Effects of the high pressure homogenization on the viability of yeast cell and volatile components in non-pasteurized rice wine

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Abstract This study investigated the effects of high pressure homogenization (HPH) to replace a thermal pasteurization on the yeast cell inactivation and the volatile components of rice wine (RW). By applying HPH (172 MPa with 5 passes), the yeast count was reduced over 4 log cycles. The inactivation of yeast cells by HPH and a thermal processing, i.e. Holder pasteurization (HP), was quantitatively analyzed. In terms of the inactivation of yeast cells, the HPH at 172 MPa with 5 passes was equivalent to the HP at 65°C for 3 min. In RW, 34 volatile components were identified, including 8 alcohols, 18 esters, 2 acids, 1 carbonyls, 3 hydrocarbons, and 2 miscellaneous. Fruity volatiles were found more in HPH samples while components having fatty and oily characters were more detected in HP treated samples.

Keywords: rice wine, high pressure homogenization, yeast inactivation, reduction factor, volatiles

Introduction

Rice wine (RW) is a product of worldwide relevance. Varied products are available in many countries. Among them, *Makgeolli* (or *Takju*) is one of the most popular traditional Korean rice wine (RW) with 6–7% alcohol, and it is popularly produced and consumed in most of the Asian countries in either pasteurized or non-pasteurized form. The nutritious and functional feature of RW differentiates from other alcoholic beverages. RW is highly nutritious and functional since it includes several bioactive components, vitamins, sugars, proteins, and various organic acids (1). Due to its unique properties, studies on the processing and storage of RW has been previously reported (2,3).

The yeast and lactic acid bacteria (LAB) play a major role in the fermentation process of RW. As the growth of LAB dominates the yeast in RW, the final products of RW show its unique taste with lower pH. However, if the yeast becomes the dominant microorganism, the final product will have higher pH values. The unique aroma characteristics of RW is mainly contributed by yeast. The fermentation process is associated with developing the volatile components of RW, such as ethyl acetate, acetaldehyde, alcohols and organic components (4,5). Nevertheless, the negative influences when the yeasts grow more than the optimum level are: the production of biofilm, the generation of CO₂, clouding of brines, and, probably, developing

negatively influenced flavors as well. Especially in the non-pasteurized rice wine (NPRW), the yeast can grow continuously after packing and affects the product quality during storage. Since the yeast in the NPRW continuously produces CO₂, the bottle cap in the final product is often not completely sealed to discharge the gas generated after bottling. It might have higher chance to get contaminated during storage and transportation (6). Thus, it is necessary to control yeast concentration once its fermentative role is completed.

Holder pasteurization (HP), a thermal pasteurization process, has long been recognized as a useful way to extend the shelf life of liquid food by reducing pathogenic cells such as milk, fruit juice, and beer (7,8). In addition, HP is a useful tool to evaluate the thermal microbial inactivation in fluid foods. However, it is inevitable to cause thermal degradation of the heat sensitive components and loss of nutrition in food. Often it leads to reduction of fresh-flavors in many beverages. Especially, designing suitable heat treatment process for RW is very complicated, because the solid particles, which are mainly composed of carbohydrates, in RW became gelatinized during heating. The flow properties after heat treatment are fully changed. Therefore, low-temperature alternatives are highly demanded, and the use of pressure has given particular attention in many food preservations (9).

High pressure homogenization (HPH) has been proven as a non-

thermal technology to reduce number of bacteria and cells without noticeable change of product quality (10–12). HPH disrupts cells by passing fluids containing cells under high pressure through a discharge slit or an orifice valve. The major operating variables to determine the performance of HPH are the pressure and the number of passes through the slit or the orifice valve (13), the temperature of the fluid (14), and homogenizer valve design (15). Cell disruptions in HPH are not related to the specific location of cell walls. Especially for yeast the impingement is a main mechanism to destroy yeast cells (16). Although few studies on inactivation microbial cell using HPH were reported, HPH has not been applied to reduce yeast count in the RW.

HP in a small scale efficiently reduces the thermal degradation, especially for minimizing the flavor changes after thermal treatment. The effect of the microorganism inactivation of the heat treatment (i.e., HP) and the non-thermal treatment (i.e., HPH) should be compared quantitatively for the industrial use of HPH. Generally, D-value and z-value are used to characterize the thermal processing. Although the antimicrobial effect of multiple-pass HPH treatments has been reported (17,18), HPH has not been applied in the RW. In addition, the effect of non-thermal treatment has not been quantitatively compared with that of thermal treatment.

Applying the HPH to RW is worth to be investigated since HPH could be a non-thermal processing which may avoid thermal degradation of RW while reducing the yeast count. The objectives of this study were to 1) investigate the effect of HPH on the reduction of the concentration of yeast cell in the RW, 2) to compare the degree of inactivation level from HP and HPH, and 3) to compare the flavor characteristics after applying HPH and HP.

Materials and Methods

Rice wine (RW) The RW was prepared in a semi-pilot scale fermentation system according to Park *et al.* (19). Cultivation of *Saccharomyces cerevisiae* (1 g) was conducted on koji (0.1 kg) with 0.13 L of distilled water at $26 \pm 1^\circ\text{C}$ for 4 days (19). The first-stage of fermentation was processed by mixing koji (0.8 kg) and distilled water (1.2 L) with fermented mother brew. After agitating, the mixture was fermented at 26°C for 2 days. The second-stage of fermentation was processed by mixing first-stage mother brew, cooked rice (2.4 kg), water (3.6 L), and purified enzyme (1.2 g). Then the mixture was fermented at 26°C for 5 days. The fermented liquor was ground and the solid particles were screened through a 100-mesh sieve. The screened sample was diluted with distilled water and the alcohol level was adjusted to 6%. The pH of the sample was 4 (± 0.2). Samples were stored at 4°C during the experiment. RW samples manufactured on the same day were used in each experiment.

High pressure homogenization (HPH) A high pressure homogenizer

(Picomax-MN400; Micronox, Seongnam, Korea) was used to homogenize RW samples. The fluid was pressurized and passed in the interaction chamber. The pressure drop was maintained during homogenizing by passing the liquid through a tube (length 90 mm, diameter 35 mm). The homogenizer was warmed up with cold tap water and operated at 100 MPa for 20 min to ensure the stability before introducing samples. Then, samples were introduced into the equipment at 10°C and were collected after water was completely substituted by each different RWs. The processing temperature (10°C) was chosen because most of RW are distributed in a cold-chain system which strictly control the storage temperature under 10°C . The residence time of RW inside the homogenizer was 15 s. The maximum outlet temperature was about 30°C and quickly cooled using an ice bath just after the homogenization. The HPH process can repeatedly be performed by consecutive pass of the fluid in the high shear fluid processor. In this study, the number of the pass (PN) and the applied pressure (P) were varied to investigate the effect of HPH on the reduction of microbial. The P was varied at 48, 69, 103, 138, and 172 MPa. The PN was varied 1, 2, 3, 4 and 5 at each P. Experiments were done in triplicate.

Pasteurization in the tube: Holder pasteurization Pasteurization of RW was undertaken, using the Holder pasteurization (HP), also called low temperature long time (LTLT) pasteurization, technique, following the procedure of the Human Milk Banking Association of North America (HMBANA) (20). The HP is used to evaluate the effect of thermal processing on the small amount of samples without applying high thermal energy for pasteurization. The HP is considered as a thermal process requiring the least thermal energy. The HP is widely applied to evaluate the changes of bioactive components with a minimum amount of thermal energy. In this study, the HP was used to compare the degree of inactivation of yeast cells after applying HPH. A small lab scale of HP system was built, and 10 mL of RW was placed in 13 mL polypropylene tubes. The tube containing RW were vertically submerged in the temperature controlled water bath at 50, 55, 60, and 65°C . The water level was reached approximately 3 cm over the level of RW. The same amount of RW was filled in all tubes used in the experiment. The temperature of RW was monitored using a thermocouple (Vernier, Beaverton, AL, USA) during heat processing. The thermocouple was located at a position of 15% of the RW volume, where usually indicates the cold point in a cylindrical geometry (21). Once the temperature had reached the target pasteurization temperature, the heat treatment continued for 20 to 40 min while maintaining the target temperature. The RW temperature was monitored and recorded. After the thermal processing, the RW was quickly cooled down to $10 (\pm 1)^\circ\text{C}$ in an ice bath. All settings were done in triplicate.

Evaluation of the equivalency of inactivation degree of HPH to HP Yeast was cultivated in the incubator at 20°C for 68 h. During culturing, anaerobic condition was maintained. A 3M™ Petrifilm™

yeast and mold count plates (3M, Minneapolis, MN, USA) was used for yeast culturing, and the colonies were blue-green and formed small defined colonies based on the 3M directions. The viable cell counts of yeast were determined by colony forming unit (CFU)/mL in triplicate independent experiments.

To assess sub-lethal injury caused by each treatment, the reduction factor was introduced. The reduction factor in log scale (log RF) was evaluated as:

$$\log \text{RF} = \log \left(\frac{\text{CFU before treatment}}{\text{CFU after treatment}} \right)$$

Polarizing Microscope To compare the degree of disruption of yeast caused by treatment, morphological characteristics of yeast were observed using a high resolution polarizing microscope (Axio Skop IIL; Carl Zeiss Vision KK Co., Gottingen, Germany). The diluted RW samples were dyed with methylene blue solution and then prepared for examination by polarizing microscopy.

Analysis of volatile components Volatile flavor components of RW were studied by solvent assisted flavor evaporation (SAFE) and gas chromatography-tandem mass spectrometry (GC-MS/MS). Re-distilled methylene chloride (60 mL) was used to extract volatile components in RW (30 mL). Dodecanoic acid methyl ester (0.1 mL of 100 ppm, v/v, in dichloromethane) was used as an internal standard. After adding the internal standard, the sample was mixed with a magnetical stirrer at 3,000 rpm for 10 min and then filtered (paper No. 41; Whatman, Maidstone, UK) under a vacuum. High-vacuum sublimation (HVS) was used to separate volatile components from the non-volatiles. HVS was operated below 10^5 Torr (22). The concentrated sample (0.1 mL) for GC-MS analysis was prepared by dehydrating over anhydrous sodium sulfate (ASS) using a Vigreux column at 40°C. The analytical conditions of GC-MS (Agilent 7890A gas chromatography-5975 mass selective detector, GC-MSD; Agilent Technologies Inc., Palo Alto, CA, USA) are described in Table 1. Authentic standard components were used to identify the volatile components in RW by comparing their mass spectra and RIs. The Wiley 275 mass spectral database was used to identify the components of which the standards are not available (23). As external references to estimate the RI of each component, n-paraffins C₇-C₂₂ were used (24). All key odorants were positively identified by comparing linear retention indices (RIs), their mass spectra, and aroma characteristics recognized at the sniffing port with those of authentic standards. The semi-quantitative analysis of volatile components was performed by comparing their peak areas to that of the internal standard component (0.1 mL of 1,000 ppm 2-ethyl-1-hexanol, w/v) on the GC-MS total ion chromatogram.

Statistical analysis The results were presented as the average and standard deviation (SD) and evaluated using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) with Duncan test

Table 1. Operating condition of GC and GC-MS for analyses of volatile components

Column	DB-5 (30 m length, 0.25 mm i.d. × 0.25 µm film thickness)
Carrier gas	Helium 0.8 mL/min, constant flow
Inlet temp.	230°C
Transfer line temp.	250°C
Oven temp.	40°C, 5 min → 4°C/min → 120°C → 16°C/min, → 220°C, 10 min
Injection volume	1 µL
Split ratio	10:1
Mass scan range	35-550 m/z
Scanning rate	2.86 scan/sec
Ionization energy	70 eV

was used to determine statistical significance ($p < 0.05$).

Results and Discussion

Effect of HPH on viability of yeast To assess the influence of operating pressure and PN for loss of viability, the changes of yeast viable cell counts by each treatment are shown in Fig. 1. As regards to the effect of pressure on the yeast cells in RW, the reduction of yeast cells at 1 PN appeared insignificant for homogenizing pressure up to 138 MPa, because a reduction less than 1 log value of CFU is not relevant microbiologically or regarding processes. The maximum reduction at 172 MPa was ca. 1 log value of CFU (Fig. 1A).

The effects of the PN at different pressures on the reduction of yeast cells were shown in Fig. 1. The reduction rate of yeast cells by the PN showed applied pressure dependence. When the pressure is lower than 69 MPa, the reduction was not significant, but at >103 MPa the reduction rate dramatically increased. At 172 MPa, each pass clearly showed a log scale reduction of yeast cells. At the PN=5 at 172 MPa, the reduction of yeast cells was over ca. 4 log cycles. It clearly demonstrated that the effect of pressure was not significant to reduce the yeast cells in RW, but as the PN increased, the reduction dramatically increased. The microscope images of the intact yeast and post-homogenization disrupted yeast were illustrated in Fig. 2. The polarizing microscope experiments indicated that a large number of yeast was considerably disrupted after 3 passes of homogenization at 172 MPa.

To evaluate the reduction of viability caused by HPH treatment, the reduction factor (eqn. 1) was introduced. The reduction factor (log RF) can quantify the effect of the operating variables such as pressure and the PN on the reduction of yeast cells in RW. The slopes of each treatment indicated the magnitude of reduction of yeast cells using the pass of HPH at different pressures. In Fig. 3, the log RF clearly increased as the PN increased at each pressure. In addition, the higher pressure showed, the higher slope reflecting the rate of

Fig. 1. Changes of yeast viable cell counts by pressure treatment at 1 pass. (A) Changes of yeast viable cell counts by number of pass. (B) 48 MPa, (C) 69 MPa, (D) 103 MPa, (E) 138 MPa, (F) 172 MPa.

reduction of yeast. Our study demonstrated that increasing operating pressure was thus concluded to improve significantly the efficiency of HPH in disrupting the yeast cells. It has been observed that several successive passes of HPH cause an additive effect on the reduction of viability of cells. Recently a similar result was reported that the microalgal cells to produce biofuel were significantly disrupted by successive passes of fluid through the HPH (25). Like a similar study, the HPH was applied to regulate the growth of foodborne molds in tomato juice (26). However, until the homogenization pressure up to 150 MPa, the inactivation of the molds in tomato juice was under 1 log unit by a single homogenization treatment, which is an unsatisfactory reduction level for food pasteurization. The inactivation level significantly increased to nearly 3 log units after applying 3 passes of homogenization. The inactivation level may be further increased by applying successive treatments. Indeed, the efficiency of a multi-step processing of HPH was found in many food applications (27,28). The reduction of cells using HPH might be non-specific so that disruption characteristics such as P or PN vary according to microbial species (28,29). Engler and Robinson (30) reported that for the yeast cells the impingement is a major mechanism of disruption. The cell disruption is attained through the high-pressure impingement of high-speed cellular jet on the static valve surface as well as through the shear stress caused by the pressure drop of fluid that the cell suspension experiences as it passes from the valve to the chamber (25). Generally, disruption of yeasts is easier than bacteria because of different cell wall structure and their larger size. These results were

successfully confirmed by Bevilacqua *et al.* (11). It clearly demonstrated that the HPH for RW is very effective to reduce the cell viability by disrupting cell walls.

Effect of thermal treatment on the inactivation of yeast To investigate the effect of thermal treatment on the inactivation of yeast in the RW (pH 4 ± 0.2), the tubes containing the RW were thermally treated in the temperature region (50 to 65°C). The prototypical thermal inactivation curves showing linear slopes between the log scale of yeast numbers and the time for the thermal treatment were obtained (Fig. 4). The slope of each curve indicates the D-value of yeast at different temperatures. The D-value of yeast is 28 min at 50°C, and it decreased to 4.18 min at 65°C. The z-value of yeast used in this study was estimated to be 19.1°C. However, these values do not properly represent the inactivation kinetic of heat treatment, because significant CUT reductions were observed during the thermal treatment. The effect of CUT on the inactivation of yeast cells significantly increased, as the pasteurization temperature increased.

Comparison of HPH and HP The reduction factor of HPH was compared with that of HP, which includes the effect of the CUT on the inactivation of yeast to determine the equivalent degree of microbial inactivation. A multiple linear regression analysis was employed using the LINEST functions in Microsoft Excel (version 2013, Microsoft Corp, Redmond, WA, USA) to develop models for 1)

Fig. 2. Microscopic images of the yeast: intact cells (A) and disrupted cells after three (B) and five (C) passes through the high shearing homogenizer. Bar: 25 μ m.

Fig. 3. Comparison of the reduction factor and regression model for yeast reduction at different treatments. (A) HPH, (B) HP.

the reduction factor of HPH as a function of the pressure (P) and the number of passes (PN), and 2) HP as a function of temperature (T) and time (t) as follows:

$$\begin{aligned} \log RF_{\text{HPH}} &= 3.18\text{E-}05(P) - 1.42\text{E-}01(PN) + 3.38\text{E-}05(P \cdot PN) - 7.39\text{E-}02 \quad (2) \\ \log RF_{\text{HP}} &= 0.22(T) - 0.23(t) + 0.01(Tt) - 10.58 \quad (3) \end{aligned}$$

Experimental data ($\pm$ standard deviation) and the regression model appear in Fig. 4. The regressions have an R^2 of 0.98 and 0.95, respectively.

Fig. 4. Survival curves of yeast in RW at different temperatures (50–65°C) in tube heating to determine the D-values (A) and z-value for yeast in rice wine (B).

Based on our analysis, the log RF value at 60°C for 19 min was equivalent to those for 3 min at 65°C. In addition, the degree of inactivation at 60°C for 19 min is equivalent to that after homogenizing for 5 passes at 172 MPa. Our approach is applicable even when the initial number of microorganism are different, and the degree of inactivation could be controlled by adjusting the temperature and time, or pressure and the number of passes.

Analysis of volatile components In our study, the chemical analysis to compare the thermal treatment and HPH were conducted using

Table 2. Volatile flavor components of RW by GC and GC-MS

No.	RI ¹⁾	Components	Relative Peak Area (%) ²⁾			Odor Description ³⁾ (Aroma Threshold value)
			GC1	GC2	GC3	
Alcohols						
1	<800	3-methyl-1-butanol	8.169±0.749	7.930±0.712	7.847±0.203	fermented, malt, wine (300 ppb)
2	<800	2-methyl-1-butanol	0.838±0.144	0.398±0.240	1.024±0.057	fermented, malt, wine (300 ppb)
3	<800	1,3-butanediol	0.152±0.007	0.145±0.032	0.115±0.017	solvent flavors
4	<800	2,3-butanediol	0.018±0.006	0.019±0.004	0.010±0.009	green, buttery (150,000 ppb)
5	837	3-ethoxy-1-propanol	0.544±0.059	0.575±0.082	0.569±0.136	
6	978	3-(methylthio)-1-propanol	0.163±0.014	0.167±0.022	0.158±0.012	vegetable, onion, cooked potato odor (500 ppb)
7	1118	benzeneethanol	7.123±0.599	6.749±0.144	7.694±0.020	floral, sweet, green (750-1,100 ppb)
8	1505	2,4-bis(1,1-dimethylethyl)-phenol	0.011±0.002	-	0.022±0.007	
Esters						
9	<800	isobutyl acetate	-	0.025±0.001	0.025±0.001	fruity, banana-apple-pear-pineapple notes (66 ppb)
10	802	ethyl butyrate	0.023±0.007	0.016±0.000	0.014±0.001	fruity odor (1ppb)
11	811	ethyl lactate	0.102±0.008	0.123±0.012	0.114±0.016	rum-buttery odor, cookie-like (14,000 ppb)
12	876	isoamyl acetate	0.194±0.023	0.212±0.006	0.185±0.010	sweet, fruity, banana, pear odor (2 ppb)
13	933	ethyl 3-hydroxybutyrate	0.038±0.003	0.041±0.004	0.038±0.003	fresh, fruity, grape-like odor
14	998	ethyl caproate	0.028±0.001	0.036±0.006	0.027±0.008	fruity, pineapple, banana with strawberry, pear & tropical notes (1 ppb)
15	1057	ethyl-4-hydroxybutanoate	1.244±0.050	1.192±0.114	1.325±0.175	
16	1177	diethyl succinate	0.020±0.005	0.017±0.001	0.021±0.002	winey, ethereal, green, grape-like odor
17	1193	ethyl octanoate	0.036±0.001	0.041±0.001	0.017±0.002	fruity, winey, sweet odor
18	1257	phenethyl acetate	0.092±0.007	0.094±0.002	0.094±0.001	sweet, rose, fruity, honey like odor (3,000-5,000 ppm)
19	1393	ethyl decanoate	0.061±0.016	0.117±0.012	0.071±0.009	sweet, fatty, nut-like, winey-cognac odor
20	1441	ethyl 1-hydroxy-3-phenylpropanoate	0.021±0.018	-	0.039±0.006	
21	1590	ethyl dodecanoate	0.051±0.028	0.054±0.008	0.060±0.010	mild fatty-waxy oily, floral fruity odor
22	1753	2-oxohexanedioic acid	0.223±0.021	-	0.021±0.009	nearly odorless
23	1795	ethyl tetradecanoate	0.110±0.104	0.056±.009	0.127±0.024	mild sweet, waxy-fatty odor
24	1991	ethyl hexadecanoate	0.360±0.236	0.123±0.018	0.346±0.044	faint, waxy, sweet odor (>2000 ppb)
25	2150	ethyl linoleate	0.021±0.007	0.015±0.002	0.048±0.010	mild oily fatty odor
26	>2200	ethyl oleate	0.031±0.006	0.020±0.002	0.072±0.004	weak, mild, sweet, oily odor
Acids						
27	1171	octanoic acid	0.062±0.007	0.011±0.003	0.031±0.008	fatty, rancid, animallic, sweaty odor (3,000 ppb)
28	1367	decanoic acid	0.030±0.002	-	-	fatty
Carbonyls						
29	907	dihydro-2(3H)-furanone	0.031±0.002	0.035±0.003	0.037±0.005	
Hydrocarbons						
30	1197	dodecane	0.018±0.007	0.012±0.001	0.012±0.005	hydrocarbon odor
31	1398	tetradecane	0.009±0.000	0.006±0.001	0.009±0.001	hydrocarbon odor
32	1473	heptadecane	0.027±0.004	0.025±0.003	0.040±0.023	hydrocarbon odor
Miscellaneous						
33	866	xylene	-	0.011±0.001	0.012±0.003	plastic (0.052-86 ppb)
34	1250	1,3-bis(1,1-dimethylethyl)benzene	0.131±0.008	0.185±.010	0.189±0.107	

¹⁾Retention indices were determined using *n*-paraffins C₇-C₂₂ as external standards.²⁾Relative peak area (peak area of component/peak area of internal standard)³⁾Flavor-Base 2010 Professional, IFFINGWELL & ASSOCIATES; Aroma threshold value is odor threshold in water.

samples treated 5 passes for 172 MPa and 3 min at 65°C. The results of GC analysis for volatile components in RW were shown in Table 2. In Table 2, GC1, GC2, and GC3 indicated the samples from the control, i.e. RW without applying HPH or pasteurization, HPH, and pasteurization, respectively.

Thirty-four volatile components were identified by GC-MS in RW (Table 2) and were grouped as 8 alcohols, 18 esters, 2 acids, 1 carbonyl, 3 hydrocarbons, and 2 miscellaneous components. Qualitative differences were observed in isobutyl acetate, ethyl 1-hydroxy-3-phenylpropanoate, and decanoic acid. Quantitative differences were found in 2-methyl-1-butanol, benzenethanol, ethyl butyrate, ethyl octanoate, ethyl decanoate, ethyl tetradecanoate, ethyl linoleate, and ethyl oleate. High contents of 3-methyl-1-butanol and benzene-ethanol were detected in all samples. 3-Methyl-1-butanol (banana-like aroma) is the main constituent of fusel oil. They form in the course of alcoholic fermentation as by-products of yeast metabolism and serve as flavor and aroma carriers in beer, wine, and spirits (31).

Benzenethanol occurs widely in nature, being found in a variety of essential oils. 3-methyl-1-butanol were detected a similar quantity in GC2 and GC3, but benzenethanol with floral, sweet, green flavor characteristics was found relatively lower levels in GC2. Relatively low amount of 2,3-butanediol(DL), an aliphatic dihydric alcohol usually found in the fermented beverages, such as beer, rice wine, and wine, were detected in GC3. Yeast mainly forms 2,3-Butanediol known as one of the characteristic orders of RW with slightly bitter taste during the carbohydrate fermentation process (32).

A group of esters was identified as wells. Ethyl decanoate, isoamyl acetate, and ethyl caproate which show floral and fruity characters were found in all samples but particularly higher amount were found in GC2 than in GC3. These components positively contribute to the overall quality of RW, and the fruity and floral sensory properties of RW are highly related to these components. Esters, such as ethyl caproate and isoamyl acetate, are widely found in a variety of food products (33). In fermented beverages such as wine and beer, they are frequently in trace amounts, such that individually they are often below aroma threshold concentrations. As such, esters are extremely important for the flavor profile of fermented beverages (34).

The highest amount of ethyl lactate generally having cookie-like and rum-buttery characters was detected in the GC3 followed by GC1 and GC2. GC2 showed the lowest level of ethyl tetradecanoate, ethyl linoleate, and ethyl oleate which generally show oily and fatty characters. Relatively higher amount of these components were detected more in GC3 compared to GC2 and GC1. According to our analysis, GC3 showed relatively higher amount of volatile components having oily, fatty and waxy characters. In addition, ethyl oleate and ethyl linoleate which found in GC3 are generally associated with the odor descriptor “rancid” and it may cause a negative impact on the quality of RW during storage and distribution.

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