

Abstract

The objective of this research is to find the substances that affect the suppression of mold growing on bread. The bread was placed in a container with lemon, grated garlic, and wasabi paste, all of which have a strong odor, and observed for about two weeks. Later, focusing on alliin, a component of garlic, an experiment was conducted on mold suppression by comparing two samples of garlic. One sample contained a clove of garlic that was cut and the other was grated, then heated. The results showed that alliin, a volatile antimicrobial component in garlic, had an effect on the inhibition of mold growth. From there, the focus shifted to elucidating the mechanism of the inhibitory effect of alliin on mold. Alliin was extracted from American peeled garlic and added to an alliinase enzyme solution to make an alliin solution. Future research should focus on the thermostability of alliin, concentrating on the variation of its inhibitory effect with temperature change.

Introduction

When I was in the third grade of junior high school, I was curious about how long it took for mold to grow on expired bread and what kind of measures were needed to prevent mold from growing, so I conducted an experiment titled "Mold Development and Control Using Bread". This experiment led me to want to investigate mold further. I wanted to study the causes of mold suppression, something which I had not been able to do before.

Materials & Method

Materials

one slice of bread, water, an atomizer, aluminum foil, wrap, a 500mL beaker, a lemon, a grated garlic, horseradish paste (tube), a fruit knife, a 3D printer, and polyethylene gloves.

Method

【STEP 1】 A slice of bread was prepared, and polyethylene gloves were worn to prevent direct contact with the bread. The bread was then cut into six squares, removing the crust. The squares were sprayed with water using an atomizer twice and left to air for 20 seconds.

【STEP 2】 Parts of the platform corresponding to the size of the bread and beaker were made using a 3D printer and assembled (Fig. 1).

【STEP 3】 The bread from **【STEP 1】** was pressed into the stand made in **【STEP 2】** as if it were stamped, and then assembled using polyethylene gloves to prevent touching the bread with hands, and a small aluminum foil case with a base of about 4 cm was placed inside a 500 mL beaker (Fig. 2). The aluminum foil case was placed in the space under the beaker (Fig. 3). Various samples were placed in the aluminum foil case.

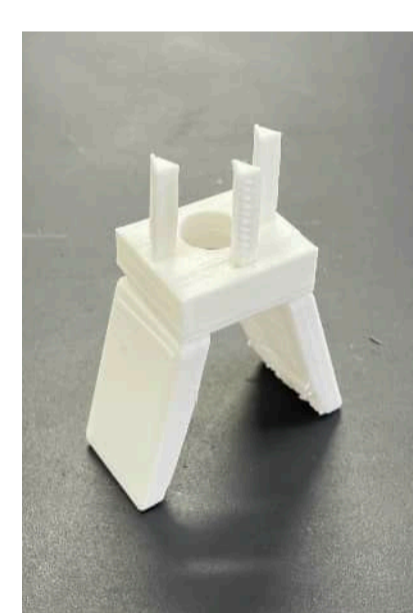


Fig. 1 3D printed platform



Fig. 2 Sealed container

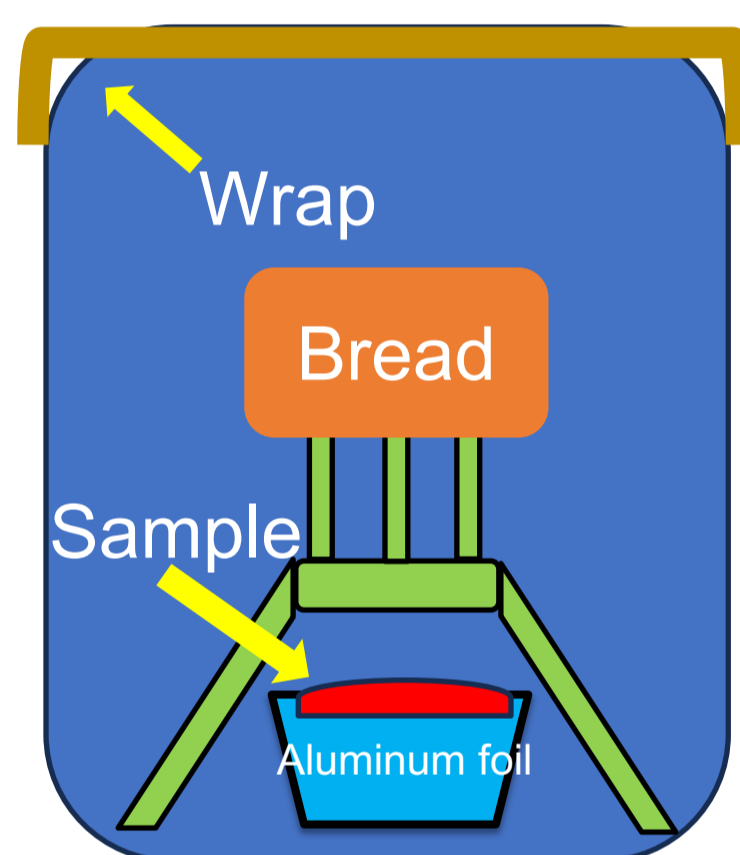


Fig. 3 Schematic diagram

Experiment 1

Sample Lemon (Fresh lemon cut into wedges), Grated garlic, Wasabi paste (from a tube)

These were placed in aluminum foil and observed at room temperature (25°C) for two weeks.

Result & Discussion

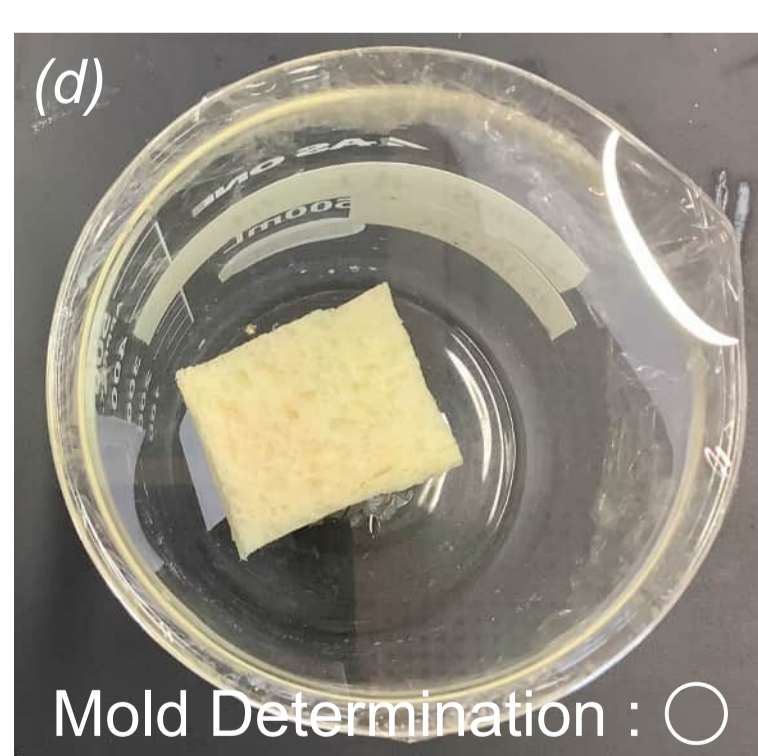
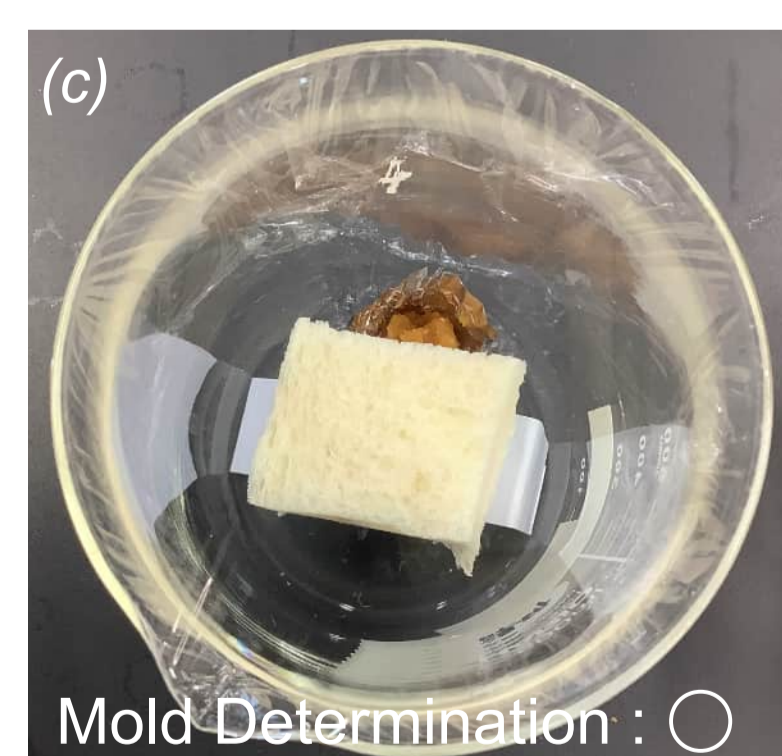
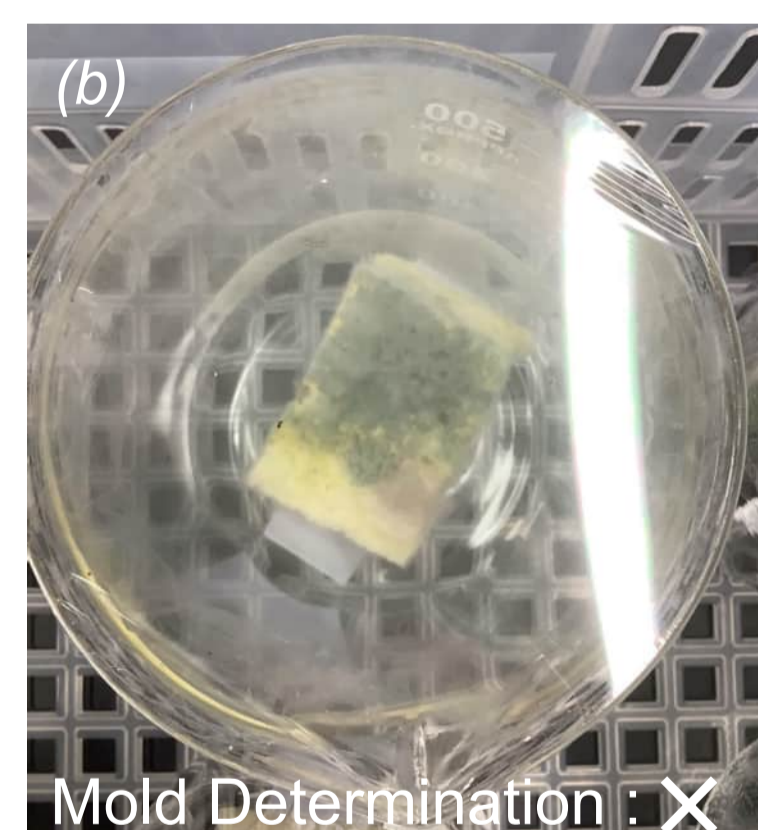
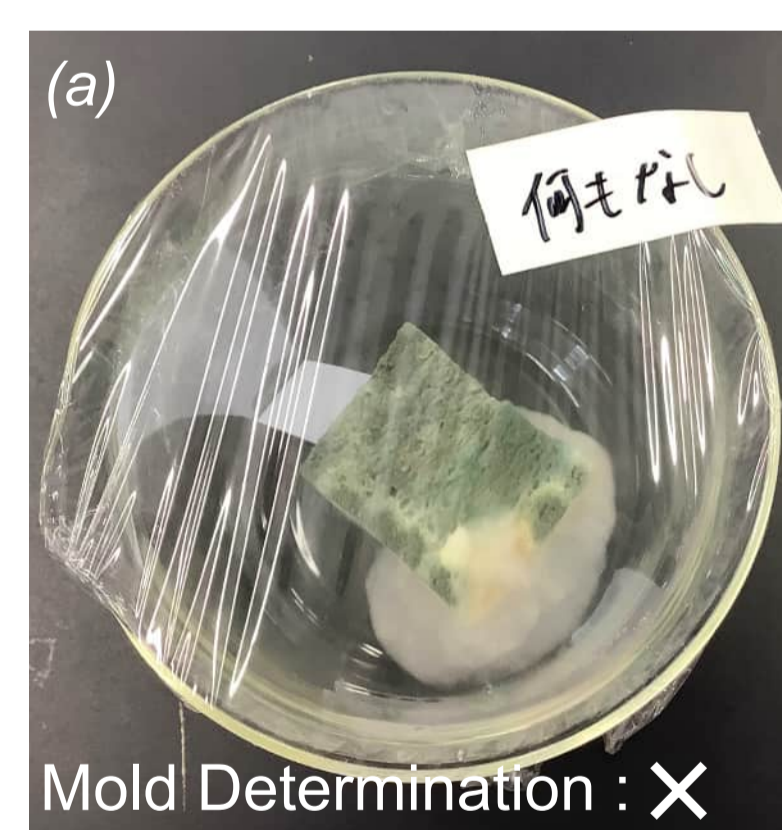


Fig. 4 Bread after two weeks [(a) Nothing (b) Lemon (c) Grated garlic (d) Wasabi paste]

Mold Determination: Moldy...X, Completely mold free...O

From the results of Experiment 1, it was found that none of the bread in the beaker with grated garlic and wasabi paste had mold, even though they were under the same conditions as the others. The bread in the beaker with grated garlic and the bread in the beaker with wasabi paste, both of which had no mold growth in this experiment, were continued to be monitored. The same experiment was conducted with grated garlic and kneaded horseradish at different times and under the same conditions. Two weeks later, the bread was again free of any mold. These results suggest that grated garlic and wasabi paste have an inhibitory effect on mold growth.

Experiment 2

Sample Focusing on whether the inhibitory effect of mold development continues even when the state is changed. Another experiment was conducted with garlic, which is easy to change its state.

Result & Discussion



Fig. 5 A clove of garlic with small cuts



Fig. 6 Heated grated garlic

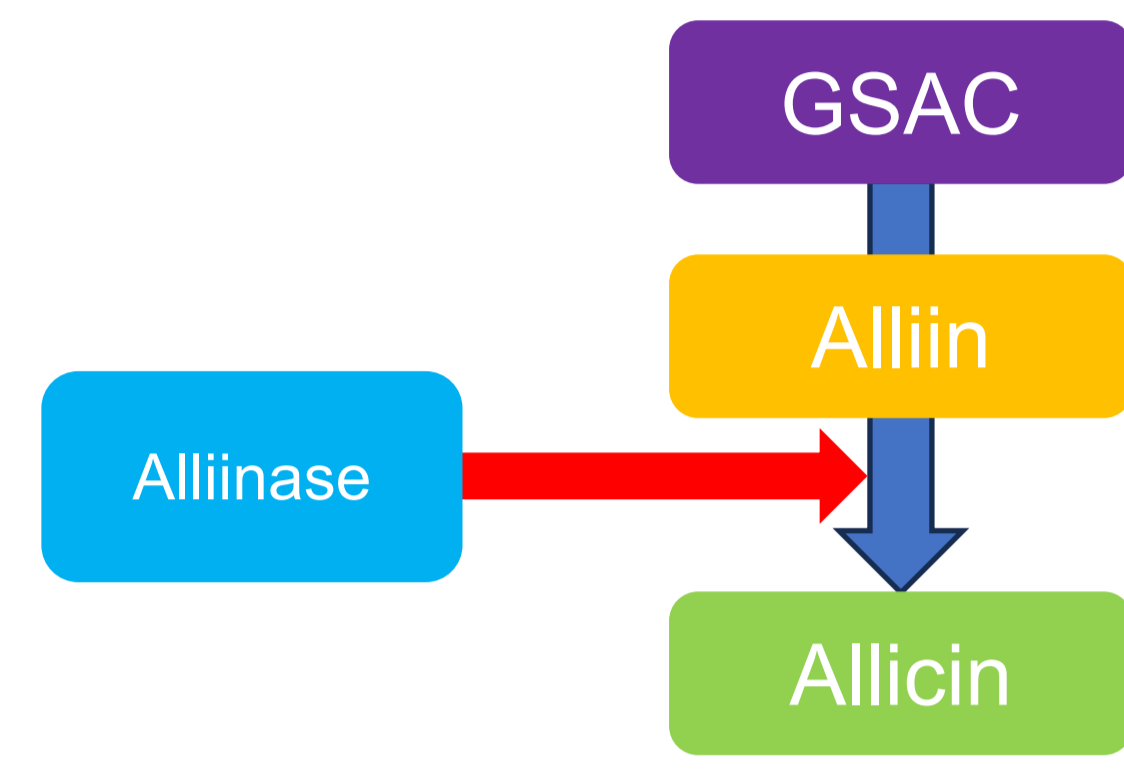


Fig. 7 Mechanism of garlic's anti-mold effect

After two weeks, the bread was found to be moldy, as was seen in Fig. 5 and Fig. 6. This is due to the mechanism of garlic's anti-mold effect. Garlic contains alliin, a "volatile antimicrobial ingredient" that is formed when alliin is activated by the alliinase enzyme, which is produced when garlic is grated. In other words, in Experiment 2, the cut garlic was not abraded, so alliin was not activated because alliinase was not generated, and the grated garlic that was heated was inactivated by the heat, so it lost its mold inhibitory effect.

Experiment 3

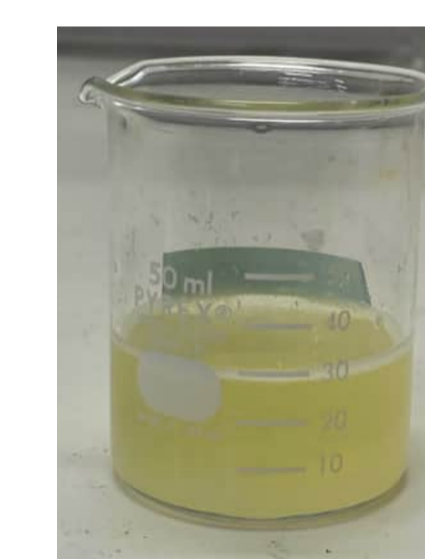
From the results of Experiment 2, it was found that alliin, a "volatile antimicrobial ingredient," was responsible for the inhibitory effect of garlic on mold, so an alliin solution was made.



Alliin solution



Alliinase enzyme solution



Alliin solution

Fig. 8 Produce of Alliin solution

To make the alliin solution, 450 g of American peeled garlic was dried, powdered, and stirred in 80% methanol for 24h×3. The extract was then concentrated under reduced pressure using a vacuum pump. For the alliinase enzyme solution, 10 g of garlic was ground, centrifuged repeatedly at 3000 rpm for 2 min, the supernatant was taken, the sediment was finely divided, adjusted to pH 4, and after the third centrifugation, 10 mL of PBS was dissolved in the sediment and 2 drops of toluene was added to keep bacteria out. The alliin solution was created using 0.5 mL of alliinase enzyme solution per 1 mL of alliin solution. The following experiment was conducted to confirm this.

Result & Discussion



Fig. 9 Garlic supernatant



Fig. 10 Alliin solution

After two weeks, the bread in the beaker with the alliin solution did not have a single mold growth (Fig. 10). It is thought that the garlic supernatant was one that contained little or no antimicrobial components.

Future Plan

Through previous experiments, it was found that alliin, a volatile antimicrobial ingredient in garlic, had an effect on the inhibition of mold growth. However, since all the experiments were conducted at room temperature (25°C), the thermostability of alliin has not been explored. Future research should elucidate the thermostability of antimicrobial activity in garlic using Ref.1 as a prior research.

References

◎Ref.1 樋口智之 [Higuchi Tomoyuki], 木村誠也 [Kimura Seiya]. (2021). 様々な温度で加熱した真空凍結乾燥ニンニクにおける抗菌活性の耐熱性に関する研究 [Heated at various temperatures. Study on the thermostability of antimicrobial activity in vacuum freeze-dried garlic], 日本冷凍空調学会論文集38巻3号 [Transactions of the Japan Society of Refrigerating and Air Conditioning Engineers, Vol. 38, No. 3], p243-249

◎Ref.2 津野貞子 [Tuno Sadako]. (1958). ニンニク属植物の栄養的価値に関する研究:(XVI) ニンニク属植物中のAlliinaseについて [Studies on the Nutritional Value of Garlic Plants: (XVI) Alliinase in Garlic Plants], ビタミン14巻 [Vitamins, vol. 14.], p659-664

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