Effects of Different Sterilization Conditions on Active Components and Flavor of Apple Vinegar

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Abstract [**Objectives**] To explore the effects of different sterilization conditions on nutrition and flavor of apple vinegar. [**Methods**] Five kinds of high temperature short time (HTST) sterilization conditions were selected to treat apple vinegar, and the volatile aroma components and the content of active components in apple vinegar before and after sterilization were analyzed. [**Results**] Compared with the control, the contents of total acid and malic acid in the samples after sterilization changed little, but the contents of citric acid increased significantly ($P<0.01$), and the contents of total phenols, ascorbic acid and total flavonoids decreased. Ethyl acetate, isopentyl acetate, ethyl caprylate, phenethyl acetate, 1-pentanol, phenylethyl alcohol, acetic acid, and sec-butyl ether were the characteristic aroma components which contributed to the flavor of apple vinegar. As sterilization temperature increased, the content of esters decreased, while the content of acids, alcohols and aldehydes increased. The contents of nutrition, active components and volatile aroma components in apple vinegar under 100 ℃ and 30 s sterilization conditions had little change compared with other sterilization conditions, so 100 ℃ and 30 s were the optimal sterilization conditions. [**Conclusions**] Under different sterilization conditions, the content of flavor components in apple vinegar will change greatly, which will affect the quality of apple vinegar.

Key words Apple vinegar, High temperature short time (HTST) sterilization, Aroma components, Nutritional components, Active components

1 Introduction

Apple is rich in nutrients, such as crude cellulose, polysaccharides, vitamins, polyphenols, essential trace elements for human body[1-5], and is one of the most popular fruits in daily life. With the continuous enrichment of apple varieties and the continuous expansion of planting area, China's apple yield is also rising, and the national apple yield reached 41 million t in 2020. Accordingly, the apple intensive processing industry is becoming more and more perfect, which not only meets people's diversified food needs, but also gradually realizes the sustainable development of the apple industry[6]. Apple vinegar is a kind of healthy drink made from fresh apple or apple juice by alcoholic fermentation and acetic acid fermentation; it not only has good flavor, but also has the nutrition and health effects of fruit and vinegar[7-8]. Apple vinegar is rich in organic acids, vitamins, amino acids, pectin, minerals and other components[9-11], which can improve blood circulation, resist bacterial invasion, resist arthritis, delay aging, promote digestion, beautify and protect skin, prevent elevated blood pressure and arteriosclerosis, eliminate toxins, regulate calcium metabolism and other health effects[12-14]. With the continuous improvement of people's requirements for food, apple vinegar products are increasingly favored by consumers. The content of volatile aroma substances, nutrients and active components in apple vinegar is the key to determine the flavor of apple vinegar, and is also an important factor to determine the quality of apple vinegar[15]. Previous studies paid more attention to the influence of fermentation process on the flavor of apple vinegar, while sterilization, as a necessary link in the production of apple vinegar, has rarely been reported on its influence on the flavor of apple vinegar. Deng Nana *et al.*[16] studied the effects of heating, ultrasonic and microwave sterilization methods on the flavor of mulberry vinegar, and the results showed that the aroma quality of mulberry vinegar after microwave sterilization was the best, but due to the high cost of microwave sterilization, it was not suitable for wide application in fruit vinegar processing industry. Thermal sterilization is the most commonly used sterilization method in the production of apple vinegar[16]. The main thermal sterilization methods include low temperature long time (LTLT) sterilization, high temperature short time (HTST) sterilization, and ultra high temperature (UHT) instantaneous sterilization, *etc.* LTLT sterilization is also known as pasteurization. The treatment temperature is usually below 100 ℃. The typical pasteurization condition is 62.8 ℃/30 min. LTLT sterilization is simple and convenient. The sterilization

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effect is 99%. Pathogenic bacteria are completely killed, but thermophilic and heat-resistant bacteria, spores and some residual enzymes can not be killed. HTST sterilization refers to the sterilization treatment above 100 ℃ and below 130 ℃, and it has the advantages of short heating time, less loss of nutrients, large treatment capacity, suitable for continuous production, saving heat source and low cost. UHT instantaneous sterilization refers to the treatment at 130 – 150 ℃ for a few seconds. UHT has high sterilization temperature, very short sterilization time, remarkable sterilization effect and little chemical change, but the equipment cost is high. Different heating methods, heating temperature and heating time will have a great impact on the volatile aroma components, nutrition and active component content of apple vinegar.

In this study, we used HTST sterilization method to sterilize the stock solution of apple vinegar, and explored the changes in flavor, nutrition and active components in apple vinegar under different sterilization temperature and time, in order to explore the effects of HTST sterilization on the flavor of apple vinegar. This study is expected to provide theoretical guidance for the sterilization technology of apple vinegar, and has practical significance for stabilizing the quality of apple vinegar in production.

2 Materials and methods

2.1 Materials and reagents

2.1.1 Materials. Harlikar apple: purchased from the market; *Saccharomyces cerevisiae* BV818: Angel Yeast Co., Ltd.; *Acetobacter suboxydans* (Shanghai Brewing 1.01): preserved in our laboratory.

2.1.2 Chemical reagents. Glucose: Sinopharm Chemical Reagent Co., Ltd.; calcium carbonate: Tianjin Damao Chemical Reagent Factory; Yeast extract: Beijing Aoboxing Biotech Co., Ltd.; potassium dihydrogen phosphate: Tianjin Guangcheng Chemical Reagent Co., Ltd.; anhydrous magnesium sulfate: Sinopharm Chemical Reagent Co., Ltd.; agar: Beijing Solarbio Science & Technology Co., Ltd.; anhydrous ethanol: Tianjin Fuyu Fine Chemical Co., Ltd.; sodium hydroxide: Tianjin Damao Chemical Reagent Factory; aluminum nitrate: Tianjin Damao Chemical Reagent Factory. The above reagents are analytically pure.

2.1.3 Culture medium. Acetobacter liquid culture medium: glucose 1%, yeast extract 1.5%, potassium dihydrogen phosphate 0.05%, anhydrous magnesium sulfate 0.05%, natural pH, 121 ℃, 20 min sterilization, cooling to room temperature, adding 3% ethanol. The solid culture medium of acetic acid bacteria was composed of glucose 2%, yeast extract 1%, calcium carbonate 2%, agar 2%, natural pH, 121 ℃, 20 min sterilization, cooling to about 50 ℃, and volume fraction 3% ethanol.

2.2 Instruments and equipment YXQ-LX-100A autoclave: Shanghai Boxun Industry & Commerce Co., Ltd.; ST-20 high temperature sterilization equipment: Shanghai Shunyi Laboratory Equipment Co., Ltd.; SW-CJ-2FD Ultra-clean Workbench: Suzhou Antai Airtech Co., Ltd.; RS-232 constant temperature shaking incubator: Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd.; BIOF-6010B/S/B/Z microbial fermentation tank: Shanghai Gaoji Bioengineering Co., Ltd.; Shanghai Gaoji Biological Engineering Co., Ltd.; 6890N-5973N gas chromatography-mass spectroscopy (GC-MS): Agilent (China) Technology Co., Ltd.

2.3 Experimental methods

2.3.1 Activation of acetic acid bacteria. Under aseptic conditions, one cycle of acetic acid bacteria was selected from the slant of strain preservation, activated on the solid medium of acetic acid bacteria by streaking, and cultured at 30 ℃ for 2 d. The activated strains were picked from the solid plate and inoculated into 50 mL of acetic acid bacteria liquid medium, and then placed in a shaker at 30 ℃ and 180 r/min for overnight shaking culture to prepare the primary seed solution. 15 mL of the primary seed solution was inoculated into 300 mL of sterilized apple juice, and placed in a shaker at 30 ℃ and 180 r/min for overnight shaking culture to prepare the secondary seed solution.

2.3.2 Preparation of apple vinegar. Fresh apples were crushed, squeezed and filtered to obtain juice, which was heated to 90 ℃ for sterilization and then poured into a fermentation tank, inoculated with active dry yeast at an inoculum size of 0.2‰, and fermented at 30 ℃ for 5 to 7 d until the residual sugar was reduced to less than 0.4% to obtain cider. The fermented cider was filtered with diatomite to remove the fermentation mud as much as possible, and then inoculated with the secondary seed liquid of acetic acid bacteria prepared in the previous step according to the inoculation amount of 5% – 10%. The fermentation tank temperature was set at 30 ℃, the stirring speed was 1 200 r/min, and the ventilation rate was 2 L/min. The fermentation lasted for 3 to 4 d until the acidity of the fermentation liquid did not rise. Finally, set different conditions to carry out sterilization treatment to obtain apple vinegar.

2.3.3 Design of sterilization conditions. As shown in Table 1, five different sterilization conditions were set, and the apple vinegar treated under the five sterilization conditions could meet the commercial sterility requirements, in which the sample S1 was the control group without sterilization treatment.

Table 1 Design of sterilization conditions of apple vinegar samples

Sample No.	Temperature//℃	Time//s
S1	–	–
S2	100	30
S3	105	15
S4	110	15
S5	115	15
S6	120	15

Note: " – " denotes not sterilized.

2.3.4 Analysis and detection. (i) Determination of volatile aroma components. Volatile aroma components in apple vinegar were determined by GC-MS method. Accurately weighed 5.00 g of apple vinegar and put it into a 20 mL sample bottle, added 2-octanol (10 μL, internal standard) with a concentration of 32.88 μg/mL, and sealed it for determination.

GC analysis conditions: DB-5 MS column (30 mm × 0.25

mm × 0.25 μm), injection temperature 250 ℃, high purity helium (99.999%) as carrier gas, flow rate 1.660 mL/min, no split flow; heating program: initial temperature 40 ℃, holding for 2 min, heating to 200 ℃ at 8 ℃/min, holding for 3 min, heating to 250 ℃ at 10 ℃/min, holding for 8 min.

MS conditions: the ion source was an electronic ionization (EI) source, the electron energy is 70 eV, the ion source temperature was 280 ℃, the quadrupole temperature was 150 ℃, the transmission line temperature was 280 ℃, and the scanning range was 50 – 600 *m/z*.

(ii) Determination of total phenols. The Folin-Ciocalteu method was used to determine the total phenolic content in apple vinegar[17].

(iii) Determination of total flavonoids. With reference to the aluminum nitrate coloration method of Fan Qi *et al.*[18], we determined the content of total flavonoids in apple vinegar. The rutin standard solution with the mass concentration of 0.1 mg/mL was prepared by accurately weighing 0.010 g of rutin standard substance, dissolving it in 50% ethanol and fixing the volume to 100 mL. Pipetted 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mL of rutin standard solution into the test tube, diluted with 50% ethanol to 2.5 mL. Added 0.15 mL of 5% $NaNO_2$ solution after mixing in a vortex, shook it up and let it stand for 6 min. Added 0.15 mL of 10% $Al(NO_3)_3$ solution, mixed it in a vortex, and let it stand at room temperature for 6 min, then added 2 mL of 1 mol/L NaOH solution, shook up, and measured the absorbance value at the wavelength of 510 nm to obtain a standard curve with the mass concentration of rutin standard solution (x) as the abscissa and the absorbance value (y) as the ordinate. The standard curve equation is:

$y = 0.005\,5x - 0.003\,6$ ($R^2 = 0.999\,5$)

The content of total flavonoids in the sample was calculated using the standard curve regression equation.

(iv) Determination of ascorbic acid. The ascorbic acid was determined with reference to 2,6-dichloro-indophenol titration in the national standard *Determination of Ascorbic Acid in Foods* (GB 5009.86-2016).

(v) Determination of organic acids. The organic acids were determined in accordance with the method in *Determination of Organic Acids in Foods* (GB 5009.157-2016).

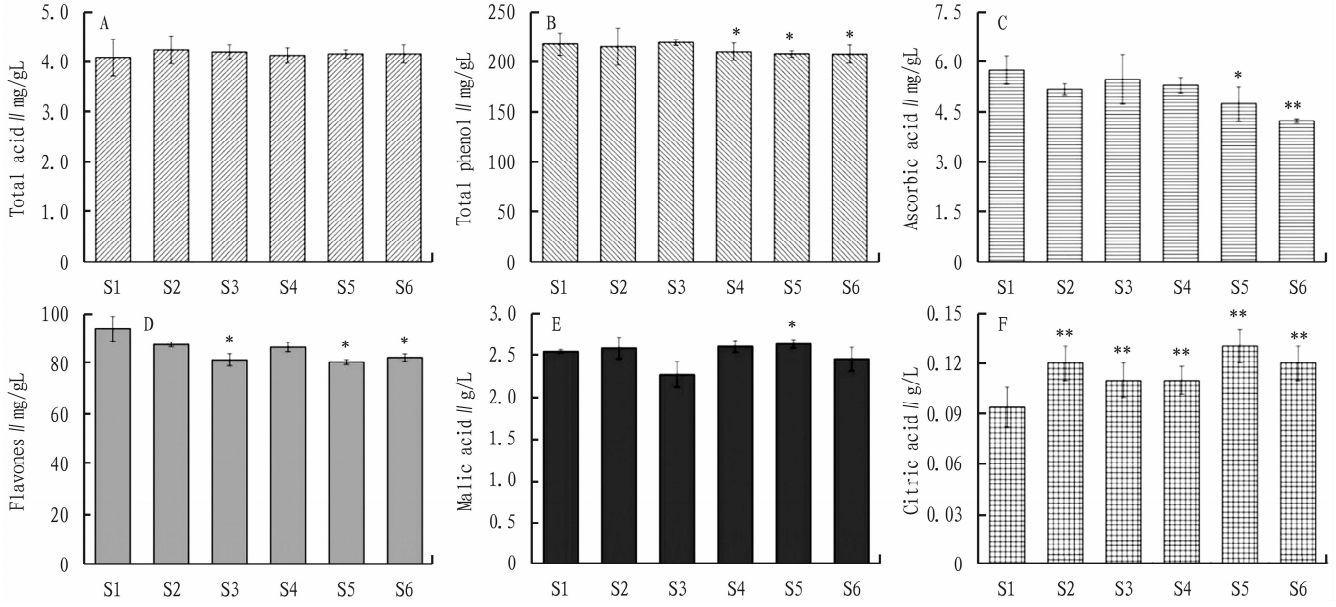
3 Results and analysis

3.1 Changes in active components in apple vinegar under different sterilization conditions After detection and analysis, the content of active components in the six samples is shown in Fig. 1. As can be seen from Fig. 1A, the total acid content of the sample after sterilization did not change significantly ($P > 0.05$) compared with the control sample S1. As shown in Fig. 1B, compared with the control sample S1, the total phenol content of samples S2 and S3 did not change significantly ($P > 0.05$), while the total phenol content of samples S4, S5 and S6 decreased significantly ($P < 0.05$). It can be seen from Fig. 1C that, compared with the control sample S1, the ascorbic acid content of samples S2, S3 and S4 did not change significantly ($P > 0.05$), the ascorbic acid content of sample S5 decreased significantly ($P < 0.05$), and the ascorbic acid content of sample S6 decreased very significantly ($P < 0.01$). As shown in Fig. 1D, compared with the control sample S1, the total flavonoid content of samples S2 and S4 did not change significantly ($P > 0.05$), while the total flavonoid content of samples S3, S5 and S6 decreased significantly ($P < 0.05$). These indicate that with the increase in temperature, phenols, ascorbic acid and flavonoids will be destroyed, resulting in the decrease of content[19]. As shown in Fig. 1E, compared with the control sample S1, the malic acid content of sample S5 increased significantly ($P < 0.05$), while the malic acid content of other samples did not change significantly ($P > 0.05$). Compared with the control sample S1, the citric acid content of all samples increased significantly ($P < 0.01$), which may be due to the decomposition of citrate caused by high temperature.

Generally, too high sterilization temperature had a greater impact on the content of active components in apple vinegar, while lower sterilization temperature had less impact on the content of organic matter in apple vinegar. Combined with the data in Fig. 1, sterilization at 100 ℃ for 30 s has little effect on the content of nutrients and active ingredients in apple vinegar, so 100 ℃ and 30 s are optimal sterilization conditions.

3.2 Changes in volatile aroma components in apple vinegar under different sterilization conditions After GC-MS detection, the determination results of volatile aroma components in 6 samples under different sterilization conditions are shown in Table 2. It can be seen from Table 2 that 50 volatile aroma components were detected in 6 apple vinegar samples under different sterilization conditions, including 35 common substances. A total of 38 volatile aroma components were detected in the unsterilized apple vinegar sample S1, including 15 esters (65.48%), 5 acids (9.96%), 8 alcohols (12.54%), 2 aldehydes (0.88%), and 2 hydrocarbons (0.19%); 1 ketone, accounting for 0.17%; 5 others, accounting for 10.78%. In sample S2, a total of 40 volatile aroma components were detected, including 16 esters, accounting for 61.53%, 5 acids, accounting for 10.00%, 7 alcohols, accounting for 13.83%, 2 aldehydes, accounting for 1.03%, 4 hydrocarbons, accounting for 1.75%, 1 ketone, accounting for 0.19%, and 5 others, accounting for 10.79%. In sample S3, 41 volatile aroma components were detected, including 15 esters, accounting for 62.22%, 5 acids, accounting for 9.93%, 8 alcohols, accounting for 13.12%, 2 aldehydes, accounting for 1.58%, 2 hydrocarbons, accounting for 0.18%, 2 ketones, accounting for 0.49%, and 7 others, accounting for 12.27%. In sample S4, 39 volatile aroma components were detected, including 15 esters, accounting for 61.32%, 5 acids, accounting for 9.83%, 7 alcohols, accounting for 13.10%, 2 aldehydes, accounting for 1.87%, 1 hydrocarbon, accounting for 0.09%, 2 ketones, accounting for 0.31%, and 7 others, accounting for 12.48%. In sample S5, 42 volatile aroma components were detected, including 15 esters, accounting for 57.91%, 5 acids, accounting for 13.39%, 9 alcohols, accounting for 13.74%, 2 aldehydes, accounting for 1.97%, 3 hydrocarbons, accounting for 0.41%, 2 ketones, accounting for 0.30%, and 6 others, accounting for 13.48%. In sample S6, 46 volatile aroma compo-

nents were detected, including 17 esters, accounting for 56.94%, 5 acids, accounting for 12.69%, 11 alcohols, accounting for 16.68%, 2 aldehydes, accounting for 2.01%, 3 hydrocarbons, accounting for 0.43%, 2 ketones, accounting for 0.29%, and 6 others, accounting for 10.97%. These indicate that esters, alcohols and acids are the main volatile aroma components in apple vinegar. Among the components, ethyl acetate, isopentyl acetate, ethyl caprylate, phenethyl acetate, 1-pentanol, phenylethyl alcohol, acetic acid, and sec-butyl ether are characteristic aroma components contributed to the flavor of apple vinegar.



Note: * denotes significant difference compared with the control ($P<0.05$), ** denotes extremely significant difference compared with the control ($P<0.01$).

Fig. 1 Effects of different sterilization conditions on the content of active components in apple vinegar

Table 2 Detection results of volatile aroma components in apple vinegar under different sterilization conditions

Retention time // sec	Compound	Sample substance content // mg/L					
		S1	S2	S3	S4	S5	S6
	Esters						
3.94	Ethyl acetate	4.43	4.08	5.92	5.53	5.53	5.57
4.84	2-Methylbutyl acetate	0.0091	0.010	0.013	0.011	0.011	0.012
5.10	n-Propyl acetate	0.042	0.038	0.052	0.050	0.052	0.051
5.68	Isobutyl acetate	0.11	0.10	0.15	0.13	0.14	0.14
6.06	Diethyl chloromalonate	0.011	0.012	0.019	0.016	0.015	0.016
6.67	Butyl acetate	0.016	0.018	0.022	0.016	0.017	0.021
7.37	2,5-Dimethyl-2-hexanol	0.015	0.015	0.026	0.027	0.028	0.026
7.55	Isopentyl acetate	1.21	1.17	1.42	1.27	1.36	1.39
9.51	Ethyl hexanoate	0.082	0.085	0.081	0.070	0.082	0.092
10.19	Hexyl acetate	0.11	0.11	0.10	0.090	0.10	0.11
12.88	Ethyl caprylate	0.54	0.39	0.36	0.29	0.34	0.42
15.56	Hexyl hexanoate	—	0.030	—	—	—	—
15.97	Ethyl caprate	0.28	0.16	0.21	0.20	0.19	0.24
16.51	Diethyl succinate	0.079	0.051	0.077	0.068	0.084	0.098
18.11	Ethyl phenylacetate	—	—	—	—	—	0.011
18.26	13,16-Octadecadiynoicacid methyl ester	—	—	—	—	—	0.011
18.50	Phenethyl acetate	0.27	0.32	0.51	0.49	0.52	0.55
18.75	Ethyl undecanoate	0.024	0.024	0.039	0.036	0.040	0.045
	Number of kinds	15	16	15	15	15	17
	Percentage // %	65.48	61.53	62.22	61.32	57.91	56.94
	Acids						
10.47	2-Amino-3-methylbutanoic acid	0.015	0.012	0.020	0.015	0.025	0.024
13.16	Acetic acid	0.92	0.86	1.22	1.16	1.68	1.57

(To be continued)

(Continued)

Retention time//sec	Compound	Sample substance content//mg/L					
		S1	S2	S3	S4	S5	S6
18.88	Caproic acid	0.033	0.032	0.025	0.023	0.036	0.047
21.52	Octanoic acid	0.11	0.12	0.13	0.10	0.17	0.24
24.37	Decanoic acid	0.020	0.036	0.041	0.037	0.057	0.075
	Number of kinds	5	5	5	5	5	5
	Percentage//%	9.96	10.00	9.93	9.83	13.39	12.69
	Alcohols						
6.92	Isobutanol	0.020	0.022	0.025	0.023	0.024	0.024
7.88	2,2,4-Trimethyl-4-penten-1-ol	0.028	—	—	0.041	0.042	0.043
8.97	1-Pentanol	0.74	0.80	0.98	0.89	0.96	0.97
9.31	3-Ethyl-4-nonanol	0.026	0.027	0.034	0.033	0.035	0.033
11.49	Hexanol	0.075	0.088	0.089	0.077	0.092	0.096
14.14	2-Nonanol	0.0072	0.0081	0.010	—	0.010	0.011
14.28	6-Methyl-1-heptanol	—	—	—	—	—	0.013
14.58	Linalool	0.023	0.022	0.016	0.014	0.030	0.031
15.02	3-Methyl-5-hexen-3-ol	—	—	0.012	—	0.016	0.018
19.28	Benzyl alcohol	—	—	—	—	—	0.011
19.72	Phenylethyl alcohol	0.47	0.49	0.73	0.70	0.81	1.34
	Number of kinds	8	7	8	7	9	11
	Percentage//%	12.54	13.83	13.12	13.10	13.74	16.68
	Aldehydes						
12.25	Nonanal	0.022	0.0077	0.024	0.013	0.016	0.021
14.44	Benzaldehyde	0.075	0.10	0.20	0.24	0.27	0.29
	Number of kinds	2	2	2	2	2	2
	Percentage//%	0.88	1.03	1.58	1.87	1.97	2.01
	Ketones						
8.34	3,4-Dimethyl-2-pentanone	—	—	0.040	0.012	0.013	0.013
18.57	β-Damascenone	0.019	0.020	0.031	0.030	0.032	0.032
	Number of kinds	1	1	2	2	2	2
	Percentage//%	0.17	0.19	0.49	0.31	0.29	0.30
	Hydrocarbons						
7.29	1-(1-Ethoxy)-pentan	0.010	0.011	—	—	0.013	0.013
8.49	3-(Prop-2-enyloxy) dodecane	—	—	0.013	—	—	—
11.14	Dodecamethylcyclohexasiloxane	—	0.012	—	—	0.034	0.039
14.73	Pentylcyclopropane	0.011	0.012	0.013	0.012	0.013	0.014
17.50	α-Farnesene	—	0.15	—	—	—	—
	Number of kinds	2	4	2	1	3	3
	Percentage//%	0.19	1.75	0.18	0.09	0.41	0.43
	Others						
15.61	Edulan	—	—	0.022	0.020	—	—
4.63	2,4,5-trimethyl-1,3-dioxolane	0.44	0.41	0.64	0.62	0.62	0.58
5.91	2,5-Dimethoxytetrahydrofuran	0.011	0.011	0.019	0.019	0.017	0.016
13.43	4,5-Dihydro-2-methyl-thiazole	—	—	0.018	0.020	0.020	0.019
13.72	Sec-butyl ether	0.59	0.58	0.93	0.98	0.96	0.88
22.97	4-Ethylphenol	0.024	0.029	0.044	0.040	0.050	0.065
31.78	2,6-di-tert-butylhydroquinone	0.13	0.12	0.13	0.13	0.14	0.13
	Number of kinds	5	5	7	7	6	6
	Percentage//%	10.78	10.79	12.27	12.48	13.48	10.97

3.2.1 Changes in acids, alcohols and esters. Organic acids are the main acidic substances in apple vinegar, including malic acid, acetic acid, tartaric acid, citric acid, succinic acid, *etc.*, and acetic acid is the main volatile acid[20], mainly produced by *Acetobacterium* using ethanol[16]. In this experiment, acetic acid was detected in all six samples, but the content was relatively small, which may be due to the conversion of acetic acid into other aroma components during fermentation[21]. Acids in most of the samples increased in different degrees after sterilization treatment, and generally showed an increasing trend with the increase in sterilization temperature, among which the contents of acids in vinegar samples S2 and S3 were close to those in vinegar sample S1, possibly because the decomposition of esters at high temperature leads to the increase in acid content. Compared with the control vinegar sample, the total content of alcohols in apple vinegar samples treated under different sterilization conditions increased, among which the content of 1-pentanol and phenylethyl alcohol increased significantly, which may be due to the thermal energy in the sterilization

process to break the glycosidic bonds in some bound alcohols[22], or the deamination and decarbonation of amino acids increase the content of alcohols in the sample[23]. Esters are the most abundant compounds detected in apple vinegar. Esters often have floral or fruity aroma, and ethyl acetate is the most abundant ester. Except for the vinegar sample S2, the content of ethyl acetate in the vinegar samples under other sterilization conditions increased, which was directly related to the content of acetic acid in the samples. However, only the vinegar sample S2 produced hexyl caproate with green, herbal and berry aroma, and only the vinegar sample S6 produced phenyl ethyl acetate. In general, the total content of esters in apple vinegar samples after sterilization treatment decreased compared with the control, which may be due to the decomposition reaction of esters by heating treatment and the production of some alcohols and acids[24].

3.2.2 Changes in ketones, aldehydes and hydrocarbons. Ketones generally have fatty and burnt flavor, and show strong floral characteristics with the growth of carbon chain. The content of ketones in apple vinegar was increased after sterilization. Except in the sample S2, the 3,4-Dimethyl-2-pentanone was detected in all the apple vinegar samples treated under the sterilization conditions. The content of β-Damascenone with strong rose aroma also increased with the increase of sterilization temperature, which may be due to the thermal effect of high temperature, which enhanced the activation energy of molecules and promoted the oxidation reaction. Compared with the control, the content of aldehydes in the samples increased significantly after sterilization. Benzaldehyde has a bitter almond flavor, which adversely affects the aroma of apple vinegar. Its content increases with the increase in temperature, which may be due to the fact that the activity of glycosidase is effectively activated after heating treatment, thus hydrolyzing glycoside substances and making benzaldehyde bound with glycoside free[21]. Therefore, the sterilization temperature of apple vinegar should not be set too high. Different sterilization conditions had a great influence on the content of hydrocarbons in apple vinegar, and only α-farnesene was detected in the vinegar sample S2, which had a strong floral scent. In the samples of this experiment, the content of esters is high and the types are various. Acids and alcohols are also the main volatile aroma components in apple vinegar, and the contents of aldehydes, hydrocarbons and ketones are less, which is consistent with previous research results[25-26]. With the increase in sterilization temperature, the proportion of esters in the volatile aroma components decreased as a whole, and the contents of esters in vinegar samples S1 and S2 were relatively high, while the contents of other substances in vinegar samples S1 and S2 changed slightly compared with the control, which were more suitable sterilization conditions.

4 Conclusions

Different sterilization conditions had a great impact on the changes of nutrition, active components and volatile aroma components in apple vinegar. In general, lower sterilization temperature had little effect on the contents of total acids, total phenols, flavonoids, ascorbic acid and malic acid in apple vinegar, and the contents of volatile aroma components fluctuated slightly. With the increase in sterilization temperature, the content of esters decreased significantly, while the content of acids, alcohols and aldehydes increased, so the sterilization temperature should not be too high to avoid destroying the flavor system of apple vinegar. Combined with the analysis of nutrition, active components and volatile aroma components in apple vinegar under different sterilization conditions, it was concluded that the content of nutrition, active components and volatile aroma components in apple vinegar under 100 ℃, 30 s sterilization conditions had little change compared with other sterilization conditions, so 100 ℃ and 30 s are the optimal sterilization condition.

The organoleptic properties of apple vinegar are produced by the coordination of various organic substances and volatile aroma components. Fifty flavor components were detected in six apple vinegar samples, but only 35 common flavor components were found. This indicates that the content of flavor components in apple vinegar would change greatly under different sterilization conditions, and would affect the quality of apple vinegar. Therefore, the effects of sterilization conditions on apple vinegar and its aroma characteristics need to be further studied in order to provide a more scientific reference for the improvement of apple vinegar quality and the establishment of sensory evaluation system.

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to visit landfills to make students feel the significance of garbage classification. Environmental protection knowledge contests are arranged in the second classroom activities, mainly competitions of some policies and regulations and some measures advocated by local governments, such as *Implementation Plan of Domestic Waste Classification System issued by the General Office of the State Council* [GuoBanFa (2017) 26], and "clean vegetables entering the city" advocated by many cities, so as to guide students to trace the source and explore the realization of garbage reduction and recycling from the source.

Through the design of each link, the ideological and political elements and professional knowledge are fully integrated, and the combination of professional education and ideological and political education is realized.

4 Conclusion

Environmental Microbiology is a professional course of science or engineering, and promoting the construction of ideological and political theories teaching in the course of Environmental Microbiology is related to the development of environmental science and engineering. It is necessary to combine ideological and political education with the cultivation of scientific spirit in course teaching, cultivate students' sense of responsibility and sense of mission of daring to explore the unknown, daring to pursue truth, and climbing the scientific peak, and improve students' ability to correctly understand, analyze and solve problems, cultivate students' craftsmanship spirit of dedication, rigorous work style, excellence, trustworthiness and innovation, and stimulate students to serve the country with patriotism, science and technology and mission. The major of Environmental and Ecological Engineering was set up late in the disciplines of the Ministry of Education, and the development process of professional construction is short. Environmental Microbiology is the basic course of Environmental and Ecological Engineering major, and the construction of ideological and political theories teaching in the course of Environmental Microbiology will help promote the speed and effectiveness of professional construction. The full exploration of ideological and political elements in curriculum, the organic combination of ideological and political theories teaching and professional knowledge, and the integration of ideological and political elements into the whole teaching process before, during and after class will make Environmental Microbiology a bioreactor for professional education and ideological and political education.

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