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Organic Acid Composition and Flavor Characteristics of Lactic Acid Fermented Cereal Beverages

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The effect of different compositions of organic acids on the flavor profile of 10% sugar solution was investigated by the response surface methodology, and the results were used to evaluate the flavor characteristics of lactic acid fermented cereal beverages. A mixture of extruded rice flour (10%) and soymilk (7.8% dry matter) was fermented with *Leuconostoc mesenteroides* (Sikhae). Depending on the substrate pretreatments, for example, the malt or amylase digestion and the proteolytic enzyme hydrolysis, the sugar and organic acid composition of the product varied. The organic acid composition of the fermented beverages was in the ranges of 0.44-0.55% lactic acid, 0.05-0.09% acetic acid and 0.07-0.09% citric acid, while that of commercial apple juice was 1.59% malic acid and 0.49% acetic acid. The flavor profiles of fermented beverages added with 10% sucrose were compared to those of apple juice and a model mixture containing 0.48% citric acid, 0.39% lactic acid and 0.12% acetic acid in 10% sugar solution. The QDA diagram of fermented beverages approached to that of apple juice, when the substrate was digested by amylase but not by protease.

The formation of acceptable flavor during lactic acid fermentation is an important technical issue in the production of high protein lactic acid fermented food and beverages from cereals and legumes (2). A strain of lactic acid bacteria, *Leuconostoc mesenteroides*, was selected for this purpose from a traditional Korean lactic acid fermented fish product, Sikhae (3). This strain produced apple juice-like flavor from rice-soymilk mixture. The mechanism of the flavor formation was studied at Korea University. Souane (6) suggested that the strain may produce ethyl 3-methylbutyrate and ethyl 2-methylbutyrate, because the addition of the precursors for these compounds, such as isopentanol, 3-methylbutanol and isovaleric acid, increased the apple-like aroma formation. On the other hand, Chung (1) claimed that the apple juice-like refreshing flavor is formed by a certain combination of organic acids produced by the heterofermentative organism during the fermentation. He suggested that the concentrations of acetic acid and lactic acid were important. The ratio of the amounts of these two organic acids was dependent on the fermentation conditions, especially on the sugar composition of the substrate.

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In the present study, the sensory quality of the mixture of three different organic acids, acetic, lactic, and citric acid, in varying concentrations was evaluated. And the flavor of lactic acid fermented cereal beverage was compared to that of apple juice and the model mixture of organic acids.

MATERIALS AND METHODS

Organic Acids Mixture

A central composite design for Response Surface Methodology was applied by using the concentrations of citric acid, lactic acid and acetic acid as independent variables. The center points and ranges were 0.5± 0.25% (v/v) for citric acid, 0.40± 0.20% (v/v) for lactic acid and 0.125± 0.125% (v/v) for acetic acid. A total of 20 combinations of organic acid mixture in 10% sugar solutions were prepared according to the central composite design (4), and were subjected to sensory evaluation.

Preparation of Lactic Acid Fermented Cereal Beverage

Lactic acid fermented cereal beverages were prepared from a extruded rice (10%) and soy milk (7.8% dry matter) mixture by inoculating with *Leuconostoc mesenteroides* (Sikhae). The effects of different pretreatments

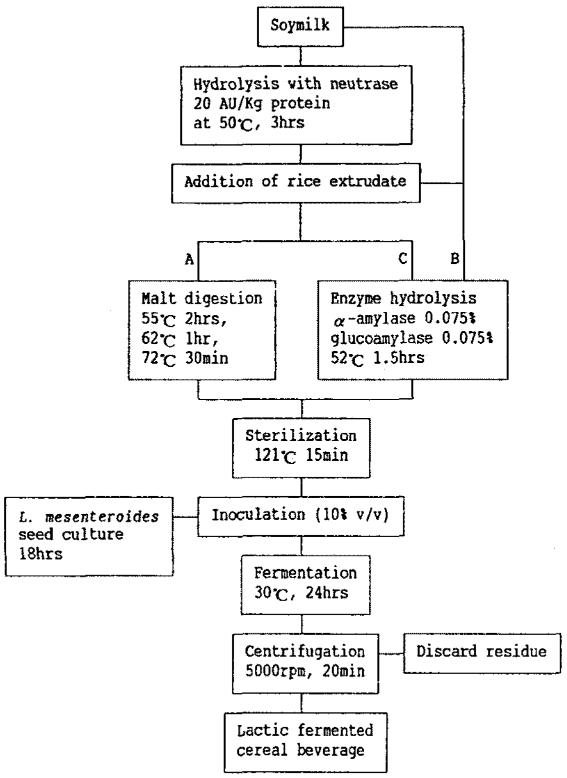

Fig. 1. Flow chart of the different pretreatments of substrate for cereal lactic fermentation.

on the vegetable substrate were tested as shown in Fig. 1. For sample A, soy milk was prepared according to the method of Souane (6), treated with Neutrase (Novo metallo protease from *Bacillus amylloliquefacience*, 20 AU/Kg protein for 2 hrs) in order to solublize the protein in acidic condition, and mixed with extruded rice which was extruded at 120℃ with 20% moisture content. The mixture was digested with malt extract according to the method of Lee *et al.* (3) and then autoclaved at 120℃ for 15 min and inoculated with *Leuconostoc mesenteroides* for fermentation at 30℃ for 24 hrs. For sample B, soymilk was not treated with proteolytic enzyme, but rice-soy milk mixture was digested only with the mixture of α-amylase (Fungamyl, Novo Ind.) and glucoamylase (Amyloglucosidase, Sigma Co.). Sample C was the same as sample B except that soy milk was hydrolized with Neutrase as in sample A. 10% sucrose was added to the fermented beverages for sensory evaluation.

Analysis of Organic Acid and Sugars

The concentrations of organic acids and sugars in apple juice and in the lactic acid fermented beverages were determined by using a HPLC using Waters carbohydrate analysis column with RI detector for sugar and a Bio-Rad Aminex HPX-87H column with UV detector for organic acids.

The total acidity was measured by titration method using 0.1 N NaOH and calculated for lactic acid as standard (3).

Sensory Analysis

In a preliminary round table discussion on apple juice and fermented cereal products, six flavor character notes were selected; sour, sweet, astringent, acidic, fresh-sour and apple juice taste. Sixteen sensory panels were selected from the graduate students of the Department of Food Technology, Korea University and asked to mark the intensities of the character notes on a 10 cm length of ruler, representing 0~100 point scores (5).

RESULTS

Effect of Organic Acid Concentration on the Sensory Taste of a Model Sugar Solution

Table 1 shows the effects of different organic acids on the sensory flavor characteristics of 10% sugar solution. Individual acid generally increased acidic, astringent and sour taste, but reduced sweet, fresh sour and apple juice-like flavor. However, the combined effects of organic acids were different from those expected from individual effect, as shown in Fig. 2, although they were not stastically significant.

Acidic taste was reduced as the concentrations of acetic acid and citric acid approach to the center points, i.e. 0.125% and 0.50%, respectively. Sourness and astringent taste increased with the increase in the concentrations of citric acid and lactic acid, but the mixture of the same amounts of these two acids tended to reduce these tastes. Sweet taste was reduced when the organic acids were added, but at the center point (0.125%) of acetic acid the sweetness intensity was higher than other two extremes. The fresh sour taste, which represented the refreshing feeling, decreased with the addition of the acids, especially with citric acid, but at the center point of acetic acid it tended to increase. Indiviual acid tended to have negative effect on apple juice flavor, while the combination of these acids, especially lactic acid and acetic acid at the center point concentration, produced the maximum intensity of apple juice flavor. It confirms the result of the previous work by Chung (1).

From the results of this experiment, a combination of organic acids, 0.48% citric acid, 0.39% lactic acid and 0.12% acetic acid, which gave the maximum apple juice-like flavor in 10% sugar solution, was chosen as a model mixture to compare the flavor profile with commercial apple juice and the laboratory made lactic acid fermented beverage from rice-soymilk mixture.

Table 1. Significance of terms in response surface analysis for the effects of organic acids to the flavor intensity of 10% sugar solution.

Variable terms	Dependent Variables					
	Acidic	Astringent	Sour	Sweet	Sour fresh	Apple
Linear:						
citrate (X_1)	(+)**	(+)**	(+)***	(−)***	(−)***	(−)**
lactate (X_2)	ns	(+)***	(+)***	(−)***	(−)*	(−)*
acetate (X_3)	(+)**	(+)**	(+)*	(−)***	(−)*	ns
Quadratic						
citrate^2	ns	ns	ns	ns	ns	(−)*
lactate^2	ns	ns	ns	ns	ns	ns
acetate^2	ns	ns	ns	(−)**	(−)***	ns
Cross product						
citrate×lactate	ns	ns	(−)**	(−)*	ns	ns
citrate×acetate	ns	(+)**	ns	ns	ns	ns
lactate×acetate	ns	ns	(−)*	(−)*	ns	ns
Number of significant terms of max 9	2	4	5	6	4	3

ns: not significant, *: significant at 5%, **: significant at 1%, ***: significant at 0.1%

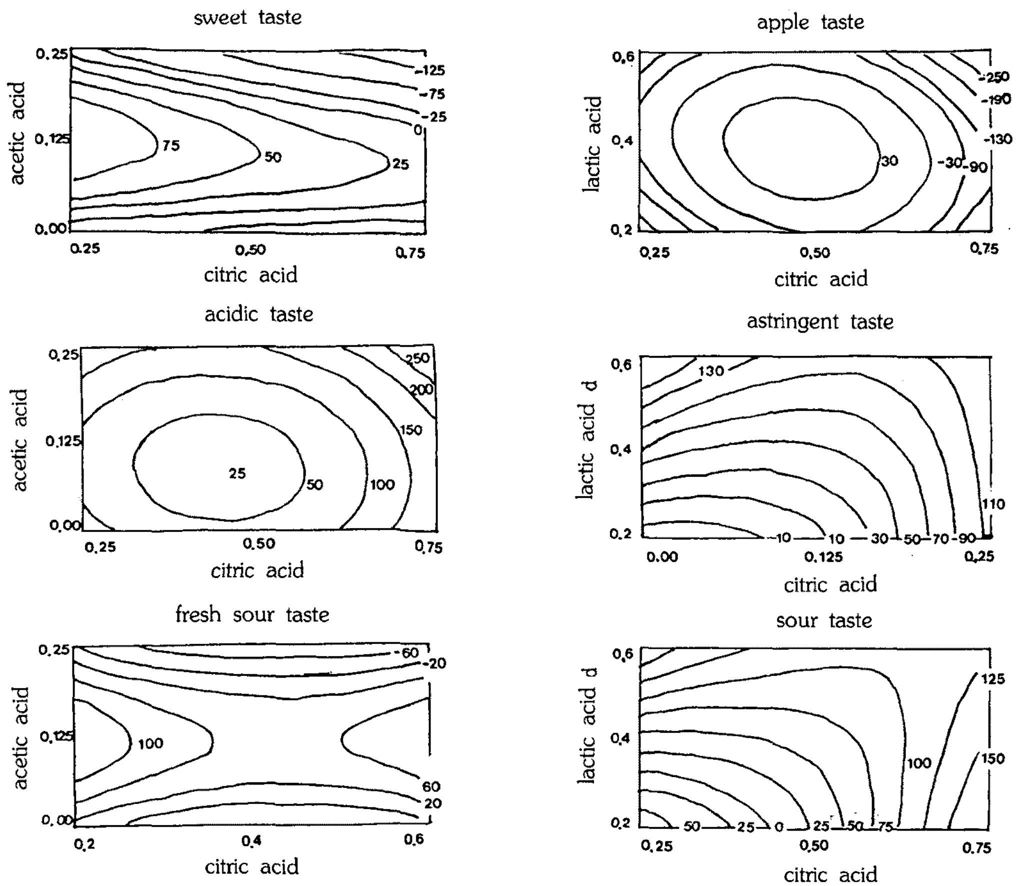

Fig. 2. RSM Contour diagrams for siginificant effects of organic acids combination on the taste character of 10% sugar solution.

Table 2. Sugar content of lactic acid fermented cereal beverages and apple-juice purchased in the market as determined by HPLC (mg/ml).

	Fructose	Glucose	Sucrose	Maltose	Total
apple juice	130.89	100.79	19.46	2.06	253.20
A	0.81	2.28	0.18	34.55	37.82
B	ns	65.76	ns	7.90	73.66
C	ns	73.69	ns	9.42	92.24

Apple juice: commercial apple juice (100%)
A: soymilk, rice extrudate+protein hydrolysis+malt treated; B: Soymilk, rice extrudate+no protein hydrolysis+amylase treated; C: Soymilk, rice extrudate+protein hydrolysis+amylase treated; ns: not significant amount.

Table 3. Organic acid compositions of lactic acid fermented cereal beverages and apple juice (%).

	Lactate	Citrate	Acetate	Malate	Acidity*
Apple juice	0.01	0.01	0.49	1.59	1.68
A	0.49	0.07	0.05	0.08	1.09
B	0.43	0.08	0.08	0.05	0.90
C	0.55	0.09	0.09	0.06	1.07
D	0.39	0.48	0.12		1.48

Apple juice: commercial apple juice (100%)
A: Soymilk,rice extrudate+protein hydrolysis+malt treated; B: Soymilk,rice extrudate+no protein hydrolysis+amylase treated; C: Soymilk,rice extrudate+protein hydrolysis+amylase treated; D: organic acid mixture of model study for sensory test; *: calculated as lactic acid from titration test.

Table 4. Means of sensory flavor intensities of lactic acid fermented cereal beverages, apple juice and organic acid mixture.

	A	B	C	D	E
Acidic taste	29.3[a]	21.1[a]	20.2[a]	56.7[b]	16.7[a]
Astringent taste	25.0[a]	15.4[a]	18.3[a]	40.5[b]	12.4[a]
Sour taste	41.7[a]	54.6[a]	48.2[a]	77.6[b]	54.8[a]
Sweet taste	36.4[b]	51.4[a]	45.9[a]	57.4[a]	54.7[a]
Fresh sour taste	35.0[b]	56.9[a]	43.8[b]	55.5[a]	60.6[a]
Apple taste	38.9[b]	72.8[b]	51.5[b]	60.9[b]	91.0[a]

A: soy milk,rice extrudate+protein hydrolysis+malt treated; B: soy milk,rice extrudate+no protein hydrolysis+amylase treated; C: soy milk,rice extrudate+protein hydrolysis+amylase treated; D: organic acid mixture (citric acid 0.48%, lactic acid 0.39%, acetic acid 0.12%); E: apple juice (Comercial product, 100%)
[a,b] significant difference between different letters.

Sugar and Acid Composition of Lactic Acid Fermented Beverages

Table 2 compares the sugar concentrations of lactic acid fermented beverages and apple juice purchased in the market. The commercial apple juice contained 13% fructose and 10% glucose. It indicated that most of the sugars added to the apple juice was inverted into fructose and glucose. On the other hand the lactic acid fermented cereal beverages showed low level of sugars. The malt digested sample contained mainly maltose while the amylase treated samples had glucose predominantly.

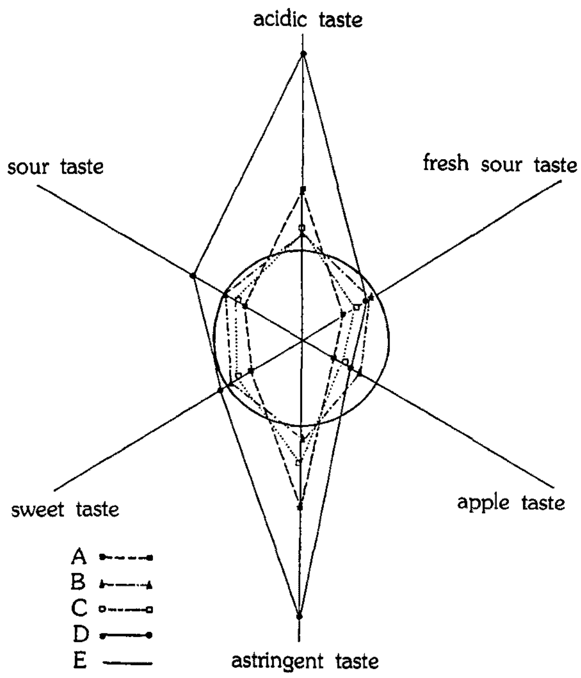

Fig. 3. QDA diagram for the flavor intensities of lactic acid fermented cereal beverages and organic acids mixture in 10% sugar solution in relation to those of apple juice. A, B, C, D, E same as in Table 4.

Table 3 shows the organic acids concentrations of apple juice and lactic acid fermented beverage from rice-soymilk mixture. In the case of apple juice, malic and acetic acids were the major acid components, with the ratio of 3 : 1, as shown in Table 4. On the other hand, the lactic acid fermented beverages were composed mainly of lactic acid (0.44~0.55%) and small amounts of acetic (0.05~0.09%) malic (0.06~0.08%) and citric (0. 07~0.09%) acids with the ratio of approximately 5:1 to lactic acid, respectively.

Sensory Quality of Lactic Acid Fermented Cereal Beverage

Table 4 summarizes the sensory intensity values of the flavor characters of lactic acid fermented cereral beverages, in comparison with those of apple juice and organic acids mixture. Fig. 3 is the QDA diagrams drawn in relation to the flavor intensities of apple juice as standard. It showed that the organic acid mixture in 10%

sugar solution had very sharp acidic and astringent taste compared to apple juice. The lactic acid fermented cereal beverage with protein hydrolysis and malt digestion (A) had quite strong acidic and astringent taste, but very low levels of sweetness, fresh sour and apple juice-like taste. The low levels of sweetness in fermented lactic beverages were consistent with their low concentration of sugar compared to apple juice. The sweetness of protease treated samples was reduced probably due to the formation of bitter peptides. Using amylase treatment instead of malt digestion improved the flavor character, reducing the acidic and astringent taste, but increasing sweetness, fresh sour and apple juice-like taste. Protein hydrolysis appeared to increase the astringent taste and decrease the fresh sour and apple juice-like taste. The lactic acid fermented product without protein hydrolysis but amylase treatment showed a flavor profile very similar to that of apple juice. But the intensity of apple juice-like taste of the fermented products was still substantially lower than that of commercial apple juice.

DISCUSSION

One of the important processing factors which influence the flavor of high protein containing cereal lactic acid fermented beverage is the pretreatment condition of the substrates, especially, the solublization of protein and the control of accompanied bitter taste formation. The hydrolysis of polysaccharides in the substrate is another factor. In the present study, the adverse effect from using malt treatment was noticed and it was found that the replacement with α-amylase and glucoamylase could solve the problem of off-flavor formation from malt. It was clear from the model study carried out in this experiment that the combination of organic acids commonly found in soft drinks, citric, lactic and acetic acids, could certainly influence the taste characteristics of the producRts. It is important to know that what extent the individual acids influence the taste of mixed products, especially when we are attempting to control the taste of fermented beverages by using heterofermentative lactic acid bacteria. *Leuconostoc mesenteroides* (Sikhae) was able to produce lactic, acetic, citric and, in some cases, malic acid and the combinations of these organic acid may attribute to the characteristic refreshing or apple juice-like flavor. In this case, the combined effect of lactic acid and acetic acid appears to be important. Chung (1) also proposed that the ratio of lactic acid and acetic acid is important in the production of apple juice-like flavor. He also suggested that the ratio of lactic/acetic acid can be manipulated by the presence of fructose in the substrate. Fructose is favorable for the production of acetic acid in the glycolytic pathway of the organism. Controlled aeration is another factor, which can change the organic acid composition of the fermentation (6). Therefore, the production of acceptable flavor of lactic acid fermented cereal beverages can be achieved by the control of organic acid composition of the product. The present study proves this possibility, and justifies further researches in this area.

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