

Effect of protease of black koji mold on sweet potato shochu brewing

メタデータ	言語: jpn 出版者: 公開日: 2019-10-25 キーワード (Ja): キーワード (En): 作成者: 瀬戸口, 翔 メールアドレス: 所属:
URL	http://hdl.handle.net/10232/00030818

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題 目	Effect of protease of black <i>koji</i> mold on sweet potato <i>shochu</i> brewing (黒麹菌のプロテアーゼが芋焼酎醸造に与える影響)
<p><i>Koji</i> mold is widely used in the traditional fermentation industry of Japan as it is called national fungus. Therefore, <i>koji</i> mold has attracted a great deal of interest, and various studies have been conducted, in particular, on yellow <i>koji</i> mold used for sake, miso and soy sauce brewing. While, at present, research has not been advanced on black and white <i>koji</i> mold used for producing <i>shochu</i>. The main roles of black and white <i>koji</i> mold in the production of <i>shochu</i> are saccharification of raw materials by secreting a large amount of enzymes and prevention of bacterial contamination with high production of citric acid. In <i>shochu</i> production, saccharification by <i>koji</i> mold and fermentation by yeast are simultaneously performed in parallel. At that time, yeast is known to be involved in the production of aroma components of <i>shochu</i>. In particular, formation of components, such as higher alcohols, esters, and sulfur compounds, is closely related to the amino acid metabolism of yeast. In this study, we focused on the acid protease of black <i>koji</i> mold that may affect the concentration of amino acids in <i>shochu</i> mash, and investigated the relation of enzyme activity and the production of aroma compounds in <i>shochu</i>.</p> <p>First, a deletion ($\Delta pepA$) and an overexpression (OE$pepA$) strain of the <i>pepA</i> gene encoding the acid protease were constructed using black <i>koji</i> mold <i>Aspergillus luchuensis</i> RIB 2604 (NBRC 4314). Rice <i>koji</i> was produced using each strain and the protease activity was measured. The $\Delta pepA$ <i>koji</i> showed 1/2 and The OE$pepA$ <i>koji</i> showed 24 times higher activity compared with the parent strain. A small-scale test of sweet potato <i>shochu</i> production was performed using each rice <i>koji</i> and the amino acid concentration of each mash was measured. The $\Delta pepA$ mash showed a lower value and the OE$pepA$ mash showed a higher value than that of parent strain, suggesting that the acid protease activity affects the amino acid concentration of mash. Furthermore, as a result of measuring the aroma component of each <i>shochu</i> obtained by distilling these mash, a negative correlation was recognized with acid protease activity regarding the amount of higher alcohol and ester. The aroma component was high when the acid protease activity was low, and was low when the activity was high. From these results, it became clear that the acid protease activity of black <i>koji</i> mold affects the aroma of sweet potato <i>shochu</i>, indicating the possibility of breeding of <i>koji</i> mold using acid protease activity as an index.</p> <p>Next, a black <i>koji</i> mold with high acid protease activity was bred by mutation treatment. <i>A. luchuensis</i> J7-2 strain was mutated by heavy ion beam irradiation and strain with high acid protease activity was screened by halo assay using casein agar plate. As a result, the APH-1 strain was obtained. Rice <i>koji</i> was prepared using each strain and the acid protease and the acid carboxypeptidase activity were measured. The APH-1 rice <i>koji</i> showed 4.5 and 2.3 times higher activity than that of J7-2 strain, respectively. Interestingly, the APH-1 mash showed high ethanol yield per raw materials. The <i>shochu</i> using APH-1 strain showed a lower amount of higher alcohol and ester than that of J7-2 strain. This result is consistent with that of OE$pepA$ strain. From these results, it was revealed that the acid protease activity plays an important role in the flavor formation and also ethanol production of sweet potato <i>shochu</i>.</p>	