Stable Method for the Recovery of γ-Poly glutamic Acid in Natto

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Abstract

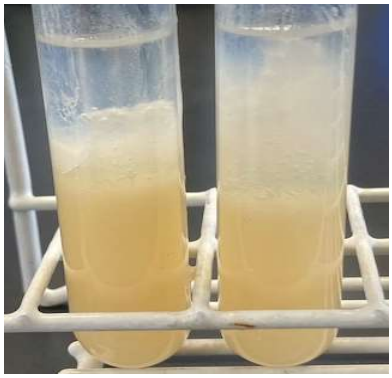
γ -Poly glutamic acid (PGA) is a viscose substance contained in natto, a traditional fermented food in Japan. It exhibits several chemical properties including water-solubility, cohesiveness, and biodegradability, rendering it useful for applications requiring these properties. However, extracting PGA is problematic. The amount of PGA that can be extracted is low, and it is unstable. The purpose of this study was to identify a stable and highly effective method for extracting a large amount of PGA.

We compared the respective PGA yields that were obtained through changing the number of times the natto was mixed as well as through changing the amount of ethanol that was added. The results clearly show that when the natto was mixed 300 times and ethanol was added to the PGA solution at a ratio of 4/5, favorable PGA yields were obtained.

Keywords: natto, γ-Poly glutamic acid (PGA), ethanol, biodegradability

Introduction

Natto is a traditional food in Japan consisting of fermented soy beans. Currently, several chemical properties of this food are researched. (Kondo,AITEC)Typically, it is overproduced, and surplus is discarded. γPGA is a viscose substance contained in natto. PGA exhibits several chemical properties including water-solubility, cohesiveness, and biodegradability, rendering it useful for applications requiring these properties. However, extracting PGA is problematic.



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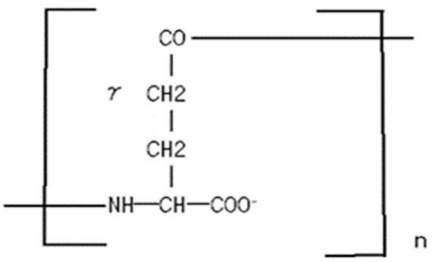


Figure 1. Structural formula of PGA.

Materials and Methods

Experiment

For all experiments, simple extraction methods were used to extract PGA.

Water was added to natto and the eluted PGA was extracted.

1. Experiment 1

1.1 Theory

We changed the amount of ethanol that was added. The more ethanol was added, the more soluble the solution became. As a result, the PGA yield increased. By contrast, if an insufficient amount of ethanol was added, PGA could not be extracted.

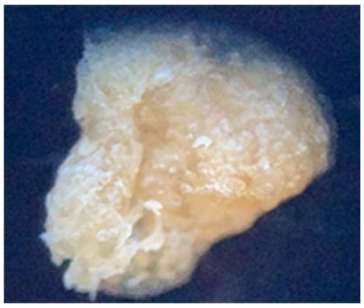


Figure 2. Recovered

1.2 Experimental operations

(1) Natto was mixed 200 times. Next, water was added to soak the mix. Then, it was strained using a gauze to remove the beans and produce a PGA solution.

(2) Next, 99.5% ethanol was added to 5 mL of the prepared PGA solution, and the water layer was separated from the ethanol layer. The amount of ethanol that was added was changed.

(3) The PGA separated on the boundary surface was extracted with a twisting motion using a glass rod.

The collected PGA was dried sufficiently and ground into a powder using a mortar and pestle.

1.3 Result

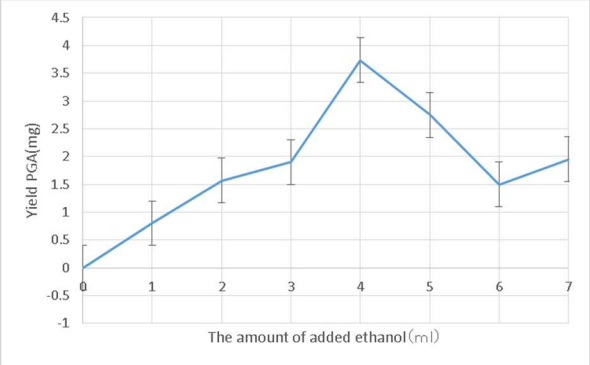


Figure 3. Relationship between the amount of added ethanol and yielded PGA.

Figure 3 shows the relationship between the amount of added ethanol and PGA yield.

The result clearly shows that when ethanol was added to the PGA solution at a rate of 4/5, the PGA yield was at a maximum.

The PGA yield decreased when 4 mL of ethanol were added.

2. Experiment 2

2.1 Theory

The number of times that the natto was mixed was changed. The more the natto was mixed, the more the viscosity increased. The viscosity is attributable to the glutamic acid. The experiment tested the hypothesis that the amount of extractable PGA would change depending on the number of times the natto was mixed.

2.2 Experimental operations

We used the method of Experiment 1.

2.3 Result

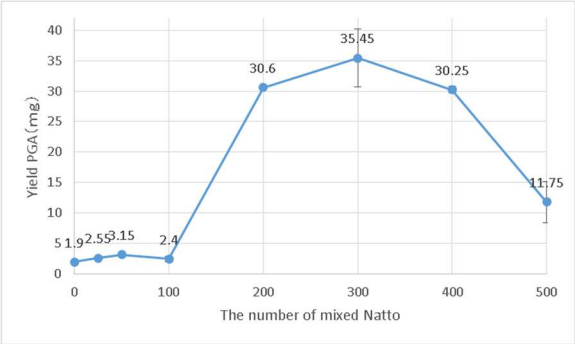


Figure 4. Relationship between the number of times natto was mixed and PGA yield.

Figure 4 shows the relationship between the number of times the natto was mixed and the PGA yield.

The result clearly shows that when the amount of ethanol was increased from 0 mL to 4 mL, the PGA yield increased. When 4 mL of ethanol were added, the PGA yield peaked at 3.7 mg. The PGA yield did not increase even if more ethanol was added.

3. Experiment 3

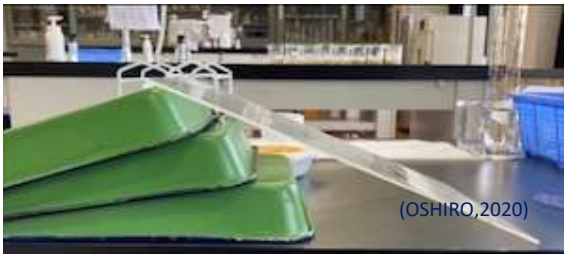
3.1 Theory

The results of experiment 2 show that the amount of extracted PGA depended on the number of times the natto was mixed.

Because the PGA is viscose, a correlation between the viscosity of the PGA solution and the number of times the natto was mixed is likely.

3.2 Experimental operations

First, the PGA solution was prepared according to Experiment 1.
Next, the PGA was placed on a customized slope (30° inclination), and the time it required to complete 20 cm was measured.
The viscosity of distilled water is 22.5 s.
Next, the correlation between the number of mixing procedures and the viscosity of the solution was analyzed.



PICTURE: Customized viscosity measuring device.

3.3 Result

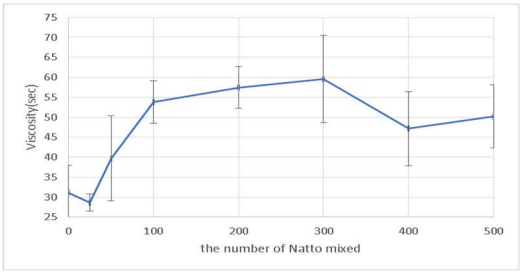


Figure 5. Relationship between viscosity and the number mixing procedures.

Figure 5 shows the relationship between viscosity and the number of mixing procedures of natto.

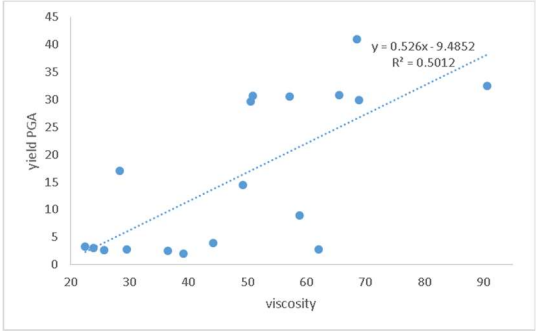


Figure 6. Correlation between viscosity and PGA yield

A strong correlation was identified between viscosity and PGA yield (correlation coefficient: 0.71).

Coefficient of determination was 0.50. So It is difficult to expect the PGA yield using this data.

4. Experiment 4

4.1 Theory

PGA has high water retention properties so it requires a lot of time to dry and powder it. The yield is also unstable.

If the approximate yield of PGA can be estimated on the basis of the characteristics of the solution, PGA can be recovered more efficiently.
To analyze the correlation between the amount of PGA yield and the turbidity of the solution, we used spectrophotometer.

4.2 Experimental operations

A spectrophotometer was used to determine the correlation between yield and turbidity of the solution. In addition, the absorbance of the PGA solution was measured.

4.3 Result

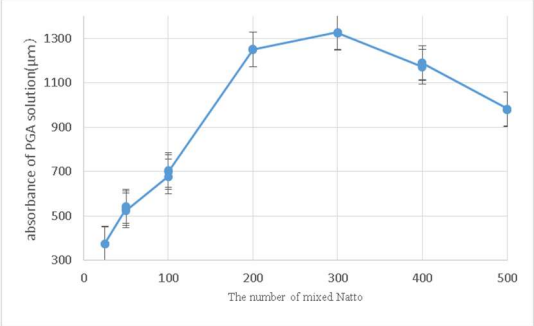


Figure 7. Relationship between absorbance of PGA solution and the number of mixing

Figure shows the relationship between the absorbance of the PGA solution and the number of mixing procedures.

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The graph has a similar shape to that of Figure 2.
This is the result of the correlation between yield and absorbance of the PGA solution.

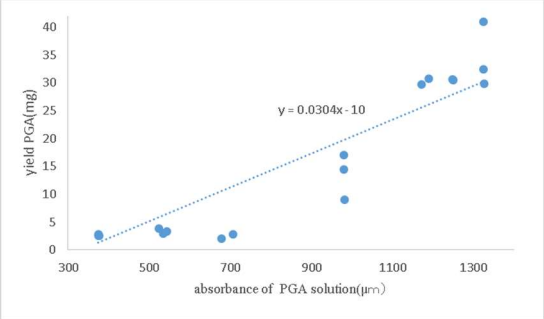

Figure 8. Correlation between the absorbance of the PGA solution and the number mixing procedures.

The correlation coefficient was 0.93 (0.932442), indicating a very strong correlation. These results suggest a very strong correlation between PGA yield and the absorbance of the PGA solution.

Conclusions

When ethanol was added to the PGS at a ratio of 4/5, the PGA yield was at a maximum.

This suggests that temperature and concentration of the solution may have had an effect. Several reasons explain a maximal PGA yield at 300 mixing procedures.

First, a layer of air formed between the beans; the PGA attached to the surface of the beans, making them more soluble in water and yielding a higher recovery. After mixing more than 400 times, the PGA bonds began to break, and the glutamic acid was released, resulting in a subsequent decrease in the amount of PGA recovered.

Second, although the PGA bond was broken by mixing the natto, the PGA bond might have been longer and altogether larger than the size of the mesh of the gauze. Therefore, the PGA could not have passed through the mesh well after only 0–100 times of mixing. In addition, after more than 400 times of mixing, many of the bonds could have been broken and the PGA might have been too fine to be extracted with the glass rod.

For the above reasons, the maximal yield of PGA was obtained when the natto was mixed 300 times.

There was positive correlation between the viscosity and PGA yield. In addition, there was a positive correlation between the amount of PGA recovered and absorbance. These results suggest the possibility that the amount of PGA powder that can be extracted can be estimated on the basis of the viscosity and turbidity (absorbance) of the solution.

$$r = \frac{\frac{1}{N}\sum_{i=1}^{N}(Xi-\overline{X})(Yi-\overline{Y})}{\sqrt{\frac{1}{N}\sum_{i=1}^{N}(Xi-\overline{X})^2}\sqrt{\frac{1}{N}\sum_{i=1}^{N}(Yi-\overline{Y})^2}}$$

Formula for calculating the correlation

Discussion

In future studies, we will examine the reasons for the maximal PGA recovery when ethanol is added to the PGA solution at a ratio of 4/5.

In addition, we will measure the purity of PGA powder and investigate cohesion.

References

[1] Masao Kondo, Acquirement and Analysis of Poly-gamma-glutamic Acid from Waste Materials of Food, Food Research Center, AITEC.

[2] Takeharu Tajima, Sachiko Sugigara, Sorption Properties of Metal Ions onto Poly-γ-glutamic Acid Nanofiber, Kyoto Institute of Technology, 2011.