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Research Article

Effects of Bio-preservative Combined with Partially Frozen Storage on Fresh-keeping of *Argopectens irradias* Meat

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Abstract: Treated with lysozyme and nisin and vacuum packed, preservation effect of the bio-preservatives on *Argopectens irradias* meat under partial freezing condition of -3°C has been studied. During the process, Total Bacterial Count (TBC) and some physical and biochemical data had been determined. Results show that after 20 days storage at -3°C, Total Volatile Basic Nitrogen (T-VBN) of the *Argopectens irradias* meat treated with lysozyme and nisin reached 13.28 and 13.98 mg/100 g, respectively which all approached upper control limit by China national sanitary standard, while the test datum of *Argopectens irradias* meat treated with combination of lysozyme and nisin reached 14.81 mg/100 g after 30 days storage which is 20 days longer than control sample. Storage time of each sample correlate to pH, TBC, T-VBN, Thiobarbituric Acid (TBA) and Ca^{2+}-ATPase activity ($p<0.05$) of the test meat. Of all test samples, combination treatment with lysozyme and nisin achieved the best preservation effect.

Keywords: *Argopectens irradias*, fresh-keeping, lysozyme, nisin, partially frozen storage

INTRODUCTION

Argopectens irradias, which biologically features strong adaptability, fast growth, short breeding cycle and high yield, is one of the widely farmed scallop species in the northern sea area of China. It is popular among consumers for their delicious meat and rich nutrition. Because of its delicate muscle texture, high water content, high enzymatic activity, it is easy to cause spoilage and lead to huge economic loss, therefore means of preservation is a crucial task.

Partially frozen storage is to store freshly captured sea food at its freezing point (around -3°C) and have it lightly frozen to keep it fresh. It has been used since 70s of 20 century to preserve sea foods and has been successful (Magnusson and Martinsdottir, 1995; Cho, 1981; Rosues *et al*., 2006). The study of partially frozen storage was started in China in 1978 for sea foods preservation (Zhang *et al*., 2011; Li *et al*., 2008; Gao *et al*., 2010). Comparing with conventional refrigeration, sea foods by partially frozen storage extend 1.5-4 times longer shelf life, hence people thinks highly of the technology (Duun and Rustad, 2007).

As people pay more attention to food safety recently, effective, low toxic, low cost and easy to use bio-preservatives tend to be a study topic (Cao *et al*., 2009; Kuwano *et al*., 2005; Cao *et al*., 2008; Xie and Yang, 2011; Gill and Holley, 2003). Lysozyme and nisin are widely used bio-preservatives in food industry. Lysozyme shows good antibacterial activity for Gram positive bacteria, *Bacillus subtilis* and *Baclicus lincheniformis*. It specially shows strong bacteriolysis for micrococcus lysodeikticus Fleming. Nisin is capable of inhibiting the growth of most Gram positive bacteria, as well as bacillus (for example *Clostridium botulinum*), heat resisting putrefying bacteria (for example *Bacillus stearothermophilus*) and *Clostridium sporogenes*. A research says that combination of the above two enhances the antibacterial effect (Chung and Hancock, 2000). It has rarely been studied to use bio-preservatives in shell fish preservation, especially in the preservation of *Argopectens irradias*, no such report has been published so far. In this report, lysozyme and nisin are used as bio-preservatives in partially frozen storage to assess the preservation effect for *Argopectens irradias* meat. For the assessment, determination and analysis of chemical and biochemical data such as pH, Total Bacterial Count (TBC), Total Volatile Basic Nitrogen (TVB-N), Thiobarbituric Acid (TBA) and Ca^{2+}-ATPase activity had been made, this may also help to develop preservation technology for *Argopectens irradias* meat.

MATERIALS AND METHODS

Materials:

- **Fresh *Argopectens irradias*:** Purchased from Qinhuangdao City aquatic products wholesale market

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- **Lysozyme:** Sigma Company
- **Nisin:** Qingdao Hope Bio-Technology Co., Ltd. (China)
- **Ca^{2+}-ATPase test kit:** Nanjing Jiancheng Bioengineering Institute (China)
- **Plate count agar:** Beijing Land Bridge Technology Co., Ltd. (China)
- PH meter PHS-3C Shanghai Precision and Scientific Instrument Ltd.
- Tissue stamp mill, Jiangsu Yitong Electronics Co., Ltd.
- Sterile console (SW-CJ-2F), Suzhou Antai Air Technology Ltd.
- Refrigerated Centrifuge, Sigma Company

Test methods: Put the fresh *Argopectens irradias* into the crushed ice, wash them with ice and water. After cleaning, Unshell to take meat and put the meat in the normal saline for cleansing, drain off. Dip the meat in the prepared preservative liquid for 3~5 min. Drain off for 3~5 min. Take 200 g of such prepared meat and vacuum seal them in a composite film bag. Mark the bags and store them at the temperature of -3°C for testing.

Based on preliminary test, nisin, lysozyme, glycine were chosen as main ingredients. By orthogonal test, formulas of preservative liquids were determined:

- **Group A:** Lysozyme-0.05%, NaCl-1.0%, glycine-7.5%
- **Group B:** Nisin-0.02%, NaCl-2.0%, glycine-6.0%
- **Group C:** Lysozyme-0.045%, nisin-0.015%, glycine-6.0%, NaCl-1.0%
- **Group K:** Control group

Determination of pH values: Take 5 g of testing meat, add nine fold double distilled water, homogenize the content, lay aside for 30 min. then measure its pH with acidometer.

Determination of TVB-N: As per Industry Standard of China: SC/T 3032-2007 (Determination of the total volatile basic nitrogen in fishery products).

Determination of TBA: Take 10.00 g of minced sample meat and put it into a 500 mL distilling flask, add 20 mL of distilled water and mix evenly, add 2 mL of HCL solution (V_{HCl}: V_{H2O} = 1:2) and 2 mL of liquid paraffin, collect 50 mL of distillate by steam distillation, take 5 mL of distillate and mix with 5 mL of TBA acetum (0.2883 g of TBA dissolve in 100 mL of 90% glacial acetic acid) in a 25 mL test tube, heat the test tube in 100°C water bath for 35 min and cool down for 10 min, measure the Absorbance (A) at 535 nm. Measure the absorbance of a control sample (substitute the distillate with distilled water):

$$TBA = A \times 7.8 \times 10^{-2}\ mg/g$$

Determination of activity of Ca^{2+}-ATPase: As per the instruction of Ca^{2+}-ATPase test kit.

Determination of TBC: As per China National standard: GB/T 4729.2.

Data processing and analysis: Draw with Excel and analyze the data with software SPSS 17.0. When $p<0.01$, there is extremely significant difference; when $p<0.05$, significant difference; when $p>0.05$, no significant difference.

RESULTS AND DISCUSSION

Variation of pH: The pH value is one of index for indicating the quality of fishery products. The pH variation of *Argopectens irradias* meat during its storage shows in Fig. 1. The pH variations of each sample groups correlated to storage time and the bio-preservative ($p<0.05$). The initial pH of sample meat was about 6.36 which is almost neutral. As time lapsed, pH of different packaged meat declined because of the accumulated lactic acid disintegrated from glycogen after death of the *Argopectens irradias*. In the first 5 days, pH of the group K (control group) dropped fast as the time past (r = -0.999), faster than other test sample groups, this may result from the acidic compounds produced by quick reproduction of acid-forming bacteria during the storage. Later on, pH of the meat ascended due to the fact that its protein started to decompose to alkaline amine and amides. Comparing with group K, pH of other test groups treated with bio-preservative ascended more slowly in the late period of the storage. Meat of the group K started to rigidify at day 5 of storage, while the meat treated with bio-preservatives started to rigidify 10 days later.

Results by Duncan Multiple Comparison indicate that pH of sample groups treated with bio-preservative displays significant difference with that of the control group ($p<0.05$). Comprehensive analysis indicates that combined treatment with lysozyme and nisin enhances inhibition for the growth of acid-forming bacteria on the basis of each function of the bio-preservative and slow the decrease of pH.

Variation of TBC: TBC not only indicates the extent that foods contaminated by bacteria, but predicts

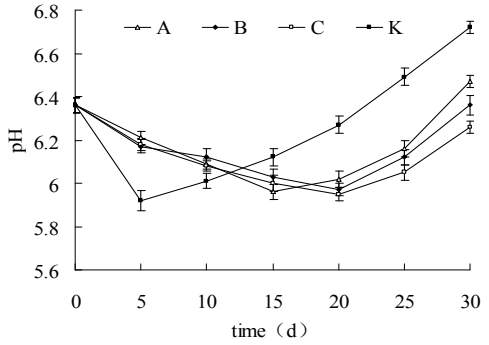

Fig. 1: Changes of pH in *Argopectens irradias* during partially frozen storage

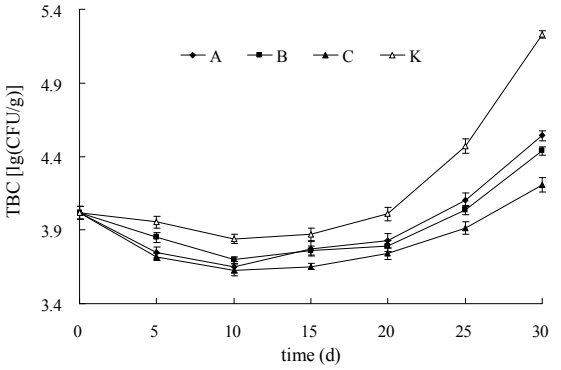

Fig. 2: Changes of TBC in *Argopectens irradias* during partially frozen storage

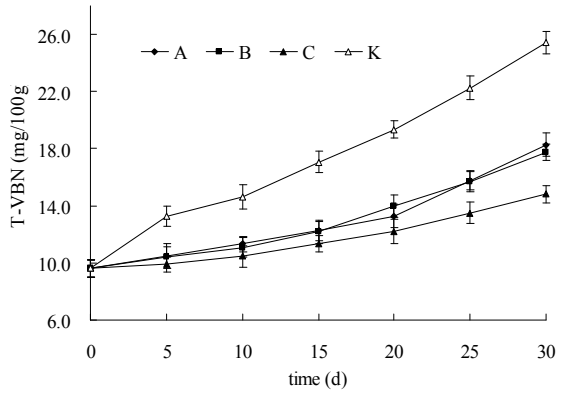

Fig. 3: Changes of T-VBN in *Argopectens irradias* during partially frozen storage

shelflife of foods as well. TBC variation of *Argopectens irradias* meat during its storage shows in Fig. 2. The graph clearly reflects TBC variation of different test groups. The initial TBC of fresh *Argopectens irradias* meat was 4.02 lg (CFU/g), TBC of control group shows obvious correlation to storage time ($p<0.05$, $r = 0.960$). In the first 5 days, TBC of control group increased slowly as time extended, while 10 days later the increase sped up, faster than other test groups. TBC variation of other test groups correlated as well to storage time ($p<0.05$). During the storage period, TBC went down initially and then climbed up, this is because bigger crystal formed at the partial freezing temperature of -3°C, the water in bacteria froze and the crystal expanded, meanwhile, water outside bacteria froze and expanded as well, the press from inside and outside led to rupture of the bacteria and killed them (Cobb, 1976).

In the late period, TBC kept increase due to the fact that protein, saccharides, lipid gradually decomposed into small molecules which supplied with nutrients for survived bacteria, therefore, bacteria multiplied and led to putrefaction.

TBC of group A and group B showed insignificant difference, while TBC of group C was obviously lower than former 2 groups ($p<0.05$), which indicates that lysozyme and nisin may have synergy effect for inhibiting the growth of bacteria. Composite bio-preservative shows good preservation effect.

Variation of T-VBN: Protein of fishery product disintegrates by bacteria and endogenous enzymes into basic substance such as ammonia and amines. Determination of T-VBN may reflect disintegration extent of protein, therefore TVB-N acts as freshness index of fishery products and other meat products. T-VBN variation of *Argopectens irradias* meat during its storage shows in Fig. 3. T-VBN variation of each test group shows correlation with storage time ($p<0.05$).

T-VBN of control group climbed up rapidly on day 5 and it reached 17.04 mg/100 g on day 15 which exceeded 15 mg/100 g, the control limit by China national sanitary standard. Besides the control group, TVB-N of other test groups showed insignificant difference at the beginning and the variation was mild. After some days, differences were getting significant. The explanation for this is that ammoniacal nitrogen released by deamination of AMP was the main factor to raise T-VBN, in this period there was less possibility that the increase of T-VBN was resulted from the decomposition of TMA and or DMA, though deamination of other substances were not excluded.

T-VBN contents of test groups treated with bio-preservative were lower than that of control group, which indicates that bio-preservative treatment inhibited effectively the growth of bacteria, hence lower the TVB-N content during the storage period. However, there was insignificant difference between the lysozyme treated group A and nisin treated group B ($p>0.05$), while T-VBN content of group C (combined treatment with lysozyme and nisin) was significantly lower than that of former 2 groups after the day 10 ($p<0.05$). On day 30, T-VBN of group C was 14.81 mg/100 g, still below the upper control limit by China national sanitary standard, which showed the best preservation effect.

Variation of TBA: TBA is widely used to assess degree of fat oxidation. The reactant TBA mainly exists in autolysis period of fat that peroxides are oxidized into aldehyde and ketone. TBA variation of *Argopectens irradias* meat during its storage shows in Fig. 4.

The graph indicates that TBA correlates apparently to storage time and preservation means ($p<0.05$). TBA of control group climbed up rapidly at beginning and reached maximum on day 10~15, then started to decline or maintained at the same level. In late period of storage, the declining trend was consistent with the studies of other researchers.

Comparing with control group, TBA of bio-preservative treated groups increased slowly and the value kept low, significantly lower than that of control group on day 5 ($p<0.01$). On day 15, TBA of the group

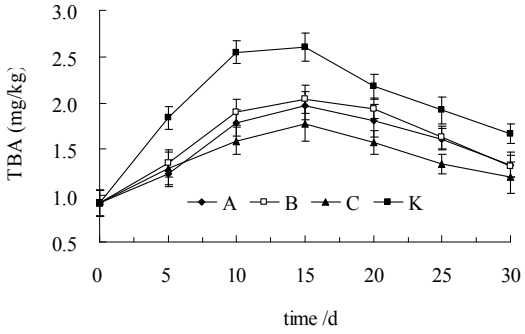

Fig. 4: Changes of TBA in *Argopectens irradias* during partially frozen storage

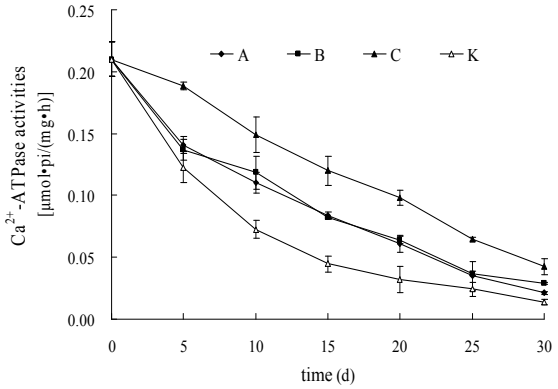

Fig. 5: Changes of Ca^{2+}-ATPase activities in *Argopectens irradias* during partially frozen storage

treated with combination of lysozyme and nisin was significantly lower than that of lysozyme treated group and nisin treated group ($p<0.05$), which indicates that combined treatment with lysozyme and nisin achieved the best effect in inhibiting the fat oxidation.

Variation of Ca^{2+}-ATPase activity: The activity of Ca^{2+}-ATPase lies in structure of the head of actomyosin, which may transform during storage, even may transform into disulfide bond, if so, its activity declines or loses (Benjakul *et al*., 1997). Activity of Ca^{2+}-ATPase accurately reflects the change of protein, thereby may judge the quality of the protein. The variation of Ca^{2+}-ATPase activity of *Argopectens irradias* meat during its storage shows in Fig. 5.

In the graph, Ca^{2+}-ATPase activities of each test groups show correlation with storage time ($p<0.05$). The activity of control group dropped rapidly within the first 10 days, then mildly after day 15 (r = -0.835). The activities of test groups treated with bio-preservative dropped more slowly than that of control group.

After day 10, Ca^{2+}-ATPase activities of control group dropped from 0.210 μmol·pi/ (mg·h), the starting point of the fresh meat, to 0.073 μmol·pi/ (mg·h), 65.24% of decrease, then after day 20, dropped to 0.032 μmol·pi/ (mg·h), 84.76% of decrease. While in group C (combined treatment with lysozyme and nisin) after 10 days storage, the activity dropped from 0.210 μmol·pi/ (mg·h), the starting point of the fresh meat, to 0.149 μmol·pi/ (mg·h), 29.05% of decrease, then after day 20, dropped to 0.098 μmol·pi/ (mg·h), 53.33% of decrease. After 5 days storage, Ca^{2+}-ATPase activities of group A, group B and group C of combined treatment were all significantly higher than that of control group K ($p<0.01$). The Ca^{2+}-ATPase activities of group A and group B showed insignificant difference ($p>0.05$). On the whole, treatment of group C retarded protein denaturation most effectively.

CONCLUSION

During partially frozen storage of *Argopectens irradias* meat, bio-preservatives such as lysozyme and nisin effectively inhibit bacteria growth, retard production of TVB-N, variation of pH, increase of TBA and drop of activity of Ca^{2+}-ATPase.

For fresh *Argopectens irradias* meat with initial TVB-N of 9.60 mg/100 g and TBC of 4.02 lg (cfu/g) under partially frozen storage at -3°C, TVB-N of control group exceeded upper control limit of 15 mg/100 g by China national sanitary standard after 12~13 days storage, while TVB-N of the meat treated with 0.02% nisin reached 13.98 mg/100 g on day 20 which approached the control limit. TVB-N of the meat treated with 0.05% lysozyme reached 15.07 mg/100 g on day 30, just exceeded the control limit, 10~11 days longer shelflife than the control group.

T-VBN of the meat treated with combination of 0.045% lysozyme + 0.015% nisin reached 14.81 mg/100 g on day 30, 17~18 days longer shelf-life than the control group and the test data was the best among the test groups, the preservation effect was significant, this may contribute to complementary antibacterial range of each bio-preservative, therefore, the antibacterial spectrum was broadened.

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