原　著

Study on Physical Properties of Poly-γ-Glutamate as a Thickener for Food Use

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＜Abstract＞

Poly-γ-Glutamate (PGA) is a sticky polymer, and one of the principal components of ***natto***, a traditional Japanese food prepared from steamed soybeans by fermentation with ***Bacillus subtilis*** (natto). PGA is a legally recognized functional material for a Ca-sorbefacient in food as a specific health food because of its strong Ca-binding capacity. Also, PGA is utilized industrially as a humectant in solid soap and cosmetic products. Other than these properties, since PGA has various characteristics (such as a high water-absorption ability, a metal-absorbing ability, antifreeze activity, biodegradability and edibility), novel applications of PGA have been receiving interest. Therefore, in this study, the physical properties of PGA were investigated to see whether PGA could be applied as a novel thickener. As a result, this study showed that the thickening ability of PGA is lower than commercial thickeners, and the viscosity of PGA solution is unstable when affected by heat, common food ingredients, and pH. Therefore, PGA was judged to be difficult to use as a thickener for food use.

Keywords: poly-γ-glutamate, thickener

Poly-γ-Glutamate (PGA) was found at first in 1937 as a component of capsule for ***Bacillus anthracis***. PGA is allowed legally by the Ministry of Health, Labour and Welfare of Japanese government to use Ca-sorbefacient in food as the specific health food, since its Ca-binding capacity is demonstrated scientifically[1]. Also it is utilized industrially as a humectant in a solid soap, a toilet water and a cosmetic gel[2].

PGA is an unusual anionic polyisopeptide in which only glutamate is polymerized via γ-amide linkages. The sticky polymer is the principal component of natto, a traditional Japanese food prepared from steamed soybeans by fermentation with ***Bacillus subtilis*** (natto)[3]. The molecular weight of PGS is maximally 7000kDa[4]. PGA is a copolymer DL-PGA[5] with a high-molecular-mass L-glutamate-rich fragment (160-400 kDa in average) and with a low-molecular-mass fragment composed mostly of D-glutamate residues (5kDa in average)[6]. PGA is produced from glutamate within the cell with the membrane-binding enzyme system called as ***pgsBCA*** and the gene for the ***Bacillus*** is coded by ***pgsBCA***. The expression of the ***pgsBCA*** gene is regulated by the Quorum Sensing of ComP pheromone and the gene expresses during the final stage of a logarithmic growth phase and the initial stage of a stable growth phase[7].

PGA has various characteristics such as a highly water absorbing ability, a metal-absorbing ability and antifreeze-activity[8,9]. PGA is tasteless, orderless, biodegradable and edible[8]. Recent study suggested that PGA

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seems to protect baker's yeast from lethal freeze injury, leading to a high leavening ability after freezing and thawing[10]. Also, novel application of PGA have been calling for interest in a wide range of industrial use such as medicine, food, cosmetics, a humectant, a water purifier, a crazing inhibitor of the concrete, an earth-water preservation agent for desert tree planting, a dew condensation inhibitor, a solid soap and biomaterials[10-12].

So far, there has been no report on the application of PGA to a thickener for food use. Therefore, in this study, the physical properties of PGA were investigated to get knowledge whether PGA is possibly expected to a novel type of thickener. Thickening agents are world-widely utilized on various kinds of food, and its required properties differ in each food types. Thus, this study was focused on the possibility of the use to a wheat flour processed food. Incidentally, emulsifiability and ability for air bubble formation of PGA were determined, since it can not be denied that PGA may have those abilities for food use.

MATERIALS AND METHODS

PGA preparation

Bacillus subtilis (natto) FW1 (gift from Shiraishi Lab., Dept. of nutritional and health science, Faculty of human environmental science, Fukuoka Women's University) was used as a PGA producer. The culture procedure was followed by Fujii's method with a little modification[13]. Nutrient medium (glutamate medium) composition consisted with 2% sucrose, 4.5% sodium L-glutamate, 0.5% NaCl, 2.7% KH_2PO_4, 4.2% $Na_2HPO_4 \cdot 12H_2O$, 0.05% $MgSO_4 \cdot 12H_2O$, 0.1% biotin. Well water was essential to dissolve these nutrients for the effective production of PGA, though the reason is unclear. 15ml pre-culture was inoculated into 150ml glutamate medium. Main cultivation was conducted at 8000rpm for 7 days under 4℃ .

PGA purification was followed by Fujii's method with a little modification[14]. Culture fluid after the culture was centrifuged at 8000rpm for 40 min. under 4℃ to separate cells from the supernatant which was collected by decantation into a glass beaker. Ethanol of the twice as much volume as to the supernatant was added little by little into the beaker with mixing slowly with a glass stick, PGA coiled itself around the glass stick. This PGA was air dried until alcohol transpired with having coiled itself around the glass stick and dissolved this PGA in sterilization water again. The PGA solution was dialyzed to cold water with the dialysis tube (UCC cellulose tubing C-110, Shiraimatsu instruments Co. Ltd, Japan) until the OD_{280} of the outer membrane solution became under 0.1. The inner membrane solution was freeze dried and the dried sample was assumed as a purified authentic PGA.

Determination of the molecular weight of purified PGA

The purified sample was dissolved into an acetic acid buffer solution to become 0.1% concentration and HPLC analysis was performed to estimate the average molecular weight of a PGA sample at the experimental condition as follows, Instrument: Shimazu LC10AD, Column: Toso TSKgel G6000PWXL (7.8mmID × 30cm), Eluant: 1M acetic acid buffer solution, Flow rate: 1ml/min, Injection: 10μℓ, temperature 40℃, Detector: RI, Molecular weight standard: polyethylene oxide.

Determination of enzymatic activity

Total α-amylase activity and total protease activity were analyzed by photometric method respectively with the Mathewson and Pomeranz's method[15]and Kruger's method with no modification[16].

Determination of viscosity

Kinematic viscosity was detected with the canon fenske viscometer (Shibata science machine industry Co. Ltd, Tokyo, Japan). B type viscosity was measured with the VT-

04 viscometer (Ryon Co. Ltd, Tokyo, Japan) exactly 30 seconds after No.3 rotor started rotation in batter solution at 20℃ .

Preaparation of wheat flour batter

Wheat flour batter was prepared with the formulation of 100g of soft wheat flour, 1g of a thickener, 20ml of de-ionized water. These materials were mixed with 5Q baking mixer (Shinagawa machinery Co. Ltd, Tokyo, Japan) for 2 min. at low speed using a beater and the batter temperature was controlled to be accurately 20 ℃ after the batter mixing finished.

Determination of emulsifiability

Emulsifiability of PGA was determined according to the Pearce and Kinsella's method with a little modification[17]. 0.1% emulsifier and 1ml of essential rape oil were added to 3ml de-ionized water. And OD_{500} change of the homogenized solution for 1 min. was measured at time. Commercial emulsifiers were used as experimental standards.

Determination of ability for air bubble formation in wheat flour batter

The standard method of Riken Vitamin Co. Ltd (Tokyo, Japan) was applied to the determination of ability for air bubble formation in wheat flour batter. The experimental batter formulation was as follows, 100g of soft wheat flour, 100g of granular sugar, 100g of whole egg and 60ml of tap water. These materials were mixed with 5Q baking mixer (Shinagawa machinery Co. Ltd, Tokyo, Japan) for 1 min. at low speed followed by 5 min. mixing at high speed using a whipper and the batter temperature was controlled to be accurately 20℃ after the batter mixing finished. Specific gravity of the mixed batter dough indicates the ability of PGA for air bubble formation in wheat flour batter. Commercial air bubble agents were used as experimental standards.

Chemicals and food additives

All the chemicals used in this study were special grade for experimental use and obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All the wheat flours, wheat bran, barley rice bran and rice bran were obtained from Torigoe Flour Milling Co., Ltd. (Fukuoka, Japan). Commercial thickeners (xanthan gum and guar gum) were gifted from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). Commercial emulsifier, Sugar ester S770 was obtained from Amato Pharmaceutical Products, Ltd. (Kyoto, Japan), Emulsie KM-500 and Emaup KM-100 (both products are mono and di-glyceride) were obtained from Riken Vitamin Co., Ltd. (Tokyo, Japan). A commercial air bubble producer for cake production, Highmer was obtained from Kaneka Corporation (Tokyo, Japan).

RESULTS AND DISCUSSION

Determination of enzymatic activity of PGA

Protease and α-amylase give unfavorable influences on the qualities of wheat flour processed foods. Protease breaks down the gluten network which is essential to form basal structure of breads and noodles. Breads influenced by protease show lower loaf volume, sticky and heavy texture of the crumb. Amylase breaks down the normal starch structure, resulting in stiff texture in the bread crumb. In the case of noodles, fragile gluten network and starch structure lead to less elasticity and quicker loss of moderate viscoelasticity in texture. Therefore these enzymatic activities should be avoided in the production of wheat flour processed foods in which gluten formation or starch structure is generally essential for better quality in finished products. For this reason, α-amylase activity and total protease activity were analyzed on the purified PGA and the results were shown in Figure 1. The result showed that no α-amylase activity was seen in the purified PGA, but fairly higher protease

activity was observed. The degree of protease activity of the purified PGA is considered to be highly critical for the normal formation of gluten network, since the activity was much higher than wheat bran whose protease activity is well known to inhibit normal gluten formation. Protease activity must be removed in order to use PGA as a food additive.

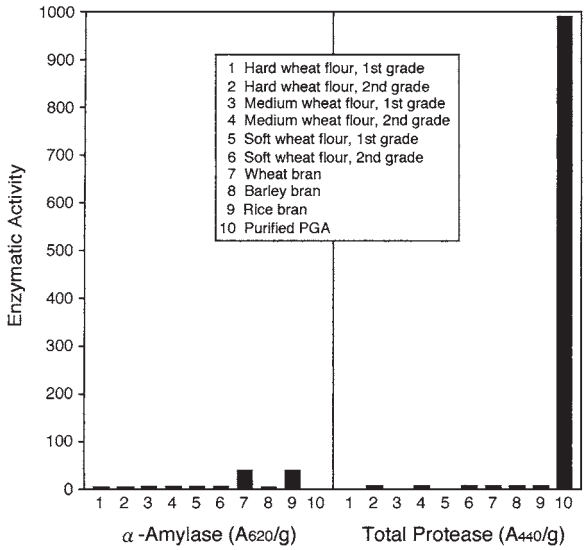

Figure 1. α-amylase activity and total protease activity for the purified PGA and several food standards. The numerical values of absorbance were shown as converted in food 1g.

Removal of protease activity from PGA by heat treatment

Heat treatment was examined to remove protease activity from the purified PGA. 5 mg /ml solution of the purified PGA was heat treated at 60, 70, 80℃ respectively for 5, 15, 30, 60 min. and the total protease activity for each sample was determined. Figure 2 shows the result. Protease activity could not be erased completely at 60℃ , but, could be removed entirely at the treatment of 70℃ for 30 min. and at the treatment of 80℃ for 5 min. It is reported that the molecular weight of PGA related to its viscosity and it did not change under 70℃ heat treatment[18]. Then, the molecular weight of the purified PGA was analyzed with the use of HPLC before and after the heat treatment at 70℃ for 30 min. And it is confirmed that the molecular weight did not change with the heat treatment showing molecular weight of 350 kDa. So, this condition of heat treatment is considered to be favorable to remove protease activity from the purified PGA without affecting its molecular weight, and also possibly without affecting the viscosity.

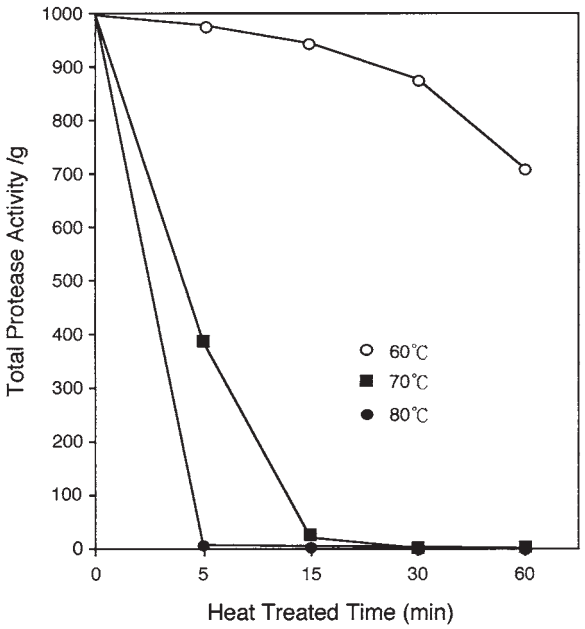

Figure 2. The effect of heat treatment on the protease activity of the purified PGA.

Effect of heat treatment on viscosity of the purified PGA solution

Viscous status of PGA solution is derived from the chemical cross-linkage among PGA molecules to some degree and the linkage is disrupted by γ-irradiation[19]. Although the heat treatment to PGA was very effective to erase the protease activity, it should be investigated that heat extinguishes the thickening ability of PGA. Then the effect of heat treatment was analyzed. The purified PGA was dissolved in de-ionized water to the concentration of 5 mg/ml, 1ml of the solution in a test tube was heat treated for 5 min. at various temperature and the kinematic viscosity was measured.

The result is shown in Figure 3. As shown, viscosity of PGA solution reduced as the treated temperature raised.

Then, the thickening ability and the viscosity stability of PGA to the wheat flour batter were compared with the commercial thickener (xanthan gum and guar gum) which are widely used in the food industry. A thickener was added to be 1 % concentration to the batter solution and the B type viscosity was measured at every ten minutes during a hour. The result is shown in Figure 4. There was no significant difference between heat treated and intact PGA, but its thickening ability and the viscosity stability were lower than the commercial thickener at the same concentration in the batter solution.

These findings suggest that PGA may not used at higher temperature in foods.

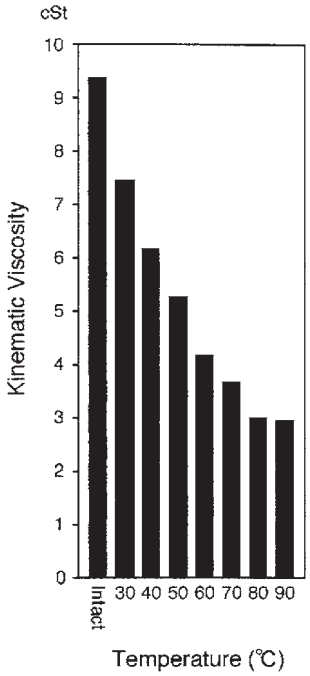

Figure 3. The effect of heat treatment to the purified PGA on its kinematic viscosity.

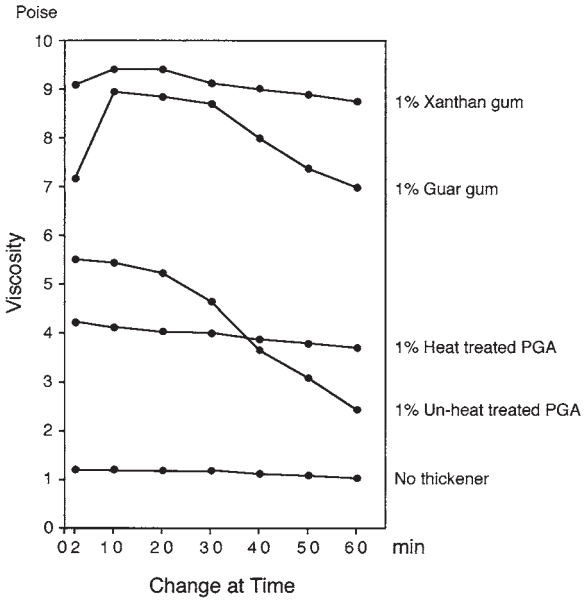

Figure 4. Comparison of the thickening ability and the viscosity stability of PGA with commercial thickening agents. Viscosity is B type value and heat treated PGA means treated PGA at 70℃ for 30min.

Effect of sugar, salt, acid and pH to viscosity of PGA

It is very important that essential properties of a food additive should not easily affected by the components in foods, since the food is comprised of a complicated mixed ingredient. Thus, the effect of representative food components such as sugar, salt, acid on the viscosity of PGA, and also the effect of pH was studied. Here, 5% sucrose, 2% salt, and 2% citric acid respectively were added to the wheat flour batter which was used to study the relation of heat treatment in this report, and pH was adjusted to pH 9 using 1N NaOH and to pH 5 using 1N HCl with the same batter. As shown in the result of Figure 5, viscosity expressed by PGA was unstable easily affected by food components and pH. These facts indicate that PGA is very limited to be used as a food ingredient.

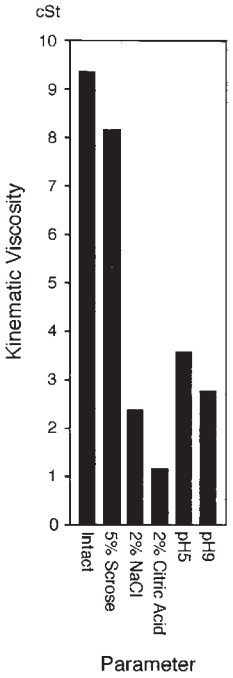

Figure 5. Effect of sugar, salt, acid and pH to viscosity of PGA batter solution.

Emulsifiability and ability for air bubble formation of PGA

The possibility that PGA may have the emulsifiability was studied and the result is shown in Figure 6. It was shown PGA has the reverse effect to emulsifiability. And PGA did not have the ability for air bubble formation as shown Figure 7.

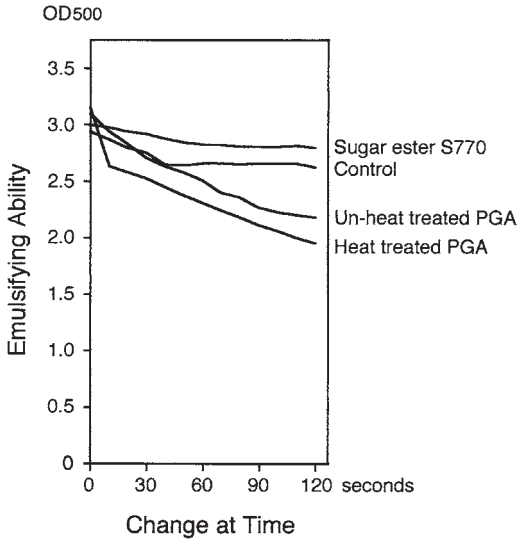

Figure 6. Comparison of emulsifying ability of PGA with commercial emulsifiers. Heat treated PGA means treated PGA at 70℃ for 30min.

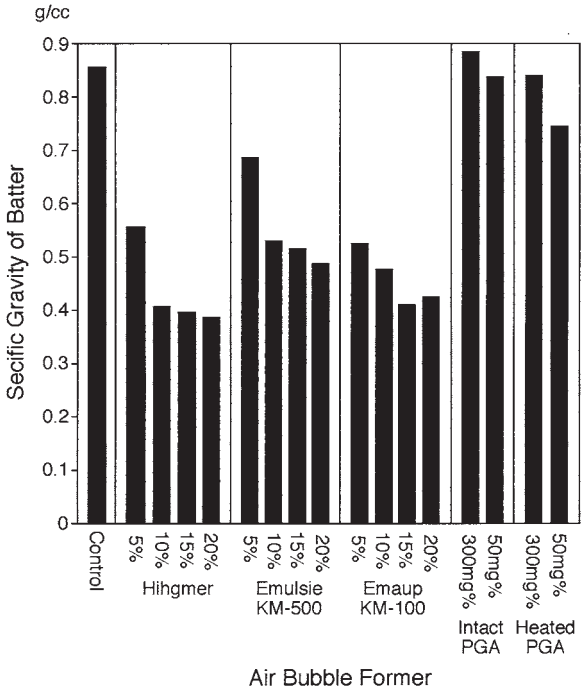

Figure 7. Comparison of the ability for air bubble formation of PGA with commercial air bubble formers. Heat treated PGA means treated PGA at 70 ℃ for 30min. Numerical value % means the concentration to the experimental batter dough.

CONCLUSION

The purified PGA prepared by a standard method showed several inferior characteristics as a thickener. Very high protease activity in PGA that gives problems to the quality in the finished food, has to be removed by heat treatment in order to be utilized as a food additive. The favorable condition for the heat treatment that won't reduce the PGA molecular weight was found and the molecular weight did not change before and after the heat treatment. Though this fact seemed to suggest viscosity of PGA solution won't change by the heat treatment, the experiment showed that the viscosity of PGA solution decreased according to the treatment temperature raises. PGA increased the wheat batter viscosity and stabilized the viscosity at least for one hour, but these abilities were lower than the commercial thickeners in the case of same amount use. The biggest problem was that

the viscosity of PGA solution decreased under the presence of sucrose, salt and citric acid. Moreover the viscosity decreased by higher and lower pH. Since food component is highly complicated, a food additive should not be affected by any ingredient and pH in food. When these facts are considered, PGA is difficult to use it as a thickener for food use.

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ポリ-γ-グルタミン酸の食用増粘剤としての物性に関する研究

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＜要　旨＞

ポリ-γ-グルタミン酸（PGA）は粘性のポリマーであり納豆の主成分のひとつである。納豆は蒸煮した大豆を*Bacillus subtilis* (natto)で発酵した日本の伝統食品である。PGAには強力なCa結合能があるため、Ca吸収促進剤として特定保健用食品の素材として認可されている。また、PGAは保湿剤として石鹸や化粧品類に産業的に利用されている。このような性質の他にも、PGAは吸水性が高く、金属吸着能があり、冷凍耐性や生分解性、可食性があるなど、さまざまな特性を有していることから、新たな用途開発に興味が持たれている。そこで本研究では、PGAが新規な食用増粘剤として利用できないか検討するために、その物性を調べることにした。その結果、PGAの増粘効果は市販の増粘剤よりも弱く、PGAを溶かした溶液の粘度は、熱や一般の食品材料、pHに影響されて不安定になることが分かった。つまり、PGAは食用増粘剤としての利用が困難であると思われた。

キーワード：ポリ－γ－グルタミン酸、増粘剤

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