Dealcoholization of Korean traditional rice wine, *takju*, combining thermal, bentonite clarification or reverse osmosis treatment

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Abstract The objective of this study was to prepare low-alcoholic *takju*. Low-alcohol (3.06%) and dealcoholized (0.04–0.28%) *takju* samples were prepared by adopting bentonite clarification, heat and reverse osmosis treatments. The dealcoholization of *takju*, in most cases, significantly ($p < 0.05$) reduced the total mineral (from 505.80 to 258.61 mg/L), free amino acid (from 989.81 to 293.58 mg/mL), antioxidant potentials and total phenolic (from 345.30 to 156.30 μg GAE/mL) contents remained in the control to RO-3 (*takju* sample obtained from 3-time reverse osmosis and heat treatment). Similarly, the content of volatile compounds was also significantly ($p < 0.05$) decreased with the dealcoholization treatments. The finding of this study clearly showed that dealcoholized *takju* could be prepared but at the expense of reduced nutritional and

antioxidant potentials. This study could be an exemplary in promoting traditional beverages in different countries by producing low or alcohol-free drinks.

Keywords Bentonite · Dealcoholization · Heat treatment · Reverse osmosis · *Takju*

Introduction

Takju, also known as *Makgeolli*, a famous Korean traditional turbid rice wine, is fermented with cereals, mostly glutinous rice. Nuruk, a fermentation starter, containing yeast and several types of fungi is essential for *takju* fermentation. *Takju* is a low-alcohol beverage with an alcohol concentration of 6–8%. It is rich in protein and carbohydrates and also possesses a small amount of organic compounds. The consumption of *takju* is increased in Korea.

In the recent years, global demand of the alcohol-free beer and wine is increasing due to increased awareness of healthy lifestyle, realization of moderate consumption of alcohol and also due to religious reasons. Consumption of such alcohol-free beverages will let one have the benefits of consuming beer or wine but avoid the disadvantageous alcohol content. Despite the increasing worldwide demand, producing such a low alcoholic ($< 3\%$ v/v) or alcohol-free ($< 0.5\%$ v/v) high quality wine is quite challenging although there are different pre-production and post-production ways to do so. The main challenge is to preserve the volatile aroma compounds, which are key factors to impart delicious flavor to the wine.

Many techniques, including bentonite clarification (Armada and Falqué, 2007) have been implied to produce low alcohol beverages. Heat treatment techniques of dealcoholization comprise evaporation and distillation under

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vacuum conditions in order to reduce the loss of aroma and flavor compounds. Further, for a large scale dealcoholization, vacuum evaporation is the most economic process among the physical methods adopted (Brányik et al., 2012). Although the thermal treatment techniques can cause a loss of organoleptic properties, nearly a complete removal of alcohol could be possible with them. Reverse osmosis (RO) is a promising alternative for dealcoholization because it can remove alcohol under mild temperature (Wenten and Khoiruddin, 2016) and thus could preserve the taste and nutritive components of the dealcoholized beverages. RO technique has been employed in removing alcohol from different fermented beverages, including wine (Gil et al., 2013).

There is a significant increase in the demand of non-alcoholic beverages. The consumption of traditional beverages like *takju* is also increased. Although several research works have been reported on dealcoholization of non-traditional wines, very limited scientific reports on the traditional wine have been published. So far it is known, this is the first report on dealcoholization of *takju*. The objectives of this study were to produce low-alcohol *takju* samples adopting bentonite clarification and RO techniques combined with heat treatment and also to investigate their physicochemical characteristics. It was hypothesized that the proposed treatments could be effective to maintain the typical characteristics of *takju* while reducing its alcohol concentration.

Materials and methods

Chemicals and reagents

All the standard chemicals and reagents; including folin-ciocalteu, phenolphthalein, and DPPH (1,1-diphenyl-2-picrylhydrazyl); were purchased from Sigma-Aldrich (St. Louis, MO, USA). *n*-Alkane standards (C₈–C₂₀ and C₂₁–C₄₀; Fluka, Steinheim, Switzerland) were used to determine the retention index and 3-octanol (Fluka, GA, USA) was used as an internal standard. Sodium sulphate was obtained from Junsei (Tokyo, Japan) and HPLC-grade solvents from J.T. Baker (Phillipsburg, NJ, USA).

Sample collection and preparation

Takju was purchased from a local store in Deagu, Korea. Alcohol content of the sample was 6–6.5%. *Takju*, typically, consists of suspended particles, which gives it a turbid appearance. The sample was kept standstill at 4 °C for 24 h for sedimentation before further processing. The sedimentation process separated the sample into two layers: a less turbid upper layer (cleared *takju*) and the other

layer of suspended particles on the bottom (uncleared *takju*). The commercial *takju* sample comprised of cleared *takju* and unclear *takju* at a ratio of around 8.5:1.5 (v/v).

The cleared *takju* was further subjected to bentonite or reverse osmosis (RO) treatment, whereas the unclear *takju* was allowed for heat treatment. There are different temperature and time combinations reported for heat treatment of beverages to reduce alcohol concentration. Since at low temperatures the dealcoholization process is not efficient while at high temperatures the antioxidant potential of wine is highly deteriorated. Therefore, in order to maintain a relatively optimum condition for an efficient dealcoholization, in the present study, the unclear *takju* was subjected for heat treatment at 60 °C for 20 min in order to reduce alcohol concentration. The heat-treated unclear *takju* was named as HT-0. Bentonite treatment was carried out to reduce the alcohol concentration (Villaño et al., 2006) with some modifications. Bentonite powder was added to the cleared *takju* at a ratio of 1:99 (w/v) and mixed at room temperature for 5 min using a shaking incubator (120 rpm). The mixture was sedimented for 6 h and then filtered through a filter paper (Whatman No. 4) using vacuum filtration. The filtrate sample was termed as BT-0. In the RO treatment, the cleared *takju* was subjected to RO system consisted of a diaphragm pump (HS-R130, Hana Science Co. Ltd., Seoul, Korea), prefilter (HS-PF1001C, Hana Science Co. Ltd.) and a RO polyamide membrane (HS-CR0125G, Hana Science Co. Ltd.). Repeated RO treatments, adopting the same system, were conducted to prepare different *takju* samples. In addition, in order to maintain the turbidity, one of the fundamental attributes of *takju*, a portion of HT-0 was also added to the final product.

The different varieties of *takju* samples prepared in this study were named as—control: *takju* obtained from a local store; BT-1: *takju* containing 85% of BT-0 and 15% of HT-0; RO-1: *takju* containing 85% of the sample obtained from 1-time RO and 15% of HT-0; RO-2: *takju* containing 85% of the sample obtained from 2-time RO and 15% of HT-0; and RO-3: *takju* containing 85% of the sample obtained from 3-time RO and 15% of HT-0. The samples (BT-1, RO-1, RO-2, and RO-3) were prepared by simply mixing the unclear *takju* (HT-0) and cleared *takju* at the proportions mentioned above. All the *takju* samples were stored at 4 °C until subsequent analyses.

pH, titratable acidity, alcohol, soluble solid content, and color measurements

The pH value of *takju* was measured using a pH Meter (Model 250; Beckman Coulter, Inc., Fullerton, CA, USA). Titratable acidity (TA) was measured following the method described earlier (Lee et al., 2017). A 9-mL of sample was

added to 10 mL of distilled water and titrated with 0.1 N sodium hydroxide using phenolphthalein as an indicator. Alcohol concentration analysis was conducted following the method described by Ough and Amerine (1988). The total soluble solid (TSS) was measured using a following a standard method described earlier (KFCT, 2006). For the color measurement, L^* (lightness), a^* (redness, + or greenness, -), and b^* (yellowness, + or blueness, -) values of *takju* samples were determined using a Chroma meter CR-300 (Minolta Chroma meter CR-300, Minolta Corp., Osaka, Japan). A Minolta calibration plate (YCIE = 94.5, XCIE = 0.3160, YCIE = 0.330) and a Hunter Lab standard plate ($L^* = 97.51$, $a^* = -0.18$, $b^* = +1.67$) were used to standardize the instrument with D65 illuminant (Kim et al., 2014).

Determination of mineral content

Mineral content of the *takju* samples was determined following the method described by Skujins (1998). The sample (0.5 mL) and HNO_3 (15.0 mL) were mixed and the mixture was diluted with equal volume of distilled water. The concentration of different minerals was determined using an inductively coupled plasma atomic emission spectrometer (ICP AES: Varian Vista, Varian Australia, Victoria, Australia). The instrument was calibrated using known standards for each mineral element.

Determination of free amino acid content

Free amino acid profile was determined following the procedure described by Je et al. (2005). A 1-mL of sample was hydrolyzed with 10 mL of 6 N HCl in a sealed-vacuum ampoule at 110 °C for 24 h. The HCl was removed from the hydrolyzed sample using a rotary evaporator and the content was mixed with 0.2 M sodium citrate buffer (pH 2.2) to make a volume of 5 mL. The mixture was passed through a Sep-Pak C_{18} cartridge (Waters Co., Milford, MA, USA) and filtered through a 0.22 μm membrane filter (Millipore, Billerica, MA, USA). The amino acid content of samples was determined using an automatic amino acid analyzer (Biochrom-20, Pharmacia Biotech Co., Uppsala, Sweden).

Determination of antioxidant potentials

The *takju* samples were subjected to freeze drying and different concentrations (5, 10, 15, 20, and 25 mg/mL) of extracts were prepared with absolute methanol.

Determination of DPPH radical scavenging activity

The DPPH free radical scavenging activity of sample was measured following the method described by Blois (1958).

A 1-mL sample was mixed with 10 mL of absolute methanol to prepare sample solution. The mixture was centrifuged (3000 rpm, 15 min), and the supernatant was filtered through a 0.2- μm syringe filter (Waters Co., Milford, MA, USA). Equal volumes of the sample solution (0.1 mL) and the freshly prepared 0.1% (w/v) DPPH solution in methanol (0.1 mL) were kept into microplate wells and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer (UV-1700, Shimadzu, Tokyo, Japan). Equal proportions of methanol and the sample solution as well as methanol and DPPH solution were mixed to measure the absorbance of control and blank, respectively. The DPPH radical-scavenging activity was calculated from the absorbance values using the following equation

$$\text{Scavenging activity}(\%) = [1 - (A - A_b)/A_o] \times 100$$

where A_o is the absorbance of methanol and DPPH solution without sample (blank), A is the absorbance of sample solution and DPPH solution, and A_b is the absorbance of sample solution and methanol (control).

Determination of superoxide anion scavenging activity

Measurement of the superoxide anion scavenging activity was based on the method described by Li (2012). A mixture of 0.3-mL of sample and 2.61 mL of 50 mM phosphate buffer (pH 8.24) was added into the freshly prepared 90 μL of 3 mM pyrogallol (Sigma-Aldrich, St. Louis, MO, USA) prepared in 10 mM HCl solution. The inhibition rate of pyrogallol auto-oxidation was measured at 325 nm using a spectrophotometer (UV-1700, Shimadzu). Absorbance value of each sample was recorded for 10 min at every 1 min interval and the increment in the absorbance was calculated by the difference.

Determination of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the 2-deoxyribose oxidation method described by Chung et al. (1997). A 0.2-mL of 0.1 M $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.1 M of EDTA was mixed with 0.2 mL of 0.1 M 2-deoxyribose, sample solution (0.7 mL) and 0.7 mL of phosphate buffer (pH 7.4) to make a total volume of 1.8 mL reaction mixture. A 0.2-mL of 10 mM H_2O_2 solution was added to the reaction mixture and incubated at 37 °C for 4 h. After this incubation, 1 mL of 2.8% TCA (trichloroacetic acid) and 1% TBA (thiobarbituric acid) each were added to the incubated mixture. The whole mixture was boiled for 10 min, cooled in ice and

absorbance value was measured at 532 nm using a spectrophotometer (UV-1700, Shimadzu).

Reducing power assay

The reducing power was determined according to the method described by Oyaizu (1986). Various concentrations of the methanolic extracts of sample (2.5 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. A 2.5-mL of 10% trichloroacetic acid (w/v) were added to the mixture was centrifuged at 1000 rpm for 8 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically (UV-1700, Shimadzu) at 700 nm. Butylated hydroxyanisole was used as standard.

Determination of total phenolic content

The total phenol content of *takju* samples was determined according to the Folin–Ciocalteu method (Singleton et al., 1999). A 250- μ L of undiluted Folin–Ciocalteu reagent was added to 50 μ L of the sample. After 1 min, 750 μ L of 20% (w/v) aqueous Na_2CO_3 was added to the mixture and the volume was made up to 5 mL with distilled water and incubated at room temperature for 2 h. The absorbance value of incubated reaction mixture was measured at 760 nm using a spectrophotometer (UV-1700, Shimadzu). Gallic acid was used to prepare a calibration curve and the total phenol content was estimated as gallic acid equivalents (μ g GAE/mL of *takju*).

Extraction and determination of volatile compounds

The volatile compounds of *takju* samples were determined by following the method described earlier (Howard et al., 2005).

For the extraction of volatile compounds, 200 μ L of 3-octanol (50 ng/mL in distilled water) was added to 5 mL of the sample. Sodium sulfate (5 g) was added to the samples (5 mL) in 20-mL screw-capped vials. The volatile compounds of the *takju* samples were extracted and absorbed at 4 °C for 20 min by using the SPME (solid-phase microextraction) fibres (50 μ m DVB/CAR/PDMS Supelco, Bellefonte, PA, USA), after shaking the vial at 40 °C for 20 min in an autosampler (Combi PAL G6504-CTC, CTC Analytics, Zwingen, Switzerland).

The profile of volatile compounds was determined using a gas chromatography/mass spectrometry (GC/MS) technique. GC/MS analysis was carried out using an Agilent 7890B GC (Agilent Technologies Inc., Santa Clara, CA, USA) system coupled to an Agilent MSD 5977B quadrupole

mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). One microliter of sample was injected into a capillary column (DB-WAX, 60 m length, 0.25 μ m i.d., 0.25 μ m film thickness, Agilent Technologies, Middleburg, OH, USA). The GC oven temperature was initially maintained at 40 °C (held for 2 min), then increased to 220 °C at a rate of 2 °C/min, and finally increased to 240 °C at a rate of 20 °C/min (held for 10 min). The carrier gas was helium at a constant flow rate of 1 mL/min. A series of *n*-alkanes (C_6 – C_{22}) was analyzed under the same conditions to obtain linear retention index (LRI) values for the reaction mixture components.

In the full-scan mode, the electron ionization mass spectra in the range of 35–350 m/z were recorded at 70 eV electron energy. The mass spectrometer was operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μ A and scanned from m/z 28 to 450 at 1.7 scans/s. The mass spectra were obtained in full-scan mode and compared with the Wiley 275 mass spectral database (Agilent Technologies, Santa Clara, CA, USA).

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using SAS 9.4 (SAS Institute Inc. Cary, NC, USA). Significant difference between the means was determined by Tukey test at 5% probability.

Results and discussion

Physicochemical characteristics

The parameters, such as pH, alcohol concentration, TA, TSS, and color values, were considered to evaluate the physicochemical characteristics of the *takju* samples.

The pH values of regular *takju* and alcohol-free *takju* were not significantly different (Table 1). The dealcoholization processes did not affect the pH value of *takju* samples as in the red wine (Liguori et al., 2013). Non-significant change in the pH value of dealcoholized *takju* implied an unaffected shelf life compared to the regular *takju* because the pH value is an important attribute influencing their shelf-life and resistance to microbial contaminations (He et al., 2014).

The alcohol content of *takju* samples was significantly varied with the dealcoholization method applied (Table 1). The initial alcohol content (6.10%) of ordinary *takju* reduced substantially, and it was halved (3.06%) with the bentonite treatment (BT-1); a further drastic reduction up to 0.04% was achieved adopting the RO treatments. It is worth noting that RO treatment dramatically reduced the alcohol content of *takju*. Reduction of alcohol with the use

Table 1 Physicochemical characteristics and total phenol content of *takju* samples after bentonite and/or reverse osmosis

Sample ¹	pH	Alcohol (% v/v)	Titratable acidity ² (g/100 mL)	Soluble solid (°Bx)	Color value ³			Total Phenolics (μg GAE/mL of sample)
					L*	a*	b*	
Control	3.73 ± 0.02 ^a	6.10 ± 0.12 ^a	0.60 ± 0.01 ^a	3.1 ± 0.2 ^a	57.57 ± 0.39 ^a	− 0.47 ± 0.02 ^a	4.22 ± 0.07 ^a	345.30 ± 3.16 ^a
BT-1	3.71 ± 0.02 ^a	3.06 ± 0.24 ^b	0.42 ± 0.02 ^b	2.7 ± 0.1 ^b	56.06 ± 0.47 ^a	− 0.90 ± 0.03 ^b	1.61 ± 0.06 ^b	315.90 ± 1.49 ^b
RO-1	3.74 ± 0.01 ^a	0.28 ± 0.13 ^c	0.12 ± 0.01 ^c	0.9 ± 0.1 ^c	56.75 ± 0.81 ^a	− 1.08 ± 0.06 ^c	1.55 ± 0.02 ^b	163.13 ± 0.95 ^c
RO-2	3.71 ± 0.01 ^a	0.08 ± 0.03 ^d	0.12 ± 0.01 ^c	0.9 ± 0.2 ^c	55.30 ± 0.05 ^b	− 1.04 ± 0.07 ^c	1.34 ± 0.17 ^c	165.75 ± 0.79 ^c
RO-3	3.71 ± 0.02 ^a	0.04 ± 0.02 ^d	0.12 ± 0.02 ^c	0.7 ± 0.1 ^c	54.54 ± 0.07 ^c	− 0.96 ± 0.12 ^{bc}	0.86 ± 0.18 ^d	156.30 ± 0.17 ^d

¹Control: *takju* obtained from a local store; BT-1: *takju* containing 85% of bentonite-treated cleared *takju* after filtration and 15% of HT-0 (heat-treated uncleared *takju*); RO-1: *takju* containing 85% of the sample obtained from 1-time RO and 15% of HT-0; RO-2: *takju* containing 85% of the sample obtained from 2-time RO and 15% of HT-0; RO-3: *takju* containing 85% of the sample obtained from 3-time RO and 15% of HT-0

²As lactic acid

³L*, lightness (100, white; 0, black); a*, redness (−, green; +, red), b*, yellowness (−, blue; +, yellow)

⁴Quoted values are mean ± SD of triplicate analyses. The values followed by different superscripts in the same column are significantly different ($p < 0.05$)

of bentonite was also reported earlier (Armada and Falqué, 2007). The reduction of aroma (Voilley et al., 1990) and possibly also of the alcohol might be due to adsorption of the molecules to the bentonite. RO also reduced the alcohol content in beer (Voilley et al., 1990) and wine (Gil et al., 2013). The reduction of alcohol by RO is due to the tangential filtration and higher transmembranar pressure than that of osmotic pressure with the use of a membrane semi-permeable to ethanol (Catarino et al., 2007).

Bentonite and RO treatments significantly reduced the TA and TSS values of dealcoholized *takju* compared to the control (Table 1). Reduced TA and TSS were also found in RO-implied fruit juice (Souza et al., 2013). As mentioned earlier, adsorption of the molecules and their wasting away with bentonite might be the reason for reduced TA and TSS in the bentonite treated *takju*.

Color values of *takju* samples were also significantly affected by the bentonite and RO treatments (Table 1). The redness and yellowness values were decreased for all the RO and bentonite treated *takju* samples, whereas the lightness value was reduced only after the repetition of RO treatment for two times. The effect of dealcoholization on color value of wine is not well reported. The possible reason for reduced color values for bentonite treatment might be due to adsorption to and subsequent loss of color compounds with the bentonite (Voilley et al., 1990) and separation out of color compounds by the semi-permeable membrane used in RO system.

Although there was significant changes in physico-chemical characteristics, no substantial yield loss in the final product of *takju* was observed due to bentonite and RO treatments.

Mineral element content

The total mineral content of *takju* samples was significantly decreased with RO (258.61–275.95 mg/L) and bentonite (472.29 mg/L) treatments compared to that of the control (505.8 mg/L) (Table 2). Interestingly, the amount of some mineral elements like Mg and K was significantly increased with the bentonite treatment compared to the control. The elements, including Mg, K, and Ca, are reported to have beneficial health effects in the prevention and treatment of essential hypertension (Houston and Harper, 2008). Previous report on wine (Nicolini et al., 2004) also showed an increased Mg and Na and decreased Zn contents due to bentonite treatment. However, the report was not in agreement for the rest of the mineral elements which might be due to the difference in the bentonite (Nicolini et al., 2004) and/or addition of 15% of HT-0 in the present study. On the other hand, the elements like As, Pb, Cd, and Hg; which are also health hazardous; were not detected in the *takju* samples (Table 2). RO, due to its operating principle (Kucera, 2015), reduces mineral and other soluble materials (Monnot et al., 2017). Since the *takju* samples (RO-1, RO-2 and RO-3) were prepared after addition of 15% of HT-0, which was not subjected to reverse osmosis in the present study, the mineral content of the samples was still high even after multiple RO.

Free amino acid profile

The free amino acids were categorized into essential, non-essential, and other free amino acids. Bentonite treatment increased the essential (279.00 mg/mL) and non-essential (602.96 mg/mL) but decreased the other free amino acid (187.19 mg/mL) contents compared to that of the control (239.33, 542.09, and 208.39 mg/mL, respectively).

Table 2 Mineral element contents (mg/L) of *takju* samples after bentonite and/or reverse osmosis

Element	Sample ¹				
	Control	BT-1	RO-1	RO-2	RO-3
As	ND ²	ND	ND	ND	ND
Ca	116.61 ± 4.13 ^{3a}	120.60 ± 4.03 ^a	59.01 ± 1.87 ^b	59.12 ± 1.38 ^b	60.89 ± 2.11 ^b
Cd	ND	ND	ND	ND	ND
Co	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.01 ± 0.01 ^a	0.02 ± 0.01 ^a	0.02 ± 0.01 ^a
Cu	0.63 ± 0.01 ^a	0.62 ± 0.01 ^a	0.50 ± 0.01 ^b	0.48 ± 0.02 ^b	0.52 ± 0.01 ^b
Hg	ND	ND	ND	ND	ND
K	118.24 ± 3.40 ^b	126.55 ± 4.55 ^a	90.44 ± 3.07 ^c	91.45 ± 2.67 ^c	76.60 ± 3.75 ^d
Mg	50.45 ± 2.05 ^b	56.73 ± 2.51 ^a	20.88 ± 0.79 ^c	21.18 ± 0.52 ^c	19.92 ± 0.97 ^c
Mn	1.94 ± 0.02 ^a	1.92 ± 0.02 ^a	0.89 ± 0.01 ^b	0.85 ± 0.01 ^c	0.72 ± 0.01 ^d
Mo	0.30 ± 0.01 ^a	0.32 ± 0.02 ^a	0.29 ± 0.02 ^a	0.30 ± 0.02 ^a	0.28 ± 0.01 ^a
Na	134.66 ± 5.11 ^b	158.28 ± 6.15 ^a	95.58 ± 3.42 ^c	99.89 ± 2.90 ^c	97.66 ± 4.38 ^c
Pb	ND	ND	ND	ND	ND
Zn	82.96 ± 0.89 ^a	7.26 ± 0.08 ^b	2.38 ± 0.01 ^d	2.66 ± 0.01 ^c	2.00 ± 0.91 ^e
Total	505.8	472.29	269.98	275.95	258.61

¹Samples are defined in Table 1²ND: non-detectable³Quoted values are mean ± standard deviation of triplicate experiments. The values followed by the different superscript letters in the same row are significantly different ($p < 0.05$)

However, RO treatment remarkably reduced the all categories of amino acids (Table 3). The reduction in the amino acid content was obvious as in the minerals (Monnot et al., 2017) due to RO (Kucera, 2015) treatment. However, the reason for increment in some of the essential and non-essential amino acids with bentonite treatment compared to the control, in the present study, was not well understood. In a previous study (Burin et al., 2016), clarification of must and wine with bentonite reduced the amino acids. The reduction in amino acid content with bentonite treatment was possibly due to the adsorption and precipitation of amino acids with bentonite (Ribéreau-Gayon et al., 2006). Moreover, addition of bentonite caused charge-charge interactions between the negatively charged bentonite and positively charged proteins that formed and precipitated out of suspension (Voilley et al., 1990).

Antioxidant potential and total phenolic content

The antioxidant potentials and total phenolic content of *takju* samples were significantly ($p < 0.05$) reduced with bentonite or RO treatments compared to the control (Tables 1 and 4). The DPPH radical-scavenging activity of BT-1 (24.31–79.83%) and RO treatments (12.65–50.77%) were significantly low as compared to the control (27.77–83.47%). As in the DPPH radical-scavenging activity, the superoxide anion (O_2^-), hydroxyl radical (HO), and reducing power were significantly reduced with RO treatments (Table 4). The dealcoholization also

reduced the total phenolic content of *takju* samples—315.90, 163.13, 165.75, and 156.30 GAE/mL for BT-1, RO-1, RO-2, and RO-3, respectively, compared to the control (345.30 GAE/mL).

Although antioxidants, including phenolic compounds, have showed numerous health benefits (Wang and Wang, 2015), RO treatment significantly reduced the health benefits of *takju* at the expense of dealcoholization. However, for some antioxidant measurements, bentonite treatment increased the antioxidant potential (Table 4). The results of increased antioxidant due to bentonite treatment found in the present study were different from those of the previous report on wine (Yildirim, 2011). The cause of increased antioxidant capacity for some of the concentrations of *takju* samples, found in the present study, was not well understood. The reason for the reduced total phenolics might be due their binding and wasting away with bentonite particles (Yildirim, 2011). A decreased polyphenol content was also found with the reducing alcohol concentrations of grape must obtained after RO (Mira et al., 2017). The dealcoholized beer collected from markets also showed lower values for antioxidant potentials and total polyphenol content (Mitić et al., 2014). The reduced phenolic content might be attributed to membrane adsorption of polyphenols (Mira et al., 2017). Reduction in the antioxidant activities of bentonite- and RO-treated *takju* samples might be due to the reduced phenolic content as the phenolic compounds possess antioxidant properties (Tyagi and Agarwal, 2017).

Table 3 Free amino acid (AA) composition (mg/mL) of *takju* samples after bentonite and/or reverse osmosis

Amino acid	Sample ¹				
	Control	BT-1	RO-1	RO-2	RO-3
Essential AA					
L-Histidine	8.63 ± 0.08 ^{2b}	9.64 ± 0.07 ^a	3.18 ± 0.36 ^d	3.80 ± 0.33 ^c	3.47 ± 0.50 ^{cd}
L-Isoleucine	18.10 ± 1.01 ^b	20.85 ± 1.55 ^a	5.68 ± 0.27 ^c	5.93 ± 0.11 ^c	5.71 ± 0.30 ^c
L-Leucine	60.97 ± 2.31 ^b	73.03 ± 3.12 ^a	17.66 ± 0.51 ^c	17.89 ± 2.03 ^c	16.85 ± 1.96 ^c
L-Lysine	45.12 ± 2.12 ^b	52.98 ± 2.51 ^a	16.60 ± 0.60 ^c	16.13 ± 0.70 ^{cd}	15.16 ± 0.77 ^{cd}
L-Methionine	13.98 ± 0.90 ^a	15.27 ± 1.68 ^a	4.51 ± 0.81 ^c	4.76 ± 0.76 ^c	4.51 ± 0.77 ^c
L-Phenylalanine	45.16 ± 3.00 ^b	50.88 ± 2.89 ^a	12.54 ± 1.22 ^c	12.29 ± 0.98 ^c	11.76 ± 1.35 ^c
L-Threonine	11.14 ± 0.81 ^b	13.53 ± 0.71 ^a	3.44 ± 0.19 ^c	3.66 ± 0.67 ^c	3.37 ± 0.59 ^c
L-Valine	36.23 ± 1.37 ^b	42.82 ± 2.31 ^a	12.73 ± 1.21 ^c	12.45 ± 0.69 ^c	11.77 ± 0.98 ^c
Sub-total	239.33	279.00	76.34	76.91	72.60
Non-essential AA					
Glycine	43.62 ± 1.01 ^b	51.14 ± 1.89 ^a	13.42 ± 1.69 ^c	13.57 ± 1.72 ^c	13.67 ± 1.61 ^c
L-Alanine	167.27 ± 1.51 ^b	175.31 ± 3.66 ^a	45.45 ± 3.03 ^c	45.91 ± 2.09 ^c	46.12 ± 1.88 ^c
L-Arginine	77.27 ± 1.90 ^b	82.81 ± 2.55 ^a	23.14 ± 2.03 ^c	21.66 ± 1.20 ^c	21.96 ± 0.96 ^c
L-Aspartic acid	28.87 ± 1.96 ^b	33.59 ± 1.11 ^a	7.99 ± 0.51 ^c	8.55 ± 0.47 ^c	8.01 ± 1.02 ^c
L-Glutamic acid	92.83 ± 2.31 ^b	106.36 ± 2.31 ^a	27.83 ± 2.31 ^c	27.77 ± 2.31 ^c	29.32 ± 2.00 ^c
L-Serine	10.29 ± 0.07 ^b	12.97 ± 0.52 ^a	3.15 ± 0.66 ^c	3.39 ± 0.21 ^c	3.15 ± 0.31 ^c
L-Tyrosine	54.15 ± 0.09 ^b	60.49 ± 2.07 ^a	13.82 ± 1.11 ^c	13.72 ± 1.69 ^c	14.06 ± 1.09 ^c
Proline	67.79 ± 2.01 ^b	80.29 ± 1.89 ^a	15.08 ± 1.20 ^c	15.91 ± 2.31 ^c	14.90 ± 3.21 ^c
Sub-total	542.09	602.96	149.88	150.48	151.19
Other AA					
1-Methyl-L-histidine	ND	ND	ND	ND	ND
3-Methyl-L-histidine	ND	ND	ND	ND	ND
Ammonia	5.47 ± 0.03 ^a	4.39 ± 0.03 ^b	3.67 ± 0.13 ^c	3.79 ± 0.20 ^c	3.67 ± 0.06 ^c
Cystathionine	10.63 ± 0.03 ^b	11.70 ± 0.57 ^a	3.85 ± 0.05 ^d	4.09 ± 0.03 ^c	3.95 ± 0.23 ^{cd}
D,L-β-Aminoisobutyric acid	20.58 ± 1.07 ^a	16.63 ± 1.81 ^b	12.63 ± 1.11 ^c	9.61 ± 1.21 ^d	9.48 ± 1.30 ^d
Ethanolamin	5.91 ± 0.05 ^a	1.94 ± 0.02 ^b	2.76 ± 0.09 ^c	2.90 ± 0.03 ^b	2.35 ± 0.03 ^d
Hydroxy proline	ND	ND	ND	ND	ND
Hydroxylysine	ND	ND	ND	ND	ND
L-Anserine	ND	ND	ND	ND	ND
L-Carnosine	ND	ND	ND	ND	ND
L-Citrulline	ND	ND	ND	ND	ND
L-Cystine	32.73 ± 1.95 ^b	35.78 ± 1.31 ^a	10.59 ± 1.02 ^c	10.18 ± 0.66 ^c	10.66 ± 0.76 ^c
L-Ornithine	77.82 ± 2.68 ^a	79.35 ± 1.76 ^a	21.49 ± 2.33 ^b	20.88 ± 2.22 ^b	21.61 ± 1.11 ^b
L-Sarcosine	ND	ND	ND	ND	ND
L-α-Aminoadipic acid	2.35 ± 0.30 ^a	2.01 ± 0.02 ^a	ND	ND	ND
L-α-Amino-n-butylic acid	ND	ND	ND	ND	ND
O-Phospho ethanol amine	ND	ND	ND	ND	ND
O-Phospho-L-serine	ND	ND	ND	ND	ND
Taurine	ND	ND	ND	ND	ND
Urea	ND	ND	ND	ND	ND
β-Alanine	5.09 ± 0.02 ^a	3.31 ± 0.08 ^b	ND	ND	ND
γ-Amino-n-butyric acid	47.81 ± 0.89 ^a	32.08 ± 1.66 ^b	18.12 ± 1.21 ^c	18.66 ± 1.31 ^c	18.07 ± 1.42 ^c
Sub-total	208.39	187.19	73.11	70.11	69.79
Total	989.81	1069.15	299.33	297.50	293.58

¹Samples are defined in Table 1²Values are expressed as mean ± standard deviation of two replicates. Values followed by different superscript letters in the same row are significantly different ($p < 0.05$)

ND non-detectable

Table 4 DPPH free-radical, superoxide anion (O_2^-), hydroxyl radical (HO) scavenging and reducing power of *takju* samples

Sample ¹	mg/mL of on a dry basis	% Inhibition			Reducing power
		DPPH	O_2^-	HO	
Control	5	27.77 ± 0.26 ^{a2}	14.67 ± 0.45 ^a	1.21 ± 0.11 ^c	0.237 ± 0.012 ^a
	10	40.33 ± 0.82 ^b	16.47 ± 0.69 ^b	3.51 ± 0.21 ^b	0.253 ± 0.003 ^a
	15	44.79 ± 0.76 ^b	34.49 ± 1.21 ^a	10.17 ± 0.43 ^a	0.310 ± 0.020 ^b
	20	72.23 ± 0.31 ^a	37.97 ± 1.32 ^b	18.71 ± 0.89 ^b	0.364 ± 0.010 ^a
	25	83.47 ± 1.25 ^a	44.02 ± 2.11 ^b	48.84 ± 0.69 ^b	0.448 ± 0.011 ^a
BT-1	5	24.31 ± 0.36 ^b	15.57 ± 1.06 ^a	2.21 ± 0.12 ^a	0.239 ± 0.011 ^a
	10	44.63 ± 0.92 ^a	20.85 ± 0.76 ^a	6.51 ± 0.20 ^a	0.277 ± 0.020 ^a
	15	49.09 ± 1.11 ^a	28.70 ± 0.69 ^b	7.12 ± 0.25 ^b	0.341 ± 0.006 ^a
	20	72.23 ± 0.69 ^a	42.34 ± 1.21 ^a	33.61 ± 1.23 ^a	0.377 ± 0.033 ^a
	25	79.83 ± 1.20 ^b	50.97 ± 0.39 ^a	62.12 ± 0.98 ^a	0.481 ± 0.050 ^a
RO-1	5	12.65 ± 0.30 ^d	8.18 ± 0.21 ^d	1.51 ± 0.17 ^b	0.212 ± 0.005 ^b
	10	23.25 ± 0.49 ^d	11.20 ± 0.76 ^d	2.52 ± 0.18 ^c	0.226 ± 0.004 ^b
	15	28.38 ± 0.56 ^d	12.78 ± 1.21 ^c	3.83 ± 0.42 ^d	0.277 ± 0.030 ^c
	20	39.15 ± 0.90 ^c	17.37 ± 1.30 ^d	6.51 ± 0.22 ^d	0.345 ± 0.026 ^a
	25	43.25 ± 1.11 ^d	25.63 ± 0.98 ^e	36.59 ± 1.92 ^c	0.361 ± 0.012 ^c
RO-2	5	16.24 ± 0.33 ^c	10.39 ± 0.76 ^c	0.71 ± 0.12 ^d	0.191 ± 0.002 ^c
	10	25.13 ± 1.25 ^c	10.54 ± 1.81 ^d	1.11 ± 0.11 ^d	0.202 ± 0.003 ^c
	15	38.63 ± 0.51 ^c	17.75 ± 1.00 ^d	3.21 ± 0.21 ^d	0.243 ± 0.002 ^c
	20	39.49 ± 0.70 ^c	23.68 ± 1.77 ^c	4.62 ± 0.19 ^c	0.313 ± 0.009 ^b
	25	50.77 ± 1.81 ^c	44.39 ± 1.88 ^c	32.27 ± 1.88 ^d	0.397 ± 0.015 ^b
RO-3	5	15.99 ± 0.32 ^c	14.41 ± 1.20 ^b	0.83 ± 0.11 ^d	0.197 ± 0.006 ^c
	10	24.24 ± 1.21 ^{cd}	19.31 ± 1.61 ^c	2.81 ± 0.13 ^c	0.218 ± 0.014 ^b
	15	28.96 ± 1.61 ^d	20.59 ± 0.89 ^c	6.12 ± 0.21 ^c	0.246 ± 0.015 ^c
	20	42.26 ± 0.77 ^b	25.48 ± 0.98 ^c	17.72 ± 1.25 ^c	0.291 ± 0.020 ^b
	25	48.48 ± 1.69 ^c	38.12 ± 0.55 ^d	30.16 ± 1.55 ^e	0.381 ± 0.042 ^{bc}

¹Samples are defined in Table 1²Values are expressed as mean ± standard deviation of three replicates. Values followed by different superscript letters for each concentration in the same column are significantly different ($p < 0.05$)

Volatile compounds

Volatile compounds of *takju* were remarkably reduced with the bentonite or RO treatments (Table 5). In most cases, RO treatment caused high reduction in the volatile compounds compared to the bentonite treatment. Loss of ester was very high with both the bentonite (1.68% peak area) and RO (0.14–0.55% peak area) treatments of dealcoholization compared to the control (6.48% peak area). Similarly, other groups of volatile compounds, including alcohol, acid, and terpene, were also reduced with the bentonite and RO treatments (Table 5). Occurrence of the volatile compounds, which were not detected in the control but were found in the BT-1 *takju* samples, might be due to addition of bentonite (Sanborn et al., 2010).

Strong linkages between bentonite and ethyl esters have been considered for the reduction of concentration of these volatile aroma compounds during bentonite fining (Voilley

et al., 1990). Bentonite has also been reported to reduce the concentration of terpene- and alcohol-based volatile aromatic compounds in wine (Armada and Falqué, 2007).

Loss of volatile compounds due to membrane-based dealcoholization process has also been reported in a previous report (Diban et al., 2008). Reduction of the volatile compounds as a result of RO treatment was most probably due to their affinity to the membrane and high volatility which favored their movement through the membrane air gap, and waste away with the stripping water, in the same manner as ethanol. In addition, a small amount of the loss could also be attributed to adsorption onto the membrane (Diban et al., 2008).

In conclusion, alcohol concentration of *takju* was reduced by adopting bentonite clarification, thermal treatment and reverse osmosis techniques. A portion (15%) of heat-treated sedimented part of the original sample was also added to the final product in order to maintain

Table 5 Volatile compounds detected in *takju* samples after bentonite and/or reverse osmosis treatments using gas chromatography time-of-flight mass spectrometry with solid-phase microextraction fibers (Unit: Peak area %)

Group	Compound	Sample ^a				
		Control	BT-1	RO-1	RO-2	RO-3
Ester	Ethyl acetate	1.09	0.31	0.13	0.03	0.09
	2-Methylpropyl acetate	0.26	0.06	ND	ND	ND
	1-Butanol, 3-methyl-,acetate	4.89	1.24	0.23	0.42	0.46
	5-Formyl-4-methylthiazole acetate	0.09	ND	ND	ND	ND
	Ethyl-4-hydroxybutanoate	0.15	0.07	ND	0.12	0.2
	Sub-total	6.48	1.68	0.36	0.57	0.75
Alcohol	Ethanol	17.2	12.61	10.08	12	13.18
	1-Propanol	0.1	ND	ND	ND	ND
	2-Methyl-1-propanol	1.45	1.12	1.4	2.87	0.94
	2-Methyl-1-butanol	1.63	0.74	0.13	0.46	ND
	3-Methyl-1-butanol	7.6	3.47	0.64	2.35	3.62
	1-Hexanol	0.08	0.03	ND	ND	ND
	3-Methylthio-1-propanol	0.12	0.06	0.05	0.11	0.15
	Benzeneethanol	10.78	6.97	2.61	8.2	10.34
	2,2'-Oxybis-ethanol	ND	0.03	ND	ND	ND
	2-Ethyl-1-hexanol	ND	ND	0.04	0.14	0.17
	1,2,3,4-Butanetetrol	ND	ND	ND	0.08	ND
	Sub-total	38.96	25.03	14.95	26.21	28.4
Acid	Butanoic acid	0.11	0.03	ND	ND	ND
	Hexanoic acid	1.77	0.37	0.12	0.23	0.27
	Benzoic acid	0.26	ND	ND	ND	ND
	Acetic acid	0.77	0.05	ND	ND	ND
	3-Methylbutyl octanoic acid	0.05	0.04	0.03	ND	0.07
	2-Hydroxymethyl benzoic acid	ND	0.03	ND	ND	0.07
	3-Amino-butanoic acid	ND	0.02	ND	ND	ND
	2-Methylbutyl octanoic acid	ND	0.04	ND	0.07	ND
	Ethyl-9-octadecenoic acid	ND	0.58	ND	ND	ND
	Ethyl octanoic acid	ND	ND	1.88	ND	ND
	Butanedioic acid	0.17	0.09	0.04	0.09	0.11
	Octanoic acid	3	2.53	ND	4.05	4.2
	Decanoic acid	2.95	2.7	2.17	4.15	5.04
	Dodecanoic acid	0.35	0.41	0.36	0.57	0.73
	Tetradecanoic acid	0.21	0.56	0.47	0.69	1.02
	Hexadecanoic acid	1.2	2.66	2.03	3.39	5.36
	2-Phenylethyl acetic acid	ND	0.31	0.05	0.11	0.15
	Ethyl-9,12-octadecenoic acid	ND	0.5	0.48	0.79	1.25
	Sulfuric acid	ND	ND	ND	0.07	ND
	2-Hydroxyl propanoic acid	ND	ND	ND	ND	ND
	Sub-total	10.84	10.92	7.63	14.21	18.27
Terpene	1,4,7,10,13,16-Hexaoxacycloocta-decane	0.67	0.2	0.09	0.27	0.35
	1-Heptene	ND	0.04	ND	ND	ND
	Hexamethylcyclotrisiloxane	ND	ND	0.06	ND	ND
	Monodeuteromethane	ND	ND	0.02	ND	ND
	Tetrahydro-2-methyl thiophene	ND	ND	0.07	ND	ND
	Sub-total	0.67	0.24	0.24	0.27	0.35

Table 5 continued

Group	Compound	Sample ^a				
		Control	BT-1	RO-1	RO-2	RO-3
Other	Ammonia	41.54	60.81	75	56.44	48.14
	1,3-Bis(1,1-dimethylethyl)-benzene	0.28	ND	ND	ND	ND
	2,4-bis(1,1-dimethylethyl)phenol	0.07	ND	ND	ND	ND
	Dimethylsilanediol	0.08	0.09	0.09	0.09	0.1
	Methyl benzene	ND	ND	0.18	0.12	0.55
	Nonanal	ND	ND	0.04	0.06	0.07
	3-Methylbutyl hexanoate	ND	ND	0.33	ND	ND
	1,3-Propanediol diacetate	ND	ND	0.03	ND	ND
	Methyl salicylate	ND	ND	0.03	ND	ND
	Ethyl oleate	ND	ND	0.4	0.71	1.23
	Isopentyl hexanoate	ND	ND	ND	0.06	0.07
	1-Phenethyl-pyrrolidine-2,4-dione	ND	ND	ND	ND	ND
	Sub-total	41.97	60.9	76.1	57.48	50.16
Compound non-identified		1.08	1.23	0.72	1.26	2.07
Total		100	100	100	100	100

The values are average of duplicate measurements

ND non-detectable

^aSamples are defined in Table 1

turbidity, one of the fundamental attributes of *takju*. The dealcoholized *takju* (0.04–0.28%) was obtained through the reverse osmosis, whereas low-alcohol (3.06%) *takju* was produced after bentonite clarification. The effect of dealcoholization was significant in altering the physicochemical properties (titratable acidity, soluble solid, color, mineral, free amino acid, antioxidant potentials, total phenols and volatile compounds) investigated, however the pH value was not significantly ($p > 0.05$) affected compared to the commercial *takju*. The results of this study showed that bentonite treatment could be appropriate to obtain low-alcoholic *takju* and reverse osmosis for preparing non-alcoholic *takju*. It is to be noted that the low-alcoholic and non-alcoholic *takju* samples, obtained in the present study, were poor in containing volatile compounds and nutritional value. This study could be a reference for further studies on producing low-alcoholic *takju* with the elevated nutritional values and antioxidant potentials for commercial application. The combination of different dealcoholization approaches adopted in this study could be useful in promoting low-alcoholic or dealcoholized traditional beverages of different countries.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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