was used to determine any significant difference among the different treatment groups at $p < 0.05$, and the data are presented as mean ± SD. Origin 2019 software (Origin Lab Inc., USA) was used to plot the data. Principle component analysis (PCA) was performed using SIMCA 14.1 software (Sartorius Stedim Biotech Inc., Germany). In hierarchical clustering analysis (HCA), the aggregation technique used was Ward's linkage, and similarity was measured using the Euclidean distance.

3. Results and discussion

3.1. Color

The result of the alteration in star anise color with storage time for various packaging procedures is depicted in **Figure 2**. Freshly dried star anise has a yellowish color to it. With the passage of time, its color deepens, turning red-brown or yellow-brown. When the storage period was less than three months, the color of the TSB samples was clearly brighter than those of the other samples, and the WB samples showed the darkest color. After more than six months of storage, the star anise color turned brown or dark brown. At 12 months, the LSB sample had the lightest star anise color, which was yellowish brown in comparison to those of the other methods. In contrast, the hue of the TSB sample was brownish-red. The other two packaging methods showed dark brown (W) and brown (TP).

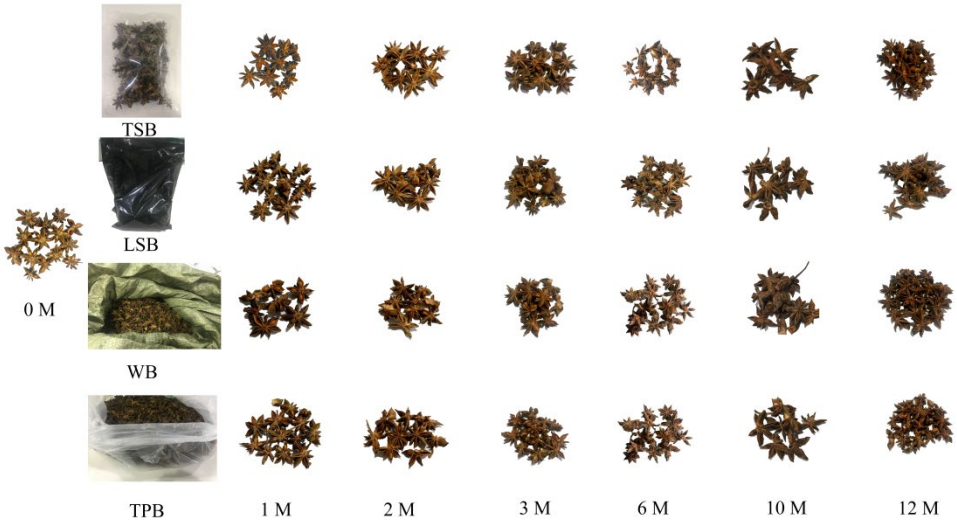

Figure 2. Changes in star anise color with storage time for different packaging methods.

The color attributes of the star anise changed over the storage period, as indicated in **Table 1**. During storage, the L* and a* values of the samples rose when packaged using the TSB, LSB, WB, and TPB methods, while the b* value declined, which might be related to the dried star anise progressively changing color from yellow-green to yellowish-brown or reddish-brown. The L* and b* values of the TSB sample were substantially greater than those of the other packaging groups ($p < 0.05$) when the storage duration exceeded eight months. After 12 months of storage, the a* values of TSB and TPB samples were substantially greater than those of the other packaging groups ($p < 0.05$). One study noted that the color of dried star anise altered during storage to become darker, which was similar to our findings[32].

Standard star anise was used as the reference, and the larger the ΔE value, the larger the color difference between a sample and the standard star anise sample. It can be observed from **Table 1** that the value of ΔE changed continuously during storage. The ΔE values of TSB and LSB samples gradually decreased, the ΔE values of WB and TPB samples first decreased and then increased, and the TSB sample had the lowest ΔE value (4.33 ± 0.03), which was significantly lower than those of other packaging groups ($p < 0.05$) after 12 months of storage. The effects of the packaging method and storage time on the ΔE value were analyzed using ANOVA and Q-test, and the result is shown in **Table 2**. There was a significant influence in the ΔE value regarding the packaging method ($p < 0.05$), and storage duration had a highly significant influence ($p < 0.01$). From the Q-test, we found that the ΔE value of the TSB sample was significantly less than those of the other groups ($p < 0.05$), followed by LSB and TPB samples, and the largest was the WB sample. From the comprehensive analysis, star anise packaged using the TSB technique was conducive to the color formation of high-quality star anise.

Table 1. Changes in L*, a*, b*, and ΔE values of star anise samples over time for different storage methods.

Packaging method	Storage period, months									
	0	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**10**	**12**
L*										
TSB	62.47 ± 0.36^{a}	57.74 ± 0.05^{b}	57.43 ± 0.09^{b}	57.08 ± 0.09^{b}	57.39 ± 0.97^{ab}	58.75 ± 0.05^{c}	57.99 ± 0.11^{b}	59.14 ± 0.09^{a}	57.25 ± 0.08^{a}	59.21 ± 0.03^{a}
TPB	62.47 ± 0.36^{a}	57.26 ± 0.05^{d}	58.08 ± 0.04^{a}	57.40 ± 0.05^{ab}	56.44 ± 0.15^{b}	58.42 ± 0.06^{d}	58.27 ± 0.10^{ab}	58.36 ± 0.03^{c}	55.19 ± 0.13^{c}	55.01 ± 0.03^{c}
LSB	62.47 ± 0.36^{a}	58.05 ± 0.02^{a}	57.15 ± 0.08^{c}	57.48 ± 0.20^{a}	56.82 ± 0.11^{b}	59.23 ± 0.07^{b}	57.55 ± 0.08^{c}	58.61 ± 0.04^{b}	55.71 ± 0.07^{b}	55.93 ± 0.04^{b}
WB	62.47 ± 0.36^{a}	57.54 ± 0.03^{bc}	57.23 ± 0.01^{bc}	55.58 ± 0.08^{c}	58.51 ± 0.08^{a}	59.86 ± 0.09^{a}	58.33 ± 0.08^{a}	58.08 ± 0.04^{d}	54.36 ± 0.10^{d}	53.67 ± 0.03^{d}
a*										
TSB	9.58 ± 0.27^{a}	9.45 ± 0.04^{b}	9.66 ± 0.10^{b}	10.52 ± 0.07^{a}	10.02 ± 0.06^{c}	9.50 ± 0.06^{c}	9.78 ± 0.07^{b}	9.46 ± 0.08^{b}	10.63 ± 0.08^{a}	11.44 ± 0.01^{a}
TPB	9.58 ± 0.27^{a}	9.54 ± 0.13^{b}	9.53 ± 0.12^{b}	10.25 ± 0.11^{a}	10.31 ± 0.02^{b}	9.85 ± 0.09^{b}	10.05 ± 0.04^{a}	10.15 ± 0.07^{a}	10.25 ± 0.03^{b}	11.28 ± 0.04^{a}
LSB	9.58 ± 0.27^{a}	9.42 ± 0.03^{b}	10.08 ± 0.02^{a}	9.90 ± 0.11^{b}	9.91 ± 0.03^{c}	9.42 ± 0.04^{c}	9.54 ± 0.03^{c}	9.62 ± 0.02^{b}	10.04 ± 0.02^{c}	10.62 ± 0.11^{b}
WB	9.58 ± 0.27^{a}	10.29 ± 0.09^{a}	9.62 ± 0.08^{b}	10.48 ± 0.07^{a}	10.49 ± 0.07^{a}	10.26 ± 0.05^{a}	9.85 ± 0.03^{b}	10.27 ± 0.10^{a}	10.23 ± 0.09^{bc}	10.76 ± 0.02^{b}
b*										
TSB	25.23 ± 0.36^{a}	21.38 ± 0.13^{b}	21.49 ± 0.05^{a}	20.05 ± 0.03^{c}	21.77 ± 0.14^{b}	21.61 ± 0.67^{ab}	21.35 ± 0.09^{b}	22.62 ± 0.02^{a}	21.15 ± 0.08^{a}	20.76 ± 0.14^{a}
TPB	25.23 ± 0.36^{a}	22.03 ± 0.11^{a}	20.71 ± 0.05^{c}	21.03 ± 0.04^{a}	21.17 ± 0.06^{c}	20.69 ± 0.05^{b}	21.54 ± 0.10^{b}	20.59 ± 0.06^{b}	19.43 ± 0.09^{c}	17.98 ± 0.01^{c}
LSB	25.23 ± 0.36^{a}	22.09 ± 0.05^{a}	20.93 ± 0.08^{b}	20.42 ± 0.11^{b}	20.84 ± 0.04^{d}	21.27 ± 0.05^{b}	21.25 ± 0.08^{b}	20.42 ± 0.03^{c}	20.62 ± 0.16^{b}	18.95 ± 0.07^{b}
WB	25.23 ± 0.36^{a}	22.27 ± 0.04^{a}	20.98 ± 0.03^{b}	19.42 ± 0.07^{d}	22.46 ± 0.07^{a}	22.41 ± 0.07^{a}	22.25 ± 0.10^{a}	20.33 ± 0.03^{c}	17.89 ± 0.06^{d}	17.13 ± 0.11^{d}
ΔE										
TSB	7.65 ± 0.47^{Aa}	6.40 ± 0.09^{Ba}	6.27 ± 0.07^{Bab}	5.86 ± 0.08^{BCa}	6.01 ± 0.33^{BCa}	6.21 ± 0.07^{Ba}	6.03 ± 0.09^{BCb}	6.24 ± 0.08^{Ba}	5.45 ± 0.09^{Cd}	4.33 ± 0.03^{Dd}
TPB	7.65 ± 0.47^{Aa}	6.44 ± 0.11^{Ba}	6.33 ± 0.13^{BCa}	5.77 ± 0.11^{CDc}	6.09 ± 0.09^{BCDa}	5.97 ± 0.10^{BCDb}	5.70 ± 0.05^{Dc}	5.71 ± 0.06^{Db}	7.19 ± 0.03^{Ab}	7.22 ± 0.05^{Ab}
LSB	7.65 ± 0.47^{Aa}	6.35 ± 0.03^{BCDa}	6.03 ± 0.02^{Db}	6.19 ± 0.12^{CDb}	6.33 ± 0.06^{BCDa}	6.24 ± 0.04^{BCDa}	6.37 ± 0.04^{BCDa}	6.22 ± 0.05^{BCDa}	6.76 ± 0.02^{Bc}	6.70 ± 0.09^{BCc}
WB	7.65 ± 0.47^{Aa}	5.65 ± 0.09^{EFb}	6.43 ± 0.08^{CDa}	6.80 ± 0.03^{Ca}	5.25 ± 0.06^{Fb}	5.45 ± 0.05^{EFc}	5.89 ± 0.05^{DEb}	5.71 ± 0.11^{EFb}	8.32 ± 0.10^{Aa}	8.81 ± 0.09^{Aa}

Note: Data are expressed as means ± SD (n = 3). Lowercase letter represents significant difference between same column ($p < 0.05$). Capital letter represents significant difference between same line ($p < 0.05$).

Table 2. Result of variance analysis and Q-test for effects of different packaging methods on star anise physicochemical indexes.

Indicator name	Variance analysis						Q-test	
	Source of variance	***SS***	***df***	***MS***	***F***	***P***	**Packaging method**	**Value**
ΔE	Storage time	34.43	9	3.82	85.21	$p < 0.01$	TSB	6.05^{b}
	Packaging method	5.076	3	1.69	37.70	$p < 0.05$	TPB	6.41^{a}
	Error	50.15	107	0.47			LSB	6.48^{a}
	Total	4977.50	120				WB	6.60^{a}
Moisture content	Storage time	145.48	9	16.16	32.03	$p < 0.01$	TSB	8.76^{c}
	Packaging method	92.14	3	30.71	60.86	$p < 0.01$	TPB	10.67^{b}
	Error	54.00	107	0.51			LSB	8.67^{c}
	Total	11297.98	120				WB	10.21^{a}
VO content	Storage time	113.30	9	12.59	149.16	$p < 0.01$	TSB	7.67^{a}
	Packaging method	3.77	3	1.26	14.88	$p < 0.01$	TPB	7.24^{c}
	Error	9.03	107	0.08			LSB	7.63^{a}
	Total	6845.85	120				WB	7.40^{b}
TA-O content	Storage time	34126.71	9	3791.86	135.69	$p < 0.01$	TSB	76.37^{a}
	Packaging method	2658.80	3	886.27	31.71	$p < 0.01$	TPB	67.64^{b}
	Error	2990.16	107	27.94			LSB	77.79^{a}
	Total	668834.24	120				WB	67.81^{b}
SA content	Storage time	12550.52	9	1394.50	203.01	$p < 0.01$	TSB	98.13^{a}
	Packaging method	38.61	3	12.87	1.87	$p > 0.05$	TPB	97.24^{a}
	Error	583.88	85	6.87			LSB	98.94^{a}
	Total	959059.04	98				WB	98.65^{a}

Note: VO: volatile oil, TA-O: trans-anethole in volatile oil, SA: shikimic acid. Lowercase letter represents significant difference between packaging methods ($p < 0.05$).

3.2. Moisture content

Figure 3A depicts the effects of packaging methods on the moisture content of star anise throughout the storage time. With the passage of time, the moisture content of star anise steadily rose, as predicted. The moisture content of samples packaged using the WB and TPB methods grew dramatically after six months of storage, eventually exceeding 12.5 percent, which was the Chinese national standard (GB/T 7652-2016), reaching 12.58 ± 0.31% and 12.51 ± 0.12% at 12 months, respectively. The moisture content of star anise stored using the sealed packaging methods of TSB and LSB also increased with time, but the increasing trend was relatively slow and the moisture contents did not exceed 12.5% after 12 months of storage, which were 10.56 ± 0.22% and 9.45 ± 0.17%, respectively. This may be due to the water vapor from the external environment, which can come into contact with the samples through the packaging materials[33]. It is noteworthy that a WB has air permeability, and so the moisture content of its star anise was easily affected by environmental temperature and humidity. The effects of packaging method and storage time on the moisture content of star anise were analyzed using ANOVA and Q-test, and the result is shown in **Table 2**. Packaging method and storage time had significant ($p < 0.01$) effects on star anise moisture content. The moisture content values for TSB and LSB packaging methods were significantly ($p < 0.05$) lower than those of the other methods during 12 months of storage. Thus, the WB and TPB methods, the two traditional packaging methods of star anise, have food safety hazards in the long-term storage of star anise. In contrast, sealed storage was proven suitable for avoiding moisture during the long-term storage of star anise.

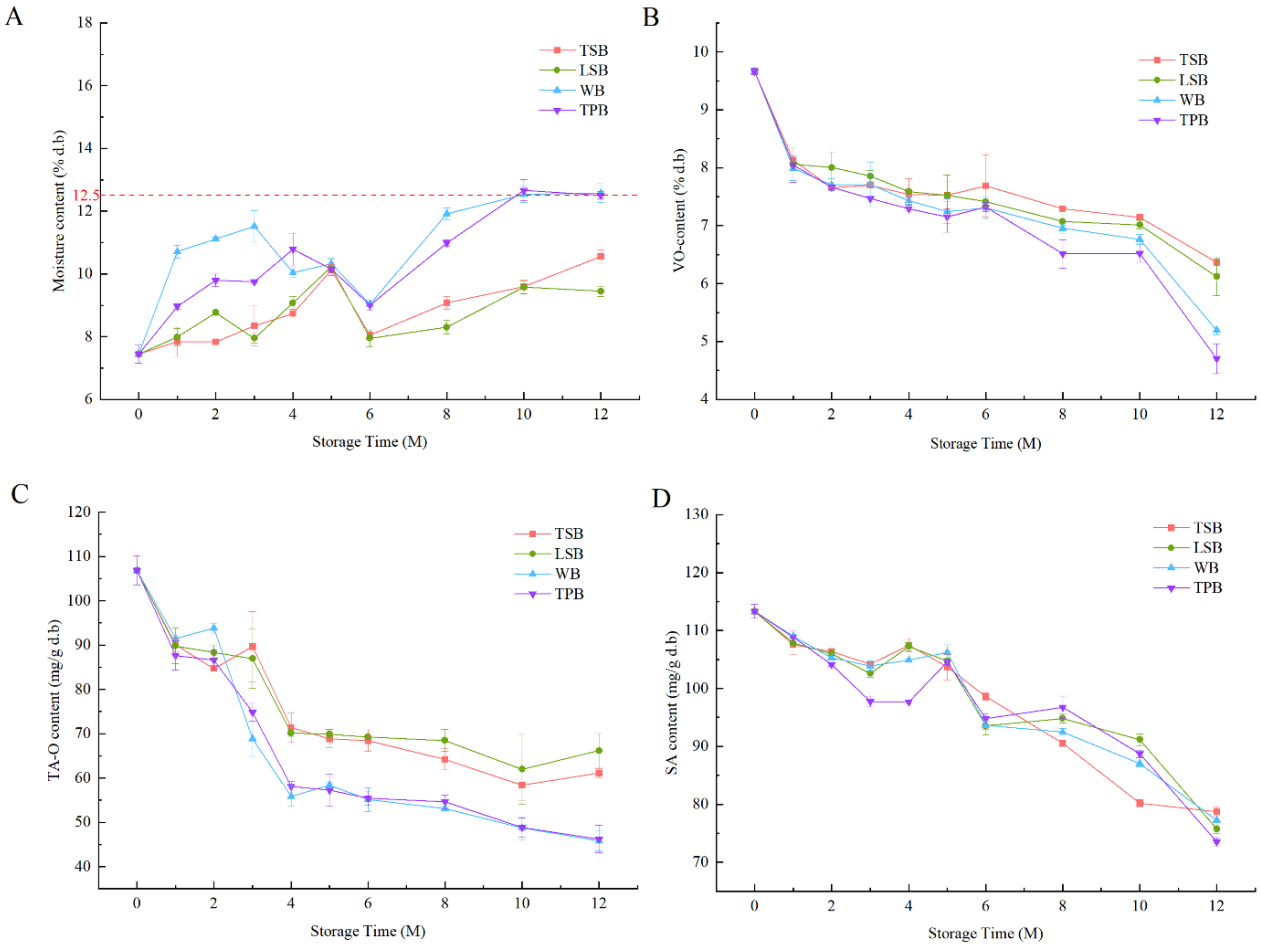

Figure 3. Changes in star anise indexes with time for different packaging methods: **A**) moisture content, **B**) volatile oil (VO) content, **C**) trans-anethole in volatile oil (TA-O) content, and **D**) shikimic acid—SA content.

3.3. VO content and TA-O content

The VO content of star anise packaged in various ways declined dramatically during long-term storage, as illustrated in **Figure 3B**. The loss of star anise's VO was modest in the first half of storage, but after more than six months, the drop trend of VO steadily increased. As seen in the changes in storage temperature and humidity in **Table S1**, the temperature and humidity in the first six months of storage were significantly lower ($p < 0.05$) than those in the final six months. Thus, the temperature and humidity of the storage environment were shown to have a substantial influence on the content of VO in star anise, and the loss of VO in star anise became more serious as ambient temperature and humidity increased. According to several studies, star anise's VO has a considerable quantity of volatile organic molecules[34]. Furthermore, Li et al.[35] found that the VO of star anise has significant volatility, which can be volatilized at ambient temperature and increases with temperature. Accordingly, it has a certain volatility, and the higher the temperature, the greater the volatility. Among the four packaging methods, the star anise VO content values for the two sealed packaging (TSB and LSB) methods were significantly higher than those of the two unsealed packaging (WB and TPB) methods ($p < 0.05$), especially at high storage temperatures and humidity. The values of VO in TSB and LSB samples were 6.36 ± 0.01% and 6.12 ± 0.33%, respectively, and the loss rates were 34.2% and 36.6%, respectively, when the storage duration was 12 months. These loss rates were substantially ($p < 0.05$) lower than those of the WB (5.19 ± 0.08%, 46.3%) and TPB (4.71 ± 0.25%, 51.2%) samples. Liang[36] also reported a similar phenomenon in Chuanxiong rhizome during storage. Furthermore, when comparing the TSB sample with the LSB sample, we found no significant difference between the storage methods ($p > 0.05$). A similar result was reported by Li et al.[35], where light had little effect on the VO content loss of star anise at a certain time.

Trans-anisole is the main substance in the flavor of star anise[8]. Several reports have also shown that trans-anethole is the main substance in the VO of star anise, accounting for more than 90%[7,37]. In **Figure 3C**, the loss in the TA-O content was seen as storage duration increased. The TA-O content of each packaging group steadily decreased. When the storage duration was shorter than two months, the four packaging options had little influence on the TA-O content. Nonetheless, after more than two months of storage, the TA-O content in WB and TPB samples began to drop substantially, while the TA-O content in TSB and LSB samples began to drop after more than three months of storage. Furthermore, the samples of the two sealed packaging methods had much greater TA-O content than those of the other methods. The changes in the TA-O content of WB and TPB samples were similar during the whole storage period. Comparing the TSB and LSB samples, the TA-O content of the TSB sample was slightly higher than that of the LSB sample with the extension of storage time. However, when the storage time was more than six months, the LSB method was more effective than the TSB method in delaying the loss of TA-O. In the end, when the storage time reached 12 months, the TSB, LSB, WB, and TPB samples showed TA-O content values of 61.08 ± 1.09, 66.17 ± 3.88, 45.75 ± 2.24, and 46.22 ± 3.10 mg/g db, respectively. The loss rates were 42.8%, 38.1%, 57.2%, and 56.8%, where the lowest was for the LSB method.

The result of the ANOVA analysis is presented in **Table 2**. Packaging method and storage time had a significant ($p < 0.01$) effect on the content of VO and TA-O, and the effect of storage time was greater than that of the packaging method. From the overall change, it was discovered that star anise packaged using the TSB and LSB methods had the greatest concentration of VO and TA-O compared with the WB and TPB methods ($p > 0.05$). However, there was no statistically significant difference between the TSB and LSB methods ($p < 0.05$). As a result, star anise packaged in sealed packages (TSB and LSB) had the highest levels of VO and TA-O after a 12-month storage period at room temperature.

3.4. Shikimic acid (SA) content

SA is a copious source for the synthesis of the antiviral drug oseltamivir, and star anise is a suitable material for obtaining it[9,38]. **Figure 3D** shows the SA content of star anise stored at room temperature for 12 months, indicating that a decrease occurred in all samples packaged using different methods. After 12 months of storage, the SA content decreased from 113.32 ± 1.21 mg/g db to 78.75 ± 0.73 mg/g db for the TSB method, 75.75 ± 0.74 mg/g db for the LSB method, 77.24 ± 0.1 mg/g db for the WB method, and 73.52 ± 0.67 mg/g db for the TPB method. Similarly, when combined with **Figure 3D** and **Table S1**, it was shown that the overall decline of the SA content in star anise decreased slowly during the first half of storage. However, it could be obviously seen that the decrease rate of the SA content of star anise increased with the rise in storage temperature and humidity. This means that storage temperature and humidity influenced the SA content of star anise. The effects of packaging method and storage time on the SA content of star anise were analyzed using ANOVA, and the result is shown in **Table 2**. Different from the indicators already mentioned, the difference between the packaging methods was found to be not significant ($p > 0.05$). However, the difference between storage times was significant ($p < 0.01$). This shows that storage time has a significant influence on the SA content. Thus, the SA content decreased with the extension of storage time regardless of the packaging method. Among the four packaging methods, the descending order of the SA content of their star anise samples is LSB > TSB > WB > TPB, but the difference between the four packaging methods was not significant ($p > 0.05$).

3.5. Changes in star anise flavor components

Star anise is a natural spice with strong aromatic as its main characteristic, and its aromatic components are mostly aromatic compounds without pungent, spicy, or other irritating odors. Therefore, it was necessary to detect the change in the flavor substances of star anise during storage. As shown in **Figure S1**, the total ion chromatograms of the flavor components in star anise under different packaging methods show that the content of the flavor substances in the samples had obvious changes after 12 months of storage. In-depth analysis found that (+)-limonene, α-pinene, linalool, 4-terpineol, estragole, trans-anethol, α-bergamotene, β-caryophyllene, cis-β-farnesene, β-sesquiphellandrene, and (S)-β-bisabolene accounted for 89.73% of star anise flavor substances, of which trans-anethole accounted for about 62.65%. This result is consistent with that of Hasegawa et al.[39] Therefore, these 11 substances were selected as representatives to study the changes in the flavor substances of star anise at different storage stages under different packaging methods.

Figure 4 presents the heat maps of changes in the flavor substances of star anise with storage time under different packaging methods. Color changes from red to yellow indicate that the relative content changed from high to low. The result shows that trans-anethole had the largest relative concentration and the reddest heat-map color, and the four packaging methods resulted in the loss of 11 major flavor components in star anise as storage time extended. With the increase in temperature and humidity, especially in the later stages of storage, the color in **Figure 4** became lighter, indicating that the loss of volatile compounds was more obvious. Further significance analysis showed that the four different packaging methods had no significant differences ($p > 0.05$) in the relative content of 4-terpineol and (S)-β-bisabolene during the whole storage process, while there was a significant difference ($p < 0.05$) for the other flavor substances. Overall, the samples packaged using the TSB and LSB methods were significantly higher in flavor substances than those of WB and TPB samples. ANOVA revealed that storage time had an extremely significant effect ($p < 0.01$) on the relative content of 4-terpineol and (S)-β-bisabolene, but no significant difference ($p > 0.05$) was found between the different packaging methods. For the relative content of trans-anethole, storage time and packaging method both had extremely significant effects ($p < 0.01$). The other substances were significantly affected by storage time ($p < 0.01$), and packaging method had a significant effect ($p < 0.05$) on them.

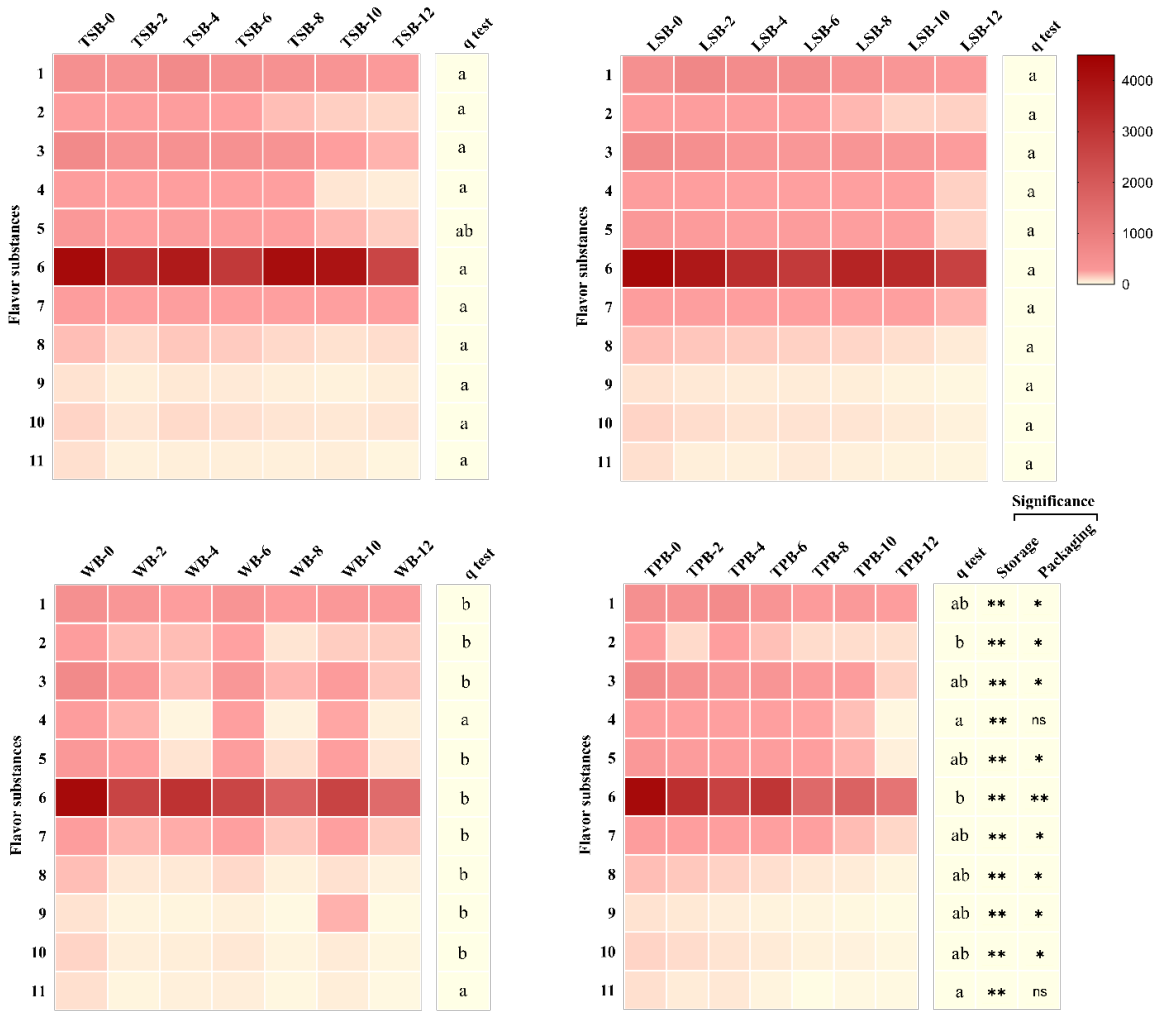

Figure 4. Heat maps comparing changes in flavor substances of star anise with storage time under different packaging methods. Note: c: control sample of freshly dried star anise. The number given after the *method* indicates the storage time, e.g., LSB-2 represents the LSB sample after two months of storage. 1: (+)-limonene, CAS/5989-28-5; 2: α-pinene, CAS/80-56-8; 3: linalool, CAS/78-70-6; 4: 4-terpineol, CAS/562-74-3; 5: estragole, CAS/140-67-0; 6: trans-anethol, CAS/104-46-1; 7: α-bergamotene, CAS/17699-05-7; 8: β-caryophyllene, CAS/87-44-5; 9: cis-β-farnesene, CAS/28973-97-9; 10: β-sesquiphellandrene, CAS/20307-83-9; 11: (S)-β-bisabolene, CAS/495-61-4; a: relative amount compared with octanol (100 μL, 4 mg/mL); ** indicates significance difference ($p < 0.01$); * indicates significance difference ($p < 0.05$); ns indicates no significance difference ($p > 0.05$); lowercase letter indicates significance difference of comprehensive effect of different packaging methods on relative content of flavor substances in the same sample ($p > 0.05$).

The result of the PCA of the flavor substances of star anise packaged using different methods at different storage times is shown in **Figure 5A** and **Figure 5B**. We determined two principal components, PC1 (81.9%) and PC2 (5.8%), which could explain approximately 87.7% of the variation in the 11 flavor components and the original information about them. This indicated that it was feasible to use the two principal components to evaluate the changes in the flavor substances of star anise with storage time under different packaging methods. From **Figure 5A**, the score scatter plot can well separate the relative contents of the 11 volatile substances of star anise with the change in storage time under different packaging methods. The greater the PC1 positive-axis value, the closer it is to the control sample (C), indicating better retention of volatile substances in samples. Star anise samples packaged using the TSB and LSB methods were largely dispersed on the positive side of PC1, while the others were mostly spread on the negative side. **Figure 5B** shows that the 11 substances were distributed on the positive side of PC1, which means that they were positively correlated with PC1. Among them, (+)-limonene, α-pinene, 4-terpineol, estragole, α-bergamotene, β-caryophyllene, cis-β-farnesene, and β-

sesquiphellandrene had large loads in PC1. Trans-anethol, β-caryophyllene, cis-β-farnesene, β-sesquiphellandrene and (S)-β-bisabolene were negatively correlated with PC2, but the other six substances were positively correlated with PC2, which were (+)-limonene, α-pinene, linalool, 4-terpineol, estragole, and α-bergamotene. Among them, α-pinene and trans-anethol had a large load on PC2.

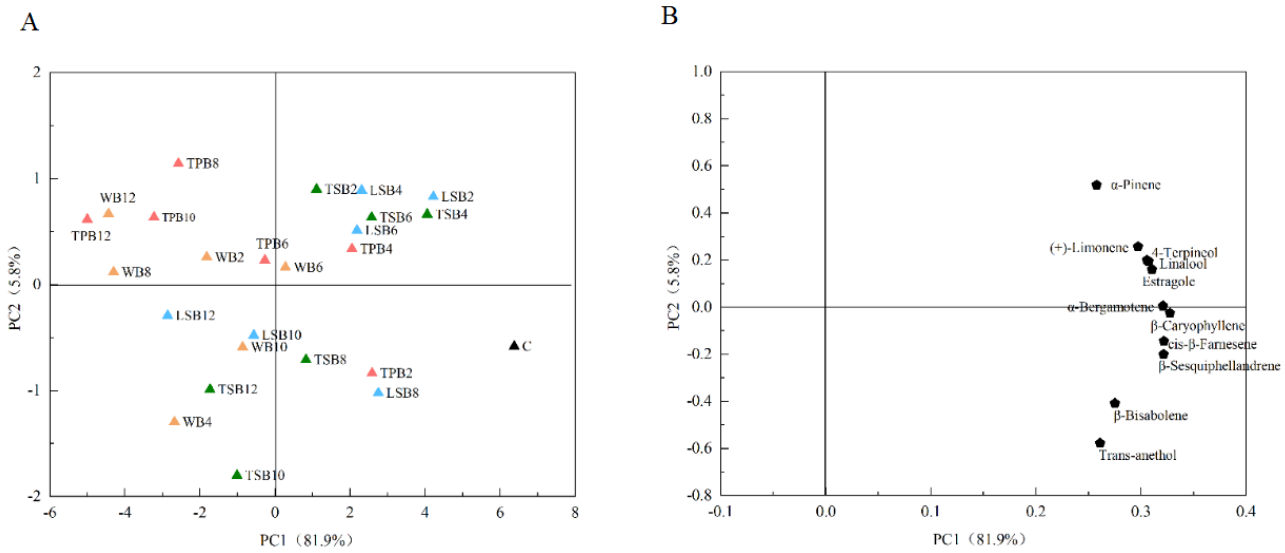

Figure 5. PCA plots of flavor substances for star anise samples packaged using different methods at different storage times: **A**) PCA score scatter plot and **B**) PCA loading scatter plot.

Furthermore, we calculated the evaluation model, which was F = 0.934F1 + 0.066F2. Then, we calculated the principal component score of the flavor substances of the samples at different storage times under different packaging methods, namely the comprehensive score, as shown in **Table 3**. The result obtained from the PCA scores indicates that the comprehensive score of the star anise samples' main flavor compounds gradually decreased with the extension of storage time. When the storage time reached 12 months, the scores of the TSB, LSB, WB, and TPB packaging methods were −1.68, −2.69, −4.10, and −4.63, respectively. The two sealed packaging methods (TSB and LSB) better retained the main flavor substances of star anise. Based on the scores of all storage periods, the descending order of the scores is LSB > TSB > TPB > WB. In summary, the relative content of the main flavor components of star anise progressively decreased over time at room temperature, regardless of the packaging method used. The loss was more severe as the ambient temperature and humidity increased. Among the four packaging techniques, the methods of sealed packaging were better at keeping the taste components in the samples, and the LSB method was the best.

Table 3. PCA scores of different samples.

Sample	Principal component scores		Comprehensive score	Samples rank	Total score	Rank
	F1	F2				
C	6.38	−0.58	5.92	1	5.92	-
TSB-2	1.11	0.90	1.10	10		
TSB-4	4.06	0.66	3.83	3		
TSB-6	2.57	0.63	2.45	5	5.35	2
TSB-8	0.82	−0.71	0.72	11		
TSB-10	−1.0	−1.80	−1.06	16		

Table 3. (*Continued*).

Sample	Principal component scores		Comprehensive score	Samples rank	Total score	Rank
	F1	F2				
TSB-12	−1.73	−0.99	−1.68	18		
LSB-2	4.22	0.83	4.00	2	7.55	1
LSB-4	2.31	0.89	2.22	7		
LSB-6	2.19	0.51	2.08	8		
LSB-8	2.76	−1.02	2.51	4		
LSB-10	−0.56	−0.48	−0.56	14		
LSB-12	−2.86	−0.29	−2.69	21		
WB-2	−1.82	0.26	−1.68	17	–12.95	4
WB-4	−2.68	−1.30	−2.59	20		
WB-6	0.28	0.16	0.27	12		
WB-8	−4.30	0.12	−4.01	23		
WB-10	−0.86	−0.59	−0.84	15		
WB-12	−4.44	0.67	−4.10	24		
TPB-2	2.59	−0.84	2.36	6	–5.86	3
TPB-4	2.05	0.34	1.94	9		
TPB-6	−0.27	0.23	−0.24	13		
TPB-8	−2.57	1.14	−2.33	19		
TPB-10	−3.22	0.64	−2.97	22		
TPB-12	−5.00	0.62	−4.63	25		

3.6. Hierarchical clustering analysis

To more thoroughly assess the differences in star anise quality across different packaging techniques as the storage period increased, HCA was performed on star anise flavor and physicochemical variables to determine the comparability of the various samples. As in Yildiz et al.[40], the PC scores of the flavor substances and the other physicochemical indicator data were used for the HCA, and the result is shown in **Figure 6**. As **Figure 6A** shows, the samples could be obviously classified into three groups: earlier storage period, middle storage period, and later storage period. This shows that the physicochemical variables were closely related to the storage time. In the group of the middle storage period, the samples packaged using the LSB and TSB methods and stored for up to 4–10 months showed similar properties in terms of being close to each other in the dendrogram. A deeper look at this region indicates that this was due to both the LSB and TSB packaging methods being able to preserve the effective substances and color of star anise. The samples packaged using the LSB and TSB methods showed lower quality losses with storage time, notably, the samples packaged using the TSB method and stored for up to 8 months and the samples packaged using the LSB method and stored for up to 10 months, as compared with WB and TPB samples. In terms of the flavor of star anise (**Figure 6B**), three clusters were established. The samples packaged using the LSB method and stored for up to 4–8 months were in the same cluster as the control group. These results certainly reveal that star anise samples packaged using the LSB method were more similar to the control sample than those of the other methods. This is a clear indicator that the LSB method of storage best preserves the flavor compounds of star anise.

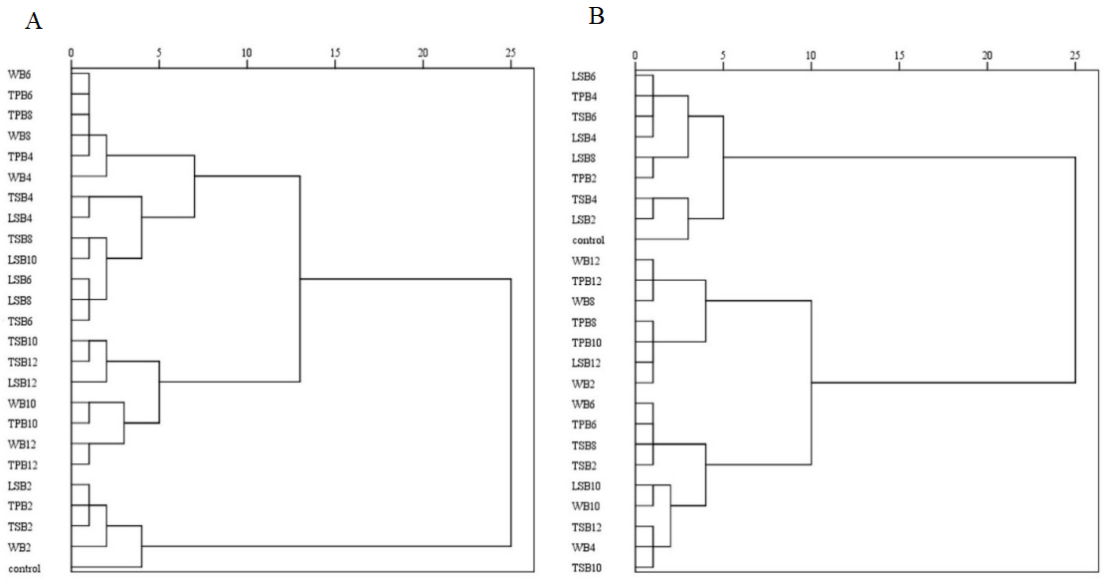

Figure 6. Dendrograms of hierarchical clustering analysis for classification of star anise stored at room temperature for 12 months under different packaging methods: **A)** physicochemical indicator data used for HCA and **B)** PC scores of flavor substance data used for HCA.
Note: Control: freshly dried star anise or standard star anise; The number given after the *method* indicates the storage time, e.g., LSB2 represents the LSB sample a*fter two months of storage.*

3.7. Determination of mycotoxins

The linear equations and correlation coefficients of the AFT and OTA standard curves are shown in **Table 4**. According to the detection result of these two types of mycotoxins for star anise stored at room temperature with ventilation in an independent environment for 12 months, no AFT (AFT-B1, AFT-B2, AFT-G1, and AFT-G2) or OTA were infected. It seems possible that this was due to the fact that the fresh star anise samples were washed before drying and then dried using a mechanical heat pump, and so the probability of fungal contamination as in the traditional drying process was greatly reduced. Furthermore, the process of storage was different from the mixed storage of various foods in markets. The star anise samples were stored separately in the experiment, which could also prevent the contamination of the mycotoxins. Thus, the star anise storage environment is of great significance in preventing mycotoxin contamination.

Table 4. Linear equations and correlation coefficients of AFT and OTA standard curves.

Name	Regression equation	R^2	LOD (ng/mL)	LOQ (ng/mL)
AFT-G1	y = 0.0706x − 0.5752	0.9999	0.004	0.012
AFT-G2	y = 0.0142x + 0.1051	0.9981	0.021	0.069
AFT-B1	y = 0.1555x − 1.4511	0.9998	0.002	0.008
AFT-B2	y = 0.0431x + 0.0759	0.9992	0.010	0.033
OTA	y = 1717.8x − 21.649	0.9960	0.001	0.004

Note: LOD—Limit of detection; LOQ—Limit of quantitation.

4. Conclusion

This study has shown that the moisture content, color, VO content, TA-O content, SA content, and the main flavor components of star anise significantly decreased as storage time increased. When stored at room temperature, the dried star anise samples packaged using different methods exhibited significant differences in the investigated indicators, except for the SA content. The TSB method better promoted the color formation of high-quality star anise, while the LSB method not only better reduced the loss of the VO content in star

anise but also better retained the TO-A, SA, and flavor substances. Combined with clustering analysis, the results show that it is advisable to use sealed packaging to ensure the quality of star anise, and light avoidance is preferable (as in LSB). In addition, star anise should be kept separate from other odorous, perishable items and should not be stored with them.

Supplementary materials

Table S1. Changes in temperature and humidity over time during storage.

Storage time (M)	Temperature			Humidity		
	Max	Min	Mean	Max	Min	Mean
0–3	19.50	13.40	16.15 ± 1.19^{c}	73.90	53.60	66.52 ± 4.98^{d}
3–6	16.80	11.60	13.57 ± 1.40^{d}	74.30	58.30	68.82 ± 3.46^{c}
6–9	27.90	15.00	23.24 ± 3.99^{b}	84.70	60.70	72.64 ± 6.84^{b}
9–12	29.70	23.00	26.55 ± 1.66^{a}	86.90	56.90	78.39 ± 7.08^{a}

Note: Lowercase letter represents significant difference between temperature or humidity ($p < 0.05$).

Table S2. Linear equations and correlation coefficients of AF and OTA standard curves.

Name	Regression equation	Correlation coefficient/R^2	LOD (ng/mL)	LOQ (ng/mL)
AF–G1	y = 0.0706x − 0.5752	0.9999	0.004	0.012
AF–G2	y = 0.0142x + 0.1051	0.9981	0.021	0.069
AF–B1	y = 0.1555x − 1.4511	0.9998	0.002	0.008
AF–B2	y = 0.0431x + 0.0759	0.9992	0.010	0.033
OTA	y = 1717.8x − 21.649	0.9960	0.001	0.004

Note: LOD—Limit of detection, LOQ—Limit of quantitation.

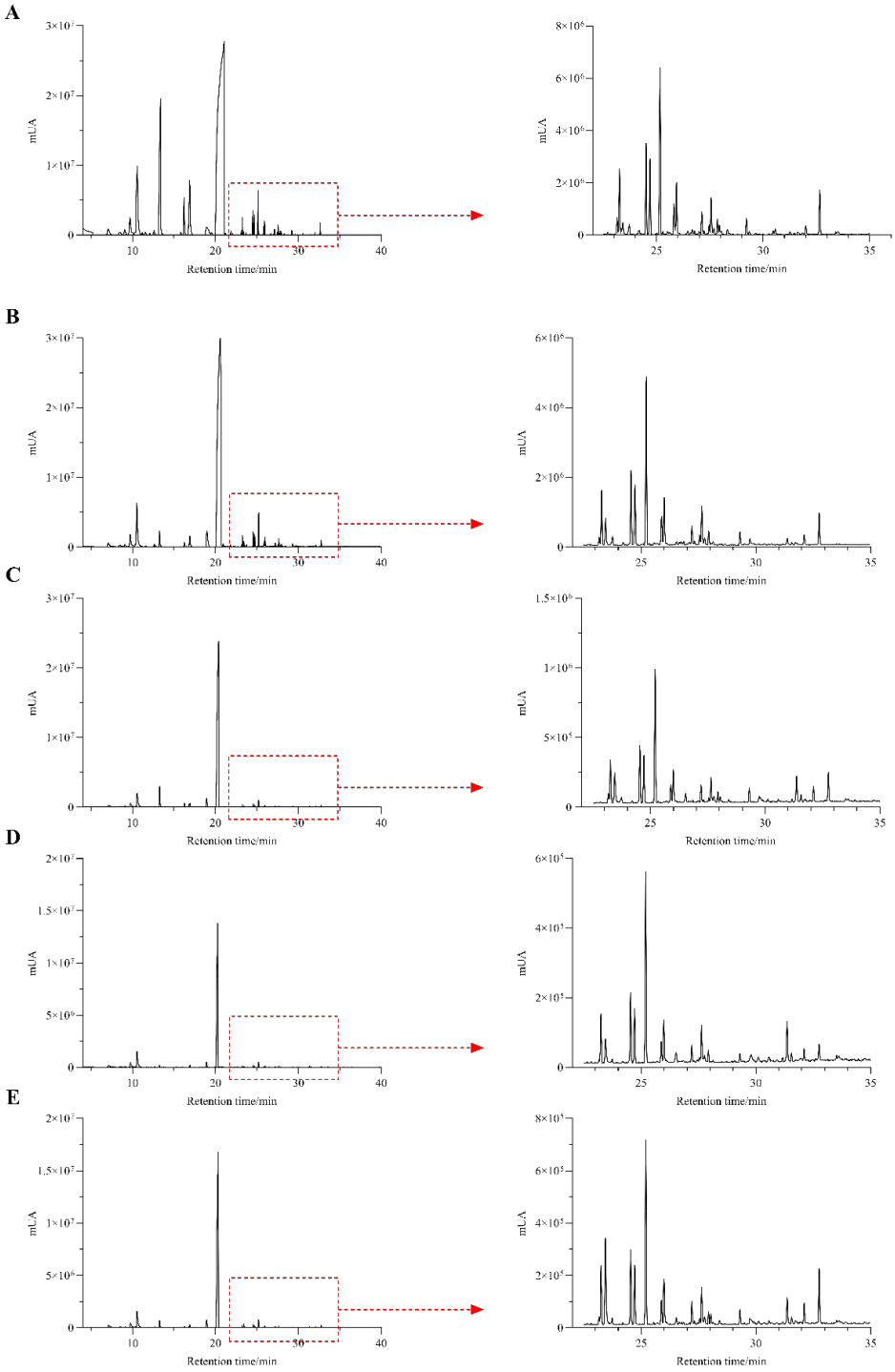

Figure S1. GC-MS total ion diagrams of flavor components of star anise under different packaging methods. Note: **A**: control sample of freshly dried star anise; **B–E**: star anise packaged using TSB, LSB, WB, and TPB for 12 months, respectively.

Author contributions

Investigation, YS, ZW, XC, KC and PH; data curation, JK, ZW, XC, KC and PH; formal analysis, YS, JK; methodology, SY and JK; software, SY and XC; writing—original draft, SY; writing—review and editing, SY and ZW; supervision, SY, XC and JK; funding acquisition, JK; resources, JK; project administration, JK; validation, ZW. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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