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Sensory and Chemical Characteristics of Worts Fermented by *Leuconostoc citreum* and *Saccharomyces cerevisiae* and Consumer Acceptability with Added Flavorings

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Abstract This study was conducted to examine the chemical and sensory characteristics of fermented worts and consumer acceptability according to added flavorings. The worts were fermented by yeast (*Saccharomyces cerevisiae*) following fermentation by lactic acid bacteria (*Leuconostoc citreum*) at different aeration conditions. Chemical and sensory descriptive analyses were conducted to examine the effects of the fermentation conditions. The consumer acceptability of the worts with added flavorings was also examined. Organic acids, functional sugars, and ethanol were produced by *L. citreum* and *S. cerevisiae*, respectively. Ethanol concentrations ranged from 10 to 25 g/L depending on the fermentation conditions. The sensory characteristics of the fermented worts were clearly differentiated by the fermentation conditions. Yeast fermentation resulted in high intensities for certain sensory attributes such as 'alcohol', 'fermented barley', 'fermented white grape', and 'grassy'. Consumer acceptability changed with different levels of sugar and lemon flavoring, and the optimum levels were determined as 14.08% sugar and 0.98% lemon flavoring. Under these conditions, it was shown that a relatively acceptable fermented wort beverage containing functional materials can be produced.

Keywords: fermented wort, chemical property, sensory characteristics, consumer acceptability, *Saccharomyces cerevisiae*

Introduction

Interest in functional foods and beverages is growing due to increased consumer demands for products with health benefits (1-3). With this trend, functional beverages are expanding in the marketplace (4,5). Fruits, vegetables, and cereals have been used as raw materials for the development of functional beverages (6-11). Malt, in particular, is considered to be a good source of amino acids, sugars, vitamins, minerals, and fiber (12); thus, malt could be a high-quality raw material for the production of fermented beverages.

In a previous study (13), the chemical and sensory properties of worts fermented by *Leuconostoc citreum* were investigated. Organic acids and functional sugars were produced during fermentation, and these metabolites as well as the sensory characteristics of the fermented worts were significantly influenced by the type of wort and the aeration conditions during fermentation.

Yeast is used to produce various foods and beverages such as bread, beer, and wine. During yeast fermentation, the major products are ethanol, carbon dioxide, and glycerol (14). Organic acids and volatile compounds such as esters, aldehydes, and sulfur compounds are also produced as secondary metabolites (8,15,16). Thus, it was thought that yeast would be a good candidate for the development of fermented beverages with various flavors. If worts are fermented by yeast following fermentation by lactic acid

bacteria (LAB), it is expected that greater varieties of metabolites and flavor compounds will be produced as compared to worts fermented by LAB only. Therefore, it is important to study the chemical and sensory properties of worts fermented by LAB and yeast for the development of functional beverages using malts. The objectives of this study were: (1) to analyze the chemical properties of worts fermented by LAB and yeast, (2) to investigate the sensory characteristics of fermented worts, and (3) to understand the consumer acceptability of fermented wort beverages with different levels of added sugar and lemon flavoring to improve the sensory quality.

Materials and Methods

Fermentation of worts The worts were prepared as reported previously (13) using malt produced by heat treatment at 120°C for 24 hr. *Leuconostoc citerum* HJ-P4 isolated from *kimchi* (17), and *Saccharomyces cerevisiae* 89-5-2 isolated from *nuruk*, were used as starters for wort fermentation. *L. citreum* and *S. cerevisiae* were cultivated in lactobacilli MRS medium (Difco Laboratories, Detroit, MI, USA) and yeast mold (YM) medium (Difco Laboratories), respectively, and stored in 50%(v/v) glycerol stock at −70°C.

The wort fermentations with *L. citreum* were carried out in a rotary shaker (Jeio Tech, Seoul, Korea) at 50 or 200 rpm, for use as control samples (L50 and L200). For further fermentation with *S. cerevisiae*, test tubes (working volume, 5-mL) were prepared with the YM medium, inoculated with a colony grown on YM agar medium, and incubated in a rotary shaker at 30°C and 200 rpm until the optical density (OD) of the culture broth at 600 nm reached

between 0.5 and 1.0. The main fermentation with *S. cerevisiae* was started by inoculating 1.0 mL of seed culture into a 500-mL flask containing 100 mL of wort, which had been fermented with *L. citreum* at agitation speeds of 50 or 200 rpm (L50 and L200) and then centrifuged to remove the cell mass. The fermentations by *S. cerevisiae* were carried out in a rotary shaker at 30°C and 50 or 200 rpm until cell growth entered the stationary phase (L50S50, L50S200, L200S50, and L200S200). Therefore, 6 fermented worts including the above 4 samples and 2 control samples (L50 and L200) were subjected to chemical and sensory descriptive analyses.

Chemical analysis of fermented worts Cell concentration was determined from the $OD_{600\ nm}$ of the culture broth. Panose concentration was determined as previously reported (13); the concentration was measured by a high performance anion exchange chromatography (HPAEC, Dionex, Sunnyvale, CA, USA) equipped with a CarboPac PA10 column and a pulsed amperometric detection (PAD) detector. As the mobile phase, a 0.6 M Na-acetate/0.15 M NaOH solution was used. The concentrations of mannitol, lactic acid, acetic acid, and ethanol were determined as previously reported (13); after the fermented worts were filtrated with a 0.45-μm pore size filter, the metabolite concentrations were measured by high performance liquid chromatography (HPLC) (Waters, Milford, MA, USA) with a HPX-87H column (300×7.8 mm) (Bio-Rad, Richmond, CA, USA). An 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 of fermented worts (SDA): 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 sensory descriptive analysis. Among them, 5 had previously participated in the evaluation of sensory characteristics of worts fermented by LAB (13). The other 3 panelists were selected using screening procedures described by Delgerzaya *et al.* (13).

Panelist training was performed in the same manner as described by Delgerzaya *et al.* (13). The training sessions were held 4 days/week for 1 month and each training session took approximately 1 hr.

SDA: Sample preparation and presentation The sample preparation and presentation were the same as described by Delgerzaya *et al.* (13), except that 30 mL of each sample was presented to the panelists instead of 25 mL.

SDA: Evaluation procedure The evaluation procedures of the fermented worts were the same as in a previous study (13). The odor, flavor, and mouthfeel attributes were evaluated first in the individual booths, and then the appearance attributes of the samples were evaluated under daylight conditions in a light box (D65 Superlight-; Boteck, Siheong, Gyeonggi, Korea). To assess the flavor and mouthfeel attributes, the panelists tasted approximately 10 mL of sample and evaluated the intensity of each attribute. The evaluations were conducted once a day at 5 p.m., for 4 consecutive days.

SDA: Statistical analysis Statistical analyses of the sensory data were performed in the same manner as described by Delgerzaya *et al.* (13).

Consumer acceptability test of fermented wort beverages (CAT): Sample preparation and presentation To improve the sensory quality of the beverage, sugar, citric acid, and lemon flavoring were added to the fermented wort, as selected from a previous descriptive analysis. According to the preliminary experiments, the level of citric acid was fixed at 0.3% in all samples, and the values for overall acceptability and the acceptability of sweet taste, sour taste, and lemon flavor were predicted within the exploration regions of sugar (4 and 16%) and lemon flavoring (0.2 and 1.0%). The second-order response surface model was assumed since curvature was expected in the system. The central composite design (CCD) with replicated center runs was used. Thus, CCD consisted of 9 factorial design points of 3 levels of sugar (4, 10, and 16%) and lemon flavoring (0.2, 0.6, and 1.0%) with another run at the center.

Sugar and citric acid were put into a flask (500-mL) and mixed with the fermented worts. The lemon flavoring was added to the mixture at the very end to prevent a loss of flavor. After mixing, the samples (20 mL) were poured into polystyrene cups (length 5.2 cm, height 4 cm; Daemyung Co., Gimpo, Gyeonggi, Korea), covered with lids, and refrigerated (4°C) until evaluation. The samples were coded with 3-digit random numbers and presented at 10±1°C, and the presentation order of all samples was randomized. Filtered tap water (20±2°C, Ceramic Filter System, Fariey Industrial Ceramics Ltd., London, UK) was also provided to rinse the mouth. For the stabilized evaluation, the center sample was presented to the panelist for warm-up before the evaluation started.

CAT: Consumer selection Sixty-four consumers (20-30 years old, female) were recruited from the campus of Ewha Womans University (Seoul, Korea) using notice boards and the University website. Any consumers who were objectionable to the fermented beverage were not included in the test.

CAT: Evaluation procedure The consumer panelists evaluated overall acceptability as well as acceptabilities for sweet taste, sour taste, and lemon flavor. The evaluation was performed using a one-to-one interview style. Before the experiment, the evaluation procedure and scale use were explained to the panelists. They were instructed to rinse their mouths twice with filtered tap water. After rinsing their mouth, a panelist first tasted the warm-up sample and then continued to evaluate the 10 experimental samples. They rinsed their mouth twice with filtered tap water before tasting the next sample. They were not allowed to taste the previous sample again, but could change their rating. The panelists evaluated 5 samples, rested for 3 min, and then continued to evaluate the remaining samples. A 15-point category scale (1: dislike very much, 15: like very much) was used for the evaluation. All evaluations were conducted between 2 and 6 p.m. and each took approximately 15 min.

CAT: Statistical analysis The consumer acceptability data were analyzed using response surface methodology (RSM). The second-order response surface model was assumed to predict the values of overall acceptability and the acceptabilities of sweet taste, sour taste, and lemon flavor in the exploration regions of sugar (4 and 16%) and lemon flavoring (0.2 and 1.0%). To find the adequate model for the data, checks were made to determine whether certain terms in the second-order response surface model were insignificant at the significance level of 0.25, and then the reduced model was constructed by dropping the insignificant terms from the full model. The reduced model is claimed to be adequate if residual plots show prototype situations. After building an appropriate response surface model for each type of acceptability, a set of operating conditions that in some way optimized all types of acceptability was found. Contour plots for each type of acceptability were described in order to graphically indicate the change in acceptability according to additions of sugar and lemon flavoring. All statistical analyses were performed using Design-Expert 7.1 software (Stat-Ease, Inc., Minneapolis, MN, USA).

Results and Discussion

Chemical properties of fermented worts The cell density of the L200S200 sample increased up to an optical density of 5.4, and ethanol was produced at a concentration of 8.5 g/L (Fig. 1A). As compared to L200, concentrations of mannitol, acetic acid, and lactic acid were slightly decreased, whereas the level of panose remained unchanged (Table 1).

In the L200S50 sample, cells grew to an optical density of 5.8, which was similar to that of fermentation conducted under more aerobic conditions (Fig. 1B). However, the

Table 1. Effect of fermentation conditions on metabolite concentrations

Sample identification	Aeration rate[1]		Final cell density (OD$_{600\,nm}$)	Ethanol (g/L)	Lactic acid (g/L)	Acetic acid (g/L)	Mannitol (g/L)	Panose (g/L)
	I (rpm)	II (rpm)						
L200	200	-[2]	1.4±0.1[3]	1.8±0.1	8.2±0.5	3.1±0.2	6.4±0.3	4.0±0.3
L200S200	200	200	5.4±0.3	8.5±0.3	6.3±0.5	2.1±0.1	5.0±0.3	4.1±0.2
L200S50	200	50	5.8±0.3	14.5±0.6	4.2±0.3	1.9±0.1	3.8±0.2	4.1±0.3
L50	50	-	1.1±0.1	2.3±0.1	7.4±0.5	2.8±0.2	5.8±0.3	3.4±0.3
L50S200	50	200	6.4±0.3	25.8±0.7	7.4±0.5	2.5±0.2	0	3.4±0.2
L50S50	50	50	7.8±0.3	25.8±0.7	7.1±0.5	2.3±0.1	0	3.4±0.2

[1]Aeration rate during fermentation with *L. citreum* (I) and *S. cerevisiae* (II).
[2]Fermentation by *S. cerevisiae* was skipped.
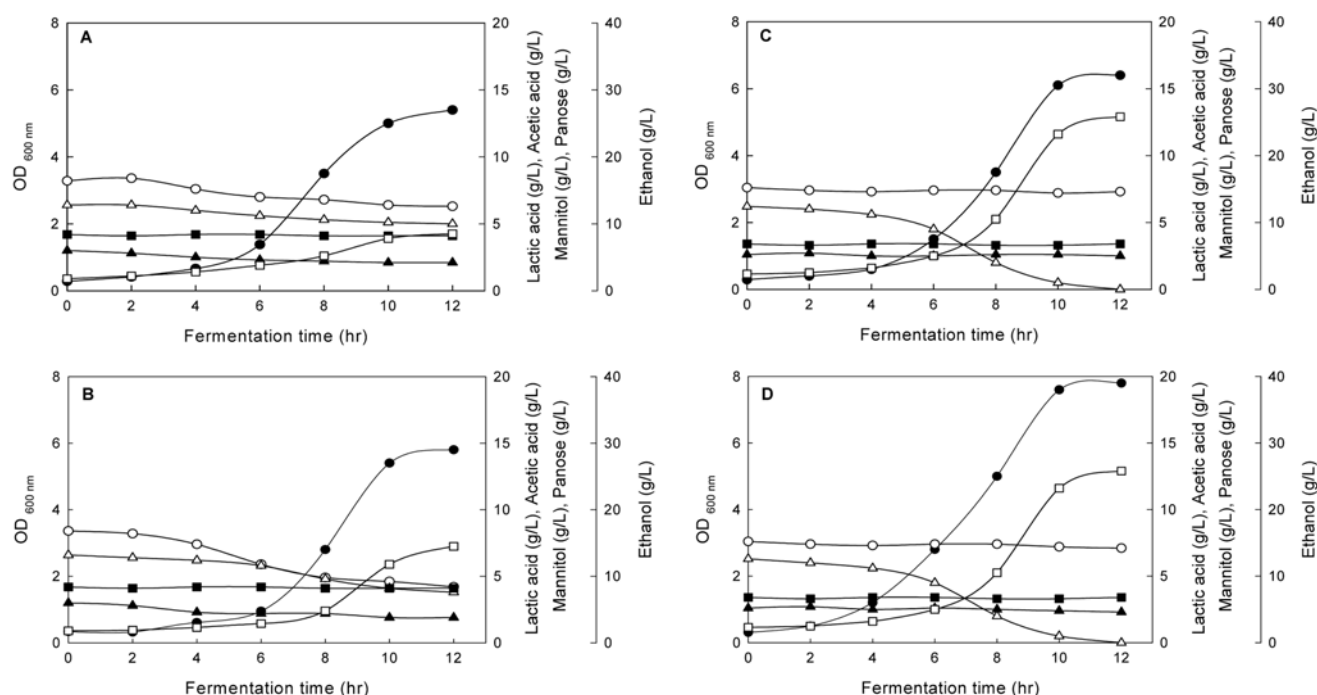[3]Means±SD of triplicate.

Fig. 1. Wort fermentation with *S. cerevisiae* 89-5-2. Wort, which had been fermented with *L. citreum* HJ-P4 at 200 rpm, was fermented with *S. cerevisiae* at 200 rpm (A) or 50 rpm (B). Wort, which had been fermented with *L. citreum* HJ-P4 at 50 rpm, was fermented with *S. cerevisiae* at 200 rpm (C) or 50 rpm (D). ●, OD; ○, lactic acid concentration; ▲, acetic acid concentration; △, mannitol concentration; □, ethanol concentration; and ■, panose concentration.

ethanol concentration of L200S50 was increased up to 14.5 g/L, and the lactic acid and mannitol concentrations were reduced at 1.9 and 3.8 g/L, respectively (Table 1).

The cell density of the L50S200 sample increased to an optical density of 6.4, and ethanol was produced to a final concentration of 25.4 g/L (Fig. 1C). As compared to L50, concentrations of lactic acid, acetic acid, and panose remained unchanged, but mannitol concentration was reduced to 0 (Table 1). Mannitol appeared to be used as a carbon source after maltose, since the major carbon source was depleted in the medium.

The fermentation pattern of the L50S50 sample (Fig. 1D) was very similar to the fermentation shown in Fig. 1C. The cells grew to an optical density of 7.8, and ethanol was produced to a concentration of 25.8 g/L. Concentrations of lactic acid, acetic acid, and panose remained constant, but mannitol was used up completely (Table 1).

Overall, these chemical analysis results indicate that the concentrations of organic acids, functional sugars, and ethanol varied greatly according to the fermentation conditions of samples.

Sensory characteristics of the fermented worts　A total of 26 attributes consisting of 1 appearance, 9 odor (o), 13 flavor (f), and 3 mouthfeel attributes were generated during the training sessions for descriptive analysis, to characterize the sensory properties of the fermented worts. The descriptions and reference samples for the sensory attributes are given
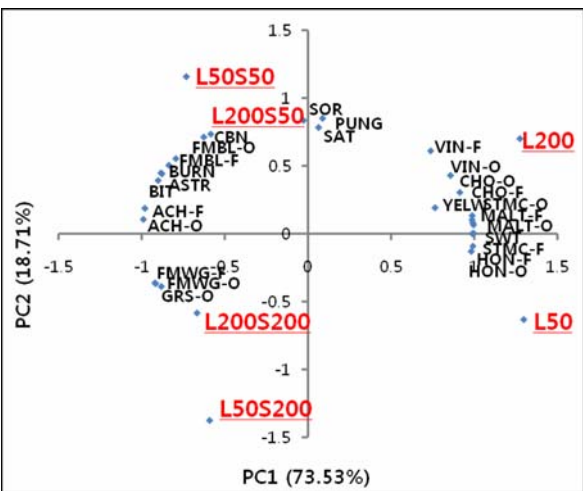

Fig. 2. Principal component (PC) loadings and scores of the sensory attributes[1] of fermented worts[2] for component 1 and 2.
[1]YELW, yellowness; MALT-O, malt odor; HON-O, honey odor; VIN-O, vinegar odor; CHO-O, *cheonggukjang* odor; STMC-O, steamed corn odor; ACH-O, alcohol odor; FMBL-O, fermented barley odor; FMWG-O, fermented white grape odor; GRS-O, grassy; SWT, sweet; SOR, sour; SAT, salty; BIT, bitter; MALT-F, malt flavor; HON-F, honey flavor; VIN-F, vinegar flavor; CHO-F, *cheonggukjang* flavor; STMC-F, steamed corn flavor; ACH-F, alcohol flavor; FMBL-F, fermented barley flavor; FMWG-F, fermented white grape flavor; PUNG, pungent; ASTR, astringent; BURN, burning; and CBN, carbonated. [2]See Table 1 for sample identification.

Table 2. Definitions of the descriptive attributes of fermented worts

Sensory attributes		Definitions
Appearance	Yellowness	Intensity of yellow color of fermented worts
Odor	Malt	Smell associated with malt
	Honey	Smell associated with honey
	Vinegar	Smell associated with vinegar
	Cheonggukjang[1]	Smell associated with *cheonggukjang*[1]
	Steamed corn	Smell associated with steamed corn
	Alcohol	Smell associated with ethyl alcohol
	Fermented barley	Smell associated with fermented barley
	Fermented white grape	Smell associated with fermented white grape
	Grassy	Smell associated with grass
Flavor	Sweet	Fundamental taste sensation of which sucrose is typical
	Salty	Fundamental taste sensation of which sodium chloride is typical
	Sour	Fundamental taste sensation of which lactic and citric acid is typical
	Bitter	Fundamental taste sensation of which caffeine and quinine is typical
	Malt	Aromatics associated with malt
	Honey	Aromatics associated with honey
	Vinegar	Aromatics associated with vinegar
	Cheonggukjang[1]	Aromatics associated with *cheonggukjang*[1]
	Steamed corn	Aromatics associated with steamed corn
	Alcohol	Aromatics associated with ethyl alcohol
	Fermented barley	Aromatics associated with fermented barley
	Fermented white grape	Aromatics associated with fermented white grape
	Pungent	Sharp irritating sensation in the throat and nasal cavity while the sample is swallowed
Mouthfeel	Astringent	Feeling which shrivels the tongue associated with tannins
	Burning	Irritating sensation in the oral cavity while the sample is in the mouth
	Carbonated	Feeling of small bubbles in the mouth associated with carbon dioxide

[1]A Korean traditional fermented soybean paste.

Table 3. Reference samples for the descriptive attributes of fermented worts

	Sensory attributes	Reference samples
Appearance	Yellowness	-
Odor[1]	Malt	10% Malt [20 g of malt (Saimdang Food Co., Ltd, Seoul, Korea) soaked in the 180 mL water for 30 min and filtered with a cloth (Dashibag, T&C Electronics, Yiwang, Gyeonggi, Korea)] dispersion
	Honey	13% Honey (Well Being Premium Honey, Gangwon Nongwon Co., Ltd., Yangju, Gangwon, Korea) solution
	Vinegar	5% Vinegar (Ottogi Vinegar, Ottogi Co., Ltd., Anyang, Gyeonggi, Korea)
	Cheonggukjang[2]	10 g *Cheonggukjang*[2] (Urirang *cheonggukjang*[2], Arirang Food, Paju, Gyeonggi, Korea)
	Steamed corn	50 g Steamed corn [100 g of corn (local supermarket, Seoul, Korea) was steamed in 300 g of water]
	Alcohol	0.5% Ethyl alcohol (Duksan Pure Chemical Co., Ltd., Ansan, Gyeonggi, Korea)
	Fermented barley	30 g Beer (Max, THE HITE Co., Ltd., Seoul, Korea)
	Fermented white grape	30 g White wine (William Fevre Chablis Grand Cru Les Clos, Chablis, France)
	Grassy	0.2% *cis*-3-Hexenal (Bolak Co., Ltd., Hwaseong, Gyeonggi, Korea)
Flavor	Sweet	2% Sucrose (Duksan Pure Chemical Co., Ltd.) solution
	Salty	0.5% Sodium chloride (Duksan Pure Chemical Co., Ltd.) solution
	Sour	0.03% Citric acid (Duksan Pure Chemical Co., Ltd.) solution
	Bitter	0.03% Caffeine (Sigma-Aldrich, St. Louis, MO, USA) solution
Flavor	Malt	10% Malt [20 g of malt (Saimdang Food Co., Ltd.) soaked in the 180 mL water for 30 min and filtered with a cloth (Dashibag, T&C Electronics)] dispersion
	Honey	8% Honey (Well Being Premium Honey, Gangwon Nongwon Co., Ltd.) solution
	Vinegar	3% Vinegar (Ottogi Vinegar, Ottogi Co., Ltd.)
	Cheonggukjang[2]	10 g *Cheonggukjang*[2] (Urirang *cheonggukjang*[2], Arirang Food)
	Steamed corn	50 g Steamed corn [100 g of corn (local supermarket, Seoul, Korea) was steamed with 300 g of water]
	Alcohol	0.5% Ethyl alcohol (Duksan Pure Chemical Co., Ltd.)
	Fermented barley	30 g Beer (Max, THE HITE. Co., Ltd.)
	Fermented white grape	30 g White wine (William Fevre Chablis Grand Cru Les Clos)
	Pungent	13% Vinegar (Ottogi Vinegar, Ottogi Co., Ltd.)
Mouthfeel	Astringent	0.1% Tannic acid (Duksan Pure Chemical Co., Ltd.) solution
	Burning	30 g *Soju* (Chamisul, Jinro Co., Ltd., Seoul, Korea) diluted to 2% alcohol content with filtered water
	Carbonated	30 g Carbonated water (San Pellegrino, San Pellegrino, Milan, Italy) diluted to 0.3% carbon dioxide content with filtered water

[1]Aliquots (30 mL) of the reference samples for the odor attributes except '*cheonggukjang*' and 'steamed corn' were put into a vial (60-mL) with lids >1 hr before the odor evaluation. For '*cheonggukjang*' and 'steamed corn' attributes, the reference samples were put into plastic air-tight containers (HPL805; Lock & Lock Co., Ltd., Ansan, Gyeonggi, Korea).
[2]A Korean traditional fermented soybean paste.

in Table 2 and 3, respectively. Multivariate analysis of variance (MANOVA) of the descriptive analysis data indicated there were significant differences among the samples ($p<0.001$). And analysis of variance (ANOVA) and Duncan's multiple range test showed that the mean values of all sensory attributes were significantly different among the samples ($p<0.05$, Table 4).

The principal component analysis (PCA) results revealed that PC1 and PC2 explained 73.53 and 18.71% of the total variance, respectively (Fig. 2). The attributes related to wort such as 'yellowness', 'sweet', 'malt (o, f)', 'honey (o, f)', and 'steamed corn (o, f)' were highly loaded on the positive side of PC1, and showed strong correlations to each other (Fig. 2). As shown in Table 4, the L50 and L200 samples fermented by LAB were evaluated as having relatively strong intensities for these attributes compared to the other samples that were further fermented by yeast (L50S50, L50S200, L200S50, and L200S200). 'Yellowness'

was rated as high in the L50 and L200 samples, but the differences between the yeast-fermented samples were small. Yeast fermentation had a minor effect on 'yellowness'. For the 'sweet' attribute, the L50 and L200 samples were rated high, while the yeast-fermented samples were rated low. The low 'sweet' intensities of the yeast-fermented samples could be due to maltose consumption, which is the major sugar in wort, during yeast fermentation (12). In a previous study (13), 'malt (o, f)' and 'honey (o, f)' were rated high in samples prepared from worts containing 100% malt and fermented by *L. citreum*. In agreement with these results, the L50 and L200 samples in this study had the highest intensities for these wort-related attributes. In addition to these attributes, the L50 and L200 samples were rated high for '*cheonggukjang* (o, f)' when compared to the yeast-fermented samples, and the L200 sample was rated high for 'vinegar (o, f)', which is assumed to be from the acetic acid produced during fermentation. When LAB

Table 4. Sensory characteristics of fermented worts

Sensory attributes		L50[1]	L200	L50S50	L50S200	L200S50	L200S200
Appearance	Yellowness	4.84[ab2]	5.28[a]	4.63[ab]	4.31[b]	4.19[b]	4.72[ab]
Odor	Malt	10.47[a]	10.75[a]	3.47[b]	3.31[b]	3.75[b]	3.28[b]
	Honey	6.44[a]	6.66[a]	3.31[c]	4.22[b]	3.59[bc]	3.81[bc]
	Vinegar	3.91[b]	4.94[a]	3.41[bc]	2.84[cd]	3.19[cd]	2.75[d]
	Cheonggukjang[3]	6.25[a]	6.56[a]	3.00[bc]	2.22[c]	4.47[b]	2.47[c]
	Steamed corn	5.53[a]	5.22[a]	2.47[b]	2.09[b]	2.66[b]	2.19[b]
	Alcohol	2.25[c]	2.28[c]	7.31[a]	6.38[b]	6.94[ab]	6.38[b]
	Fermented barley	2.72[c]	3.03[c]	6.97[a]	4.19[b]	6.22[a]	4.41[b]
	Fermented white grape	1.72[c]	1.56[c]	4.94[b]	6.91[a]	4.56[b]	6.41[a]
	Grassy	1.53[c]	1.56[c]	2.81[b]	3.91[a]	3.06[b]	4.19[a]
Flavor	Sweet	8.06[a]	8.06[a]	4.09[b]	4.41[b]	3.87[b]	3.53[b]
	Sour	4.50[c]	6.97[a]	6.38[ab]	4.91[c]	6.09[ab]	5.59[bc]
	Salty	3.28[c]	5.47[a]	4.94[ab]	3.59[c]	4.22[bc]	4.16[bc]
	Bitter	2.09[c]	2.75[c]	6.66[a]	4.09[b]	5.81[a]	5.72[a]
	Malt	9.41[a]	9.38[a]	3.50[b]	2.84[c]	3.19[bc]	3.06[bc]
	Honey	5.69[a]	5.69[a]	2.19[c]	2.84[b]	2.47[bc]	2.69[bc]
	Vinegar	4.13[b]	4.94[a]	4.09[b]	3.03[c]	3.59[bc]	3.50[bc]
	Cheonggukjang[3]	5.44[a]	5.94[a]	2.47[b]	2.22[b]	2.62[b]	2.25[b]
	Steamed corn	5.47[a]	5.38[a]	2.00[b]	2.09[b]	2.09[b]	2.16[b]
	Alcohol	2.06[c]	2.59[c]	7.53[a]	6.03[b]	6.91[ab]	6.53[ab]
	Fermented barley	2.97[d]	3.41[d]	7.78[a]	4.75[c]	6.41[b]	5.31[bc]
	Fermented white grape	2.09[c]	1.91[c]	4.72[b]	6.72[a]	4.94[b]	5.88[a]
	Pungent	3.12[b]	5.87[a]	4.84[a]	3.41[b]	5.13[a]	3.78[b]
Mouthfeel	Astringent	3.41[d]	4.59[c]	7.00[a]	5.28[bc]	6.22[ab]	6.16[ab]
	Burning	1.94[c]	2.47[c]	4.84[a]	3.41[b]	4.84[a]	3.91[ab]
	Carbonated	1.66[c]	2.78[ab]	3.47[a]	2.25[bc]	3.59[a]	3.09[ab]

[1]See Table 1 for sample identification.
[2]Means of 4 replicates with 8 panelists; Means within a row not sharing a superscript letter are significantly different ($p<0.05$, Duncan's multiple range test).
[3]A Korean traditional fermented soybean paste.

Table 5. Sensory acceptability of fermented wort beverages containing different levels of sugar and lemon flavoring

Independent variables		Response variables			
Sugar (%)	Lemon flavoring (%)	Overall acceptability	Sweet taste acceptability	Sour taste acceptability	Lemon flavor acceptability
4	0.2	4.17	4.73	5.75	5.22
4	0.6	4.77	5.47	5.89	6.41
4	1.0	5.17	5.59	6.63	6.83
10	0.2	6.92	7.77	7.50	7.36
10	0.6	8.44	8.30	8.58	8.92
10	0.6	8.50	8.03	8.58	8.56
10	1.0	8.36	8.67	8.50	8.87
16	0.2	8.30	8.34	8.42	7.94
16	0.6	8.72	8.94	8.80	9.13
16	1.0	9.03	8.61	8.28	9.25

is used for fermentation under aerobic conditions, acetic acid production is usually increased, while under anaerobic conditions, it is decreased (18). The results of an earlier study (13) and this study indicate that the L200 sample had a higher acetic acid concentration and greater 'vinegar (o, f)' value than the L50 sample.

Attributes such as 'alcohol (o, f), 'bitter', 'fermented barley (o, f)', 'fermented white grape (o, f)', 'grassy (o)', 'astringent', and 'burning' were highly loaded on the negative side of PC1 (Fig. 2). The yeast-fermented samples were evaluated as being strong in these attributes compared to the samples fermented by LAB alone (Table 4). Thus, it can be said that these attributes are mainly affected by yeast fermentation. Since ethanol is produced during yeast

fermentation (14), it is assumed that the intensity of 'alcohol (o, f)' would be affected by the concentration of ethanol produced during yeast fermentation. The ethanol concentrations of the L50S50 and L50S200 samples were approximately twice as much as those of the L200S50 and L200S200 samples (Table 1). However, sensory differences in the 'alcohol (o, f)' attribute were relatively small among these samples (Table 4). The attributes of 'bitter', 'astringent', and 'burning' were closely located with 'alcohol (o, f)', indicating that all these attributes were affected by ethanol production (Fig. 2). In evaluations of sensory characteristics for alcoholic beverages such as wine and beer, 'bitter' and 'astringent' attributes were generated (19-23). Also, Paterson and Piggott (24) reported that the pain sensation of whiskey was related to the alcohol. These reports could partly explain the high correlations between the 'alcohol (o, f)', 'bitter', 'astringent', and 'burning' attributes in this study.

'Fermented barley (o, f)', 'fermented white grape (o, f)', and 'grassy (o)' were also rated high in the yeast-fermented samples as compared to the samples fermented by LAB only. Thus, these attributes are assumed to be affected by aroma compounds produced during yeast fermentation. The samples were clearly separated along the PC1 according to the types of fermentation.

'Sour', 'salty', and 'pungent' were highly loaded on the positive side of the PC2 dimension and were located closely, showing strong correlations to each other (Fig. 2). The L200, L50S50, and L200S50 samples were highly loaded on the positive PC2, while the L50, L50S200, and L200S200 samples were loaded relatively high on the negative PC2. The intensities of the 'sour' and 'pungent' attributes could be affected by organic acids and acidic volatiles produced during fermentation, respectively (25). However, the intensity of 'sour' did not clearly relate with the concentration of organic acids (Table 1 and 4). The samples were separated along the PC2 according to their intensities of 'sour', 'salty', and 'pungent' attributes. Meanwhile the L50S50 and L200S50 samples were highly loaded on the positive PC2 and were closely located with 'alcohol (o, f)', 'bitter', 'fermented barley (o, f)', 'astringent', and 'burning', indicating they had strong correlations to each other. However, the L50S200 and L200S200 samples were highly loaded on the negative PC2 and were closely located with 'fermented white grape (o, f)' and 'grassy (o)', showing strong correlations to each other. Thus, the yeast-fermented samples were separated along the PC2 dimension according to their differences in sensory characteristics, which were due to different aeration conditions (50 or 200 rpm) during yeast fermentation.

The fermented wort that had low intensities of undesirable attributes was selected to prepare the fermented wort beverage used to examine consumer acceptability with added flavorings. Thus, sample L50S200, which rated relatively low for 'alcohol (o, f)', 'bitter', '*cheonggukjang* (o, f)', and 'burning' attributes was chosen.

Descriptive analysis showed that the sensory characteristics of the worts fermented by LAB and yeast were significantly affected by the fermentation conditions.

Consumer acceptability of fermented wort beverages
The mean values for overall acceptability and the

Table 6. Analysis of variance showing the significance of the effects of variables on sensory acceptability of fermented wort beverages

Source[1]	df	Sum of squares	*p*-value
Overall acceptability			
A	1	1,520.04	<0.0001
B	1	107.32	<0.0001
C	63	1,422.50	<0.0001
A^2	1	250.73	<0.0001
B^2	1	23.57	0.0553
Lack of fit	508	3,401.54	0.0053
Sweet taste acceptability			
A	1	1,086.76	<0.0001
B	1	44.01	0.0111
C	63	1,458.19	<0.0001
A^2	1	237.51	<0.0001
Lack of fit	509	3,538.12	0.0998
Sour taste acceptability			
A	1	558.25	<0.0001
B	1	32.09	0.0356
C	63	2,008.18	<0.0001
AB	1	16.50	0.1315
A^2	1	134.63	<0.0001
Lack of fit	508	3,728.63	0.2517
Lemon flavor acceptability			
A	1	658.88	<0.0001
B	1	210.04	<0.0001
C	63	1,870.00	<0.0001
A^2	1	115.80	<0.0001
B^2	1	41.79	0.0137
Lack of fit	508	3,530.94	0.2138

[1]A, sugar; B, lemon flavoring; and C, consumer panelists.

acceptabilities of sweet taste, sour taste, and lemon flavor for the fermented wort beverages at each run of CCD are shown in Table 5.

Since each consumer panelist evaluated overall acceptability and the acceptabilities of sweet taste, sour taste, and lemon flavor at all runs of CCD, the panelists were treated as blocks. The consumer test data were analyzed by assuming the second-order response surface model with 64 blocks (panelists). The reduced model, by dropping the insignificant terms from the full model, was claimed to be adequate since the residual plots showed prototype situations. The analysis of variance table for the resulting response surface model for each type of acceptability is summarized in Table 6. Significant block effects ($p<0.0001$) for each type of acceptability indicated that significant differences existed in the average scores at all 10 runs of CCD among the consumer panelists. Since the CCD had 2 replicate runs at the center, the residual sum of squares can be partitioned into pure error and lack-of-fit components. The lack-of-fit test in Table 6 is testing for the lack of fit for the resulting response surface model. The *p*-value for this test, with the exception of the overall acceptability, seemed to be satisfactory, implying that the resulting response surface model was adequate. The scatter plots of studentized residuals and predicted values for each type of acceptability showed a
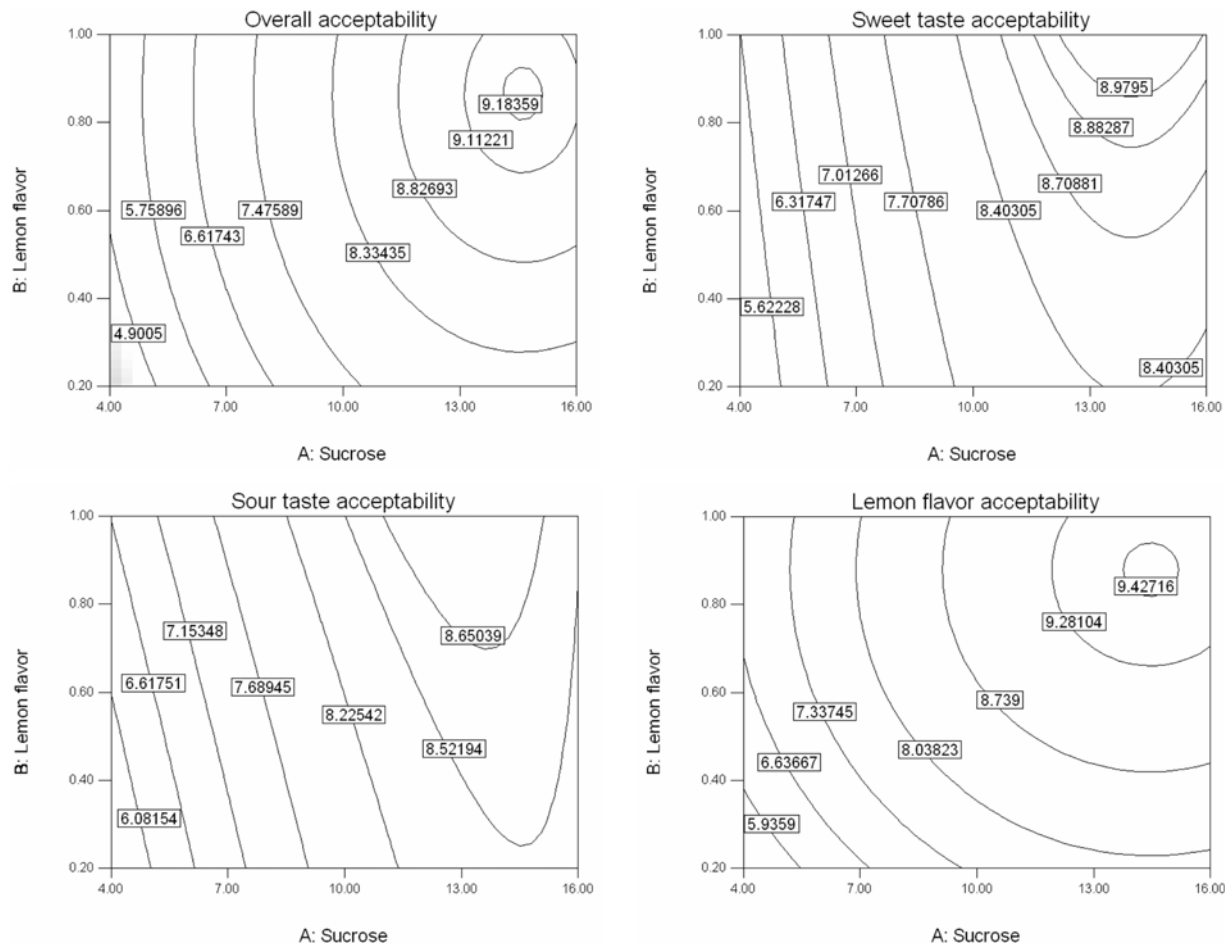
Fig. 3. Contour plots for overall acceptability and acceptability of sweet taste, sour taste, and lemon flavor of fermented wort beverages containing different levels of sugar and lemon flavoring.

random pattern, which supports that the developed model was reasonably adequate (figures not shown). It was interesting to see that there was no interaction effect between the sugar and lemon flavoring for all types of acceptability, with the exception of sour taste. For sour taste acceptability, the *p*-value for the interaction effect was 0.1315, which indicated that the interaction effect might be negligible. Thus, the sugar and lemon flavoring seemed to have additive effects on all types of acceptability. Also, sugar exerted a highly significant quadratic effect on all types of acceptability. On the other hand, lemon flavoring had a significant quadratic effect only on lemon flavor acceptability and had a significant linear effect on the other types of the acceptability.

Defining the coded variables X_1 and X_2 corresponding to the sugar and lemon flavoring as $X_1 = (\text{sugar} - 10)/6$ and $X_2 = (\text{lemon flavoring} - 0.6)/0.4$, the regression equations for the different types of acceptability in terms of the coded variables are as follows:

$$\hat{Y}_1 = 8.25 + 1.99X_1 + 0.53X_2 - 1.30X_1^2 - 0.40X_2^2$$

$$\hat{Y}_2 = 8.19 + 1.68X_1 + 0.34X_2 - 1.24X_1^2$$

$$\hat{Y}_3 = 8.23 + 1.21X_1 + 0.29X_2 - 0.25\ X_1X_2 - 0.94X_1^2$$

$$\hat{Y}_4 = 8.69 + 1.31X_1 + 0.74X_2 - 0.88X_1^2 - 0.53X_2^2$$

where $\hat{Y}_1$, $\hat{Y}_2$, $\hat{Y}_3$, and $\hat{Y}_4$ are the predicted values of overall acceptability and the acceptabilities of sweet taste, sour taste, and lemon flavor, respectively.

As an approach to the numerical optimization of multiple responses, the simultaneous optimization technique popularized by Derringer and Suich (25) was used. Their procedure utilizes desirability functions. First each response y_i was converted into an individual desirability function d_i over the range [0, 1],

$$d_i = \begin{cases} 0 & y < 1 \\ \dfrac{y_i - 1}{15 - 1} & 1 \leq y_i \leq 15 \end{cases}$$

Then the coded variables were chosen to maximize the overall desirability

$$D = (d_1 \times d_2 \times d_3 \times d_4)^{\frac{2}{4}}$$

which was equivalent to maximizing the geometric mean of the predicted values of the 4 responses. The optimal conditions were found to be 14.08% sugar and 0.98% lemon flavoring. The predicted values at the optimal condition were 9.15, 9.08, 8.73, and 9.41 for overall acceptability and the acceptabilities of sweet taste, sour
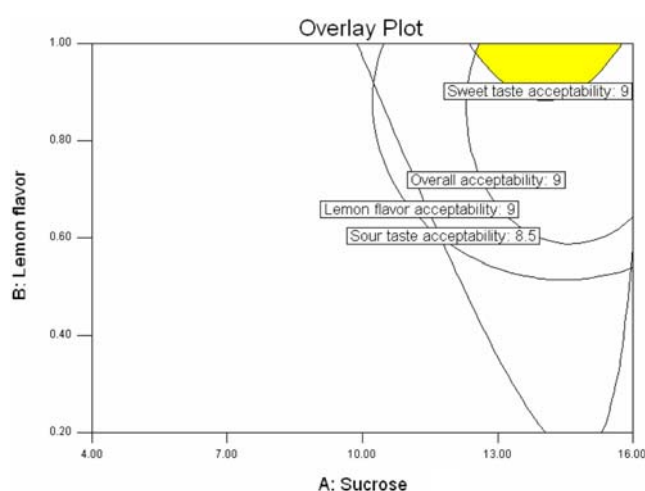
Fig. 4. Overlay plot for overall acceptability and acceptability of sweet taste, sour taste, and lemon flavor of fermented wort beverages containing different levels of sugar and lemon flavoring.

taste, and lemon flavor, respectively. Contour plots for each sensory acceptability are presented in Fig. 3. Overall acceptability and lemon flavor acceptability were highest at 14-15% sugar and 0.8-0.9% lemon flavoring and then decreased gradually. The acceptabilities of both sweet taste and sour taste scored highest at around 13-14% sugar, and they gradually increased as the proportion of lemon flavoring increased within the experimental range. Figure 4 shows an overlay plot for 4 responses with contours, for which $Y_1 \geq 9.0$, $Y_2 \geq 9.0$, $Y_3 \geq 8.5$, and $Y_4 \geq 9.0$ and the feasible region were found around the optimal condition.

The consumer acceptability tests indicate that a relatively acceptable fermented wort beverage can be developed with added flavorings used in this study. Although sucrose was used as the starting point to examine the desirable level of sweetness, sucrose can be replaced with other sweeteners such as intensive sweeteners for added health benefits. Thus, further studies are necessary to choose a desirable sweetener and the optimal level of sweetness.

Acknowledgments

References

1. Luckow T, Delahunty C. Consumer acceptance of orange juice containing functional ingredients. Food Res. Int. 37: 805-814 (2004)
2. Temelli F, Bansema C, Stobbe K. Development of an orange-flavored barley β-glucan beverage with added whey protein isolate. J. Food Sci. 69: 237-242 (2004)
3. Verbeke W. Consumer acceptance of functional foods: Socio-demographic, cognitive, and attitudinal determinants. Food Qual. Prefer. 16: 45-57 (2005)
4. Beucler J, Drake M, Foegeding EA. Design of a beverage from whey permeate. J. Food Sci. 70: S277-S285 (2005)
5. Deshpande RP, Chinnan MS, McWatters KH. Nutritional, physical, and sensory characteristics of various chocolate-flavored peanut-soy beverage formulations. J. Sens. Stud. 20: 130-146 (2005)
6. Jeong J-S, Kim S-H, Kim M-L, Choi K-H. Acidic beverage fermentation using citrus juice and antimicrobial activity of the fermented beverage. J. Korean Soc. Food Sci. Nutr. 37: 1037-1043 (2008)
7. Kim S-Y, Choi E-H. Optimization for the lactic acid fermentation of mixed fruit and vegetable juices. Korean J. Food Sci. Technol. 34: 303-310 (2002)
8. Jung D-S, Lee Y-K, Lim KW. Characteristics of fermented fruit and vegetable mixed broth using by bacteriocin-producing lactic acid bacteria and yeast. Korean J. Food Sci. Technol. 32: 1358-1364 (2000)
9. Shin D-H. A yogurt like product development from rice by lactic acid bacteria. Korean J. Food Sci. Technol. 21: 686-690 (1989)
10. Yu T-J, Rhi J-W. Studies on preparation of lactic acid fermented beverages from a malt syrup. Korean J. Food Sci. Technol. 14: 57-62 (1982)
11. Jo S-J, Oh S-M, Jang E-K, Hwang K, Lee S-P. Physicochemical properties of carrot juice fermented by *Leuconostoc mesenteroides* SM. J. Korean Soc. Food Sci. Nutr. 37: 210-216 (2008)
12. Briggs DE, Stevens R, Young TW, Hough JS. Malting and Brewing Science: Malt and Sweet Wort. Vol. 1. Kluwer Academic/Plenum Publishers, London, UK. pp. 1-192 (1981)
13. Delgerzaya P, Shin JY, Kim K-O, Park J-B. Wort fermentation by *Leuconostoc citreum* originated from *kimchi* and sensory properties of fermented wort. Food Sci. Biotechnol. 18: in press (2009)
14. Pronk JT, Steensma HY, Van Dijken JP. Pyruvate metabolism in *Saccharomyces cerevisiae*. Yeast 12: 1607-1633 (1996)
15. Kim YR, Moon ST, Park SK. Effects of yeast strains and fermentation temperatures in production of hydrogen sulfide during beer fermentation. Korean J. Food Sci. Technol. 40: 238-242 (2008)
16. Lee H, Lee T-S, Noh BS. Volatile flavor components in the mashes of *takju* prepared using different yeasts. Korean J. Food Sci. Technol. 39: 593-599 (2007)
17. Eom H-J, Seo DM, Han NS. Selection of psychrotrophic *Leuconostoc* spp. producing highly active dextransucrase from lactate fermented vegetables. Int. J. Food Microbiol. 117: 61-67 (2007)
18. Neves AR, Pool WA, Kok J, Kuipers OP, Santos H. Overview on sugar metabolism and its control in *Lactococcus lactis* − The input from *in vivo* NMR. FEMS Microbiol. Rev. 29: 531-554 (2005)
19. Gutiérrez Afonso VL. Sensory descriptive analysis of red wines undergoing malolactic fermentation with oak chips. J. Food Sci. 68: 1075-1079 (2003)
20. Mirarefi S, Menke SD, Lee SY. Sensory profiling of chardonel wine by descriptive analysis. J. Food Sci. 69: S211-S217 (2004)
21. Daems V, Delvaux F. Multivariate analysis of descriptive sensory data on 40 commercial beers. Food Qual. Prefer. 8: 373-380 (1997)
22. Guinard J-X, Yip D, Cubero E, Mazzucchelli R. Quality ratings by experts, and relation with descriptive analysis ratings: A case study with beer. Food Qual. Prefer. 10: 59-67 (1999)
23. Etaio I, Pérez Elortondo FJ, Albisu M, Gaston E, Ojeda M, Schlich P. Development of a quantitative sensory method for the description of young red wines from *Rioja Alavesa*. J. Sens. Stud. 23: 631-655 (2008)
24. Paterson A, Piggott JR. The contributions of the process of flavor in Scotch malt whisky. pp. 151-170. In: Distilled Beverage Flavour. Piggott JR, Paterson A (eds). Ellis Horwood, Chichester, UK (1989)
25. Derringer G, Suich R. Simultaneous optimization of several response variables. J. Qual. Technol. 12: 214-219 (1980)