Food and Chemical Toxicology 134 (2019) 110833

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Exogenous bacterial composition changes dominate flavor deterioration of dried carrots during storage

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ARTICLE INFO

Keywords:
Dried carrots
Water status
Flavor deterioration
Bacteria composition
volatile compounds

ABSTRACT

Flavor deterioration is a serious problem in dried carrots during storage and is frequently accompanied by water absorption and bacterial growth. To explore the underlying mechanism of flavor deterioration, relationship among water status, exogenous bacterial composition and flavor changes in dried carrots were analyzed at different water activities (a_w, 0.43, 0.67, 0.76 and 0.84). Results suggested that the water molecules mobility significantly increased in the dried carrots at higher a_w levels (0.67, 0.76 and 0.84), this was attributed to the raised content of bound water, rather than immobilized or free water. Consequently, this accelerated microbial growth and flavor deterioration. At $a_w = 0.84$, the characteristic flavor compounds including 2,3-butanediol, pentanoic acid, hexanoic acid, heptanoic acid and nonanoic acid were lost. The disagreeable flavor compounds including terpenes were produced during the storage period. These were the main contributors of flavor deterioration in the dried carrots. Lactic acid bacteria, as the dominant bacteria in dried carrots during storage, were proved to be closely related to the production of o-cymene, β-pinene and β-myrcene. Moreover, the emergence of *Pediococcus* spp. was the major factor leading to the increase of γ-terpinene in dried carrots.

1. Introduction

Carrot is an important root vegetable rich in bioactive compounds (Melough et al., 2018). Processed carrot is widely consumed all over the world because of its highly desirable taste and special flavor and aroma. Drying is the most efficient method for long time preservation, convenient transportation and consumption (Murcia et al., 2009; Oliveira et al., 2015). Diced or sliced carrot is often dried to provide raw materials for food industry, especially for the instant food industry (Demiray and Tulek, 2015). Moreover, dried carrot slices or dices are the second popular dried vegetables used for snacks (Huang and Zhang, 2012). However, quality deterioration occurs to dried carrots because of moisture absorption during inappropriate storage conditions, including discoloration, off-flavors and loss of antioxidative properties. Several deteriorative reactions that cause these changes are influenced by storage conditions (Koca et al., 2007). An important aspect of concern in dried foods is their chemical stabilization and physical changes as well as microbial growth, which could occur during storage (Caparino et al., 2017; Miranda et al., 2014).

Discoloration and loss of characteristic flavor are the significant quality deterioration in dried carrots during storage. In turn, these greatly reduce consumers acceptability and commercial value of the dried carrots (Salehi, 2018). It is reported that moisture absorption of dried food materials, such as freeze-dried *Agaricus bisporus*, significantly promote growth of spoilage microorganism and flavor deterioration (Yang et al., 2019). Some spoilage microorganisms were contributed to the loss of some characteristic flavor compounds and to the production of off-flavor compounds (Rousseaux et al., 2014; Yang et al., 2019). However, information on flavor quality deterioration in dried carrot during storage, and the type of microorganisms causing the deterioration is still limited and unclear.

Here, we investigated the quality deterioration of dried carrots at different water activities, and analyzed the relationship among water status, bacterial composition and flavor changes during storage. This might provide strategies for maintaining better quality of dried carrots.

2. Materials and methods

2.1. Sampling

Dried carrots were purchased from Xinghua Dehydrated Foods Group Co., Ltd. (Taizhou, Jiangsu Province, China). Samples were

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https://doi.org/10.1016/j.fct.2019.110833
Received 3 August 2019; Received in revised form 17 September 2019; Accepted 20 September 2019
Available online 28 September 2019
0278-6915/

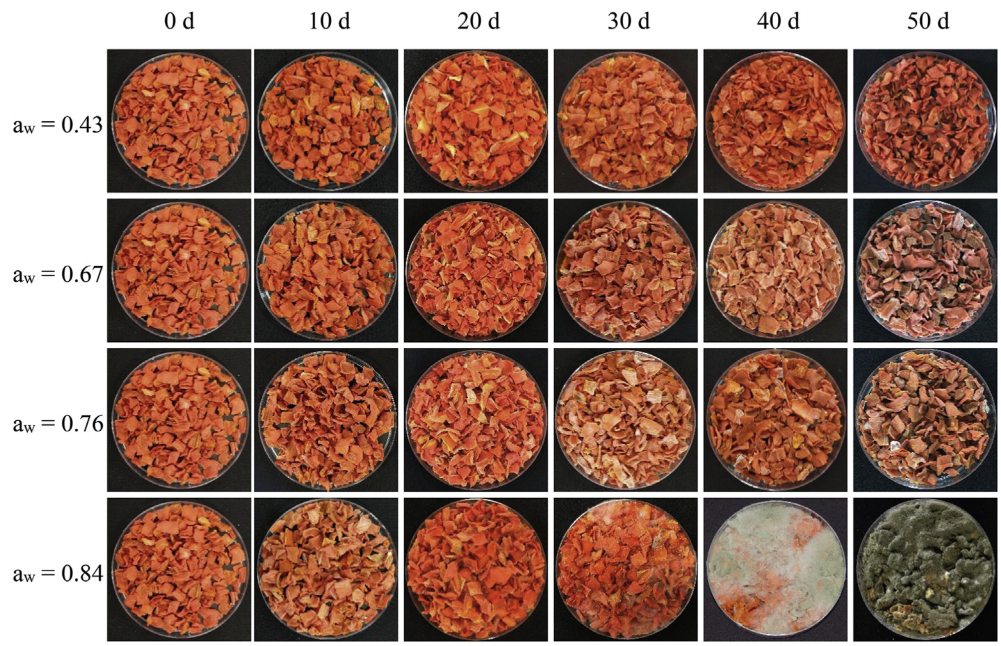

Fig. 1. Appearance changes of dried carrots at different a_w levels.

placed in sealed desiccators for 50-day storage period. Five saturated salt solutions ($MgCl_2$, K_2CO_3, $NaNO_2$, $NaCl_2$ and KCl) were used to achieve different relative humidity at 25 °C (0.33, 0.43, 0.67, 0.76 and 0.84, respectively) (Wang et al., 2013). Sample portions were taken out and analyzed every 10 days during storage period.

2.2. Moisture content and water status

Dried carrots (5 g) were taken from the desiccators and dried at 105 °C. Moisture content was calculated by using weight loss as a percentage of the initial weight. Low-field nuclear magnetic resonance (LF-NMR) was used to monitor water status and molecular mobility according to our previous study (Yang et al., 2017). Pulse parameters were set as follows: corresponding resonance frequency (SF) for protons: 19 MHz; spectral width (SW): 200 kHz; echo time (TE): 0.2 ms; pulse widths at 90° (P1) and 180° (P2): 12 and 24 ms, respectively; waiting time (TW): 3500 ms; number sampling (NS): 15; radio frequency delay time (RFD): 0.5 ms; analog gain (RG1): 20 db; and digital gain (DRG1): 3.

2.3. Total bacterial counts and sequencing libraries construction

Total bacterial counts in dried carrots at different a_w were monitored according to our previous study (Yang et al., 2019). Samples were homogenized and gradiently diluted (1:10) with phosphate buffer solution (10 mmol/L, pH = 7.2). The homogenate was used for the determination of total bacterial counts, which were determined using colony counting method.

Dried carrots at 0 and 50 d at different a_w were chosen for 16s rDNA sequencing analysis. Total DNA was extracted from the bacterium solution by the TIANamp Bacteria DNA kit (TIANGEN Biotech, Beijing, China) following the manufacturer's instruction (Mulla et al., 2018). The V4 region primer set was chosen to obtain the best coverage of most exogenous organisms. The 16S rDNA gene was amplified by polymerase chain reaction amplification of V4 library and sequencing.

Forward primer: 341F CCTACGGGNGGCWGCAG;

Reverse Primer: 805R ACTACHVGGGTATCTAATCC

The polymerase chain reaction program included one denaturing step at 94 °C for 2 min, 25 cycles of 94 °C for 20 s, 55 °C for 40 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min and 4 °C forever. Then, the 16S rDNA from the samples were examined with MiSeq sequencing by Genesky Biotechnology Inc. (Shanghai, China). High-throughput sequencing was performed on the Illumina Miseq platform with 2 × 250 bp paired-end method after the library was quantified. Operational taxonomic units (OTUs) were clustered with a similarity of 97% sequence similarity cut-off for total identified bacterial 16S rDNA sequences by UPARSE. Mothur was used for taxonomical assignments at 80% confidence level based on the Ribosomal Database Project database. Cluster analysis was performed by using the Bray-Curtis dissimilarity index and the unweighted pair-group method with arithmetic means (UPGMA) linkage method. Heatmap plot was used to depict the relative abundance of each bacterial family (variables clustering on the Y- axis) within each sample (X-axis clustering) (Xie et al., 2017). Metastats was used to identify the members within a community responsible for differences between communities (Fang et al., 2018).

2.4. Solid phase micro-extraction combined with gas chromatography–mass spectrometry (HS–SPME–GC–MS) analysis

Volatile compounds in dried carrots were analyzed by HS–SPME–GC–MS according to our previous study (Yang et al., 2016). Dried carrots (1 g) were put in a 20-mL headspace vial and then sealed. A 75 μm carboxen/polydimethylsiloxane fiber was used to collect the volatile compounds at 60 °C for 40 min, which were then analyzed by GC–MS (7890A/5975C, Agilent Technologies, Santa Clara, CA). Volatile compounds were separated on a DB-5MS capillary column (30 m × 0.25 mm, 0.25 mm) (J&W Scientific, Folsom, CA, United States). Mass spectra was taken at 70 eV ionization energy in the 35–550 amu mass range, with the ion source temperature at 230 °C. C7–C30 n-alkanes were purchased from Sigma–Aldrich, St. Louis, MO, USA. The retention times of n-alkanes were used to calculate retention indices of volatile compounds according to Skalicka-Woźniak et al. (2018). The compounds were identified based on matching of the recorded mass spectral data with MS library (NIST 08, Washington DC), literature-reported retention index and comparison of the fragmentation patterns with those reported in previous literatures (Cornara et al., 2018).

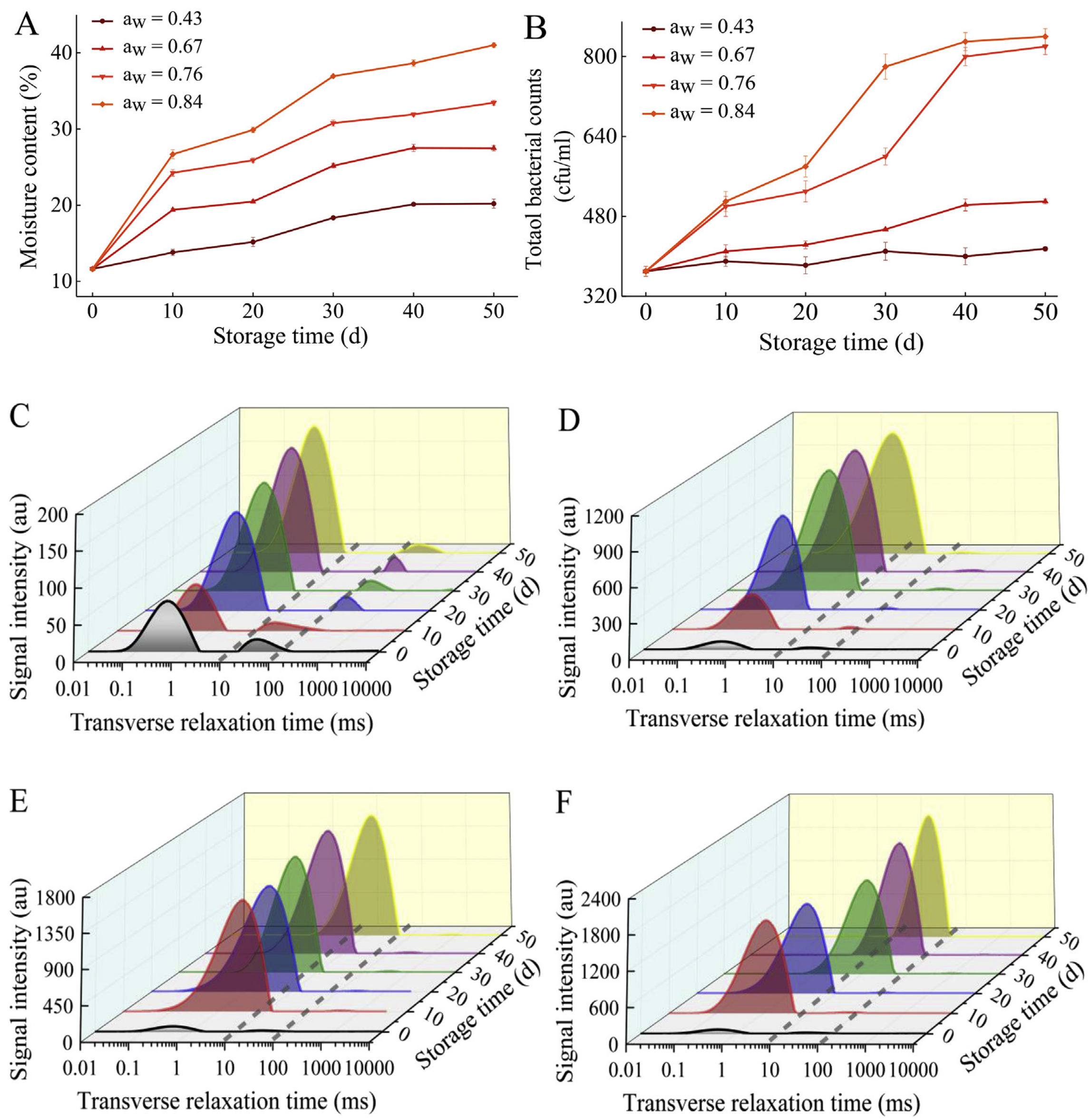

Fig. 2. Moisture content (A) and total bacterial counts (B) in dried carrots at different a_w. Distribution of LF-NMR T_2 relaxation times of dried carrots at a_w = 0.43 (C), a_w = 0.67 (D), a_w = 0.76 (E) and a_w = 0.84 (F).

2.5. Orthogonal partial least squares (OPLS) analysis

OPLS can integrate multiple data blocks to improve interpretation and identification of relevant information (Sharif et al., 2014). OPLS was used to describe the correlation between volatile compounds identified by GC-MS analysis and microbial composition at genus level for exogenous bacteria in dried carrots. An OPLS model has been previously described (Sharif et al., 2014).

2.6. Statistical analysis

Experimental data from the HP-SPME-GC-MS was analyzed using the statistical software, PASW statistic 18. The data were expressed as the mean ± standard deviation (SD). The obtained data were analyzed by SAS system, Version 9.0 (SAS 153 Institute, Cary, NC). SIMCA 14.2 software was used for OPLS analysis. Least significant differences (LSD) multiple comparison tests were then performed with a 95% confidence level.

3. Results and discussion

3.1. Appearance quality

Fig. 1 shows the appearance quality of dried carrots stored at different a_w. Discoloration, wilting and shrinkage of the dried carrots stored at different a_w were observed after 50 days of storage. Compared with samples at a_w = 0.43, dried carrots were soft and wet at higher a_w (0.67, 0.76 and 0.84). Furthermore, pleasant, carrot-like flavor was lost at a_w = 0.76 and 0.84 accompanied with bacterial growth after 30 days of storage.

3.2. Moisture content, water status and total bacteria colony counts

Fig. 2A shows moisture content in dried carrots at different a_w. Moisture content increased with increasing a_w for all treatments. In addition, the final values for all samples exceeded safe moisture level (15%) for dried foods (Afzal et al., 1999).

Fig. 2B shows the total bacterial counts in dried carrots during storage. Compared with samples at a_w = 0.43, significant bacterial

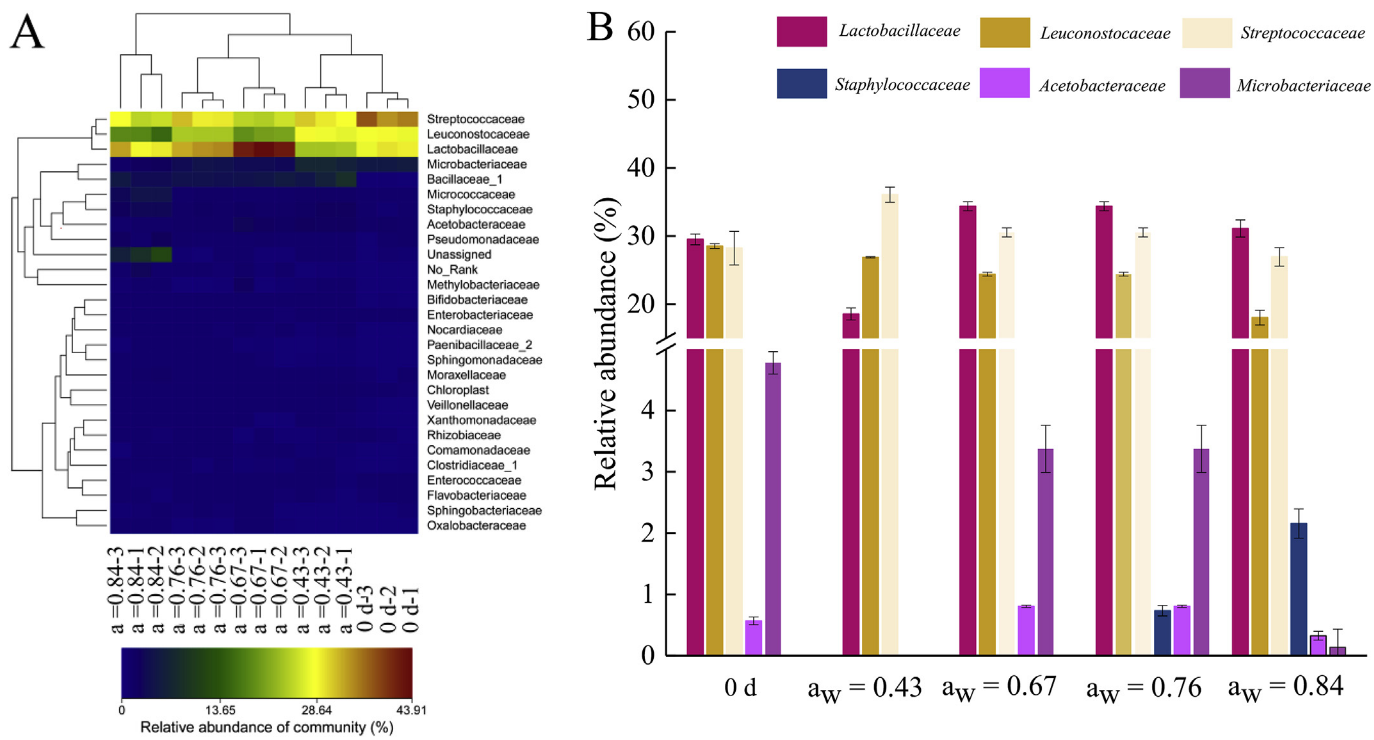

Fig. 3. Heatmap analysis (A) and significant difference ($p < 0.05$) among groups (B) at family level of the exogenous bacterial composition at 0 and 50 d in dried carrots stored at different a_w.

growth was observed ($p < 0.05$) in dried carrots at $a_w = 0.67$, 0.76 and 0.84. Furthermore, the total bacterial colonies at $a_w = 0.76$ and 0.84 exceeded 800 at the end of storage, much larger than those at the other two a_w levels. This indicated that $a_w = 0.76$ and 0.84 presented better growing conditions for bacteria on dried carrots.

NMR signal intensity is proportional to the proton density and moisture content (Sanchez-Alonso et al., 2014). Fig. 2C–F showed the water status detected by LF-NMR spectroscopy. Three relaxation times and their corresponding peak area were recorded. For samples at 0 d, the signals in the range between 0 and 10 ms (T_{21}) represented the bound water, between 10 and 100 ms (T_{22}) represented the immobilized water and those between 100 and 1000 ms (T_{23}) represented the free water (Yang et al., 2017). The transverse relaxation time of bound water increased for treatment samples during storage.

The peak area of T_{21} in all samples increased significantly ($p < 0.05$), whereas that of T_{22} and T_{23} did not change significantly ($p > 0.05$). This confirms that bound water was the dominant water status in dried carrots. In addition, T_{21} peak area increased significantly ($p < 0.05$) in all treatments, suggesting that mobility of bound water increased (Pitombo and Lima, 2003). In addition, the transverse relaxation time of bound water increased for samples at $a_w = 0.76$ and 0.84 during storage and was located in the range between 10 and 100 ms at the end of storage. This result indicated that molecular mobility of bound water increased and could be utilized by microorganisms to reactivate their growth and propagation (Li et al., 2015).

3.3. Bacterial community at the family level

Taxonomy heatmap reflects the actual similarities and differences in community composition of these samples. The bacterial community structure in dried carrots was visualized using a heatmap by employing the data of relative abundance of each family. The findings were compared using a hierarchical dendrogram (Fig. 3A). The similarity between samples decreased from higher to lower taxonomic levels. At the family level, *Streptococcaceae*, *Lactobacillaceae* and *Leuconostocaceae* were detected as the main families in all samples. Furthermore, all the three families belonged to the order *Lactobacillales* (Dehler et al., 2017). *Lactobacillales* shows varying potential to cause spoilage in foods by production of off-flavor compounds (Casaburi et al., 2015).

Cluster analysis showed that bacterial communities in samples at $a_w = 0.43$ were clustered closer to the samples at 0 d. However, the communities between dried carrots at $a_w = 0.84$ and other samples displayed a further relationship. Fig. 3B shows exogenous bacterial profiles in dried carrots with significant difference between groups. Compared with samples at 0 d, relative abundance of *Lactobacillaceae* significantly increased for dried carrots at $a_w = 0.67$, 0.76 and 0.84 ($p < 0.05$). Zhang et al. (2017) reported that *Lactobacillaceae* is positively correlated with some volatile compounds that affect flavor in fermentation system.

3.4. Bacterial composition at the genus level

Fig. 4 shows the bacterial composition in the dried carrots at the genus level. Although different samples markedly had different bacterial communities, five groups had relatively similar community composition. For samples at 0 d, the dominant genera were *Streptococcus* spp., *Lactobacillus* spp., *Pediococcus* spp., *Weissella* spp. and *Leuconostoc* spp., some of which had a sum relative abundance of more than 80%. All the bacteria were classified into lactic acid bacteria (LAB), a gram-positive, acid-tolerant bacterial group (Alvarez-Sieiro et al., 2016). In most cases, spoilage LABs originate from the production line or from raw materials and are resistant to environmental stresses. Some spoilage *Lactobacilli* have high heat resistance, which makes them more likely to survive after a drying process (Ferrando et al., 2015).

After 50 days of storage, relative abundance of *Weissella* spp., *Leuconostoc* spp. and *Lactobacillus* spp. decreased whereas that of *Pediococcus* spp. increased. In addition, *Fructobacillus* spp. was detected in dried carrots at all a_w levels after 50 days of storage, and its relative abundance increased with the increase in a_w. In this study, LABs were responsible for spoilage and have been shown to produce off-flavors which frequently affect the quality of fruits, vegetables and juices (Rawat, 2015).

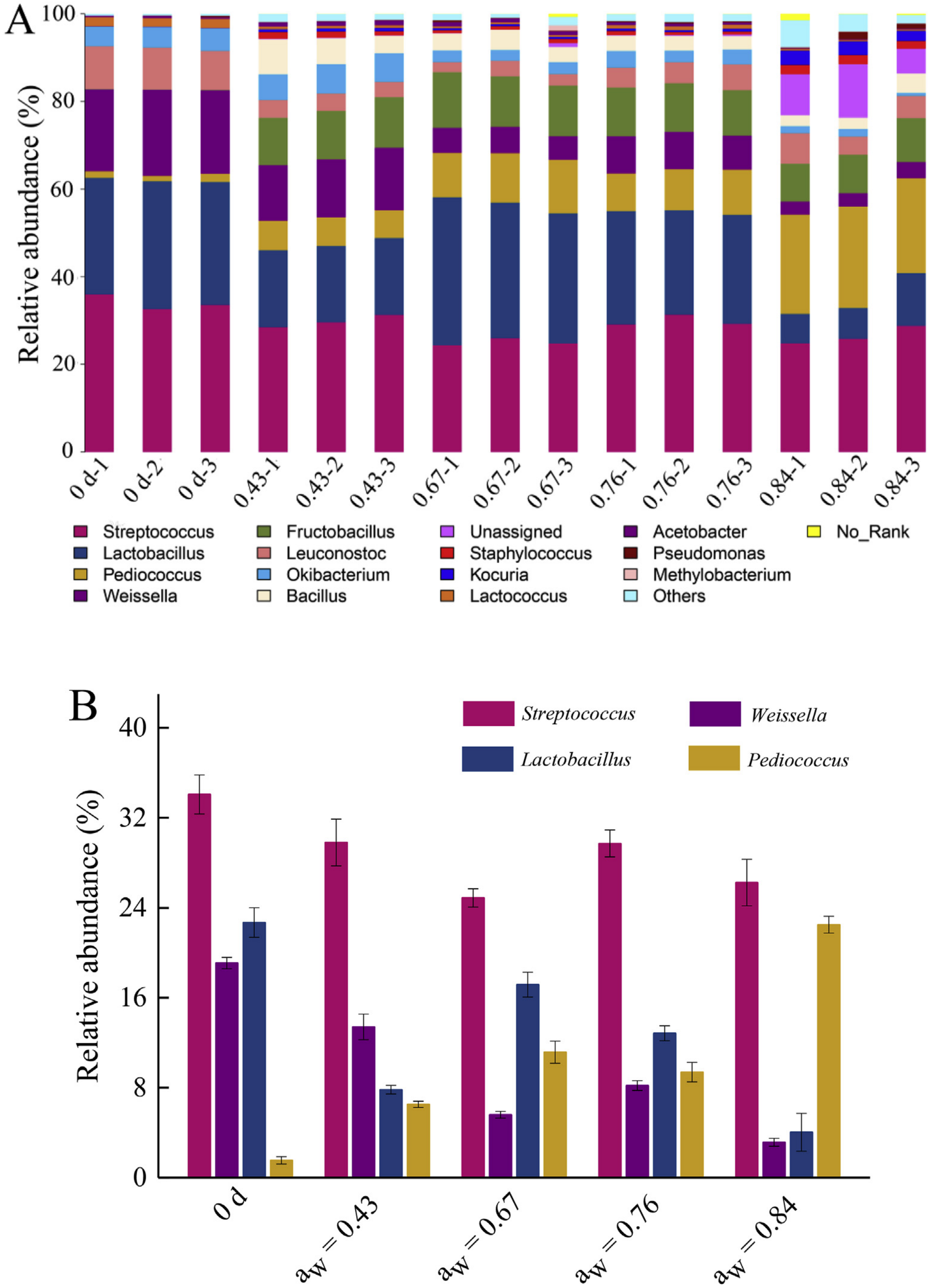

Fig. 4. Bacterial composition of the exogenous bacterial composition at the genus level (A) and relative abundance of *Streptococcus* spp., *Weissella* spp., *Lactobacillus* spp. and *Pediococcus* spp. (B) at 0 and 50 d in dried carrots stored at different a_w.

3.5. GC-MS analysis

Table 1 lists volatile compounds in dried carrots at 0 and 50 d stored at different a_w. Fifty volatile compounds were identified, including seven aldehydes, six acids, 20 hydrocarbons, four alcohols, three esters, three ketones and seven heterocyclic and aromatic compounds.

For samples at 0 d, acids and hydrocarbons were the main volatile compounds. For example, acetic acid, hexanoic acid and β-caryophyllene were the dominant volatile compounds, and had a relative content of 15.90, 26.44 and 8.07%, respectively. Some typical volatile compounds in dried carrots were also detected, including limonene, 2,3-butanediol and γ-terpinene (Kjeldsen et al., 2003). However, four of the five acids, namely pentanoic acid, hexanoic acid, heptanoic acid and nonanoic acid, were lost at higher a_w after 50 d of storage as well as 2,3-butanediol. It was reported that 2,3-butanediol is a typical volatile compound which gives a cocoa butter (sweet chocolate) flavor to dried carrots (Moreira et al., 2017). Moreover, relative content of terpenes increased at higher a_w levels after 50 d of storage. These included α-pinene, o-cymene, β-pinene and γ-terpinene. The increased content of terpenes was suggested to correlate with off-flavors in carrots (Rajkumar et al., 2017). Nevertheless, bacterial growth was observed on dried carrots at a_w = 0.67, 0.76 and 0.84 after 50 d of storage. Bacteria are known to produce a range of volatile organic compounds that affect flavor of foods (Tait et al., 2014). However, the correlation between the bacteria and the volatile compounds in dried carrots is still unclear.

Table 1
Volatile compounds content in dried carrots at different a_w.

No.	Category	Compound name	Retention index	Relative content (%)				
				0 d	$a_w = 0.43$	$a_w = 0.67$	$a_w = 0.76$	$a_w = 0.84$
	Aldehydes			9.23	7.31	9.4	6.61	6.33
1		Hexanal	800	3.63	4.19	4.24	0.81	1.05
2		Furfural	860	0.22	0.65	0.42	1.20	0.73
3		Octanal	1008	1.20	0.43	2.94	1.72	1.68
4		Nonanal	1102	1.70	—	—	—	—
5		Decanal	1207	1.48	1.33	—	—	—
6		β-cyclocitral	1225	1.00	0.71	1.02	2.47	2.41
7		2-Heptenal, (E)-	961	—	—	0.78	0.41	0.46
	Acids			47.21	28.37	12.49	6.24	4.87
8		Acetic acid	641	15.90	18.22	12.49	6.24	4.87
9		Pentanoic acid	925	2.09	—	—	—	—
10		Hexanoic acid	1019	26.44	10.15	—	—	—
11		Heptanoic acid	1076	1.30	—	—	—	—
12		Octanoic acid	1186	1.04	—	—	—	—
13		Nonanoic acid	1237	0.44	—	—	—	—
	Hydrocarbon			15.55	6.16	28.67	36.46	41.97
14		o-cymene	1018	1.31	1.48	14.76	11.74	12.47
15		Limonene	1028	0.90	—	—	1.61	1.73
16		γ-terpinene	1056	2.51	0.48	9.56	15.10	18.84
17		Naphthalene	1211	0.70	—	—	—	—
18		Tetradecane	1400	0.30	—	—	—	—
19		Longifollene	1403	1.76	2.68	0.85	0.49	0.46
20		β-caryophyllene	1415	8.07	1.52	3.50	7.52	8.47
21		α-caryophyllene	1455	0.52	2.47	0.46	0.78	0.93
22		(S)-β-bisabolene	1508	0.79	1.86	0.24	0.23	0.39
23		α-pinene	917	—	3.96	2.44	6.14	6.07
24		Benzaldehyde	980	—	2.11	5.01	1.22	1.13
25		β-pinene	981	—	1.67	8.08	9.58	8.04
26		β-myrcene	992	—	2.45	4.49	4.68	2.20
27		Terpinolene	1085	1.42	1.09	3.95	7.17	8.20
28		4-isopropenyltoluene	1090	—	0.59	1.20	0.72	0.68
29		Dodecane	1000	—	0.48	0.24	—	—
30		Dodecane, 2,6,10-trimethyl-	1375	—	6.52	—	—	—
31		α-terpinene	1015	—	0.84	0.30	0.33	0.51
32		Pentadecane	1500	—	1.11	—	—	—
33		2,4-dimethylbenzaldehyde	1190	—	—	0.36	0.27	0.30
	Alcohol			1.94	12.31	0.51	0.57	0.15
34		2,3-butanediol	802	1.94	0.97	—	—	—
35		Benzyl alcohol	1060	—	8.71	—	—	—
36		1-hexanol	855	—	2.63	0.51	0.41	——
37		1-nonanol	1166	—	—	—	0.16	0.15
	Esters							
38		Bornyl acetate	1283	0.22	0.27	1.12	1.81	1.82
39		Dihydroactinidioli	1532	13.22	6.14	—	—	—
40		Myristicin	1521	—	0.80	1.30	1.07	1.06
	Ketone			0	1.29	4.14	2.8	2.49
41		Geranylacetone	1430	—	1.29	3.02	2.29	1.98
42		Acetophenone	1044	—	—	0.53	—	—
43		o-methylacetophenone	1170	—	—	0.59	0.51	0.51
	Aromatic compounds			10.69	12.2	15.6	12.55	10.92
44		β-ionone	1486	2.43	4.59	2.23	2.39	2.34
45		β-ionone 5,6-epoxide	1455	7.38	0.73	4.10	4.64	4.06
46		Caryophyllene oxide	1581	0.88	0.16	2.10	2.27	1.88
47		Pyrazine, 2,5-dimethyl-	928	—	1.04	1.94	1.17	0.83
48		Pyrazine, 2,6-diethyl-	1081	—	5.68	0.73	0.31	0.33
49		Pyrazine, 2-ethyl-5-methyl-	981	—	—	4.50	—	—
50		3,4-Dimethylthiophene	917	—	—	—	1.77	1.48

3.6. Correlation analysis

OPLS derived a model which was used to describe the correlation between microbial composition at the genus level and the 50 volatile compounds in dried carrots at $a_w = 0.67$, 0.76 and 0.84. These volatile compounds were numbered and showed in Table 1. R^2 and Q^2 of the model were 0.788 and 0.816 respectively, suggesting that OPLS method was well fitted for analysis and prediction (Hemeryck et al., 2018). Based on the correlation coefficient between volatile compounds and bacteria at the genus level, 20 bacteria and 35 volatile compounds were moderately and highly correlated ($|\rho| > 0.7$) (Wang et al., 2016). Fig. 5B shows the correlation between volatile compounds and bacteria. The red line meant a positive correlation while the blue line represented a negative correlation.

Streptococcus spp., *Weissella* spp., *Lactobacillus* spp. and *Leuconostoc* spp. were the dominant genera and were moderately and highly correlated with 5, 7, 4 and 21 volatile compounds, respectively. Most terpene contents were higher in samples stored after 50 d, including o-

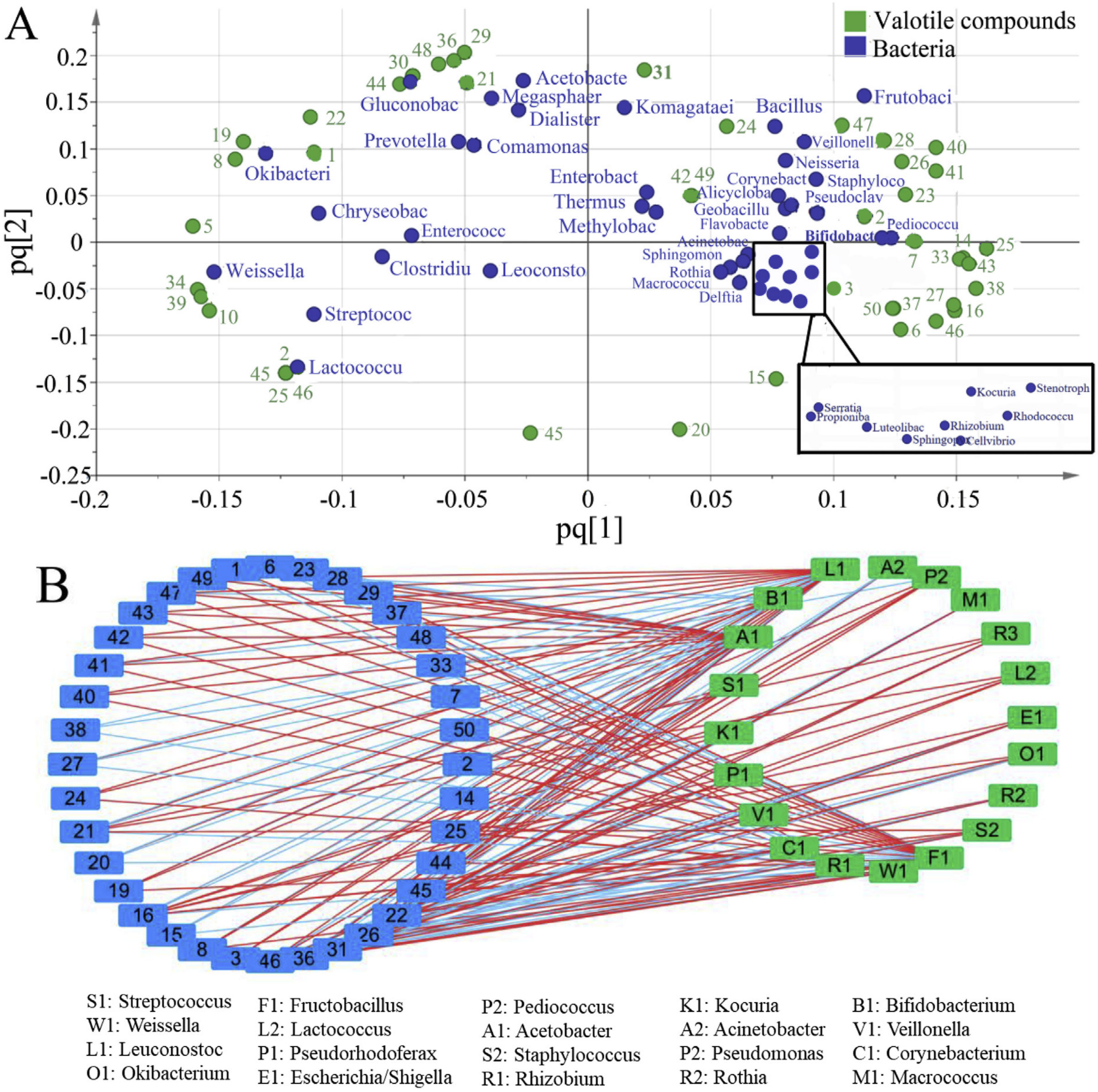

Fig. 5. Orthogonal partial least squares loading scatter plot of volatile compounds (numbered in Table 1) and exogenous bacterial composition at genus level (A) and the correlated network between volatile compounds and bacteria at genus level (B). The left-side circle represents volatile compounds correlated with bacteria (|ρ| > 0.7). The right-side one represents bacteria correlated with volatile compounds (|ρ| > 0.7). The red lines linking the circles represent a positive correlation while the blue lines represent the a negative correlation between bacteria and volatile compounds. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cymene, γ-terpinene, β-pinene and β-myrcene. Terpenes exist in glycoconjugated forms in several fruits and vegetables. They contribute to a large part of the volatile compounds and are the most diverse class of compounds (Martins et al., 2017). In addition, most volatile terpenes were found in glycosidic forms as precursors of various volatiles. They can be released from glycosides by acidic and enzymatic hydrolysis (Lee et al., 2018). In this study, o-cymene, β-pinene and β-myrcene had a highly positive correlation with LABs, including *Streptococcus* spp., *Weissella* spp., *Leuconostoc* spp. and *Lactobacillus* spp. LAB metabolism can result in β-glucosidase breaking terpene–sugar bonds and enhancing the release of terpenes (Lee et al., 2018). This could be a possible reason to the domination of LAB and the increase of terpene. In addition, owing to an increase in γ-terpinene, and *Pediococcus* spp. and their highly positive correlation (|ρ| > 0.7), we hypothesized that the increase of *Pediococcus* spp. led to an increase of γ-terpinene, contributing to a harsh and oily flavor in carrots (Ulrich et al., 2015).

4. Conclusion

In the present study, the exogenous bacterial composition and volatile compounds in dried carrots were investigated at different a_w levels (0.43, 0.67, 0.76 and 0.84) for 50 days. High environmental water activity increased the bound water content and the mobility of water molecules in dried carrots, which accelerated microbial growth and flavor deterioration during storage. Lactic acid bacteria, such as *Streptococcus* spp., *Weissella* spp., *Lactobacillus* spp. and *Leuconostoc* spp. contributed to the increase of terpenes including o-cymene, β-pinene and β-myrcene. Moreover, *Pediococcus* spp. caused an increase in γ-terpinene content which in turn contributed to off flavor in carrots. In summary, controlling these bacteria might be helpful to maintain the typical flavor compounds and overall sensory acceptability of dried carrots during storage.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work is financially supported by the Foundation of National Key R & D Program of China (2017YFD0400903), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX18_1437).

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