

**BREAD COMPRISING SACCHARIFIED RICE SOLUTION AND METHOD FOR
PREPARING SAME**

Technical Field

[1] The present invention relates to bread containing a saccharified rice solution and a method for preparing the same and more specifically, to bread containing a saccharified rice solution prepared using *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees, and a method for preparing the same.

Background Art

[2] In general, bread refers to a product by fermenting a mixture of wheat flour as a main ingredient with water, and then baking the resulting product in an oven. Flour, salt, water, yeast, and dairy products are blended to prepare a dough. In this case, nuts or dried fruits are also added thereto. The quality of bread may depend on the ratio of ingredients and the preparation process. The fermentation process is very important in order to produce high-quality bread and greatly affects the physical properties and flavor of the bread.

[3] Recently, research has been conducted on baking using saccharified rice. In the prior art, Korean Patent No. 10-1750154 discloses a rice fermented composition and rice bread

having superior effect of aging-retardation and a method of preparing the same, and Korean Patent No. 10-1926741 entitled "natural fermented bread using rice" discloses a method of improving the quality of bread using fermented rice broth. Therefore, the present invention discloses a method of preparing a saccharified rice solution having improved taste and flavor while solving the problems of conventional saccharified rice solutions, and a method of producing bread containing the same.

Disclosure

Technical Problem

[4] Conventionally, a saccharified rice solution has been prepared using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji. In this case, the sweetness increases depending on saccharification. However, there is a recent trend in which consumers are averse to sweet flavors. Therefore, the present invention aims at preparing a saccharified rice solution that has improved various and rich flavors including umami and reduced sweetness compared to a conventional saccharified rice solution prepared using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji, and developing bread with improved various and rich flavors including umami, containing the saccharified rice solution.

Technical Solution

[5] In accordance with one aspect of the present invention, the above and other objects can be accomplished by the provision of a saccharified rice solution prepared by adding *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees to rice flour or rice, followed by hydrolysis.

[6] Meanwhile, the rice flour is preferably alpha rice flour.

[7] Meanwhile, the saccharified rice solution is preferably prepared by further adding glucose or rice syrup to the rice flour or rice, followed by hydrolysis.

[8] In accordance with another aspect of the present invention, provided is a flour dough for baking containing the saccharified rice solution.

[9] In accordance with another aspect of the present invention, provided is bread prepared by baking the dough.

Advantageous effects

[10] The present invention provides a saccharified rice solution that is prepared using *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees, and thus is capable of reducing sweetness, providing a further enhanced umami taste based on increased contents of glutamic acid and aspartic acid, and improving various and rich flavors (aroma) compared to a conventional saccharified rice solution

prepared using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji.

Description of Drawings

[11] FIG. 1 is a graph showing the results of analysis of the physical properties of bread produced using the saccharified rice solution of the present invention.

[12] FIG. 2 is a graph showing the results of analysis of aroma components of bread produced using the saccharified rice solution of the present invention.

Best Mode

[13] Conventionally, a saccharified rice solution has been prepared using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji. In this case, the sweetness increases due to saccharification. However, there is a recent trend in which consumers are averse to sweet flavors. Therefore, the present inventors made extensive efforts to develop a saccharified rice solution that reduces sweetness and improves various and rich flavors including umami, while maintaining the advantages of conventional saccharified rice solutions prepared using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji.

[14] The present invention provides a saccharified rice solution prepared by hydrolyzing rice flour or rice to which

Aspergillus oryzae koji, *Aspergillus kawachii* koji and lees have been added.

[15] In the present invention, an optimum process of preparing a saccharified rice solution having a high sensory score was established by mixing *Aspergillus oryzae* koji with *Aspergillus kawachii* koji at an optimum ratio of 2.5 to 3.5:6.5 to 7.5, further adding lees thereto and performing efficient starch pre-treatment and sugar supplementation (treatment with glucose and rice syrup). At this time, more preferably, *Aspergillus oryzae* koji is mixed with *Aspergillus kawachii* koji at a ratio of 3:7. As described above, the saccharified rice solution prepared using *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees is capable of reducing sweetness to an appropriate level and improving various and rich flavors (fragrance) including umami compared to a saccharified rice solution prepared using only *Aspergillus oryzae* koji, or *Aspergillus kawachii* koji.

[16] In addition, the contents of aspartic acid and glutamic acid showing the umami in the saccharified solution containing only *Aspergillus oryzae* koji or *Aspergillus kawachii* koji are 0.016% and 0.033%, respectively, but the contents of aspartic acid and glutamic acid in the saccharified solution containing *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees are 0.023% and 0.049%,

which correspond to 69.5% and 67.3% increases, respectively. As described above, the saccharified rice solution prepared using *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees according to the present invention exhibits remarkably improved umami compared to a saccharified rice solution prepared using only *Aspergillus oryzae* koji, or *Aspergillus kawachii* koji.

[17] Meanwhile, any rice flour may be used as the rice flour without limitation as long as it is known in the art, but the rice flour is preferably alpha rice flour. The alpha rice flour means alpha (α)-starch rice flour (i.e., rice flour gelatinized into alpha-starch).

[18] Meanwhile, in the present invention, the saccharified rice liquid is preferably prepared by hydrolyzing rice flour or rice to which glucose or rice syrup has been further added.

[19] The *Aspergillus oryzae* koji derived from *Aspergillus oryzae* and the *Aspergillus kawachii* koji derived from *Aspergillus kawachii* were used.

[20] The term "lees" refers to the residue left behind after the liquor ingredient is filtered from raw rice wine or sake (unfiltered liquor), which is also called "sake lees". Lees contain large amounts of dietary fibers, proteins, amino acids, organic acids and vitamins, as well

as enzymes such as amylase and protease. Here, saccharification of *Rhizopus oryzae* left over lees was used.

[21] Meanwhile, the present invention provides a flour dough for baking containing the saccharified rice solution.

[22] Meanwhile, the flour dough for baking is preferably obtained by performing primary fermentation using strong flour, water, and yeast *Saccharomyces cerevisiae* SPC-SNU 70-1 (KCTC 12776BP) to prepare a sponge dough, adding the saccharified rice solution of the present invention to the sponge dough and then performing secondary fermentation on the resulting mixture.

[23] Meanwhile, in the present invention, the sponge dough may be prepared under any fermentation conditions that allow yeast strains to grow. Preferably, the dough is mixed at a temperature of 24 to 26°C, is allowed to stand at 22 to 28°C for 20 to 40 minutes, and is fermented at a low temperature of 4 to 8°C for 14 to 18 hours. The fermentation at a low temperature for a long time as in the above conditions has advantages of causing accumulation of metabolites and increasing extensibility and flavors of the product. In addition, such a method has a low production risk and high efficiency compared to a straight method.

[24] Meanwhile, in the process of preparing the dough of the present invention, the fermentation is preferably performed such that the dough is mixed at a temperature of

26 to 28°C, intermediate fermentation is performed at a temperature of 25 to 28°C and a relative humidity of 80 to 90% for 20 to 40 minutes, rounding is performed and aging is performed at a temperature of 25 to 28°C and a relative humidity of 80 to 90% for 10 to 20 minutes. Through the above process, gluten is rearranged and stabilized to prepare for fermentation.

[25] Also, the present invention provides bread prepared by baking the dough.

[26] Also, the present invention provides bread prepared by baking the dough.

[27] Any dough may be used as the dough for baking as long as it is generally prepared by adding water and the like to wheat flour, and if necessary, by adding salt (preferably refined salt), refined sugar, shortening, or the like thereto.

[28] The term "baking" used herein generally means a process of baking to produce bread, and specifically refers to a method of baking using dry heat in an oven. Since a known conventional method can be used, a detailed description thereof will be omitted.

[29] As can be seen from the following example, the present invention provides a saccharified rice solution that is prepared using an *Aspergillus oryzae* koji, an *Aspergillus kawachii* koji, and lees and thus is capable of reducing

sweetness and improving various and rich flavors (fragrance) including umami taste, compared to a conventional saccharified rice solution prepared using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji. Also, bread having excellent various and rich flavors (fragrance) including umami taste can be produced using the saccharified rice solution.

[30] Hereinafter, the present invention will be described in more detail with reference to the following examples. The scope of the present invention is not limited to the examples, and encompasses modifications of the technical concept equivalent thereto.

[31] **[Example 1: Preparation of saccharified rice solution using *Aspergillus oryzae* koji and *Aspergillus kawachii* koji of present invention]**

[32] In this example, an optimal process for preparing a saccharified rice solution having excellent sensory scores was established by setting an optimal mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji and then further performing efficient starch pre-treatment and sugar supplementation (treatment with glucose and rice syrup).

[33] **1) Analysis method**

[34] For analysis of the saccharified rice solution, pH, TTA (measurement of acidity), Brix (measurement of sweetness), organic acid, free sugar, and free amino acid were analyzed and sensory evaluation was performed.

[35] Specifically, pH analysis was performed by homogenizing a mixture of 5 g of a sample with 95 mL of distilled water and then measuring the pH of the resulting sample three times using a pH meter. For TTA analysis, the appropriate amount of NaOH was measured in accordance with 6.8.1.1 Acidity, 6. the Food Standards Test Method, 8. General Test Method, in Food Codex. For Brix analysis, the refractive index of the saccharometer was measured in accordance with 2.1.4.1 saccharide, 2. Food Ingredients Test Method, 8. General Test Method in Food Codex. For sensory evaluation, properties, taste, texture, and color were evaluated in accordance with 1.1 Property (sensory evaluation), 1. Food General Test Method, 8. General Test Method in Food Codex.

[36] In addition, the organic acids, free sugars, and free amino acids were assayed by instrumental analysis, and detailed analysis conditions are shown in Tables 1 to 3.

[37] [Table 1]

Organic acid assay

Analytical Instrument	UPLC (Waters, USA) Model name: Acquity UPLC
Column	Unison US-C18 (Imtakt, USA) 250 x 4.6 mm, 5 µm
Detector	PDA (Waters, USA)

Analysis conditions	. Mobile Phase: 0.1% phosphoric acid . Flow rate: 0.7 ml/min . Column Temperature: 25°C . Sample Temperature: 20°C
Sample preparation	. 2g of sample is diluted 10x with tertiary DW (w/w%), followed by mixing under vortexing. . After centrifugation, the supernatant is diluted at a suitable ratio for analysis (final dilution ratio of 1/20), and then filtered through 0.45 µm filter. . Instrumental analysis

[38] [Table 2]

Free sugar analysis

Analytical Instrument	HPLC (Shiseido, USA) Model name: Nanospace S1-2
Column	Carbohydrate High Performance (Waters) 4.6 x 450 mm, 4 µm
Detector	RID (Shiseido, USA)
Analysis conditions	. Mobile Phase: 80% Acetonitrile . Flow rate: 1 ml/min . Column Temperature: 35°C
Sample preparation	. 2g of sample is diluted 10x with tertiary DW (w/w%), followed by mixing (under vortexing, product (bread): Sonication 5 min) . After centrifugation, the supernatant is diluted at a suitable ratio for analysis and then filtered through 0.45 µm filter. (final dilution ratio : material 1/100, product (bread) 1/10) . Instrumental analysis

[39] [Table 3]

Free amino acid assay

Analytical Instrument	HPLC (Thermo Dionex, USA) Model name: Dionex Ultimate 3000
Column	Inno C18 column (YoungJin Biochrom, Korea) 4.6 x 450 mm, 5 µm
Detector	FL Detector (Agilent, USA)
Analysis conditions	. Mobile Phase A: 40 mM Sodium phosphate, pH 7 . Mobile Phase B: 3DW/Acetonitrile/Methanol (10:45:45 v/v%) . Flow rate: 1.5 ml/min . Column Temperature: 40°C . Sample Temperature: 20°C
Sample preparation	. 2 g of sample is diluted 10x with tertiary DW (w/w%), followed by mixing under vortexing . After centrifugation, the supernatant is diluted at a suitable ratio for analysis and then filtered through 0.45 µm filter. . Instrumental analysis

[40] 2) Setting optimal mixing ratio of saccharified rice solution using *Aspergillus oryzae* koji and *Aspergillus kawachii* koji

[41] In the present invention, a saccharified rice solution exhibiting excellent sensory properties was prepared using *Aspergillus oryzae* koji and *Aspergillus kawachii* koji, instead of malt, which is generally used as an enzyme source. During the saccharification process, *Aspergillus oryzae* formed in *Aspergillus oryzae* koji, produces saccharides, decomposes rice proteins to produce amino acids, and creates a mild sweetness. In addition, *Aspergillus kawachii* formed in *Aspergillus kawachii* koji produces various organic acids, especially fresh acidic flavor (citric acid). Based thereon, *Aspergillus kawachii* can create a variety of tastes (sour taste and umami taste) than conventional saccharified liquids (e.g., Sikhye (Korean traditional rice beverage)).

[42] Meanwhile, in the present invention, it was attempted to set the optimal mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji. For this purpose, saccharified rice solutions were prepared by varying the mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji, the pH, TTA, Brix, total organic acid content, total free sugar content, and total free amino acid content of the saccharified rice solutions were measured and sensory

evaluation was performed. The results are shown in Table 4. In addition, the results for detailed components of metabolites, organic acids, free sugars, and free amino acids are shown in Table 5. Based thereon, it was possible to calculate the optimal mixing ratio (2.5-3.5: 6.5-7.5) of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji, which had the best sensory score.

[43] [Table 4]

	T1	T2	T3	T4	T5
Hard rice	21.74	21.74	21.74	21.74	21.74
<i>Aspergillus oryzae</i> koji	10.87	15.22	21.74	0	6.52
<i>Aspergillus kawachii</i> koji	10.87	6.52	0	21.74	15.22
Feed water	56.52	56.52	56.52	56.52	56.52
Total	100	100	100	100	100
Remarks	<i>Aspergillus oryzae</i> koji: <i>Aspergillus kawachii</i> koji =5:5	<i>Aspergillus oryzae</i> koji: <i>Aspergillus kawachii</i> koji =7:3	<i>Aspergillus oryzae</i> koji 100%	<i>Aspergillus kawachii</i> koji 100%	<i>Aspergillus oryzae</i> koji: <i>Aspergillus kawachii</i> koji =3:7
Brix (Sweetness measurement)	43.3	43.4	45.3	42.8	42.1
pH	4.47	4.79	5.94	3.92	4.18
TTA (Acidity measurement)	5.98/12.29	4.03/10.02	1.07/5.73	12.14/19.38	8.35/14.82
Total organic acid (%)	0.217	0.156	0.033	0.300	0.273
Total free sugar (%)	30.328	30.244	32.482	28.795	26.891
Total free amino acid (%)	0.378	0.414	0.395	0.338	0.371
Sensory score	5.84	5.59	3.41	4.52	7.21

(9-point scale)					
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[44] [Table 5]

Analysis items		T1	T2	T3	T4	T5
Organic acid (%)	Malic acid	0.063	0.058	0.024	0.041	0.021
	Lactic acid	0.021	0.006	0.000	0.099	0.067
	Acetic acid	0.000	0.000	0.000	0.000	0.000
	Citric acid	0.000	0.000	0.000	0.000	0.000
	Succinic acid	0.124	0.084	0.000	0.152	0.186
	Fumaric acid	0.009	0.009	0.009	0.008	0.000
	Total	0.217	0.156	0.033	0.300	0.273
Free sugar (%)	Fructose	0.000	0.000	0.000	0.000	0.000
	Glucose	23.369	25.381	27.353	23.951	22.528
	Sucrose	0.000	0.000	0.000	0.000	0.000
	Maltose	6.959	4.862	5.129	4.844	4.363
	Lactose	0.000	0.000	0.000	0.000	0.000
	Total	30.328	30.244	32.482	28.795	26.891
Free amino acid (%)	Aspartic acid	0.017	0.019	0.027	0.014	0.016
	Glutamic acid	0.036	0.041	0.047	0.028	0.033
	Asparagine	0.019	0.019	0.014	0.015	0.017
	Serine	0.015	0.018	0.025	0.009	0.013
	Glutamine	0.028	0.033	0.028	0.016	0.022
	Histidine	0.011	0.010	0.007	0.009	0.010
	Glycine	0.009	0.011	0.015	0.005	0.007
	Threonine	0.011	0.015	0.019	0.006	0.009
	Arginine	0.002	0.001	0.001	0.062	0.038
	Alanine	0.024	0.028	0.032	0.017	0.021
	GABA	0.002	0.003	0.005	0.002	0.002
	Tyrosine	0.028	0.030	0.024	0.024	0.026
	Valine	0.022	0.026	0.027	0.011	0.018
	Methionine	0.011	0.012	0.011	0.009	0.010
	Ornithine	0.000	0.000	0.000	0.000	0.000
	Tryptophan	0.008	0.009	0.008	0.008	0.009
	Phenylalanine	0.030	0.032	0.016	0.026	0.028
	Isoleucine	0.015	0.019	0.020	0.007	0.011
	Leucine	0.048	0.050	0.039	0.041	0.045
	Lysine	0.025	0.023	0.014	0.020	0.024
	Proline	0.017	0.016	0.017	0.019	0.014
	Total	0.378	0.414	0.395	0.338	0.371

[45] 3) Preparation of saccharified rice solution at optimum mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji using addition of sugar supplementation

[46] The sugar content in the saccharified rice solution prepared at the optimal mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji (2.5 ~ 3.5 : 6.5 ~ 7.5) was corrected and sugar supplementation was performed to prevent quality deviation. In order to confirm the optimal sugar supplementation, pH, TTA, Brix and total free sugar content of the saccharified rice solution prepared by changing the mixing ratio of supplemented sugar components (glucose, sugar, rice syrup) were measured and sensory evaluation was performed. The results were as shown in Table 6, which shows that the experimental group supplemented with glucose and rice syrup had the best sensory score as the harmony of sweetness was the best.

[47] [Table 6]

	T6	T7	T8	T9	T10	T11
Hard rice	21.74	21.74	21.74	21.74	21.74	21.74
<i>Aspergillus oryzae</i> koji	6.52	6.52	6.52	6.52	6.52	6.52
<i>Aspergillus kawachii</i> koji	15.22	15.22	15.22	15.22	15.22	15.22
Feed water	56.52	56.52	56.52	56.52	56.52	56.52
Total	100	100	100	100	100	100
Glucose	25	0	0	12.5	0	12.5
Rice syrup	0	25	0	0	12.5	12.5
Sugar	0	0	25	12.5	12.5	0
Remarks	Glucose	Rice syrup	Sugar	Glucose,	Sugar, rice	Glucose,

				sugar	syrup	rice syrup
Brix	41.6	40.3	43.9	43.2	42.1	42.1
pH	4.04	4.07	4.02	4.04	4.01	4.18
TTA (Acidity measurement)	6.44/11.13	6.71/12.02	6.57/11.31	6.33/11.01	7.13/12.50	8.35/14.82
Total free sugar (%)	31.850	22.790	31.110	31.090	27.030	26.891
Sensory score (9-point scale)	5.25	5.09	5.46	6.84	6.45	7.14

[48] 4) Preparation of saccharified rice solution prepared at optimum mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji, while performing sugar supplementation and varying starch pre-treatment process

[49] A saccharified rice solution was prepared at the optimal mixing ratio of *Aspergillus oryzae* koji to *Aspergillus kawachii* koji (2.5 ~ 3.5 : 6.5 ~ 7.5), while performing sugar supplementation and changing starch pre-treatment (gelatinization, α -starch). The pH, TTA, Brix and total free sugar content of the saccharified rice solution were measured and sensory evaluation thereof was performed. The moisture content of hard rice prepared by the gelatinization process was calculated, the content of alpha rice flour (rice flour) prepared by the α -starch preparation process was reduced, and the feed water content was increased to adjust the content of the starch used for saccharification to a similar level thereto. The results of total organic acids, total free sugars, total free amino

acids and sensory scores after starch pretreatment (gelatinization, α -starch) were overall similar (Table 7). Therefore, it is possible to use alpha rice flour (rice flour) instead of hard rice in the preparation process. Based thereon, it is possible to shorten the process of preparing hard rice and pulverize raw materials and thereby to improve shelf life and production workability.

[50] [Table 7]

	T12	T13
Hard rice	21.74	0
<i>Aspergillus oryzae</i> koji	6.52	6.25
<i>Aspergillus kawachii</i> koji	15.22	14.58
Feed water	56.52	64.58
Alpha rice flour (Rice flour)	0	14.58
Total	100	100
Glucose	12.5	12.5
Rice syrup	12,5	12.5
Total	125	125
Brix	42.1	41.9
pH	4.18	4.31
TTA (acidity measurement)	8.35/14.82	8.16/14.97
Total organic acid (%)	0.273	0.316
Total free sugar (%)	26.891	25.881
Total free amino acid (%)	0.371	0.378
Sensory score (9-point scale)	7.21	7.29

[51] [Example 2: Preparation of saccharified rice solution using *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees of present invention]

[52] In this example, a saccharified rice solution was prepared by adding lees in order to supplement the saccharified rice solution using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji in Example 1. In the saccharification process of *Rhizopus oryzae* remaining in lees, starch is decomposed to produce free sugars and amino acids.

[53] The pH, TTA, Brix, total organic acid content, total free sugar content, and total free amino acid content of the saccharified rice solution prepared by varying the amount of lees while *Aspergillus oryzae* koji and *Aspergillus kawachii* koji are used at the optimal mixing ratio were measured and sensory evaluation was performed. The results are shown in Table 8. In particular, as the alpha rice flour content of the starch material increases, the metabolite content and the sensory evaluation score increase. Therefore, among the saccharified rice solutions to which lees were added, T15 was set as the optimal experimental group.

[54] [Table 8]

	T14	T15	T16	T17
<i>Aspergillus oryzae</i> koji	2.65	2.29	2.65	2.39
<i>Aspergillus</i>	6.35	5.72	6.35	5.74

<i>kawachii</i> koji				
Feed water	31.75	29.85	42.33	33.49
Lees	52.91	45.72	42.33	52.63
Alpha rice flour (rice flour)	6.35	16.43	6.35	5.74
Total	100	100	100	100
Glucose	5.3	5.1	5.3	5.3
Rice syrup	5.3	5.1	5.3	5.3
Total	110.58	110.58	110.58	110.58
Brix (Sweetness measurement)	27.7	34.9	26.7	27
pH	4.42	4.27	4.16	4.14
TTA (Acidity measurement)	7.46/13.40	9.11/14.10	7.34/13.10	8.33/13.86
Total organic acid (%)	0.3231	0.218	0.280	0.309
Total free sugar (%)	14.859	16.390	15.641	13.797
Total free amino acid (%)	0.394	0.379	0.399	0.391
Sensory score (9-point scale)	4.86	6.72	5.18	5.54
Remarks	Low alpha rice flour content		Low lees content	High lees content

[55] Meanwhile, in order to perform sensory evaluation upon addition of lees, the contents of ingredients of free amino acids were further analyzed. As a result, as shown in Table 9, the contents of glutamic acid and aspartic acid, which are amino acids related to umami, in T15 (saccharified rice solution containing lees, Table 8) compared to T5 (saccharified rice solution without lees, Table 4) were high, and the contents of proline, ornithine, and leucine, which

were used to create a strong flavor felt in the bread crust, were high. This indicates that the umami taste and flavor are enhanced by the addition of lees.

[56] [Table 9]

Analysis items		T5	T15
Free amino acid (%)	Aspartic acid	0.016	0.023
	Glutamic acid	0.033	0.049
	Asparagine	0.017	0.023
	Serine	0.013	0.019
	Glutamine	0.022	0.027
	Histidine	0.010	0.014
	Glycine	0.007	0.018
	Threonine	0.009	0.014
	Arginine	0.038	0.073
	Alanine	0.021	0.042
	GABA	0.002	0.003
	Tyrosine	0.026	0.030
	Valine	0.018	0.021
	Methionine	0.010	0.011
	Ornithine	0.000	0.006
	Tryptophan	0.009	0.027
	Phenylalanine	0.028	0.011
	Isoleucine	0.011	0.000
	Leucine	0.045	0.042
	Lysine	0.024	0.036
	Proline	0.014	0.032
	Total	0.371	0.52

[57] Overall, by addition of lees, it was possible to prepare a saccharified rice solution with enhanced various rich flavor, including umami, while appropriately reducing the sweetness created in the saccharified rice solution using *Aspergillus oryzae* koji or *Aspergillus kawachii* koji.

[58] [Example 3: Preparation of bread using saccharified rice solutions of Examples 1 and 2 and testing]

[59] In this example, bread (plain bread) was prepared using the saccharified rice solutions of Example 1 (containing *Aspergillus oryzae* koji and *Aspergillus kawachii* koji) and Example 2 (containing *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees) and tested.

[60] 1) Preparation of bread (plain bread) using saccharified rice solutions of Examples 1 and 2

[61] The components of the sponge dough (see Table 10 below) were put into a mixer (product name: SK101S MIXER: Japan), kneaded in the second stage for 2 minutes and in the third stage for 1 minute, and mixed such that the final temperature of the dough was 25°C. Then, the dough was allowed to stand at room temperature for 30 minutes, and then primarily fermented in a fermenter at 6°C for 16 hours to prepare sponge dough. Then, the components of the dough (see Table 10 below) are put into a mixer (product name: SK101S MIXER: Japan), kneaded in the first stage for 1 minute, the sponge dough was added thereto, and the resulting mixture was kneaded in the second stage for 3 minutes and in the third stage for 3 minutes, and mixed such that the final temperature of the dough was 27°C. The dough was intermediate-fermented in a fermenter at 27°C and a

relative humidity of 85% for 30 minutes, was divided into uniform segments, rounded, and aged in a fermenter at 27°C and a relative humidity of 85% for 15 minutes. After aging, the dough was molded and placed in a bread case. Then, the dough placed in the bread case was fermented at 37°C and at a relative humidity of 85% for 50 minutes to prepare bread dough. The prepared bread dough was baked in an oven at an upper heat of 170°C and at a lower heat of 210°C for 35 minutes, and then was cooled at room temperature until the internal temperature reached 32°C.

[62] [Table 10]

		Control group	Addition of Example 1 saccharified rice solution	Addition of Example 2 saccharified rice solution
Sponge dough	Strong flour	70	70	70
	Commercial yeast : <i>Saccharomyces cerevisiae</i> (SPC- SNU 70-1 (KCTC 12776BP) ^a	0.7	0.7	0.7
	Rimusoft ^b	0.3	0.3	0.3
	Feed water	42	42	42
Dough	Strong flour	3	30	30
	Refined salt	1.8	1.8	1.8
	White sugar	7	7	7
	Whole milk powder	3	3	3
	Butter	10	10	10
	Commercial yeast	0.6	0.6	0.6
	Feed water	13	7	8
	Example 1 saccharified rice solution (T13)	0	10	0
	Example 2	0	0	10

	saccharified rice solution (T15)			
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[63] a: *Saccharomyces cerevisiae* SPC-SNU 70-1 (KCTC 12776BP) is derived from Korean Patent No. 10-1551839 (Registration date: September 3, 2015) b: Rimusoft is an emulsifier as a food additive (glycerin fatty acid ester) and represents "Rimusoft super (v)".

[64] 2) Texture and ingredient analysis and sensory evaluation of bread

[65] The texture and component analysis, and sensory evaluation of bread prepared using the saccharified rice solutions of Examples 1 and 2 above were performed.

[66] First, the texture (physical property) was compared using a texture analyzer (Stable Micro Systems texture analyzer). As analysis indicators, hardness, cohesiveness, springiness, gumminess, chewiness, and resilience were measured. A higher hardness value indicates higher hardness. Springiness means the degree to which a substance returns to its original state, cohesiveness means the ability of food to retain its original state, gumminess means stickiness, chewiness refers to the degree of swallowing, and resilience refers to a force at which an object is restored after pressed. Each analysis parameter was repeatedly measured in 3 replicates.

[67] As a result, as can be seen from FIG. 1 and Table 11, both bread produced using the saccharified solutions of Examples 1 and 2 had improved overall texture (decreased hardness, increased cohesiveness and resilience, and maintained springiness).

[68] [Table 11]

	Hardness	Springiness	Cohesiveness	Gumminess	Chewiness	Resilience
Control group	1.336	0.863	0.489	0.654	0.564	0.181
Example 1 saccharified rice solution	0.84	0.746	0.513	0.712	0.349	0.194
Example 2 saccharified rice solution	0.92	0.592	0.542	0.498	0.295	0.213

[69] Then, the free sugar component was analyzed (see Table 2), and the fragrance component was compared using the GS/MS system (Table 12).

[70] [Table 12]

Analysis system	Operation conditions
GC/MS analysis	*GC Model name: Agilent 7890A *Inlet temperature: 230°C *Column: DB-WAX (60 m X 250 μ m X 0.25 μ M) *Carrier gas: helium *Flow rate: 1 ml/min *Oven temperature program : from 40°C (5 min) $\rightarrow$ 8°C/min $\rightarrow$ 230°C (10 min) *MS detector: Agilent 5975C MSD (EI mode)
SPME analysis	* Fiber: DVB/Carboxen/SPME (Supelco Co.) * Sample equilibration time - incubation temp. 85°C - incubation time 30 min

[71] As a result, as can be seen from Table 13, the bread made using the saccharified solution of Example 2 exhibited an increase in various types of sweetness (increased content of several free sugars) compared to the control group, and exhibited an appropriately reduced sweetness compared to bread made using the saccharified solution of Example 1. That is, the bread made using the saccharified solution of Example 2 had an appropriate sweetness.

[72] [Table 13]

Free sugar (%)	Control	Example 1 saccharified rice solution	Example 2 saccharified rice solution
Fructose	0.75	1.22	1.10
Glucose	0.46	2.26	1.14
Sucrose	0.00	0.00	0.00
Maltose	2.10	2.04	1.83
Lactose	0.82	0.89	0.94
Total	4.13	6.42	5.01

[73] Meanwhile, sensory evaluation was performed on the saccharified rice solutions of Examples 1 and 2. As a result, as can be seen from Table 14, the sensory score was higher in bread made using the saccharified solutions of Examples 1 and 2 compared to the control group, and in particular, the saccharified rice solution of Example 2 exhibited an improved umami taste compared to the saccharified rice solution of Example 1. This indicates that the umami taste and flavor are enhanced by the addition of lees.

[74] [Table 14]

Analysis items		Control	Saccharified rice solution of Example 1	Saccharified rice solution of Example 2
Sensory evaluation (9-point scale)	Sweetness	4.85	8.26	6.45
	Saltiness	5.16	5.03	5.71
	Sourness	4.96	5.24	5.69
	Umami	5.48	6.33	8.17
	Overall	5.11	7.21	7.94

[75] Meanwhile, as shown in FIG. 2 and Table 15, the bread prepared using the saccharified solutions of Examples 1 and 2 were detected to have a greater variety of flavor ingredients (Example 1: 17 types, Example 2: 14 types) than the control group (12 types). Bread prepared using the saccharified rice solution of Example 1 was found to have a great amount of alcohol having fruity, floral, and whiskey flavors, and a great amount of aldehyde having savory flavors such as roasted, bread, and almond flavors. Bread made using the saccharified rice solution of Example 2 was found to have great amounts of alcohol having fruity, floral, and whiskey flavor characteristics, aldehyde having savory flavors such as roasted, bread, and almond flavors, and ester having sweet and fresh flavors such as fruity and apple flavors. This is considered to be due to various metabolites produced during the preparation of the saccharified rice solution of Example 2.

[76] [Table 15]

Flavor Area		Control group	Saccharified rice solution of Example 1	Saccharified rice solution of Example 2	Description
Alcohol	Ethanol	38,496,132	91,670,582	160,923,927	Strong, Alcohol, Ethereal
	Isobutyl alcohol	6,379,861	6,427,139	9,415,984	Wine, malty
	3-Methyl-1-butanol	-	122,146,206	-	Whiskey fruity banana
	2-Heptanol	1,469,788	-	-	Fresh lemongrass herbal floral fruity
	1-Hexanol	-	-	4,042,512	Green, Fruity, Sweet, Woody, Floral
	Phenethyl alcohol	-	73,224,730	-	Rose- honey-like
	Total	46,345,781	293,468,657	174,382,423	
Aldehyde	3-Methylbutane	-	1,674,724	-	Chocolate, Roasted Bread, Fruity, Corn flakes
	Hexanal	2,813,624	2,146,948	2,120,518	Fresh, Green, Fruity, Sweaty
	2-Amylfuran	2,631,942	2,355,922	2,251,848	Fruity, Green, Earthy
	Acetoin	2,934,085	4,467,510	3,029,328	Sweet buttery creamy dairy milky fatty
	Nonanal	3,296,232	1,681,528	2,714,667	Waxy, green, fatty
	Furfural	-	3,909,770	-	Sweet,

					Almond, Bread
	Benzaldehyde	-	12,784,843	10,714,977	Almond, Strong, Bitter, Cherry
	Benzeneacetaldehyde	-	3,012,631	-	Honey, floral
	Total	1,675,883	32,033,876	20,831,338	
Ketone	2-Heptanone	32,696,033	22,106,778	15,733,276	Fruity, Spicy, Sweet, Grass, Coconut, cinnamon
	2-Nonanone	59,845,480	32,634,616	29,141,357	Fruity fresh sweet green weedy earthy herbal
	2-Undecanone	-	11,149,345	8,590,500	Floral and fatty pineapple
	Total	92,541,513	65,890,739	53,465,133	
Ester	Isoamyl formate	93,602,735	-	146,191,846	Fruity green apple
	Ethyl hexanoate	1,516,859	-	2,071,142	Sweet, Fruity, Green, Creamy, Milky, Balsamic
	Hexyl formate	-	2,531,475	-	Green waxy floral herbal plum apple cucumber
	Ethyl octanoate	14,551,853	4,392,463	-	Fruity, Wine, Pear brandy, Banana, Sweet, Fresh
	Diethyl succinate	-	-	5,521,844	Fruity apple, cooked apple,

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	Total	109,671,447	6,923,938	153,784,832	
	Total	360,234,624	398,317,210	402,463,726	

[77] Overall, it can be seen from the above results that, using the saccharified rice solution (Example 2) containing *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees, it is possible to prepare a saccharified rice solution that is capable of reducing sweetness and improving various and rich flavors including umami due to the high contents of glutamic acid and aspartic acid, which are umami taste-related amino acids, compared to the saccharified rice solution containing *Aspergillus oryzae* koji and *Aspergillus kawachii* (Example 1).

【CLAIMS】

【Claim 1】 A saccharified rice solution prepared by adding *Aspergillus oryzae* koji, *Aspergillus kawachii* koji and lees to rice flour or rice, followed by hydrolysis.

【Claim 2】 The saccharified rice solution according to claim 1, wherein the rice flour is alpha rice flour.

【Claim 3】 The saccharified rice solution according to claim 1, wherein the saccharified rice solution is prepared by further adding glucose or rice syrup to the rice flour or rice, followed by hydrolysis.

【Claim 4】 A flour dough for baking containing the saccharified rice solution according to claim 1.

【Claim 5】 Bread prepared by baking the flour dough according to claim 4.