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Note

High-yield production of ethyl α-D-glucoside in shochu brewing and evaluation of its functionality

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We developed a brewing method for rice and barley shochus, the main mash of which contains 3-times and 10-times more ethyl α-D-glucoside (α-EG), respectively, compared with that from conventional methods. The 2.5% α-EG extract prepared from a distillation residue of rice shochu was found to have a moisture-retaining effect.

Key words: ethyl α-D-glucoside (α-EG); moisturizing effect; functional food; skin care

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Alpha-EG is a glycoside produced by transglycosylation of starch-derived maltose or maltooligosaccharides by

α-glucosidase of koji-mold using ethanol as an acceptor in brewing of sake (Japanese rice wine) (1). Since Imanari *et al*. discovered the presence of α-EG in sake (2), influences of α-EG on the flavor of sake and its production mechanism have been studied (1,3,4) because α-EG is contained in sake at about 0.1–0.7% (5), which is the fourth highest following water, ethanol, and glucose in this order among the ingredients of sake. Alpha-EG has instantaneous sweetness as well as slow-acting and mild bitterness.

Alpha-EG, an ingredient deeply involved in the taste of sake, is also reported to have a moisture-retaining effect and roughness-improving when applied on skin. Kitamura *et al*. applied a sake concentrate (approximately 2-fold or 0.2-fold concentrated sake) to the skin of hairless mice for 5 days and then irradiated the mice with ultraviolet B (UVB), followed by further application of the concentrate for 3 days. Consequently, they found that Transepidermal water loss (TEWL) levels was significantly reduced compared with a control (0.5% Triton X-150) and that 0.05% α-EG reagent also showed a similar effect (6).

Moreover, orally ingested α-EG has also been reported to have a moisture-retaining effect. Hirotsune *et al*. found that α-EG and organic acids, which are components of sake, reduce TEWL levels of UVB-irradiated hairless mice (7). As a result of their subsequent test of dose dependence of these substances, they confirmed that α-EG shows efficacy. Thus, α-EG is an ingredient expected to be useful for skin beauty through oral ingestion as well as skin application. However, no effective manufacturing method of α-EG produced by various brewing methods has been reported.

For the production of α-EG, coexistence of ethanol, maltose, maltooligosaccharides, and α-glucosidase that catalyzes the reaction is necessary in the process of fermentation. Sake brewing, a parallel double-fermentation process in which saccharification and fermentation progress simultaneously, satisfies these

requirements. On the other hand, α-EG is not produced enzymatically in wine brewing, which has no saccharification process, and in beer brewing, in which α-glucosidase is inactivated by the boiling following saccharification (8). Sato *et al* suggested that a small amount of α-EG, which is included in wine, is formed by a nonenzymatic condensation reaction of glucose and ethanol (5). Although not detected in shochu, a type of spirit, α-EG is likely to be produced in its main mash because it is produced by parallel double-fermentation. On the other hand, the distillation residue of shochu is a waste liquid containing a high concentration of organic matters generated in the process of shochu manufacturing. Because this residue contains abundant moisture and nutrients, including protein, fat, and saccharides, a variety of methods for effective utilization of this residue have been studied, such as feed, food, and human consumption related to its specific functions, such as the hepatopathy inhibitory effect (9). In the present study, in order to effectively utilize the distillation residue of shochu, we exploited a manufacturing method of shochu in which a material with a moisture-retaining effect that can be used in cosmetics, dietary supplements, and drinks was produced from the residue. For this purpose, we aimed a high-yield production of α-EG in shochu's main mash and addition of an extra effect, the moisture-retaining effect of α-EG, to the residue. First, we checked whether α-EG was produced in a general manufacturing method of shochu and then examined brewing methods leading to a high α-EG content in the distillation residue, while maintaining the quality of the distillation product (shochu).

The brewing was conducted with the following materials and conditions. Briefly, koji-mold (*Aspergillus awamori*), yeast (*Saccharomyces cerevisiae* NBRC2373 for shochu), and barley (Borden variety) were used. The enzymes used were "Sumizyme" (Shin Nihon Kagakukogyo Co. Aichi, Japan) for α-amylase and "α-glucosidase Amano" (Amano Enzyme Inc., Nagoya, Japan) for α-glucosidase. In addition, the water

for brewing was used after its pH was adjusted to 3.5 with lactic acid. For rice shochu, 12 g of koji (rice covered with a growth of koji fungus) was mixed with 21 mL of water and 5.3×10^6 cells/mL of yeast and was subjected to primary fermentation for 5 days at 30°C, followed by addition of 40 g of steamed rice and 96 mL of water, and secondary fermentation for 8 days at 20°C or 30°C. For barley shochu, 16 g of koji was mixed with 28 mL of water and subjected to primary fermentation for 5 days in the same conditions as those of rice shochu, followed by the addition of 40 g of steamed barley and 66 mL of water, and secondary fermentation for 8 days at 20°C or 30°C. In the brewing in which koji was reduced and enzymes were added, the amount of koji was reduced to 1/2 (6 g), 1/4 (4 g), and 1/8 (3 g) of the normal amount in the case of rice shochu and 1/8 (2 g), 1/10 (1.6 g), and 1/12 (1.3 g) in the case of barley shochu. Dried pregelatinized rice of the amount equivalent to the reduced amount was added so that the total amount of rice used in the brewing remained the same. A preliminary experiment that investigated enzyme quantities added indicated that α-EG productivity was higher when enzymes were added to the secondary main mash compared with that observed when enzymes were added to the primary main mash and that α-EG productivity was higher when 4140 units of α-glucosidase and 2520 units of α-amylase were added compared with that when 8280 units of α-glucosidase and 5040 units of α-amylase were added. Based on these data, 4140 units of α-glucosidase and 2520 units of α-amylase were added to the secondary main mash in koji-reduced brewing to determine the amount of koji to be used. The analysis of α-EG was performed using High performance liquid chromatography (HPLC) (Alliance HPLC system, Waters Co., MA, USA) with the conditions described in Fig. 1.

The rice shochu and barley shochu showed similar tendencies. Alpha-EG was produced at a higher yield in the cooler (20°C) brewing condition than in the brewing condition at 30°C, as well as in the brewing condition in which koji

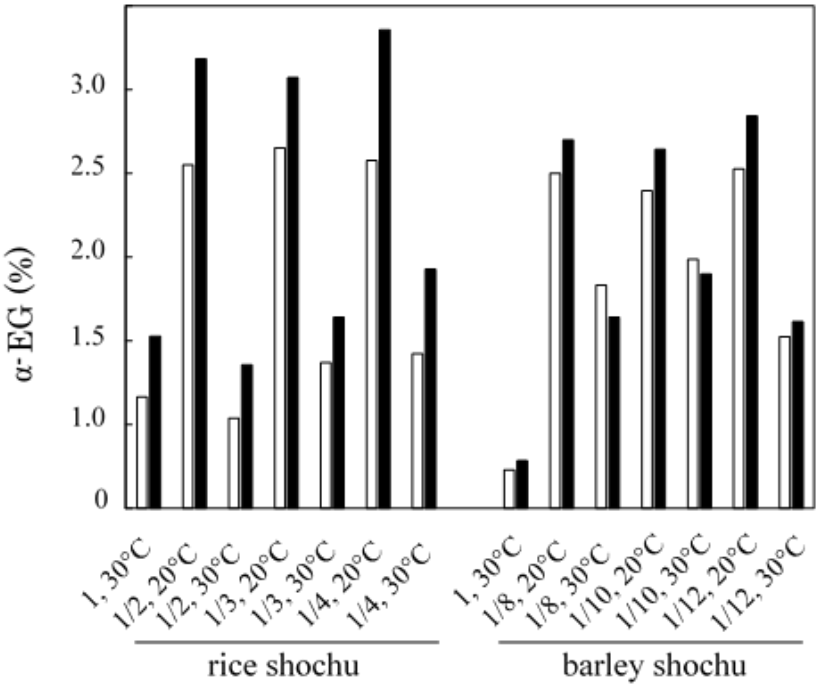

FIG. 1. Alpha-EG concentration in the main mash and distillation residue preparations of rice and barley shochus brewed with various koji contents. Alpha-EG was analyzed using the following conditions. HPLC system; Alliance HPLC system (Waters Co., MA, USA), detector; 2414 RI detector, (Waters Co., MA, USA), column; Shodex SPO 810 (Showa Denko, Tokyo, Japan), mobile phase; H_2O, flow rate; 400μl/min, column temperature; 65°C. The temperatures represent those of the secondary main mash. The numbers represent the amount of koji used, wherein 1, 1/2, 1/3, 1/4, 1/8, 1/10, and 1/12 denote 12 g, 6.0 g, 4.0 g, 3.0 g, 2.0 g, 1.6 g and 1.3 g, respectively. Open bars, fermentation broth, closed bars, shochu residue. Data represent mean values of three experiments.

content was reduced and α-amylase and α-glucosidase were added.

We successfully developed a brewing method in which the α-EG content in the brewing of rice shochu at 20°C with enzyme supplementation was 2.1%, which was more than 3-times higher than 0.6% in the brewing at 30°C, and the

content in the brewing of barley shochu in the same conditions at 20°C was 2.2%, 10-times higher than 0.2% of the brewing at 30°C. This was likely due to the better transglycosylation efficiency of α-glucosidase at 20°C at which degradation of starch in the raw material by glucoamylase becomes slower compared with the brewing at 30°C at which glucose supply from the starch by glucoamylase activity becomes predominant. On the other hand, because the α-amylase and α-glucosidase preparations have glucoamylase activity which degrade maltose and other substrates of α-glucosidase but does not contribute to α-EG production (10), the amount of enzyme preparations is a future subject of examination.

To check the quality of shochu brewed in the conditions of high yield α-EG production, rice shochu and barley shochu containing 30% alcohol were produced by vacuum distillation, diluted to 25% with water, and subjected to tasting separately (Table 1). A panel of

Table 1. Sensory evaluation of shochu brewed under high α-EG producing conditions.

	rice shochu		barley shochu	
koji ratio	1	1/4	1	1/12
fermentation temp.	30°C	20°C	30°C	20°C
odor	2.9±0.3	3.1±0.5	2.5±0.5	2.7±.5
taste	3.1±0.5	3.1±0.5	2.7±0.5	2.7±0.5
overall quality	3.1±0.5	3.0±0.4	2.7±0.5	2.6±0.5

The shochu samples were evaluated by a 5-point scale, in which 1 is the best and 5 is the worst. Flavor is scored for its acceptability and intensity. Taste is scored for its overall impression of whether sample is light or heavy, the extent of a sweet or acid taste, presence or absence of an abnormal taste, and the balance between the taste-components. Overall quality is evaluated with flavor, taste, and color. Values are the mean ±SD (n=8).

8 specialists judged that there were no differences in both flavor and taste and no issues in quality between the shochus obtained by conventional brewing and brewing with a high content of α-EG. In addition, because no change in the total amount of α-EG was observed before and after distillation as a result of the comparison of α-EG concentration between the distillation residue and the main mash, α-EG was confirmed to neither react with any other ingredient in the main mash nor evaporate and be recovered in shochu during distillation.

Fig. 2 shows the result of the pach test of the skin moisture-retaining effect of the α-EG-containing extract prepared from the shochu residue. The samples used in this test were prepared as follows: The residue of rice and barley shochus brewed with 1/4 and 1/12 amount of koji, respectively, compared with normal was completely dried to remove water and alcohol, mixed with an equal volume of reverse osmosis water (RO water), and centrifuged to extract α-EG, which was then prepared at a 2.5% concentration. After cleaning and moisture removal of the inner upper arm of subjects in the early 20s, followed by 20 min of acclimation, a patch sheet containing 100 μL of the sample was attached. After being left to stand for 2 min, the sheet was removed and the moisture contained mainly in the horny layer of the skin surface was measured every 15 min until 120 min using a skin moisture meter. The results showed that the extraction residue of rice shochu and α-EG reagent caused a significantly different skin moisture content after 2 h in the skin test compared with that observed in the RO water. On the other hand, because the moisture content was not significantly different despite the 2.5% α-EG content in the extraction residue of barley shochu, a substance negatively influencing the moisture retention might have been present.

We have successfully developed a brewing method that increases the content of α-EG, which has been confirmed to have a moisture-retaining effect, in the distillation residue of rice

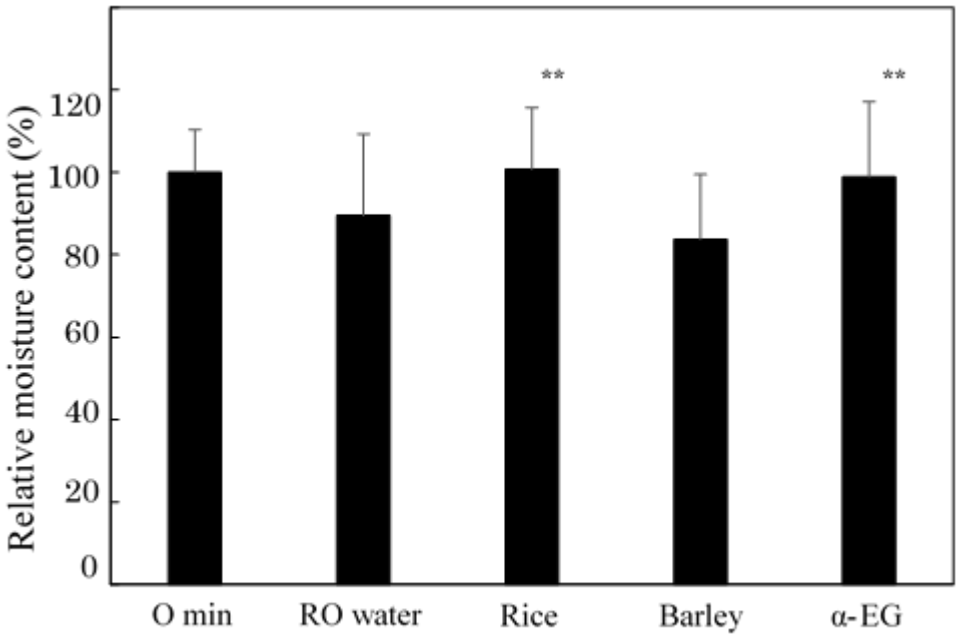

FIG. 2. The rate of change of skin moisture after application of the α-EG-containing shochu residue extract. The relative value of the skin moisture content 120 min after application of each sample is shown. Skin moisture content before treatment with sample (0 min) was set at 100%. Fourteen subjects were studied, and each sample was tested on 3 sites per subject. Rice; rice shochu residue extract, Barely; barely shochu residue extract, α-EG; 2.5% α-EG reagent. Results are mean ± SD (n=14). **$p<0.05$, significant difference in comparison to the RO water (t test).

and barley shochus, thus adding extra value to the residue, which is mainly discarded a waste material. We consider that this technical development can lead to the effective utilization of the distillation residue of rice shochu based on the following results: In the moisture retention test in which the extract (2.5% α-EG concentration) of the distillation residue of rice shochu was applied to subjects in their early 20s, the extract showed a moisture-retaining effect as indicated by a remarkable difference in the retained moisture content 120 min after the application compared with that observed in the control. Based on the

result that no remarkable difference in the moisture-retaining effect was observed between the α-EG solution extracted from the distillation residue of rice shochu and the α-EG reagent, α-EG is likely to be significantly involved in the moisture-retaining function. In the test to assess the effect of α-EG to improve UVB-caused rough skin in mice, α-EG has been reported to improve the balance between growth and cornification of epidermal cells (6). However, because this is the result of the third day after UVB irradiation in the test, the moisture-retaining effect observed in our study, which was measured in a period as short as 120 min, was likely to have been generated by a mechanism different from the growth/cornification balancing of epidermal cells.

Because the present study used 2.5%, a relatively high concentration of α-EG, we intend to examine whether the extract is also effective at lower concentrations. In addition, we expected a synergistic effect of α-EG and sphingolipids (11), a moisture-retaining substance found in the barley shochu residue, but unfortunately, we did not find the moisture-retaining effect with the barley shochu residue. Therefore, we would like to examine whether we can improve this technique by further modifying the fermentation method.

Also in the past, development of materials from the shochu residue for cosmetics and drinks has been conducted for its effective utilization. In our case, we intend to develop a new moisture-retaining material that is effective for beauty purposes through both oral ingestion and skin application using the α-EG-enriched shochu residue that we developed in this study and apply this to foods or cosmetics.

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