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Wort Fermentation by *Leuconostoc citreum* Originated from *Kimchi* and Sensory Properties of Fermented Wort

Purev Delgerzaya, Jin-Yeong Shin, Kwang-Ok Kim, and Jin-Byung Park*

Department of Food Science & Engineering, Ewha Womans University, Seoul 120-750, Korea

Abstract Fermentation of wort was investigated with an ultimate goal to develop a fermented beverage rich in prebiotics and functional ingredients as well as desirable in flavors. Wort was fermented with *Leuconostoc citreum* HJ-P4 originated from *kimchi* and subjected to sensory descriptive analysis. *L. citreum* HJ-P4 produced various organic acids (e.g., lactic acid, acetic acid) as well as functional sugars (e.g., mannitol, panose) during wort fermentation. The concentration and ratio of lactic acid and acetic acid were significantly influenced by roasting conditions of malts used for wort preparation and aeration conditions during fermentation. The concentration of mannitol and panose varied depending on the sucrose content of wort and aeration conditions. Sensory characteristics of the fermented worts were clearly differentiated according to the roasting conditions of malts used for wort preparation and aeration conditions during fermentation. These results indicate that metabolite concentration of fermented wort and its sensory properties can be manipulated with roasting conditions of malts and fermentation conditions.

Keywords: wort fermentation, *Leuconostoc citreum*, sensory property, panose, mannitol

Introduction

Barley is widely used for the production of tea, beverage, and beer. A variety of aroma and flavor compounds are generated by pyrolysis and the amino-carbonyl reaction during heat processing of barley (e.g., kilning). In addition, the high activities of hydrolytic enzymes such as amylases, proteases, and β-glucanses allow its storage compounds to be degraded into sugars, amino acids, peptides, and other small molecules during the sporulation (1).

Wort, which is prepared from enzymatic digestion of malt followed by mashing and steeping in hot water, contains various nutritional ingredients such as maltose, maltotriose, amino acids, peptides, vitamins, and fibers (1). Thus, wort seems to be suitable as a raw material to produce fermented beverages containing prebiotics [Prebiotics are a category of functional food, defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating growth of the specific bacteria in colon, and therefore improve health of organisms (2). For instance, oligosaccharides and dietary fibers are included in this category (3)].

Leuconostoc sp. which are involved in fermentation of *kimchi*, sauerkraut, and dairy products, are heterofermentative lactic acid bacteria to produce various organic acids (e.g., lactic acid, acetic acid), sugar alcohols (e.g., mannitol), dextrans, and vitamins (vitamin K and B_9) (4). Particularly, some of *Leuconostoc* sp. were reported to produce dextransucrase (E.C. 2.4.1.5), which catalyzes the transfer of glucose from sucrose to other carbohydrates by mainly linking an α-(1,6)-glucosyl bond (5,6). When the acceptor is a monosaccharide or disaccharide, a series of oligosaccharide acceptor-products are produced. Since wort includes maltose and sucrose, panose (6^2-α-D-glucopyraosylmaltose) will be produced by the enzyme.

There are only a few reports to investigate development of malt-based beverages and their sensory characteristics (7,8). In particular, development of malt-based beverages via fermentation was not reported yet in our knowledge.

The objective of this study was to examine fermentation of wort by *Leuconostoc citreum* HJ-P4, isolated from *kimchi* and reported to produce dextransucrase (5). Sensory properties of the fermented wort were also investigated.

Materials and Methods

Microorganisms and medium *Leuconostoc citerum* HJ-P4 isolated from *kimchi* (5) was used as a starter for wort fermentation. The bacteria were cultivated in lactobacilli MRS medium (BD Difco, Franklin Lakes, NJ, USA) or in wort and stored in 50%(v/v) glycerol stock at −70°C.

Preparation of wort Three types of malt (i.e., malt, caramel malt, and roast malt), which were purchased from a local microbrewery, were used for preparation of wort; caramel malt and roast malt were produced after additional heat treatment of malt at 200 and 250°C, respectively, for 2 hr. Wort was produced according to the protocols used generally in microbrewery (1); 15 kg of malts were mashed and steeped in 90 L of 38°C water for 20 min. The broth was heated with agitation so that the temperature was increased stepwise to 72°C for 85 min. After filtration through malt husk layer, the broth was boiled for 1 hr. After cooling, the broth or wort was used for fermentation or stored in −20°C.

Fermentations Test tubes (working volume: 5-mL) were prepared with the MRS medium, inoculated with a colony grown on MRS agar medium, and incubated in a rotary

*Corresponding author: Tel: +82-2-3277-4509; Fax: +82-2-3277-4213
E-mail: jbpark06@ewha.ac.kr
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shaker at 28°C and 200 rpm until optical density (OD) of culture broth measured at 600 nm reached 0.5 to 1.0 (9). The main fermentation was begun by inoculating 1.0 mL of seed culture into a 500-mL flask containing 100 mL wort and incubated in a rotary shaker at 28°C and 200 rpm until cell growth ceased. Effects of sucrose supplementation and aeration conditions on metabolite formation were examined by varying sucrose concentrations from 0 to 8%(w/v) and agitation speed from 50 to 200 rpm.

Analytical methods Cell concentration was determined from the $OD_{600\,nm}$ of culture broth. Concentrations of glucose, fructose, sucrose, maltose, panose, and maltotriose were determined by high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA) equipped with the CarboPac PA10 column and the PAD detector (Dionex), in which 0.6 M Na-acetate/0.15 M NaOH solution was used as the mobile phase. Concentrations of mannitol, lactic acid, and acetic acid were measured by high performance liquid chromatography (HPLC, Waters, Milford, MA, USA) equipped with the HPX-87H column (300×7.8 mm) (Bio-Rad, Richmond, CA, USA). H_2SO_4 solution (5 mM) was used as the mobile phase at a flow rate of 0.6 mL/min at 60°C.

Sensory descriptive analysis (SDA) of fermented worts: Panel selection and training Eight panelists (24-27 years old, female) from the Department of Food Science and Engineering at Ewha Womans University (Seoul, Korea) participated in the descriptive analysis. These panelists were selected using a screening procedure which was based on their discrimination, description, and ranking ability. The screening procedure was based on Meilgaard's suggestion (10) with some modification. The discrimination test consisted of 10 sets of triangle tests discriminating between the 4 basic tastants and different flavors. The description test and the ranking test consisted of 7 common odors and 4 basic tastants solutions, respectively.

During the training session, the panelists were familiarized to various types of fermented worts and developed objective and efficient evaluation procedure for the samples. They generated sensory descriptors and defined them in consensus. They selected the reference samples for each sensory attribute. The training sessions continued until they achieved consistency in the evaluation of the samples. The training sessions were held 3 days/week for 2 months and each training length was approximately 1 hr.

SDA: Sample preparation and presentation As shown in Table 1, 6 worts, fermented by *L. citreum* were used for descriptive analysis. They were different in the types of wort and agitation speed during fermentation with sucrose contents fixed at 20 g/L.

The worts fermented by *L. citreum* were prepared a day before the experiment and refrigerated (4°C) until sample preparation. One and half hr before the experiment, aliquots (25 mL) samples were poured, closed in containers with lids, and left in the room temperature for 1 hr to form headspace. The brown bottle (60 mL) was used for the container because of variance in the color of the samples. Samples were refrigerated again until the evaluation and presented at 10±1°C. The same brown bottle (10±1°C) was provided for tasting and samples were poured into the bottle until the marking line for 10 mL. The samples were designated with 3-digit random numbers and the presentation order was randomized. Filtered tap water (Ceramic Filter System, Fariey Industrial Ceramics Ltd., London, UK; 20±2°C) was presented to rinse the palate between samples. The separate samples (40 mL) were presented in 50-mL glasses for the evaluation of appearance.

SDA: Evaluation procedure The quantitative descriptive analysis (QDA®) (11) procedure was used with partial adoption of the spectrum descriptive analysis method (12). The odor and flavor attributes were first evaluated in the individual booth under dim red light (a 30 W incandescent lightbulb with red color; wave length 600 to 760 nm, 100 lumen) to avoid the bias that might result from differences in color. The panelists evaluated the odor and flavor attributes monadically as one samples was completed before the next sample was tasted. For the odor evaluation, the panelists opened the lid of the container, sniffed 2 times, closed the lid immediately, and marked on the scale. The flavor attributes of the samples were assessed by tasting approximately 10 mL of sample which was applied to the tasting container. The panelists rinsed their mouth once with the filtered water before tasting each sample. The appearance attribute of the samples was evaluated under daylight conditions, with the use of a light box (D65 Superlight-III; Boteck, Siheong, Gyeonggi, Korea) and the each sample was coded with a different random numbers. The intensity of sensory attributes were evaluated using 15-point category scales (1='weak', 15='strong') (13). The evaluation session was conducted in 4 replications and the evaluation was conducted twice a day, at 10 a.m. and 5 p.m., for 2 consecutive days. Each session length was approximately 20 min. Panelists were not allowed to eat or drink anything except for water, or to brush their teeth or gargle, for 1 hr before the evaluation session.

SDA: Statistical analysis Multivariate analysis of variance (MANOVA) was conducted to evaluate significance of overall differences among the samples. Since the MANOVA revealed that a significant difference among the samples, analysis of variance (ANOVA), which estimates the effects of variance on each of the attributes, was performed. When the samples had significantly different intensities on each attribute, Duncan's multiple range test was conducted to compare the significant differences (α=0.05) between the samples for each attribute. A principal component analysis (PCA) was performed to summarize the relationships between the mean values for the sensory attributes and the samples. All of the statistical analyses were performed using SPSS for Windows software (version 12.0, SPSS, Chicago, IL, USA).

Results and Discussion

Preparation of wort Three types of wort were prepared with commercially available malts (i.e., malt, caramel malt, and roast malt). Wort A, which was made of malt only, was yellow-colored and had typical malt aroma. Wort C, made of 80 g/L of malt, 83 g/L of caramel malt, and 3.3 g/L of roast malt, was dark-colored and had burnt flavor and

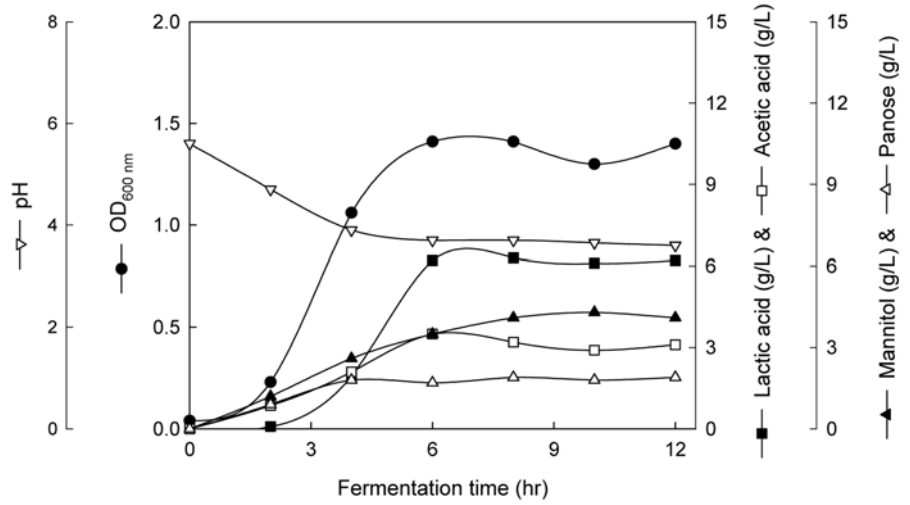

Fig. 1. Wort fermentation with *L. citreum* HJ-P4. Wort was fermented with *L. citreum* HJ-P4 at a rotary shaking incubator at 200 rpm and 28°C.

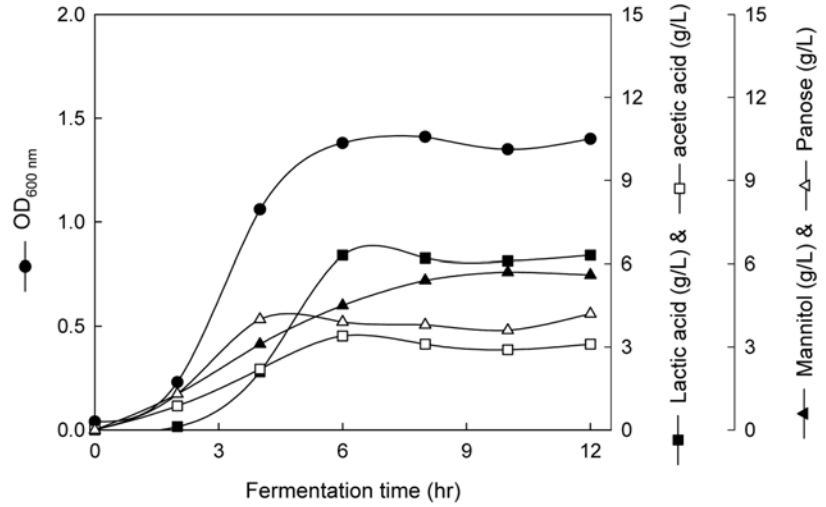

Fig. 2. Effect of addition of sucrose on wort fermentation. Wort was fermented after sucrose was added to a concentration of 2%(w/v).

aroma in addition to typical malt aroma. Wort B, made of 123 g/L of malt, 42 g/L of caramel malt, and 1.7 g/L of roast malt, showed intermediate properties of Wort A and C.

The sugar compositions of Wort A, B, and C were similar each other; Wort A contained 5.8 g/L of maltotriose, 40.5 g/L of maltose, 5.4 g/L of sucrose, 8.2 g/L of glucose, and 7.8 g/L of fructose.

Wort fermentation with *L. citreum* HJ-P4 Wort A was fermented with *L. citerum* HJ-P4, which had been shown to produce highly active dextransucrase and thereby oligosaccharides (5,6), at a rotary shaker (200 rpm and 28°C) (Fig. 1). *L. citreum* HJ-P4 began to grow just after inoculation and its concentration reached 1.4 at $OD_{600\ nm}$ (t=6 hr) in Wort A. The fermentation pH reduced sharply from 5.6 to below 3.7 within 6 hr fermentation time. Concentrations of lactic acid, acetic acid, and mannitol increased in parallel with cell density to 6.2, 3.5, and 3.5 g/L, respectively. Formation of panose ceased earlier than other metabolites resulting in a final concentration of 1.8 g/L at t=4 hr (Fig. 1). The earlier cessation of panose formation appears due to low stability of dextransucrase at low pH, as reported in the previous study (14,15).

Fermentation of Wort B and C showed growth and metabolite production profiles very similar to fermentation of Wort A. However, fermentation of Wort C exhibited the highest final cell density and the lowest panose concentration (Table 1). In addition, lactic acid was the major organic acid produced in fermented Wort A, whereas concentration of acetic acid was over 3 fold higher than that of lactic acid in fermented Wort C. Wort B showed cell density and metabolite concentrations between Wort A and C. These results indicate that the metabolite concentrations of fermented wort are significantly influenced by the type of malt used for preparation of wort.

Effects of addition of sucrose In order to promote production of panose and mannitol during fermentation, which are known as prebiotics and functional sugars, the effect of sucrose addition was investigated.

Table 1. Effect of fermentation conditions on metabolite concentrations

Fermentation conditions			Final cell density ($OD_{600\ nm}$)	Lactic acid (g/L)	Acetic acid (g/L)	Mannitol (g/L)	Panose (g/L)
Wort type[1)]	Sucrose (g/L)	Shaking (rpm)					
A	0	200	1.4±0.1[2)]	6.2±0.5	3.1±0.2	4.1±0.3	1.9±0.2
A[i)]	20	200	1.4±0.1	6.2±0.5	3.1±0.2	5.4±0.3	3.8±0.3
A	40	200	1.4±0.1	6.2±0.5	3.2±0.2	6.6±0.4	4.5±0.3
A	80	200	1.4±0.1	6.4±0.5	3.5±0.2	8.4±0.5	5.5±0.4
A[ii)]	20	50	1.1±0.1	7.4±0.6	2.8±0.2	5.8±0.3	3.4±0.3
B	0	200	2.4±0.2	1.9±0.1	2.9±0.2	2.1±0.2	1.2±0.1
B[iii)]	20	200	2.4±0.2	1.8±0.1	3.2±0.2	2.8±0.2	4.3±0.3
B[iv)]	20	50	1.8±0.1	2.9±0.2	2.4±0.2	3.2±0.3	3.8±0.3
C	0	200	2.8±0.2	1.4±0.1	5.0±0.3	2.7±0.2	0.5±0.4
C[v)]	20	200	2.8±0.2	1.7±0.1	5.4±0.3	3.7±0.3	2.8±0.3
C[vi)]	20	50	1.8±0.1	2.7±0.2	2.4±0.2	5.1±0.3	2.4±0.2

[1)]Used for sensory evaluation. The samples [(i-vi)] were designated as A200, A50, B200, B50, C200, and C50, respectively, in the text.
[2)]Mean±SD.

When sucrose was added into the Wort A to a concentration of 2 to 8%(w/v), cell growth and organic acid formation of *L. citreum* were not significantly influenced (Fig. 1, 2, and Table 1). However, the concentrations of mannitol and panose were increased from 4.1 and 1.9 g/L to 8.4 and 5.5 g/L, respectively. The same trends in growth and metabolite production were observed in fermentation of Wort B and C (data not shown).

The increase in concentration of mannitol and panose seemed because sucrose served as an inducer of dextransucrase of *L. citreum* and provided glucosyl donor to maltose as well as provided fructose to mannitol dehydrogenase to produce mannitol, as previously reported (5,16). Thus, the production of panose and mannitol during wort fermentation could be promoted by supplementation of sucrose into wort.

Effects of aeration rate Carbon metabolism of *Leuconostoc* sp., including formation of organic acids and sugar alcohols, was markedly influenced by oxygen availability (4,16). Therefore, we investigated the effects of aeration conditions on cell growth and metabolite formation of *L. citreum* HP-J4 during wort fermentation.

When the rotation speed of shaking incubator was decreased from 200 to 50 rpm during fermentation of Wort A supplemented with 2% sucrose, growth rate was slightly reduced resulting in a final cell density of 1.1 at $OD_{600\ nm}$ (Fig. 3). Concentrations of acetic acid and panose were also a little decreased. However, the concentration of lactic acid and mannitol increased from 6.2 and 5.4 g/L to 7.4 and 5.8 g/L, respectively, with reduced oxygen availability. The same trends in growth and metabolite production were observed in fermentation of Wort B and C (Table 1). This result indicates that oxygen availability has an effect on formation of lactic acid and mannitol during fermentation of wort.

Sensory properties of fermented worts Concentration of fermentation metabolites, which might have impact on

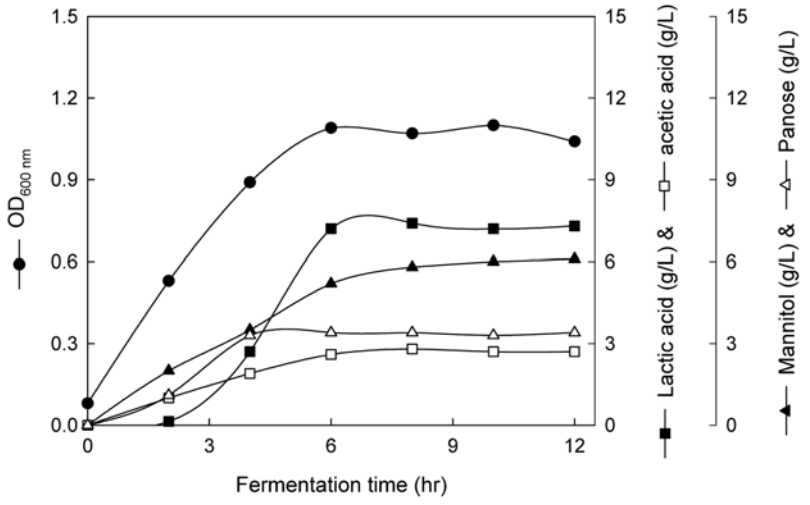

Fig. 3. Effect of agitation speed on wort fermentation. Wort was fermented with *L. citreum* HJ-P4 at a rotary shaking incubator at 50 rpm. Sucrose was added to a concentration of 2% and culture temperature was kept at 28°C.

Table 2. Definitions and the reference samples for the sensory attributes of worts fermented by *L. citreum*

Sensory attributes		Definition	Reference samples
Appearance	Brownness	Intensity of brown color of fermented wort	-
Odor[1)]			
	Malt	The smell associated with malt	10% Malt [20 g of malt (Saimdang Food Co., Ltd., Seoul, Korea) was soaked in the 180 mL water for 30 min and filtered by a cloth (Dashibag, T&C Electronics, Yiwang, Gyeonggi, Korea) made of polyethylene and polypropylene complex fibers] solution
	Honey	The smell associated with honey	20% Honey (Well Being Premium Honey; Gangwon Nongwon Co., Ltd., Yangju, Gangwon, Korea) solution
	Vinegar	The smell associated with vinegar	15% Vinegar (Ottogi Vinegar, Ottogi Co., Ltd., Anyang, Gyeonggi, Korea) solution
	Brown sugar	The smell associated with brown sugar	10% Brown sugar (Dark Brown Sugar, CJ Cheiljedang Corp., Incheon, Korea) solution
	Cheonggukjang[2)]	The smell associated with *cheonggugjang*	3% *Cheonggukjang* (*Cheonggukjang* powder, Gyoha Food, Paju, Gyeonggi, Korea) solution
	Roasted barley	The smell associated with roasted barley	30 g Roasted barley tea (CHA ESAY BORI; Dong Suh Foods Co., Ltd., Siheong, Gyeonggi, Korea)
	Burnt	The smell associated with burnt grain such as barley	5 g Barley (Rice Korea Co., Ltd., Seoul, Korea) burnt at strong heat for 5 min
	Cumin	The smell associated with cumin	0.03 g Cumin (McCormick Ground Cumin; McCormick Co., Inc., Hunt valley, MD, USA)
Flavor	Sweet	Fundamental taste sensation of which sucrose is typical	0.3% Sucrose (Duksan Pure Chemical Co., Ltd., Ansan, Gyeonggi, Korea) solution
	Salty	Fundamental taste sensation of which sodium chloride is typical	0.1% Sodium chloride (Duksan Pure Chemical Co., Ltd.) solution
	Sour	Fundamental taste sensation of which lactic and citric acid is typical	0.05% Citric acid (Duksan Pure Chemical Co., Ltd.) solution
	Bitter	Fundamental taste sensation of which caffeine and quinine is typical	0.07% Caffeine (Sigma-Aldrich, Ltd., St. Louis, MO, USA) solution
	Malt	Aromatics associated with malt	10% Malt [20 g of malt (Saimdang Food Co., Ltd.) was soaked in the 180 mL water for 30 min and filtered by a cloth (Dashibag; T&C Electronics, Yiwang, Gyeonggi, Korea) solution
	Honey	Aromatics associated with honey	20% Honey (Well Being Premium Honey; Gangwon Nongwon Co., Ltd.) solution
	Vinegar	Aromatics associated with vinegar	15% Vinegar (Ottogi Vinegar, Ottogi Co., Ltd.) solution 3% *Cheonggukjang* (*Cheonggukjang* powder; Gyoha Food) solution
	Cheonggukjang	Aromatics associated with *cheonggugjang*	30 g Roasted barley tea (CHA ESAY BORI; Dong Suh Foods Co., Ltd.)
	Roasted barely	Aromatics associated with roasted barley	5 g Barley (Rice Korea Co., Ltd., Seoul, Korea) burnt at strong heat for 5 min
	Burnt	Aromatics associated with burnt grain such as barley	10% Molasses (GRANDMA'S Molasses; B&G Foods, Inc., Roseland, NJ, USA) solution
	Molasses	Aromatics associated with molasses	25% Vinegar (Ottogi Vinegar, Ottogi Co., Ltd.) solution
	Pungent	The sharp irritating sensation in the throat and nasal cavity while the sample is swallowed	40 g Malt [10 g of malt (Saimdang Food Co., Ltd.) was soaked in the 200 mL water for 30 min and filtered by a cloth (Dashibag; T&C Electronics)] solution

[1)]Aliquots (30 mL) of the reference samples for the odor attributes were put into vial (60-mL) with lids >1 hr before the odor evaluation.
[2)]A Korean traditional soy bean paste.

Table 3. Sensory characteristics of worts fermented by *L. citreum*

Sensory attributes[1)]		A50[2)]	A200	B50	B200	C50	C200
Appearance	Brownness	3.72^{c}	3.63^{c}	7.16^{b}	6.84^{b}	10.28^{a}	10.16^{a}
Odor	Malt	8.13^{a}	7.69^{ab}	7.03^{b}	5.81^{c}	5.75^{c}	5.41^{c}
	Honey	7.03^{a}	6.53^{ab}	6.75^{ab}	6.00^{b}	6.00^{b}	5.09^{c}
	Vinegar	4.28^{a}	4.69^{a}	4.59^{a}	4.81^{a}	4.00^{a}	4.81^{a}
	Brown sugar	5.38^{c}	5.28^{c}	6.69^{b}	6.47^{b}	7.66^{a}	6.84^{b}
	Cheonggukjang	4.13^{d}	4.81^{cd}	5.41^{bc}	6.44^{a}	6.00^{ab}	6.66^{a}
	Roasted barley	6.41^{bc}	5.84^{c}	7.34^{ab}	7.53^{a}	7.87^{a}	6.91^{ab}
	Burnt	5.91^{c}	5.44^{c}	7.25^{b}	7.28^{b}	8.78^{a}	7.91^{ab}
	Cumin	4.62^{b}	4.62^{b}	4.87^{b}	5.75^{ab}	5.06^{ab}	6.03^{a}
Flavor	Sweet	5.78^{b}	6.75^{a}	4.94^{c}	6.50^{a}	3.97^{d}	5.16^{bc}
	Salty	5.03^{c}	5.59^{bc}	5.69^{bc}	6.59^{a}	5.09^{bc}	5.91^{ab}
	Sour	5.16^{bc}	6.09^{a}	5.75^{ab}	6.44^{a}	4.75^{c}	5.19^{bc}
	Bitter	3.41^{c}	4.03^{c}	5.06^{b}	5.16^{b}	6.81^{a}	6.41^{a}
	Malt	7.59^{a}	6.94^{ab}	6.62^{bc}	5.44^{d}	5.78^{cd}	5.41^{d}
	Honey	5.53^{a}	5.41^{ab}	4.69^{bc}	4.91^{abc}	4.31^{c}	4.34^{c}
	Vinegar	5.44^{bc}	6.84^{a}	5.78^{b}	7.03^{a}	4.88^{c}	5.72^{b}
	Cheonggukjang	3.94^{bc}	3.72^{c}	4.09^{bc}	4.50^{ab}	4.47^{ab}	4.84^{a}
	Roasted barley	6.09^{bc}	5.50^{c}	6.59^{ab}	6.97^{ab}	7.34^{a}	6.75^{ab}
	Burnt	4.53^{d}	4.66^{d}	6.09^{c}	7.00^{bc}	8.16^{a}	7.47^{ab}
	Molasses	4.63^{b}	5.25^{ab}	5.16^{ab}	5.69^{a}	5.50^{a}	5.94^{a}
	Pungent	4.19^{d}	5.59^{bc}	5.03^{cd}	6.72^{a}	4.50^{d}	6.13^{ab}

[1)]Means of 4 replicates from 8 panelists; Means within a row not sharing a superscript letter are significantly different (p<0.05, Duncan's multiple range test).
[2)]See Table 1 for abbreviation.

sensory properties, was influenced by type of wort, sucrose supplementation, and aeration conditions used during fermentation. The concentration of functional sugars (e.g., mannitol, panose) was markedly increased when sucrose was added to a concentration of 2%, whereas the concentrations of functional sugars and organic acids were not significantly changed with respect to concentration of sucrose added at over 2% (Table 1). Thus, 6 samples different in type of wort and aeration conditions during fermentation and identical in concentration of sucrose supplemented to 2% were chosen for sensory descriptive analysis (Table 1).

A total of 21 appearance, odor, and flavor attributes were generated during training sessions for descriptive analysis to characterize the sensory properties of the fermented worts. The descriptions and the reference samples for the attributes are given in Table 2. MANOVA performed on the descriptive analysis data revealed significant differences among the samples (p<0.001) and ANOVA showed that mean values of all the sensory attributes except 'vinegar odor' differed significantly between the samples (p<0.05, Table 3).

Principal component analysis (PCA) revealed that PC1 and PC2 explained 61.87 and 29.06% of the total variance, respectively (Fig. 4). The main attributes defining PC1 dimension included 'brownness', 'brown sugar odor', 'bitter', 'burnt odor', 'burnt flavor', 'roasted barley odor', and 'roasted barley flavor' on the positive side of PC1 and 'malt odor', 'malt flavor', 'honey odor', and 'honey flavor' on the negative side of PC1. The PC2 dimension was mainly defined by the descriptors such as 'sour', 'vinegar odor', 'vinegar flavor', 'pungent', and 'salty', which were located on the positive side of PC2.

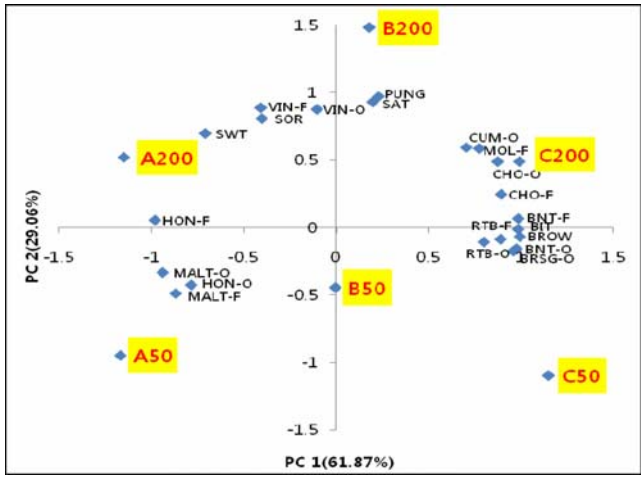

Fig. 4. Principal component (PC) loadings and scores of the sensory attributes[1)] of worts[2)] fermented by *L. citreum* for component 1 and 2. [1)]BROW, brownness; MALT-O, malt odor; HON-O, honey odor; VIN-O, vinegar odor; BRSG-O, brown sugar odor; CHO-O, *cheonggukjang* odor; RTB-O, roasted barley odor; BNT-O, burnt odor; CUM-O, cumin odor; SWT, sweet; SAT, salty; SOR, sour; BIT, bitter; MALT-F, malt flavor; HON-F, honey flavor; VIN-F, vinegar flavor; CHO-F, *cheonggukjang* flavor; RTB-F, roasted barley flavor; BNT-F, burnt flavor; MOL-F, molasses flavor; PUNG, pungent. [2)]See Table 1 for abbreviation.

The 'brownness', 'brown sugar odor', 'bitter', 'burnt

odor', 'burnt flavor', 'roasted barley odor', and 'roasted barley flavor' attributes, which were loaded on the positive PC1 dimension, showed high correlation to each other (Fig. 4) and strong in samples C50 and C200 prepared from Wort C (Table 3). This was probably because the samples C50 and C200 were made of malts containing large amount of roast malt, which had been produced at high temperature. It was reported the intensity of brown color, roasted flavor, and burnt flavor was influenced by roasting time and temperature (17,18). For instance, the intensity of the roasting-related attributes was increased by formation of Maillard reaction compounds such as pyrazines and pyrazine derivatives, which was promoted at high temperature (18,19).

The 'malt odor', 'malt flavor', 'honey odor', and 'honey flavor' attributes, which were loaded on the negative PC1 dimension, showed high correlation to each other (Fig. 4) and strong in samples A50 and A200 prepared from Wort A (Table 3). The sample A200 was also closely located with 'sweet' attribute. The 'malt odor' appeared to result from 3-methylbutanal and 2-methylbutanal, which were reported as major compounds involved in odors of barley malt (20).

The samples B50 and B200 were loaded between the samples A50, A200 and the samples C50, C200 along the PC1, indicating those samples had intermediate sensory properties of A and C.

In summary, the fermented wort samples were separated along the PC1 according to the intensity of 'brownness', 'brown sugar odor', 'bitter', 'burnt odor', 'burnt flavor', 'roasted barley odor', 'roasted barley flavor', 'malt odor', 'malt flavor', 'honey odor', and 'honey flavor', which was influenced by the roasting conditions of malts used in wort preparation.

The 'sour', 'vinegar odor', 'vinegar flavor', 'pungent', and 'salty' attributes were closely correlated to each other and loaded on the positive side of PC2 (Fig. 4). The samples A200, B200, and C200 were loaded highly on the positive side of PC2, while samples A50, B50, and C50 were loaded on the negative side of PC2. In detail, samples A200 and B200 were rated high on 'sour' attribute (Table 3). Samples A200, B200, and C200 were rated high on 'vinegar flavor'. This was probably due to concentration of organic acids, in particular, acetic acid produced during fermentation. According to fermentation results (Table 1), acetic acid concentration was higher at A200, B200, and C200, which had been produced under more aerobic conditions than at A50, B50, and C50, respectively.

The samples A200, B200, and C200 were also rated high on 'pungent' attribute. The intensity of 'pungent' was reported to depend on content of acidic volatiles such as acetic acid, 2- and 3-methyl butanoic acid, and 4-hydroxy-2, 5-dimethyl-3(2H)-furanon (20). Thereby, we assumed that the 'pungent' attribute of the samples resulted from such acidic volatile compounds.

The samples B200 and C200 were rated high on 'salty' attribute compared to other samples. These samples were also rated high on '*cheonggukjang* odor', '*cheonggukjang* flavor', and 'cumin odor'. The intensity of '*cheonggukjang*' attribute, which was known to be influenced by concentration of tetramethyl pyrazine, 3-methyl butanoic acid, butyric acid, and valeric acid (21), might negatively affect consumer acceptability of the fermented worts because most of Korean consumers do not expect '*cheonggukjang*' attribute in beverages. Cumin is commonly used as spice in Indian, Greek, and Turkish cuisine. However, most of Koreans are not familiar with cumin odor and might feel unpleasant when they perceive the odor. Therefore, cumin odor might also negatively affect the consumer acceptability of the fermented worts.

In summary, the fermented wort samples were separated along the PC2 according to the intensity of 'sour', 'vinegar odor', 'vinegar flavor', 'pungent', and 'salty', which was mostly affected by aeration conditions during fermentation.

This study showed that concentration of organic acids (e.g., lactic acid, acetic acid) as well as functional sugars (e.g., mannitol, panose) was significantly influenced by roasting conditions of malts used in wort preparation, sucrose content and/or aeration conditions during fermentation. Sensory properties of the fermented worts were also dependent on roasting conditions of malts and aeration conditions. We assume that these results could make a contribution to development of a fermented beverage rich in prebiotics and functional ingredients as well as desirable sensory properties.

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