

## Utilization of Enokitake and Shiitake in fiber-bread processing and their characteristics

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Bread containing 5% enokitake (*Flammulina velutipes*) produced manually the expanded most, however, in the case of shiitake (*Lentinus edodes*), the loaf volume of bread decreased with an increasing concentration of shiitake. In the case of dough without sugar, the bread containing 10% enokitake the expanded most, however, that of more than 10% enokitake decreased with an increasing concentration of enokitake. The loaf volume of shiitake bread without sugar also decreased with an increasing concentration of shiitake because shiitake strongly inhibited the growth of baker's yeast. The firmness of the bread increased with an increasing concentration of enokitake or shiitake until the concentration was 30%. Fermentable sugars such as glucose, mannitol, maltose and trehalose from enokitake and glucose from shiitake were consumed with fermentation by yeast (*Saccharomyces cerevisiae*) during the first 4 hours of incubation.

### INTRODUCTION

Many attempts have been made to popularize bakery products because they are easy, convenient and an inexpensive means of taking in hygienically prepared ready-to-eat nutrition<sup>1)-5)</sup>.

Enokitake (*Flammulina velutipes*), shiitake (*Lentinus edodes*), maitake (*Grifola frondosa*), bunashimeji (*Hypsizygus marmoreus*), kikurage (*Auricularia auricula*) and nameko (*Pholiota nameko*) are edible mushrooms, of which commercial cultivation has begun in many countries<sup>6)-9)</sup>. Mushrooms are rich in fiber, and thus can be used in fiber bread processing<sup>10)</sup>.

We previously reported various types of mushroom breads in which fiber-rich mushrooms were added to wheat flour, and bread made in an automatic bread baker. The characteristics of these breads were compared with those of white

bread. Because the addition of mushrooms such as maitake, bunashimeji, kikurage and nameko had effects on the loaf volume, we recently reported the mechanism of gas production in mushroom bread processing and suggested that the optimum bread-baking performance may be achieved by an automatic bread baker if it is possible to control gas production by baker's yeast (*Saccharomyces cerevisiae*) or fermentation time. The loaf volume of enokitake and shiitake breads was the same as that of standard white bread, unlike that of mushrooms such as maitake, bunashimeji and nameko, made in an automatic bread baker. These breads were preferred to other types of breads containing maitake, bunashimeji, kikurage and nameko. Especially, mushroom breads containing 5% enokitake or 5% shiitake were preferred in total evaluation to standard white bread.

We describe herein a conventional type of bread in which some of the water is manually replaced with a fiber-rich material, namely enokitake or shiitake. The characteristics of enokitake and shiitake bread without sugar in the dough are also reported. Furthermore, we describe the relationship between the fermentable sugars derived from enokitake or shiitake, and gas production by baker's yeast in enokitake and shiitake bread processing.

## MATERIALS AND METHODS

### Materials

The wheat flour used was a commercial product with protein, lipid, carbohydrate and water contents of 12, 1.8, 69 and 14.5%, respectively. "Camellia" wheat flour and "Super Camellia" dry yeast were obtained from Nisshin Flour Milling Co., Tokyo, Japan. Raw enokitake (*F. velutipes*) mushrooms with a water content of 89.7% and raw shiitake (*L. edodes*) mushrooms with a water content of 91.1% were purchased at a local market in Nishinomiya, Hyogo Prefecture, Japan.

### Measurement methods

Table 1 shows the dough ingredients of white bread and mushroom breads containing enokitake or shiitake. Raw mushrooms were homogenized into small pieces (approximately 0.1mm to 1mm

particles), and added at 0 to 30% (mushroom weight / total weight, w/w) to white bread dough, mixed, fermented at 30°C for 120min to 180min, and then baked at 180°C for 20 min. The loaf volume was measured with a rapeseed displacement method.

### Effects of mushroom concentration on dough expansion

Portions of 150.2g of white bread and mushroom bread dough, which were prepared by the ingredients shown in Table 1, were placed in beakers at 300ml. The dough expansion in the beakers was measured by determining the height of the dough during development (fermentation) at 30°C.

### High pressure liquid chromatography

Qualitative and quantitative analyses of the fermentable sugars from raw enokitake and shiitake were performed by HPLC with an TSK gel Amide 80 column (4.6 I.D. X 250mm, Tosoh Co., Japan) in a Tosoh HPLC system. The column was developed with a 20% acetonitrile solution at 80°C at a flow rate of 0.7ml/min. Sugar detection was performed with a reflective index (RI) detector (Tosoh RI-8012).

## RESULTS AND DISCUSSION

### Making enokitake and shiitake breads

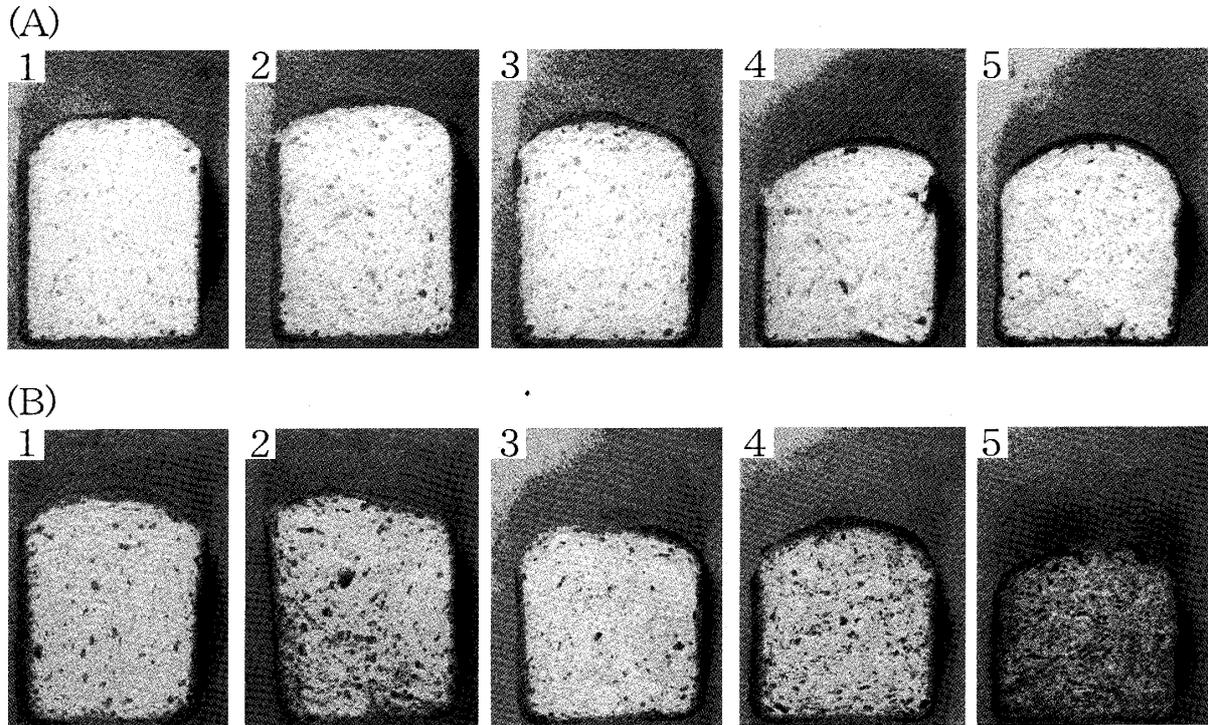
As shown in Figs. 1 and 2, the loaf volume

Table 1. White bread and mushroom bread ingredients

Ingredient	Mushroom concentration (%)									
	0*		5		10		20		30	
	Amount(g)	Ratio(%)	Amount(g)	Ratio(%)	Amount(g)	Ratio(%)	Amount(g)	Ratio(%)	Amount(g)	Ratio(%)
Wheat flour	84.0	100.0	84.0	100.0	84.0	100.0	84.0	100.0	84.0	100.0
Sugar	5.1	6.1	5.1	6.1	5.1	6.1	5.1	6.1	5.1	6.1
Butter	3.3	3.9	3.3	3.9	3.3	3.9	3.3	3.9	3.3	3.9
Dried non-fat milk	1.8	2.1	1.8	2.1	1.8	2.1	1.8	2.1	1.8	2.1
Salt	1.5	1.8	1.5	1.8	1.5	1.8	1.5	1.8	1.5	1.8
Water	53.6	63.8	46.1	54.9	38.6	46.0	23.6	28.1	8.6	10.2
Dry yeast	0.9	1.1	0.9	1.1	0.9	1.1	0.9	1.1	0.9	1.1
Mushroom	0.0	0.0	7.5	8.9(5%)	15.0	17.9(10%)	30.0	35.7(20%)	45.0	53.6(30%)
Total	150.2	—	150.2	—	150.2	—	150.2	—	150.2	—

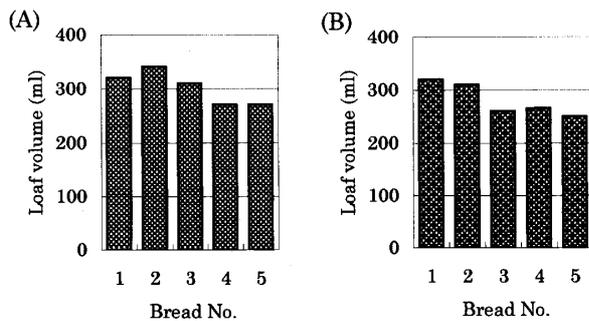
\*The control was standard white bread.

Homogenized mushrooms were added at 0 to 30% concentrations (mushroom weight/total weight) to dough, mixed, fermented, and then baked into bread.



**Fig. 1.** Photographs of white bread, enokitake breads and shiitake breads

(A), enokitake bread;(B), shiitake bread. 1, 0%(white bread(control)); 2, 5% mushroom bread; 3, 10% mushroom bread ; 4, 20% mushroom bread; 5, 30% mushroom bread. Mushrooms were added at 5~30%(mushroom weight/total weight)to the dough in 2~5. Photographs show a middle cutaway view of each bread.



**Fig. 2.** Effect of the concentration of mushroom homogenate on loaf volume in baking

(A), Enokitake bread; (B), Shiitake bread.

- 1, 0% (white bread(control));
- 2, 5% mushroom bread;
- 3, 10% mushroom bread ;
- 4, 20% mushroom bread;
- 5, 30% mushroom bread.

Mushrooms were added at 5 ~30% (mushroom weight/total weight) to the dough in 2 ~5.

of the breads containing 5 and 10% homogenized enokitake was almost the same as that of standard white bread, when an automatic bread baker was used, as described in previous reports<sup>11)</sup>. The bread contains 5% enokitake the expanded most. The loaf volume of bread increased until the addition of enokitake was 5% concentration, however, that of more than 10% enokitake decreased with an increasing concentration of enokitake. In the case of shiitake, standard white bread expanded the most, and the loaf volume of bread decreased with an increasing concentration of shiitake(0 to 30%).

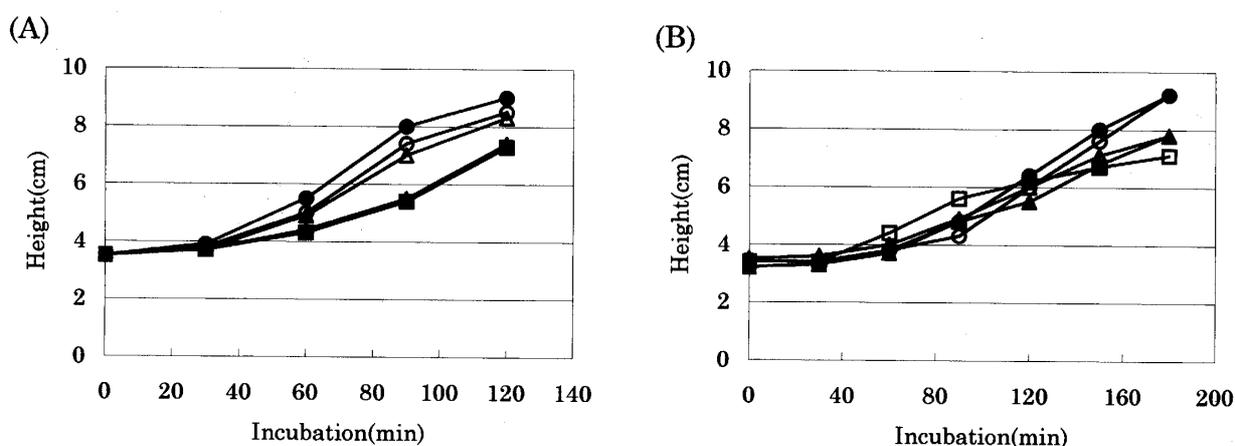
We previously reported that in the medium containing enokitake or shiitake, baker's yeast grew less than that containing any other type of mushrooms such as maitake, bunashimeji and nameko. Especially, shiitake completely inhibited the growth of baker's yeast. Enokitake may also

**Table 2.** Effect of mushrooms on the physical properties

Mushroom (%)	Enokitake		Shiitake	
	Hardness $\times 10^4$ (N/m <sup>2</sup> )	Cohesiveness	Hardness $\times 10^4$ (N/m <sup>2</sup> )	Cohesiveness
0*	1.8741	0.8249	1.4837	0.8947
10	2.1084	0.8384	1.3661	0.8229
20	2.2251	0.8460	2.0300	0.7962
30	2.2341	0.8475	3.1234	0.7988

\*The control was standard white bread.

Homogenized mushrooms were added at 0 to 30% concentrations (mushroom weight/total weight) to the dough without sugar, mixed, fermented, and then baked into bread.

**Fig. 3.** Effect of the concentration of mushroom homogenate on dough expansion

(A), Enokitake bread; (B), Shiitake bread. ○, 0% (white bread (control)); ●, 5% mushroom bread; △, 10% mushroom bread; ▲, 20% mushroom bread; □, 30% mushroom bread. Mushrooms were added at 5~30% (mushroom weight/total weight) to the dough. Portions of 150.2g of white bread and mushroom bread dough which was prepared by the ingredients shown in Table 1, was placed in beakers at 300ml. The dough expansion in the bakers was measured by determining the height of the dough during dough development (fermentation) at 30°C.

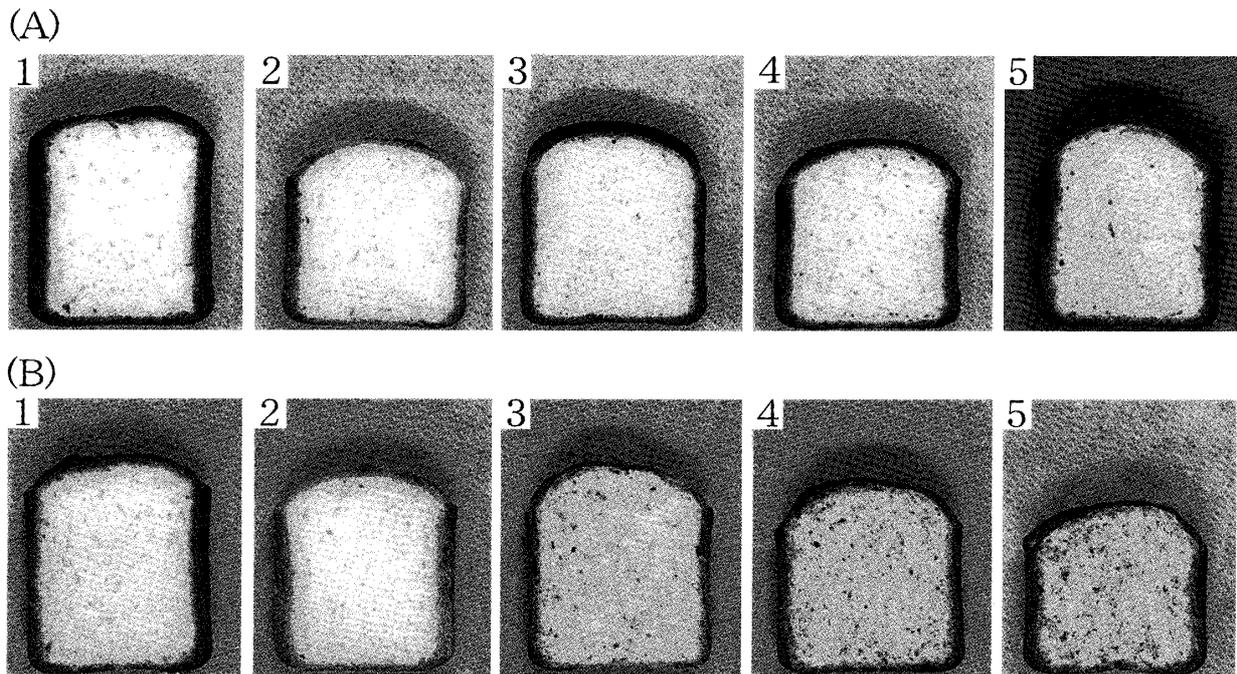
have a yeast-inhibitory effect like that of shiitake<sup>12</sup>). Thus, shiitake breads and breads containing more than 10% enokitake expanded less than standard white bread.

The hardness and cohesiveness of the 0, 10, 20 and 30% mushroom breads were measured by reolometer, as shown in Table 2. The firmness and cohesiveness of the enokitake bread increased with added enokitake until the concentration was 30%. In the case of shiitake, 30% shiitake bread was the hardest, followed by the bread containing 20% shiitake. The hardness of bread containing 10% shiitake was almost the

same as that of standard white bread.

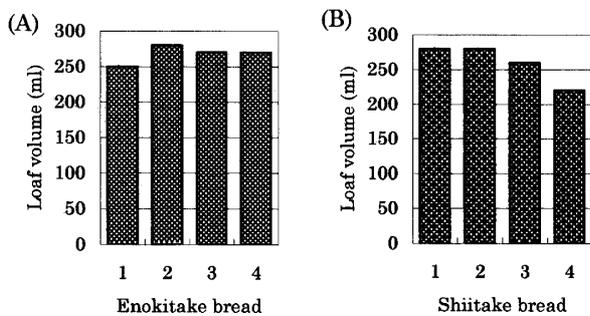
#### Effects of the concentration of mushroom breads on dough expansion

The standard white dough and the dough containing 5% to 30% enokitake or shiitake were expanded by incubation at 30°C. As shown in Fig. 3, the volume (height in cm) of more than 20% enokitake dough and more than 10% shiitake dough were decreased. The same trend existed with the breads.



**Fig. 4.** Photographs of enokitake and shiitake breads without sugar in the dough

(A), enokitake bread;(B), shiitake bread. 1, 0%(white bread without sugar(control)); 2, 10% mushroom bread; 3, 20% mushroom bread; 4, 30% mushroom bread. Mushrooms were added at 10~30% (mushroom weight/total weight)to the dough without sugar in 2~4. Photographs show a middle cutaway view of each bread.



**Fig. 5.** Effect of the concentration of mushroom homogenate on loaf volume after baking without sugar

(A), Enokitake bread;(B), Shiitake bread. 1, 0%(white bread(control)); 2, 10% mushroom bread; 3, 20% mushroom bread ; 4, 30% mushroom bread. Mushrooms were added at 10%~30% (mushroom weight/total weight)to the dough in 2~4.

**Effect of the concentration of mushrooms on dough expansion and loaf volume without sugar in the dough**

Usually, sugar is extensively used in bakery bread. Mushrooms such as enokitake and shiitake are rich in fiber, minerals, vitamins and fermentable sugar, which are low calorie, whereas sugar is high calorie(4Kcal/g). It was suggested that sugar might possibly be replaced by fermentable sugar of mushrooms in the dough for a good size loaf volume. Thus, making enokitake and shiitake bread without sugar was attempted.

As shown in Figs. 4 and 5, unfortunately, the loaf volume of bread containing 10% to 30% enokitake without sugar expanded less than the same as that of standard bread with sugar(Fig. 2). Until 10% concentration, the loaf volume increased, but that of more than 20% enokitake decreased with an increasing concentration of enokitake. However, the bread containing 10% to 30% enokitake without sugar expanded more than the standard bread without sugar. Thus, the addition of enokitake supplies additional carbohydrates to baker's yeast and has

promoted alcohol fermentation under anaerobic conditions.

The breads containing shiitake without sugar expanded less than that of standard bread without sugar. The loaf volume of bread decreased with an increasing concentration of shiitake. It seems that shiitake strongly inhibits the growth of yeast, so shiitake bread could not expand. These results showed the same tendency of that of enokitake or shiitake bread with sugar (Figs. 1 and 2).

#### The effects of the supply of fermentable sugar from mushrooms on the fermentation

Table 3 shows the change in fermentable sugar in the presence of enokitake and shiitake. Enokitake contained fermentable sugars, such as glucose, mannitol, maltose and trehalose. The concentrations of glucose, mannitol, maltose and trehalose in 1ml-extracted solution were 240 $\mu$ mol, 315 $\mu$ mol, 225 $\mu$ mol and 230 $\mu$ mol, respectively. All fermentable sugars were consumed with fermentation by yeast during the first 4 hours of incubation.

**Table 3.** Change in fermentable sugar in the presence of enokitake(A) and shiitake(B)

(A)		
Fermentable sugar in enokitake	Concentration ( $\mu$ mol)	
	0h	4h
Glucose	240	25
Mannitol	315	145
Maltose	225	20
Trehalose	230	20
(B)		
Fermentable sugar in shiitake	Concentration ( $\mu$ mol)	
	0h	4h
Galactose	1470	1815
Glucose	441	115
Arabinose	329	403
Trehalose	201	197
Fructose	107	165
Sucrose	21	18

Therefore, it seems that the addition of enokitake to white bread dough supplies additional fermentable sugar as a carbohydrate source to baker's yeast and promotes alcohol

fermentation under anaerobic conditions, thus increasing gas production. We concluded that gas production caused by adding enokitake to white bread dough leads to expansion of the dough.

On the other hand, shiitake contained fermentable sugars such as galactose, glucose, arabinose, trehalose, fructose and sucrose. The concentrations of galactose, glucose, arabinose, trehalose, fructose and sucrose in 1ml-extracted solution were 1727.3 $\mu$ mol, 530.2 $\mu$ mol, 520.0 $\mu$ mol, 202.4 $\mu$ mol, 157.6 $\mu$ mol and 22.2 $\mu$ mol, respectively.

Almost all glucose was consumed with fermentation by yeast during the first hour of incubation. It seems that the addition of shiitake to white bread dough supplies additional glucose as a carbohydrate source to baker's yeast, however, it seems that shiitake strongly inhibits the growth of baker's yeast. Consequently, the loaf volume of shiitake bread expanded less.

In general, wheat flour contains a low level of fermentable sugars (about 0.5%); a level that is too low to support yeast activity throughout the proof period. Usually, when sugar is added to the dough, it is sufficient to produce gas (carbon dioxide) for good-sized, well-aerated loaves. We previously reported that those excessive fermentable sugars from mushrooms (maitake, bunashimeji, nameko and kigurage) result in excessive gas (carbon dioxide) production, producing sticky crumbs in the resulting bread with a decreased loaf volume. However, the optimum bread-baking performance was achieved by controlling the gas production of baker's yeast<sup>10),12)</sup>. In this experiment, we manually produced a conventional type of enokitake and shiitake. The mushroom breads are rich in fiber, minerals, vitamins and  $\beta$ -D-glucan which acts as an anti-cancer agent. Furthermore, the mushroom breads are low calorie food.

Many papers have been published on the significance of dietary fiber for human nutrition. An increased cereal fiber intake is

desirable, and this can be achieved with bread. We studied the making of bread in which enokitake and shiitake were added to the wheat flour. A fiber content of 5.63g/100g, 5.72g/100g, and 5.81g/100g of dough were calculated in baked bread containing 10%, 20%, and 30% of enokitake and shiitake, respectively (Okamura et al. 1998). Standard white bread had a fiber content of 5.54g/100g (baked bread). Therefore, enokitake and shiitake bread is a good bread additive providing dietary fiber.

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