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Effects of CO_2 pretreatment on the volatile compounds of dried Chinese jujube (*Zizyphus jujuba* Miller)

Kai CHEN[1], Lei GAO[1], Qiong LI[1], Huan-Rong LI[1]*, Yanyan ZHANG[2]

Abstract

The aim of this study was to investigate whether anaerobic metabolites could induce volatile compounds and improve aroma of dried jujube (*Ziziphus jujuba* Miller). Jujube fruits were incubated in a polyvinyl chloride bag containing 5% CO_2 and 95% N_2 for up to 168 h at 25 °C and 3 samples were randomly removed every 6 h and oven dried to a moisture content of ≅ 20%. The volatile compounds of control and 5% CO_2-pretreated Chinese jujube fruits were extracted by simultaneous distillation extraction and identified by gas chromatography-mass spectrometry(GC-MS). The acetaldehyde and ethanol contents were determined by gas chromatography (GC). The results indicated that a large accumulation of acetaldehyde and ethanol caused changes in aroma composition of dried jujube products and 5% CO_2 pretreatment led to an increase in the levels of some compounds, particularly esters, acetaldehydes, and ethanol, whereas the amount of acids were decreased significantly. Principal component analysis showed that integrative scores of 5% CO_2 pretreatment at 120 h were the highest, and aroma quality was better than that of the control. Relatively low concentrations of anaerobic respiration metabolites are good for jujube fruit aroma composition.

Keywords: simultaneous distillation extraction; anaerobic metabolites; volatile compounds; dried jujube; PCA.

Practical Application: Control of anaerobic respiration induces volatile compounds that can improve the aroma.

1 Introduction

The jujube is mainly distributed in the subtropical and tropical regions of China and America (Wu et al., 2012). It is commonly used in folklore medicine to cure various diseases (Chen et al., 2015). Chinese jujube (*Zizyphus jujuba Miller*) is indigenous to China and has a history spanning over 4000 years (Li et al., 2009). In recent years, the yearly output of fresh Chinese jujube has increased significantly (Jin et al., 2015). According to the Xinjiang Statistical Yearbook 2015, there was a total planting area of 483,628 hectares and yearly production of >2.5 million tons. Fruit rot after harvesting has always been a serious problem for Chinese jujube, leading to a short shelf life, inferior quality, and poor economic benefit. As a result, processing of its products is necessary (Okutsu et al., 2007; Kamiloglu et al., 2009; Zozio et al., 2014; Bao et al., 2014). Hot-air drying of jujube is an efficient and popular method for preservation and further processing of the fruits (Fang et al., 2009; Ding et al., 2012; Lewicki, 2006; Zhang et al., 2015; Boudhrioua et al., 2003).

Volatile compounds are important constituents of natural products and play an important role in fruit quality (Lu et al., 2013). Anaerobic conditions surrounding jujubes affect the quality, particularly the production of aroma in the fruit during the post-harvest period. Production of the two anaerobic metabolites or anaerobic conditions induces certain aroma volatile that may enhance the flavor quality of jujube fruits (Golias et al., 2010; Lurie & Pesis, 1992; Wendakoon et al., 2006; Pesis, 2005). Therefore, the application of anaerobic metabolites may be beneficial for post-harvest fruit quality through increasing the relative amount of aromatic components.

Gas chromatography-mass spectrometry (GC-MS) is an effective way to determine the volatile components in flavoring agents. The repeatability of simultaneous distillation extraction (SDE) is greater than that of other methods (Wang et al., 2014; Hernández et al., 2016). Therefore, it is considered as the most reliable method for the quantitative analysis of volatile compounds (Cai et al., 2001). Other studies mainly report on the characteristics of the shape and type of species. However, little information is available about the effects of pretreatment before drying on the composition of volatile compounds in the Chinese jujube. In our previous studies, we found that bitter taste and aroma deficiency were the main disadvantages of hot-air drying when fresh jujube is dried in hot-air directly without any after-ripening pretreatment (Wei, 2011).

The objectives of this research were to investigate the effect of pretreatment with different anaerobic metabolites on the contents of ethanol, acetaldehyde and volatile compounds in fresh and subsequently dried jujube. The main aim of this study was to provide guidance on pretreatment before drying the Chinese jujube in terms of volatile compounds.

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[1] *School of Food Science and Pharmacy, Xinjiang Agriculture University, Urumqi, Xinjiang Uygur Autonomous Region, China*
[2] *School of Food and Biological Engineering, Zhengzhou University of Light Industry, Zhengzhou, Henan, China*
*Corresponding author: chenk117@sohu.com

2 Materials and methods

2.1 Materials

The full-red stage fruits of *Ziziphus jujube Mill*er were collected from the Hami district of Xinjiang Uygur Autonomous Region, China. The initial moisture content of fresh samples was 68 ± 0.44% (wb) and it was determined as described by (Fang et al., 2009). In practice, the moisture content of dried jujube is required to be <25% (wb). In our study, the final moisture content of dried jujube was about 20% (wb).

2.2 CO_2 pretreatment

Selected jujube weighing 0.2 kg was packed in a polyvinyl chloride bag (210 × 297 mm) containing 5% CO_2 and 95% N_2 by modified atmosphere packaging machine (multi-functional modified atmosphere packaging Machine,DQB-360W,Shang Hai Qing Pa Food Packaging Machine Co., Ltd, China). The 5% CO_2 concentration was based on an earlier study from our laboratory that found 5% CO_2 was the optimum concentration to induce metabolic changes in jujube during storage (Zhang et al., 2013). In total, 84 bags (16.8 kg) were stored at a temperature of 25 ± 2 °C for 168 h, removing 0.6 kg every 6 h (three bags). Selected jujube weighing 4.2 kg were randomly unpackaged and kept at 25 ± 2 °C for 168 h, removing 0.6 kg every 24 h as control samples. Then, jujube sample was oven-dried at 45 °C for 96 h (the moisture content of dried jujube was ≅20% wb).

2.3 Determination of acetaldehyde and ethanol content

The content of acetaldehyde and ethanol was determined by GC (Zhang et al., 2012). Slices of jujube (20 g) were homogenized in a homogenize at 6000 rpm for 5 min with ultrapure water (50 mL) at room temperature and placed into volumetric flasks (100 mL) with ultrapure water. After 10 min, the aqueous extracts were filtered under suction through Whatman No. 4 filter paper. A 5 mL filtered sample was added into a 30 mL headspace vial and sealed with a PTFE-faced silicone septum (Supelco, USA). The vial was left at 60 °C in a water-bath for 30 min to equilibrate its headspace. A 1 mL gas sample was withdrawn from vial by using a plastic hypodermic syringe through a silicone septum and analyzed by gas chromatography, with a flame-ionization detector (FID) and a Capillary column (GSBP-LNOWAX-MS, 30 m × 0.25 mm × 0.25μm, USA). The acetaldehyde and ethanol contents of jujube fruit were expressed according to the following Equation 1 and 2:

$$Acetaldehyde\ content\ (\%) = \frac{c \times v \times 0.783}{m} \times 100 \quad (1)$$

$$Ethanol\ content\ (\%) = \frac{c \times v \times 0.789}{m} \times 100 \quad (2)$$

where C is concentration of acetaldehyde or ethanol in extracting solution (%), v is constant volume (mL), m is sample weight (g), 0.783 is relative density of acetaldehyde (g/mL), 0.789 is relative density of ethanol.

Acetaldehyde and ethanol were detected using a flame-ionization detector at 200 °C, 200 °C injection temperature, and 45 °C oven temperature, N_2 as carrier gas, flow rate is 25 mL/min.

2.4 Volatiles analysis

Isolation of volatile compounds

SDE was used for the extraction of volatiles from Chinese jujube. Samples (150 g) after crushing were blended with ultra-pure water (150 mL) and then extracted for 2 h using Likens-Nickerson apparatus with dichloromethane (50 mL) as the extraction solvent. At the end of extraction, the dichloromethane was dried over anhydrous Na_2SO_4, concentrated to ca. 2 mL in volume by rotary evaporation at a temperature of 38 °C, and stored in a sealed vial at 4 °C for GC-MS analysis.

GC-MS analysis

Quantitative and qualitative analysis of volatile compounds were performed using a GC-MS system (Trace, Thermo Fisher ITQ900, Waltham, MA, USA) equipped with a HP-20MS capillary column (25 m × 0.2 mm × 0.1 μm). Helium gas was used as a carrier gas, the flow rate was 1 mL/min and the split ratio was 20:1. An initial oven temperature of 40 °C was maintained for 4 min, then raised to 180 °C at a rate of 5 °C/min for 2 min, then increased to 200 °C at a rate of 2 °C/min for 2 min, then raised to 260 °C at a rate of 3 °C/min and held for 20 min. The injection volume was 0.5 μl. The MS was operated in EI mode at 70 eV, Temperature at injection port: 220 °C, the source temperature was kept at 200 °C, and the temperature of the GC-MS transfer line was 280 °C. The mass acquisition range was 33-650 amu.

Qualitative and quantitative analysis

From the total ion chromatogram, peaks with the degrees of matching and reverse matching >800 (maximum: 1,000) based on NIST/Wiley Libraries were selected and identified according to relative molecular masses, chemical formulas and molecular structures. The percentage of each component was calculated by normalizing the corresponding peak area.

2.5 Statistical analysis

All statistical analyses were performed using SPSS Base 19.0 (IBM Corporation, NY, USA). The graphs were created in OriginPro 8.5 (OriginLab Corporation, Northampton, MA, USA). The average value of aroma compound content in different samples was evaluated using principal component analysis (PCA) with maximum variation rotation.

3 Results and discussion

3.1 The effects of 5% CO_2 pretreatment on acetaldehyde and ethanol content

As fruits age, the ability of cells to carry out oxygen transmission is reduced, leading to the occurrence of anaerobic respiration in jujube. During anaerobic respiration, the pyruvate generated from glycolysis is unable to enter the Krebs cycle. Pyruvate is instead decarboxylated into acetaldehyde and reduced to ethanol, Acetaldehyde and ethanol are precursors in aroma synthesis, and accelerate the synthesis of ester and acids (Gao et al., 2006). Therefore, we defined acetaldehyde and ethanol contents as indicators for the degree of anaerobic respiration being performed.

The variation in the content of acetaldehyde and ethanol in fresh Chinese jujube following pretreatment with 5% CO_2 for 168 h and control samples is shown in Figure 1.

The changes in acetaldehyde and ethanol content of fresh jujube without CO_2 pretreatment are showed (Figure 1a). The acetaldehyde content increased with time and the highest value was 0.0076%. The ethanol content also increased with time and reached the highest value of 0.0217% at 72 h and then decreased. The changes in acetaldehyde and ethanol content of fresh jujube with 5% CO_2 pretreatment are showed in Figure 1b. The acetaldehyde content increased rapidly in the first 84 h, and began to fluctuate after this time. The highest value was 0.0290% and occurred at 162 h. The ethanol content changed slightly in the first 90 h, and increased sharply after that. The highest value of 0.1824% was obtained at 162 h.

The acetaldehyde and ethanol content of dried jujube without pretreatment remained at a relatively low level (acetaldehyde: 0.0010-0.0056%, ethanol: 0.0007-0.0044%) (Figure 1c). The acetaldehyde and ethanol contents of dried jujube with pretreatment remained at a high level (acetaldehyde: 0.0010-0.0230%, ethanol: 0.0007-0.0099%), particularly for samples pretreated for >90 h (Figure 1d).

It was previously suggested that the accumulation of acetaldehyde and ethanol before drying is the main cause of flavor perception after drying as these two products of anaerobic metabolites are considered as jujube aroma compounds that have a large effect on the flavor of dried jujube (Lurie & Pesis, 1992).

3.2 The effects of different pretreatment on aromatic composition of Chinese jujube after drying

A total of 89 volatile compounds were identified in control samples and the main aroma compounds were (Z)-7-tetradecenoic acid, tetradecenoic acid, lauric acid, n-decanoic acid, 3-furaldehyde

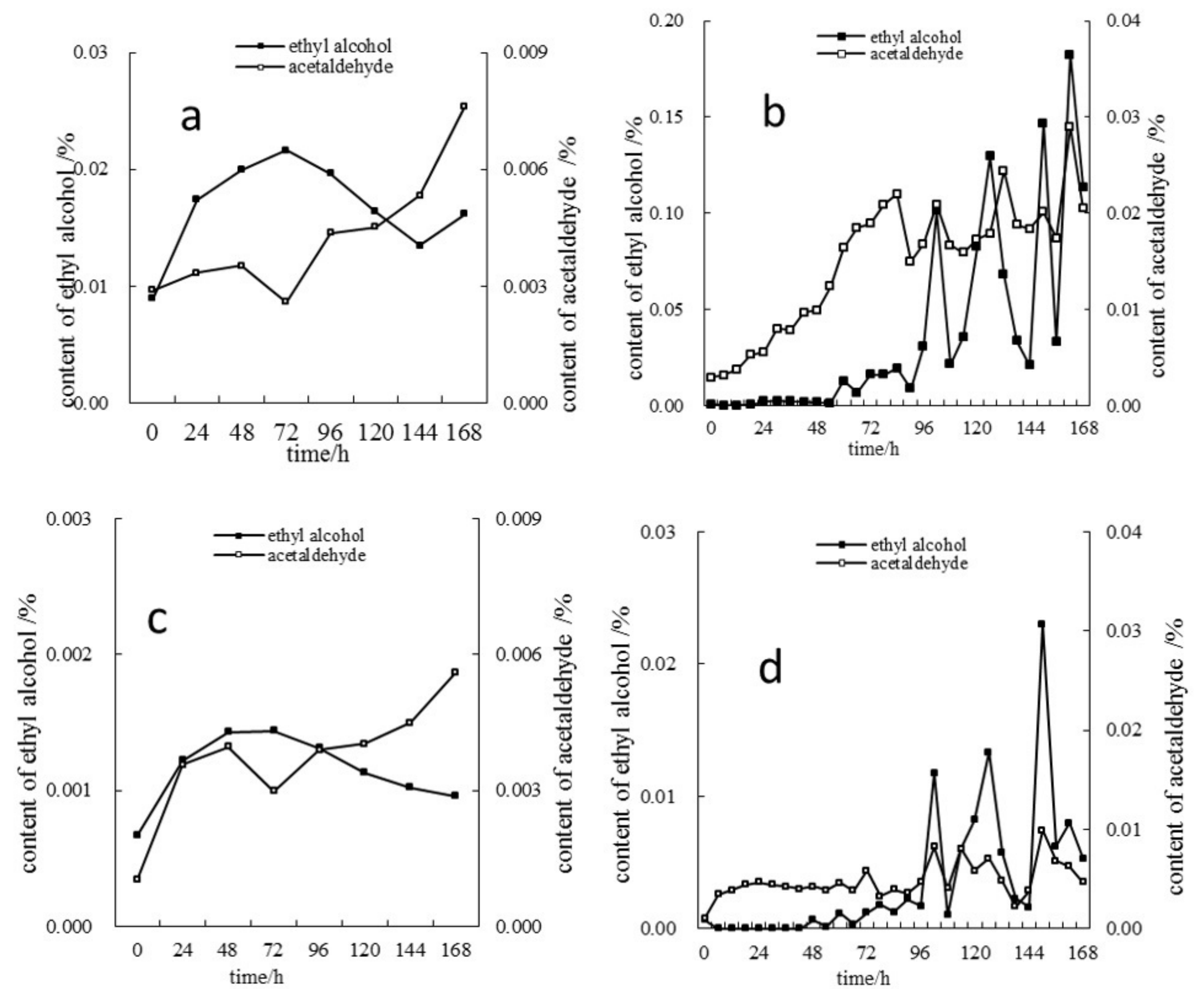

Figure 1. The changes of alcohol and acetaldehyde content. (a) control; (b) 5% CO_2 pretreatment jujube; (c) control after drying jujube; (d) 5% CO_2 pretreatment after dried jujube.

and ethyl 9-hexadecenoate. For samples pretreated with 5% CO_2, according to the change rule of ethanol content, 10 samples were determined and 112 types of volatile compound were identified. The main aroma compounds were 3-furaldehyde, n-decanoic acid, lauric acid, myristic acid, cis-9-hexadecenoic acid, nutmeg acid methyl ester, ethyl 9-tetradecenoate, methyl hexadec-9-enoate, ethyl 9-hexadecenoate, phthalic acid and 3,6-dimethyl-2-ethylhexyl ester.

Esters

A total of 25 types of ester compound were detected in control samples, including 9 types of methyl ester compound and 7 types of ethyl ester compound. In control samples, during the first 4 days of treatment, the levels of methyl ester compounds decreased and the levels of ethyl ester compounds increased.

In samples pretreated with 5% CO_2, a total of 38 ester compounds were identified, including 15 types of methyl ester compound and 10 types of ethyl ester compound. With the extension of pretreatment time, the number of ester compound types and their levels increased in varying degrees. The level of methyl ester compounds increased within 120 h (from 4.829% to 16.856%), and the level of ethyl ester compounds increased from 4.918% to 40.894%. Thus, after pretreatment with the appropriate amount of 5% CO_2 during sample drying, the relative percentage and number of esters contributing to the aroma of samples increased significantly compared with those of the control sample. The main increase in content and number of types identified was observed for the ethyl ester compounds. The increase in ethyl ester content was due to the higher ethanol content, which was caused by anaerobic respiration (Grant et al., 1992). After drying, esters were not affected despite the changes of aroma compounds.

Acids

In the control samples of dried product, 20 types of acid compound were detected, including n-decanoic acid, lauric acid, (Z)-7-tetradecenoic acid and tetradecenoic acid. In total, 10 types of saturated fatty acid and 7 types of unsaturated fatty acid were detected. The relative percentage of content of acid compounds increased slightly during the process, particularly n-decanoic acid content.

For samples pretreated with 5% CO_2, a total of 23 types of acid were detected, including 13 types of saturated fatty acid and 8 types of unsaturated fatty acid. Within 72 h, the content of acid compounds in the treatment group decreased significantly, then maintained at about 40%, and began to decline after 114 h. The relative content changed from 67.302% to 18.739% in 126 h. N-decanoic acid content in the dried jujube did not change and the content of lauric acid and nutmeg acid decreased, particularly after 114 h. Therefore, compared to 5% CO_2-pretreatment, acid content of the control sample (from 45.093% to 0.461%) decreased significantly during the whole drying time, which was mainly caused by a decrease in lauric acid and nutmeg acid content.

Aldehydes and alcohols

In the control samples of dried product, 10 types of alcohol and 5 types of aldehyde were detected. In addition, the content of alcohol increased during 5% CO_2 pretreatment.

In total, 10 samples were selected from 5% CO_2-pretreated jujubes, in which 11 types of alcohol and 7 types of aldehyde were detected. The C_4-C_6 alcohols smell approximately similar to anesthetic compounds. The content of four C_6 alcohols detected in the experiment increased after treatment for 120 h, which is most likely associated with the bitter spicy taste of late processing dried jujube. The C_7 alcohol had an odor. Thus, as pretreatment time is extended, the relative content of alcohol increases, which contributes to jujube aroma. E-11,13-Tetradecadienal and (Z,Z)-10,12-hexadecadienal have an odor, with both increasing in content within 48 h, rising to around 24% until 120 h, up to 40%. This finding illustrated that after 5% CO_2 pretreatment, the quality of aroma from the dried jujube decreased after 120 h.

Ketone, alkene, and alkanes

In the control samples of dried product, 6 types of ketone, 3 types of alkene and 14 types of alkane were detected. In contrast, in the 5% CO_2 pretreatment samples, 9 types of ketone, 5 types of alkene and 11 types of alkane were detected. Alkane compounds have a much weaker contribution to the aroma than ketones and alkenes.

Other compounds

Other compounds detected mainly included 3 amine compounds, 1 phenol, and 2 types of naphthalene. Most of the amine compounds had a stench, while phenolic compounds provided aroma.

With differing lengths of treatment time, the content of all types of aroma compounds were changed. The extension of pretreatment time led to a slight decline in acid content of the control samples within 72 h, then increased slowly until it reached 15.76%. The content of esters increased sharply in 72 h, and then started to decrease down to 27.91%. Within 48 h, the content of aldehydes increased slightly, and was then maintained at a relatively stable level. Ketone content increased slightly after 96 h (Figure 2a). The relative content of alkenes, alcohols, alkanes, and other compounds had little change, with only slight increases during late processing (Figure 2b).

The changes in ester and acid content of 5% CO_2-pretreated samples were more marked (Figure 3a). Within 72 h, ester content increased markedly and after 72 h, it increased more steadily, which similar results were found by Yan et al. (2011).With the extension of time, acid content decreased gradually. Alkane and alcohol content increased slightly during late processing and the content of ketones and alkenes remained almost unchanged (Figure 3b).

The results indicated that different lengths of 5% CO_2 pretreatment lead to different trends in the content of alcohol, acid, aldehyde, and ester compounds. Compared with the control sample, the content of acids decreased, aldehyde and esters content increased, and ketones reached peak levels.

3.3 Evaluation of flavor quality on dried Chinese jujube with CO_2 pretreatment

Table 1 shows the relative content of flavor composition in dried Chinese jujube after pretreatment with 5% CO_2. Esters, alcohols, aldehydes, acids, ketones, alkenes, and alkanes were the major constituents.

As outlined in Table 2, the three principal components of the PCA model account for 87.392% (>85%) of the total variance among the Chinese jujube samples, which mainly reflects the correlation of Chinese jujube aroma compounds.

By the comprehensive score (Table 3), samples that were pretreated with 5% CO_2 for 120 h demonstrated the highest score and jujube aroma quality was the best. The direct dried samples had the lowest score, which suggested the worst quality

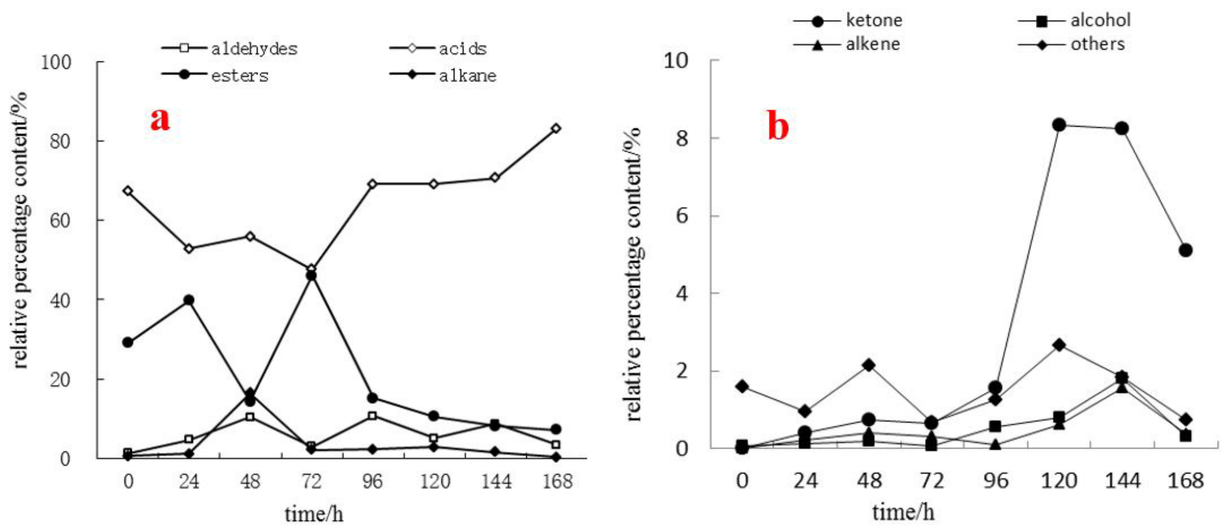

Figure 2. The changes of each aroma type and content of the control sample after drying. (a) the relative percent content of acid, aldehydes, esters and alkane; (b) the relative percent content of ketone, alkene, alcohol and others volatiles.

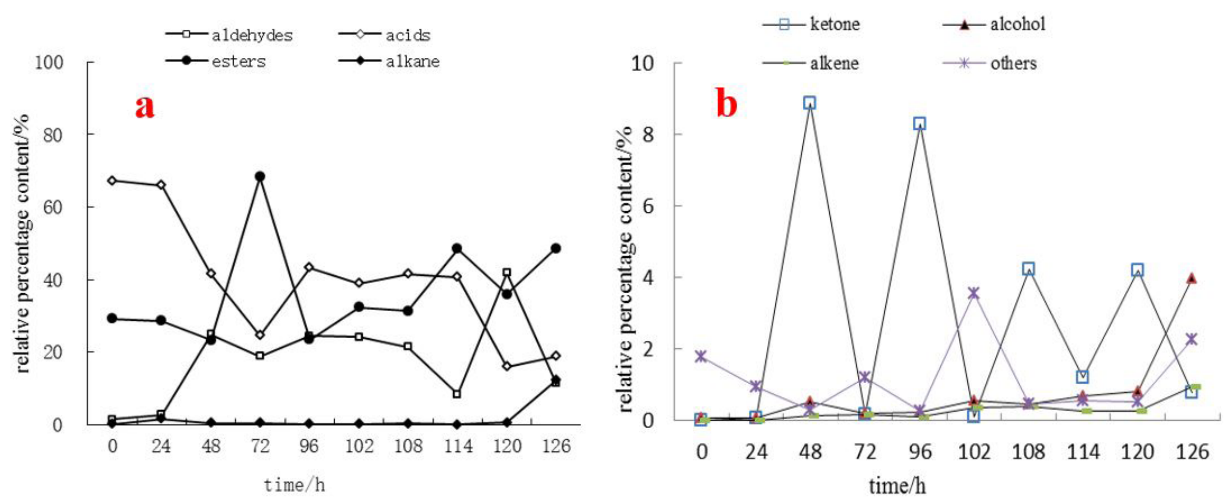

Figure 3. The changes of each aroma type and content of the 5% CO_2 sample after drying. (a) the relative percent content of acid, aldehydes, esters and alkane on 5% CO_2-pretreated sample; (b) the relative percent content of ketone, alkene, alcohol and others on 5% CO_2-pretreated sample.

Table 1. Relative content of aroma composition in dried Chinese jujube after 5% CO_2 pretreated.

aroma component	Relative content %									
	00h	24h	48h	72h	96h	102h	108h	114h	120h	126h
aldehydes	1.350	2.632	24.843	18.857	24.330	24.041	21.361	8.250	41.766	11.523
ketone	-	0.063	8.895	0.180	8.286	0.113	4.248	1.208	4.195	0.786
acids	67.302	65.887	41.501	24.480	43.293	38.964	41.570	40.616	15.965	18.739
alcohols	0.063	0.056	0.530	0.197	0.242	0.547	0.463	0.683	0.810	3.991
esters	29.082	28.505	23.271	68.222	23.387	32.245	31.274	48.440	35.886	48.539
alkane	0.092	1.581	0.422	0.376	0.085	0.168	0.233	-	0.573	12.198
alkene	-	-	0.147	0.178	0.113	0.354	0.380	0.256	0.271	0.947
others	1.795	0.931	0.297	1.190	0.266	3.567	0.471	0.564	0.534	2.265

Table 2. Eigenvalues of the principal components and their contribution and cumulative contribution.

Component	Eigenvalue	/% of Variance	Cumulative/%
1	3.656	45.700	45.700
2	2.163	27.032	72.732
3	1.173	14.660	87.392

Table 3. Principal component scores after standardization.

No	PC1 score	PC2 score	PC3 score	Integrative scores	Rank
00h	-1.165	-1.599	1.173	-1.590	10
24h	-1.091	-1.479	1.185	-1.385	9
48h	-0.646	0.585	0.672	0.611	3
72h	1.351	0.198	-1.943	-0.394	7
96h	-0.721	0.485	0.690	0.453	4
102h	-0.062	0.156	-0.009	0.085	6
108h	-0.330	0.204	0.246	0.120	5
114h	0.242	-0.545	-0.440	-0.742	8
120h	0.667	1.801	-0.902	1.566	1
126h	1.753	0.193	-0.670	1.277	2

of jujube. Therefore, postharvest Chinese jujube pretreated with 5% CO_2 and subsequently hot-air dried can effectively improve the quality of aroma.

4 Conclusions

Compared with the control jujube, pretreated with 5% CO_2 and subsequently dried produced, the content of acids decreased, aldehyde and esters content increased, and ketones reached peak levels. Via PCA analysis, jujube pretreated for 120 h with 5% CO_2 and subsequently dried produced the highest score and best aroma quality.

In conclusion, Pretreatment with 5% CO_2 is more desirable than directly drying the jujube as it induces the appropriate amount of anaerobic respiration, and subsequently makes the dried jujube have a stronger and richer flavor.

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