

# Effect of Gamma Polyglutamic Acid( $\gamma$ -PGA) on the Processing Property of Seafood

林昭邦、柯文慶；謝昌衛

E-mail: 9708428@mail.dyu.edu.tw

## ABSTRACT

$\gamma$ -polyglutamic acid (  $\gamma$ -PGA) is a water soluble, edible and biodegradable substance and has been proven to be non-toxic to our environment and human body. Examples of food applications of  $\gamma$ -PGA and its derivatives have been very limited. Fresh tilapia has been used as the material in this research, and three pieces of fish meat have been taken from it. Based on the method of three piece filleting, tilapias were dissected into pieces and skinned fish steaks that the size of 3 (length)  $\times$  2 (width)  $\times$  1 (high). They are soaked in three different kinds of molecular weight  $\gamma$ -PGA solutions which are 0.5% of the Na form with high molecular weight(Na/HM), the Na form with low molecular weight(Na/LM) and the Na form with hydrogel (Na/Hy), and also immersed in sodium tripolyphosphate (P) and RO water (R) with the same concentration for comparison, in order to compare the influence of different types of  $\gamma$ -PGA solutions on the appearance of the fish meat and the change of its freshness during the storage. The results could be obtained as follows: The effect of glazes of various  $\gamma$ -PGA upon the storage stability and the quality of tilapia fillet and its associated storage deterioration are examined. Fresh tilapia fillets were glazed using RO water (R), sodium tripolyphosphate (P) and  $\gamma$ -PGA( Na/HM and Na/Hy ). Ice glazing enhanced the storage quality of the tilapia fillet as compared to an untreated sample. Some of the  $\gamma$ -PGA glazing treatments did provide some degree of protection, although some did not. The antioxidant activity of  $\gamma$ -PGA upon tilapia flesh related substantially to the  $\gamma$ -PGA species used for glazing.  $\gamma$ -PGA afforded better protection lipid oxidation, therefore they maintained a better quality of preserved tilapia fillet. The combination of a glazing treatment and the application of  $\gamma$ -PGA at a 0.5% concentration was able to greatly increase the storage quality of the frozen tilapia fillets. In the aspect of the surimi products, different forms of  $\gamma$ -PGA (Na/HM, Ca/HM, Ca/LM and Na/SM) in the sailfish surimi sold to the market have been adopted in the experiment. The result could be seen as the following: comparing different forms of  $\gamma$ -PGA with high molecular weight (above 1,000,000), the added form, Ca/HM  $\gamma$ -PGA, (with gel strength of 835 g  $\times$  mm) has better gel strength than 0.2% of the form of Na/HM (with gel strength of 680 g  $\times$  mm). Also, comparing the differences between the same Ca form of  $\gamma$ -PGA products with various molecular content added, the result shows that the form of Ca/HM (with gel strength of 830 g  $\times$  mm) has better gel strength than that of Ca/LM (with gel strength of 680 g  $\times$  mm). Observing from the above experiment, the high molecular  $\gamma$ -PGA is more helpful than the lower molecular  $\gamma$ -PGA to enhance the gel strength of surimi;  $\gamma$ -PGA of the Ca form is more helpful than that of the Na form to strengthen the gel strength of fish surimi. To understand the mechanism of surimi gel-forming of  $\gamma$ -PGA, Calcium lactate are added to surimi infusion to investigate the effects of gel strength, and water holding capacity. At the same concentration, Ca/HM  $\gamma$ -PGA produced 7 times gel strength formation as compared to control. Therefore, this result has a remarkable contribution to the promotion of adding  $\gamma$ -PGA to the fish processing products.

Keywords :  $\gamma$ -polyglutamic acid ; glazing ; surimi ; gel strength

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## REFERENCES

1. 邱萬敦。2002。漁獲物的保鮮與處理。第 95-98 頁。翠柏林企業股份公司。台中。台灣。
2. 行政院農委會漁業署。2006。中華民國台灣地區漁業年報。台北。台灣。
3. 行政院衛生署。1998。食字第 87032655 號公告修正。行政院衛生署。台北。台灣。
4. 牟敦剛、江昇榮，2000，幾丁質在生技產品-醫療、食品及環保上之應用。
5. 邵廣昭。1996。台灣常見魚介貝類圖說(下)-魚類。第 174-175 頁台灣省漁業局。
6. 吳熊清、邱思魁。1996。水產食品學。國立編譯館。台北。台灣。
7. 柯文慶 1993。高壓在食品加工上之應用。國立中興大學教材。
8. 柯文慶、張瑞、賴滋漢。2003。食品加工。第 33-35 頁。富林出版社。台灣。
9. 胡興華。1996。拓漁台灣。第 33-35 頁。行政院農委會漁業署。台北。台灣。
10. 張為憲。2001。食品化學。華香園出版社。台北。
11. 曾明義。2005。吳郭魚出口加工產業現況分析。國立台灣海洋大學水產養殖研究所碩士論文。基隆。台灣。
12. 劉蕙菁。2003。花腹鯖與虱目魚在不同溫度中生物胺及鮮度品質之變化。國立台灣海洋大學食品科學研究所碩士論文。基隆。台灣。
13. 蔡佳玲。2006。收穫後處理與包裝對海鱸、吳郭魚與鱸魚品質與 5 儲藏期限之影響。國立台灣海洋大學食品科學研究所碩士論文。基隆。台灣。
14. 蘇遠志，2003，納豆菌代謝產物的開發與應用。生物產業14(2):117-30。
15. An, H., Hartley, P. S., Fan, X. and Morrissey, M. T. 1995. Activity staining of pacific whiting (*Merluccius productus*) protease. *J. Food Sci.* 60:1228-1232.
16. AOAC. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washing, D.C. USA.
17. Asghar, A., Samejima, K. and Yasui, T. 1984. Functionality of muscle proteins in gelation mechanisms of structured meat products. *CRC Crit. Rev. Food Sci. Nutr.*22:27-32.
18. Autio, K., Kiesvaara, M., and Polvinen, K. 1989. Heat-induced gelation of minced rainbow trout (*Salmo gairdneri*): effect of pH, sodium chloride and setting. *J. Food Sci.* 54:805-808, 823.
19. Biliaderis, C. G. 1983. Differential scanning calorimetry in food research-A review. *Food Chem.* 10:239.265.
20. Bhattacharyya, D., Hestekin, J.A., Brushaber, P., Cullen, L.G., and Sikdar, S.K. 1998. Novel poly-glutamic acid functionalized microfiltration membranes for sorption of heavy metal at high capacity. *J. Membrane* 141: 121-135.
21. Boye, S. W. and Lanier, T.C. 1988. Effects of heat-stable alkaline protease activity of Atlantic menhaden (*Brevoortia tyrannus*) on surimi gels. *J. Food Sci.* 53:1340-1342, 1398.
22. Bramsnaes, F. 1981. Maintaining the quality of frozen foods during distribution. *Food Technol.* 35: 38.
23. Busconi, L., Folco, E. J. E., Martone, C. B. and Sanchez, J. J. 1989. Fish muscle cytoskeletal network: Its spatial organization and its degradation by an endogenous serine proteinase. *Arch. Biochem. Biophys.* 368:203-208.
24. Carballo, J., Cavestany, M. and Jimenez Colmenero, F. 1992. Rheological changes during thermal gelation of meat batters containing surimi from Alaska pollack (*Theragra chalcogramma*) or sardine (*Sardina pilchardus*). *J. Sci. Food Agric.* 59 (1), 117-122.
25. Chan, J. K., Gill, T. A., Thompson, J. W. and Singer, D. S. 1995. Herring surimi during low temperature setting, physicochemical and textural properties. *J. Food Sci.* 60:1248-1253.
26. Cheng, C. S., Hamann, D. D., Webb, N. B. and Sidwell, V. 1979. Effects of species and minces fish gel texture. *J. Food Sci.* 44:1087-1092.
27. Choi, H. J. and Kunioka, M. 1995. Preparation conditions and swelling equilibria of hydrogel prepared by irradiation from microbial poly (L-glutamic acid). *Radiat Phy. Chem.* 46: 175-179.
28. Chung, P., Jae-Chul, C., Yoon-Ho, C., Hisaaki, N., Kazuya, S., Terumi, H., Haruo, M., Tomomitsu, S., Kenji, S., Makoto, A. and Moon-Hee, S. 2005. Synthesis of super-high-molecular-weight poly-L-glutamic acid by *Bacillus subtilis* subsp. *Chungkookjang*. *J. Molecular*

Catalysis B: Enzymatic. 35 : 128 – 133. 29. Cromwick, A. M., Birrer, G. A. and Gross, R. A. 1996. Effect of pH and aeration on gamma poly(glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures. *Biotechnol Bioeng.* 50(2), 222-227. 30. Deng, J., Toledo, R. T. and Lillard, D. A. 1976. Effect of temperature and pH on protein-protein interaction in actomyosin solutions. *J. Food Sci.* 41: 273-277. 31. Fantasia, L. D. and Duran, A. P. 1969. Incidence of *Clostridium botulinum* type E in commercially and laboratory dressed white fish chugs. *Food Technol.* 23: 793. 32. Ferry, J. D. 1948. Protein gels. *Adv. Prot. Chem.* 3:1-78. 33. Fleming, S. E., Sosulski, R. W., Kilara, A. and Humbert, E. S. 1974. Viscosity and water absorption characteristics of slurries of sunflower and soybean flours, concentrates and isolates. *J. Food Sci.* 39:188-191. 34. Fukao, T., Ohyabu, S., Sawada, H., and Ohta, Y. 1998. Effects of addition of powdered protein to kamaboko on growth of *Pseudomonas fluorescens*. *Fisheries Sci.* 64:57-61. 35. Fukuda, Y., Tarakita, Z., Kawamura, M., Kakehata, K., and Arai, K. 1982. Denaturation of myofibrillar protein in chub mackerel. *Bull. Jap. Soc. Sci. Fish.* 48:1672-1676. 36. Gorman, B. M., Sofos, J. N., Morgan, J. B., Schmidt, G. R. and Smith, G. C. 1995. Evaluation of hard-trimming, various sanitizing agent and hot water spraying – washing as decontamination interventions for beef brisket adipose tissue. *J. Food Protect.* 58: 899-907. 37. Hermansson, A. M. 1978. Physico-chemical aspects of soy proteins structure formation. *J. Texture Studies* 9:33-41. 38. Hollender, R., Bender, F. G., Jenkins, R. K. and Black, C. L. 1993. Research note: Consumer evaluation of chicken treated with a trisodium phosphate application during processing. *Poultry Sci.* 72:755-759. 39. Ho-Nam C., Sang-Yup L, and In-Hwan D. 2001. Process for preparing gamma poly glutamic acid from high-viscous culture broth. US patent 2001/001634A1, 2001-08-23. 40. Honikel, K. O. 1987. The water binding of meat. *Fleischwirtsch.* 67(9): 1098-1100. 41. Ikeuchi, Y., Tanji, H., Kim, K. and Suzuki, A. 1992. Mechanism of heat-induced gelation of pressurized actomyosin: pressure-induced changes in actin and myosin in actomyosin. *J. Agric. Food Chem.* 40:1756-1761. 42. Ito, Y. 1996. Glutamic acid independent production of Poly ( -glutamic acid) by *Bacillus subtilis* TMA-4. *J. biosci Biotechnol Biochem.* 60(18): 1239-1242. 43. Jayasingh, P. and Cornforth, D. P. 2003. Comparison of antioxidant effects of milk mineral, butylated hydroxytoluene and sodium tripolyphosphate in raw and cooked ground pork. *Meat Sci.* 66:83-89. 44. Jiang, S. T., Wang, J. H. and Chen, C. S. 1991a. Purification and some properties of calpain II from tilapia muscle (*Tilapia nilotica* × *Tilapia aurea*). *J. Agric. Food Chem.* 39:237-241. 45. Johnson, L. N., Phillips, D. C. and Rupley, J. A. 1968. The activity of lysozyme: An Interim review of crystall ographic and chemical evidence. *Brookhaven Symp. Biol.* 21: 120~138. 46. Kamath, G. G., Lanier, T. C., Foegeding, E. A., and Hamann, D. D. 1992. Nondisulfide covalent cross-linking of myosin heavy chain in "setting" of Alaska pollock and Atlantic croaker surimi. *J. Food Biochem.* 16:151-172. 47. Katoh, N., Nozaki, H., Komatsu, L. and Arai, K. 1979. A new method for evaluation of the quality of frozen surimi from Alaska pollack relationship between myofibrillar ATPase activity and kamaboko forming ability of frozen surimi. *Bull. Japan. Soc. Sci. Fish.* 45: 1027-1032. 48. Ko, Y. H. and Gross, K. A. 1998. effect of glucose and glycerol on gamma poly(glutamic acid) formation by *Bacillus licheniformis* ATCC 9945A. *Biotechnol Bioeng.* 57(4): 430-437. 49. Kunioka, M. 1993. Properties of hydrogel prepared by irradiation in microbial poly( -gutamic acid) aqueous solution. *Kobunshi Ronbunshu.* 50:755-760. 50. Kutota, H., Nambu, Y., Takeda, H. and Endo, T. 1992. Poly-gamma- glutamic acid ester and shaped boby hereof. US Patent 5: 118,784. 51. Kutota, H., Nambu, Y. and Endo, T. 1993. Convenient and quantitative esterification of poly( -gutamic acid) produced by microorganism. *J. Polym. Sci. Part A Polym Chem.* 31: 2877-2878. 52. Labuza, T. P. 1985. An integrated approach to food chemistry. In " Food Chemistry ", Fennema, O. R. Ed., p. 766-772. Dekker, New York. 53. Lanier, T. C., Lin, T. S., Hamann, D. D. and Thomas, F. B. 1981. Effects of alkaline protease in minced fish on texture of heat-processed gels. *J. Food Sci.* 46:1643-1645. 54. Lin, T. S. and Lanier, T. C. 1989. Properties of an alkaline protease from the skeletal muscle of Atlantic croaker. *J. Food Biochem.* 4:17-28. 55. Liu, Y. M., Lin, T. S., and Lanier, T. C. 1982. Thermal denaturation and aggregation of actomyosin from Atlantic croaker. *J. Food Sci.* 47: 1916-1920. 56. Makinodan, Y., Toyohara, H. and Niwa, E. 1985. Implication of muscle alkaline proteinase in the textural degradation of fish meat gels. *J. Food Sci.* 50:1351-1355. 57. Makinodan, Y., Yokoyama, Y., Kinoshita, M. and Toyohara, H. 1987. Characterization of an alkaline proteinase of fish muscle. *Comp. Biochem. Physiol.* 87B:1041-1046. 58. Martone, C. B., Busconi, L., Folco, E. J. and Sanchez, J. J. 1991. Detection of a trypsin-like serine protease and its endogenous inhibitor in hake skeletal muscle. *Arch. Biochem. Biophys.* 289:1-5. 59. Matsuda, Y. 1979. Influence of sucrose on the protein denaturation of lyophilized carp myofibrils during storage. *Bull. Jap. Soc. Sci. Fish.* 45: 573-579. 60. Matsukawa, M., Sato, R., Kimura, S. and Arai, K. I. 1998. Interacting mechanism of sodium pyrophosphate with gel forming ability of NaCl-ground meat from walleye pollack. *Nippon Suisan Gakkaishi* 64:1034-1045. 61. Ogawa, M., Tamiya, T., and Tsuchiya, T. 1995. Thermal stability of -helical structure of fish myosin rods. *Comp. Biochem. Physiol.* 110B:367-370. 62. Ogawa, Y., Yamaguchi, F. and Yuasa, K. 1997. Effect production of gamma polyglutamic acid by *Bacillus Subtilis* (natto) injar fermenters. *Biosci Biochem.* 61(10): 1684-1687. 63. Oguni, M., Inoue, N., Ohi, K. and Shinano, H. 1987. Denaturation of crap myosin B during frozen and super cooled storage at -8 . *Nippon Suisan Gakkaishi.* 53: 789-794. 64. Orawan, J., Soottawat, B. and Wonnop, V. 2006. Effect of phosphate compounds on gel-forming ability of surimi from bigeye snapper (*Priacanthus tayenus*). *F. Hydrocolloids* 20: 1153 – 1163. 65. Otani, Y. and Tabata, Y. 1996. Rapidly curable biological glue composed of gelatin and poly(L-glutamic acid). *Biomaterials.* 17: 1387-1391. 66. Owusu-Anshah, Y. O. and Hultin, H. O. 1986. Chemical and physical changes in red hake fillets during frozen storage. *J. Food. Sci.* 51: 1402-1406. 67. Perez-Camero, G., Congregado, F., and Bou, J. 1999. Biosynthesis and ultra- sonic degradation of bacterial poly -glutamic acid. *Biotechnol. bioeng.* 63: 110-115. 68. Perez-villarreal, B. and Pozo, R. 1990. Chemical composition and ice spoilage of albacore (*Thunnus alalunga*). *J. Food. Sci.* 55: 678. 69. Post, L. S., Lee, D. A., Solberg, M., Furgang, D., Specchio, J. and Garham, C. 1985. Development of botulinal toxin and sensory deterioration during storage of vacuum and modified atmosphere packaged fish fillets. *J. Food. Sci.* 50: 990. 70. Price, R. J., Melvin, E. F. and Bell, J. W. 1991. Postmortem changes in chilled round, bled and dessed albacoe. *J. Food. Sci.* 56:318-321. 71. Ritchie, S. M. C., Bachas, L. G., Olin, T., Sikdar, S. K. and Bhattacharyya, D. 1999. Surface modification of silica and cellulose-based

microfiltration membranes with functional polyamino acids for heavy metal sorption. *Langmuir*. 15:6346-6357. 72. Roussel, H. and Cheftel, J. C. 1990. Mechanisms of gelation of sardine proteins: influence of thermal processing and of various additives on the texture and protein solubility of kamaboko gels. *Inter. J. Food Sci. Technol.* 25:260-280. 73. Saeki, H., Iseya, Z., Sugiura, S. and Seki, N. 1995. Gel forming characteristics of frozen surimi from chum salmon in the presence of protease inhibitors. *J. Food Sci.* 60:917-921, 928. 74. Saito, T. and Arai, K. 1959. A new method for estimating the freshness of fish. *Bull. Jap. Soc. Sci. Fish.* 24: 749~750. 75. Sanda, F., Fujiyama, T. and Endo, T. 2001. Chemical synthesis of polygamma glutamic acid by polycondensation of gamma glutamic acid methylester. *J. Polym Science Part A-1.* 39(5): 732-741. 76. Sano, T., Ohno, T., Otsuka-Fuchino, H., Matsumoto, J. J. and Tsuchiya, T. 1994. Carp natural actomyosin: thermal denaturation mechanism. *J. Food Sci.* 59: 1002-1008. 77. Sathivel, S. 2005. Chitosan and Protein Coatings Affect Yield, Moisture Loss, and Lipid Oxidation of Pink Salmon (*Oncorhynchus gorbuscha*) Fillets During Frozen Storage. *J. Food. Sci.* 70:455-459. 78. Scannell, A. G., Ross, R. P. and Hill, C. 2000. An effective lacticin biopreservative in fresh pork sausage. *J. Food Port.* 63(3): 370-375. 79. Shih, I. L. and Van, Y. T. 2001. The production of poly-( $\gamma$ -glutamic acid) from microorganisms and its various applications. *Bioresource Technology.* 79: 207-225. 80. Shih, I. L., Van, Y. T., Yeh, L. C., Lin, H. G. and Chang, Y. N. 2001. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. *Bioresour Technol.* 78: 267-271. 81. Shyu, Y. S., Hwang, J. Y. and Hsu, C. K. 2008. Improving the rheological and thermal properties of wheat dough by the addition of  $\gamma$ -polyglutamic acid. *LWT* 41 : 982 – 987. 82. Siger, J. W., De, V. P., Bhatt, R. 2000. Conjugation of camptothecin to poly(L-glutamic acid). *Ann NY Acad Sci.* 922: 136-150. 83. Stanley, D. W. 1983. Relation of structure to physical properties of animal material. In "Physical Properties of Foods." M. Peleg, and EB. Bagley(Ed). p.157-206. AVI Publishing Company Inc. Westport, CT. USA. 84. Stoknes, I., Rustad, T. and Mohr, V. 1993. Comparative studies of the proteolytic activity of tissue extracts from cod (*Gadus morhua*) and herring (*Clupea harengus*). *Comp. Biochem. Physiol.* 106B:613-619. 85. Su, H., Lin, T. S. and Lanier, T. C. 1981. Investigation into potential sources of heat-stable alkaline protease in mechanically separated Atlantic croaker (*Micropogon undulates*) *J. Food Sci.* 46:1654-1656,1664. 86. Taguchi, T., Ishizaka, H., Tanaka, M., Nagashima, Y. and Amano, K. 1987. Protein-protein interaction of fish myosin fragments. *J. Food Sci.* 52:1103-1104. 87. Tanford, C. 1968. Protein denaturation. *Adv. Prot. Chem.* 23:121-129. 88. Tanimoto, H. H., Kuuraishi, C., Kido, K. and Seguto, K. 1995. High absorption mineral-containing composition and food. US patent US Patent US5447: 732. 89. Toyohara, H. and Shimizu, Y. 1998. Relation between the modori phenomenon and myosin heavy chain breakdown in threadfin-bream gel. *Agric. Biol. Chem.* 52:255-257. 90. Tsukamasa, Y., Sato, K., Shimizu, Y., Imai, C., Sugiyama, M., Minegishi, Y. and Kawabata, M. 1993.  $\gamma$ -( $\gamma$ -glutamyl)lysine crosslink formation in sardine myofibril sol during setting at 25 °C. *J. Food Sci.* 58:785-787. 91. Watabe, S., Kamal, M. and Hashimoto, K. 1991. Postmortem changes in ATP, creatine phosphate, and lactate in sardine muscle. *J. Food. Sci.* 56: 151-153. 92. Yamanaka, S. 1991. New gamma-polyglutamic acid, production therefore and drinking agent containing the same. JP Patent 3047087. 93. Yokoi, H., Natsuda, O., Hirose, J., Hayashi, S. and Takasaki, Y. 2001. Characteristics of a biopolymer flocculant produced by *Bacillus* sp. PY-90. *J. Ferment Bioeng.* 79: 378-380. 94. Zhang, Y., Lu, H. and Levin, R. E. 2003. Enhanced storage-life of fresh haddock fillets with stabilized sodium chlorite in ice. *Food Microbiology.* 20: 87-90.