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Research Article

Quality comparison of non-thermal sterilized raw apple vinegar and commercial apple vinegar products

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Abstract A nonthermally sterilized raw apple vinegar was manufactured using an ultra-fine filtration process (0.2 μm membrane filter) and its quality was comparable to commercially available vinegar products. First, using apple concentrate as a raw material, it was possible to produce non-thermal sterilized Using a two-stage fermentation process of alcohol and acetic acid fermentations, a non-thermally sterilized raw apple vinegar with pH 2.94 and an acidity of 6.20% was produced from an apple concentrate. The fermentation process increased the browning index significantly. However, the fundamental quality parameters of the non-thermal sterilized raw apple vinegar (A) with sterilized apple vinegar (B) did not differ significantly. The pH (2.92-2.95) of apple vinegar (A and B) was higher than that (pH 2.65-2.70) of commercial vinegar (C and D), and the total acidity, which is in the range of 6.20-6.21% and 6.53-6.90%, respectively, was higher in samples C and D than in samples A and B. However, four kinds of organic acids were detected in non-thermal sterilized raw apple vinegar (A), and its total organic acid content (6,245.00 mg%) was significantly higher than that of other samples (B, C, D) ($p<0.05$). In particular, malic acid content, as a main organic acid in apples, was very high in sample (A) (244.83 mg%) and sample (B) (210.21 mg%), compared to commercial products C (125.78 mg%) and D (86.90 mg%). The total polyphenol content and antioxidant activity of fermented apple vinegar (A, B) were more than twice as high as those of commercial products (C, D). Vinegar A had higher total polyphenol content than vinegar B. The above results suggest it is possible to manufacture and commercialize non-thermal sterilized raw apple vinegar with higher organic acid content and antioxidant properties using ultra-fine filtration.

Keywords non-thermal sterilized raw apple vinegar, two-step fermentation, ultra-fine filtration process, quality comparison

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1. Introduction

Apple (*Malus pumila* var. *dulcissima*) is the fruit of a perennial woody plant classified in the Rosaceae family. Apples are one of the most cultivated fruits in Korea (Kim et al., 2021), and contain a variety of functional ingredients such as water-soluble solids, organic acids, vitamins, and polyphenol compounds with biological activity. Apples are known as a health food that prevents degenerative diseases such as arteriosclerosis, diabetes, and high blood pressure, and their consumption is increasing (Shin, 2011; Yun et al., 2007). According to the 2022 Processed Food Market Status (Vinegar Edition), vinegar production in 2021 is 129,000 tons, of which fermented vinegar accounts for 95.8%. Search terms related to 'vinegar' are reported in the order of vinegar efficacy, vinegar raw materials, and use of naturally fermented vinegar (Korea Agro-Fisheries and Food Trade Corporation aT, 2022). In particular, apple cider vinegar is a representative fermented

beverage that has been used since ancient times, regardless of the East or West. It is not only used as an acidulant to improve the taste of food, but is also widely used in medicine and beauty fields (Jeong et al., 1998).

Vinegar is largely classified into synthetic vinegar, fermented vinegar, and others depending on the manufacturing method (Chung et al., 2015). Starting with persimmon vinegar in the 1990s, fermented vinegar has lower acidity than synthetic vinegar, but consumer preference is increasing due to its rich taste and nutritional differentiation (Kwon et al., 2000). Fruit vinegar before the 1990s was brewed vinegar containing a certain amount of fruit juice by diluting alcohol, mixing mineral salts, etc. and fermenting it with acetic acid, and fruit vinegar defined as 100% persimmon fruit (acidity 2.6% or more) (KFDA, 2012).

However, with the revision of the traditional food standard for vinegar in 2020, vinegar according to the Food Code is fermented and manufactured using grains, fruits, and alcoholic beverages as main ingredients. Meanwhile, it is divided into fermented vinegar and diluted acetic acid, which are made by mixing and maturing grain saccharification liquid and fruit juice, and fermented vinegar includes grain vinegar and fruit vinegar (Full text of revision of traditional food standard specifications, April 10, 2020) .

Vinegar is a product with a long shelf life, and foreign substances such as cellulose are generated due to *Acetobacter xylinum* remaining during the storage period and distribution process. This causes the vinegar product to become cloudy and produce sediment, but most microorganisms are controlled through heat treatment during the manufacturing process (Jang et al., 2003). However, during the heat treatment process, the effective components of vinegar are destroyed or the volatile components are reduced, causing the vinegar to lose its flavor, so the development of technology that can replace heat treatment is required. Therefore, research and development has been conducted on methods of combining photocatalysts (TiO2/UVC) and high-voltage pulses (Pulsed Electric Fields, PEF). However, since it is difficult to apply to actual farms or manufacturers, continuous research and development is necessary to develop simpler and more economical non-heat treatment technology (Lee, 2011). Research related to vinegar is limited to research to compare and improve the quality of vinegar, research on raw materials and manufacturing methods, and research on fermentation strains. Therefore, research on improving the quality of vinegar and developing technology using non-sterilizing processes is still insufficient for industrial use.

Therefore, in this study, a non-thermal sterilizing ultra-fine filtration process (0.2 μm membrane filter) is used, which has a simple manufacturing process without a heat sterilization process, has energy saving effects, and can minimize the destruction of the active ingredients of vinegar by heat, thereby providing high-quality, non-thermal sterilizing products. To this end, we attempted to establish a manufacturing process for high-quality non-thermal sterilized raw apple vinegar by comparatively analyzing the physicochemical, microbiological, and antioxidant properties of non-thermal sterilized apple vinegar and commercially available apple vinegar products.

2. Materials and methods

2.1. Materials and strains

Apple concentrate (72 °Brix) was provided by Natural Food Co., Ltd. and stored frozen for use in the experiment. Two types of commercially available apple vinegars (products from Company O and Company D) were purchased at a large supermarket and used as samples (samples C and D). The yeasts used for alcohol fermentation were *Saccharomyces cerevisiae* R12 (KCTC 17798, KMF Co., Ltd., Daegu, Korea) and *Saccharomyces cerevisiae* Fermivin (No. 7013, DSM Food Specialties, Seclin, France) which was purchased from Wine Kit Korea Co., Ltd. (Yesan, Korea). They were used for subculture and seed culture in YPD medium (1% yeast extract, 2% peptone, 2% glucose, 2% agar, pH 6.0) for 24 h at 30℃. The acetic acid bacterium *Acetobacter pasteurianus* KFC 819 is subcultured on solid medium (glucose 3%, yeast extract 0.5%, $CaCO_3$ 1%, ethanol 3%, agar 2%, pH 7.0) at 30℃ for 48 h, then seed cultured and refrigerated at 4℃.

2.2. Manufacture of non-thermal sterilized raw apple vinegar

Apple vinegar was manufactured using apple concentrate as a raw material in two stages: alcohol fermentation and acetic acid fermentation. The first stage of alcohol fermentation is to inoculate 16 °Brix apple concentrate with 5% (v/v) of seed culture and ferment in a constant temperature incubator (JSMI-04C, JSResearch Inc., Gongju, Korea) at 30℃ for 48 h for acetic acid fermentation. In the second stage of acetic acid fermentation, acetic acid bacteria culture is inoculated into the apple alcohol fermentation broth, fermented in a

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shaking incubator (HB-201SL, Hanbaek Scientific Co., Bucheon, Korea) at 30℃, 200 rpm, 18 days, and filtered with a membrane filter (0.2 μm). Thus, non-thermal sterilized raw apple cider vinegar (sample A) was prepared. At this time, apple cider vinegar prepared under the same conditions as above and sterilized at 95℃ for 10 min was used as a sample of sterilized apple cider vinegar (sample B).

2.3. Physicochemical quality measurement during fermentation of non-thermal sterilized raw apple vinegar

The pH of non-thermal sterilized raw apple vinegar was measured at room temperature using a pH meter (STAR-A111, Thermo Fisher Scientific Inc., Waltham, MA, USA). To measure total acidity, 2-3 drops of 1% phenolphthalein indicator were added to 1 mL of sample and then converted to acetic acid by neutralization and titration with 0.1 N NaOH. Browning index was measured by measuring absorbance at 420 nm using a UV-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Sugar content was measured using a digital refractometer (PAL-1, Atago Co., Ltd., Tokyo, Japan). The alcohol content was calculated by distilling 100 mL of fermentation broth using the National Tax Service liquor analysis method and converting the value measured with an alcohol hydrometer at 15℃ to the Gay Lussac Table.

2.4. Analysis of organic acid and free sugar contents during fermentation of non-thermal sterilized raw apple vinegar

Vinegar samples such as non-thermal sterilized raw apple vinegar were treated with a Sep-pak C18 cartridge, filtered through a 0.45 μm membrane filter, and analyzed for organic acid and free sugar contents using a high performance liquid chromatograph (HPLC, Waters 2487, Waters Co., Milford, PA, USA). Organic acids were analyzed using an Atlantis TM dC18 column (3.9×150 mm, Waters Co.) under the conditions of mobile phase 10 mM NaH_2PO_4 (pH 2.7), flow rate 1.0 mL/min, injection volume 10 μL, and the detector was UV (210 nm). Organic acid standards, such as oxalic, malic, citric, tartaric, succinic, lactic, and acetic acid (Sigma-Aldrich Co., St. Louis, MO, USA), were used for the calibration curve to calculate each content (mg%). To analyze the free sugar content, 25 mL of distilled water or 50% ethanol solution was added to each sample, the weight was checked, and the sugars were extracted by heating at 85℃ for 25 min. After cooling, an extraction solvent was added to reach the weight of the initial extraction solvent, and then filtered and used as a measurement sample. The analysis column was an amino (NH_2) column (3.9×300 mm, 10 μm, Waters Co.) and detected at 30℃. The mobile phase solvent (80% acetonitrile) was used for isocratic elution. At this time, the dissolution rate was 1.0 mL/min, the injection volume was 10 μL, and a differential refractometer (RI) detector was used (Kang et al., 2023). Sugar standard substances used were fructose, glucose, maltose, and sucrose (Sigma-Aldrich Co.), and the content (%) was calculated from each calibration curve by analyzing under the same conditions as the sample.

2.5. Determination of physicochemical and microbiological qualities of non-thermal sterilized raw apple vinegar during storage

non-thermal sterilized raw apple vinegar stored at room temperature (20±5℃) for 12 months was used for determining the changes in soluble solids (°Brix. refractometer, PR-101, Atago Co.), pH (pH meter), total acidity (acetic acid content), browning index (UV spectrophotometer), and microbial counts of total aerobic bacteria (TAB), coliforms, and *Escherichia coli* at 2 month intervals. TAB, coliforms and *E. coli* were spread on dry film medium (3M Petrifilm, 3M Co., St. Paul, MN, USA) and cultured at 35℃ for 24-48 h to confirm their stability during storage.

2.6. Determination of total polyphenol content of non-thermal sterilized raw apple vinegar and commercially available vinegar

The total polyphenol content of the sample was quantitatively analyzed according to the method of Choi et al. (2003), which uses the principle that Folin-Ciocalteu reagent is reduced by phenolic compounds in the extract and turns blue. Add 2 mL of 7% Na_2CO_3 solution to 100 μL of each sample, leave for 3 min, add 100 μL of 1 N Folin-Ciocalteu reagent, react for 30 min, and measure absorbance at 720 nm using a UV-visible spectrophotometer (UV-1800). The total polyphenol content was calculated by comparing with the standard gallic acid calibration curve (r^2=0.990).

2.7. Determination of antioxidant activity of non-thermal sterilized raw apple vinegar and commercially available vinegar

To measure the antioxidant activity of a sample, the method of measuring the scavenging ability of free radicals was modified (Blois, 1958). That is, 0.8 mL of 0.004% 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich Co.) dissolved in methanol and 0.2 mL of sample were homogeneously mixed and reacted in the dark for 30 minutes, followed by UV-visible spectrophotometer (UV-1800) was used to measure the absorbance at 517 nm. Antioxidant activity using ABTS radical was measured by modifying the method of Re et al. (1999) by using the principle that ABTS free radicals generated by reacting with potassium persulfate discolor the radical's characteristic blue-green color by antioxidant substances. That is, 7.4 mM of 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich Co.) and 2.45 mM of potassium persulfate were dissolved in distilled water and left in the dark for 12-16 h to obtain $ABTS^{\bullet+}$ cation radical formed.

2.8. Statistical analysis

All the results were expressed as the mean value (mean) and standard deviation (SD) of triplicate experiments, and statistics were analyzed for each experiment result using the statistical program SPSS (V22.0, SPSS Inc., USA). Significant differences between the average values of each experimental group were verified by Duncan's multiple range test ($p<0.05$).

3. Results and discussion

3.1. Selection of yeast strains for non-thermal sterilized raw apple vinegar

To select yeast suitable for alcohol fermentation of apple concentrate, the first stage of alcohol fermentation was performed using *S. cerevisiae* Fermivin, which is known to have excellent alcohol fermentation properties, and *S. cerevisiae* R12, a yeast isolated from persimmon vinegar. The apple concentrate was diluted to 16.00 °Brix, inoculated with *S. cerevisiae* R12 and *S. cerevisiae* Fermivin, respectively, and subjected to alcohol fermentation for 48 h. Table 1 shows the changes in sugar content and alcohol content before and after fermentation. The sugar content of the initial sample fermented by two types of yeast was 15.20-15.30 °Brix, and after fermentation, it decreased overall to 7.30-8.10 °Brix. These results show that when producing grape wine, yeast uses sugar as a substrate to produce alcohol, so after fermentation, the total sugar content decreases and the alcohol content increases. This trend of low residual sugar content and high alcohol content means that active fermentation occurred, which is similar to the results of Kim and Han (2011). The alcohol content before and after fermentation using two types of yeast tended to increase (5.10-5.80%), which was the opposite of the change in sugar content, but the two types of yeast showed a similar pattern. However, the amount of sugar reduction and alcohol increase differ depending on the yeast, and the alcohol production yield compared to the sugar reduction rate was 70.83% and 73.42% in fermentations using *S. cerevisiae* R12 and *S. cerevisiae* Fermivin, respectively, and higher alcohol production yield was confirmed in fermentation using *S. cerevisiae* Fermivin. These results are similar to reports that yeast used in fermentation for vinegar production consumes soluble solids and reduces sugar to produce alcohol (Hwang, 2015). Therefore, in this study, non-thermal sterilized raw apple vinegar was prepared using *S. cerevisiae* Fermivin, which has a high alcohol production yield.

3.2. Changes in physicochemical quality during fermentation of non-thermal sterilized raw apple vinegar

The vinegar manufacturing process can be broadly divided

Table 1. Changes in alcohol and °Brix before and after fermentation using different yeasts

Yeasts	Fermentation stage	°Brix	Alcohol content (%)
S. cerevisiae R12	Before fermentation	15.30±0.00[1)]	0.00±0.00
	After fermentation	8.10±0.00	5.10±0.10
S. cerevisiae Fermivin	Before fermentation	15.20±0.00	0.00±0.00
	After fermentation	7.30±0.00	5.80±0.20

[1)]Values are mean±SD (n=3).

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into the first stage of alcohol fermentation, in which alcohol is produced, and the second stage of acetic acid fermentation, in which acid is produced. During the first stage of alcohol fermentation by inoculating apple concentrate with *S. cerevisiae* Fermivin, the changes in physicochemical quality and free sugar content before and after fermentation are shown in Tables 2 and 3. The pH change before and after alcohol fermentation decreased from pH 3.81 to pH 3.74, and total acidity increased from 0.32% to 0.40%. The change in sugar content before and after fermentation decreased from 14.90 °Brix to 7.30 °Brix. The alcohol content was 0.00% before fermentation, but was 6.00% after fermentation. These results were similar to the results reported by Seo (2001) in which acetic acid fermentation produces organic acid (acetic acid) using alcohol as a substrate, resulting in a decrease in alcohol content and an increase in total acid content. The free sugar content before and after fermentation was highest at 2,071.45 mg% of fructose, followed by 1,133.39 mg% of glucose and 146.08 mg% of sucrose, and maltose was not detected regardless of fermentation. After fermentation, sugar was converted to alcohol by yeast, and the overall free sugar content decreased. In addition, sucrose showed a reduction rate of over 90.0%, confirming that it is used as a main substrate for fermentation. This result was consistent with reports by Kim et al. (2008) and Sung et al. (2014) that yeast's sugar utilization was high in the order of sucrose, glucose, and fructose during the alcohol fermentation process. In order to set the period of acetic acid fermentation, which is the second stage of fermentation, changes in total acidity and pH are shown in Fig. 1. The total acidity at the beginning of the second stage fermentation was 1.02% and pH was 3.62. The total acidity after fermentation was 6.20% and pH was 2.95. As fermentation progressed, total acidity increased and pH tended to decrease in inverse proportion (r^2=0.98), confirming that fermentation proceeded normally. The point at which the total acidity content no longer increased was considered the end point of fermentation, and fermentation was carried out for 21 days. As a result, it was confirmed that it was possible to produce non-thermal sterilized raw apple vinegar by using an ultra-fine filtration (0.2 μm membrane filter) process to sterilize the fermented broth after fermentation without undergoing a heat sterilization process.

Table 2. Changes in °Brix, pH, total acidity and alcohol content during alcohol fermentation of apple juice

	Before fermentation	After fermentation
°Brix	14.90±0.00[1)]	7.30±0.00
pH	3.81±0.01	3.74±0.01
Total acidity (%)	0.32±0.01	0.40±0.01
Alcohol content (%)	0.00±0.00	6.00±0.10

[1)]Values are mean±SD (n=3).

Table 3. Changes in free sugar content during alcohol fermentation of apple juice (unit: mg%)

Sugars	Before fermentation	After fermentation
Fructose	7,738.00±28.12[1)]	2,086.76±39.55
Glucose	3,963.39±16.48	1,124.16±42.54
Sucrose	2,629.42±84.30	142.89±12.72
Maltose	ND[2)]	ND

[1)]Values are mean±SD (n=3).
[2)]ND, not detected.

3.3. Comparison of quality properties of non-thermal sterilized raw apple vinegar during storage

Since the manufactured non-thermal sterilized raw apple vinegar is stored and distributed at room temperature, quality characteristics were compared every two months for 12 months while stored at room temperature (Table 4). Sugar content, pH, and total acidity did not show significant changes in all samples over 12 months. The browning index increased from 0.11 to 0.27 after 12 months. These results

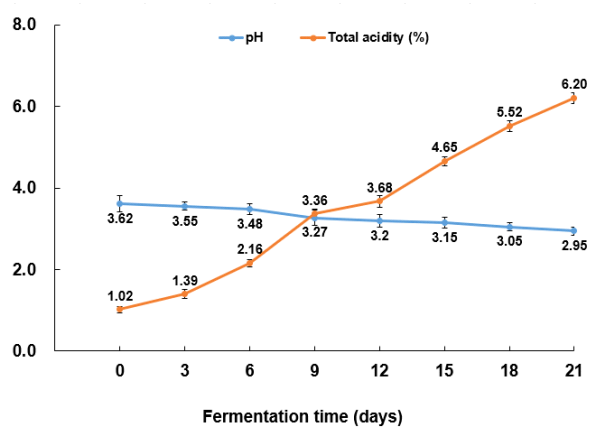

Fig. 1. Changes in pH and total acidity during acetic acid fermentation of apple vinegar. Values are mean±SD (n=3).

Table 4. Changes in °Brix, pH, total acidity, browning index and microbial counts of non-thermal sterilized raw apple vinegar during storage

	Storage period (months)						
	0	2	4	6	8	10	12
°Brix	6.20±0.00[1)NS2)]	6.20±0.00[NS]	6.20±0.00[NS]	6.20±0.00[NS]	6.20±0.00[NS]	6.20±0.00[NS]	6.20±0.00[NS]
pH	2.95±0.00[NS]	2.95±0.00[NS]	2.95±0.00[NS]	2.95±0.00[NS]	2.95±0.01[NS]	2.94±0.01[NS]	2.94±0.01[NS]
Total acidity (%)	6.18±0.00[NS]	6.18±0.01[NS]	6.18±0.01[NS]	6.18±0.00[NS]	6.18±0.00[NS]	6.18±0.00[NS]	6.18±0.01[NS]
Browning index (420 nm)	0.11±0.01[a3)]	0.15±0.00[b]	0.22±0.00[c]	0.26±0.01[d]	0.26±0.01[e]	0.27±0.01[f]	0.27±0.00[g]
Total aerobic bacteria (CFU/mL)	ND[4)]	ND	ND	ND	ND	ND	ND
Coliforms (CFU/mL)	ND	ND	ND	ND	ND	ND	ND
E. coli (CFU/mL)	ND	ND	ND	ND	ND	ND	ND

[1)]Values are mean±SD (n=3).
[2)]NS, not significant.
[3)]Different superscript letters ([a-g]) indicate significant differences ($p<0.05$) by Duncan's multiple range test.
[4)]ND, not detected.

are consistent with the report that the browning degree of commercially available apple vinegars is in the range of 0.022 to 0.327 (Kim et al., 2010). Our findings support the report that browning was severe when alcohol fermentation and acetic acid fermentation were carried out consecutively (Kim et al., 2013). As mentioned above, it was confirmed that there was negligible changes in the physicochemical qualities of non-thermal sterilized raw apple vinegar during the entire storage of 12 months. In addition, the results of testing for total bacteria, coliforms, and *E. coli* were confirmed to be non-detectable, ensuring the microbiological safety of the manufactured vinegar samples. Therefore, the possibility of producing non-thermal sterilized raw apple vinegar with stable physicochemical quality and microbial safety was confirmed without a sterilization process through an ultra-fine filtration process.

3.4. Comparison of physicochemical properties between non-thermal sterilized raw apple vinegar and commercially available apple vinegar

To support the commercialization of non-thermal sterilized raw apple vinegar manufactured in this study, quality comparison with commercial products was conducted. Table 5 shows the results of examining changes in physicochemical quality of manufactured unpasteurized raw apple vinegar (A), apple vinegar that went through a sterilization process (B), and two types of commercially available products (C, D). The manufactured apple vinegars (A, B) had the same sugar content of 6.20 °Brix regardless of whether it was sterilized or not, and the commercially available vinegar (C and D) had 6.90 and 6.60 °Brix, respectively, indicating that the samples A and B had a lower sugar content than samples C and D. The change in total acidity was lower in samples A (6.20%)

Table 5. Comparison of °Brix, pH and acidity among apple vinegars

	Samples[1)]			
	A	B	C	D
°Brix	6.20±0.00[2)c3)]	6.20±0.00[c]	6.90±0.00[a]	6.60±0.00[b]
pH	2.95±0.02[a]	2.92±0.03[a]	2.70±0.02[b]	2.65±0.03[c]
Total acidity (%)	6.20±0.03[c]	6.21±0.03[c]	6.53±0.01[a]	6.29±0.02[b]

[1)]A, non-thermal sterilized raw apple vinegar; B, sterilized apple vinegar; C and D, commercial apple vinegar.
[2)]Values are mean±SD (n=3).
[3)]Different superscript letters ([a-c]) indicate significant differences ($p<0.05$) by Duncan's multiple range test.

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and B (6.21%) than in commercial products C (6.53%) and D (6.29%), but it complied with the vinegar standard (acetic acid content 4.0-20.0%) of the Korean Food Code. These results were consistent with the report (Jeong et al., 1999) that apple cider vinegar manufactured through a two-step fermentation process had higher pH and lower total acidity compared to commercial products. In the production of non-thermal sterilized raw apple vinegar, it was confirmed that sterilization treatment had no significant effect on the sugar content, pH, and total acidity of the vinegar sample.

3.5. Comparison of organic acid content between non-thermal sterilized raw apple vinegar and commercially available apple vinegar

Organic acids in vinegar are a major factor affecting acidity and umami and can be an important indicator of the quality of vinegar. Acetic acid produced by the action of acetic acid bacteria during the vinegar brewing process becomes the standard and standard for vinegar fermentation management (Kim and Han, 2011). The results of comparing the organic acid composition and content of the non-thermal sterilized raw apple vinegar manufactured in this study and commercial products are shown in Table 6. In addition to acetic acid, oxalic, malic, and succinc acids were also detected. The content of acetic acid, which is the standard and standard for fermentation, in each vinegar was the highest at 5,682.39- 5,945.07 mg%, and trace amounts of oxalic acid were detected at 25.83-32.45 mg%. Malic acid is the main organic acid that determines the flavor of apple vinegar, and its content was significantly highest in non-thermal sterilized raw apple vinegar (A) at 244.83 mg%. In samples (B), (C), and (D), 210.21, 125.78, and 86.90 mg% of malic acid was detected, respectively, confirming that the malic acid content of non-thermal sterilized raw apple vinegar is more than twice that of commercially available apple vinegar. Succincic acid content of 77.61 and 81.01 mg% was detected in samples A and B, respectively, but was not detected in commercial products. The total organic acid content of non-thermal sterilized raw apple cider vinegar (A) was 6,245 mg%, the highest among the four samples. These results showed a similar trend to the report by Seo et al. (2001) that acetic acid was the highest organic acid in apple cider vinegar, followed by malic acid. Kwon et al. (2015) reported that malic acid has the greatest effect on the acidity of fruit juice among wines when total acidity and pH are the same, and apples are known to be rich in malic acid. The above results showed that the non-thermal sterilized raw apple vinegar (A) prepared in this study had a significantly higher total organic acid content than the sample (B) that had been fermented using the same method and sterilized. Therefore, it was confirmed that it was possible to manufacture apple vinegar with a richer organic acid content by excluding the sterilization process.

3.6. Comparison of total polyphenol content between non-thermal sterilized raw apple vinegar and commercial apple vinegar

Phenolic substances are natural antioxidants and secondary

Table 6. Comparison of organic acid content in apple vinegars

Organic acids	Samples[1)]			
	A	B	C	D
Oxalic acid	26.55±0.21[2)b3)]	25.42±0.29[d]	25.86±0.06[c]	27.85±0.08[a]
Tartaric acid	ND[4)]	ND	ND	ND
Malic acid	244.83±0.23[a]	210.21±1.38[b]	125.78±0.30[c]	86.90±0.84[d]
Acetic acid	5,896.02±0.71[a]	5,724.27±84.84[b]	5,682.39±30.99[b]	5,945.07±3.53[a]
Citric acid	ND	ND	ND	ND
Succinic acid	77.61±0.23[b]	81.01±1.29[a]	ND	ND
Total	6,245.00±1.38[a]	6,040.91±87.80[b]	5,834.03±31.35[c]	6,059.82±4.45[b]

[1)]A, non-thermal sterilized raw apple vinegar; B, sterilized apple vinegar; C and D, commercial apple vinegar.
[2)]Values are mean±SD (n=3).
[3)]Different superscript letters ([a–d]) indicate significant differences ($p<0.05$) by Duncan's multiple range test.
[4)]ND, not detected.

metabolites widely distributed in plants. Because this compound has a phenolic hydroxyl group, it has the property of binding to proteins and other macromolecules, and has various physiological activities such as intestinal regulation, cholesterol-lowering, anticancer, and antioxidant effects (Kim et al., 2000). In this study, the total polyphenol content of non-thermal sterilized raw apple vinegar and commercial products was investigated (Fig. 2). The total polyphenol content of the manufactured unpasteurized raw apple cider vinegar (A) was 331.68 ppm, which was more than two times higher than that of commercial products (C and D), and was significantly higher than that of the apple vinegar (B) that went through a sterilization process. ($p<0.05$). These results are similar to those of Lee et al. (2012), who compared the total polyphenol content and radical scavenging activity of apple juice according to sterilization temperature, and found that pasteurized apple juice showed significantly higher functional ingredient content and activity, making it suitable for pasteurization. Therefore, it was suggested that a manufacturing method that does not undergo a heat sterilization process in the manufacture of apple vinegar is a method that can minimize the degradation of functional ingredients.

3.7. Comparison of antioxidant activity between non-thermal sterilized raw apple vinegar and commercially available apple vinegar

Representative methods for measuring antioxidant effects include measuring DPPH radical scavenging ability and ABTS cation scavenging ability. The results of comparing the DPPH and ABTS radical scavenging abilities of the non-thermal sterilized raw apple cider vinegar manufactured in this study and commercial products are shown in Fig. 3. The antioxidant activity measured by both methods was more than two times higher in apple vinegar A and B prepared through this two-step fermentation than in commercial products C and D, which was similar to the total polyphenol content. These results are similar to the report by Jo et al. (2012) that the DPPH radical scavenging ability (27.75%) and ABTS radical scavenging ability (36.77%) of commercial apple cider vinegar (total acidity 6.61%) are related to the total polyphenol and total flavonoid contents. Kim et al. (2013) found that vinegar that underwent continuous alcohol and acetic acid fermentation had higher antioxidant properties, and the non-thermal sterilized raw apple vinegar produced in this study showed relatively high antioxidant properties because it was manufactured by fermenting 100% apple concentrate.

4. Conclusions

In this study, non-thermal sterilized raw apple vinegar was manufactured using an ultra-fine filtration process (0.2 μm membrane filter) and its quality was compared with commercially available vinegar products. First, it was possible

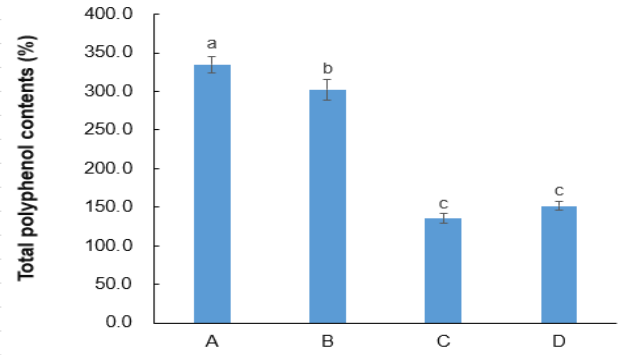

Fig. 2. Comparison of total polyphenol contents among various apple vinegars. A, non-thermal sterilized raw apple vinegar; B, sterilized apple vinegar; C and D, commercial apple vinegars. Values are mean±SD (n=3). Different superscript letters ($^{a-c}$) on the bars indicate significant differences by Duncan's multiple range test ($p<0.05$).

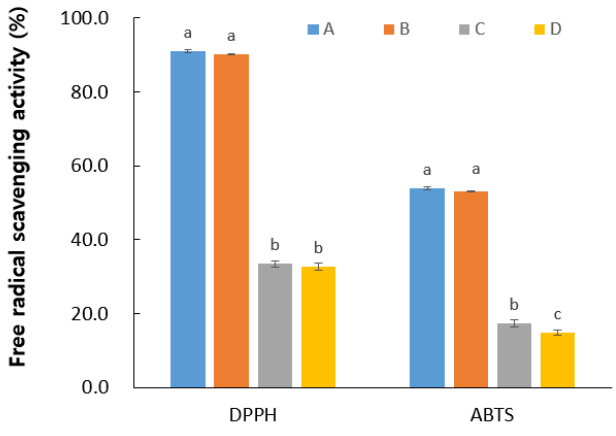

Fig. 3. Comparison of DPPH and ABTS radical scavenging activities in apple vinegars. A, non-thermal sterilized raw apple vinegar; B, sterilized apple vinegar; C and D, commercial apple vinegars. Values are mean±SD (n=3). Different superscript letters ($^{a-c}$) on the bars indicate significant differences by Duncan's multiple range test ($p<0.05$).

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to produce non-thermal sterilized raw apple cider vinegar with pH 2.94 and acidity 6.20% using apple concentrate as a raw material through a two-step fermentation process of alcohol fermentation and acetic acid fermentation. There was no significant difference in basic quality indicators of non-thermal sterilized raw apple vinegar (A) compared to sterilized apple vinegar (B). The pH (2.92-2.95) of the manufactured apple vinegars (A and B) was higher than the pH (2.65-2.70) of the commercially available vinegars (C and D), and the total acidity was ranged from 6.20-6.21% and 6.53-6.90%, respectively, showing it was higher in commercially available vinegar. However, four types of organic acids were detected in non-thermal sterilized raw apple vinegar (A), and its total organic acid content (6,245.00 mg%) was the highest compared to other samples (B, C, D) ($p<0.05$). In particular, the content of malic acid, the main organic acid in apples, was very high in Sample A (244.83 mg%) and Sample B (210.21 mg%) compared to commercial products C (125.78 mg%) and D (86.90 mg%). The total polyphenol content and antioxidant activity of samples A and B were confirmed to be more than twice that of commercial products (C, D). non-thermal sterilized raw apple cider vinegar (A) had a significantly higher total polyphenol content than heated apple vinegar (B). The above results suggest the possibility of manufacturing and commercializing non-thermal sterilized raw apple vinegar with high organic acid content and antioxidant functionality through the use of ultra-fine filtration process in apple vinegar production.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Kim SH, Jeong YJ. Methodology: Kim SH, Seo JH. Writing - original draft: Kim SH, Jeong YJ. Writing - review & editing: Seo JH, Jeong YJ.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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