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Microbiological and Biochemical Characterization of the Traditional Steeping Process of Waxy Rice for *Yukwa* (a Korean Oil-Puffed Snack) Production

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Abstract

Selected microbiological and biochemical characteristics of the steeping process for the production of *yukwa*, a traditional Korean oil-puffed snack made of waxy rice, were investigated during steeping of waxy rice in water for 15 days. The lengthy steeping process was largely predominated by lactic acid bacteria (LAB), particularly, *Lactobacillus* and *Leuconostoc*. The predominat type of bacterium isolated was the Y26 strain tentatively identified as *Lactobacillus plantarum*. The titratable acidity of the steeping medium increased from 0.01 to 1.13%, in parallel with the decrease in pH ranging from 6.3 to 4.2 as the steeping period increased from 0 to 15 days. A high amount of lactic acid and to a much lesser extent, butyric acid, acetic acid, propionic acid, and succinic acid were detected during the steeping process. The amount of reducing sugars in the steeping medium increased from 0.61 to 10.43 mg/mL, whereas sucrose decreased from 0.46 mg% to an undetectable level. Starch degradation products including glucose, maltose and oligosaccharides ranging G3-G7 were not initially noticed, but their content increased during the steeping process until completion. However, no oligosaccharides larger than G8 were detected in the steeping medium. The activities of α-amylase, β-amylase and protease in the steeping medium of waxy rice tended to rise increase with time during the steeping process. From these results, the lengthy steeping process in *yukwa* production can be characterized as the spontaneous fermentation, dominated by lactic acid bacteria, which is a necessary process for inducing biochemical modification of waxy rice.

Key words: *yukwa*, steeping process, waxy rice, lactic acid bacteria, spontaneous fermentation

INTRODUCTION

Yukwa is a Korean traditional oil-puffed snack made of waxy rice (*Oryza sativa*). It has long been consumed as a popular snack due to its soft texture and unique taste, and has become a factory-made product due to its large increase in consumption. The main procedures for its traditional manufacture are 1) water-steeping of waxy rice for 1~2 weeks, 2) milling, 3) steaming, 4) pounding, 5) kneading, 6) molding and drying, and 7) deep-frying and coating with starch syrup (1-3). The quality of *yukwa* depends primarily on the process variables, including the steeping duration of waxy rice, the milling method, and the drying and frying conditions. Among these, the steeping process is believed to be necessary for obtaining the desirable quality features of *yukwa*, such as soft texture, porous intrastructure with even air cells, and volume expansion (1,3). However, the reason for the lengthy steeping duration for *yukwa* production has yet to be clearly defined.

Generally, the major objective of rice steeping or soaking is to hydrate the kernel for sufficient gelatinization on subsequent cooking (4,5). Considering that steeping for 30~60 min in water appears sufficient to reach the equilibrium state (6,7), the extraordinary lengthy steeping period of waxy rice in *yukwa* preparation is required for hydration of the waxy kernel and for induction of biochemical changes via natural fermentation. It is well known that fermentation is usually a desirable process for biochemical modification of primary food products resulting from the activity of microorganisms and their enzymes. Fermentation is a commonly used food process throughout most of the world for the purpose of enhancing food properties such as taste, flavor, texture, nutritional value and shelf life. The preparation of many traditional or indigenous cereal-based foods such as *ogi*, *kwunu*, *burukutu* and *togwa* is normally carried out by natural fermentation involving mixed cultures of bacteria, yeast and/or fungi (8-11).

In a previous study, *yukwa* made from waxy rice with longer steeping periods was found to have desirable volume expansion, mechanical and sensory textural prop-

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erties as the steeping period of waxy rice changed from 0 to 15 days (3). Additionally, contents of sugar, protein, lipid and minerals of raw waxy rice were found to decrease, and the volatile organic compounds were detected in steeping medium by increasing the steeping time (12). These facts imply that natural fermentation could be involved in *yukwa* production. However, there is inadequate information on the microbiological aspects and biochemical changes that occur during steeping of waxy rice to provide a comprehensive understanding of this process.

The traditional lengthy steeping process is still used in South Korea, based on years of experience. Moreover, this process is time-consuming, unpredictable and often leads to *yukwa* with varied qualities. In an attempt to optimize the steeping process and to omit or shorten this lengthy process for *yukwa* production with improved quality on a commercial scale, it is necessary to understand which changes occur in waxy rice during the steeping process and how they affect the quality of *yukwa*.

Thus, the aims of this study were to investigate the selected microbiological and biochemical characteristics associated with the steeping process of waxy rice to produce *yukwa*.

MATERIALS AND METHODS

Samples

Milled waxy rice (*Shinsunchalbyeo* cultivar), with a total milling yield of 74%, was obtained from a commercial source. Forty kg of the milled waxy rice was steeped in 64 L of tap water at 25°C in an 80 L tank for 15 days. This steeping process was repeated twice. Samples weighing 30~50 g taken in triplicate throughout the steeping period were collected into sterile plastic containers (Nunc, London, UK), placed in a chilled carrier and taken immediately to the laboratory for analysis. For evaluation of unsteeped milled waxy rice (zero days), the milled waxy rice was steeped at 25°C for 30 min.

Characterization of dominating microorganisms

Enumeration and isolation : Duplicate samples of the steeping media (25 g) were homogenized in 225 mL sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, 1000 mL distilled water, pH 7.0±0.2). The homogenate was then decimal diluted and spread on each selective media. For the enumeration and isolation of general-type lactic acid bacteria (LAB), MRS agar medium was used. Modified Lactobacillus selection agar (m-LBS), phenyl ethyl alcohol agar (PES) and KF Streptococcus agar (KFS) were used for the selective isolation of *Lactobacillus*, *Leuconostoc* and *Enterococcus*, respectively (13). *Bacillus* was enumerated and isolated on tryptone glucose extract agar (TGE) after 10 mL of each sample had been treated at 80°C for 20 min. For selective isolation of yeast and fungi, potato dextrose agar (PDA) containing 0.007% (w/v) rose Bengal (r), 0.04% (w/v) cyclohexamide, 0.005% (w/v) chloramphenicol and 0.01% (w/v) tartaric acid (t) was used. EMB agar (EMB) was used for the isolation of *E. coli* form, and *Corynebacterium* agar (CRA) for *Corynebacterium* was also used. Aerobic mesophilic bacteria in a waxy slurry for *yukwa* production were enumerated on plate count agar (PCA, Merck) after incubation for 2 days at 30°C. The individual isolates from the countable plates of each selective media were maintained in sterile cryovials containing MRS broth plus 10% (v/v) glycerol.

Characteristics of isolates : Isolates obtained at different steeping days were characterized by microscopic examination and by conventional biochemical and physiological tests. The cultures were examined for colony and cell morphology; Gram reaction; catalase reaction; growth pattern and fermentation test. These tests were performed according to the procedures described by Gerhardt et al. (14). In order to confirm that the counted colony of each morphology type on selective medium originated from a strain, plasmid DNA was extracted from each isolate and was electrophoresed on 0.6% agarose gel (15). The fermentation pattern among carbohydrates was determined by using the API 50 CH gallery with the API 50 CHL medium (BioMérieux, Marcy-l'Etoile, France). The inoculated galleries were incubated at 30°C and the observations were made after 24 and 48 hr. Identification of the isolates was facilitated by the use of the computer program, APILAB PLUS, version 3.2.2. (BioMérieux).

pH and titratable acidity

The pH of the steeping media was measured with a pH meter (Orion, Model 420, USA) in triplicate. The titratable acidity (TA) was determined according to the AOAC method (16). The results were expressed as a percentage of lactic acid {(mL of 0.1 N NaOH / weight of sample titrated)×0.009×100}.

Organic acids

Organic acids in steeping medium were analyzed by JASCO 900 series high performance liquid chromatograph (PU-987 pump, UV-975 detector, CO-965 column oven, AS-950-10 auto sampler, JASCO Co., Japan). Fifty milliliters of each steeping medium were dried on Speedvac concentrator (Savant AS 260, Savant Instrument Inc., USA), and redissolved in 1 mL of 0.008 N H_2SO_4. After filtration through a 0.22 μm membrane filter, samples were injected into a HPLC system equipped with a Waters Aminex HPX-42A column (300×7.8 mm), maintained at 35°C. The organic acids were eluted with 0.008

N H_2SO_4 with a flow rate of 0.6 mL/min, and monitored by UV absorption at 210 nm. HPLC chromatograms and quantification were obtained using JASCO Borwin software. Standard curves based on the peak area were calculated for the individual organic acids covering a broad range of concentrations.

Sugar analysis

The reducing sugar in the steeping medium of waxy rice samples was measured by the AOAC method (16). The oligosaccharide profile in the steeping medium was analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Aliquots (50 mL) of steeping medium were treated with mixed resin (Sigma Co., USA) for 16 hr at ambient temperature. After concentration using a rotary evaporator at 40°C, the resulting solutions were membrane-filtered and applied to a CarboPac PA1 column (4×250 mm) mounted on a Doinex BioLC chromatograph unit (Dionex Ltd., UK). The oligosaccharides were eluted with a gradient of 75~250 mM sodium acetate in 150 mM NaOH over 60 min. Mixtures of maltodextrins including sucrose were run as standards.

Enzyme assays

α-, β-Amylase activities in steeping medium were assayed as described by Yamamoto et al. (17) with a slight modification. In the α-amylase assay, a mixture containing 1.0 mL of sample and 2 mL of 0.5% potato starch solution (pH 5.9) was prepared and incubated for 10 min at 40°C. A 0.3 mL aliquot of the reaction mixture was then combined with 0.1 mL of 0.01 N iodine containing 0.1 N hydrochloric acid. After the addition of 10 mL of distilled water, the color intensity was measured with a spectrophotometer at 660 nm. When the blue color intensity decreases by half in 10 min under the specified condition, the activity is defined as one unit activity of α-amylase. To assay β-amylase activity, 1.0 mL of sample and 2 mL of 0.5% potato starch solution (pH 5.9) were incubated as described in α-amylase assay. Then, the amount of reducing sugar produced was estimated by the Nelson-Somogyi method (18) with glucose as a standard. One unit of β-amylase activity was defined as the enzyme amount that produces reducing sugar equivalent to 10 μmol of glucose under the assay conditions. Protease activity was measured by a modified Anson's method (19) using casein as a substrate. The reaction mixtures, containing 2.6 mL of 0.6% (w/v) casein and 0.5 mL of steeping medium were incubated for 10 min at 30°C. The reaction was stopped by 2.5 mL of trichloroacetic acid and kept for 30 min at room temperature. After centrifugation at 13,000 rpm for 10 min, 2.0 mL aliquot of the supernatant was taken into a test tube. Then, 5.0 mL of 0.55 M Na_2CO_3 and 1.0 mL of 0.067 N Folin reagent were added to the test tube and incubated for 30 min at 30°C. Absorbance of the solution at 660 nm was measured. One unit of protease activity was defined as the amount of enzyme required to produce 1 μg of tyrosine in 1 mL of sample per min at 30°C.

Statistical analysis

Mean separation and significance were analyzed using the SAS package (20). Analysis of variance (ANOVA) was performed for measured variables with Duncan's multiple range tests to determine the significant differences among the mean values.

RESULTS AND DISCUSSION

Microbiological characteristics

The nine selective media for the measurement of the spectrum and amounts of microorganisms associated with the steeping process of waxy rice were chosen among 32 selective media (data not shown). The media chosen were MRS agar for general-type LAB, m-LAB for *Lactobacillus*, PES for *Leuconostoc*, KFS for *Enterococcus*, PDArt for yeast or fungi, TGE for *Bacillus*, EMB for coliform bacteria, CRA for *Corynebacterium* and PCA for total bacterial count.

Changes in the microbial population during the steeping of waxy rice for *yukwa* production are represented in Fig. 1. Collectively, counts on the various selective

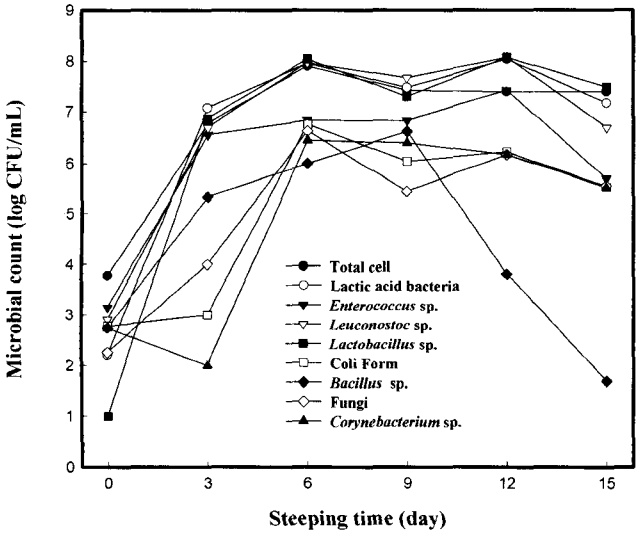

Fig. 1. Changes in microflora found in the steeping medium of waxy rice during the steeping process. Values are the mean of triplicate determinations. Each bacterial population was counted by growing colony on selective media. The selective media used for counting total cell, general type of lactic acid bacteria, *Enterococcus*, *Leuconostoc*, *Lactobacillus*, coli form, *Bacillus*, fungi and *Corynebacterium* were PCA, MRS, KF *Streptococcus* agar, PES, m-LBS, EMB, TGE, PDArt and CRA, respectively.

media, monitored throughout the steeping process, rapidly increased during the three days, peaking at 6 days, and then remaining constant until the end of steeping. Total bacterial count on PCA increased from 6.0×10^3 CFU/mL at the beginning to 8.0×10^7 CFU/mL at 6 days of steeping. Numbers of LAB, *Lactobacillus*, *Leuconostoc* and *Entrococcus* rapidly increased from 1.6×10^2, 1.0×10^1, 8.0×10^2 and 1.4×10^3 CFU/mL at the beginning, to 9.2×10^7, 1.1×10^8, 9.3×10^7 and 3.6×10^6 CFU/mL at 6 days of steeping, respectively, showing that this group rapidly dominates the total microflora. Counts of coliform bacteria, fungi and *Corynebacterium* also increased from 6.0×10^2, 1.8×10^2 and 5.5×10^2 CFU/mL at the beginning, to 5.8×10^6, 4.3×10^6 and 2.8×10^6 CFU/mL at 6 days of steeping, respectively, then slightly decreased until the end of steeping. In contrast, numbers of *Bacillus* increased from 5.5×10^2 CFU/mL at the start, to 4.2×10^6 CFU/mL at 9 days of steeping, thereafter rapidly decreasing to 5.0×10^1 CFU/mL. From these results, the steeping process was shown to be a complex microbial process in which a small amount of LAB rapidly replaced the epiphytic microflora and governed the steeping process of waxy rice. This is similar to what occurs in other fermented foods; LAB is the dominant type of organism and is commonly isolated from a wide range of indigenous fermented foods including *togwa* (fermented sorghum, maize and millet), *fufu* (fermented cassava), *iru* (fermented African locust bean), *kenkey and ogi* (fermented maize) (21-25).

Generally, the use of selective media cannot provide conclusive selectivity for the enumeration and isolation of specific microorganisms, and colony form on the selective medium is more or less different depending on each strain. Hence, plasmid DNA profiles obtained from each isolate was compared by electrophoresis on an agarose gel to confirm that the counted colony on selective medium was originated from a particular strain (data not shown). Sixteen tentative LAB strains, which were observed as the dominating microorganisms during steeping process, were isolated and further characterized. The characterization of dominating colony type and number of isolated LAB strains on the selective medium and their identification result are shown in Table 1. After 3-days of steeping, the Y74 strain was predominant, at the level of 1.2×10^7 CFU/mL. This was a Gram-positive and catalase-negative coccus, tentatively identified as *Leuconostoc citreum* with a 95.8% identification level by API 50-CH. Y75, Y73 and Y12 strains with 87, 57 and 42% population size, respectively, were identified as *Brevibacillus laterosporus*, *Paenibacillus mocerans* and *Lactobacillus pentosus*. As a dominant type, the Y15 strain grown on m-LBS was present at levels of 1.0×10^8 (91%), and followed by Y37 (85%), Y58 (61%) and Y68 (47%) strains at 6-days of steeping. The Y15 strain, which was found to be a Gram-positive and catalase-negative rod, was tentatively identified as *Lactobacillus pentosus* with 98.5% identification level. After 9-days of steeping, YB4, YB18, YB1 and YB7 strains isolated were present at levels of 1.8×10^7 (90%), 2.7×10^7 (90%), 3.3×10^7 (70%) and 3.1×10^6 (46%), respectively. The YB4 strain, Gram-positive and catalase-negative coccus, was the most dominating population and tentatively identified as *Lactobacillus plantarum* by API CH50 kits. However, this is questionable in that its cellular form is mostly rod, not a coccus, suggesting a need for further identification. YB1 strain grown on mLBS was also tentatively identified as *Lactobacillus plantarum*, but YB18 and YB7 were not confidently typed using API CH50 kits. The Y26 strain was the most dominant type with a level of 1.2×10^8 CFU/mL (86%) after 12-days of steeping. This strain was a Gram-positive and catalase-negative rod and was tentatively identified as *Lactobacillus plantarum* with a 99.9% identification level. Y25 and Y40 strains, which were Gram-positive and catalase-negative rods, were also tentatively identified as *Lactobacillus plantarum*. In contrast, Y69, Gram-positive and catalase-negative coccus, was not confidently typed by means of API CH50. Throughout the steeping process, the most dominant type was the Y26 strain tentatively identified as *Lactobacillus plantarum* during the later stage of the steeping process. This is in agreement with observations made during *togwa* fermentation; in which the majority of the bacteria found belonged to the *Lactobacillus plantarum* group (26). On the other hand, several studies have indicated that fermentation of African traditional foods, including cassava starch, *togwa*, *kenkey*, *mawe* and *fufu* has been due to the action of yeasts (9,10,27-30). This disagreement could be explained by differences in food preparation as well as variations in the microflora ecotypes in different regions.

Chemical and enzymatic characteristics

The changes in pH, titratable acidity, organic acids and reducing sugar during the steeping process are shown in Table 2. The initial titratable acidity of the steeping medium, expressed as a percentage of lactic acid, was 0.01%, but reached a final level of 1.13% after a 15-days steeping period. In parallel with the increase in titratable acidity, the pH of the steeping medium decreased from 6.3 to 4.2 as the steeping period increased from 0 to 15 days. A high amount of lactic acid and, to a much lesser extent, butyric acid, acetic acid, propionic acid and succinic acid were detected during the steeping process. The lactic acid, propionic acid and butyric acid in the

Table 1. Characterization and identification of major strain appearing on selective media at different steeping periods

Day	Strain	Selective medium and expected genus of isolated strain	Colony form on selective medium		Colony count (mL)		Characteristics			APIS0CHL	
			Color	Size/Other	Isolated uniformed colony	Total colony	Gram staining	Cell form	Catalase	Scientific name	% ID[1]
3	Y75	mLBS/*Lactobacillus*	Ivory	Middle	7.4×10^6	8.5×10^6	+	C[3]	+	*Brev. Laterosporus*[5]	99.3
	Y74	MRS/GLAB[2]	Ivory	Middle	1.2×10^7	1.2×10^7	+	C	-	*Leuconostoc citreum*	95.8
	Y73	PES/*Leuconostoc*	Transparence	Middle/Mucus	5.0×10^6	8.8×10^6	+	C	+	*Paenib. Mocerans*[6]	-
	Y12	KF/*Entrococcus*	Red	Middle	1.5×10^6	3.6×10^6	+	C	-	*Lacto. pentosus*[7]	72.3
6	Y15	mLBS/*Lactobacillus*	White	Small	1.0×10^8	1.1×10^8	+	R[4]	-	*Lacto. pentosus*	98.5
	Y58	MRS/GLAB	White	Large	9.2×10^7	1.5×10^8	+	C	-	*Lacto. plantarum*	81.0
	Y37	PES/*Leuconostoc*	Transparence	Large/Mucus	9.3×10^7	1.1×10^8	+	C	-	*Lacto. plantarum*	99.0
	Y68	KF/*Entrococcus*	Red	Middle	7.0×10^6	1.5×10^7	+	C	-	*Lacto. plantarum*	76.9
9	YB4	mLBS/*Lactobacillus*	White	Small	1.8×10^7	2.0×10^7	+	C	-	*Lactov plantarum*	82.4
	YB18	MRS/GLAB	White	Small	2.7×10^7	3.0×10^7	+	R	-	Uninterpretable profile	-
	YB1	PES/*Leuconostoc*	Transparence	Large/Mucus	3.3×10^7	4.7×10^7	+	C	-	*Lacto. plantarum*	60.0
	YB7	KF/*Entrococcus*	Red	Middle	3.1×10^6	6.8×10^6	+	C	-	Unacceptable profile	-
12	Y26	mLBS/*Lactobacillus*	White	Small	1.2×10^8	1.4×10^8	+	R	-	*Lacto. plantarum*	99.9
	Y25	MRS/GLAB	White	Large	8.2×10^7	1.4×10^8	+	R	-	*Lacto. plantarum*	99.9
	Y40	PES/*Leuconostoc*	Transparence	Small/Mucus	1.0×10^8	1.2×10^8	+	R	-	*Lacto. plantarum*	99.9
	Y69	KF/*Entrococcus*	Red	Middle	1.9×10^7	2.7×10^7	+	C	-	Uninterpretable profile	-

[1]% ID: Percentage of identification.
[2]GLAB: General type of lactic acid bacteria.
[3]C: Coccus.
[4]R: Rod.
[5]*Brev.*: *Brevibacillus.*
[6]*Paenib.*: *Paenibacillus.*
[7]*Lacto.*: *Lactbacillus.*

Table 2. Changes in pH, titratable acidity, organic acids and reducing sugar in the steeping medium of waxy rice during 15 days of steeping

Steeping period (day)	pH	Titratable acidity (%)	Reducing sugar (mg/mL)	Organic acid (mg/mL)				
				Lactic acid	Propionic acid	Butyric acid	Succinic acid	Acetic acid
0	6.32[a1)]	0.01[g]	0.61[g]	ND[2)]	ND	ND	ND	ND
1	6.01[b]	0.03[f]	1.60[e]	ND	ND	ND	ND	0.03[d]
3	4.75[c]	0.06[ef]	0.93[f]	ND	ND	ND	0.02[c]	0.25[ab]
6	4.25[d]	0.16[d]	1.98[d]	0.35[d]	0.02[c]	0.04[d]	0.02[c]	0.18[b]
9	4.11[d]	0.54[c]	4.23[c]	2.19[c]	0.06[bc]	0.28[c]	0.09[b]	0.19[b]
12	4.13[d]	0.82[b]	6.82[b]	3.13[b]	0.12[b]	0.35[b]	0.03[c]	0.19[b]
15	4.22[d]	1.13[a]	10.43[a]	5.71[a]	0.18[a]	0.52[a]	0.19[a]	0.31[a]

[1)]Values followed by the same letter in the same column are not significantly different ($p<0.05$).
[2)]ND, not detected.

steeping medium remained undetected until the third day of steeping, and thereafter rapidly increased to reach 5.71, 0.18 and 0.52 mg/mL, respectively, at the end of steeping. Throughout steeping duration, the concentration of succinic and acetic acids in the steeping medium increased ranging from 0.02~0.19 and 0.03~0.31 mg/mL, respectively. These results indicate that high amounts of organic acids in the steeping medium can lead to a rapid drop in pH and an increase in the titratable acidity, at which point the environment becomes selective against less acid-tolerant microorganisms.

The concentration of reducing sugars in the steeping medium increased from 0.61 to 10.43 mg/mL, with a 17-fold increase throughout the 15 days of steeping. The levels of sugar and oligosaccharide during steeping of waxy rice were analyzed by HPAEC based on the assumption that there could be some by products of enzymatic starch-degradation or non-enzymatic action in the steeping medium. As shown in Table 3, the concentration of sucrose was 0.46 mg% initially and thereafter decreased to an undetectable level, suggesting that sucrose is the preferred substrate for the growth of microorganisms. On the other hand, glucose and maltose were unnoticed at the beginning, and thereafter increased with the steeping duration (0.11 to 7.61 mg% for glucose and 0.18 to 5.22 mg% for maltose). The oligosaccharides ranging from G3 to G7 were undetected in the steeping medium at the early stage of steeping, and then increased until the end of steeping. Particularly, the G3 and G5 oligosaccharides (odd number of glucose units), appeared to accumulate in the steeping medium as compared to oligosaccharides with even numbers (G4 or G6). These results revealed that oligosaccharides with an even number of glucose units were further hydrolyzed by β-amylase (1,4- α -D-glucan maltohydrolase). Throughout the steeping process, no oligosaccharides larger than G8 were detected in the steeping medium. The presence of oligosaccharides, together with the rapid increase in the concentration of reducing sugars, indicated that enzyme activity associated with degradation of starch and/or protein possibly increased during the steeping process. Fig. 2 illustrates the changes in α -amylase, β-amylase and protease activities in the steeping medium of waxy rice throughout the steeping process. Significant α -amylase activity was found after 1-day of steeping and proportionally increased until the end of steeping. β-Amy-

Table 3. Distribution of oligosaccharides in the steeping medium of waxy rice during 15 days of steeping at ambient temperature

Sugar (mg%)	Steeping period (day)						
	0	1	3	6	9	12	15
Glucose	ND[1)]	0.11	0.20	0.19	1.17	3.80	7.61
Sucrose	0.46[2)]	0.22	0.15	0.10	ND	ND	ND
Maltose (G2)	ND	0.18	0.59	1.57	3.09	4.89	5.22
Maltotriose (G3)	ND	ND	0.03	1.42	2.43	3.65	5.10
Maltotetraose (G4)	ND	ND	Tr[3)]	0.15	0.30	0.42	0.60
Maltopentaose (G5)	ND	ND	0.08	0.15	0.60	0.90	1.25
Maltohexaose (G6)	ND	ND	ND	ND	0.39	0.40	0.45
Maltoheptaose (G7)	ND	ND	0.06	ND	0.14	0.20	0.30

[1)]ND, not detected.
[2)]All values are mean of duplicate.
[3)]Tr, below than 0.01 mg%.

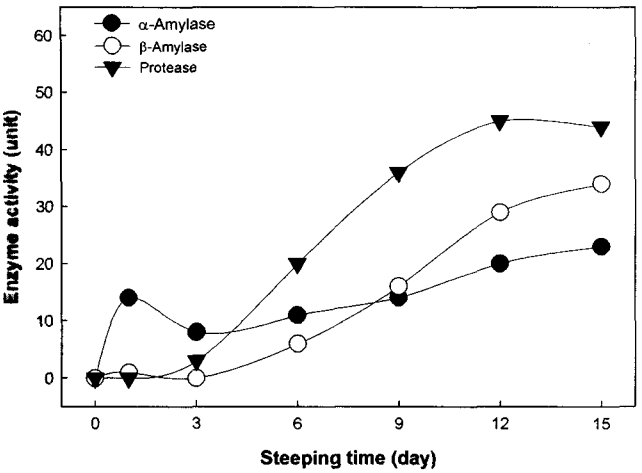

Fig. 2. Changes in enzyme activity of the steeping medium during the steeping process.

lase activity was also detected from the 6th day of steeping and increased until the end of steeping. Protease activity rapidly increased from the 3rd day of steeping, reaching a maximum at 12 days, and then remained constant until the 15th day of steeping. These results suggest that the long-term steeping of waxy rice in water for *yukwa* production can be characterized, at least in part, by the degradation of starch or protein that leads to grain softening and specific physicochemical and functional modification of waxy rice via fermentation.

The results presented here provide the microbiological and biochemical characteristics associated with the steeping process for *yukwa* production, in which waxy grain is steeped in water for 1~2 weeks. From these results, the long-term steeping process for *yukwa* production can be seen as the combination of typical natural heterolactic acid fermentation of cereal grains such as sorghum or millet or maize and a spontaneous cassava retting. These data could be used to control this lengthy process and to develop starter culture for the production of *yukwa* with improved quality on a commercial scale. Additional research is needed to identify the precise strains by means of joint phenetic-genetic tools and to investigate which microorganisms contribute to the desirable functional and organoleptic properties of the *yukwa*.

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