

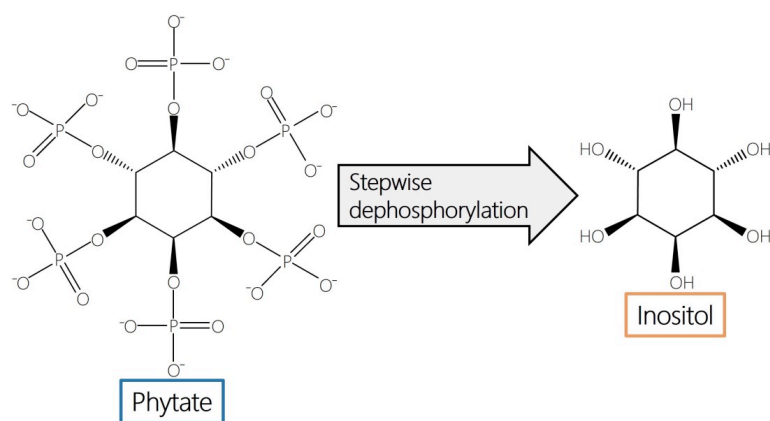
## Enhanced dephosphorylation of phytate to inositol in brown-rice *koji-amazake* saccharified with rice-*koji* made with *Aspergillus oryzae* and *Aspergillus luchuensis*

Brown rice provides B vitamins, minerals, and dietary fiber, but its bran layer contains phytate (inositol hexakisphosphate), which binds minerals and can reduce their bioavailability depending on processing and intake conditions. Therefore, brown rice products require processing approaches that enhance nutritional quality. *Koji-amazake* is a sweet, fermented rice drink produced through enzymatic saccharification by rice-*koji* and contains glucose, amino acids, and B vitamins. While *Aspergillus oryzae* is typically used for rice-*koji* production, *Aspergillus luchuensis*, which produces citrate, is also utilized. Phytase and acid phosphatase from both fungi contribute to phytate dephosphorylation. Complete phytate dephosphorylation yields nutritionally important inositol (Fig. 1). This study evaluated acidification resulting from citrate produced by *A. luchuensis*-derived rice-*koji* and quantified phytate dephosphorylation and inositol formation in brown-rice *koji-amazake* saccharified with rice-*koji* derived from *A. oryzae* or *A. luchuensis*, applied either individually or as a mixture.

When brown-rice *koji-amazake* was prepared using 5 g of brown rice (Himenomochi), 10 mL of water, and 3 g of dried rice-*koji* and incubated at 55°C for 8 h, the product made solely with *A. oryzae*-derived rice-*koji* (Condition 1) showed a Brix value of 37.2%, a pH of 5.98, and no detectable citrate (Table 1). Increasing the ratio of *A. luchuensis*-derived rice-*koji* (Conditions 2–5) lowered Brix values and increased citrate content, leading to a decrease in pH (Table 1). In non-fermented mixtures with heat-inactivated *A. oryzae*-derived rice-*koji*, phytate and inositol contents were 652 mg and 2.15 mg per 100 g, respectively, whereas Condition 1 yielded 516 mg of phytate and 9.26 mg of inositol (Fig. 2). Mixed-rice-*koji* Conditions 2–4 significantly enhanced phytate degradation and inositol production compared with Condition 1 (Fig. 2). Condition 5, using only *A. luchuensis*-derived rice-*koji*, produced phytate (158 mg/100 g) and inositol (47.9 mg/100 g) levels similar to those of Condition 4 (Fig. 2).

The incorporation of *A. luchuensis*-derived rice-*koji* effectively promotes phytate dephosphorylation in brown rice-*koji-amazake*, supporting product designs aimed at improving nutritional value. Citrate from *A. luchuensis* acidifies the product, which may enhance microbial stability and broaden flavor options, although consumer preference for sourness should be considered when adjusting formulations. Because this study used commercially available dried rice-*koji* and enzyme activities vary among fungal strains, the practical application of these findings requires evaluating strain-specific enzymatic properties of the rice-*koji* used.

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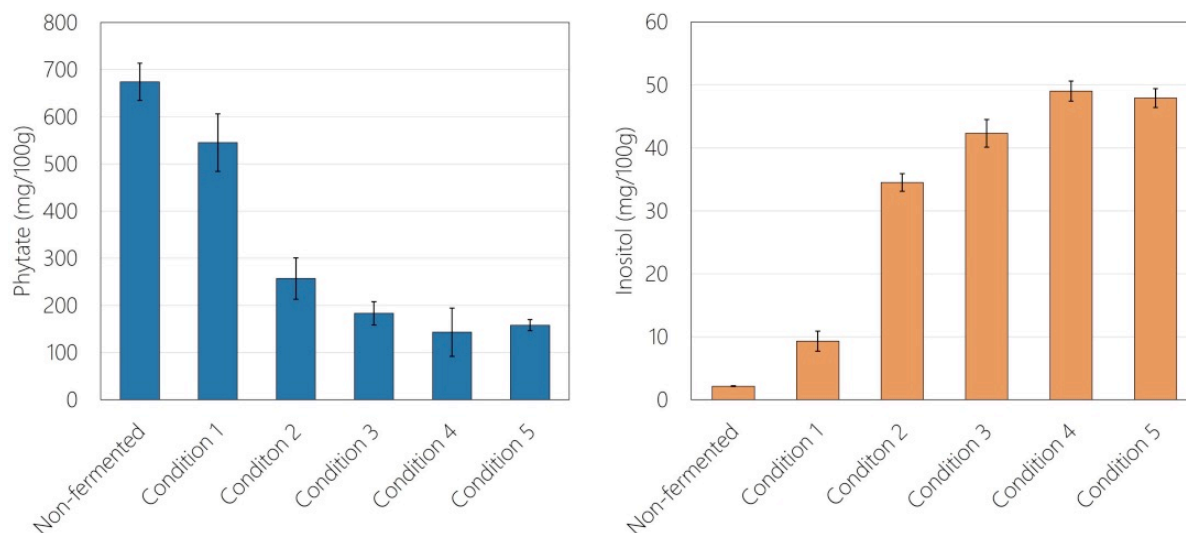


**Fig. 1. Chemical structures of phytate and inositol formed through its stepwise dephosphorylation**

**Table 1. Brix values, citrate contents, and pH of brown-rice *koji-amazake* saccharified with rice-*koji* made with *Aspergillus oryzae* and *Aspergillus luchuensis***

Conditions	Ratio of rice- <i>koji</i> derived from <i>A. oryzae</i> and <i>A. luchuensis</i> (%)		Brix (%)*	Citrate (%)*	pH*
	<i>A. oryzae</i>	<i>A. luchuensis</i>			
1	100	0	37.2 (0.17)	Not detected	5.98 (0.04)
2	75	25	36.7 (0.24)	0.18 (1.6)	4.74 (0.02)
3	50	50	36.0 (0.12)	0.26 (1.4)	4.17 (0.03)
4	25	75	35.6 (0.19)	0.44 (2.2)	3.88 (0.05)
5	0	100	35.4 (0.42)	0.56 (1.6)	3.68 (0.05)

\*Values represent means of three replicates, with standard deviations in parentheses.



**Fig. 2. Phytate and inositol contents in brown-rice *koji-amazake* saccharified with rice-*koji* made with *Aspergillus oryzae* and *Aspergillus luchuensis***

Values represent means of three replicates obtained from the pre-fermentation sample and from brown-rice *koji-amazake* prepared under Conditions 1–5 shown in Table 1, with error bars indicating standard deviations.

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