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Effect of Soy Peptide on Brewing Beer

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Here, we examined the effect of soy peptides (SPs) on the fermentation and growth of Yeast Bank Weihenstephan 34/70 (W34/70), a bottom-fermenting yeast. We compared fermentation for SP with that for a free amino acid (FAA) mixture having the same amino acid composition as SP, as a nitrogen source. Maltose syrup was used as a carbon source, and the medium contained excess amounts of essential minerals and vitamins. We observed that SP was better than FAA mixture at promoting fermentation and growth and that much more β-phenylethyl alcohol was produced during fermentation with SP than with FAA mixture. Subsequently, we compared fermentations with the FAA mixture and selected mixtures containing various dipeptides of Phe as a nitrogen source. We found that the rates of Phe metabolism and β-phenylethyl alcohol generation were much higher when Phe was presented as a dipeptide (Phe-Asp, Phe-Leu, or Phe-Phe) than when presented as FAA. These results show that amino acids such as Phe are absorbed more rapidly when presented as a peptide than as FAA, resulting in a more rapid production of β-phenylethyl alcohol.

[**Key words:** soy peptide, free amino acid, phenylalanine (Phe), β-phenylethyl alcohol, bottom-fermenting yeast]

Recently, no-malt beer is becoming more and more popular in the Japanese market for beerlike alcohol beverages. In this category, malt and wort cannot be used as sources of nutrition for yeasts and, thus, proven carbon sources, including corn syrup and maltose syrup, are used. Other sources of nutrition, particularly a nitrogen source, are also necessary for fermentation. One study has shown that the use of soy peptides (SPs) results in high performance for fermentation while preventing off-flavor development in the production of no-malt beer (1).

In traditional brewing, amino acids and peptides in wort are utilized as a nitrogen source. The importance of amino acids in wort for brewery fermentation has been studied for many years (2–5). Jones and Pierce classified them into groups A to D according to their rate of absorption from wort under brewery conditions (2). Although peptides in wort appear to be assimilated by yeasts, the extent to which they are absorbed and how they affect the fermentation are not well known (6–10).

On the other hand, SPs, which are obtained by the hydrolysis of soy protein isolates and whose amino acid composition is optimal, are utilized widely for isotonic drinks and functional foods. In healthy adult males, the absorption of SPs is quicker and more efficient than that of protein or amino acid mixtures (11). In addition, SPs are widely used for various fermented food products. Izawa *et al.* confirmed that SPs promote the effectiveness of bakers' yeast and increase tolerance to freeze-thaw stress (12).

In this study, we examined the use of SP in the fermentation and growth of brewing yeasts and the production of flavor.

MATERIALS AND METHODS

Materials SP was produced on a pilot scale in our laboratory. The main components, as well as some components that have a considerable effect on yeast fermentation, are shown in Table 1,

TABLE 1. Profile of SP

Composition	%
Dry matter	94.1
Protein content	83.7
Ash	7.3
Free amino acid content	27.2
Vitamin	mg/100 g
Biotin	0.0421
Pantothenate	0.15
Inositol	530
Niacin[a]	0.71
Vitamin B6	0.13
Riboflavin	0.13
Thiamine	0.11
Mineral	mg/100 g
Zn	3.52
Mn	1.51

[a] Equivalent amount to nicotinic acid.

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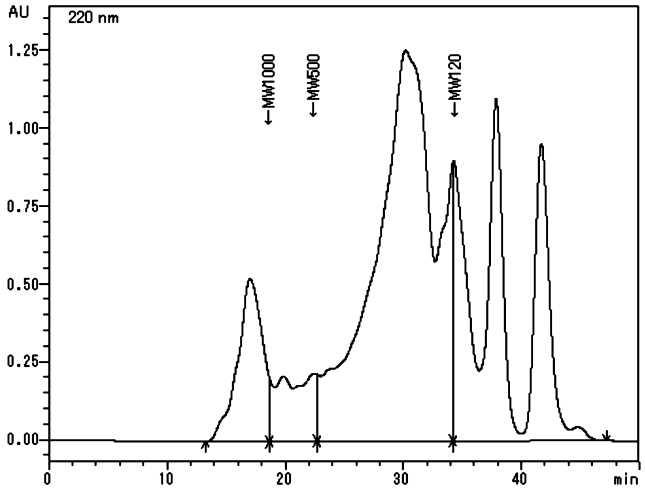

FIG. 1. Distribution of molecular weights of SP.

and the distribution of the molecular weights of the SP is shown in Fig. 1. Maltose syrup, MC-55, was purchased from Nihon Shokuhin Kako (Tokyo). Dipeptides were purchased from Kokusan Chemical (Tokyo).

SP analysis Dry matter was measured by drying the SP to a constant weight in a forced air oven at 105°C for 5 h. Protein content was determined by the Kjeldahl method using a 2400 Kjeltec Auto Analyzer Unit (FOSS Analytical AB, Hoganas, Sweden). The distribution of the molecular weights of the SP was analyzed using the method described by Maebuchi *et al.* (11) using Superdex Peptide 7.5/300 GL (Amersham Biosciences, Piscataway, NJ, USA) and 10 mM sodium phosphate buffer (pH 8.0) containing 1% (w/w) sodium dodecyl sulfate (SDS) at a flow rate of 0.25 ml/min as the mobile phase. Eluted peptides were detected from their absorbance at 220 nm. Ash content was measured by the dry ash method using an electric muffle furnace at 600°C. Free amino acid content was calculated after analysis using an L-8500 amino acid analyzer (Hitachi, Tokyo) after deproteinization with 3% (w/w) sulfosalicylic acid. The following components were analyzed by the Japan Food Analysis Center, Osaka; riboflavin and thiamine as thiamine hydrochloride were quantified by HPLC; vitamin B6 was measured by microbiological assay using *Saccharomyces cerevisiae* (*S. uvarum*) ATCC 9080; biotin, pantothenate, and niacin (measured as the equivalent amount of nicotinic acid, exclusive of tryptophan) were measured by microbiological assay using *Lactobacillus plantarum* ATCC 8014; and Mn and Zn were quantified by ICP atomic emission spectrometry. The profile of the SP is shown in Table 1. The amino acid composition (except for tryptophan) of the SP was analyzed using a JLC-500/V amino acid analyzer (JEOL, Tokyo) after hydrolysis. Tryptophan content was quantified by HPLC. The amino acid composition of the SP is shown in Table 2.

Preparation of free amino acid (FAA) mixture with the same amino acid composition as SP preparation A 2% solution with the same composition of amino acids as the SP (Table 2) was prepared from reagent-grade free amino acids. The solution was adjusted to pH 1.5 with 6 N HCl to fully dissolve the amino acids. Because glutamine, asparagine, and cysteine are unstable under acidic conditions, the concentration of glutamic acid added to the solution is the sum of the concentrations of glutamic acid and glutamine, and the concentration of aspartic acid added to the solution is the sum of the concentrations of aspartic acid and asparagines; also cystine was added to the solution instead of cysteine. The solution was used to prepare a medium containing FAA mixture with the same amino acid composition as the SP. The 2% solution used for the experiment on the absorption of Phe in medium with FAAs or dipeptides was prepared by the same method.

TABLE 2. Amino acid composition of SP

Amino acid	g/100 g SP
Asp	9.71
Thr	3.19
Ser	4.28
Glu	16.00
Gly	3.41
Ala	3.46
Val	4.01
Cys	1.00
Met	1.04
Ile	3.85
Leu	6.50
Tyr	3.12
Phe	4.41
His	2.22
Lys	5.18
Arg	6.36
Pro	4.40
Trp	1.05

Media and growth conditions The brewing yeast used in this study was Yeast Bank Weihenstephan 34/70 (W34/70). The cells used in this study were first cultured at 25°C for 24 h with shaking (120 rpm) in an Erlenmeyer flask (300 ml) containing 120 ml of YPD medium and then cultured at 25°C for 72 h in an Erlenmeyer flask (2000 ml) containing 900 ml of YPD medium. The yeast cells were harvested by centrifugation (1690×*g* for 10 min), washed with sterile water, and centrifuged again. The slurry was suspended in three volumes of sterile water, and the cell suspension was used to inoculate the test medium.

Fermentation tests were carried out as described by Dixon (13). Each medium was adjusted to pH 5.0 and autoclaved at 121°C for 20 min. The cell suspensions described above were added to each medium in a tube (1135 mm length×55 mm internal diameter) and aerated by agitating up and down. Each fermentation test was carried out at 15°C for 10 or 11 d. Cell density was analyzed from turbidity readings at 660 nm after dilution with water. An aliquot of the culture medium was sampled and filtered through no. 5A filter paper (Advantec, Tokyo). The specific gravity of the filtrate was measured, and the alcohol content of the culture medium was estimated.

Analysis of flavor components by GC-MS The flavor components (*i.e.*, ethyl acetate, *n*-propanol, isobutanol, isoamyl acetate, isoamyl alcohol and β-phenylethyl alcohol) in the medium were measured using a static balance pressure headspace system PerkinElmer HS40 (PerkinElmer Analytical Instruments, Uberlingen, Germany) coupled to a GC/MS-QP2010 (Shimadzu, Kyoto). A 3 g sample from each medium after filtration through no. 5A filter paper (Advantec) was taken and introduced into 22-ml round-bottomed vials; then the vials were sealed with aluminum-rubber septa. The vials with samples were held at 40°C for 45 min, purged and pressurized with helium at 130 kPa. The flavor components were driven through the transfer line, which was held at 200°C, to the injector of the gas chromatograph and separated on an Rtx-WAX capillary column (30 m length×0.32 mm internal diameter 0.50 μm film thickness) (Restek, Bellefonte, PA, USA) under the following conditions: injector temperature, 150°C; carrier gas helium, 3.06 ml/min; temperature program: 40°C held for 2 min, increased to 200°C at a rate of 10°C/min and 200°C held for 15 min. The GC column was directly connected without splitting to the ion source of a QP2010 quadrupole mass spectrometric detector, which was operating in the scan and SIM modes (monitor ion: 42, 59, 70 and 91). The components were identified by computer-matching of their mass