

Effects of Glycinin Basic Polypeptide on Sensory and Physicochemical Properties of Chilled Pork

Ying-Qiu Li*, Man Hao, Jie Yang, and Hai-Zhen Mo¹

School of Food Science and Engineering, Qilu University of Technology, No. 3501 University Road of Changqing District, 250353, Jinan, Shandong Province, China

¹School of Food Science, Henan Institute of Science and Technology, Xinxiang, 453003, China

Received September 21, 2015
Revised January 9, 2016
Accepted January 21, 2016
Published online June 30, 2016

*Corresponding Author
Tel: +86-531-89631195
Fax: +86-531-89631195
E-mail: lyq@qlu.edu.cn; sdililyq@126.com

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Effects of glycinin basic polypeptide (GBP) on sensory and physicochemical properties of pork during chilled storage were investigated. Pork treated with GBP was analyzed periodically for sensory properties, pH, total volatile base nitrogen (TVB-N), α -thiobarbituric acid (TBA), and total viable count (TVC) values. Compared with controls, TBA values of pork treated with GBP did not change. TVB-N, pH, and TVC values of pork showed reductions with increasing concentrations of GBP during 8 days of storage. However, there were increases in sensory scores. TVC values of treated pork showed a positive linear relationship with both pH and TVB-N values. GBP at 0.16 and 0.20% efficiently inhibited bacterial growth, and enhanced chilled pork sensory scores. Therefore, GBP has potential as a pork biological preservative for extension of shelf life during chilled storage.

Keywords: glycinin basic polypeptide, chilled pork, sensory properties, physicochemical properties, biological preservative

Introduction

Generally, meat and meat products are highly vulnerable to microbial contamination on account of a high water activity, high protein content, and relatively large content of free amino acids (1,2). In order to maintain good meat and meat products quality and extend the shelf life during storage, preservatives and antioxidants such as nitrites, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) have been widely used in meat products (3). However, these chemical and synthetic preservatives are harmful to human health due to potential toxicity if consumed over a prolonged period. Thus, there are increasing demands for natural preservatives in meat products (4-6).

Glycinin basic polypeptide (GBP), a basic subunit of soybean glycinin stemming from natural soybeans, is a cationic peptide with a molecular weight of approximate 20 kDa that is linked to the acid subunit of soybean glycinin by a single disulfide bond. GBP is a white powder lacking both color and odor with a low solubility in water and hydro-phobicity. GBP has a cationic nature that can attack the bacterial cell wall membrane (7-11). Sitohy *et al.* (11) showed that GBP exhibited antibacterial activities against *Listeria monocytogenes*, *Bacillus subtilis*, and *Salmonella enteritidis* at a minimum inhibitory concentration of 50 μ g/mL. Moreover, GBP inhibited propagation of *Listeria mono-cytogenes*, *Salmonella enteritidis*, and *Bacillus subtilis* inoculated into pasteurized milk after 16-20 days of storage at 4°C

(11). Sitohy *et al.* (11) also reported that the antibacterial mechanism of GBP might be from the reaction with the bacterial cell wall membrane due to a cationic and hydrophobic nature. Li *et al.* (12) reported that GBP interacted with the cell surface of *Escherichia coli* by electrostatic attraction, then changed the morphology and damaged the cell membrane structure of *E. coli* cells, leading to membrane permeability and, eventually, cell death. Therefore, GBP can be used as a substitute or supplement for synthetic preservatives in meat and meat products due to bacteriostasis.

Sensory properties, pH, α -thiobarbituric acid (TBA), total volatile base nitrogen (TVB-N), and total viable count (TVC) values of meat and meat products have been used for evaluation of pork quality (13). Lipid oxidation is a critical factor in food deterioration. The α -thiobarbituric acid (TBA) value is widely used as index of lipid oxidation based on measurement of the malonaldehyde content. The TBA value increases with a rising degree of lipid oxidation (14-16). The TVB-N value, a parameter that quantifies compounds composed of ammonia and primary, secondary, and tertiary amines, is widely used as an indicator of muscle tissue deterioration (3). An increase in TVB-N values is connected with activities of spoilage bacteria and endogenous enzymes. During storage, pork deterioration occurs due to growth and reproduction of spoilage bacteria, which contribute to degradation of pork proteins into nitrogenous alkaline autolysis compounds and production of bacterial metabolites (3,16,17). Thus, both pH and TVB-N values increase.

The relationship of TBC, pH, and TVB-N values is relevant. Li *et al.* (18) measured pH, TVB-N, TBA, and TBC values of pork for study of the preservation effect of ϵ -PL on the shelf life of chilled pork. Duan *et al.* (2) reported preservative characteristics of a compound antiseptic for improvement of physicochemical properties of fresh lingcod fillets based on observation of changes in pH, TBA, and TVC values of lingcod. Wang *et al.* (19) determined pH, TVB-N, and TVC values of chilled pork for evaluation of the suitability of extracted housefly pupae peptides for prolongation of shelf life. In addition, Mahgoub *et al.* (20) showed that methylated soybean proteins counteracted re-contamination and extended the shelf life of pasteurized milk during cold storage based on analysis of pH and TVC values. Rodríguez-Carpena *et al.* (21) indicated that avocado by-products could be used as outstanding inhibitors of color deterioration, lipid oxidation, and protein denaturation during cold storage of raw porcine patties based on investigation of changes in TBA values.

No reports are known regarding effects of GBP on qualities and physicochemical properties of chilled pork. The main purpose of this study was determination of indices of physicochemical sensory properties, pH, TVB-N, TBA values, microbiological characteristics of TVC values for chilled pork treated with GBP, and evaluation of preservative and antimicrobial effects of GBP in chilled pork during storage.

Material and Methods

Preparation and purification of GBP and treatment of chilled pork

Defatted soybean flakes purchased from Scents Holding Co., Ltd., (Jinan, China), were ground using an attritor (JP-300A-8; Jiupin Industry and Trade Co., Ltd., Yongkang, China) for passage through a 1 mm sieve and obtained powder was used for isolation of glycinin following the method reported by Nagano *et al.* (22). Glycinin was dissolved in 30 mM Tris buffer (pH=8.0) containing 15 mM β -mercaptoethanol to a final concentration of 2% (w/v). The protein solution was heated by a water bath kettle (DK-8D; Jinyi Instrument Technology Co., Ltd., Jiangsu, China) to 90°C for 30 min, then centrifuged using a high-speed refrigerated centrifuge (TGL-16/TGL16; Xiangyi Instrument Co., Ltd., Changsha, China) at 11,000 \times g at 4°C for 15 min. The precipitate of crude GBP was washed thrice with a small amount of 30 mM Tris buffer (pH=8.0) dispersed in distilled water, then freeze-dried by a lyophilizer (DS-0.7HTF; Dingsheng Machinery Co., Ltd., Shanghai, China). Crude GBP was dissolved in 10 mM phosphate buffer (pH=7.2) to a final concentration of 1 mg/mL, then eluted using the same phosphate buffer (pH=7.2) on a Sephadex G-150 apparatus (2 \times 35 cm) (Shanghai Troody Analytical Instruments Co., Ltd., Shanghai, China) at 2 mL/min. The eluent containing purified GBP was collected and freeze-dried, then ground into a powder using an attritor (JP-300A-8; Jiupin Industry and Trade Co., Ltd.) for subsequent experimentation.

Chilled pork was procured from a local supermarket in Jinan, China

in June of 2014 and cut into uniform 1 \times 1 \times 1 cm blocks with an average weight of approximately 5 g, and blocks were evenly divided into 6 groups. Blocks of each group were dipped into 0, 0.04, 0.08, 0.12, 0.16, and 0.20% GBP dispersion liquids for 1 min, then drained for 1 min on a sterile stainless steel mesh screen. Blocks were then packed in sterile plastic bags and stored at 4°C for 8 days. Block samples were taken from each package and minced using a mincer (JYS-A850; Joyoung Co., Ltd., Jinan, China) before sensory properties, pH, TVB-N, TBA, and TVC values were determined at regular intervals.

Sensory Evaluation Chilled pork treated with different GBP concentrations for 8 days was subjected to consumer acceptability testing. Seventy-two consumer panelists from the staff and student body, both undergraduate and graduate, of Qilu University of Technology consisting of 35 females and 37 males of 22-35 years of age were recruited. Chilled pork treated with 0, 0.04, 0.08, 0.12, 0.16, and 0.20% GBP was baked in a microwave oven for 4 min at 700 W and the same amount of pork per treatment was immediately served to panelists, who were provided with fresh water for palate cleansing and removal of residual flavors between pork samples. Panelists evaluated appearance, odor, texture, taste, and overall acceptability of chilled pork using a 9 point hedonic scale of 1=extremely dislike, 2=dislike very much, 3=dislike, 4 = slightly dislike, 5=neither like nor dislike, 6=a bit like, 7=like, 8=like very much, 9=extremely like (23).

Determination of pH For pH measurement, as described by Duan *et al.* (2) and Souza *et al.* (16) with slight modification, 5 g of chilled pork was homogenized in 95 mL of distilled water using an homogenizer (Stomacher 80; Seward Medical, Worthing, UK) and the homogenate was filtered through 4.5 μ m filter paper (Whatman International Co., Ltd., Maidstone, England). The filtrate pH was measured using a Sartorius PB-10 digital pH meter (Sartorius AG, Gottingen, Germany).

Determination of total volatile basic nitrogen (TVB-N) contents

TVB-N values of chilled pork were determined following a modified method of Goulas and Kontominas (24). Five g of chilled pork was placed into a conical flask with addition of 50 mL of distilled water. After shaking for 30 min by a constant temperature shaker (THZ-103B; Yiheng Co., Ltd., Shanghai, China) at 150 r/min, the mixture was filtered through 4.5 μ m filter paper (Whatman) and the filtrate was stored at 4°C. A 5 mL aliquot of an absorbing solution of 20 g/mL boric acid (Lanyu Reagent Co., Ltd., Jinan, China) and 3 to 4 drops of a mixed indicator solution of 2 g/L methyl red-ethanol (Lanyu Reagent Co., Ltd.) and 1 g/L methylene blue (Lanyu Reagent Co., Ltd.) were placed into a conical flask and blended evenly by shaking. The conical flask was then placed at the bottom of the condenser of a distillation device. A 2 mL aliquot of the previous filtrate was mixed evenly with an equal volume of a 10 g/L magnesia suspension, followed by distillation for 5 min in the reaction chamber of the distillation device (FSZ2-1000; Yuecheng Drug Chemical Equipment Co., Ltd., Changzhou,

China). The resulting distillate was dropped into the previous conical flask, then the mixture in the flask was titrated using 0.01 M hydrochloric acid (HCl). A reagent blank was analyzed simultaneously. TVB-N contents expressed as mg/100 g in samples were calculated as:

$$\text{TVB-N (mg/100 g)} = \frac{(V_1 - V_2) \times c \times 14}{m \times 0.05} \times 100$$

where V_1 =HCl volume of samples in mL; V_2 =HCl volume of a reagent blank in mL, c =concentration of HCl (M), and m =samples weigh (g).

Determination of 2-thiobarbituric acid (TBA) values TBA values as malonaldehyde were determined following the method of Souza *et al.* (16) with modification. Four hundred mg of chilled pork along with a 2 mL aliquot of 1-butanol (AR) was placed into a 50 mL volumetric flask, then distilled water was added up to 50 mL with mixing by shaking. A 5 mL aliquot of the resulting mixture and 5 mL of TBA reagent prepared via dissolution of 200 mg of TBA in 100 mL of 1-butanol followed by filtration through filter paper were pipetted into a dry, stoppered test tube, which was then placed in a water bath at 95°C for 120 min, followed by chilling in a refrigerator (DW-86L058; Dawei Science Instrument Co., Ltd., Hangzhou, China). Absorbance (A_s) values of samples were measured at 530 nm using a spectrophotometer (UV1902PC; Aucy Technology Instrument Co., Ltd., Shanghai, China). The absorbance value of a mixture of 5 mL of sterile water with 5 mL of TBA reagent (A_b) was expressed as mg of malonaldehyde per kg of meat, and calculated as:

$$\text{TBA (mg/kg)} = \frac{50 \times (A_s - A_b)}{200}$$

Microbiological analysis TVC values of minced pork were measured following modification of the method of Ojagh *et al.* (25). Five g of chilled pork was transferred to a 100 mL beaker and 95 mL of sterilized peptone water was added. The mixture was homogenized for 2 min using a homogenizer (Stomacher 80; Seward Medical). Then, plate count agar consisting of 0.5 g of tryptone, 0.25 g of yeast extract, 1 g of glucose, and 1.5 g of agar dissolved in 100 mL of distilled water and Petri dishes were prepared and autoclaved using a pressure steam sterilizer (LDZH-100KBS; Shenan Medical Instrument Factory, Shanghai, China) at 121°C for 22 min. A 0.1 mL aliquot of serial dilutions of homogenates was spread on the surface of solidified medium in Petri dishes and incubated in an incubator (LRH-70F; Silan Instrument Co., Ltd., Shanghai, China) at 37°C for 10 days for determination of total numbers of bacteria on plate count agar, which were counted at regular intervals of 0, 2, 4, 6, 8, and 10 days after incubation. Values were transformed into logarithms of numbers of colony-forming units (CFU/g).

Statistical analysis All experiments were performed in triplicate. Mean values and standard errors of triplicate data were reported. An analysis of variance (ANOVA) was performed using SPSS17.0

software (SPSS Inc., Chicago, IL, USA). Graphs were constructed using Excel 2003 (Microsoft Office Excel 2003 for Windows, Microsoft, Redmond, WA, USA). The ANOVA was used for determination of significance of treatments. Duncan's method was used for multiple comparisons. Significance was defined at $p < 0.05$.

Results and Discussion

Effect of GBP on sensory properties of chilled pork Sensory properties of chilled pork treated with different concentrations GBP during 8 storage days at 4°C are shown in Fig. 1. Pork would not be accepted by consumers until the sensory score of overall acceptability was greater than 4.0 (4=slightly dislike). All sensory scores displayed a downward trend with storage time (Fig. 1). Moreover, there was a significant ($p < 0.05$) decline in all sensory scores for control pork samples with increasing storage time when compared with treatment samples. On the fourth day, control pork samples received an average sensory score of 4.0, indicating forthcoming deterioration. Control pork samples were evaluated as putrefied with an average overall acceptability score of 2.2 (2=dislike very much, 3=dislike) on day 6. Sensory scores of pork samples treated with 0.04, 0.08, and 0.12% GBP were approximately 4.4 on day 6, that were similar to controls on day 4.0. However, on day 6, pork samples treated 0.16 and 0.20% GBP exhibited average scores of 7.1, an acceptable evaluation that was a significant ($p < 0.05$) increase, compared with controls with average scores of 2.3. All sensory properties, including appearance, texture, odor, taste, and overall acceptability of chilled pork samples received unacceptable scores on day 8 with all scores lower than 4 (slightly dislike). Thus, GBP improved sensory properties of meat during chilled storage. Moreover, 0.16 and 0.20% GBP resulted in a significant ($p < 0.05$) preservative effect for chilled pork on day 6 of storage, compared with controls. No significant ($p > 0.05$) differences between 0.16 and 0.20% GBP treated pork samples in sensory scores were identified. Therefore, 0.16% GBP was the optimal treatment concentration with an overall acceptability score of 7.0 (like) on day 6 of storage.

Similar results have been reported. Fan *et al.* (3) reported rapid decreases in all sensory scores for fish with an increasing storage time. Li *et al.* (18) reported that 1.25 and 1.50% ϵ -polylysine treatments markedly improved sensory properties during storage and prolonged the shelf life of chilled pork to 6 days, similar to results reported in this study. A similar observation was reported by Osman *et al.* (26) where raw milk supplementation with the soybean 11S subunit enhanced the storage quality of bovine raw milk at room temperature and under cold conditions without significantly affecting sensorial properties. Osman *et al.* (27) reported that methylated soybean proteins could extend the storage time of raw milk at room temperature during manufacture of yoghurt without affecting the sensorial qualities of the final product, similar to results presented herein.

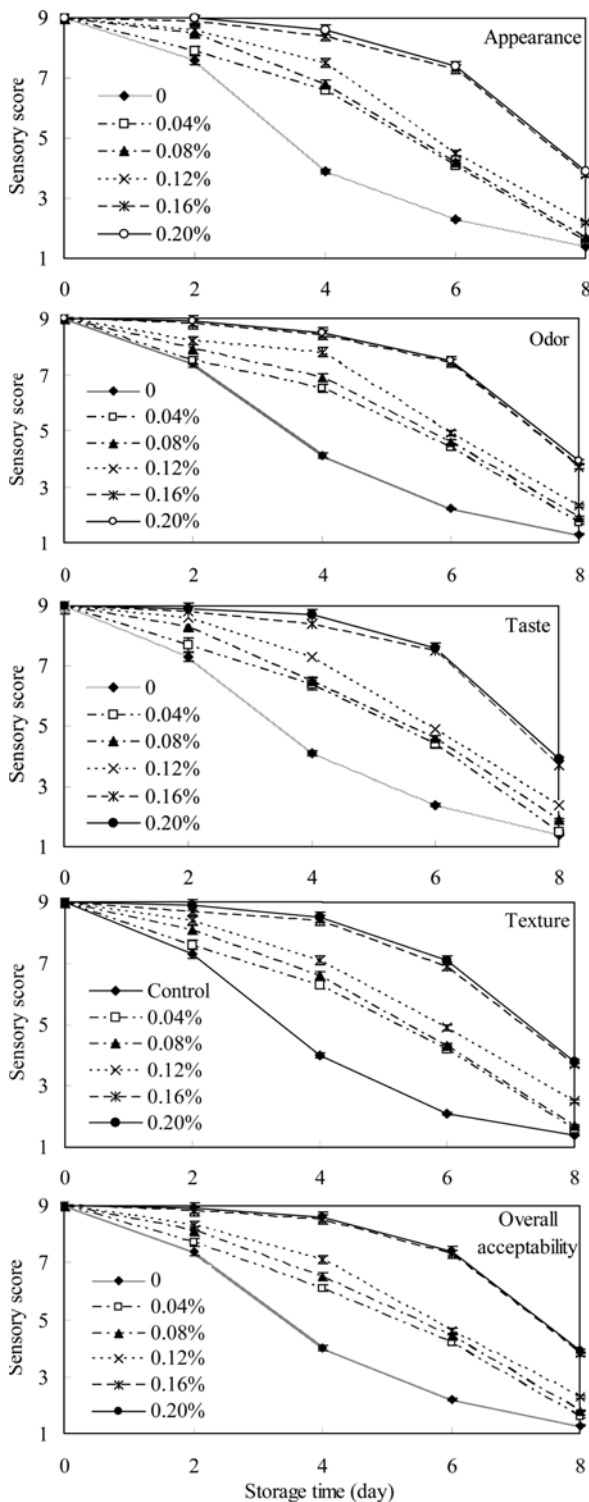


Fig. 1. Effect of glycine basic polypeptide (GBP) on sensory properties of chilled pork during storage at 4°C.

Effects of GBP on pH values of chilled pork An increase in pH values influences the quality of pork during chilled storage, particularly the sensory properties of odor, taste, and texture. Generally speaking, an increase in pH is connected with rapid pork deterioration

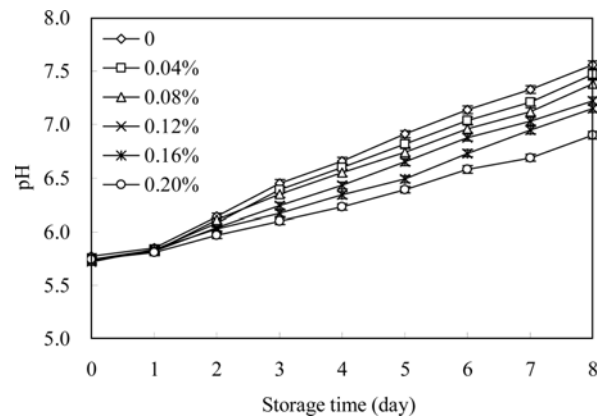


Fig. 2. Effect of GBP on pH values of chilled pork during storage at 4°C.

involving formation of nitrogenous alkaline autolysis compounds and post-mortem production of bacterial metabolites in muscle tissues (28).

Effects of different concentrations of GBP on pH values of chilled pork during storage at 4°C are shown in Fig. 2. pH values of controls increased from 5.77 to 7.56 with storage time. However, pH values of all pork samples treated with GBP increased more slowly, from 5.77 to 6.90, than control values, which showed consistently higher values during storage. Thus, GBP inhibited a pH increase in pork. On days 3 and 4, pH values of controls reached 6.45 and 6.66, showing forthcoming deterioration of pork, while pH values of pork samples treated with 0.20% GBP only reached 6.58 on day 6, indicating that 0.20% GBP extended the shelf life of chilled pork to 6 days. On day 5, pH values of pork samples treated with 0, 0.04, 0.08, 0.12, 0.16, and 0.20% GBP were 6.91, 6.82, 6.74, 6.65, 6.49, and 6.39, respectively, showing that pH values decreased with increasing GBP concentrations. Treatments with 0.16 and 0.20% GBP decreased pH values of pork samples more significantly ($p < 0.05$) than GBP concentrations of 0, 0.04, 0.08, and 0.12%. Thus, 0.16 and 0.20% GBP treatments prevented deterioration of chilled pork and prolonged the effective storage time.

The tendency of pH increase reported herein was coincidental with the observation of Li *et al.* (18) who showed that pH values of chilled pork increased with an increase in ϵ -PL concentrations, similar to the report of Wang *et al.* (19) that pH values for pork samples treated with extracted housefly pupae peptides were lower than for untreated chilled pork samples during chilled storage. Mahgoub *et al.* (20) reported that methylated soybean proteins significantly prevented changes in pH values of pasteurized milk, inhibited growth of bacteria, and lengthened the shelf life of pasteurized milk from 6 to 16 days at 4°C, and Mahgoub *et al.* (29) also revealed that esterified soybean proteins maintained the pH level of raw milk at pH 6.4 after 8 days of refrigerated storage, compared with 4 days for untreated raw milk, both reports in agreement with results reported herein.

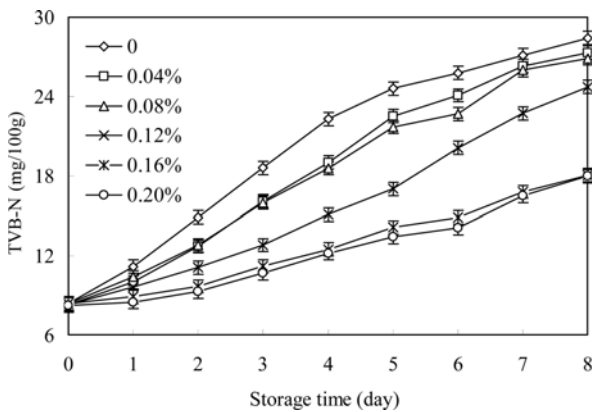


Fig. 3. Effect of GBP on total volatile base nitrogen (TVB-N) values on chilled pork during storage at 4°C.

Effect of GBP on total volatile base nitrogen (TVB-N) values of chilled pork The impact of GBP at different concentrations on TVB-N values of chilled pork during 8 days storage at 4°C is shown in Fig. 3. The initial TVB-N value of pork samples was approximately 8.42 mg/100 g. TVB-N values of controls rose from 8.42 to 28.44 mg/100 g with an increasing storage time from 0 to 8 days. However, TVB-N values of treated pork samples increased significantly ($p < 0.05$) more slowly from 8.30 to 17.98 mg/100 g than those of controls during 8 days storage, indicating that GBP retarded an increase of pork TVB-N values. On day 3 of storage, TVB-N values of 0.04, 0.16, and 0.20% GBP treated pork samples were 16.10, 11.21, and 10.70 mg/100 g, respectively, whereas, the control value already reached 18.62 mg/100 g. On day 6, TVB-N values of 0.04, 0.08, 0.12, 0.16, and 0.20% GBP treated pork samples were 24.00, 22.70, 20.12, 14.90, and 14.10 mg/100 g, respectively. However, the control value was 25.79 mg/100 g. TVB-N values of pork samples treated with 0.16 and 0.20% GBP at on day 6 were significantly ($p < 0.05$) lower than values for other treated pork samples. Control and treated pork samples were not considered acceptable for consumption on days 7 and 8 due to TVB-N values higher than 15.00 mg/100 g (30). Thus, 0.16 and 0.20% GBP treatments prevented deterioration in chilled pork quality and prolonged the product shelf life to 6 days.

Souza *et al.* (16) showed that TVB-N values of salmon fillets increased from 8.06 to 27.04 mg/100 g with an increasing storage time, similar to results of this study and similar to the report of El Bassi *et al.* (31) that TVB-N values for pork samples treated with antimicrobial agents were consistently lower than for untreated pork samples, and in agreement with the report of Wang *et al.* (19) that TVB-N values of pork samples treated with 1.2 g/L extracted housefly pupae peptides were significantly less than for controls.

Effect of GBP on α -thiobarbituric acid (TBA) values of chilled pork Changes in TBA values of control and pork samples treated with GBP at different concentrations during 8 days storage at 4°C are shown in Fig. 4. All TBA values showed a slight increase with storage time. On day 7 of storage, TBA values of chilled pork were 1.45, 1.50, 1.51,

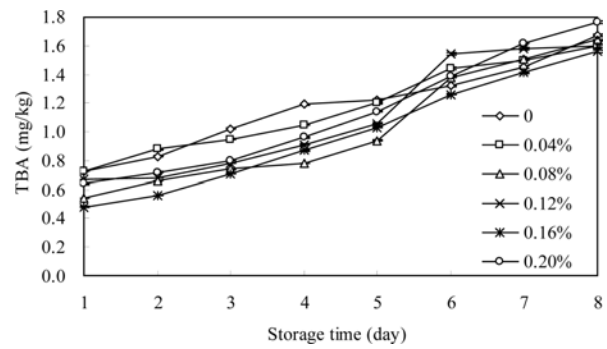


Fig. 4. Effect of GBP on TBA values on chilled pork during storage at 4°C.

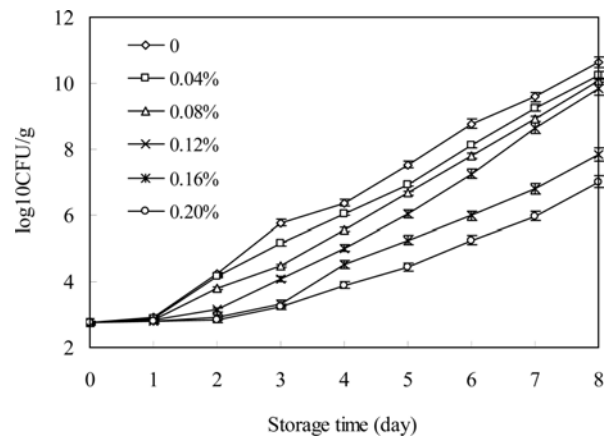


Fig. 5. Changes in total viable count (TVC) values of chilled pork treated with different concentrations of GBP during storage at 4°C.

1.58, 1.41, and 1.62 mg/kg for all pork samples treated with 0, 0.04, 0.08, 0.12, 0.16, and 0.20% GBP, respectively. There were no significant ($p > 0.05$) differences in TBA values of pork samples with an increasing GBP concentration. Therefore, GBP had no effect for reduction of TBA values in chilled pork, which indicated that GBP had no apparent anti-oxidant activity in chilled pork.

According to Fan *et al.* (3), fish flesh usually takes on an objectionable odor when TBA values are higher than 1-2 mg/kg. Pearson *et al.* (32) found that pork has higher saturated fatty acid contents than other meats and fish. Therefore, TBA values of chilled pork in this study were higher than for values of fish reported by Fan *et al.* (3) during chilled storage. No significant ($p > 0.05$) of GBP anti-oxidation effect between treated and untreated pork samples reported herein was probably related to a low temperature of 4°C and short storage time of 7 days, which prevented apparent oxidation of pork fat.

Effect of GBP on total viable counts (TVC) in chilled pork TVC values of chilled pork were affected by use of 0, 0.04, 0.08, 0.12, 0.16, and 0.20% GBP during 8 days of storage (Fig. 5). There was an upward trend in values from an initial value of 2.75 of log₁₀ CFU/g for all pork samples with increasing storage time. TVC Values for treated pork samples increased from 2.75 to 7.01 at a slower rate than for control sample values that increased from 2.75 to 10.63 during

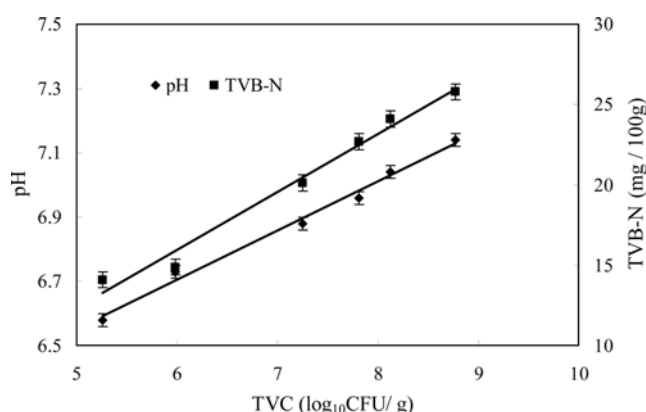


Fig. 6. Correlations between pH, TVB-N, and TVC values of chilled pork during storage at 4°C.

storage. Moreover, the values of log₁₀ CFU/g for chilled pork decreased with an increase of GBP concentrations during the same storage period. Thus, GBP prevented growth of bacteria. On day 5, chilled pork samples treated with 0.04, 0.08, 0.12, 0.16, and 0.20% GBP exhibited TVC values of 6.93, 6.67, 6.04, 5.26, and 4.43 log₁₀ CFU/g, respectively, whereas control values had already reached 7.54 log₁₀ CFU/g. Treatments with 0.16 and 0.20% GBP had significantly ($p < 0.05$) stronger antibacterial effects than concentrations of 0.04, 0.08, 0.12%. In addition, sensory analysis also demonstrated that chilled pork treated with 0.16 and 0.20% GBP was acceptable on days 5, 6, and 7. On day 8, TVC values of all pork samples exceeded 7.01 log₁₀ CFU/g with unacceptable sensory scores of less than 4.0. Thus, 0.16 and 0.20% GBP treatments inhibited growth and reproduction of bacteria and extended the shelf life of chilled pork to 6 days.

Sitohy *et al.* (11) reported that GBP was more effective at killing pathogenic and spoilage bacteria than penicillin, and GBP effectiveness was associated with the basicity of the protein fraction, in agreement with results reported herein. The basicity of protein participated in empowering protein molecules with a specific reactivity for reactions with the bacterial cell wall membrane, similar to the report of Dhatwalia *et al.* (33) where wheat puroindoline protein exhibited activities against both Gram-positive and Gram-negative bacteria, and inhibited a wide range of microbial pathogens in crop plants. Das *et al.* (34) also reported that sesame peptides had bacteriostatic effects against pathogen growth.

Correlations between pH, TVB-N, and TVC values of chilled pork

Correlations between pH, TVB-N, and TVC values of chilled pork treated with 0, 0.04, 0.08, 0.12, 0.16, and 0.20% GBP on storage day 6 are shown in Fig. 6. pH values of controls increased from 6.58 to 7.14 with an increase of TVC values from 5.26 to 8.77 log₁₀ CFU/g. A plot of pH as a function of TVC values showed a positive linear relationship with $R^2 = 0.9910$ (Eq. 1):

$$\text{pH} = 0.1532 \times (\text{TVC}) + 5.7855 \quad (1)$$

The correlation between TVB-N and TVC values was similar to the correlation between pH and TVC values. A plot of TVB-N values vs. TVC values showed a positive linear relationship ($R^2 = 0.9825$) (Eq. 2):

$$\text{TVB-N} = 3.6011 \times (\text{TVC}) - 5.6369 \quad (2)$$

This study showed that GBP had effects on sensory scores, pH, TVB-N, and TVC values of chilled pork during storage at 4°C with increasing concentrations of GBP. However, TBA values of chilled pork treated with GBP showed no change. Treatments with 0.16 and 0.20% GBP can be used for effective inhibition of growth and reproduction of microorganisms and prevention of changes in physicochemical properties of chilled pork. GBP treatments at 0.16 and 0.20% improved quality attributes of chilled pork. Analysis of sensory properties demonstrated that a 0.16% GBP treatment exhibited good effects. Therefore, GBP has potential for use as a natural preservative and for extension of the shelf life of chilled pork.

Acknowledgments This study was supported by the National Natural Science Foundation of China (31371839), the Major Plan of Study and Development (2015GSF120006), the Science and Technology Agency, Shandong Province, Science and Technology Development Plan, Shandong Province Colleges and Universities (J13LE02), and the Program for Science and Technology Innovation Team in Universities of Henan Province (16IRTSTHN007).

Disclosure The authors declare no conflict of interest.

References

- Jeyasekaran G, Ganesan P, Anandaraj R, Jeya Shakila R, Sukumar D. Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaes indicus*) stored in dry ice. *Food Microbiol.* 23: 526-533 (2006)
- Duan J, Cherian G, Zhao Y. Quality enhancement in fresh and frozen lingcod (*Ophiodon elongates*) filets by employment of fish oil incorporated chitosan coatings. *Food Chem.* 119: 524-532 (2010)
- Fan W, Sun J, Chen Y, Qiu J, Zhang Y, Chi Y. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food Chem.* 115: 66-70 (2009)
- Chang WT, Chen YC, Jao CL. Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. *Bioresource Technol.* 98: 1224-1230 (2007)
- Chung YC, Chen CY. Antibacterial characteristics and activity of acid-soluble chitosan. *Bioresource Technol.* 99: 2806-2814 (2008)
- Song Z, Liu Q, Guo H, Ju R, Zhao Y, Li J, Liu X. Tostadin, a novel antibacterial peptide from an antagonistic microorganism *Brevibacillus brevis* XDH. *Bioresource Technol.* 111: 504-506 (2012)
- Damodaran S, Kinsella JE. Effect of conglycinin on the thermal aggregation of glycinin. *J. Agr. Food Chem.* 30: 812-817 (1982)
- Delwiche SR, Pordesimo LO, Panthee DR, Pantalone VR. Assessing glycinin (11S) and β -conglycinin (7S) fractions of soybean storage protein by near-infrared spectroscopy. *J. Am. Oil Chem. Soc.* 84: 1107-1115 (2007)
- Hu SD, Liu H, Qiao SY, He PL, Ma X, Lu WQ. Development of immunoaffinity chromatographic method for isolating glycinin (11S) from soybean proteins. *J. Agr. Food Chem.* 61: 4406-4410 (2013)
- Mo XQ, Zhong ZK, Wang DH, Sun XZ. Soybean glycinin subunits: Characterization of physicochemical and adhesion properties. *J. Agr. Food Chem.* 54: 7589-7593 (2006)
- Sitohy MZ, Mahgoub SA, Osman AO. *In vitro* and *in situ* antimicrobial action and mechanism of glycinin and its basic subunit. *Int. J. Food Microbiol.* 154: 19-29 (2012)

12. Li YQ, Sun XX, Feng JL, Mo HZ. Antibacterial activities and membrane permeability actions of glycinin basic peptide against *Escherichia coli*. *Innov. Food Sci. Emerg.* 31: 170-176 (2015)
13. Cao Y, Gu W, Zhang J, Chu Y, Ye X, Hu Y, Chen J. Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-pork during refrigerated storage. *Food Chem.* 141: 1655-1660 (2013)
14. Srikar LN, Hiremath JG. Fish preservation I. Studies on changes during frozen storage of oil sardine. *J. Food Sci. Tech.* 9: 191-193 (1972)
15. Kanatt SR, Chander R, Sharma A. Chitosan and mint mixture: A new preservative for meat and meat products. *Food Chem.* 107: 845-852 (2008)
16. Souza BW, Cerqueira MA, Ruiz HA, Martins JT, Casariego A, Teixeira JA, Vicente AA. Effect of chitosan-based coatings on the shelf life of salmon (*Salmo salar*). *J. Agr. Food Chem.* 58: 11456-11462 (2010)
17. Campos CA, Rodríguez Ó, Losada V, Aubourg SP, Barros-Velázquez J. Effects of storage in ozonised slurry ice on the sensory and microbial quality of sardine (*Sardina pilchardus*). *Int. J. Food Microbiol.* 103: 121-130 (2005)
18. Li YQ, Feng JL, Han Q, Dai ZY, Liu W, Mo HZ. Effects of ϵ -polylysine on physicochemical characteristics of chilled pork. *Food Bioprocess Tech.* 7: 2507-2515 (2014)
19. Wang Y, Dang X, Zheng X, Wang J, Zhang W. Effect of extracted housefly pupae peptide mixture on chilled pork preservation. *J. Food Sci.* 75: 383-388 (2010)
20. Mahgoub SA, Sitohy MZ, Osman AO. Counteracting recontamination of pasteurized milk by methylated soybean protein. *Food Bioprocess Tech.* 6: 101-109 (2013)
21. Rodríguez-Carpena JG, Morcuende D, Estévez M. Avocado by-products as inhibitors of color deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage. *Meat Sci.* 89: 166-173 (2011)
22. Nagano T, Hirotsuka M, Mori H, Kohyama K, Nishinari K. Dynamic viscoelastic study on the gelation of 7 S globulin from soybeans. *J. Agr. Food Chem.* 40: 941-944 (1992)
23. Kim, HW, Hwang, KE, Song, DH, Kim, YJ, Ham, YK, Yeo, EJ, Kim, CJ. Effect of soy sauce type on the quality characteristics of emulsion sausages. *Food Sci. Biotechnol.* 24: 1309-1315 (2015)
24. Goulas AE, Kontominas MG. Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): Biochemical and sensory attributes. *Food Chem.* 93: 511-520 (2005)
25. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chem.* 120: 193-198 (2010)
26. Osman AO, Mahgoub SA, Sitohy MZ. Preservative action of 11S (glycinin) and 7S (β -conglycinin) soy globulin on bovine raw milk stored either at 4 or 25°C. *J. Dairy Res.* 80: 174-183 (2013)
27. Osman A, Mahgoub S, El-Masry R, Al-Gaby A, Sitohy M. Extending the technological validity of raw buffalo milk at room temperature by esterified legume proteins. *J. Food Process. Pres.* 38: 223-231 (2014)
28. Shenderyuk VI, Bykowski PJ. Salting and marinating of fish. pp. 147-162. In: *Seafood: Resources, Nutritional Composition, and Preservation*. Sikorski ZE (ed). CRC Press, Inc., Boca Raton, FL, USA (1990)
29. Mahgoub S, Osman A, Sitohy M. Inhibition of growth of pathogenic bacteria in raw milk by legume protein esters. *J. Food Protect.* 74: 1475-1481 (2011)
30. Chinese standard GB 2707 (2005): Hygienic standard for fresh (frozen) meat of livestock.
31. El Bassi L, Hassouna M, Shinzato N, Matsui T. Biopreservation of refrigerated and vacuum-packed *Dicentrarchus labrax* by lactic acid bacteria. *J. Food Sci.* 74: M335-M339 (2009)
32. Pearson AM, Love JD, Shorland FB. Warmed-over avor in meat, poultry and fish. *Adv. Food Res.* 23: 1-74 (1977)
33. Dhatwalia VK, Sati OP, Tripathi MK, Kumar A. Isolation, characterization and antimicrobial activity at diverse dilution of wheat puroindoline protein. *World J. Agri. Sci.* 5: 297-300 (2009)
34. Das R, Dutta A, Bhattacharjee C. Preparation of sesame peptide and evaluation of antibacterial activity on typical pathogens. *Food Chem.* 131: 1504-1509 (2012)