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DISSERTATION

Development of technology of squid meat preparation
with new consumer properties

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(Signature, name, and surname of the applicant)

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(Name and surname of the supervisor, scientific degree, scientific title)

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ANNOTATION

Cui Zhenkun **Development of technology of squid meat preparation with new consumer properties**

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Dissertation for the degree of the Doctor of Philosophy in the specialty 181 − «Food technologies» − Sumy National Agrarian University, Sumy, 2022.

Based on scientific demonstration and experimental research, this thesis is devoted to improving the industrial technology to produce squid products and developing and applying the blue light (Blu-ray) cold sterilization technology using the vacuum and low-temperature synergistic heat treatment.

With the improvement of economic development and the change of the people's diet concept, aquatic products with high protein and low fat are more and more popular among the people. Squid can meet this demand of consumers to a certain extent. The annual catch of squid in China is on the rise, and the catch of *Molluscs* in 2019 was 16324799.7 tons (live weight) (FAO). However, foodborne pathogenic bacteria contamination seriously threatens food safety and human health. In addition, the use of antibiotics promotes the emergence of drug-resistant bacteria and even multidrug-resistant bacteria, which brings severe challenges to food safety control. In particular, with the improvement of people's living standards and the improvement of logistics and transportation conditions, people's consumption demand for seafood is showing an increasing trend. Reducing the threat of food safety is still an issue in food public health today. one of the significant challenges. Thus, the hot issue is producing safe squid products with high-quality properties and preserving them for as long as possible. People admire green, natural, healthy food, hoping that casual food usually tastes the original flavor and texture of the product and its nutrition. Cooking meat produces physical and chemical changes that negatively impact meat product quality, such as cooking loss, nutrient loss, reduction in volume, color change, and toughness in texture. Different cooking temperatures will have different effects. However, although the traditional thermal processing method can kill the pathogenic microorganisms of squid products, such high-temperature heating will cause

the loss of moisture and nutrients in squid products and cause the squid products to become too hard to chew, reducing the consumption attributes of squid products.

Therefore, this paper aims to demonstrate the sous vide cooking (SV) scientifically squid and apply Blu-ray cold sterilization technology to its products to develop the high-quality, high-nutritive value of aquatic products industrialization technology.

In this paper, based on the systematic study of the raw material characteristics of squid, we further investigated the effects of different heat treatments (boiling, steaming and sous vide cooking) on physicochemical and microbiological indicators of squid. Then on the basis, the fingerprint of heat-treated squid was constructed. Furthermore, through the effects of cooking temperature, heating time, and marination time on the quality of SV squid, we optimized the process parameters of SV squid and explained the mechanism of tenderization of SV squid. Meanwhile, we developed a Blu-ray sterilization technology and determined the feasibility of this technology in SV squid.

The introduction substantiates the topic research rationale, reflects its connection with scientific programs and topics, defines and substantiates the purpose and objectives of the research, object, subject, and critical techniques, indicates the novelty and practical significance of the results, determines the personal contribution of the candidate in research, the approbation of the results and gives the publications on the topic of thesis paper, structure, and load.

The first chapter analyzes the current situation of squid fishery and processing, the factors affecting the quality of meat products (including changes in heat treatment methods and storage period) and summarizes that the deep processing of squid products still needs to be further improved. Literature studies have shown that SV is a gentler heat treatment method that can effectively improve the quality of meat products (reducing cooking losses, improving color, and preventing oxidation during heating and storage), thereby evaluating the feasibility of the SV heat treatment of squid. Although scholars believe that SV heat treatment can ensure food safety, we still consider applying blue light sterilization technology to ensure the edible safety of squid products further.

The second chapter formulates the overall plan of scientific research, reflects the primary direction of the thesis, and gives the research materials and research topics. This

section describes all experimental methods and equipment. Standard and generally accepted research methods are employed to study physicochemical properties, microbiology, sensory parameters, microstructure, and nutritional and biological value. Scientific mathematical-statistical methods process the experimental results. The research was carried out at Henan Institute of Science and Technology in China, Sumy State Agricultural University in Ukraine, and Sanjia Food Co., Ltd. in Henan Province, China.

The third chapter systematically studies Argentine squid's physicochemical properties and nutritional indicators. On that basis, discuss the effect of different cooking methods (boiling, steaming, and SV) on squid quality and construct squid's fingerprints treated with different cooking methods through sensory evaluation, physical and chemical indicators, and microbial indicators. The results show that SV squid has different characteristics from other cooking methods. Although SV squid has a lower rating for flavor and odor, it scored higher for preference, appearance, and texture. According to the different characteristics of its cooking flavor (43 specific volatile substances), we constructed the fingerprint of the squid heat treatment method and summarized the best method of squid.

In order to further study the advantages and characteristics of SV squid, according to the single-factor experimental results (cooking temperature, cooking time, and salinity), we optimized the process of SV squid by response surface methodology and determined the best process parameters (cooking temperature 60.7℃, cooking time 30 min, salinity 4.5%) of SV squid.

Our findings and the scholars show that SV meat is more tender than traditionally cooked meat. Moreover, we found that SV squid is the more tender the cooking time longer. Based on the above studies, we revealed the tenderization mechanism of SV squid. SV heating did not inactivate the endogenous enzymes of squid. During the SV heating process, the action of protease accelerated the degradation of protein, leading to the deterioration of various protein indicators, leading to the squid being tender.

In order to ensure the safety of SV squid, we explored the feasibility of Blu-ray sterilization. The study showed that Blu-ray irradiation had little effect on the quality of SV squid, and the dose of 216 J/cm^2 had the best sterilization effect. During storage at 0°C,

5°C, and 10°C, the quality of SV squid after Blu-ray sterilization was not significantly different from that of the control group. Moreover, during the storage process, the microorganisms (total viable count and *Psychrobacter*) in the SV squid after Blu-ray irradiation were lower than those in the control group.

Finally, we constructed the industrial process of SV squid and calculated the economic benefits of SV squid. The pilot production of SV squid was approved under Sanjia Food Co., Ltd., Henan Province, China.

Keywords: squid, heat treatment, quality, shelf life, flavor, sensory evaluation, sterilize, nutrition, protein, microstructure, combination, food products, aquatic, technology

LIST OF THE APPLICANT'S PUBLICATIONS

SCOPUS / Web of Science publications

1. **Cui, Z**., Dubova, H., & Mo, H. (2019). Effects of sous vide cooking on physicochemical properties of squid. *Hygienic Engineering and Design*, 29, 35-40. (*The applicant participated in research, analysis of the results and writing the article*)

2. **Cui, Z**., Manoli, T., Nikitchina, T., & Mo, H. (2020). Trends in the manufacture of processed squid products. *Food Science and Technology*, 14(1), 89-97. https://doi.org/10.15673/fst.v14i1.1650 (*The applicant participated in research, analysis of the results and writing the article*)

3. **Cui, Z.**, Yan, H., Manoli, T., Mo, H., Bi, J., & Zhang, H. (2021). Advantages and challenges of sous vide cooking. *Food Science and Technology Research*, 27(1), 25-34. https://doi.org/10.3136/fstr.27.25 (*The applicant participated in research, analysis of the results and writing the article*)

4. **Cui, Z.**, Yan, H., Manoli, T., Mo, H., Li, H., & Zhang, H. (2020). Changes in the volatile components of squid (*illex argentinus*) for different cooking methods via headspace-gas chromatography-ion mobility spectrometry. *Food Science & Nutrition*, 8(10), 5748-5762. https://doi.org/10.1002/fsn3.1877 (*The applicant participated in research, analysis of the results and writing the article*)

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12. **Cui Z. K.**, Halyna Dubova. (2018). China's food industry economy status. Proceedings of the International Scientific and Practical Internet Conference in Memory of the Professor, 364-366. (November 22, 2018, Sumy) (*PhD participant in carrying out of experimental research, processing of results, and writing the article*)

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19. **Zhenkun Cui**, Halyna Dubova, Haizhen Mo (2019). Effects of sous vide cooking on physicochemical properties of squid. Food Quality and Safety, Health and Nutrition Congress, 37-38. (June 12-14, 2019, Ohrid, Macedonia) (*PhD participant in carrying out of experimental research, processing of results, and writing the article*)

20. **Cui, Z.**, Yan, H., Manoli, T., Fan, C. & Zhang, H. (2021). Effect of blue light sterilization on the quality of sous vide cooking squid. Food Quality and Safety, Health and Nutrition Congress, 176-177. (June 9-11, 2021, Ohrid, Macedonia) (*PhD participant in carrying out of experimental research, processing of results, and writing the article*)

21. **Cui Zhenkun**, Tatiana Manoli, Tataina Nikitchina (2021). The relevance of the study of biogenic amines in aquatic products. The 81st Food Technologies Scientific Conference, 61-63. (April 27-30, 2021, Odessa) (*PhD participant in carrying out of experimental research, processing of results, and writing the article*)

22. **Cui Zhenkun**, Tatiana Manoli, Tataina Nikitchina (2021). Influence of the newest methods of culinary processing and biotechnological features of hydrations on the change of dish quality indicators. "Technologies of food and compound feed" International Scientific and Practical Conference, 28-31. (September 21-24, 2021, Odessa) (*PhD participant in carrying out of experimental research, processing of results, and writing the article*)

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24. **Cui Zhenkun**, Tatiana Manoli (2021). Study on optimal sous vide squid (*Illes argentinus*). Sustainable Development Trends and Challenges under COVID-19, 20-21. (November 29-30, 2021, Sumy) (*PhD participant in carrying out of experimental research, processing of results, and writing the article*)

25. **Cui Zhenkun**, Tatiana Manoli (2021). Analysis of flavor substances of squid via electronic nose. Innovative and resource-saving food production technologies. (December 21, 2021, Poltava) (*PhD participant in carrying out of experimental research, processing of results, and report presentation*)

Patents

26. **Cui, Z.,** Yan, H., Zhang, H., Liu, B., Zhao, Y., …, Zhang, Z. (2021). Refrigeration type blue light sterilization aquatic product display cabinet. (Authorized) [ZL202022638458.3] (*PhD participant in carrying out of experimental research, processing of results, and writing the patent*)

27. Yan, H., **Cui, Z.,** Wu, S., Liu C. (2021) Blue light sterilization box for experiment. (Submitted and accepted) [202120510074.5] (*PhD participant in carrying out of experimental research, processing of results, and writing the patent*)

Pactical recommendations

28 **Cui Zhenkun.** (2021). Proof of production and application of sous vide cooking squid

CONTENTS

INTRODUCTION

Actuality of theme.

The total resources of squid in the world's oceans are about $4.2\times10^8 - 6.5\times10^8$ tons, which are widely distributed in the Atlantic, Indian and Pacific Oceans. Due to the short maturation time and fast growth of cephalopods, and with the development of pelagic fisheries, squid fishery is an emerging fishery that is being developed and is one of the most promising fishery resources.

Squid is rich in nutrition, high in protein and low in fat, rich in essential amino acids, and the composition of essential amino acids is close to that of whole egg protein, which is a kind of nutritional health and good flavor aquatic resource. However, the proteins of invertebrates such as squid are different from those of ordinary fish. In addition, squid contains paramyosin that is unique to invertebrates, which makes it unique from other fish. After harvesting, improper pre-treatment or traditional heat treatment can significantly impact squid muscle and product quality. Therefore, at present, squid is mainly sold as frozen products, but the quality (flavor, texture, gel-forming ability, etc.) can also change with long-term frozen storage, mainly related to the denaturation of its muscle proteins during the freezing process (freezing denaturation).

At present, the research on squid is mostly in physiological functions, living environment, distribution of squid resources and freshness. Meanwhile, the enterprises do not process the squid more deeply, but only simply and roughly, such as freezing. That causes waste of resources and reduces enterprises' economic and social benefits.

Therefore, it is the purpose of this doctoral thesis to investigate a heat treatment method that is suitable for the characteristics of squid raw materials and can be industrially produced and applied.

Connection of work with scientific programs, plans, topics.

"Innovative technological solutions in food production", registration number 0119U101237, 2019-2023

The aim of the study.

The aim of the dissertation is the scientific justification and development of the technology for preparing squid meat with high consumer properties and studying the effect of various heat treatments on the organoleptic, physicochemical, and microbiological indicators of squid.

Research objectives.

1. To analyze literary sources according to the selected research topic.

2. To investigate the peculiarities of the biochemical composition and physicochemical properties of the Argentine squid (*illex argentines*).

3. To investigate the influence of various heat treatment methods on the safety and quality indicators of squid.

4. Justify the method of cooking squid according to sensory characteristics.

5. To optimize the SV process of squid processing; and investigate the tenderization mechanism.

6. To study the mechanism of tenderization of squid muscle tissue prepared by sous vide technology.

7. To investigate the effect of heat treatment conditions on squid weight loss.

8. Justify the possibility of extending the storage period of SV squids by means of Blu-ray sterilization.

9. To investigate the changes in quality indicators of squid treated with Blu-ray irradiation during storage.

10. To develop technological and equipment schemes for the production of products from squid, obtained by the technology of SV.

11. Determine the socio-economic efficiency of scientific and technical development and implement the results of the work in practical production.

For the purpose were assigned the following tasks:

1. The characteristics of squid raw materials.

(1) Squid shape characteristics.

(2) Basic composition analysis and nutritional evaluation of squid meat

2. Study on the effect of cooking methods on squid quality.

(1) Sensory evaluation of squid by different cooking methods.

(2) The effect of different cooking methods on the flavor of the squid.

(3) The effect of different cooking methods on the physical and chemical properties of squid.

3. The influence of sous vide cooking squid processing characteristics

(1) The sous vide cooking heating intensity affects the physical properties of squid.

(2) The sous vide cooking heating intensity affects the chemical properties of squid.

4. The tenderization mechanism of sous vide cooking squid.

(1) Changes in the shear force of squid during heating.

(2) Changes in squid protein composition during heating.

(3) Study the relationship between the change of squid protein composition and the shear force during heating.

5. The effect of Blu-ray sterilization on sous vide cooking squid

(1) The effect of Blu-ray sterilization on the quality of sous vide cooking squid.

(2) The effect of sous vide cooking squid storage period after Blu-ray irradiation.

6. Economic benefits of SV squid

Research methods.

Food science, food engineering, microbiology, and statistics.

The object of the study

To develop technology for squid meat preparation with new consumer properties.

Scientific novelty of the obtained results.

1. A systematic analysis of the effects of different heat treatment methods on the quality of squid has proved the feasibility of sous vide cooking as industrial production of squid.

2. Based on optimizing the technology of sous vide cooking squid, we revealed the mechanism of tenderization of this cooking method.

3. Evaluated the feasibility of blue light irradiation cold sterilization technology applied to sous vide cooking squid and provided theoretical and practical basis.

4. The feasibility of the industrial production of SV squid was determined by economic analysis.

Practical significance of the obtained results.

Based on comparing the advantages of sous vide cooking and traditional heat treatment methods. This study optimized sous vide cooking process parameters and explained its tenderization mechanism, providing a theory for the deep processing of sous vide cooking technology in the food field. Furthermore, this technology's application in industrialized food production will surely bring considerable economic benefits. In addition, through the research of blue light cold sterilization technology, explore the feasibility of blue light in the field of food sterilization, and provide a new method for food cold sterilization technology.

Personal contribution of the applicant.

The Ph.D. student has searched and analysed literature sources on the topic of the work, selected methods and techniques, experimental and laboratory research, statistical processing, and analysis of the results. Interpretation and generalization of the obtained results, drawing up the conclusions of the dissertation, practical recommendations were carried out under the supervisor's guidance.

Approbation of dissertation results.

The main provisions and results of the research were reported and received general scientific approval at the annual scientific reports and conferences of faculty and graduate students at Sumy National Agrarian University, Faculty of Food technologies, Sumy (2018-2021); Proceedings of the International Scientific and Practical Internet Conference in Memory of the Professor, Sumy (2018); Food Quality and Safety, Health and Nutrition Congress, Ohrid, Macedonia (2019 and 2021); University of the All-Ukrainian Student Scientific Conference, Sumy (2019); State and Prospects of Food International Scientific and Technical Conference, Ternopil (2019); The Annual Scientific and Practical Conference of Lecturers, Postgraduates, and Students of the SNAU, Sumy (2019); Development of Food Products International Scientific and Practical Conference, Kharkiv (2019);"Technologies of food and compound feed" International Scientific and Practical Conference, Odessa (2020-2021); The 80th Food Technologies Scientific Conference, Odessa (2020); The 81st Food Technologies Scientific Conference, Odessa (2021); New Tendencies and Global Trends in Technologies for Raw Material Storage and Food

Production, Poltava (2021); Sustainable Development Trends and Challenges under COVID-19, Sumy (2021); Innovative and Resource-Saving Food Production Technologies, Poltava (2021).

Publications.

The main provisions of the dissertation are set out in 12 scientific papers, which are 9 SCOPUS / Web of Science publications and 3 foreign publications of other countries (China).

The structure and scope of the dissertation.

The dissertation is presented on 136 pages of computer text, illustrated with 18 tables and 35 figures and consists of annotation, introduction, review of literature, materials and methods, results of own research, generalization, analysis and discussion of research results, conclusions, proposals, list used sources, applications. The list of used sources of literature includes 203 names, all from far abroad.

CHAPTER 1 LITERATURE REVIEW ON THE TOPIC AND CHOICE OF RESEARCH DIRECTIONS

1.1 Development status of squid processing

1.1.1 Fishery status

Since its inception in 1985, China's offshore fishing industry has continued to develop rapidly, especially during the "Twelfth Five-Year Plan" period, and its equipment level and overall strength have improved significantly. By the end of 2016, there were 162 national offshore fishing enterprises, an increase of 46% over 2010; nearly 2900 offshore fishing vessels (including fishing vessels under construction), the number of operating vessels increased by 66% compared with 2010; the total output of offshore fishing was 1.99 million tons, compared with 2010. The annual growth rate is 78%. The operational sea area covers the jurisdictional waters of 42 countries (regions) and the Pacific Ocean, the Indian Ocean, the Atlantic Ocean, and the Antarctic waters. Among them, 1329 fishing vessels on the high seas, accounting for 6% of the world's high seas fishing vessels, with a production of 1.32 million tons, accounting for 12% of the world's high seas fishery production, the number of ships, are among the highest in the world. The development of the offshore fishing industry guarantees the supply of aquatic products in the domestic market and improves the national nutritional structure and is also conducive to the protection of China's offshore fishery resources. The position of squid in China's offshore fishing industry is becoming more critical, and squid products are gradually becoming more and more popular on the consumers' table. At present, China's high seas squid fishing fleet and squid production rank first globally [1].

For five years since 2010, the catch of cephalopods was increasing. In 2015, the catch volume stabilized, and in 2016 it decreased [2]. The catch of the three main species – Humboldt squid (*Dosidicus gigas*), Argentine shortfin squid (*Illex argentinus*), and Pacific flying squid (*Todarodes pacificus*) – decreased, respectively, by 26.86 and 34%, and in general, a decrease in the volume of catch in 2016, compared to 2015, amounted to 1.2 million tons. China and Morocco remained the largest shellfish producers in the past two

years, while China, Peru, and India were the top three suppliers of squid and cuttlefish. The main markets for cephalopods were Japan, the United States of America, and the largest countries in Southern Europe, particularly Spain and Italy. The worldwide demand, primarily for octopuses and squid, was fuelled by the growing popularity of Japanese cuisine, Hawaiian poké (fish salad), and Spanish tapas. However, the catch in 2016-2017 was small, which led to a significant increase in prices. The main areas for catching these squids are the Pacific Ocean, Peruvian waters, and the Atlantic Ocean. Aquaculture is a promising activity, too. Figure 1.1 shows the favorable growth rates of world aquaculture in 1980-2020 (FAO).

The average annual growth rate of aquaculture is slowing down: in 2003–2016, the production grew, on average, by 5.7%, but in 2017–2019, this Figure did not exceed 2.1%. Despite the slowdown, aquaculture in this regard remains the leader among all sectors producing food of animal origin. Figure 1.2 shows the production of marine molluscs from 1980 to 2019 (FAO). Cephalopods as an object of aquaculture are only characteristic of Asia (Table 1-1). In 2016, aquaculture production in inland waters amounted to 51.367 thousand tons in live weight [2]. No other region of the world is engaged in this type of production, while the share of molluscs amounted to 286 thousand tons. In total, the volume of aquaculture amounted to 80.031 thousand tons, the share of molluscs is 17.139 thousand tons.

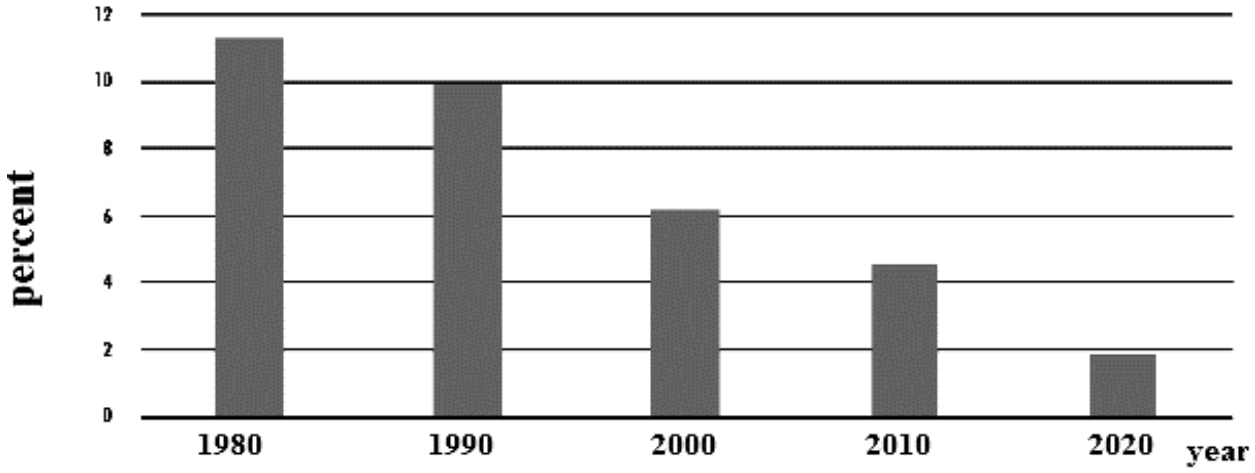
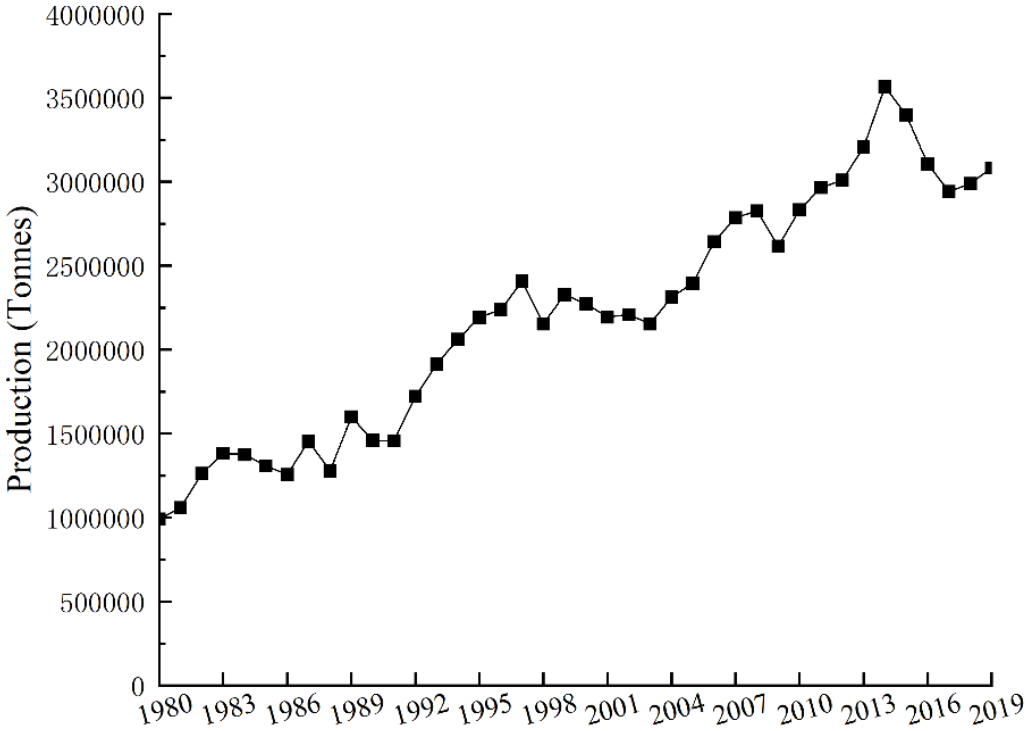


Figure 1.1 – Growth rates of world aquaculture, 1980–2020

Source from FAO (1980-2020)



Source from FAO (1980-2020)

Figure 1.2 – Marine molluscs production from 1980-2019

Table 1.1 – Manufacture of aquaculture products by continent, 2016 (thousand tons in live weight) (FAO)

Category	Africa	Americas	Asia	Europe	Oceania	Worldwide
Inland aquaculture						
Molluscs	-	-	286	-	-	286
Total	1954	1140	47765	502	5	51367
Marine and coastal aquaculture						
Molluscs	6	574	15550	613	112	16853
Total	28	2207	23781	2443	205	28664

The geographical distribution of aquaculture and the most significant producers are shown in Figure 1-3. According to the FAO, aquaculture is currently present in the economies of 202 countries and territories, with production being actively developed in recent years in 194 countries. The imbalance in the geographical distribution of aquaculture by region and by the government within particular areas, as before, is quite pronounced. Despite the increase in production in absolute terms, this situation has remained for ten years. For two decades, 89% of global aquaculture production has been in

China. Over the years, Africa, North and South America have slightly increased their shares of global output, while Europe and Oceania have reduced somewhat.

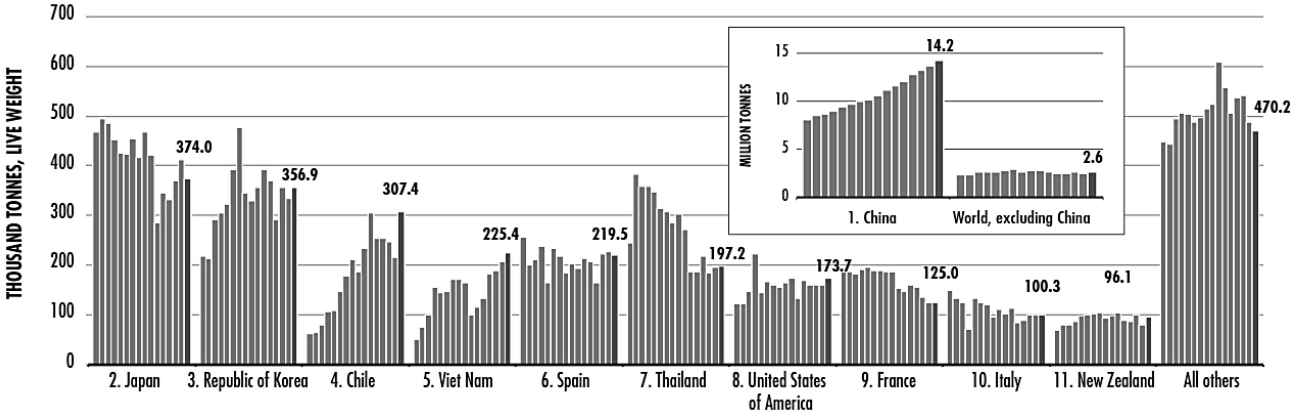


Figure 1.3 – Largest producers of cultured marine molluscs

Source from FAO (1980-2020)

1.1.2 Current status of squid processing

Argentinian squid, *Illex Argentines*, belongs to *Mollusc Cephalopoda Ommastrephidae Illex* [3]. The biological characteristics of squid are that its body is cylindrical, which is divided into a head, foot, and carcass. The head is short, the trunk is long, up to 600 mm, and the end is thin. There are ten arms and feet in front of the head, arranged around the mouth. When the head and trunk are folded, they look like a rocket Jane. The forward or backward speed can reach 40 ~ 50 km per 1 h [4]. At present, Commercial types of large-scale development include *Illex argentinus*, *Symplectoteuthis oualaniensis*, *Dosidicus gigas*, *Ommastrephes bartramii*, *Todarodes pacificus* ,and *Nototodarus sloani* [5]. The volume of cephalopods commercialized in the world during 2017 was 3772567 t (including Squids, cuttlefishes, octopuses) (data from FISHSTAT, FAO). Recent studies have shown that the Argentine squid in the Argentine waters has a wide distribution range and an enormous resource advantage, with typical distribution characteristics across continental slopes and deep seas. In addition, the quality of Argentine squid is also outstanding among soft fish, and the output is relatively considerable. As one of the essential components of the world's cephalopod resources, Argentine squid is being developed and utilized on a large scale worldwide [6]. Squid is extremely nutritious, low in fat and high in protein. Argentine squid carcass is rich in amino acids, and the ratio is close to the amino acid pattern provided by the U.S. Food and

Drug Administration (FDA). It is rich in two flavor amino acids, glutamic acid, and aspartic acid. From the perspective of lipid composition, the phospholipid content of Argentine squid is very high, accounting for 57.6% of the lipid, and the cholesterol content accounts for 21.3% of the lipid, which is higher than that of general fish and pork. Excessive cholesterol concentration in the blood can cause diseases such as atherosclerosis. However, the constant intake of squid does not need to worry about the increased cholesterol concentration in the blood [7]. The squid muscles do not accumulate fat, so the corpus callosum has low-fat content, and the main component of the lipid is the phospholipid, which is 40% to 45%. The fat of the squid mainly accumulates in the internal organs. The viscus of the squid is extremely high in oil content. The oil is mainly highly unsaturated fatty acids, from which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be extracted. EPA and DHA have the effect of enhancing brain function, improving memory, and preventing brain aging. In addition, the squid is also rich in taurine, lysine, diced carnitine, betaine, guanidine, nucleotides, sugars, trimethylamine oxide, and other flavor substances [7].

As a kind of invertebrate, the muscle protein of squid is slightly different from that of ordinary fish. The muscle protein of squid contains some paramyosin that has no ATPase activity to influence the protein stability, gel strength, and other properties of squid products [8], limiting the processing of squid. At present, the research on the processing technology of squid can divide into two categories. One is the optimization and improvement of traditional squid processing technology such as squid rings [9], dried squid [10], frozen squid products [11]. Another is the comprehensive processing and utilization of squid by-products or minced meat leftovers to extract biologically active peptides [12; 13] or squid flavor condiments [14]. Although many squid products have appeared in the consumer market, there are not many deep processing methods for Argentine squid, and there is a lack of variety.

In recent years, consumers worldwide have higher and higher requirements for food, not limited to delicious food, but also paying more attention to food quality, safety, and health. For processed products, people are more advocating green and natural. Healthy food, for aquatic products, people hope to enjoy the original flavor and texture of the

product and its nutrition from the everyday foods they usually eat. Long-term observations show that traditional aquatic products are processed by high temperature, high pressure, high salt, and dryness to kill microorganisms and inhibit their growth, affecting aquatic raw materials' soft texture and tasty flavors rich range of nutrients. Usually, the squid is frozen on the fishing boat immediately after salvage and then goes to the market for sale after landing. During transportation and storage, the squid protein undergoes structural changes, causing the squid muscle to become stiff and lose moisture due to prolonged freezing [15]. In addition, Kagawa et al. [16] reported that squid was considered in the rigid stage when refrigerated for four h, and the firmness decreased sharply with the extension of the refrigeration period. Squid muscles are composed of several layers of laterally extending muscle fibers covered with connective tissue [17]. Morales et al. [18] found that the differences were attributed to the distribution of myofibrils in squid muscles and their collagen content. Squid has a lot of edible parts, about 60% to 80%, and cooking loss is about 25-42%, which generally occurs in the first 15 minutes of the heating process [17]. Traditionally processed aquatic products can no longer meet people's requirements for recreational aquatic products. Over the years, efforts have been made to improve the moisture content of dried products, and to obtain the soft and juicy sensory properties of the products has been the pursuit of the aquatic products processing industry. To satisfy consumers' pursuit of returning to nature, high-value ready-to-eat aquatic products using mild heat treatment and other gentle processing methods have become research and development hotspots.

1.2 Effect of processing on meat quality

1.2.1 Quality changes during cooking treatment of meat products

Cooking meat produces physical and chemical changes that negatively impact meat product quality, such as cooking loss, nutrient loss, reduction in volume, color change, and toughness in texture [19]. Different cooking temperatures will have different effects. The core temperature inside the meat during the cooking process can be roughly divided into three stages.

In the first stage, the core temperature of the muscle ranges from 40 to 60°C. Myosin, the least thermally stable in this temperature range, is denatured first [20]. Subsequently, the higher-order structure of myofibrils and sarcoplasmic proteins changes and begins to degenerate [21]. At this stage, the main reason for the change in meat texture is the contraction of myofibrillar proteins [22], and the meat's firmness increases as the meat's juices are expelled from the cells. Degeneration of myofibrillar proteins results in shortened sarcomeres [23]. In addition, partial denaturation, and contraction of collagen, straightening the initially curled muscle fibers [24] and compressing the muscle fibers to reduce their diameter [25], also increase muscle stiffness.

In the second stage, the core temperature of the muscle is in the range of 60-80°C. In this temperature range, actin in muscle tissue begins to denature at 71-83 °C [26], and collagen also denatures at 60-70 °C. However, the denaturation temperature of muscle protein varies from animal to animals, such as that of beef at 69°C [27] and that of chicken at 65 °C [25]. The change of connective tissue is the main reason for the texture changes in the range of 60-80°C [22].

At this stage, the collagen in muscle tissue is further denatured, the helical structure is decomposed, the binding force of muscle fibers is gradually lost, and the diameter of muscle fibers gradually increases [25]. As the temperature increases, the myofibrils contract, and the muscle fibers separate from the endomysium and fascia, at which point the shearing force of the meat reaches a maximum [23]. Subsequently, the myofibrillar protein degrades, the fibrous structure becomes blurred, and the firmness of the meat begins to decrease. In this temperature range, the muscle will be the inner and outer sarcolemma contracted, the actin contract and dehydrated, and the muscle fiber diameter will become smaller. These lead to increased cooking losses [21].

In the third stage, the core temperature of the muscle is higher than 80°C. In this temperature range, collagen begins to gel [21; 28], muscle shear force decreases, and the meat becomes tender. The endomysium and fascia are almost completely destroyed [23] and flaky [21]. In the industrial production of meat, the water retention, muscle tissue structure, adhesion, and emulsifying properties of meat products are all affected by muscle protein [29]. Animal muscle proteins mainly include myofibrillar protein, sarcoplasmic

protein, and muscle matrix protein. Myofibrillar proteins are soluble in salt. Sarcoplasmic proteins are water-soluble. Muscle matrix proteins are insoluble in high ionic strength neutral buffers. Therefore, sarcoplasmic and muscle matrix proteins cannot form gels, and the influence on the quality of meat products can be ignored.

1.2.2 Quality changes of meat products during storage

Lysosomes are membrane organelles used to degrade cellular components in mammalian cells. They contain high concentrations of acid hydrolases, such as proteases, glycosidases, lipases, nucleases, and phosphatases. When these enzymes are released from the lysosome, enzymes degrade the components inside or outside the cell.

Histoproteinases are mainly found in lysosomes and are therefore also called lysosomal proteases. When $5 < pH < 7$, histone proteases are readily activated, while they become unstable when $pH > 7$ or $pH = 7$ (except for histone D, E, S). Histases are all hydrolyzed from an inactive precursor zymogen, consisting of a signal peptide, a precursor peptide, and a catalytic domain containing the active center of the mature protease. The signal peptide is responsible for transporting the ribosomally expressed precursor zymogen protein to the endoplasmic reticulum. It is hydrolyzed to form the zymogen containing only the precursor peptide and the catalytic domain. The precursor peptide is a high-affinity reversible inhibitor of the zymogen activity. It occupies the enzyme's active center, rendering the zymogen catalytically inactive. Subsequently, it is transported to the intracellular lysosome, automatically hydrolyzed under lysosomal acidic conditions to produce active cathepsin [30].

At present, more than 10 cathepsins have been purified and identified from fish and shellfish muscles and viscera, such as cathepsins A, B, C, D, E, H, L, L-like, X, and S. However, when studying the relationship between cathepsin and aquatic products gel degradation, most of the research focused on cathepsin B, H, L, and L-like enzymes. Compared with other cathepsins, cathepsins B, H, L, and L-like are the most active lysosomal proteases in the muscles of many fish species after death. In short, the effect of cathepsin on the processing characteristics of aquatic products, especially the rheological properties of the muscles after death, has attracted more and more attention from scientific and technological workers on the contribution of cathepsin to muscle degradation [31; 32].

In the process of stiffening fish muscles after death, the pH drops rapidly. That provides conditions for cathepsin to function. The role of cathepsin B, L, and D in the degradation of muscle protein varies. Some people think that the optimal pH of cathepsin B and L is closer to the pH of the postmortem muscle (6.0-7.0), while the optimal pH of cathepsin D is about 3.0, and it is impossible to reach such a low pH in the postmortem muscle. Therefore, cathepsin B and L may play a more significant role in muscle than cathepsin D [33; 34]. However, some scholars have proposed that cathepsin D has a more critical role in muscle degradation because cathepsin is content D is much higher than cathepsin B and L [35; 36]. In addition, studies have also found that cathepsin can also degrade collagen in muscle connective tissue [37]. The myofibrillar protein that cathepsin can degrade and its effect on muscle tissue structure are shown in Table 1-2.

Table 1.2 – The cathepsins degrading myofibrillar proteins in muscle and the effects on the muscle structure

Myogenic fibronectin degradation	Tissue Protease	Impact on organizational structure
Alpha-coactivator	B、D、L	Z-line weakening
Myoglobulin	B、D、L	Disruption of myogenic fiber structure
Promyoglobulin	L	Disruption of myogenic fiber structure
Troponin T	B、L	Disruption of myogenic fiber structure
30kDa fragments appear	B、L	Disruption of myogenic fiber structure
Actin	L	Disruption of myogenic fiber structure

1.3 Research progress of sous vide cooking and blue light sterilization

1.3.1 Sous vide cooking (SV)

In the 1970s, a Hungarian physicist, Professor Nicholas Kurti at the University of Oxford, United Kingdom, and a French physical chemist, Herve, organized a team of scientists and professional chefs, including Pierre Gilles de Gennes, a Nobel Prize winner in physics, to study cooking [38] which led to the proposal of the concept of molecular gastronomy in 1988. Hospitals first used SV technology to disinfect packaged foods to improve food safety and storage. In 1974, SV technology began to use in the production of gourmet food. Chefs Pralus G. and Troisgros P. found that the foie gras in the plastic bag was sealed and cooked at a precise temperature to minimize fat and moisture loss, thus

maintaining the best taste [39]. Simultaneously, the French scientist Goussault B. cooperated with fast food companies and hospitals to formally propose SV technology. In 1986, Goussault B. teamed up with the famous French chef Robuchon J. to create the first national catering project for the French National Railways and bring SV technology to other sizeable commercial foodservice organizations [39]. Later, this new cooking technique began to be introduced to the United States, the United Kingdom, and Canada and used by some of the world's top restaurants. At the beginning of the 21st century, Michelin-starred restaurants used SV technology in the kitchens. Despite these breakthroughs, SV technology is still only appearing in the field of professional chefs. Around the year, SV technology was taken seriously, and countries began to introduce cheaper and more portable immersive circulators, and the number of people using SV technology in restaurants and homes has increased dramatically. In the past 20 years, studies on SV cover various research interests, such as food safety [40], storage time [41; 42], quality improvement [43; 44], effects on nutrients [45], nutritional bioavailability [46], and various other technical approaches [47]. The research has also conducted in different types of meat and plant products (Table 1).

SV is a cooking method in which food is put into a vacuum bag and cooked under controlled temperature and time conditions [48]. SV is different from traditional cooking in many aspects. First, the cooking materials are put into the bag, vacuumed, and cooked at low temperatures. Such pasteurization conditions can avoid the risk of bacterial contamination while inhibits the growth of anaerobic bacteria in food during storage [49; 50]. Thus, the cooked food can be stored for a more extended period and rapidly cool down after cooking. Second, the cooking temperature and time can be precisely controlled. Vacuum-sealed packaging can effectively transfer heat while preventing oxidation and loss of volatile substances and moisture [51], resulting in a better flavor of foods [52]. The precisely controlled temperature and time not only reduce the negative effect of cooking on nutrients (e.g., proteins, lipids, vitamins) but also increase total phenolic content and antioxidant activity [53] and improve the overall texture and color of foods [48; 54-57]. Christensen et al. [58] found that low temperature and long time (LTLT) heating can reduce the strength of connective tissue and increase the solubility of collagen, which

makes the beef as soft as veal. Roldan et al. [59] found the lamb loins tenderness positively correlated with cooking time (6 h, 12 h, and 24h). However, as the cooking temperature increased, the weight loss of lamb loins increased, and the moisture content decreased. SV can get the lamb loins for better brightness and redness, and safety at 60 °C. Besides, the beef cooked by SV (59 °C, 5 h) did not increase thiobarbituric acid reactive substances (TBARS) and warmed-over flavor (WOF) after 30 d of storage [60]. Scholars have also evaluated the safety of SV. After cooking at 50-62 °C, the number of mesophiles and *psychrotrophic* bacteria in beef decreased significantly [49; 60; 61]. Moreover, the number of *Escherichia coli* and mesophiles in pork cooked at 53°C for several hours also decreased significantly [62; 63]. To reduce the nutrient loss while optimizing the overall quality and shelf life of meat during cooking, lots of food processors use the SV technique to replace the traditional cooking methods (frying, microwaving, and grilling) [64; 65]. So far, scholars have only focused on the research of SV cooking technology on protecting food nutrients, quality improvement, and food safety. In addition, they think that the lower cooking temperature of SV produces less flavor [66; 67].

For decades, SV has proved to be safe by some scientists. The precisely controlled temperature and time of SV technology have been demonstrated to improve the flavor, texture, and color of food and preserve its nutritional values and prolong its storage time, a quality that cannot be obtained from traditional cooking methods. However, vacuum and heating at a lower temperature reduce or hinder fat oxidation and Maillard reaction, reducing food flavor. Besides, packaging also prevents the spillage of more defective volatile components. SV's challenges are enriching the flavor of SV food and improving the appearance color of SV animal-derived food to replace traditional cooking methods. The future trend is from restaurants to food factories, adopting SV technology at all levels of catering services and industrializing and standardizing it. Therefore, studies on SV in various aspects, such as structural changes of food and the mechanism of food quality, to prolong food storage time while maintaining its quality can increasingly become new research interests.

1.3.2 Blu-ray sterilization technology

In recent years, the incidence of foodborne diseases caused by pathogenic microorganisms has increased gradually [68]. People have paid significant attention to food safety and quality, mainly during global food shortages after natural disasters and epidemic outbreaks. Because microorganisms are susceptible to temperature, heating has been employed mainly as one of the conventional sterilization techniques to kill microorganisms in food. Heat sterilization technology is widely used in the food industry, which further guarantees food safety. For example, the canning process uses heat treatment technology to sterilize. Heat sterilization technology has been used in the food industry for many years to ensure food safety, extend shelf life, and maintain food quality [69-71]. However, heat sterilization technology could also harm the quality of the food. The high temperature during the sterilization process may change the food's color, flavor, and nutrient loss [72]. Consumers' demand for sensory and nutritional food quality increases day by day, requiring safety and not compromising food quality. Control of microorganisms in food without compromising nutritional and organoleptic properties. Non-thermal disinfection technology to minimize the loss of various nutrients in food, try to maintain the original flavor of food and improve the economy of disinfection technology, convenience, improve food packaging and storage conditions, extend the shelf life of food to meet the needs of the growing material life of consumers.

Light sterilization is a non-pharmacological technology, including photodynamic therapy (PDT) and ultraviolet radiation (UVC), and has been widely studied as an alternative to traditional antibiotics [73]. The advantage of light sterilization is that the killing effect is the same regardless of the bacteria's antibiotic resistance. Blu-ray (Blu-ray) sterilization technology selects Blu-ray for light sterilization, and its wavelength is 405-470 nm. Blu-ray can sense by Gram-positive bacteria, Gram-negative bacteria, and fungi and induce Blu-ray receptors to cause physiological reactions [74]. It has potential antibacterial or bactericidal ability without photosensitizers. Also, Blu-ray is less harmful to mammalian cells [75]. The mechanism of Blu-ray sterilization is cell death induced by oxidative stress caused by reactive oxygen species (ROS) generated by the endogenous photosensitizers of bacteria after absorbing Blu-ray [76; 77]. Photosensitizers in the

ground state are converted to their single or trilinear states upon irradiation with Blu-ray, in the presence of oxygen, undergoing two types of energy transfer: type I that produces toxic oxygen species, such as hydrogen peroxide (H_2O_2), superoxide, or hydroxyl radicals; (2) type II that generates $^1O_2$ [78; 79].

Blu-ray sterilization in the medical field is more research application. Hamblin [80] et al. found that the killing rate of *Helicobacter pylori* was 99.9% when irradiated with 405 nm Blu-ray, and Fukui [81] et al. showed that Blu-ray (405 nm) had a significant inhibitory effect on the growth of *Porphyromonas gingivalis* (more than 75% inhibition compared to unirradiated), while Blu-ray at 430 nm and longer wavelengths did not significantly inhibit their growth. In clinical studies, pulsed Blu-ray also showed significant improvement in the treatment of *acne vulgaris* [82; 83]. Because of the effectiveness of Blu-ray sterilization and the absence of any thermal effect, scholars noticed it in the food field. Dos Anjos [84] et al. showed that after Blu-ray irradiation (413 nm, <2 h, 720 J/cm^2), all *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella Typhimurium*, and *Mycobacterium fortuitum* presented a 5 log inactivation in milk. Blu-ray irradiation (460 nm, 4°C, irradiance 92 mW/cm^2) reduced *Salmonella* in orange juice by 3.3 lg CFU/mL, and the same irradiance reduced *Salmonella* by 3.6 lg CFU/mL when the sterilization temperature was increased to 12°C. At the same time, *Salmonella* achieved a reduction of 4.8 lg CFU/mL when the temperature was increased to 20°C [85]. Blu-ray sterilization is still effective for solid foods. It was found that Blu-ray irradiation (460 nm) can sterilize fresh-cut fruits without any food additives, and Blu-ray irradiation (irradiance 92, 147.7, and 254.7 mW/cm^2) when used at ambient temperatures of 7°C and 16°C, can inhibit *Salmonella spp*. inoculated on the surface of fresh-cut pineapples [86]. Josewin et al. [87] used Blu-ray (460 nm) in combination with riboflavin (25, 50, and 100 μM) for sterilization and found that Blu-ray at a dose of 2.4 kJ/cm^2 reduced *Listeria monocytogenes* on the surface of smoked salmon by 0.7-1.2 lg CFU/cm^2 at 4°C and 12°C. Thus, it can be seen that the Blu-ray non-thermal sterilization technology in the food field is a relatively wide range of applications. However, although Blu-ray is harmless to the operator's skin, visual impairment is a concern, and operational standards should be developed to ensure safety.

1.4 Chapter summary

1. We analyzed the current situation of squid fishery and processing, the factors affecting the quality of meat products (including changes in heat treatment methods and storage period), and summarizes that the deep processing of squid products still needs to be further improved.

2. The advantages and characteristics (reducing cooking losses, improving color, and preventing oxidation during heating and storage) of SV as a new cooking method are reviewed, and the research progress.

3. The application research, advantages, characteristics, and sterilization principle of Blu-ray cold sterilization technology was reviewed.

CHAPTER 2 METHODOLOGY

This chapter introduces the experimental protocol in the study, defines the research topic and materials, describes the research method, and analyzes and processes the experimental data.

The research was carried out at Henan Institute of Science and Technology in China, Sumy State Agricultural University in Ukraine, and Sanjia Food Co., Ltd. in Henan Province, China.

2.1 Materials of research

The thesis work was carried out in conjunction with the Sumy National Agrarian University and Henan Province Key R&D and Promotion Projects (212102110022 and 212102110017).

Consumables

Centrifuge tube, petri dish, gloves, syringes and needles, cotton wool, methyl alcohol, test tubesgas ,nitrogen gas (99.99% purity), FS-SE-54-CB-1 capillary column (15 m × 0.53 mm, 1 μm).

Equipment

1. furnace (SXZ-12-10, Shanghai Jingsheng Scientific Instrument Co., Ltd., China); 2. Soxhlet extractor (Zhejiang Sayin Scientific Instruments Co., Ltd., China); 3. electric blast dryer (DHG-9146A, Shanghai Jinghong Experimental Equipment Co., Ltd., China); 4. gas chromatography analyzer (Shimadzu JC-7A); 5. vacuum sealer (DZ-260, Dajiang holding group electric co. LTD, China); 6. SV machine (Anova Culinary, A3.2-120V, United State of American); 7. electronic nose (PEN 3, Germany AirSense); 8. GC-IMS Flavor analyzer (FlavorSpec®, Dortmund, Germany); 9. colorimeter (CR-400, Konica Minolta Holding Company, Japan); 10. spectrophotometer (7200, Unico Shanghai Instruments Co., Ltd.); 11. texture meter (TA.XTC, Shanghai Baosheng Industrial Development Co., Ltd.); 12. light-emitting diode (LED) (460−475 nm); 13. pH meter (PHS-3C, Shanghai Yidian Scientific Instruments Co., Ltd.); pH meter (PHS-3C, Shanghai Yidian Scientific Instruments Co., Ltd.).

Chemicals and solvents

The following chemical reagents are of analytical grade and purchased in Sinopharm Chemical Reagent Beijing Co., Ltd, China. Ethanol, magnesium acetate solution, glucose solution, anthrone reagent, sulfuric acid solution, boric acid solution, sodium hydroxide solution, phosphate buffer solution, trichloroacetic acid, 2-thiobarbituric acid, casein solution and water (ISO 10304-3: 1997 Water quality - Determination of dissolved anion by liquid chromatography of ions - Part 3: Determination of chromate, iodide, sulfite, thiocyanate and thiosulfate).

2.2 Research Methods

Food science, bacteriological, pharmacological, toxicological, and statistical.

2.2.1 Sample preparation

The Argentinian squid (about net weight 400 g) was chosen the same size and purchased from an aquatic products market in Qingdao city, China's Shandong Province. The squid specimens were kept refrigerated with flake ice inside polystyrene boxes provided with a lid and holes for drainage and transported to the laboratory at -18°C. Afterward, and just before cooking, squid specimens separated into the head, foot (wrist), and ketone body with scissors after thawing and washing. The average weight of the ketone body of squid was 20±4 g (n=16), respectively. Each squid specimen's length, width, and thickness of the ketone body of squid were 4±1 cm, 4±1 cm, and 1±0.4 mm. All the squid were randomly divided into four groups to prepare for processing and kept at 4°C until further analysis. From each specimen, one specimen was kept raw (RAW) as a control, and the others were submitted to cooking.

2.2.2 Processing method

1. Boiling (BO)

The samples were cooked in hot water (100°C, 5 min).

2. Steaming (ST)

The samples were cooked in the steam oven (100°C, 5 min).

3. SV

The samples were into a plastic vacuum bag and sealed using a vacuum sealer (DZ-260, Dajiang holding group electric co. LTD, China) and using SV machine (Anova

Culinary, A3.2-120V, United State of American) heated in water baths (preheating experiment temperature)[88], timed experiment time.

All the samples were soaked in ice water after cooking for 20 min and stored at -20°C until analysis., including raw materials as a control group.

2.2.3 Sensory evaluation

Refer to the ISO standards (ISO 11136:2014, Sensory analysis—Methodology—General guidance for conducting hedonic tests with consumers in a controlled area). The sensory evaluation team consists of 20 people between 18 and 25 years old, all of whom have undergone sensory evaluation training. A hedonic scale method is adopted from 1 to 9 points, where 1 means extremely disliked and 9 means extremely liked [89].

2.2.4 Determination of meat harvesting rate

The squid was thawed, weighed (W_1), and weighed (W_2) after removing the head, bones, skin, and internal organs. The formula was calculated as follows.

$$Meat\ harvesting\ rate = \frac{W_2}{W_1} \times 100\%$$

In the formula, W_1: the whole squid weight (g), W_2: remove the head, tail, bones, skin, and internal organs after the weight (g).

2.2.5 Moisture content determination

Refer to the ISO standard (ISO 1442:1997) and slightly modified. Samples of 200 g were ground at least twice in a meat grinder to homogenize them and mix them well. The ground samples were stored in a sealed container to prevent deterioration and compositional changes during storage, and the samples were analyzed no later than 24 h. The squid (3-4 times the weight of the sample) and the glass rod and the weighing bottle were placed in a drying oven at 103±2 °C, with the cap diagonally supported on the side of the bottle, heated for 30 min, removed, and covered, placed in a desiccator, cooled to room temperature, and weighed to the exact size. Add 5-10 mL of ethanol, place the weighing bottle and its contents in water (60-80℃), stir continuously, and wait for the ethanol to evaporate completely. The weighing bottle and contents were moved into a drying oven for 2 h. The bottle was removed and placed in a desiccator to cool to room temperature, weighed precisely in m3, and then placed in a drying oven for 1 h until the difference

between the two consecutive weighing results did not exceed 0.1%. The calculation formula is as follows.

$$Moisture\ content = \frac{m_2 - m_3}{m_2 - m_1} \times 100\%$$

In the formula, m_1: bottle weight (g), m_2: total weight of sample and bottle (g), m_3: total weight of sample and bottle after drying (g).

2.2.6 Ash content determination

Refer to the ISO standard (ISO 936:1998) and slightly modified. The 200 g sample was homogenized in a meat grinder twice, mixed well, and then packed into a sealed container to prevent spoilage and compositional changes (within 24 h). The crucible was cauterized in a muffle furnace (SXZ−12−10, Shanghai Jingsheng Scientific Instrument Co., Ltd., China) at 550−600°C for 30 min, removed when the furnace temperature dropped below 200 °C, cooled to room temperature in a desiccator, weighed accurately to 0.0001 g, and repeatedly cauterized to a constant weight. Then put 5 g of the specimen into the crucible, spread it evenly, and weigh it again to 0.0001 g. Precisely aspirate 1 mL of magnesium acetate solution and add it evenly dropwise onto the specimen in the crucible. Put the crucible into a slightly boiling water bath for 30 min, and then gradually heat it on an electric or gas furnace to make the specimen fully carbonized until it is smokeless, and then move the crucible into a muffle furnace with the temperature-controlled at 550-600 °C, and let the specimen burn at this temperature for at least 30 min to make it ash completely. If the ash contains black particles, put the crucible back into the Muffle furnace and re-operate according to the above procedure so that the difference of successive weighing shall not exceed 1 mg. The same specimen is measured three times, and a blank test is done at the same time. The calculation formula is as follows.

$$Ash\ content = \frac{m_3 - m_1 - m_0}{m_2 - m_1} \times 100\%$$

In the formula, m_0: mass of magnesium oxide produced by adding magnesium acetate (g), m_1: mass of crucible (g), m_2: mass of crucible and sample (g), m_3: mass of crucible and ash (g).

2.2.7 Determination of crude fat content

Refer to the ISO standard (ISO 1443:1973) and slightly modified. All parts of the Soxhlet extractor (Zhejiang Sayin Scientific Instruments Co., Ltd., China) were washed and dried with distilled water, and the bottom vial was dried to a constant weight (the difference between the first and second weighing was not more than 0.002 g) in an electric blast dryer (DHG-9146A, Shanghai Jinghong Experimental Equipment Co., Ltd., China) at 103±2°C. Add the appropriate amount of sea sand and stir continuously with a flat glass rod at one end until it is loose. Wipe the weighing dish and glass rod and put them into the filter paper tube together. Plug the top of the filter paper cylinder with a small amount of skimmed cotton. Place the dried cartridge with the sample into a drying oven at 103±2°C for 2 h. Place the dried cartridge with the sample into a Soxhlet extraction cartridge, connect the bottom vial, which has been dried to a constant weight, and inject the extraction solution to above the height of the siphon tube. After the extraction solution flowed, the extraction solution was added to one-third of the siphon tube height and connected to the reflux condenser. Submerge the bottom flask in a water bath and heat it. Gently plug the upper opening of the condenser tube with a small piece of skimmed cotton. The water bath temperature should be controlled so that the extraction solution refluxes every 6-8 min. At the end of extraction time of 6-12 h, pick up a drop of an extract with a hairy glass plate, and if there is no oil spot, the extraction is completed. After extraction, the extraction solution was recovered. Remove the bottom vial, evaporate on a water bath, and remove the residual extract. Wipe the exterior of the bottom vial with degreasing filter paper, dry in a drying oven at 103±2 °C for 1 h, remove, cool to room temperature in a desiccator, and weigh. Repeat drying for 0.5 h, cooling, and weighing until the difference between the two weighings does not exceed 0.002, which is the constant amount. The minimum weighing shall prevail. The calculation formula is as follows.

$$Fat\ content = \frac{m_2 - m_1}{m} \times 100\%$$

In the formula, m_1: mass of bottom bottle (g), m_2: mass of bottom bottle and crude fat (g), m: mass of sample (g).

2.2.8 Determination of crude protein content

Refer to the Chinese Food Standard (GB 5009.5-2010) and slightly modified. The sample should be at least 200 g, ground at least twice with a meat grinder, mixed well, weighed 0.5-5 g of the sample, and determined according to GB 5009.5-2010 (Chinese National Standards) method.

2.2.9 Determination of total sugar content

Referring to the method of Somani [90] et al. with minor modifications. Make a standard curve: accurately pipette 0.10 g/L glucose solution 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 mL, distilled water to 1.00mL; add 4.00mL anthrone reagent, respectively. Quickly immerse in an ice water bath to cool down. After adding each tube, immerse it in a boiling water bath together, and cover the mouth of the tube with a glass ball to prevent evaporation. Re-boil from the water bath, boil accurately for 10 minutes, take it out, cool with tap water, leave it at room temperature for about 10 minutes, and compare the color at 620 nm. Take the double-distilled water treated in the same way as a blank for color comparison. Take the optical density as the ordinate and the micrograms of the sugar content as the abscissa to make a standard curve. The regression equation is y=10.302x-0.0075, and the correlation coefficient R^2=0.9992.

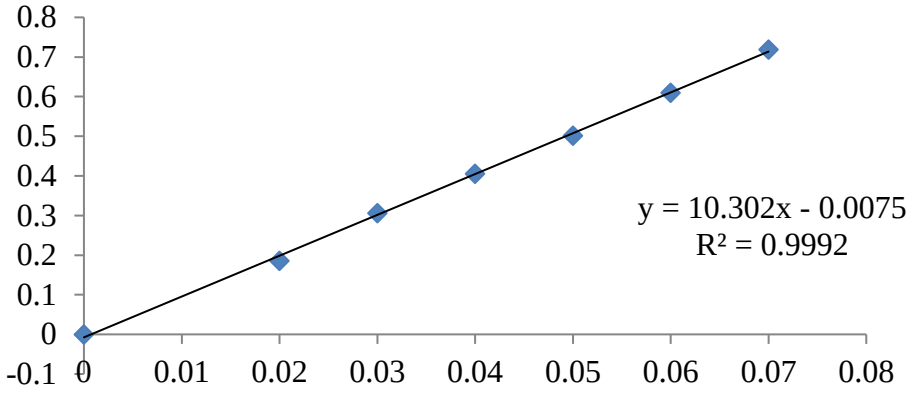


Figure 2.1 – Standard curve of total sugar

Take 200 g of the sample, put it in a meat grinder at least twice to mix it well, and store it in an airtight container at 4°C in a refrigerator. Weigh about 10 g (accurate to 0.001 g) of the above-ground squid meat in a beaker, add 100 mL of distilled water, heat it

on a boiling water bath for 30 min, cool it, and then fix the volume to 150 mL. After mixing, filter the filtrate and set it aside.

Determination of total sugar content: Take 1.00 mL of the above sample filtrate, add anthrone reagent, operate the same as the standard curve, and determine colorimetrically. The content was calculated according to the standard curve and the concentration of the sample.

2.2.10 Protein content determination

Protein content was determined using the Kjeldahl method to determine the protein content in squid. The sample (0.5g) was put into a 100mL nitrogen-fixing flask, mixed catalyst and 20mL sulfuric acid solution were added, transferred to a fume hood, and heated on an electric furnace or graphite furnace for digestion. After the sample was carbonized, the temperature was increased, and the liquid in the bottle was kept at a slight boil until the liquid was light green and transparent and then digested for 1 h. After decomposition, cool, rinse the bottle wall with water, calm, and fix the volume to 100 mL. The sample was then cooled down, rinsed with water, cooled and volumized into a 100 mL volumetric flask, and shaken. Do the blank at the same time. Connect the nitrogen-fixing distillation apparatus, add water to 2/3 in the 500mL distillation bottle, put a few glass beads, add three drops of methyl red indicator and 1mL of sulfuric acid distilled water acidic, heat the water in the distillation bottle to keep it boiling.

Open the device condensate and the receiving bottle, add 10.0mL boric acid solution and three drops of mixed titration indicator solution. The receiving bottle is placed in the lower end of the condensate tube inserted into the liquid level. Precisely aspirate 10.0mL of the pretreatment digestion solution into the nitrogen-fixing device bottle, rinse the bottle wall with a bit of water, add 20mL of 40% sodium hydroxide solution along the bottle wall of the nitrogen-fixing device, and seal the device immediately. Open the distillation for about 10 min, move the receiving bottle to make the lower end of the condenser tube leave the liquid surface, and continue to distill for 2 min. After distillation, rinse the lower part of the condenser tube with a bit of water, remove the receiving bottle, and titrate with sulfuric acid standard titration solution until the mixed indicator solution is light gray-red. Also, do the blank control.

2.2.11 Isolation and determination of various protein components in squid muscle

Refer to Hashimoto's [91] method with slight modifications. Weigh 5 g of squid muscle, add 50 mL of phosphate buffer solution (0.05 mol/L, pH 7.5), and mash with a high-speed tissue masher for 2 min. The supernatant was collected, and the precipitate *A1* was added to 50 mL of the above phosphate buffer. The supernatant was then collected, and precipitate *A1* was added to 50 mL of the above phosphate buffer solution and the above operation was repeated, and the supernatant obtained by combining the two centrifuges was added to 15% trichloroacetic acid solution. The supernatant from the two centrifugations was combined, and a final concentration of 5% was obtained by adding 15% trichloroacetic acid solution and then centrifuged at 9000 rpm for 15 min at 4 °C to obtain precipitate *A2* (i.e., sarcoplasmic protein fraction). To precipitate *A1* obtained above, add ten times the phosphate buffer solution (0.05 mol/L, pH 7.5) containing 0.6 mol/L NaCl. The above operation was repeated once, and the supernatant was combined twice to obtain fraction *B* (myofibrillar protein fraction) and precipitate. The supernatant was combined twice to obtain fraction *B* (myofibrillar protein fraction) and precipitate *C* (matrix protein fraction). After the above proteins were isolated, their contents were determined by micro Kjeldahl. The contents of the above proteins were determined by the micro Kjeldahl method.

2.2.12 Fatty acid determination

Refer to the ISO Standard (ISO 1443:1973) and slightly modified. Fatty acids were measured using a gas chromatography analyzer (Shimadzu JC-7A) according to AOAC Method (11th Edition) (AACC International Approved Method 58-18.01).

2.2.13 Amino acid determination

Samples were analyzed using an automated amino acid analyzer (Hitachi Model 835-50).

2.2.14 Electronic nose analysis

The electronic nose (PEN 3, Germany AirSense) was used to preliminarily evaluate the aroma profile of the squid samples. It was conducted by the following procedure. Taking the center of the squid sample to be tested, each sample (1 g) was prepared in a 20

mL gas chromatographic analysis bottle at room temperature for 30 min. The injection rate was 600mL/min, the rate of carrier gas was 600mL/min, the measurement time was 60 s. The cleaning time varied from 180 s due to the different sample odours. The parameters were optimized in detail, and each analysis was repeated 15times.

The types of sensitive substances corresponding to the ten sensors of the electronic nose are as follows: W1C: aromatic hydrocarbon compounds; W5S: nitrogen oxide compounds; W3C: ammonia, aromatic molecules; W6S: hydride; W5C: olefins, aromatic, polar molecules; W1S: alkane Class; W1W: sulfur compounds; W2S: alcohols, partially aromatic compounds; W2W: aromatic compounds, organic sulfur compounds; W3S: alkanes and fats.

2.2.15 Determination of volatile components by HS-GC-IMS

2.2.15.1 Isolation of volatile organic compounds in squid

Volatile compounds of squid in different cooking methods identified by a GC-IMS Flavor analyzer (FlavorSpec®, Dortmund, Germany). Each squid sample (2 g) package was placed in a 20 mL headspace vial sealed and subsequently incubated at 60 °C for 15 min. Finally, 500 μL headspace was injected automatically by using a heated syringe (65 °C) into the heated injector of the GC-IMS equipment under conditions reported below. Three parallel samples took from the same processing for analysis.

2.2.15.2 HS-GC-IMS apparatus

After that, the carrier gas (nitrogen gas, 99.99% purity) that passed through the injector inserted the sample into the FS-SE-54-CB-1 capillary column (15 m × 0.53 mm, 1 μm) which heated at 60 °C for timely separation.

2.2.15.3 Chromatographic Conditions

For analysis, a headspace volume of 500 μL sampled at a speed of 150 μL/s and a syringe temperature of 65 °C to avoid condensation effects. The flow rate of carrier gas programmed: 2 mL/min held for 2 min, linear rose to 100 mL/min within 18 min, total running time 20 min. For avoiding cross-contamination, the syringe automatically flushed for 30 s using nitrogen before each analysis and 5 min after each analysis.

The n-ketones C4-C9 (Sinopharm Chemical Reagent Beijing Co., Ltd, China) was as external references to calculate the retention index (RI) of each volatile compound.

According to comparisons of RI and the drift time (DT) with the GC × IMS Library Search (FlavorSpec®, Dortmund, Germany), the volatile compounds from samples identified in different cooking methods. Volatile compounds were identified by comparing IMS drift time (the time it takes for ions to reach the collector through drift tube, in milliseconds) and retention index with those of the authentic reference compounds. The signal intensity represents the height or the peak area. Using Laboratory Analytical Viewer, Reporter, Gallery Plot, Dynamic PCA, and GC × IMS Library Search database supported by HSGC-IMS instrument, three-dimensional (3D) and two-dimensional (2D) fingerprint maps of the volatile organic components of squid in different cooking methods constructed respectively. The Gallery map used for principal component analysis (PCA) analysis of volatile substances of squid in different cooking methods.

2.2.16 Determination of cooking loss

Cooking loss was expressed as a percentage of the initial weight [92] :

$$\text{Cooking loss (\%)} = \frac{\text{Weight loss after cooking}}{\text{Initial sample weight}} \times 100$$

2.2.17 Color measurement

The samples were measured using a colorimeter (CR-400, Konica Minolta Holding Company, Japan). The color was described using the CIELAB scale: L^*, a^*, and b^*. Measurements were performed five times per sample on different surface points.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

$$\text{Whiteness} = \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

2.2.18 Determination of TBARS

For extraction, the sample (20 g) was first mixed with 25 mL of aqueous 20% trichloroacetic acid (TCA) solution and 15 mL of distilled water. The mixture was homogenized and allowed to stand at 25°C for 1 h. After centrifugation for 10 min (3000 rpm), the filtrate was diluted with distilled water to 50 mL. Next, 2 mL of the fresh filtrate was mixed with 2 mL of 0.02 M aqueous 2-thiobarbituric acid (TBA) solution, placed in a water bath in a cuvette containing a stopper at 95°C lasting 30 min. And then cooled under tap water. The spectrophotometer was calibrated at 532 nm with distilled water, and then the sample absorbance was measured. The colorimetric absorbance obtained from the

spectrophotometer (7200, Unico Shanghai Instruments Co., Ltd.) was converted to mg malonaldehyde/kg meat to represent TBA content.

2.2.19 pH determination

Refer to the ISO standard (ISO 2917:1999) and slightly modified.Sample (5g) was homogenized three times with 50 ml of distilled water for 30 s each. The filtrate (25 mL) was taken after homogenization, and pH was measured using a pH meter.

2.2.20 Microbiological analyses

Refer to the ISO standard (ISO 4833-1:2013, Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique, the following used this method) and slightly modified. The samples were placed in 10 mL of phosphate buffer saline (10 mM, pH=7.4, NaCl 8 g, KH_2PO_4 0.24 g, $Na_2HPO_4 \cdot 12H_2O$ 3.63 g, KCL 0.2 g, distilled water 1 L) in a centrifuge tube and sonicated for 5 min. They were then centrifuged at 5000 rpm. The supernatant after gradient dilution (10^0-10^7) was taken for 5 min and incubated (37°C, 7-9 h) in Luria-Bertani medium (pH=7.4-7.6, tryptone 10, yeast extract 5 g, NaCl 10 g, agar 15 g, distilled water 1 L) and counted.

The samples were placed in 10 mL of phosphate buffer saline (10 mM, pH=7.4, NaCl 8 g, KH2PO4 0.24 g, Na2HPO4·12H2O 3.63 g, KCL 0.2 g, distilled water 1 L) in a centrifuge tube and sonicated for 5 min. They were then centrifuged at 5000 rpm. The supernatant after gradient dilution (100-107) was taken for 5 min and incubated (37°C, 7-9 h) in Luria-Bertani medium (pH=7.4-7.6, tryptone 10, yeast extract 5 g, NaCl 10 g, agar 15 g, distilled water 1 L) and counted. The number of Psychrobacter was determined after incubating the plates at 5°C for 72 h.

2.2.21 Response surface methodology

2.2.21.1 Single-factor experiments

The cooking temperature was set to 60°C, and the sauce (salinity 5%) and the effect of cooking time (10 min, 20 min, 30 min, 40 min, 50 min) on the sensory evaluation of SV squid were investigated.

The cooking time was set to 60 min and the sauce (salinity 5%), and the effect of cooking temperature (50℃, 55℃, 60℃, 65℃, 70℃) on the sensory evaluation of SV squid was investigated.

The cooking temperature was set to 60°C and the cooking time was set to 30 min, and the effect of the sauce salt concentration (0%, 2.5%, 5%, 7.5%, 10%) on the sensory evaluation of SV squid was investigated.

2.2.21.2 Response surface optimization test

According to the principle of Box-Behnken central combination design, a three-factor, three-level response surface analysis was conducted based on a single-factor test with cooking temperature, cooking time, and sauce salinity as independent variables and sensory scores as response values, and the test factor levels are shown in Table 2.1.

Table 2.1 – Response surface test design

	Factor		
	-1	0	1
A-Cooking temperature（℃）	55	60	65
B-Cooking time（min）	20	30	40
C-Salinity（%）	2.5	5	7.5

2.2.22 Shear force (SF) determination

Referring to the method of Baublits et al. [93], the cooked samples were divided into 2×2×0.5 cm cubes, and samples were sheared along the muscle fibers in a vertical direction using a texture meter (TA.XTC, Shanghai Baosheng Industrial Development Co., Ltd.) at 20°C. The force required to shear the samples were recorded in Newton (N).

2.2.23 TCA-soluble peptide determination

Refer to the method of Sriket et al. [94] with a slight modification. Weighed 2 g of minced squid sample and added 18mL of 5% trichloroacetic acid solution. Homogenized the mixture with a high-speed disperser for 1 min and then ice bath for 30 min—centrifuged at 11000 g for 10 min at 4°C. The Lowry method determined the content of

peptides in the supernatant [95], and the results were expressed in μmol Tyr/g meat. Each group of samples should be made with 3 parallel samples.

2.2.24 Measurement of myofibril fragmentation index (MFI)

Refer to the method of Li et al. [96] of measuring MFI with slight modifications. Weighed 2 g minced sample, put it into a 100 mL centrifuge tube, added 20 times volume of MFI buffer (100 mmol/L KCL, 11.2 mmol/L K_2HPO_4, 8.8 mmol/L KH_2PO_4, 1 mmol/L EGTA, 1 mmol/L $MgCl_2$, 1 mmol/L NaN_3) at 2℃, homogenized 3 times at high speed, the 20 s each time (interval 1 min) and then filtered it. Removed the connective tissue in the homogenate, then placed it in a 4000 r/min, 2℃ refrigerated centrifuge for 10 min, removed the supernatant, and added 20 times the volume of 2℃ MFI buffer to the centrifuge tube, Fully dissolved, shook well, made a liquid suspension, and then centrifuged for 10 min in a 4000 r/min, 2℃ refrigerated centrifuge, took it out, poured out the supernatant, and dissolved the precipitate with 2.5 times the volume of 2℃ MFI buffer as a suspension, poured it into a volumetric flask, then rinsed the centrifuge tube with 2.5 times the volume of MFI buffer at 2℃, and poured the cleaning solution into the volumetric flask, mixed well, and took 1 mL of the suspension measured the concentration of protein in the suspension by the double constriction method, and then diluted it with MFI buffer at 2℃ to made the concentration of the suspension protein 0.5±0.05 mg/mL, then took 1 mL of the diluent and used the biuret. Measured the absorbance value *A* at 540nm by the method, and multiplied the result by 200 to get the MFI value.

$$MFI = A_{540} \times 200$$

2.2.25 Determination of the myofibril apparent diameter

Refer to the method of Culler [97] with slight modification. After adding 2 g of sample to 30 mL of pre-cooling buffer (100 mmol/L KCl, 20 mmol/L K_3PO_4, 1 mmol/L EDTA, 1mmol/L $MgCl_2$, pH 7.2): Homogenized at 4℃ for 1min. Then centrifuged at 1000 g for 15min. Repeat the above operation for the resulting pellet. Suspend the resulting pellet in 15 mL of the above buffer. After taking 1.5 mL and measuring the particle size distribution with a particle size analyzer, the particle size is calculated according to the refractive index of a regular sphere 1.54. The apparent diameter of myofibrils was expressed by the average particle size (MA) calculated by the surface area.

2.2.26 Total proteolytic activity assay

Refer to the method of Sriket et al. [98] with slight modification. Added 3 times the volume of phosphate buffer (pH 7.6) to the ground meat sample, placed it in an ice bath (<4°C) after homogenization, and stirred continuously for 30 min with a magnetic stirrer. The supernatant after centrifugation at 10000 g for 30 min was the crude enzyme solution. Pipetted 200 μL of crude enzyme solution, added 200 μL distilled water and 625 μL reaction buffer (0.2 mol/L Na_2HPO_4, 0.1 mol/L citric acids, pH=5.0), and finally, added 200 μL of 10 g/L casein solution, and reacted in a constant temperature water bath at 50°C for 15 min. Added 200μL of 50% TCA solution to stop the reaction, centrifuged at 10000 g, 4℃ for 10 min, and determined the peptide content in the supernatant by Lowry method [95]. The amount of hydrolyzing casein and releasing 1 μmol of tyrosine per minute at 50°C was defined as 1 unit of enzyme activity (U).

2.2.27 Microstructure observation

SV squid samples (5mm×5mm×2mm) were fixed in 2.5% glutaraldehyde solution at 4°C for 48 h and then rinsed 3 times with 0.2 mmol/L phosphate buffer (pH 7.2) for 15 min each. Then, gradient dehydration was performed with ethanol (50%, 15 min; 70%, 15 min; 90%, 15 min), and finally dehydrated 3 times with anhydrous ethanol for 10 min each. The samples were pre-frozen overnight in a deep-freeze refrigerator at -50°C and then dried under vacuum in a freeze dryer for 24 h. The samples were taken out and sprayed with gold, and the microstructure was observed by electron microscope.

2.2.28 Blu-ray irradiation

We used a light-emitting diode (LED) (460−475 nm) to treat samples. The LED luminance (Lux) was measured using a digital light meter (Lutron-LX-101A, Lutron electronic enterprise Co. ltd, Taiwan, China). Further, the irradiance (W/cm^2) of the LED bulb was calculated using photometric conversion., Eq. (1)

$$P = L * [Km * V(\lambda)]^{-1}$$

where, *P*=irradiance (W/cm2), *L*=luminance (lux) K m =maximum value of spectral luminous efficacy and V (λ) =photopic spectral function at the wavelength at 470 nm.

The energy per unit area (fluence) applied for each experiment was calculated using *Eq* [99].

$$E = P * t$$

where, E=fluence (J/cm^2), P= irradiance/power density (W/cm^2) and t= duration of treatment (s).

2.2.29 Blu-ray irradiated SV squid sample storage

The storage temperatures were controlled at 0±0.1°C, 5±0.1°C, and 10±0.1°C. According to the pre-experiment results, the samples were randomly taken at appropriate intervals (adjusted based on the spoilage rate at different storage temperatures, and the frequency was increased later according to the spoilage rate) for physicochemical and microbiological tests.

2.2.30 Sensory evaluation of Blu-ray irradiated SV squid samples

The sensory evaluation group consisted of 15 trained panelists aged between 18 and 25. The color, odor, body mucus, and muscle elasticity of squid were evaluated and scored, with 20 being the best quality, 12 being the high-quality period endpoint, and 4 being the endpoint quality. The sensory scoring criteria are shown in Table 2.2.

Table 2.2 – Criterion of sensory evaluation for squid

Score	Color	Odor	Body surface mucus	Muscle elasticity
5	Standard color and very shiny muscle cuts	With the unique flavor of squid, no off-flavor	No mucus, clean	Firm and elastic muscles, depressions disappear immediately after finger pressure
4	Standard color, shiny muscle cut surface	With the unique flavor of squid, no obvious off-flavor	Slight mucus, no decay	Firm and elastic muscles, depressions disappear faster after finger pressure
3	Slightly dull color, slightly shiny muscle cut	Slightly fishy smell	More mucus, slightly decayed	The muscles are elastic, and the depression disappears slightly slowly after finger pressure
2	Dull color and lusterless muscle cut	Obvious fishy smell	Much mucus and obvious decay	The muscles are slightly elastic, and the depression disappears very slowly after finger pressure

1	Dull color, no luster on muscle cut surface	Strong fishy odor or ammonia smell	Overflowing with mucus and completely rotten	Muscle inelasticity, finger depression is apparent after pressure

2.2.31 Statistic analysis

The volatile organic components (VOCs) analyzed by the instrumental analysis software LAV (Laboratory Analytical Viewer), three plug-ins (reporter, gallery plot, and dynamic PCA) and GC×IMS Library Search. Determination of VOCs by GC-IMS.

Response surface methodology analysis: Origin 2021 software (OriginLab Corporation, Massachusetts, USA) was used for data analysis. All assay samples were replicated at least three times independently, and experimental data were expressed as mean and standard deviation, and Tukey's multiple polar deviation test compared means. Based on the single-factor test, the process conditions were optimized by response surface analysis based on Box-Behnken's central combination test design principle with sensory scores as response values. The optimal process conditions were obtained using Design-Expert 13 software to analyze.

The data were analyzed with SPSS13.0 software (IBM). Results were expressed as mean±standard deviation and drawn with OriginLab Origin 2021. All the control and treatment groups were repeated three times.

2.3 Technical route figure

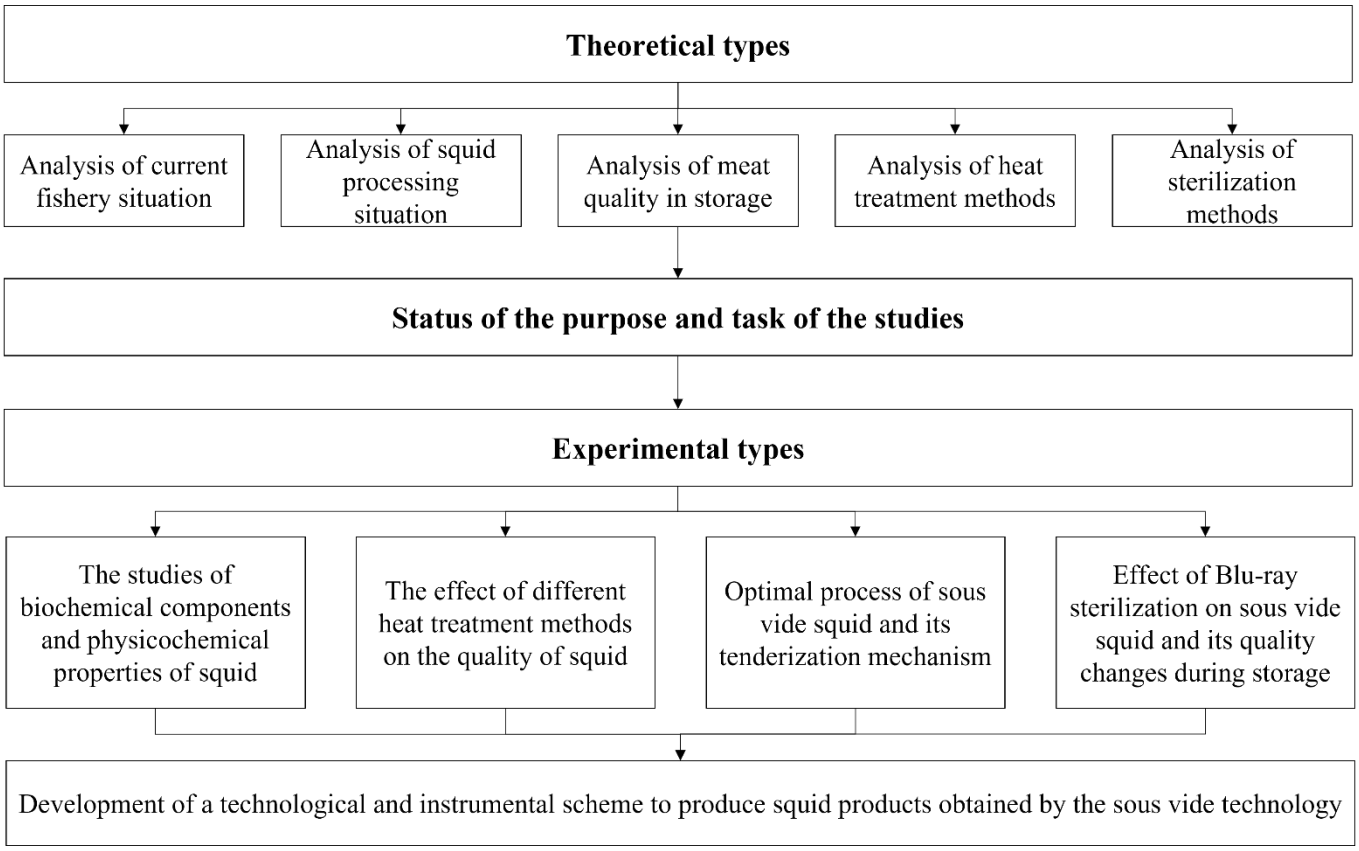


Figure 2.2 – Technical route

2.4 Chapter summary

1. Established a protocol for developing a pilot study of SV squid.

2. Identify research materials, equipment, and methods.

3. Select and provide descriptions of physicochemical, microbiological, microstructural, sensory methods for experimental research and nutritional and biological value calculation.

CHAPTER 3 DEVELOPMENT OF SOUS-VIDE TECHNOLOGY OF SQUIDS WITH HIGH CONSUMER PROPERTIES

3.1 The biochemical components and physicochemical properties of Argentine squid (*Illex argentines*)

The species composition of squids is quite diverse and includes about 200 species. Commander (*Berryteuthis magister*), Pacific (*Todarodes pacificus*) squids, as well as squids of the genus *Loligo, Ilex*, and others are of industrial importance. The development of a new or improvement of an existing technology is based on the chemical and mass composition, biochemical characteristics of raw materials. Therefore, we analysed the physical and chemical properties and nutritional components of squid to provide a reference for the deep processing of squid.

3.1.1 Meat extraction rate of squid

Table 3.1 – Weight and proportion of different parts and ratio of net meat of squid

	Weight (g)	Specific gravity (%)
Head and feet (wrist)	96.76±6.32	24.36
Tail	38.65±4.21	9.73
Ketone body	175.36±8.25	44.14
Bone spur	0.511±0.078	0.13
Skin	3.85±0.22	0.97
Viscera	82.12±7.03	20.67
Gross weight	397.25±18.652	24.36
Meat collection rate		44.14

Note: This statistic is the average of 6 squids. Results are mean ± standard deviation.

In order to justify the feasibility of using squid for the production of culinary products, the mass composition was investigated, and the yield of the edible part was determined. The results of our own research are presented in Table 3.1. The meat extraction rate refers to the net weight of meat taken from the carcass after skinning and bone removal to the stripped weight. The Argentine squid bone spurs only accounted for 0.13% of the stripped weight, indicating that the squid had few bone spurs and had the advantage of being easy to process (Table 3.1). The proportion of head, tail, and viscera

was 24.36%, 9.73%, and 20.67%, respectively, and the total of the three accounted for 54.76% of the stripped weight, and the meat extraction rate was 44.14%, indicating that the by-products of Argentine squid accounted for a more significant proportion, which affected the meat extraction rate, and the meat extraction rate was lower than the proportion of by-products. Despite Argentine squid's low meat extraction rate and its advantages of fewer bone spines, white meat, and easy meat extraction, Argentine squid still has a high processing value.

3.1.2 Squid Nutritional Composition

Table 3.2 – General nutrient components in squid

Component (g/100g)	Moisture	Crude fat	Ash	Crude protein	Total sugar
Wet material	80±0.86	1.99±0.08	1.37±1.03	15.54±1.03	0.10±0.25
Dry material		10.47±0.08	7.21±1.03	81.80±1.03	0.54±0.25

Note: This statistic is the average of 3 samples. Results are mean ± standard deviation.

The raw material muscle's fat, ash, and carbohydrates content are critical indicators to judge raw material's processing and storage quality. For this reason, the available nutrient content of Argentine squid was determined. Table 3.2, which is the available nutrient content of Argentine squid muscle, shows that the crude fat, ash, and total sugar contents were: 1.99%, 1.40%, and 0.10%, respectively, which shows that the sugar content of Argentine squid fish was very low, almost absent, and the fat and ash contents are also low. It can be seen that Argentine squid has high protein and low-fat content, while suitable to meet the trend of consumer demand for high protein and low-fat products, and low ash and sugar content, which is conducive to processing and storage.

3.1.3 Protein fractions of squid muscle

Table 3.3 – Protein components in the muscle of squid

Protein composition (g/100g)	Myoplasmic proteins	Myogenic fibronectin	Matrix proteins	Other
Component content	2.718±0.007	11.373±0.006	0.558±0.009	0.891±0.004
Percentage of total protein content	17.490±0.007	73.185±0.006	3.590±0.009	5.735±0.004

Note: This statistic is the average of 3 samples. Results are mean ± standard deviation.

Muscle protein mainly includes sarcoplasmic protein, myofibril protein, and muscle matrix protein [100]. Table 3.3 shows that the crude protein of Argentine squid has the highest myofibrillar protein content, reaching 73.2%, followed by sarcoplasmic protein. Therefore, the high content of muscle myofibril protein in the Argentine squid is also conducive to its processing and utilization. Myofibrillar protein is the leading research object in aquatic food processing. In addition to participating in muscle contraction, its role is also closely related to the rheological properties of meat products such as cohesiveness, water retention, elasticity, and texture. The higher the content of the main components that form the protein gel, the greater the strength and elasticity of the gel.

3.1.4 Squid lipid composition

Table 3.4 –Analysis of lipid composition of squid

Lipid type	Phospholipids	Cholesterol	Glycerides	Free fatty acids
Content (%)	58.2	20.5	20.1	1.2

Table 3.5 – Composition analysis of ether extract in squid

	Extraction amount	Iodine value	Acetone soluble matter	Acetone insoluble matter	Unsaponifiable matter
Carcass （%）	1.47	106.3	59.1	41.9	27.5

The phospholipid content of Argentine squid is exceptionally high (Table 3.4), and the cholesterol in the squid muscle is relatively higher than that of ordinary fish, but there is no need to worry about the increase of cholesterol value in blood when eating squid. Research shows that as long as the ratio of taurine to cholesterol in the food consumed: T/C>2 or more, the cholesterol value in the blood will not increase because taurine can inhibit the accumulation of cholesterol in the blood of the body. Squid muscle is rich in taurine, with 10.8-53.8 mg [101] of taurine per 100g of fresh product. Therefore, the cholesterol contained in squid is precisely involved in physiological nutrition and metabolism.

The fat content of squid is between 1% and 2% (Table 3.5), of which 59.1% is soluble in acetone, and the unsaponifiable matter is obtained after strong saponification. The acetone insoluble are mainly lipids, lecithin, and cephalin. The unsaponifiable part is

mainly cholesterol. It can be seen that the combination of lower fat content in squid and vacuum packaging can effectively prevent the fat oxidation of food during heating.

3.1.5 Amino acid composition of squid carcass

Table 3.6 – Amino acid composition of squid carcass (g/100g fresh)

Amino acids	Content	Amino acids	Content	Amino acids	Content
Phenylalanine *	0.100±0.008	Lysine *	0.221±0.020	Aspartic acid #	0.261±0.021
Isoleucine *	0.108±0.014	Tryptophan *	0.030±0.003	Glycine #	0.130±0.012
Valine *	0.115±0.013	Serine	0.117±0.015	Histidine	0.064±0.002
Threonine *	0.117±0.013	Tyrosine	0.057±0.004	Arginine	0.198±0.013
Leucine *	0.198±0.020	Cystine	0.014±0.001	Alanine #	0.155±0.010
Methionine *	0.082±0.009	Glutamate #	0.395±0.032	Proline	0.151±0.014
DAA	1.098	EAA	0.971	NeAA	1.542
TAA	2.513	DAA/TAA (%)	43.693	EAA/TAA (%)	38.639
EAA/NEAA (%)	62.970				

*Note: TAA，total amino acids；EAA，essential amino acids；NEAA，nonessential amino acids；DAA，delicious amino acids；*essential amino acids，#flavor amino acids.*

Table 3.7 – Comparison of essential amino acids in squid carcasses with egg protein and recommended by FAO/WHO for human body model（mg/g）

Amino acids	FAO mode	Egg protein mode	Argentine squid
Isoleucine	2.50	3.31	2.69
Leucine	4.40	5.34	4.92
Lysine	3.40	4.41	5.50
Methionine ＋ Cystine	2.20	3.86	2.39
Phenylalanine ＋ Tyrosine	3.80	5.65	1.42
Threonine	2.50	2.92	2.91
Tryptophan	0.60	0.99	0.75
Valine	3.10	4.10	2.86
Total	22.5	30.58	23.44

To evaluate protein quality, it is generally necessary to analyze its amino acid content and composition. Table 3-6 shows the composition and content of amino acids in three kinds of squid carcasses. It can be seen from the Table 3.6 that the total amount of amino acids in Argentine squid is 2.513 g/100 g. Glutamic acid is not only the amino acid

with the best umami taste but also an essential amino acid in the biochemical metabolism of brain tissue. It participates in synthesizing various physiologically active substances and is a vital amino acid required by the human body. According to the ideal model of FAO/WHO, the amino acid EAA/TAA of the protein with better quality is about 40%, and the EAA/NEAA is about 60%. The essential amino acids of Argentine squid accounted for 38.639% of the total amino acids, and the ratio of essential amino acids to non-essential amino acids was 62.970%. It indicated that Argentinian squid belongs to a better quality protein source.

Generally, the content of umami amino acids can determine the deliciousness of squid. The higher the content of umami amino acids, the more delicious the squid, and the strong flavor of seafood comes from these umami amino acids. They were converting the amino acid content of Argentine squid into milligrams of amino acids per gram of protein. Table 3.7 lists the comparison of the essential amino acids of squid with egg protein and the standard model of human amino acids recommended by FAO/WHO. The amino acid pattern of a protein refers to the type and quantity of amino acids, among which essential amino acids are the most important indicators for evaluating nutritional value. Argentine squid's total essential amino acids were 23.44 mg/g, which was lower than the egg protein standard model (30.58 mg/g).

3.2 The effect of different heat treatment methods on the quality of squid

The muscle protein of squid contains some paramyosin that has no ATPase activity to influence the protein stability, gel strength, and other properties of squid products [8], limiting the processing of squid. At present, research on the processing technology of squid can be divided into two categories. One is the optimization and improvement of traditional squid processing technology, such as squid rings [9], dried squid [10], and frozen squid products [11]. The other is the comprehensive processing and utilization of squid by-products or minced meat leftovers to extract biologically active peptides [12; 13] or squid flavor condiments [14]. Thus, the intensive processing of aquatic products and the extension of the storage period are significant issues.

To the excessive pursuit of shape and color, traditional cooking methods such as high-temperature cooking, high-temperature stewing, deep-fried baking, mostly centered on cooking. In the process of overheating, not only does the quality of aquatic products decline, and the loss of nutrients may also produce toxic and harmful substances such as heterocyclic amines, benzopyrenes [102]. The emergence of SV technology solves these problems to a certain extent and can better preserve the quality and nutrition of aquatic foods and reduce the production of harmful substances to ensure food safety. Therefore, convenient, and healthy SV products are immediately welcomed by working families, singles, and the elderly. Foods cooked under vacuum and at low temperatures will concern the food industry due to their convenience, nutritional safety, low cooking loss, and high product yield. The catering industry, food retail, restaurant, the transportation system (aviation, railway, and shipping), the military, hospitals, health food markets, and schools will also become potential markets for SV technology [103].

Therefore, the main objective of the present section was to use sensory evaluation, electronic nose, and GS-IMS to analyze the volatile components of squid. Through principal component analysis, the establishment of fingerprints, and heat map analysis, to explore the differences and correlations of volatile flavor substances heated by squid in different cooking methods. In addition, it explores the effects of different heat treatment methods on squid's physical and chemical properties to provide a reference for SV technology to replace traditional cooking squid.

3.2.1 Justification of the method of cooking squid based on sensory characteristics

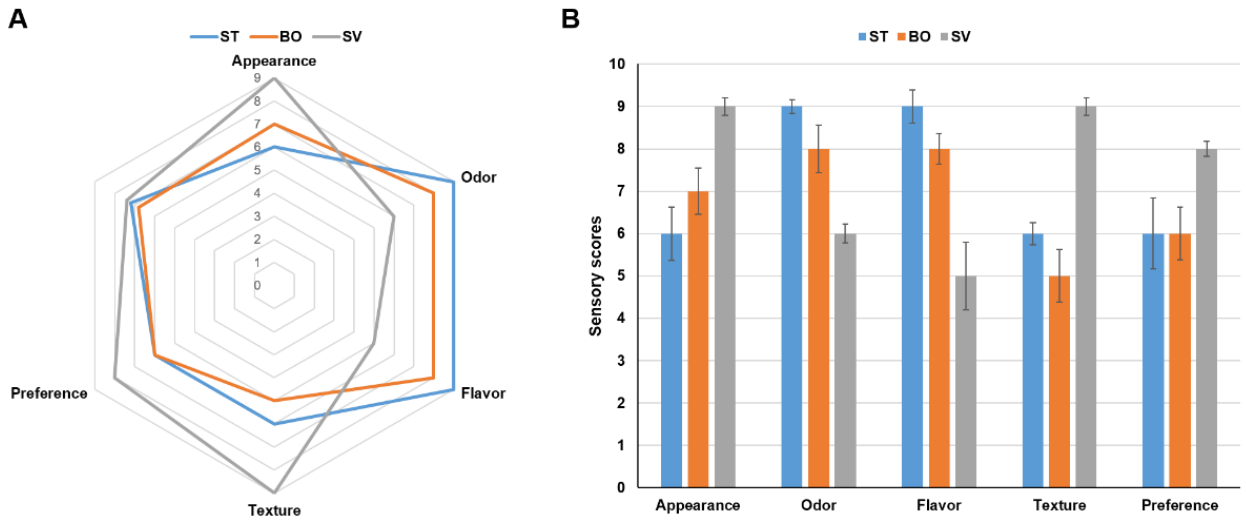


Figure 3.1 – Sensory scores of squid in different cooking methods. (A) Sensory radar chart of squid in different cooking methods. (B) Sensory scores of appearances, aroma, flavor, texture, and preference of squid prepared from different cooking methods. (BO: boiled squid; ST: steamed squid; SV: sous vide cooking squid.)

Sensory evaluation is one of the crucial criteria for evaluating the quality of squid products. The samples of squid prepared according to traditional technology (boiling and steaming), as well as samples prepared using sous-vide technology, were subjected to the study (see details 2.2.2). The ST and BO samples' sensory scores were relatively close, while the SV sample's sensory scores and the other two samples differed, especially in preference, appearance, and texture (Figure 3.1). The high temperature (100°C) heating of ST and BO caused the sample to shrink and curl, which reduced the appearance and preference score, while the appearance of the SV sample was intact without curling, which may be due to the lower cooking temperature (60°C). However, ST and BO samples performed well in both flavor and odor, and their scores were higher than those of SV samples. It is worth mentioning that the ST sample had the highest scores for flavor and odor. The difference in flavor and odor is that the fat in the SV sample heated under vacuum conditions cannot be completely oxidized to produce more aldehydes [88], resulting in less volatile substances. In terms of texture, SV samples had a higher softness than the other two heating methods. It may be that the endogenous enzymes in the sample are not wholly inactivated at lower cooking temperatures and still play a role [29].

3.2.2 Physicochemical and microbial differences of squid by cooking methods

Table 3.8 – Physicochemical and microbial differences of squid by cooking methods.

Treatment	Cooking loss (%)	Whiteness	TBARS (mg MDA/kg)	TVC (lg CFU/g)
RAW	—	18.10±0.048d	0.099+0.0016a	2.74+0.036a
BO	26.76±0.016b	23.24±0.013c	0.042+0.0008b	1.70+0.011b
ST	32.44±0.019a	23.69±0.016b	0.059+0.0005c	2.16+0.025c
SV	15.36±0.001c	37.36±0.061a	0.036+0.0008d	1.26+0.007d

Note: Results are mean ± standard deviation (n=3), values within a column with different superscript letters are significantly different (P<0.05). RAW: raw squid; BO: boiled squid; ST: steamed squid; SV: sous vide cooking squid. TBARS: Thiobarbituric acid reactive substance. TVC =Total viable count.

Any technological impact leads to a change in the physical and chemical properties of the processed product. The results of the study of the influence of the type of heat treatment on the change in the main physical and chemical indicators are shown in Table 3.7. SV was the most minimal 15.36% in cooking loss, followed by BO (26.76%) and ST (32.44%). The results are because SV has a low cooking temperature and places in a vacuum packaging bag for heating. The sample's fat will melt during the cooking process, the juice will be lost, and the water will evaporate [104]. The high-temperature causes myofibril protein and collagen's denaturation [105], so ST and BO samples' cooking loss was more. Generally, the cooking loss is related to the texture of aquatic products. The cooking temperature significantly affects the shear force, springiness, cohesiveness, and chewiness of animal-derived foods, and the change of time is not statistically significant [59; 106; 107]. It should emphasize that SV of animal source foods should give sufficient cooking time to completely dissolve the collagen in the sample to reduce the finished product's hardness. SV at 65°C for 20 min, the scallops have a good taste and full shape, and the nutrient content of taurine, protein, and vitamins is significantly higher than that of traditional cooking [108]; the SV sea cucumber cooked at 70°C for 60 min has moderate hardness, crisp and smooth taste, and flexibility [109]. Also, SV technology can replace tender meat technology pre-treatment [110]. Espinosa [111] found that the flavor and texture of the *Sparus aurata* were best when cooked at 60°C for 12-15 min.

Color is one of the most direct and essential sensory evaluation indicators for evaluating the food. The color of squid can be evaluated very well by the whiteness. Table 3.7 shows that the whiteness of the squid after cooking had increased, and the whiteness of

the SV sample was the highest ($P<0.05$). These results are consistent with the data of the authors [112]. The higher whiteness values of the sous-vide samples are explained that SV sample was heated in a vacuum package, and the sample's myoglobin was in a hypoxic myoglobin state. Compared with oxygenated myoglobin in BO and ST samples, deoxymyoglobin in the SV samples had higher thermal stability [112]. So, the SV samples showed a redder color and a higher whiteness. The oxygen-free SV has a significant improvement in product color and is superior to traditional boiling products. SV salmon cooking at 65°C and 90°C for 15 min, the color difference is noticeable, in which the color of the salmon cooked at 90°C is not as well as cooking at 65°C, while the color of the salmon cooked at 65°C remains unchanged [113; 114]. Tang [115] found that the color of catfish cooked at 60, 70, and 80°C was significantly brighter than that of raw fish, and it was stable at 4°C for 24 d. Dong [116] found that using SV technology, the scallop adductor muscle cooked at 55°C for 32 h, the brightness was not affected by the increase of cooking time, and the redness and yellowness decreased with the prolongation of cooking time. Our results were consistent with Roldan et al.'s [59] reported that SV lamb loins exhibit a more reddish color at lower temperatures. SV technology has different effects on the color of different aquatic products, and for the same aquatic products, using different cooking temperatures and cooking time will also have a significant impact on the color of aquatic products.

Aquatic products are rich in nutrients, which cause the oxidation of proteins and lipids during heating, which leads to changes in the nutritional value of aquatic products [117]. TBARS is one of the critical methods to detect the oxidation degree of animal fats and vegetable oils [118], and it is also suitable for aquatic products. TBARS is related to the content of lipid oxidation compounds, mainly aldehydes [119]. Table 3.7 shows that the SV sample has the lowest degree of oxidation because it was heated under vacuum conditions, effectively preventing fat oxidation [120]. Similar studies indicated that at low-temperature processing temperatures of 65°C, the retention of unoxidized unsaturated fatty acids in SV water products is generally more excellent than that of conventional cooking at temperatures up to 85°C [48]. Besides, both triglycerides and phospholipids of fish contain long-chain polyunsaturated acyl groups, which are particularly susceptible to

oxidation due to their high degree of unsaturation. Espinosa [111] developed ready-to-eat seabream (SV at 60°C, adding acid sauce and storage at 2°C) and found that it achieved lower TBARS throughout the storage process. Our result also proved the reason for the lack of flavor and odor of SV samples. Also, lower TBARS levels and vacuum packaging can prevent fat oxidation, which is more conducive to long-term squid storage.

The total viable count (TVC) is displayed in Table 3.8. In this study, the TVC of raw squid was 2.74 lg CFU/g. After heating, the TVC in the squid samples decreased, and the TVC of SV samples was the most minimal ($P<0.05$). Because SV is to vacuum encapsulate the raw materials in the bag, which can effectively prevent the proliferation of bacteria [54]. Besides, SV processed products have high resistance to microbial growth [121] and extended shelf life. Díaz et al. [122] found that salmon treated (80°C, 45 min) effectively prevent the growth of aerobic and anaerobic psychrophilic bacteria, lactic acid bacteria, mold, and yeast, and Enterobacteriaceae during cold storage. Espinosa et al. [111] discussed the effect of SV treatment on the microbial content of seabream and found that *Salmonella* and *Listeria monocytogenes* were not detected. Different SV temperatures and times used for the same aquatic product and the microbiological safety and shelf life are different. Gonzalez-fandos et al. [113] studied cooking salmon with different temperature and time combinations (65°C for 5 min, 90°C for 10 min, and 90°C for 15 min) and found that the shelf life was 21 d (cooking at 65°C for 5 min, storage at 2°C), the shelf life was also 21 d (cooking at 90°C for 5 min, storage at 10°C); if SV salmon cooked at 90°C for 5 min or 15 min, the shelf life was 45 d refrigerated at 2°C. Also, scholars have combined SV technology with other technologies to control pathogenic microorganisms in aquatic products and further extend the shelf life, e.g., increased brine [123] and irradiation [124].

3.2.3 Analysis of flavor substances of squid via electronic nose

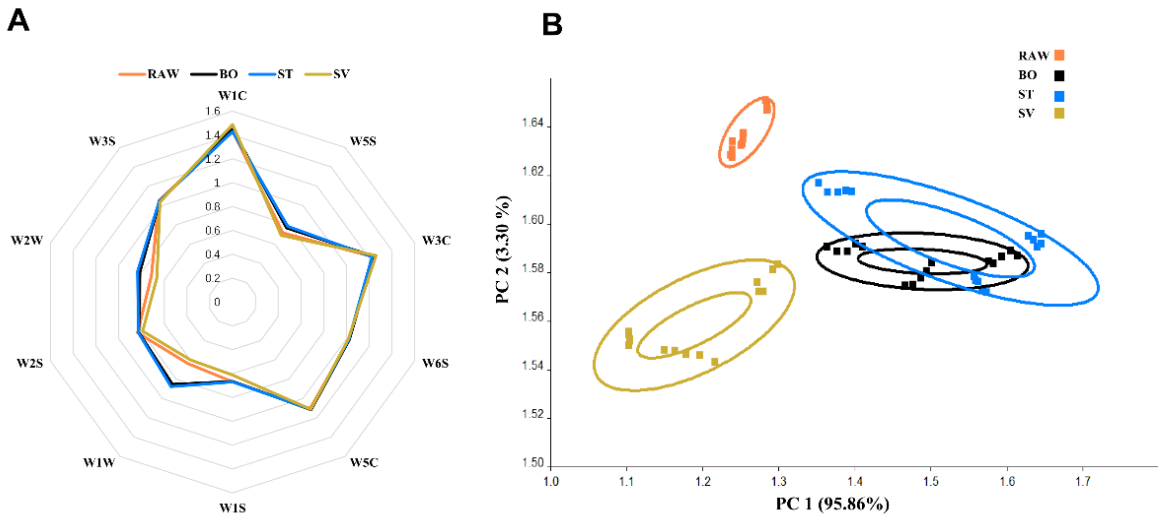


Figure 3.2 – Electronic nose analysis. (A) Radar charts of volatile compounds in squid from different cooking methods. (B) Analysis of the predominant volatile compounds in squid samples. RAW: raw squid; BO: boiled squid; ST: steamed squid; SV: sous vide cooking squid.

Sensory evaluation is one of the critical indicators of food quality. In this study, the sensory evaluation could distinguish the squid samples with different cooking methods. The squid samples did not need other pre-treatments, so only the professionalism and consistency of the evaluation were considered in the sensory assessment, ignoring the importance of consumers' demand for the product. Consumers' perceptions can be used for the assessment in the product development, which can significantly save time and labor costs [125]. It also suggested that evaluation based on preferred attribute elicitation methodology had the advantage of providing data on the importance of attributes for product acceptance [126]. Besides, in the sensory evaluation, the combination of the projective method [127], descriptive analysis method [128], and temporal methods [129] is better to the development of new squid products.

The aroma of meat products is an important evaluation index. Aroma contributes to the acceptability of the meat, and it is the result of a combination of various volatile

compounds and chemical reactions that form flavor components, such lipid oxidation, Maillard reaction, and the interaction between lipid oxidation products and Maillard products. [130]. Different cooking methods affect the flavor of meat products [131], especially the volatile components [132]. The types of VOCs present in the BO and ST samples were similar. These techniques utilize the same temperature and pressure. SV heats the sample for a long time, and it utilizes low temperature under vacuum conditions. Thus, the VOCs are significantly different from BO and ST. Studies have shown that different heating methods affect the quality of food [133]. Even for SV, product quality is different under different vacuum degrees [134].

Compared with traditional sensory analysis, electronic nose is simple, fast, objective, and intuitive. According to the different response values of the electronic nose sensor to the aroma components of squid in different cooking methods, an intuitive radar chart was established, as shown in Figure 3.2A. The radar chart analysis method is mainly employed to study the sensors. Using this method, the contribution rate of each sensor to the squid sample can be distinguished, so as to investigate which type of volatile components play predominant roles in distinguishing the sample. From Figure 3.2A, the difference in volatile flavor compounds from different squid samples was mainly in the sensors W2W, W5S, W1W, and W1C, which are aromatic compounds, sulfur organic compounds, and nitrogen oxide compounds. It can be seen from the radar chart that the flavor of squid is greatly influenced by aromatic compounds. Conversely, the BO and ST squid samples had more obvious responses at W1W and W2W, which can be contributed to sulfide. Specific volatile components need further verification.

In order to highlight the aroma differences in squid from different cooking methods, a PCA scatter diagram (Figure 3.2B) was generated according to electronic nose data of the overall flavor substance composition. PCA is a multivariate statistical analysis technique that examines the correlation between multiple variables and reveals the internal structure between multiple variables through a few principal components [135]. Generally, the PCA model is selected as the separation model when the cumulative contribution rate reaches 60% [136]. PCA of aromatic substances from different heat-treated squids (5 detection signals from 48s to 52s) was performed, and the result shows that the first

principal component contribution rate was 95.86%; the second principal component contribution rate was 3.30%; and the cumulative contribution rate was 99.16%. This indicates that the two principal components can adequately represent predominant characteristics of the sample. Each sample in the group was relatively concentrated in a specific range and has a precise distance from the clustered areas of other groups. RAW samples and SV samples were clustered separately (the farthest distance in the PCA chart), while BO and ST were clustered together. This suggests that these methods have a high degree of similarity. Moreover, the results indicate that different cooking methods lead to differences in squid aroma.

The results of electronic nose test found that the aroma and taste of SV squid were different from BO and ST samples, which was consistent with the results of sensory evaluation (Figure 3.1), which may be related to the heating under vacuum conditions and the lower heating temperature. We next further investigate further research on flavor variability and the reasons that influence flavor variability.

3.2.4 Differences in VOCs in squid samples

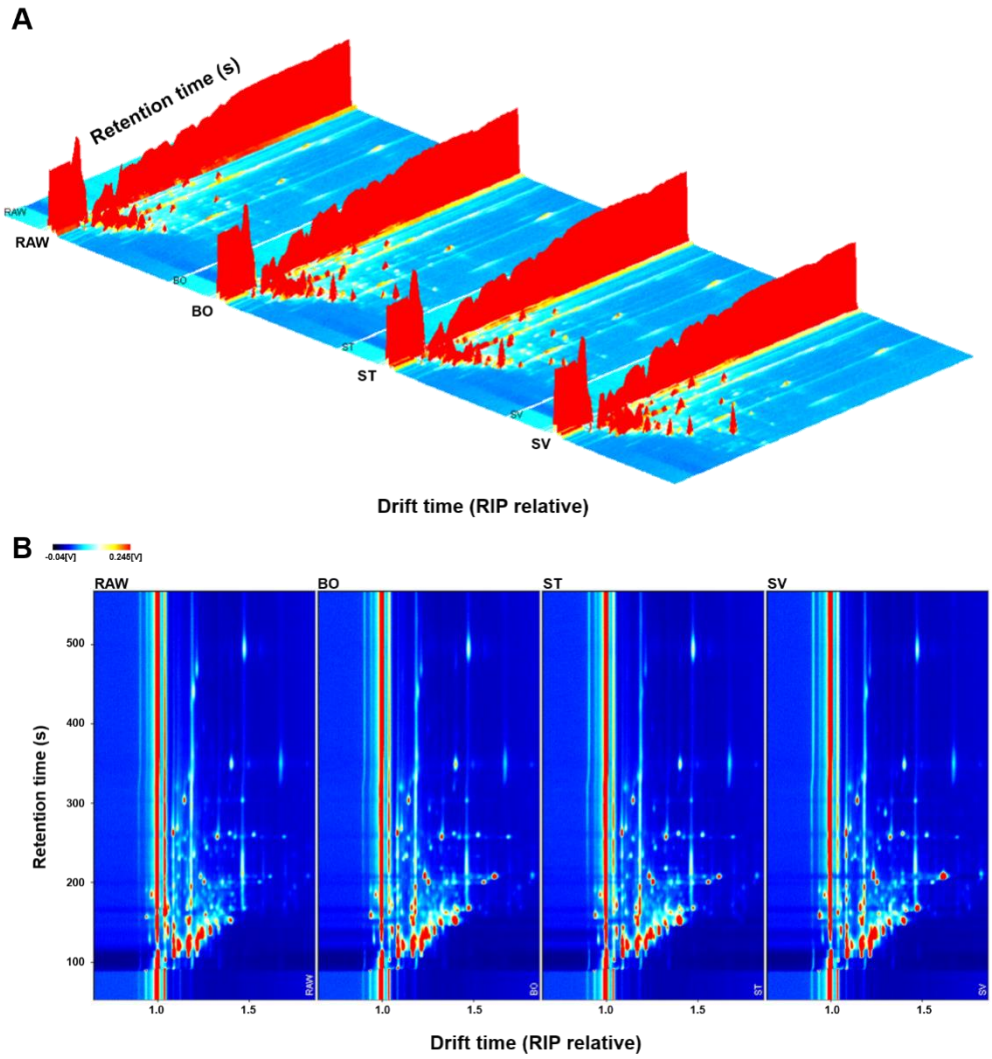


Figure 3.3 – Gas chromatography-ion mobility spectrometry of squid from different cooking methods. (A) Three-dimensional topographic plots and chromatograms of squid samples. The y-axis represents the retention time of the gas chromatograph; the x-axis represents the ion migration time for identification, and the z-axis represents the peak height for quantification. (B) Two-dimensional topographic plots and chromatograms of squid samples. RAW: raw squid; BO: boiled squid; ST: steamed squid; SV: sous vide cooking squid. The ordinate represents the retention time, and the abscissa represents the migration time. The red vertical line represents the reaction ion peak (RIP), and the migration time after normalization was 8.0 ms. Each point on the right of RIP represents a volatile substance. Color represents the signal intensity of the substance. White indicates low intensity, and red indicates high intensity. The darker the color, the greater the intensity.

A series of physical and chemical changes occur after meat is heated, which significantly affects the quality of the processing [137]. HS-GC-IMS analyzed the differences between volatile compounds in heat-treated squid samples. Figure 3.3A shows the 3D topographic plot spectrum of volatile flavor compounds. From Figure 3.3A, the VOCs of squid from different cooking methods are very similar, but the signal intensities are slightly different. After heat treatment, the content of most flavor compounds changes to varying degrees.

Although the differences in volatile flavor compounds can be visualized in the 3D spectrum, a 2D plot spectrum is more evident. Figure 3.3A shows a top view of the 3D GC-IMS spectrum of Figure 3.3B projected onto a 2D plot, which can directly compare the differences in flavor substance for various heat-treated squid. However, a volatile compound may produce one or more bright spots, representing monomers or dimers and trimers, depending on the concentration of volatile compounds. The entire spectrum represents all components found in the headspace sample. Most of the signals in the topographic plot of squid samples from different cooking methods appeared between 100 and 200 s, while in SV samples the signals were distributed between 100 s and 300 s. Moreover, the signal intensity was stronger than that the other heat treatments.

Both 2D and 3D plot spectrums showed that the VOCs were different in different cooking methods, and the content was different, consistent with the results of electronic nose and sensory evaluation.

3.2.5 Identification of VOCs in squid from different cooking methods

Table 3.9 – Identified compounds in squid prepared from different cooking methods

No.	Compound	CAS	Molecule Formula	MW	RI1	Rt2	Dt3	Comment
1	Ethanol	C64175	C2H6O	46.1	433.3	103.409	1.0443	
2	Acetone	C67641	C3H6O	58.1	519.7	122.144	1.1168	
3	2-butanone	C78933	C4H8O	72.1	604.1	140.442	1.0581	mono
4	Isopropyl alcohol	C67630	C3H8O	60.1	514	120.912	1.1701	mono
5	Acetic acid ethyl ester	unidentified	*	0	622.6	144.529	1.092	mono
6	1-propene-3-	C10152768	C4H8S	88.2	694.8	163.592	1.0421	

	methylthio							
7	3-hydroxybutan-2-one	C513860	C4H8O2	88.1	720.4	172.421	1.0573	mono
8	Prop-1-ene-3,3'-thiobis	C5928841	C6H10S	114.2	853.3	231.52	1.119	
9	3-methylthiopropanal	C3268493	C4H8OS	104.2	909.9	264.103	1.0867	mono
10	Cyclohexen-2-one	C930687	C6H8O	96.1	918	269.439	1.1098	
11	N-nitrosodiethylamine	C55185	C4H10N2O	102.1	897.9	256.562	1.1479	
12	Benzaldehyde	C100527	C7H6O	106.1	963.7	304.911	1.1488	mono
13	Octanal	C124130	C8H16O	128.2	1007	349.69	1.4063	mono
14	Benzaldehyde	C100527	C7H6O	106.1	962.8	304.102	1.4666	dimer
15	Heptanal	C111717	C7H14O	114.2	900.9	258.372	1.3292	mono
16	3-methylthiopropanal	C3268493	C4H8OS	104.2	907.3	262.447	1.3961	dimer
17	Furfural	C98011	C5H4O2	96.1	827.7	218.686	1.3297	
18	Pentan-1-ol	C71410	C5H12O	88.1	763.3	189.603	1.2521	
19	3-pentanone	C96220	C5H10O	86.1	694.4	163.468	1.1109	mono
20	n-propyl acetate	C109604	C5H10O2	102.1	711.7	169.31	1.1648	mono
21	1-butanol	C71363	C4H10O	74.1	647.6	150.411	1.1741	
22	Pentanal	C110623	C5H10O	86.1	697.4	164.416	1.1858	
23	2-butanone	C78933	C4H8O	72.1	587.4	136.814	1.2451	dimer
24	Acetic acid ethyl ester	C141786	C4H8O2	88.1	618.6	143.642	1.3379	dimer
25	2-methyl-1-propanol	C78831	C4H10O	74.1	636.8	147.822	1.3716	
26	3-pentanone	C96220	C5H10O	86.1	693.5	163.194	1.3562	dimer
27	Hexanal	C66251	C6H12O	100.2	790.5	201.506	1.2571	mono
28	Acetic acid butyl	C123864	C6H12O2	116.2	806.2	208.622	1.2375	mono

	ester							
29	N,N-diethylethanamine	C121448	C6H15N	101.2	689.6	161.95	1.2289	
30	Hexanal	C66251	C6H12O	100.2	792.5	202.431	1.561	dimer
31	Acetic acid butyl ester	C123864	C6H12O2	116.2	805.9	208.485	1.6192	dimer
32	n-Propyl acetate	C109604	C5H10O2	102.1	711.3	169.144	1.4752	dimer
33	Ethyl 2-hydroxypropanoate	C97643	C5H10O3	118.1	805.1	208.089	1.5362	
34	Octamethylcyclotetrasiloxane	C556672	C8H24O4Si4	296.6	1009	352.022	1.6762	
35	Octanal	C124130	C8H16O	128.2	1007.2	349.831	1.8216	dimer
36	Heptanal	C111717	C7H14O	114.2	901	258.418	1.6948	dimer
37	Isopropyl alcohol	C67630	C3H8O	60.1	523	122.845	1.219	dimer
38	3-hydroxybutan-2-one	C513860	C4H8O2	88.1	712.9	169.706	1.328	dimer
39	Nonanal	C124196	C9H18O	142.2	1109.5	494.176	1.4776	
40	Compound from HS-Vial Septum	GAS_00002	*	0	836	222.729	1.4664	
41	(E, E)-2,4-heptadienal	C4313035	C7H10O	110.2	1017.6	362.652	1.1919	
42	2-heptanone	C110430	C7H14O	114.2	891	252.342	1.2602	
43	Furaneol	C3658773	C6H8O3	128.1	1074.1	441.089	1.1985	
44	Linalool	C78706	C10H18O	154.3	1093.8	470.625	1.217	
45	Nonanoic acid	C112050	C9H18O2	158.2	1266.9	730.255	1.5409	

CAS is the registration number of chemical substances by Chemical Abstracts Service.

1. Represents the retention time in the capillary GC column.

2. Represents the retention index calculated on FS-SE-54-CB column using n-ketones C4-C9 as external standard.

3. Represents the drift time in the drift tube

Figure 3.3 shows the differences in VOCs in squid prepared from different cooking methods intuitively, but it is difficult to accurately determine the specific substance in the

2D and 3D spectrum maps. Through GC-IMS separation, the differences in volatile substances in the squid samples are elucidated. According to the retention time and ion migration time of volatile substances in gas chromatography, 43 specific volatile substances were determined (Table 3.9). Due to different concentrations of VOCs, certain compounds might produce multiple signals or spots (dimers or trimers).

3.2.6 Fingerprints of squid samples

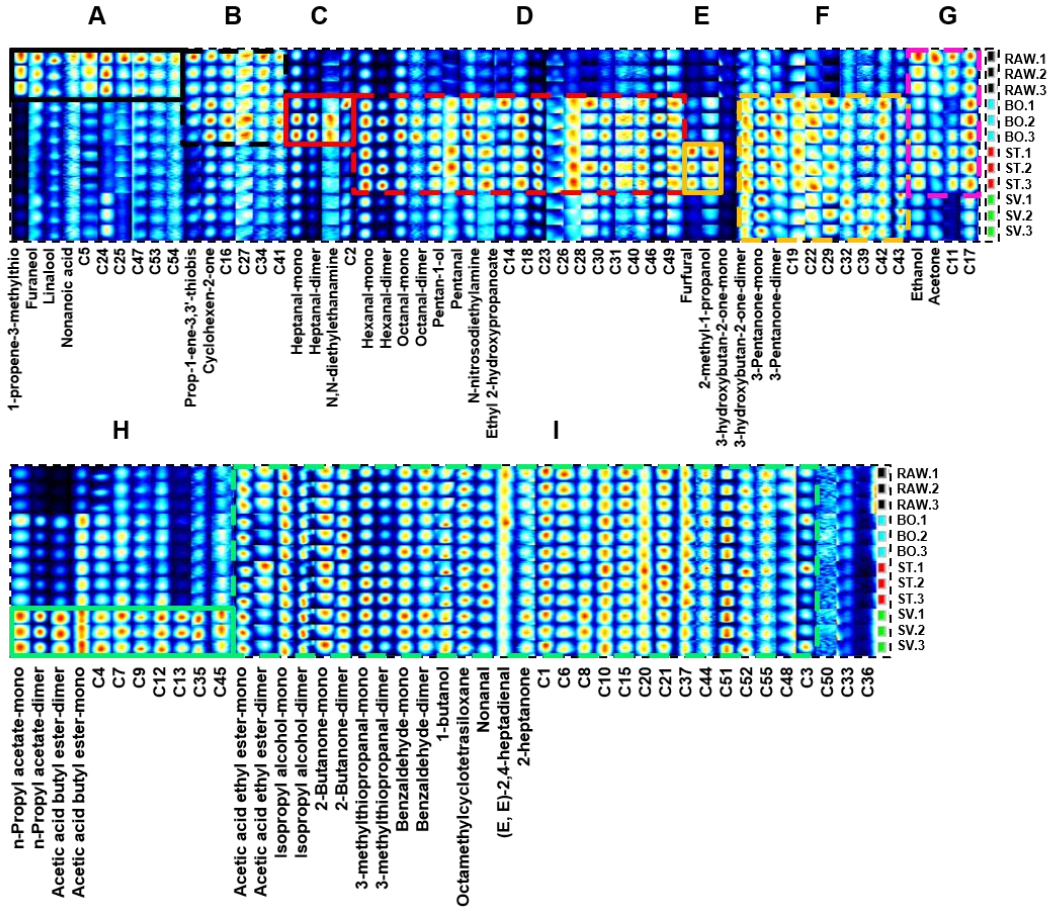


Figure 3.4 – Fingerprint of squid in different cooking methods. RAW: raw squid; BO: boiled squid; ST: steamed squid; SV: sous vide cooked squid.

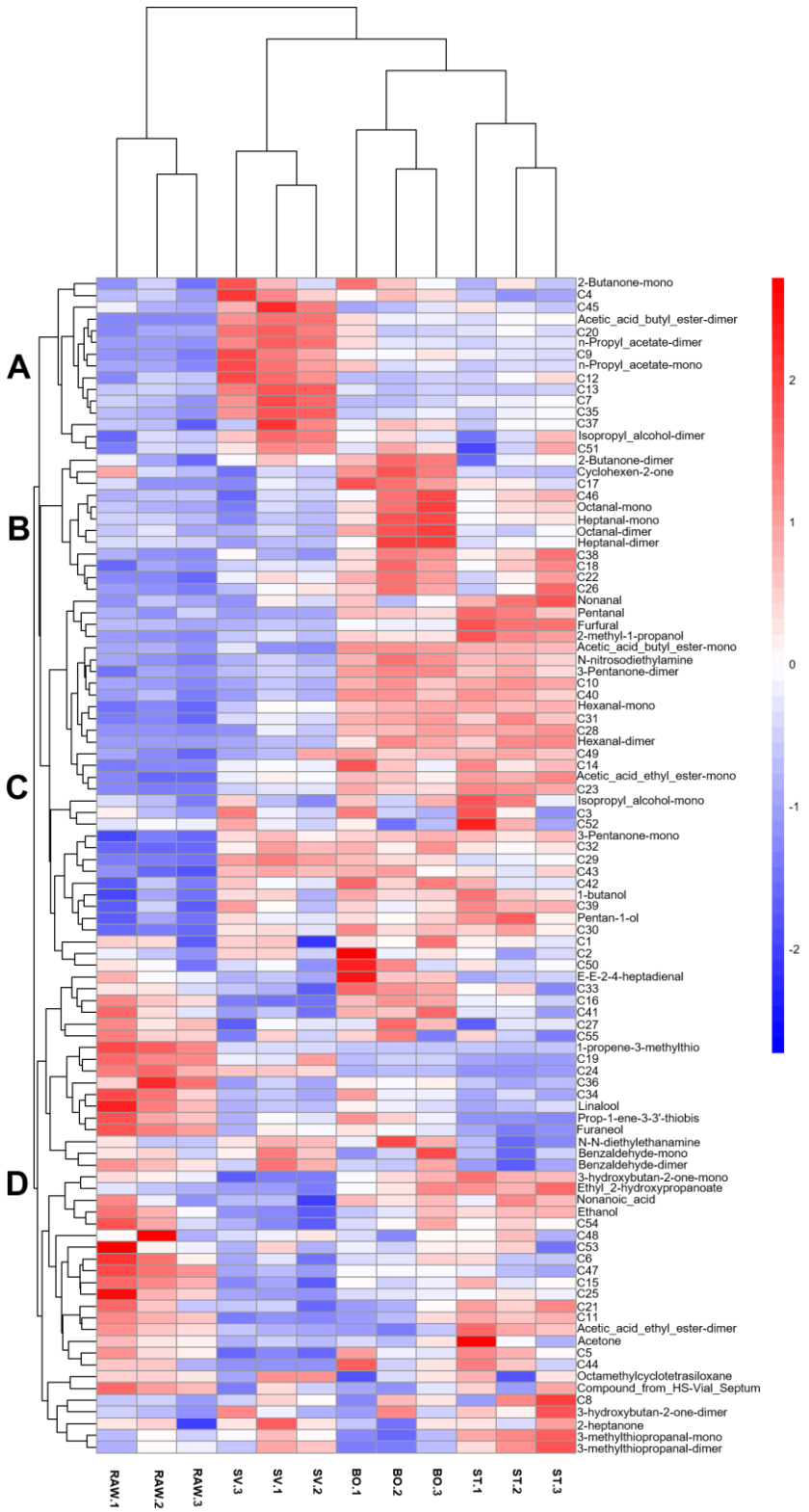


Figure 3.5 – Heat map of squid from different cooking methods. RAW: raw squid; BO: boiled squid; ST: steamed squid; SV: sous vide cooked squid.

In order to compare the differences in VOCs more comprehensively, the gallery plot plug-in of LAV software was used to generate the peak signal of the topographic plots of squid samples (Figure 3.4). In Figure 3.4, each row represents all selected signal peaks

from a squid sample, and each column represents the signal peak of the same volatile organic compound. Brightness indicates the content of a substance. The higher the brightness, the higher the content of the substance.

Complete VOC information of each sample and its differences between the samples are depicted in Figure 3.4. The material marked by the solid black line in area A has the highest content in RAW samples, such as 1-propene-3-methylthio, furaneol, linalool, and nonanoic acid. The substances marked by the solid red line in area C had the highest content in the BO squid, such as heptanal and N, N-diethylethanamine. The substances marked by the solid yellow line in area E were found in ST squid. The compounds with the highest content include furfural and 2-methyl-1-propanol. The solid green line in region H had the highest content in SV squid, including compounds such as n-propyl acetate and acetic acid ethyl ester.

The substances marked by the black dotted line in area B were the highest in RAW and BO squid, such as prop-1-ene-3, 3'-thiobis, and cyclohexen-2-one. The substances marked by the red dotted line in the area D were the highest in BO and ST squid, including hexanal, octanal, pentan-1-ol, pentanal, N-nitrosodiethylamine, and ethyl 2-hydroxypropanoate. The substances marked by the yellow dotted line in area F were the highest in BO, ST, and SV squid, such as 3-hydroxybutan-2-one and 3-pentanone. The substances marked by the red dotted line in the area G were the highest in RAW, BO, and ST squid, including ethanol and acetone. The green dashed line in area I indicates the flavor substances that were common to the four kinds of the samples, such as acetic acid ethyl ester, isopropyl alcohol, 2-butanone, 3-methylthiopropanal, benzaldehyde, 1-butanol, octamethylcyclotetrasiloxane, nonanal, (E,E)-2,4-heptadienal, and 2-heptanone.

The heat map is an intuitive and visual method for analyzing the distribution of experimental data. It can cluster data and samples to determine the quality of the samples [138]. There were four treatment groups: RAW, BO, ST, and SV. The VOCs were classified into four clusters: group A, group B, group C, and group D in Figure 5. The volatile compounds in group A were mainly represented in RAW. Volatile compounds in group B were mainly represented in BO and ST. Volatile compounds in group C were represented in BO, and volatile compounds in group D were only represented in SV. From

Figure 3.5, SV samples had particular volatile components that are different from ST samples, BO samples, and RAW samples, such as acetic acid butyl ester, n-propyl acetate-mono, and n-propyl acetate-dimer. Also, BO samples and ST samples had acetic acid ethyl ester-mono, hexanal-mono, hexanal-dimer, N-nitrosodiethylamine, 3-pentanone-dimer, and acetic acid butyl ester-mono. This result was different from SV samples. Some unknown volatile compounds need to be further studied and determined.

3.2.7 Discussion

Volatile compounds of cooked meat include lipids and fatty acid oxidation products, such as alcohols, aliphatic hydrocarbons, ketones, carboxylic acids, aldehydes, and esters [139]. Aldehydes are the main products of the oxidative degradation of fatty acids and the characteristic aroma components of meat [139]. In this study, 43 VOCs were identified, including 14 aldehydes. The threshold value of aldehydes is relatively low, which contributes significantly to the flavor in cooked squid. In the experiment, 3-methylthiopropanal was present in all four sample types. 3-Methylthioprapanol was first identified as a volatile component in cooked squid by Kubota [140] It is a “baked potato” descriptor and considered an essential contributor to the scent of squid [141] as well as cooked lobster [142]. However, the difference in the contents of aldehydes in squid from different heating methods has an important impact on the sample odor. Ketones are also the products of fatty acid oxidation, which are usually the products of automatic oxidation of unsaturated fatty acids [143]. There are ten ketones in the 43 VOCs detected in ST, BO, and SV samples, such as 3-hydroxybutan-2-one, 3-pentanone, acetone, 2-butanone, and 2-heptanone. In particular, furaneol (4-hydroxy-2,5-dimethyl-3(2H)furanone) in RAW samples is described as a “caramel-like” flavor, and it is a flavor component of strawberry [144] and pineapple [145]. Kubota [140] considered furaneol a substance that contributes to the sweetness of squid after cooking. Ester compounds are produced by the esterification of alcohols and acids [146] and esters are found in many flavors of crustacean fish products that are cooked and heated [147]. SV squid has the highest content of n-propyl acetate and acetic acid ethyl ester of all samples tested. The volatile substance n-propyl acetate is a short-chain aliphatic ester that produces a pleasant fruity aroma. It is a component of natural flavors [148]. This indicates the SV conditions are

conducive to the esterification reaction and can yield more desirable flavor in squid. Flavor variability and deficiencies are due to sous-vide, but new and pleasing flavors are noteworthy.

The type of volatile components was determined by the degree of oxidative degradation of fatty acids under different cooking temperatures, heating pressures, and transfer medium. The results show that the aldehydes were more varied and in higher abundance when squid was heated at 100°C under normal pressure. Conversely, the aldehydes of SV were less abundant than ST and BO. The reason is high-temperature and high-pressure conditions are conducive to the thermal oxidative degradation of fatty acids [149] and more volatile components are generated when heated. The increase in cooking temperature increased the degree of oxidative degradation of fatty acids, thus increasing the content of volatile components. Compared with ST and BO at normal pressure and 100°C, SV is heated under vacuum at 60°C, and the degree of fatty acid oxidation degradation is lower during heating [150]. This reduces the content of volatile substances generated during heating. Falowo [151] reported that there was no pronounced effect of SV cooking temperature on fatty acid of beef and liver compared to raw samples. Generally, low cooking temperature is indeed a weakness of SV cooking, but long-term heating sometimes compensates for this weakness. Long-time heating increases the volatile substances derived from the degradation of amino acids and/or thiamine [152; 153], such as 2-methyl-thiophene, carbon disulfide, benzothiazole, and dimethyl disulfide [67]. Mortensen et al. [154] indicated that the effect of SV heating time on flavor has a greater effect than the cooking temperature. For beef and pork cooked in SV, the longer the cooking time, the better the flavor [155]. SV cooking still develops a pleasant flavor at a low temperature. 3-Methylbutanal, a meaty-nutty flavor of dry-cured ham [156], was found in SV cooked meat after 24 h of heating at 60°C [105; 152]. Of course, the prolonged heating time of SV reduces the lipid oxidation of carbonyl compounds in food, which indicates that they further react with other compounds (proteins, amino acids, etc.) to produce new, more desirable volatiles [105; 152]. In this study, n-propyl acetate and acetic acid ethyl ester (highlighted in group H in Figure 4) were produced after SV heating;

however, they were not detected in BO and ST. Moreover, by adding reducing sugar or other flavor precursors, SV food flavor can also be enhanced [157].

In addition to this, SV technology produces high-quality sensory characteristics of meat products. Studies have shown that a slow heating rate is key to generating tender meat [158] and maintaining the meat core temperature closed to 60°C for a long time [159]. These observations have been further confirmed in the SV technique [59; 62; 160]. SV means not only higher sensorial quality but LTLT heating can also minimize the nutrient loss [45], improve bioaccessibility [46], ensure the safety of food [161; 162], and extend the shelf life [162-164]. Given the many advantages of SV and the growing demand for nutritious and convenient foods, the application of SV technology in the industrial production of squid is feasible.

Overall, SV shows good heat treatment advantages. SV squid outperformed BO and ST squid in cooking loss, whiteness, TBARS, and microbiological indicators. Through sensory evaluation and electronic analysis, it was found that different cooking methods have a great influence on the flavor of the squid. SV squid flavor is different from that of ST and BO. Additionally, a method was developed to evaluate the characteristic volatile compounds of squid samples from different cooking methods by establishing the fingerprint with HS-GC-IMS. A total of 43 volatile substances, including some dimers, were detected by GC-IMS analysis from samples of squid from different cooking methods. The predominant compounds were mainly aldehydes, ketones, alcohols, and esters. Given the many advantages of SV technology, it could be used for the industrial production of squid.

3.3 Optimal process of SV squid and its tenderization mechanism

Changes in physicochemical properties from fresh ingredients to processed pre-cooked foods involve sensory properties, nutrition, and quality [165]. In particular, after a long and rigorous process of meat ingredients, curing and various heating steps lead to shrinkage and oxidation reactions, affecting the final meat product's organoleptic properties and nutritional value [166]. Therefore, the effect of using suitable heat treatment on the quality of pre-cooked meat products is studied in-depth, not only to ensure the

quality, flavor, and nutrition of the products. However, it also achieves energy-saving and efficiency by optimally controlling the heat treatment conditions.

The previous section's research showed that SV squid outperformed BO and ST squid in texture, appearance, and preference, except for flavor and odour. SV has excellent potential for aquatic product processing. However, the best process for SV squid has not been reported in the literature. In addition, the reason why the tenderness of SV squid is better than the traditional cooking method still deserves further investigation.

Therefore, this section uses the response surface method to optimize the production process of SV squid. This basis explores the tenderization mechanism of SV squid to provide theoretical and data references for SV in the industrial production of aquatic products.

3.3.1 Determining the optimal technological regimes for cooking squid using sous vide technology

1. Single-factor experimental results

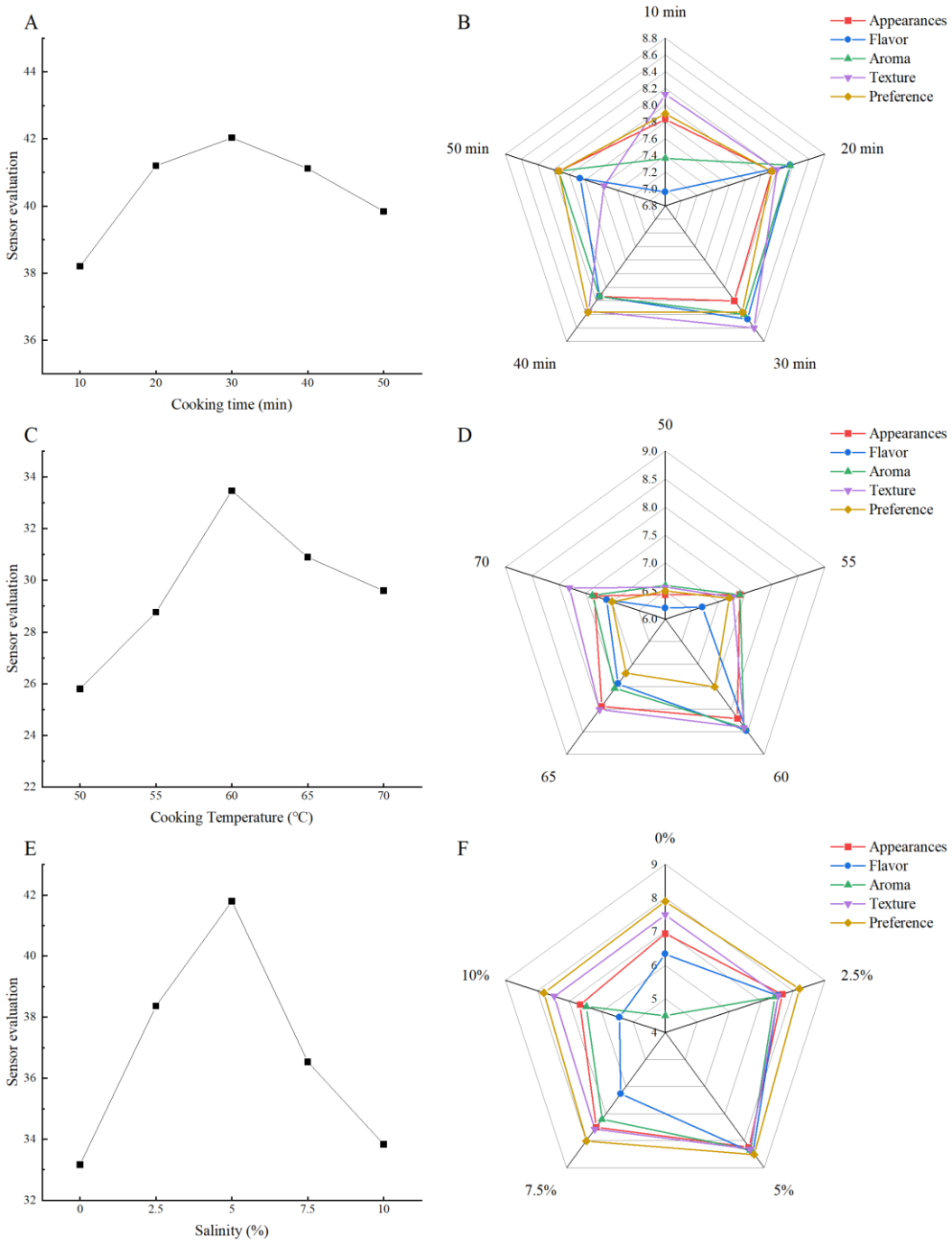


Figure 3.6 – Single-factor experiment of sous vide squid (A. The influence of cooking time on sensory evaluation; B. The sensory evaluation radar chart of cooking time; C. The influence of cooking temperature on sensory evaluation; D. The sensory evaluation radar chart of cooking temperature; E. The influence of salinity on sensory evaluation; F. Radar chart for sensory evaluation of salinity)

First, the influence of processing time on the formation of squid consistency was studied at a constant temperature of 60°C. Set the cooking temperature to 60°C and perform a single factor test on the cooking time. The results showed (Figure 3.6A and

3.6B) that when the cooking time was 20 min and 30 min, the taste and aroma were excellent, especially when the cooking time was 30 min, the texture was the best. With the prolonged cooking time, the sensory evaluation showed a downward trend. In the end, the best cooking time was 30 min.

The next step was to study the effect of temperature on the formation of consistency at a fixed processing time of 30 minutes. Set the cooking time to 30 min and perform a single factor test on the cooking temperature. The results showed (Figure 3.6C and 3.6D) that the sensory scores first rose and then fell as the cooking temperature increased. When the cooking temperature was 60°C, the overall score was the highest, and the final cooking temperature was 60°C.

The difference between SV and traditional cooking is that there is no stir-frying process during the heating process. Therefore, preliminary seasoning is required before cooking. The squid and sauce should be placed in a vacuum packaging bag together. In this study, the single-factor experiment on salinity showed that (Figure 3.6E and 3.6F) when the salinity was 0% and 2%, the aroma and taste of squid were insufficient, and there was a significant improvement as the marinating time increased. When the salinity exceeded 2%, the sensory score appeared to decrease, and the taste of squid was too heavy when the salt was too high. In addition, as the marinating time was prolonged, the water in the squid would be lost to varying degrees under the osmotic pressure of the salt, which reduced the quality of the squid. Finally, chose 2% salinity.

2. Response surface method optimization test results

Based on the results of the single-factor test for SV squid, a response surface design test was conducted to determine the best process parameters for SV squid using three factors that have an influence on the process: cooking temperature (A), cooking time (B) and salinity (C) according to the Box-Behnken central combination test design principle, and a response surface analysis test was conducted to determine the best process parameters for SV squid, and the experimental design and results are shown in Table 3.10.

Table 3.10 – Box-Behnken response surface test design and results

No.	A: Cooking temperature (°C)	B: Cooking time (min)	C: Salinity (%)	Y: Sensory score

1	55	40	2	36
2	65	20	2	38
3	60	40	3	38
4	60	30	2	43
5	55	30	3	38
6	65	40	2	39
7	55	20	2	37
8	60	30	2	43
9	60	30	2	43
10	60	20	3	39
11	60	40	1	40
12	60	30	2	43
13	60	30	2	44
14	55	30	1	37
15	65	30	3	38
16	60	20	1	40
17	65	30	1	40

The quadratic multinomial regression model of sensory ratings (*Y*) on cooking temperature (*A*), cooking time (*B*) and salinity (*C*) was obtained by fitting the data information in Table 3.9 by multiple regression with Design-Expert 13 software as:

$$Y = 43.20 + 0.875A - 0.125B - 0.50C + 0.50AB - 0.75AC - 0.25BC - 3.35A^2 - 2.35B^2 - 1.60C^2$$

According to this model to predict the original data, the correlation coefficient of the equation is R^2=0.9850, and the correction coefficient of determination is 0.9656 (0.9656>0.80), indicating that the correlation of the model is good. The signal-to-noise ratio is 19.95 (19.85>4), meaning the equation can reflect the actual experiment well; the coefficient of variation is 1.18%, and the model cannot explain only 3.44% of the variation of the model. The model has a good fit and can be used for preliminary analysis and prediction of the SV squid process. As shown in Table 3.11, the regression model P<0.0001, indicating that the model is highly significant. Among the influencing factors of sensory evaluation, the cooking temperature has the most significant impact (P<0.01), followed by salinity (P<0.05) and cooking time (P>0.05); in cross-effects, cooking temperature and salinity have significant effects on the senses (P<0.05). In addition, the *p*

values of A^2, B^2, and C^2 are all less than 0.001, indicating that the influence of test factors on the response value is not a simple linear relationship, and the quadratic term also has a significant influence on the response value. According to the multiple regression fitting analysis and processing of three factors, the response surface of the sensory evaluation was analyzed (Figure 3.7, Figure 3.8, and Figure 3.9). The results showed that the interaction between cooking temperature and salinity was strong, consistent with the analysis of variance in Table 3.10.

It is worth noting that the cooking temperature of SV technology has a more significant impact on the food than the cooking time. The texture, color, aroma, and flavor of squid significantly lacked after heating treatment at lower temperatures. In contrast, the color, aroma, and flavor evaluation of squid decreased significantly when the cooking temperature was too high, in addition to texture. The vacuum packaging form and low-temperature heating can effectively reduce the water loss of squid during the heating process, ensuring a tender and juicy taste. The higher the cooking temperature (central temperature), the greater the cooking loss. That is because as the cooking temperature of meat products increases (40°C), the contraction of myogenic fibrous proteins leads to a decrease in interfibrillar volume, the water retention capacity of myogenic fibers decreases, and the loss of water in muscle tissue during heating increases the hardness of cooked meat. Our study found that the tenderness of squid decreased at 60°C as the heating time continued to grow, despite the gradual increase in cooking losses. That is inconsistent with the results of other scholars' studies. Becker [62] et al. and Christensen et al. [106] found that extended cooking time (5-17 h, 48-63°C) did not change the low-temperature vacuum cooking pork shear force. Similarly, Sanchez Del Pulgar et al. [65] showed that pork cheek meat's texture (hardness, viscosity, elasticity, adhesion, and chewiness) did not increase with low-temperature vacuum cooking (60°C, 5-12 h of continuous heating). Different types of muscles have different properties and compositions. The muscle structure of aquatic products and animal products is even more different. Therefore, the cause of SV squid tenderization still needs further investigation.

3. Prediction and verification experiments of optimal process conditions

Table 3.11 – Response surface test results of variance analysis

Source	Sum of Squares	df	Mean Square	*F*-value	*P*-value	Significance
Model	101.51	9	11.28	50.94	< 0.0001	**
A-Cooking temperature	6.13	1	6.13	27.66	0.0012	**
B-Cooking time	0.1250	1	0.1250	0.5645	0.4769	#
C-Salinity	2.00	1	2.00	9.03	0.0198	*
AB	1.0000	1	1.0000	4.52	0.0712	#
AC	2.25	1	2.25	10.16	0.0153	*
BC	0.2500	1	0.2500	1.13	0.3233	#
A^2	47.25	1	47.25	213.40	< 0.0001	**
B^2	23.25	1	23.25	105.01	< 0.0001	**
C^2	10.78	1	10.78	48.68	0.0002	**
Residual	1.55	7	0.2214			
Lack of Fit	0.7500	3	0.2500	1.25	0.4028	
Pure Error	0.8000	4	0.2000			
Cor Total	103.06	16				
			R^2=0.9850 Adjusted R^2=0.9656			

Note: "" indicates significant difference (P<0.05), "**" indicates highly significant difference (P<0.01), "#" indicates insignificant difference (P>0.05).*

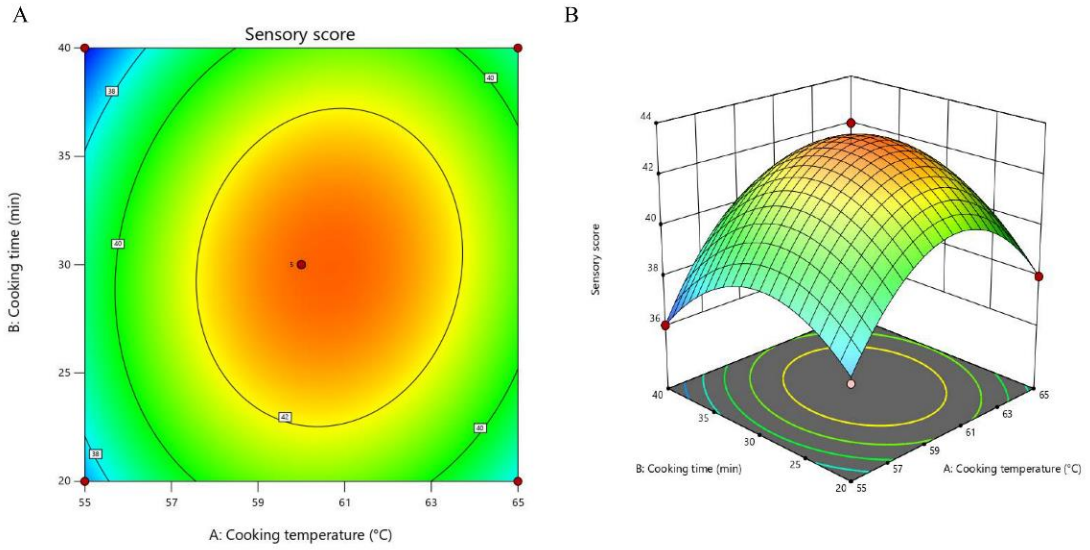


Figure 3.7 – Contour maps and surface maps for each factor interaction (A. Contour graph of cooking time and cooking temperature; B. Surface graph of cooking time and cooking temperature.)

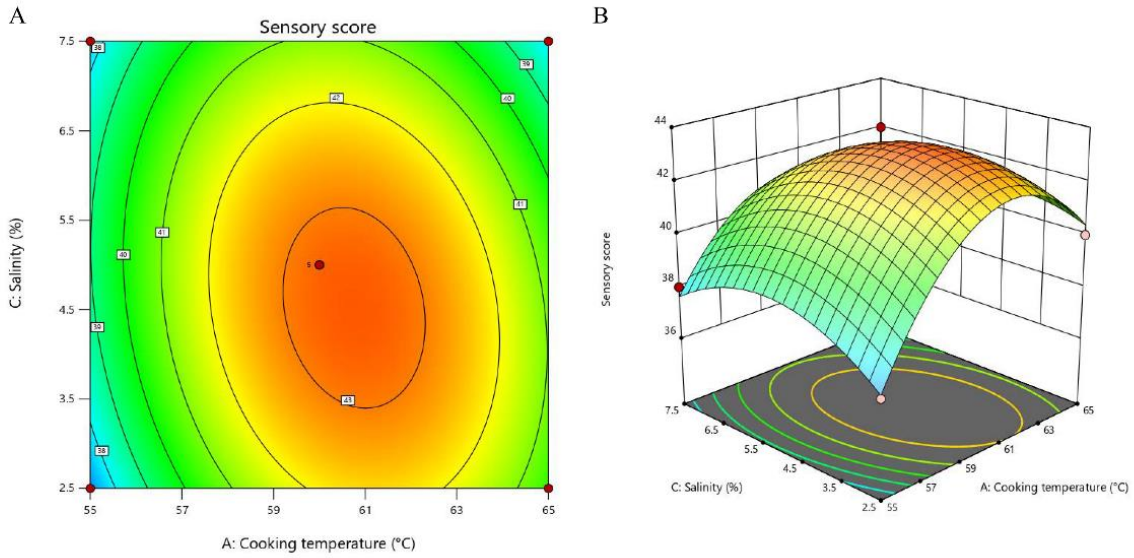


Figure 3.8 – Contour maps and surface maps for each factor interaction (A. Contour graph of salinity and cooking temperature; B. Surface graph of salinity and cooking time.)

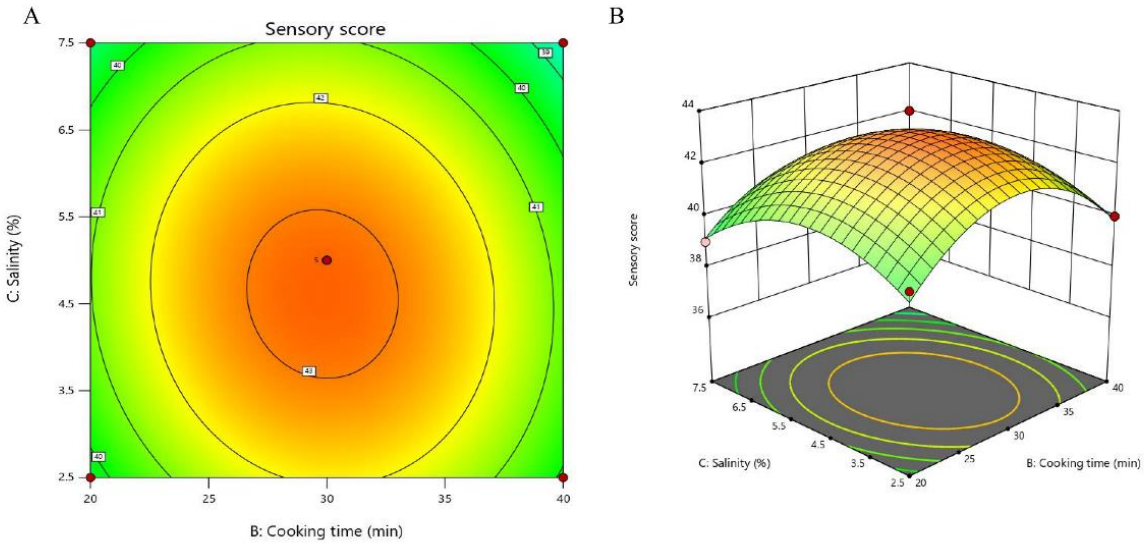


Figure 3.9 – Contour maps and surface maps for each factor interaction (A. Contour graph of cooking time and salinity; B. Surface graph of cooking time and salinity.)

The above analysis showed that the best technological test plan for SV squid is cooking temperature 60.7°C, cooking time 30 min, salinity 1.8%, and the predicted sensory score was 43.32. The sensory score of 44.12±0.52 (n=3) obtained from the experiments according to this optimal process parameter is consistent with the

theoretically predicted value, which indicates that the response surface analysis method is feasible for SV squid process optimization. The actual sensory scores were higher than the predicted values, which may be part of the interference factors in the evaluation process. The following study can refer to several criteria for judging to make the results more objective and comprehensive.

However, why the lower heating temperature can make the squid tender compared with the traditional cooking method, and what is the mechanism of the tenderization of SV squid? We conduct further research to make the results more objective and comprehensive.Based on the above research, the next we explore the SV squid tenderization mechanism through cooking loss, TVC, TCA-soluble peptide determination, and endogenous enzyme activity to provide a theoretical basis for the intensive processing of aquatic products.

3.3.2 Effects of cooking treatment duration on SF

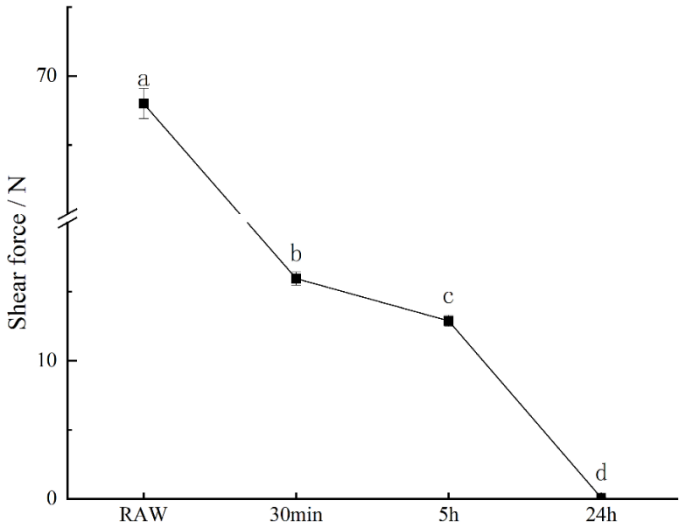


Figure 3.10 – Effect of heating time on the shear force of SV squid.

SF is an essential index for judging the tenderness of meat products, and SF is inversely proportional to tenderness. On Figure 3.10 shows the results of a study of the effect of heating time on changes in shear force at 60°C. In this study, it can be seen that the SF of the squid became smaller as the heating time of SV was prolonged (P<0.05), and when the heating time reached 24 h, the SF of the sample reached 0.059 N (Figure 3.10). At this time, the appearance of the sample was effortless to break, although it was still intact. That is consistent with the findings of scholars that meat products become

increasingly tender as SV heating time increases. When the core temperature of the meat product is close to 60°C and the heating time is extended, a more tender meat product can be obtained. Laakkonen et al. [167] and Machlik et al. [168] found that cooking for a long time at a temperature close to 60°C not only avoided the increase in meat toughness observed at higher temperatures but also improved the tenderness of the meat after keeping it for 4 h. These observations were further confirmed in scallops. SV at 65°C for 20 min, the scallops had a good taste and full shape, and the nutrient content of taurine, protein, and vitamins is significantly higher than that of traditional cooking [108]; the SV sea cucumber cooked at 70°C for 60 min has moderate hardness, crisp and smooth taste, and flexibility [109]. Espinosa [111] found that the flavor and texture of the *Sparus aurata* were best when cooked at 60°C for 12-15 min. In addition, SV technology could replace the pre-treatment of tender meat technology [110]. SV is known to tenderize meat products compared to other cooking methods, as it has a better ability to retain moisture in meat products during heating. More prolonged heating of SV makes squid softer, and whether the reason is still related to retaining moisture or whether other reasons cause squid to soften requires further research.

3.3.3 Effects of cooking treatment duration on cooking loss

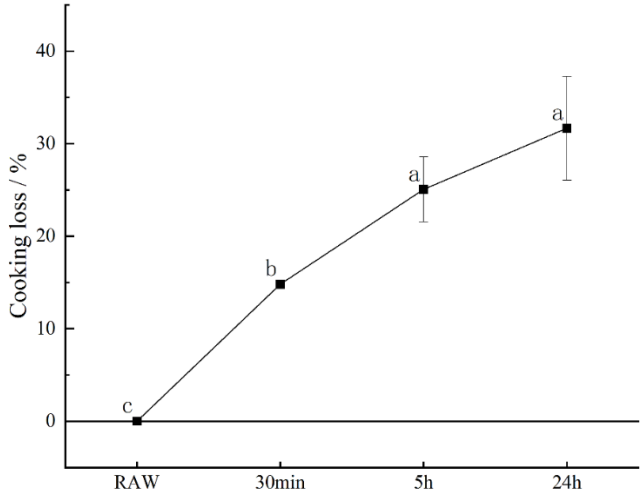


Figure 3.11 – Effect of heating time on the cooking loss of SV squid.

Cooking loss is a measure of moisture loss from meat products during heating. We found that the cooking loss of the samples gradually increased with heating time ($P<0.05$). When heated for 24h, the samples' cooking loss was maximum (Figure 3.11). It is noteworthy that the difference was not significant ($P>0.05$), although the cooking loss

increased from 25.05% to 31.66% during the heating of the samples from 5 h to 24 h. Qiu et al. [169] found that the cooking loss of large yellow croaker (*Larimichthys crocea*) increased as the cooking temperature (60 and 70°C) increased, but the increase in time (5 and 10min) had little effect on the cooking loss. We found that as the heating time of SV increased, the cooking loss became increased, but the sample became tender, which contradicts the results of Cui [88], who found that squid cooking loss and hardness increased with increasing SV temperature (55-85°C). It can be seen that the reason for the tenderness of SV squid after prolonged heating at 60°C (30 min to 24 h) is not related to moisture. Instead, as the heating time increases, the cooking loss increases. Taking 24 hours as the endpoint of the experiment is because the squid has lost the taste of the modified product by continuing to prolong the heating time. In addition, the purpose is to explore the root cause of the tenderness of the SV squid.

3.3.4 Investigation of factors influencing the formation of the structure of squid muscle tissue

Protein is an essential component of squid, and protein degradation is an important cause of softening the meat texture. Researchers generally believe that the protein changes of squid after death are closely related to the degradation of proteases. However, the detailed mechanism of the texture change of SV squid is still not fully understood. Therefore, it is of great significance to understand the changes of proteins in squid in the process of SV and to study the role of protease in SV squid.

1. TVC

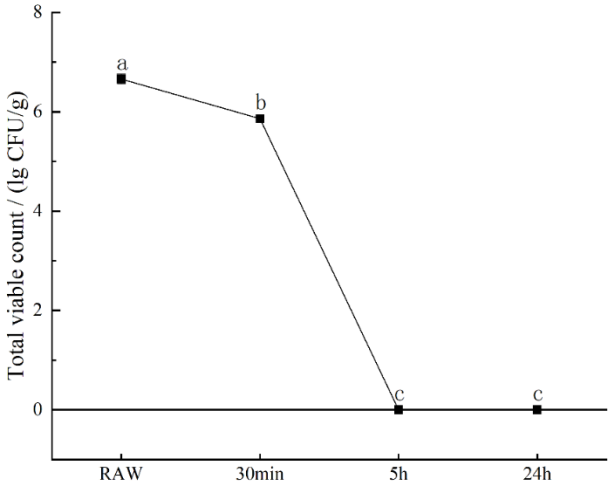


Figure 3.12 – Effect of heating time on the TVC of SV squid.

The softening of texture is generally accompanied by the degradation of major proteins in muscle. The leading cause of protein degradation is enzymes, including muscle endogenous enzymes and enzymes of microbial origin [170]. The reason for the tenderness of SV squid is the action of endogenous enzymes or enzymes derived from microorganisms. The number of microorganisms surviving during the heating process of SV is a key factor.As the heating time was extended in this study, the TVC decreased (P<0.05) (Figure 3.12). When heated to 5 h, TVC was no longer visible, which shows that SV at 60℃ was safe. In addition, that result also proved that the reason for the long-term heating and tenderization of the sample at a lower temperature was not caused by spoilage microorganisms, which grown and reproduced, penetrated the muscle tissue, and destroyed the tissue structure.

2. TCA-soluble peptide

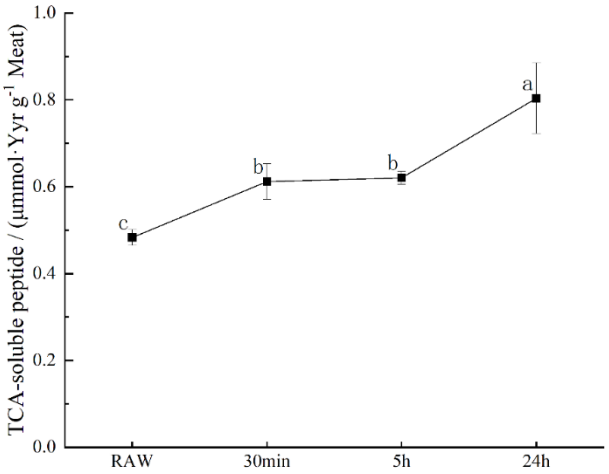


Figure 3.13 – Effect of heating time on the TCA-soluble peptide of SV squid.

There are two central proteolytic systems in the tenderization process of aquatic products: cathepsin and calpain. Unlike mammals, calpastatin in fish muscles is still active during the storage process after the fish dies [171]. During the micro-freezing process, part of the water in the muscle tissue of the fish freezes, and the concentration of cytoplasm causes the concentration of calcium ions to increase. It activates calpain, causing the enzyme activity under micro-freezing conditions about 50% higher than ice-stored samples [172]. In contrast, cathepsin has more excellent thermal stability than calpain [167; 173]. Calpain is completely inactivated at temperatures above 55°C, while cathepsins, especially B and L, have been reported to remain active after being heated at 55°C for 24 h

[106; 174]. Cathepsins B and L are endopeptidases, which may increase the solubility of collagen by weakening the connective tissue, thereby promoting the softness of the meat [58; 175; 176]. It has been reported that incubating connective tissue with cathepsin B can significantly reduce the denaturation temperature of connective tissue in calves and steers [177]. In addition, a study by Burleigh et al. [178] showed that cathepsin B promotes the degradation of soluble and insoluble collagen by eliminating intermolecular crosslinks.

The TCA-soluble peptide is one indicator that reflects the degree of protein degradation by endopeptidases and microorganisms [179]. The higher the content of TCA-soluble peptide, the higher the degree of protein degradation. The TCA-soluble peptide showed an upward trend ($P<0.05$) with the extension of heating time, indicating that squid protein had undergone autolysis during the heating process (Figure 3.13). Cai et al. [180] and Jiang et al. [181] found similar results during the ice storage of red sea bream fillets and grass carp fillets. The content of TCA-soluble peptides increased significantly, indicating that the samples were affected by endogenous enzymes during storage, resulting in the nitrogen-containing degradation products of myogenic increased and the softening of meat products. In particular, the results might indicate the activity of endogenous enzymes during heating protein degradation.

3. MFI

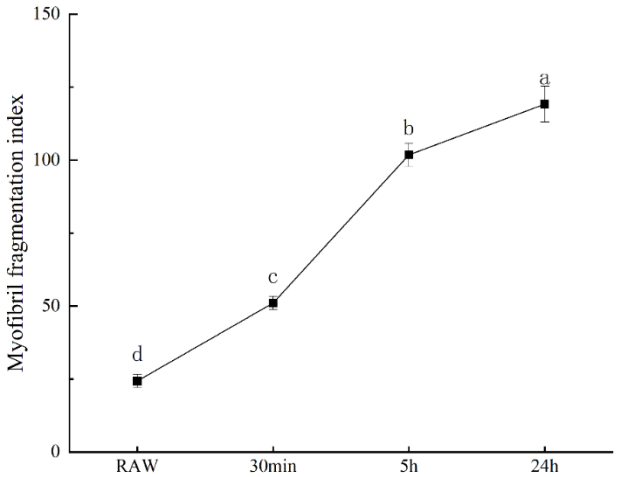


Figure 3.14 – Effect of heating time on the MFI of SV squid.

During the post-mortem maturation process of aquatic products, the following changes have taken place in the structure of myofibrils: Z-line degradation, breakage of the connection between myosin and actin, degradation of connexins, and breakage of the

original intact myofibrils into different numbers of muscles. This process is called MFI [182]. The MFI index refers to the proportion of myofibrils with a length of 1-4 sarcomeres in the total number of myofibril fragments. MFI reflects the degree of destruction of myofibrils and their skeletal proteins in muscle cells, and it can also be considered to reflect the degree of muscle maturation [183]. Figure 3.14 shows that as the heating time increases, the MFI value showed an upward trend, and the MFI value varied significantly at each time (P<0.05). In this study, heating was performed at 60°C. The endogenous enzyme was not wholly inactivated at this temperature, and it was beneficial to enzyme activity. Myofibrillar protein was broken down into small fragments under the action of endogenous enzymes, which was consistent with this study result of TCA-soluble peptides. In addition, the fragmentation of myofibrils led to a decrease in water holding capacity, and cooking loss decreased with the extension of the heating experiment. To observe the changes of myofibrils of snakehead fish fillets at -20°C, Jiang et al. [181] found that the myofibrils of snakehead fish fillets with different freezing methods and temperatures showed different degrees of flaking during freezing and hypothesized that this change was caused by protein dehydration, and these changes would directly cause changes in the texture and water-holding capacity of fish flesh. The larger MFI showed that the more the myofibrils were broken down, the greater the degree of damaged to the integrity of the internal structure of the myofibrils. The smaller the MFI showed that, the better the integrity of the internal system of the myofibrils [182]. Studies have shown a significant correlation between MFI and meat tenderness [184; 185].

4. Myofibril apparent diameter

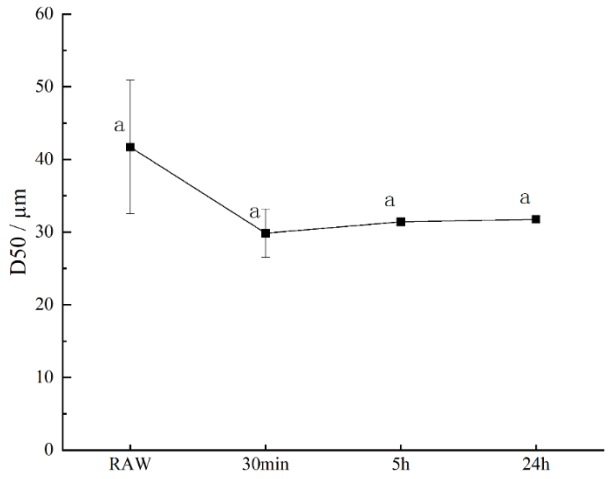


Figure 3.15 – Effect of heating time on the myofibril apparent diameter of SV squid.

The apparent diameter of myofibril is used to quantify the index of the degree of destruction of myofibril during the storage of meat products. As shown in Figure 3.15, with the extension of heating time, the apparent diameter of squid myofibril protein showed a downward trend. The result was consistent with the result that MFI and TCA-soluble peptides increased with increasing heating time.

5. Total proteolytic activity

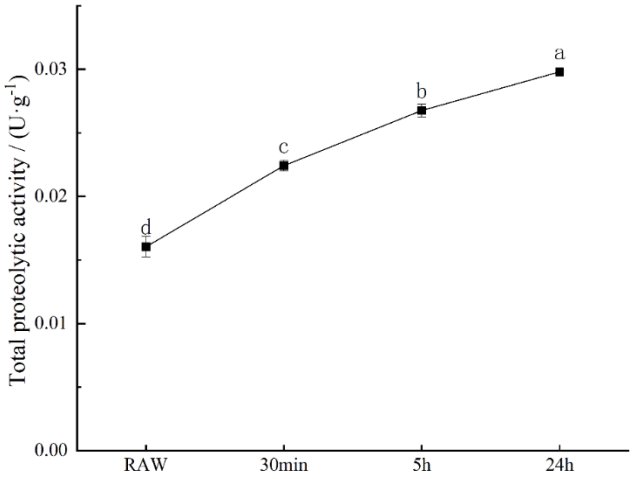


Figure 3.16 – Effect of heating time on the total proteolytic activity of SV squid.

After the death of aquatic products, the muscles will initiate the process of cell apoptosis, which the activation of endogenous enzymes will accompany [186]. The main endogenous enzymes in muscles are cathepsin, calpain, and matrix metalloproteinases, which degrade protein after the death of aquatic products [187]. Figure 3.16 shows that total proteolytic activity gradually increased as the heating time increased ($P<0.05$). The result indicated that heating did not entirely inactivate endogenous enzymes, but heating increased enzyme activity. Generally, the softening of raw meat quality is caused by protein degradation caused by cell apoptosis [188]. After the death of the aquatic product, the muscle tissue initiates the process of programmed cell apoptosis, the endogenous protein degrading enzyme in the aquatic product [189]. The endogenous enzyme in aquatic products has an impact on texture. The activities of cathepsin B, cathepsin D, cathepsin L, calpain, and collagenase are closely related to the quality of aquatic products [190]. Bahuaud et al. [170] explained that the muscle fiber gap of Atlantic salmon under micro-freezing conditions increases and accelerates the loss of juice and pointed out that tissue

protease B and L play a role in it. Godiksen et al. [35] investigated the changes in the electrophoresis pattern of myofibrillar protein after cathepsin B, D, and L treatment. They pointed out that cathepsin D was the central protease that caused the texture deterioration of rainbow trout. Hultmann et al. [191] found that the effect of collagenase on the texture and protein changes of Atlantic salmon and cod during ice storage was the main factor that caused cod texture softening. The hardness loss of salmon during ice storage was closely related to cathepsin D. In this study, the endogenous enzyme activity in SV squid increased and degraded protein, which was one of the reasons for the tenderness of squid. Further research is needed as to which endogenous enzyme is the main reason for the tenderness of SV squid.

6. Microstructure

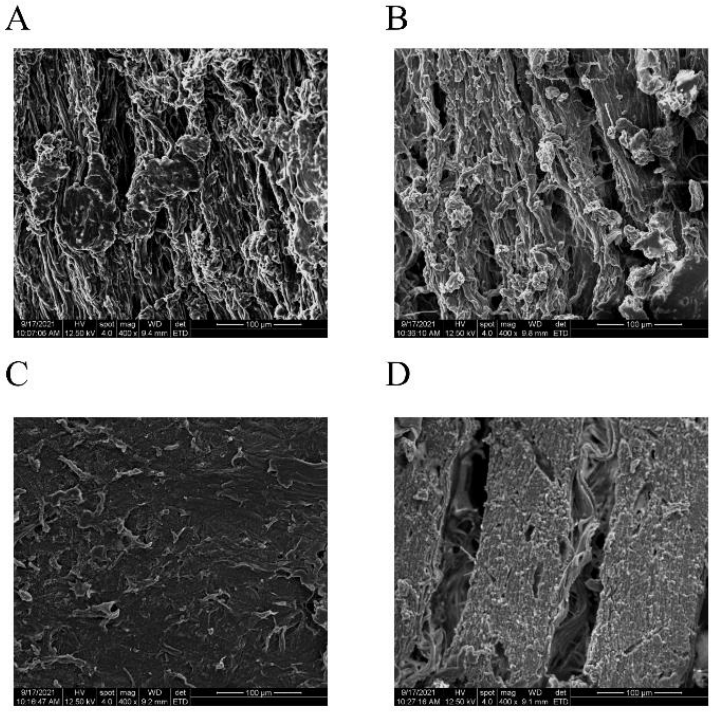


Figure 3.17 – Changes in the microstructure of SV squid at different heating times.

Scanning electron microscopy is used to detect the microscopic state of muscle fibers. Through the high resolution of the microscope, the muscle fibers can be degraded by cathepsin and the separated state from the endomysium. That can be displayed in the form of images to analyze the microstructure. Figure 3.17 shows the changes in the microstructure of SV squid at different heating times. The muscle fiber bundles of raw squid, the endomysium, and the perimysium were closely connected, and the gaps between

the muscle fiber bundles were tiny. Compared with raw squid, after heating for 30 min, differences appeared between different treatment groups. The boundaries between the squid muscle fiber bundles became blurred, the arrangement was disordered, the gaps between the muscle fibers increased, and the particulate matter appeared on the surface. These granules may be produced by the degrading connective tissue, such as endomysium and perimysium, and some sarcoplasmic proteins [192]. With the prolongation of heating, the degree of deterioration of muscle fibers was also increasing. Collagen fibers and connective tissue connect muscle fibers. Both muscle fibers and collagen fibers are degraded by endogenous enzymes, which leads to the separation of muscle fibers and intramuscular membrane and the formation of a gap [193]. In addition, the gaps between muscle fiber bundles were likely to occur with the loss of muscle juice. The microstructure changes of SV squid were consistent with their increased cooking loss, increased endogenous enzyme activity, and increased MFI.

7. Correlation analysis

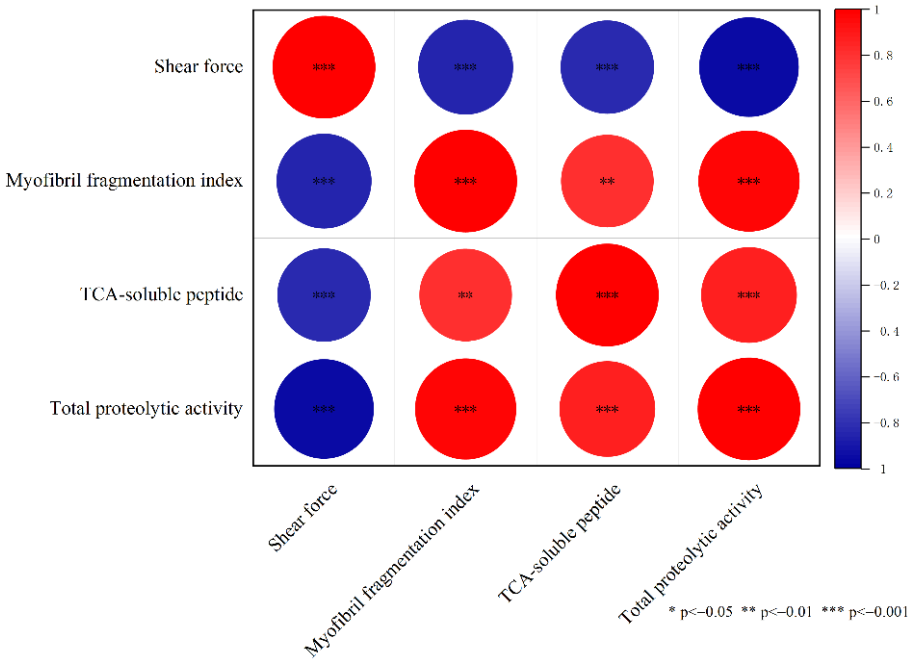


Figure 3.18 – Pearson, correlation analysis of indexes such as shear force, TCA-soluble peptide, and total proteolytic activity during heating of SV squid

We analyzed the correlation between the changes in the SF, MFI, TCA-soluble peptides, and total proteolytic activity of SV squid during heating. As shown in Figure 3.18, SF has negatively correlated with MFI, TCA-soluble peptides, and total proteolytic

activity (P<0.001), indicating a particular relationship between shear force, protein degradation, and proteolytic activity. The total proteolytic activity was positively correlated with MFI and TCA-soluble peptides (P<0.001). Moreover, MFI is positively correlated with TCA-soluble peptides (P<0.01). It shows that there was a particular connection between enzyme activity and protein degradation. These results can be inferred from the above that SV heating did not inactivate the endogenous enzymes of squid. During the SV heating process, the action of protease accelerated the degradation of protein, leading to the deterioration of various protein indicators, leading to the SF decrease.

With the increase of heating time of SV squid (60℃), squid SF and myofibril apparent diameter decreased, while cooking loss, TCA-soluble peptide, MFI, and Total proteolytic activity all increased. Scanning electron microscopy found that with the increase of heating time, the space between muscle fibers increased, and the boundaries between muscle fiber bundles began to become blurred and arranged disorderly. It can be inferred that the reason for the tenderness of the SV (60°C) squid is that the squid's endogenous enzymes degrade myofibril. The results of this study also explained why SV squid was more tender than conventionally (ST and BO) cooked squid.

3.4 Effect of Blu-ray sterilization on SV squid and its quality changes during storage

Compared with livestock and poultry meat, aquatic products are highly susceptible to spoilage, and microbial activity is the leading cause of spoilage of aquatic products during storage. The number of bacteria in aquatic products is an essential indicator of the degree of spoilage. Although many studies have shown that SV can ensure food safety, further sterilization can be sufficient to ensure food safety and reduce the probability of foodborne diseases. In addition, the addition of sterilization technology can also prolong the shelf life of SV squid and reduce the negative impact on quality caused by the storage period. As a result, enterprises can reduce production costs and increase their operating income. After SV, the food needs to be cooled quickly to reduce the temperature and microbial damage to the food. In this way, heat sterilization technology cannot be applied

to SV food. The Blu-ray sterilization technology has proven to be an effective non-thermal sterilization technology. Moreover, the Blu-ray sterilization effect is better in low-temperature conditions. The combination of Blu-ray (435 nm, 33.8 J/cm^2) and curcumin (50 μM) reduced the bacteria of *Staphylococcus aureus* on the surface of cucumber and pepper by 2.5-2.6 log CFU [194]. Kim et al. [195] found a 0.8-0.9 log CFU reduction of *Salmonella* on the cooked chicken after Blu-ray (405±5 nm) irradiation at 4°C, while Blu-ray inhibited bacterial growth at 10°C and 20°C, indicating that low-temperature environments are more suitable for Blu-ray sterilization. However, studies have shown that while sterilizing the milk, Blu-ray causes a bleaching effect visible to the naked eye [84] and can also cause discoloration of orange juice [85]. Currently, the effectiveness of sterilization of SV squid using Blu-ray has not been reported in the literature, and no scholar has studied whether Blu-ray affects the quality of cooked squid.

Next, we investigated the effectiveness of Blu-ray sterilization on SV squid and its effect on the quality of the squid by measuring the TVC, sensory evaluation, colour, pH, shear force, and TBARS. Furthermore, we explored and analyzed their quality changes and shelf-life during storage at 0, 5, and 10°C.

3.4.1 The effectiveness of Blu-ray on the sterilization of SV squid

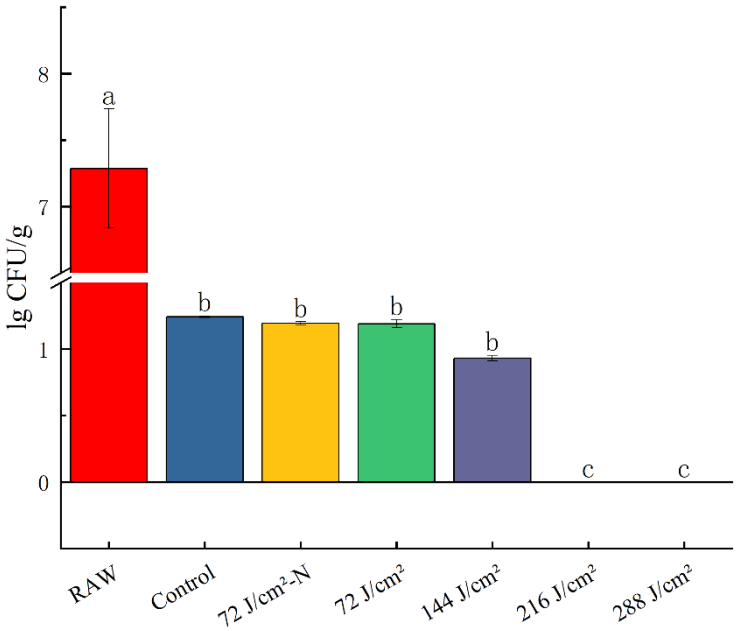


Figure 3.19 – Effect of Blu-ray irradiation dose on the total number of bacterial colonies. Different letters indicate differences ($P < 0.05$).

We prepared squid samples according to the results in 3.3.1 (60℃ for 30 min, salinity 1.8%). The samples for studying the effect of Blu-ray irradiation on changes in quality indicators and determining the shelf life of sous-vide squids were prepared in accordance with the developed optimal sous-vide processing parameters. The Blu-ray (460−475 nm) was irradiated according to the parameter requirements of 2.2.28 (dose 72 J/cm^2, 144 J/cm^2, 216 J/cm^2).

The cooking temperature of SV at 60°C, it could also reduce the number of bacteria in the samples and meet the safety standards for consumption (Figure 3.19). In this experiment, as the dose of Blu-ray increased, it could effectively reduce the number of TVC (P<0.05). When the irradiation dose reached 216 J/cm^2, no bacteria were detected. Josewin et al. [196] evaluated the efficacy of Blu-ray (405 nm and 460 nm) to kill *Listeria monocytogenes*（*L. monocytogenes*）and *Salmonella monocytogenes* on the epidermis of cantaloupe and showed that Blu-ray was more effective at 4°C and 20°C, with inactivation of both bacteria ranging from 1.1 to 3 lg CFU/g. Besides, using riboflavin-assisted Blu-ray (460 nm) sterilization technology could minimize *L. monocytogenes* in smoked salmon, reducing the risk posed during storage [87]. However, SV's food is placed in vacuum bags for heating, and further research is needed to determine whether the packaging blocks Blu-ray to reduce sterilization effectiveness.

In order to explore whether the packaging bag can affect the Blu-ray sterilization effect, the experiment used the same dose of Blu-ray (72 J/cm^2) to irradiate the samples with the vacuum bag and without the bag (72 J/cm^2-N), respectively. It was found that there was no difference in the irradiation effect (P>0.05), indicating that the vacuum bag did not block the Blu-ray sterilization effectiveness. Hyun et al. [197] indicated the bactericidal effect of Blu-ray (460-470 nm) on nylon/polyethylene film (0.08 mm and 0.06 mm) wrapped sliced cheese and found that this material was significantly effective in killing *Pseudomonas fluorescens*. Thus, Blu-ray reduced and killed bacteria in SV squid, and the bacteria in the samples were dose-dependent on Blu-ray sterilization. It is because that the excitation of endogenous porphyrins and the subsequent production of cytotoxic reactive oxygen species (ROS) by bacteria under the irradiation of Blu-ray leads to cell death [80; 198]. Therefore, we used Blu-ray at a dose of 216 J/cm^2 for all the following

experiments to irradiate the samples and investigate Blu-ray's effect on the quality of SV squid.

3.4.2 Sensory effects of Blu-ray irradiation on SV squid

The introduction of innovative technological methods in food technology requires a study of their effect on the organoleptic characteristics of the finished product. No one has previously investigated the effect of Blu-ray irradiation on the quality of SV squid. To investigate whether Blu-ray has a quality effect on the squid samples. We chose to irradiate the samples at a dose (216 J/cm^2) that effectively killed the bacteria and compared the study with unirradiated samples. As shown in Figure 3.20, the Blu-ray irradiated samples had higher color scores and lower flavor than the control group, while there was no difference in appearance and texture. Overall, the Blu-ray irradiated samples were judged to be few differences from the control group and had high acceptability.

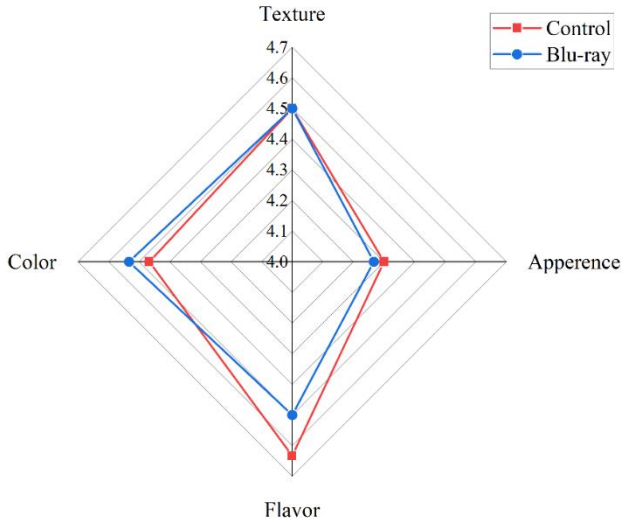


Figure 3.20 – Effect of Blu-ray irradiation (dose 216 J/cm^2) on the sensory of sous vide cooking squid (60℃ for 30 min).

3.4.3 Effects of Blu-ray irradiation on SF of SV squid

Tenderness is an essential parameter for evaluating the edible quality of meat, reflecting the structural characteristics of protein in meat, the distribution state, and fat content. As Figure 3.21 showed, the SF of samples irradiated by Blu-ray(19.22±0.31 N) decreased compared to the control group (17.24±0.32 N) (P<0.05). To further investigate the effect of Blu-ray on the samples, we examined the effect of different doses on the SF of the samples and found that the SF of the samples decreased as the irradiation dose

increased. Moreover, we irradiated single-sided (shows as 72 J/cm^2-S in Figure 3.22) and double-sided (72 J/cm^2 per side irradiation) (shows as 72 J/cm^2-D in Figure 3.22) of the samples (equivalent to double dose), and the results also showed that the SF of the samples irradiated on both sides (17.06±0.56 N)was smaller than that of the samples irradiated on one side (18.55±0.65 N). There was no difference in the shear force between single-sided and double-sided irradiated samples (P>0.05), except for the samples irradiated at 216 J/cm^2, where there was a difference in SF between single-sided (15.24±0.56 N) and double-sided irradiation (13.04±0.82 N) (P<0.05). As the dose of Blu-ray exposure increases, the result may be because the Blu-ray energy provided causes the sample to become tender. However, there was no difference in the quality of the samples judged by sensory evaluation only.

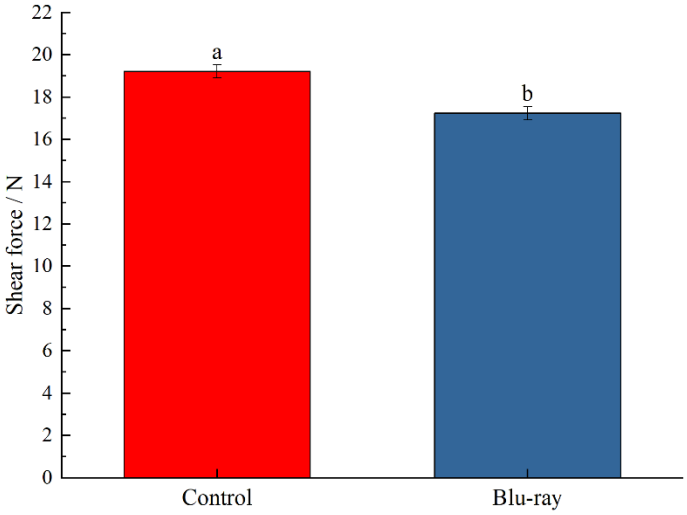


Figure 3.21 – Effect of Blu-ray irradiation (dose 216 J/cm^2) on the shear force of sous vide cooking squid (60℃ for 30 min). Different letters indicate differences (P < 0.05).

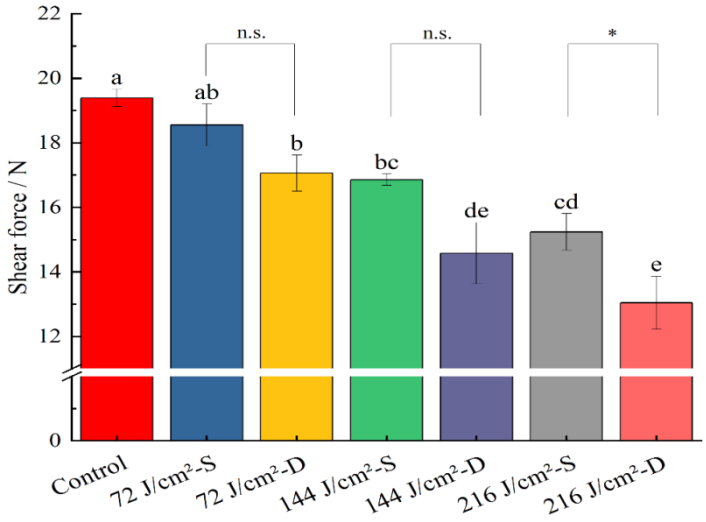


Figure 3.22 – Effect of Blu-ray irradiation dose on the sensory of sous vide cooking squid. Different letters and * indicate differences ($P < 0.05$), n.s. indicate indifference.

3.4.4 Effects of Blu-ray irradiation on color of SV squid

Table 3.12 – Effect of Blu-ray irradiation on the color of sous vide cooking squid

	*L**	*a**	*b**
Control	82.51±0.89Aa	0.40±0.09Bb	9.67±0.16Aa
Blu-ray	84.13±0.76Aa	3.06±0.10Aa	9.82±0.17Aa
ΔE	3.13±0.46		

Note: Values are mean ± standard deviation (SD). Different letters in the same column with different shoulder labels indicate significant differences; lowercase letters indicate (P<0.05) uppercase letters indicate (P<0.01).

Color is one of the essential indicators for the evaluation of squid products. In this study, it was found that *L** and *b** of the samples irradiated by Blu-ray did not differ from the that of unirradiated ($P>0.05$) (Table 3.12). It is noteworthy that the redness of the samples after Blu-ray irradiation became larger and differed from that of unirradiated, and it is significant ($P<0.01$). *ΔE* indicates the difference between the two colors. When *ΔE* is between 2 and 3.5 [199], inexperienced observers also notice the color variability. The *ΔE* of 3.13 in this study indicates a color difference between the samples irradiated by Blu-ray and those of the control group, consistent with the color results in sensory evaluation. It has been studied that irradiation of sliced cheese with Blu-ray (460 nm) at 4°C did not affect the color (*ΔE*) during storage and the *ΔE* of the irradiated sliced cheese was only 2.24 ± 0.84 by the seventh day of storage. However, irradiation of sliced cheese with the

same wavelength of Blu-ray at 25°C resulted in a ΔE of 4.47 ± 2.04 by the second day of storage [197]. Similar to these findings, Ghate et al. [85] showed that the effect of Blu-ray at 4°C (460 nm) on orange juice quality was less than that of Blu-ray irradiation at 25°C. Although the samples were kept at low temperature (4±2°C) during the irradiation in our study, the results indicated that Blu-ray (460 nm) had effects on the color of SV squid (ΔE=3.13±0.46). The discoloration of squid after illumination may be due to the absorption of Blu-ray by carotenoids in squid, whose absorption spectrum is between 400–500 nm, and their subsequent oxidative degradation[200; 201].

3.4.5 Effects of Blu-ray irradiation on pH of SV squid

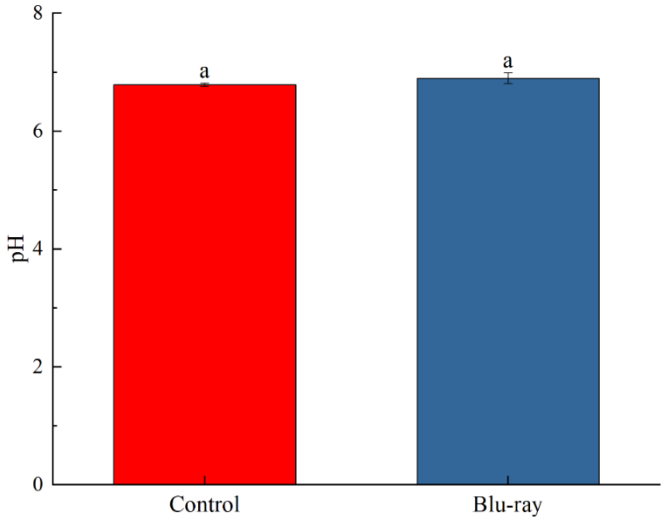


Figure 3.23 – Effect of Blu-ray irradiation (dose 216 J/cm^2) on the pH of sous vide cooking squid (60℃ for 30 min).

The pH value is one of the essential indicators of meat quality. In this study, the changes of pH of SV squid irradiated with Blu-ray were comparatively analyzed. The results showed that the pH value of the samples before irradiation was 6.79±0.02 and after irradiation was 6.89±0.07, and there was no difference between them (P>0.05) (Figure 3.23). It indicated that Blu-ray irradiation does not affect the pH of SV squid.

3.4.6 Effects of Blu-ray irradiation on TBARS of SV squid

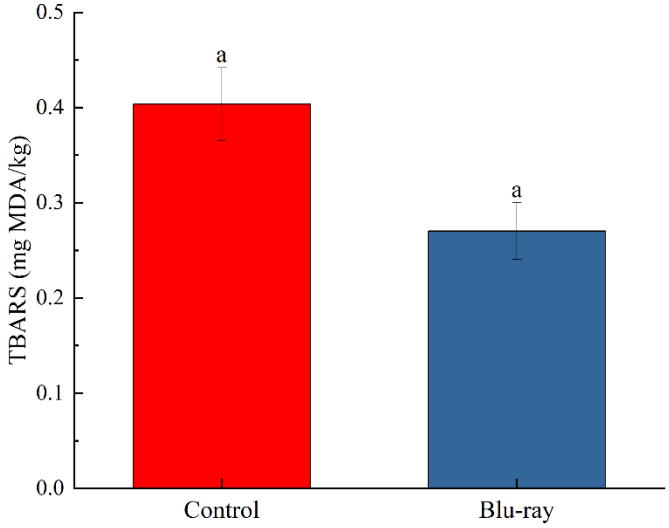


Figure 3.24 – Effect of Blu-ray irradiation on the thiobarbituric acid reactive substances of sous vide cooking squid.

Fats are subjected to light, heat, and oxygen and undergo an acidification reaction, decomposing aldehydes and acid compounds. In this study, we compared the degree of fat oxidation in the samples after Blu-ray irradiation, and the TBARS in the samples decreased from 0.241±0.027 to 0.175±0.021 before irradiation (Figure 3.24), but there was no difference between the two groups (p>0.05). Fat oxidation is due to the oxidation of double bonds of fatty acids to hydroperoxides, which are unstable and further decomposed to produce small molecules such as aldehydes, ketones, alcohols, and acids [202]. Light induces the activation of oxygen molecules and the generation of free radicals in the auto-oxidation chain reaction, thereby accelerating the rate of fat auto-oxidation [203]. Besides, the photosensitizer in meat (hemoglobin) converts from ground state oxygen($^3O_2$) to excited state oxygen ($^1O_2$) to attack the unsaturated double bonds of fat, resulting in a photosensitive oxidation reaction [204]. However, our results indicated that after Blu-ray irradiation, the SV squid did not undergo photo-oxidation.

3.4.7 Sensory changes of SV squid during storage after Blu-ray sterilization

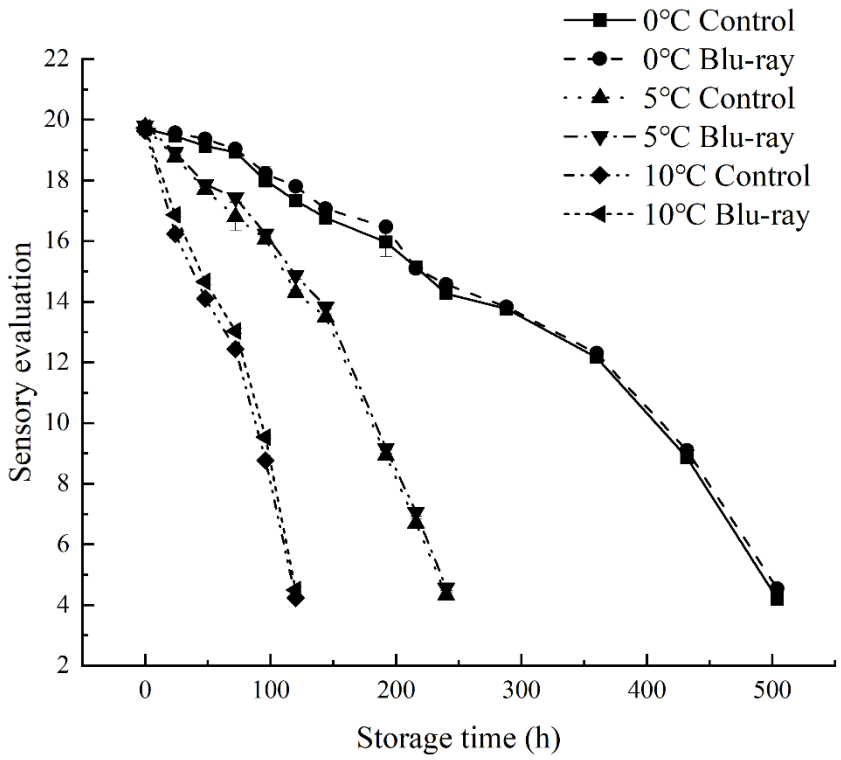


Figure 3.25 – Sensory evaluation of squid during storage.

This study chose 0, 5, and 10°C because the conventional refrigerated storage temperature is around 5°C due to temperature fluctuations during storage and transportation. We conducted the sensory evaluation following 2.2.30, and the scoring details are shown in Table 2.2. The color, odor, body mucus, and muscle elasticity of squid were evaluated and scored, with 20 being the best quality, 12 being the high-quality period endpoint, and 4 being the endpoint quality.The time required to reach the end of the high-quality period (control group and irradiated group) was 360, 144, and 72 h, and the sensory score was 12, while those taken to reach the end of shelf-life (control group and irradiated group) was 504, 240, and 120 h, and the sensory score was 4 during storage at 0, 5, and 10°C, respectively. The quality of squid at different temperatures varied greatly. Other than this, there was no significant difference in sensory scores between the Blu-ray treated and control groups. However, the sensory scores of the Blu-ray group were higher than those of the control group (Figure 3.25).

3.4.8 Color changes of SV squid during storage after Blu-ray sterilization

Table 3.13 – The value of L^*， a^*， b^* of squid during storage

Storage temperature	Time	*L**	*a**	*b**
0°C Control	Initial time	75.61±0.24b	-1.58±0.43a	2.32±0.35a
	The end of high-quality period	78.37±0.75a	-2.04±0.57ab	2.40±0.43a
	The end of shelf period	79.69±0.57a	-2.62±0.12b	2.50±0.47a
0°C Blu-ray	Initial time	78.79±0.72b	-1.24±0.09a	1.32±0.46a
	The end of high-quality period	79.39±0.47ab	-1.69±0.36a	2.30±0.34a
	The end of shelf period	80.32±1.19a	-2.42±0.26b	2.33±1.28a
5°C Control	Initial time	79.55±1.32a	-1.47±0.28a	1.54±0.11c
	The end of high-quality period	81.05±0.36a	-1.61±0.06a	4.02±0.42b
	The end of shelf period	81.57±0.17a	-1.62±0.43a	6.42±1.08a
5°C Blu-ray	Initial time	78.03±0.67b	-0.65±0.13a	0.11±0.26b
	The end of high-quality period	78.42±0.23b	-0.74±0.02a	3.56±0.16a
	The end of shelf period	80.46±0.25a	-0.78±0.16a	3.68±0.20a
10°C Control	Initial time	75.49±0.24c	-2.39±0.12b	0.76±0.53c
	The end of high-quality period	78.49±0.72b	-2.11±0.10ab	5.73±0.24b
	The end of shelf period	80.84±0.46a	-1.91±0.04a	9.57±1.08a
10°C Blu-ray	Initial time	77.48±0.26b	-4.10±0.37c	1.47±0.08c
	The end of high-quality period	77.75±0.17b	-1.84±0.30b	4.34±0.09b
	The end of shelf period	83.25±0.53a	0.35±0.02a	5.69±0.14a

Note: Results are mean±standard deviation (n=3), values within a column with different superscript letters are significantly.

The *L**, *a**, and *b** values of squid were measured using a LAB colorimeter as recommended by the International Commission on Illumination. The *L** and *b** values of squid at different storage temperatures showed an increasing trend. The trend of *a** was not noticeable; except for a more significant change at 10°C, the other values were more stable. With an increase in storage time, the color of squid displayed increased brightening and yellowing (Table 3.13). Although the skin of the squid samples was peeled, the surface was still protected by a film, and their oxidation rate was low. Therefore, the change in redness was small.

Moreover, the mucus moisture produced during the storage period covered the surface layer, resulting in progressive production of specular reflection by the film, thus increasing its brightness. Ramirez-Suarez et al. [205] found a similar gradual yellowing of color during storage (0°C, for 15 days) of squid (*Dosidicus gigas*). Our results, to some extent, indicated that with the prolongation of time, the squid surface mucus increased, and the quality decreased, which was consistent with the sensory evaluation.

Furthermore, in our study, the color of the samples treated with Blu-ray irradiation had some differences from the control group. Moreover, the samples after Blu-ray irradiation were whitish compared to the control group from the sensory observation. Thus, these changes might be caused by the loss of riboflavin in the squid samples after Blu-ray irradiation [84]. Besides, Ghate et al. [85] showed that the color of orange juice changed after exposure to Blu-ray (460 nm). However, the samples irradiated using Blu-ray did not reduce the sensory characteristics of the squid.

3.4.9 SF changes of SV squid during storage after Blu-ray sterilization

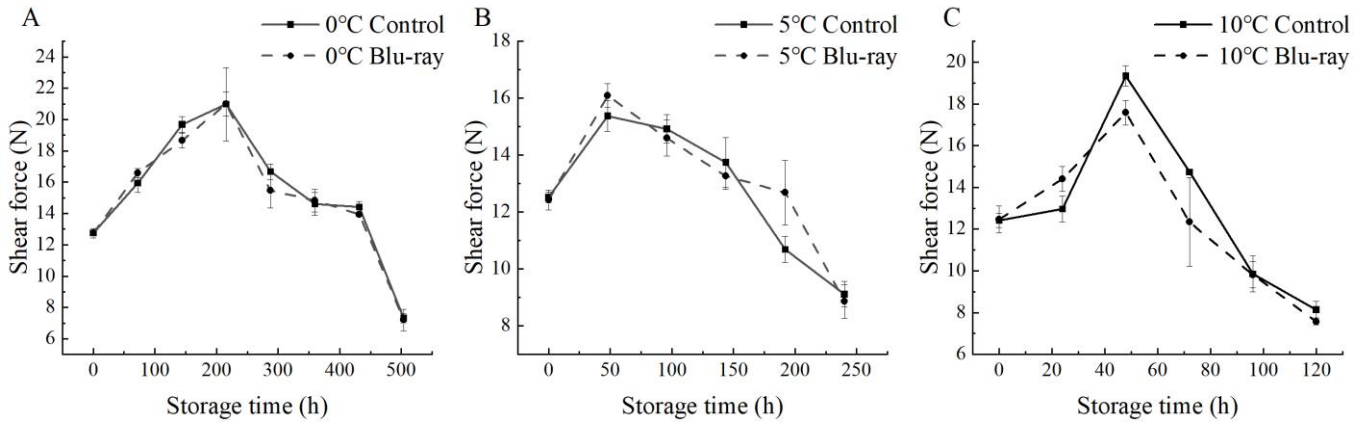


Figure 3.26– Shear force of squid changes during storage at (A) 0°C, (B) 5°C, and (C) 10°C.

SF value is widely used to measure the tenderness of aquatic food products, and it reflects the internal structure of the meat; the structural properties of various proteins in the muscle determine the tenderness of the meat and average meat shear value. The SF at all three temperatures increased and then decreased. Under anaerobic conditions, the vacuum-packed squid generates energy from glycolysis and produces lactic acid, which causes the pH of the muscle tissue to drop rapidly. The pH changes the homeostasis of protons and reduces the electrostatic repulsion between myofibrillar proteins. It results in irreversible lateral contraction of muscle fibers, making the squid stiff during the initial storage period.And then because of storage time, wherein the squid flesh softened due to decomposition (Figure 3.26). The storage of squid at 10°C exhibited the fastest rise and fall in SF value, suggesting that storage in ice could maintain the meat quality. However, the SF at 0°C was the highest, with a value larger than the initial SF, probably because the meat was slightly harder than fresh squid after storage at 0°C. Squid is rich in protein and has high elasticity, and as stiffness occurs, it can increase various indicators such as elasticity, hardness, and cohesion. In this study, the temperature of heat treatment was only 60°C. Therefore, the endogenous enzymes in the squid might not have been wholly inactivated and still functioned in the subsequent low-temperature storage. With the prolongation in storage time, the squid cell structure, muscle tissue, and protein stereo-structure were gradually destroyed by microorganisms, resulting in less SF [205; 206]. Our results showed that the Blu-ray irradiation treatment had little effect on the SF of the squid compared to each control group during storage.

3.4.10 TBARS changes of SV squid during storage after Blu-ray sterilization

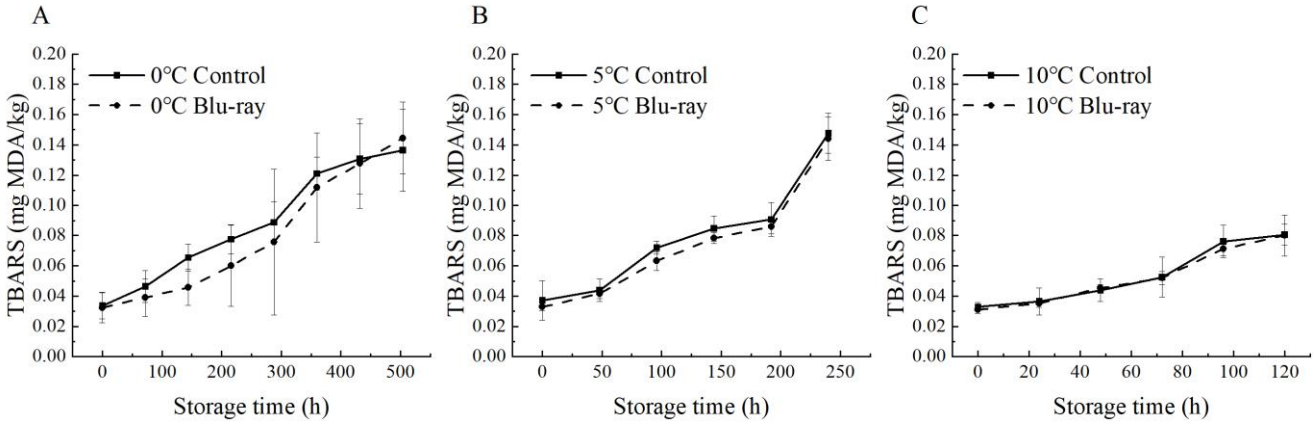


Figure 3.27 – The TBARS changes in squid during the storage at (A) 0°C, (B) 5°C, and (C) 10°C.

The lower the TBARS value, the lower the degree of fat oxidation and the better the quality of the product. Lipid degradation products can cause off-flavors in fresh fish during storage. As shown in Figure 3.27, the values of TBARS in squid in both the control and treatment groups showed an increasing trend as the storage time increased. The increasing trend of TBARS of stored squid at 0, 5, 10°C was consistent.Furthermore, Blu-ray treatment did not induce photosensitive oxidation in squid. Compared with the control group, Blu-ray irradiation reduced the fat oxidation of the squid stored at low temperature, enhanced the anti-bacterial effect, and resulted in a longer shelf-life.

3.4.11 TVC changes of SV squid during storage after Blu-ray sterilization

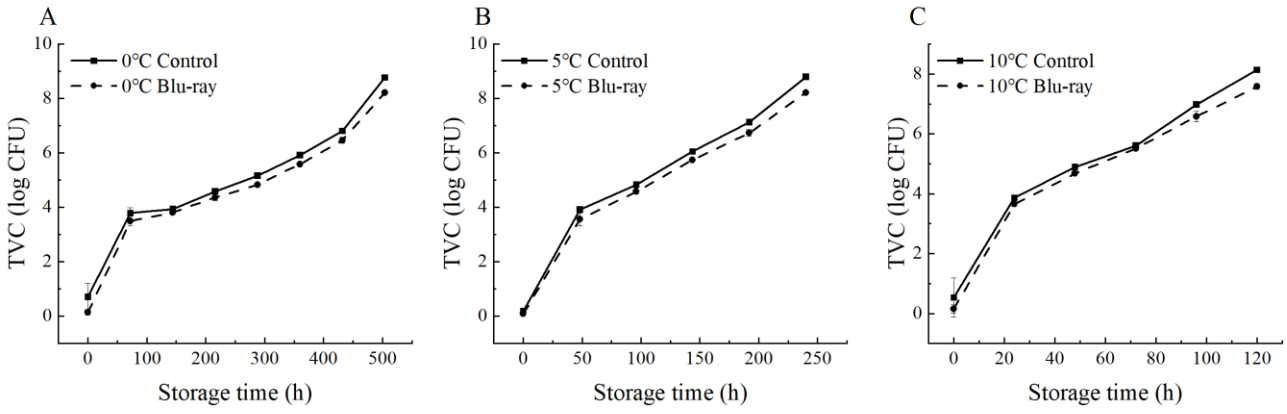


Figure 3.28 – Changes in TVC during the storage at (A) 0°C, (B) 5°C, and (C) 10°C.

Table 3.14 – Correlation between the total viable counts and sensory evaluation

Storage temperature	Correlation between the total viable counts and sensory evaluation (R^2)
0°C Control	0.95
0°C Blu-ray	0.95
5°C Control	0.93
5°C Blu-ray	0.92
10°C Control	0.92
10°C Blu-ray	0.99

The main factors that cause spoilage of aquatic products are microorganisms, as well as enzymatic and chemical changes, and the degree of spoilage of aquatic products through the growth of spoilage microorganisms [207]; therefore, TVC is known as a conventional indicator of aquatic product quality [208]. The total number of bacterial colonies in all samples showed an increasing trend with increased storage time. The growth rate of microorganisms was higher under storage at 10°C, almost directly entering the logarithmic phase, while there were obvious delay periods during storage at 0 and 5°C. Since microbial metabolism requires enzyme catalysis and the catalytic rate of enzymes depends on temperature, low temperature causes microbial growth to be delayed. There was a good correlation between TVC and sensory scores in both control and Blu-ray irradiated groups at 0, 5, and 10°C. In Figure 3.28, TVC at the end of the high-quality period was 5.92, 6.05, and 5.61 lg CFU/g in the control group and 5.58, 5.74, and 5.50 lg CFU/g in the Blu-ray irradiated group at 0, 5 and 10°C, respectively. At the end of shelf life, the total number of colonies was 8.79, 8.79, and 8.13 lg CFU/g in the control group and 8.21, 8.22, and 7.57 lg CFU/g in the Blu-ray irradiated group, respectively. The microbial counts reached 5.5 lg CFU/g or more at the end of the high-quality period in both Control and Blu-ray groups. In microbiological quality guides for ready-to-eat foods in Australia and New Zealand, the microbial limit for Class A food (after heat treatment) is proposed to be 5 lg CFU/g [209]. In China, *Salmonella* cannot be detected in aquatic products, the limit for *Vibrio parahaemolyticus* is below 100 MPN/g, and the limit for *L. monocytogenes* is below 100 CFU/g (GB 29921−2021). Therefore, in terms of the high-quality sensory period of squid, the lower the temperature, the more likely it causes the illusion of food safety, good sensorial characteristics, or high quality; however, its total

bacterial count is high, with the total number of colonies at the end of high-quality periods at 0 and 5°C more than that at 10°C. In addition, there was a good correlation (R^2＞9) between TVC and sensory scores at 0, 5, and 10°C (Table 3.14).

3.4.12 *Psychrobacter* changes of SV squid during storage after Blu-ray sterilization

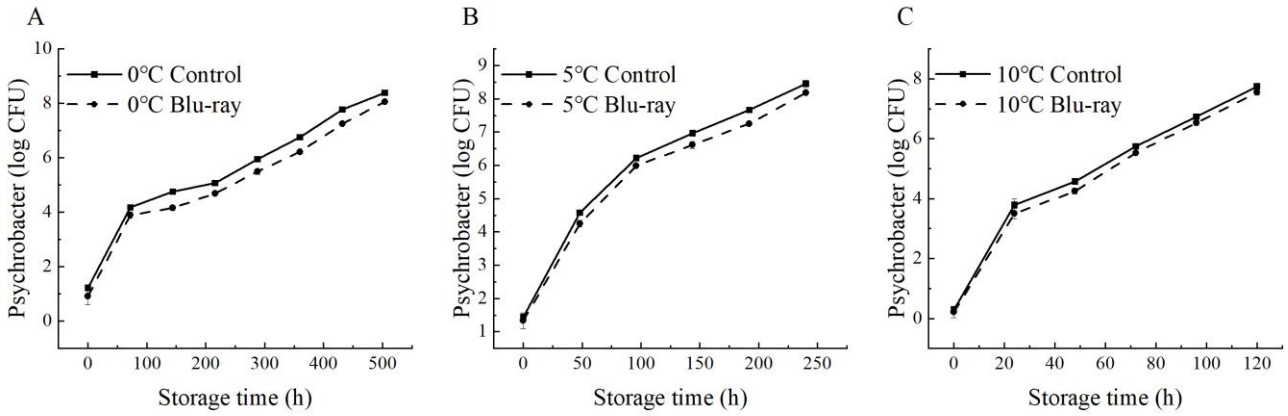


Figure 3.29 – Changes in *Psychrobacteria* during storage at (A) 0°C, (B) 5°C, and (C) 10°C

Typical bacteria grow at 25 to 40°C, while *Psychrobacter* generally grows best between −15 to 20°C. The most common species of cold-loving bacteria are *Yersinia pestis*, *Listeria monocytogenes*, and *Pseudomonas spp*. Aquatic products and meat are more vulnerable to contamination of food by these species. The squid growing in the ocean depths is more likely to become infected with this type of *Psychrobacter*. From Figure 3.29, we could see that the growth trend of *Psychrobacter* was similar to that of TVC, and the growth of the number of *Psychrobacter* and TVC was the same in all temperature conditions throughout the storage process, and the correlation between the two was good (R^2 ＞ 0.9). During low-temperature storage, the growth of non-*Psychrobacter* might be inhibited, while the *Psychrobacter* had an advantage over that of non-*Psychrobacter*. Our results suggested that at the initial time and during storage, the Blu-ray treatment group had a lower number of TVC and colonies of *Psychrobacter* than the control group, indicating that Blu-ray sterilization was effective and performed well during the whole storage period.

Overall, Blu-ray irradiation was able to kill bacteria in SV squid, and the deadly effect of Blu-ray on bacteria was dose-dependent. When the dose of Blu-ray reached 216

J/cm2, the bacteria in SV squid were undetectable. Meanwhile, the effects of Blu-ray irradiation on the samples' sensory, pH, and TBARS were not significant compared with the control group. It is worth noting that Blu-ray irradiation reduced the SF of SV squid, and Blu-ray irradiation affected the color of SV squid, especially the yellowness. In addition, our results demonstrated the positive effect of Blu-ray treatment during the storage of SV squid by inhibiting microbial growth and reducing fat oxidation. Although Blu-ray irradiation affected the color of the squid, it did not affect the sensory characteristics of the squid. Moreover, our results showed that the quality of squid decreases as the storage time increases. However, storage at lower temperatures could extend the storage time of SV squid. Our study notes that the evaluation of food quality cannot be done only by sensory evaluation but also requires a comprehensive evaluation of other physical and chemical indicators. Future research should focus on the mechanism of Blu-ray for the destruction of specific food-borne micro-organisms and the application of Blu-ray for sterilization of aquatic products.

3.5 Development of a technological and instrumental scheme to produce squid products obtained by the SV technology

Table 3.15 – Intake of aquatic products by residents of some countries

	China	India	United States	United Kingdom	South Africa	Turkey	Sweden
Aquatic Products	40-75 g/day	> 100-200 g/week	226-283g/ week	280 g/week	160-270 g/ week	2 times/week	2-3 times/week

The "2021 Edition of Chinese Resident Dietary Guidelines Scientific Research Report" is based on literature, book retrieval, and comparison studies in the past five years from 2016 to 2020. The full text of 46 English dietary guidelines and 91 graphics from different countries (regions) were sorted and analyzed. Critical information such as relevant recommendations, food recommendations, restrictions, consumption guidance, and graphic design are collected and analyzed. The research report indicates that the protein source of Chinese residents is mainly animal meat, which accounts for about 85% of the total protein intake, while the protein intake of aquatic products only accounts for 24.3. Table 3.15 is the recommended intake of aquatic products in some countries.

However, actually the intake of aquatic products of Chinese residents is much lower than that of other countries. Therefore, developing a squid product is feasible to increase the proportion of aquatic products in the diet, thereby increasing protein intake.

3.5.1 SV squid production process

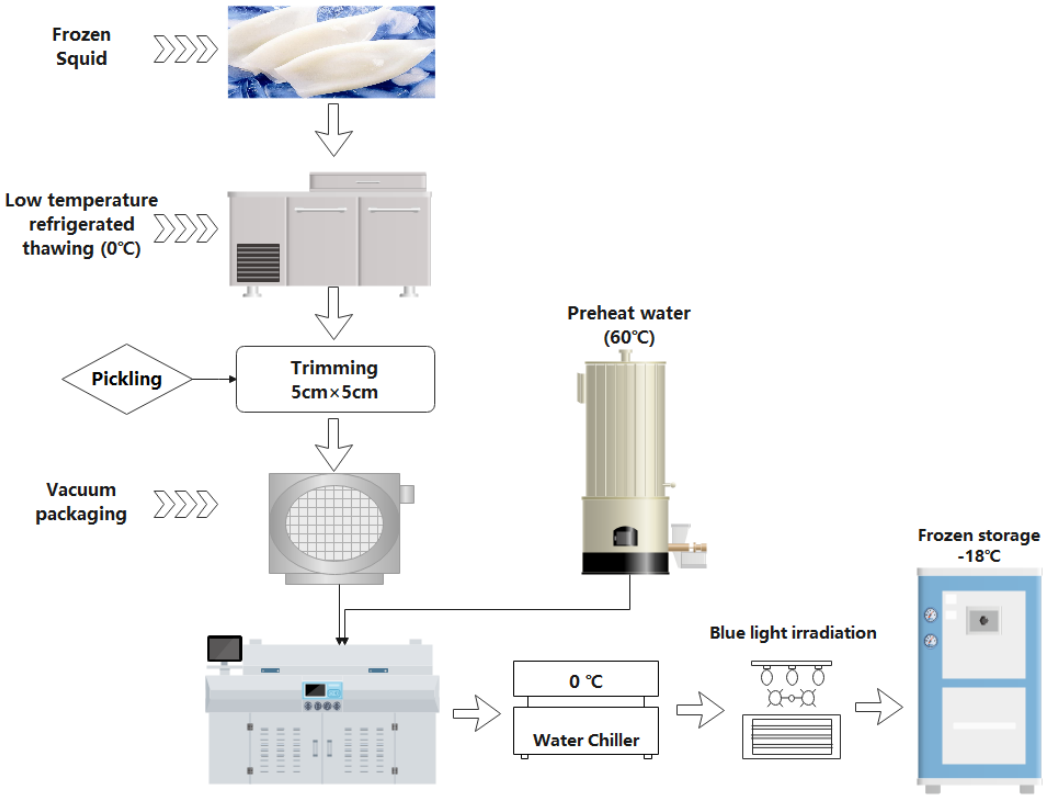


Figure 3.30 – Sous vide cooking squid industrialized production flow chart.

Among the various categories of China's national economy, the food industry is the largest industry, closely related to the lives of residents, and has an irreplaceable key position. With the rapid economic development and the continuous improvement of residents' living standards, China's food consumption structure is changing from subsistence consumption to health and enjoyment consumption. Consumers' requirements for food are no longer limited to "eat enough to eat well," but hope that food. It is rich in nutritional value and affects health care.

The safety control of industrialized food is incomparable to that of small handmade workshop food. Industrialized food is from raw material control and supply chain control to hazard analysis of each processing/storage process, key points control, detailed food safety records, traceability systems, and recall mechanisms. These are all things that small

workshops are not capable of doing. Develop a squid product with new consumer properties based on the results of our research (Figure 3.30). All production processes should comply with ISO standards (ISO 22000:2018 Food safety management systems — Requirements for any organization in the food chain and ISO/TS 22002-1:2009 Prerequisite programmes on food safety — Part 1: Food manufacturing).

1. Choose high-quality frozen squid products (ГОСТ 20414-2011 Frozen squid and cuttle. Specifications).

2. Frozen squid is thawed under low temperature refrigeration (0℃).

3. Trim the squid (5cm×5cm) after thawing and washing. (ISO 10304-3: 1997 Water quality - Determination of dissolved anion by liquid chromatography of ions - Part 3: Determination of chromate, iodide, sulfite, thiocyanate and thiosulfate).

4. Put the prepared 1.8% brine solution and the cut squid (material-liquid weight ratio 1:1) into the container, and marinate at 4°C for 90min (GOST 2874-82 *Drinking water. Hygiene requirements and quality control). Then all the squid without saltwater is vacuum packed (vacuum packaging bag −20℃ to 121℃, material: Polyamide+cast Polypropylene) (vacuum −0.1Mpa). (ISO/TS 22002-4:2013 Prerequisite programmes on food safety — Part 4: Food packaging manufacturing)

5. Put all the packaged squid into the preheated water, keep 60℃ for 30 min. And then, remove all the squid and put them in ice water (0℃) to cool (cool down to 5°C).

6. Blue light irradiation for sterilization (dose 216 J/cm^2) followed by cold storage (5℃) (ISO/TS 22002-5:2019 Prerequisite programmes on food safety — Part 5: Transport and storage) .

3.5.2 Economic benefits

Table 3.16 – Funds utilization table

No.	Items	Amounts (RMB Yuan)
1	Construction investment	5,800,000
(1)	Project cost	3,780,000
A	Construction cost	1,620,000
B	Equipment acquisition cost	2,000,000
C	Installation engineering cost	160,000

(2)	Fixed assets and other expenses	120,000
A	management fee	26,000
B	Pre-consultation fee	10,000
C	Exploration and design fee	30,000
D	Engineering Insurance	20,000
E	Joint commissioning fee	18,000
F	Inspection fees	18,000
(3)	Intangible asset expense	1,200,000
A	land use fee	480,000
B	Technology research and development and technology intangible assets	720,000
(4)	Other fee	200,000
(5)	Reserve fee	500,000
2	Liquidity	1,000,000
Total		6,800,000

1. Financial evaluation basis

(1) According to "Methods and Parameters of Economic Evaluation of Construction Projects" (Second Edition) prepared by China Development and Reform Commission;

(2) China's relevant fiscal and taxation systems.

(3) The present depreciation rate of this project is 10%.

(4) The project calculation period is 10 years.

(5) Data provided by the project unit.

2. Funding

The total investment of this project is RMB 6,800,000 (US dollar 1,046,153), the company finances itself RMB 1,000,000 (US dollar 153,846), and the financing amount is RMB 5,800,000 (US dollar 892,307) (Table 3.16).

3. Financial analysis

(1) Operating cost

Table 3.17 – Operating cost table

No.	Items	Content	Operating cost (RMB Yuan)
1	Raw and auxiliary materials	250 t ×34,000 Yuan/t	8,500,000

2	Fuel and power		200,000
3	Salary and welfare fee	2800 Yuan/person•month ×20 persons × 12 months	840,000
4	Depreciation		400,000
5	Promotion fee		146,000
6	Repair fee		314,000
7	Management fee		50,000
8	Manufacturing fee		950,000
9	Other fee		600,000
Total			12,000,000

(2) Tax

Table 3.18 – Tax payment form

No.	Items	Amount (RMB Yuan)
1	Corporate income tax	3,400,000
2	Business tax	1,000,000
3	Education surcharge	600,000
	Total	5,000,000

(3) Sales revenue

Table 3.19 – Sales income statement

No.	Product name	Quantity (ton)	Price (RMB per ton)	Sales revenue (RMB Yuan)
1	Sous vide squid	250	80,000	20,000,000
Total				20,000,000

According to the Chinese tax law for joint ventures, from the year when the enterprise starts to make a profit, the corporate income tax is calculated and levied at a rate of 17%, the business tax is paid at 5%, and education fees and surcharges are paid at 3%.

Corporate income tax = 20,000,000 × 17% = 3,400,000

Business tax = 20,000,000 × 5% = 1,000,000

Education surcharge = 20,000,000 × 3% = 600,000

Total tax = corporate income tax + business tax + urban construction tax + education surtax

=3,400,000 + 1,000,000 + 600,000 = 5,000,000 (4) Income measurement

Table 3.20 – Income table

No.	Sales revenue (RMB Yuan)	Operating cost (RMB Yuan)	Tax (RMB Yuan)	Profit (RMB Yuan)
1	20,000,000	12,000,000	5,000,000	3,000,000

4. Financial indicators

(1) Payback period

The payback period refers to the time required for the project to repay the entire investment with net income and is an important indicator to reflect the project's investment recovery capability. The payback period is calculated from the starting point of the investment, and the year when the accumulated net cash flow is equal to zero or a positive value is the year when the payback ends. The calculation formula is:

$$\sum_{t=1}^{R} C_t - C_0 = 0$$

In the formula, T is the investment payback period, C_t is the cash inflow in the t period, and C_0 is the initial investment amount.

The payback period is expressed in years. Based on this formula, the project's payback period is 2.27 years.

$$T = \frac{6{,}800{,}000}{3{,}000{,}000} = 2.27$$

(2) Return on investment (ROI)

The ROI refers to the total annual net profit of a typical operating year when the project reaches the designed production capacity to the project's total investment. It is a static indicator to examine the investment profitability of the project unit. The ROI reflects whether the investment project can obtain more income with less investment. The calculation formula is:

$$\text{ROI} = \frac{P_y}{I_t} \times 100\%$$

In the formula, P_y is total net profit for the year, I_t is total project investment. Based on this formula, we can get

$$\text{ROI} = \frac{3{,}000{,}000}{6{,}800{,}000} \times 100\% = 44.12\%$$

(3) Breakeven point (BEP)

The BEP is the zero-profit point or the earnings turning point. Usually refers to the output when all sales revenue equals all costs (the intersection of the sales revenue line and the total cost line). With the boundary of BEP, when the sales revenue is higher than the break-even point, the enterprise makes a profit; otherwise, the enterprise loses money. The calculation formula is:

$$BEP = \frac{C_f}{P_u - C_u}$$

In the formula, C_f is fixed costs, P_u is sales price per unit of product, and C_u is variable cost per unit of product. Based on this formula, we can get the BEP is 100 (Figure 3.31).

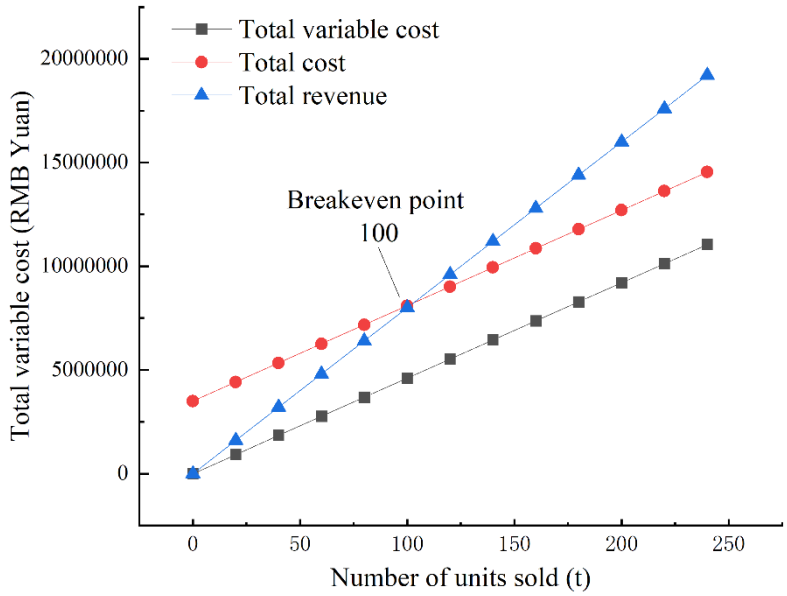


Figure 3.31 – Breakeven chart

Table 3.21 – Table of main economic indicators

No.	Indicators	Unit	Amount
1	Total investment	RMB Yuan	6
2	Annual sales revenue	RMB Yuan	6,800,000
3	Annual operating costs	RMB Yuan	12,000,000
4	Annual profit and tax	RMB Yuan	8,000,000
5	Annual tax	RMB Yuan	5,000,000
6	Annual profit	RMB Yuan	3,000,000
7	Payback period	Year	2.27

8	Return on investment	%	44.12
9	Breakeven point	t	100

According to the benchmark of Chinese food processing enterprises, the general investment payback period is 8.3 years, the investment profit rate is 16%, and the investment tax rate is 21%. The indicators calculated in this study are higher than the industry benchmark requirements, indicating that the project's benefits are higher than the industry average. The comprehensive analysis of the project's profitability and financial viability proves that the economic benefits and financial results of the project are promising, indicating that the project has high investment profit, low risk, promising economic and social benefits, and is a project of great investment value, and the project is financially feasible.

3.6 Chapter summary

1. The physicochemical and nutritional indicators of Argentine squid were systematically studied.

2. The effects of different cooking methods on the sensory, physicochemical properties, and microbial indicators of squid were studied. Comprehensive comparison, SV is beneficial to the application research of the industrial production of squid.

3. The process parameters of SV squid were optimized based on response surface methodology. Moreover, on this basis, the mechanism of SV squid tenderization was revealed.

4. The blue light sterilization technology was applied in the production process of SV squid. The results showed that blue light irradiation had no effect on the quality of squid and had better storage properties during refrigeration.

5. The industrial production process of SV squid is established, and economic analysis is carried out. The results show that the industrial production of SV squid has excellent economic prospects, and the benefits are great, which is worthy of promotion.

CONCLUSIONS

1. The current state of squid fishing and processing is analyzed. China is the undisputed leader in the production of shellfish, which is insufficiently used to produce healthy food products for consumers in the Chinese domestic market. The main ways of processing squid for food purposes are defined. Traditional methods of heat treatment of muscle tissue were analyzed; identified shortcomings of existing technologies. The relevance of the development of the SV technology of shellfish processing as a new and effective cooking method has been established. Ways to extend the shelf life of squid products by cold sterilization of Blu-ray were analyzed.

2. Peculiarities of the biochemical composition and physicochemical properties of the Argentine squid (*Illex argentines*) were studied. The meat extraction rate was 44.14%, indicating that the by-products of Argentine squid accounted for a more significant proportion. Despite Argentine squid's low meat extraction rate and its advantages of fewer bone spines, white meat, and easy meat extraction, Argentine squid still has a high processing value. The crude Muscle protein, fat, ash, and total sugar contents were: 17.49, 1.99%, 1.40%, and 0.10%, respectively. It can be seen that Argentine squid has high protein and low-fat content, while suitable to meet the trend of consumer demand for high protein and low-fat products.

3. By studying the effect of different heat treatment methods on the quality of squid, SV showed good heat treatment advantages. Firstly, SV heat-treated squid is safe to eat. After heating, the TVC in the squid samples decreased, and the TVC of SV samples was the most minimal ($P<0.05$). Sencond, the cooking loss of SV squid was 15.36%, while that of BO squid and ST squid were 26.76% and 32.44%, respectively. Third, the whiteness of SV squid was 37.36±0.061, while that of BO squid and ST squid were 23.24±0.013 and 23.69±0.016, respectively. Also SV squid outperformed BO and ST squid in TBARS, and microbiological indicators. Regarding sensory evaluation, SV squid outperformed BO and ST squid in texture, appearance, and preference, except for flavor and odor. Due to the long-term low-temperature heating conditions under vacuum, the flavor of SV squid is different from steamed and boiled squid, and it has unique special flavor compounds. The

aroma and volatile substances of squid samples from different cooking methods (boiling, steaming, sous vide) were determined and analyzed by headspace–gas chromatography–ion mobility spectrometry. A total of 43 characteristic flavor compounds were identified.

4. In the study of the sensory effects of different heat treatments on squid, we found that the sensory scores of the ST and BO samples were relatively close. In contrast, the sensory scores of the SV sample differed from the other two samples, particularly in terms of superior scores for preference, appearance, and texture. The high temperature (100°C) heating of ST and BO caused the sample to shrink and curl, which reduced the appearance and preference score. In contrast, the appearance of the SV sample was intact without curling, which due to the lower cooking temperature (60°C).

5. After the single-factor experiment, the SV squid was optimized by response surface methodology, and the results showed that the best technological test plan for SV squid is cooking temperature 60.7℃, cooking time 30 min, salinity 1.8%, and the predicted sensory score was 43.32. The sensory score of 44.12±0.52 (n=3) obtained from the experiments according to this optimal process parameter is consistent with the theoretically predicted value.

6. With the increase of heating time of SV squid (60℃), squid SF and myofibril apparent diameter decreased, while cooking loss, TCA-soluble peptide, MFI, and Total proteolytic activity all increased. Scanning electron microscopy found that with the increase of heating time, the space between muscle fibers increased, and the boundaries between muscle fiber bundles began to become blurred and arranged disorderly. It can be inferred that the reason for the tenderness of the SV (60°C) squid is that the squid's endogenous enzymes degrade myofibril. The results of this study also explained why SV squid was more tender than conventionally (ST and BO) cooked squid.

7. Experiments showed that SV was the most minimal 15.36% in cooking loss, followed by BO (26.76%) and ST (32.44%). The results are because SV has a low cooking temperature and places in a vacuum packaging bag for heating. The high-temperature causes myofibril protein and collagen's denaturation, so ST and BO samples' cooking loss was more. In addition, We found that the cooking loss of the samples gradually increased with heating time (P<0.05). When heated for 24h, the samples' cooking loss was

maximum. It is noteworthy that the difference was not significant ($P > 0.05$), although the cooking loss increased from 25.05% to 31.66% during the heating of the samples from 5 h to 24 h.

8. Blu-ray irradiation was able to kill bacteria in SV squid, and the deadly effect of Blu-ray on bacteria was dose-dependent. When the dose of Blu-ray reached 216 J/cm^2, the bacteria in SV squid were undetectable. Meanwhile, the effects of Blu-ray irradiation on the samples' sensory, pH, and TBARS were not significant compared with the control group. It is worth noting that Blu-ray irradiation reduced the SF of SV squid, and Blu-ray irradiation affected the color of SV squid, especially the yellowness.

9. The positive effect of Blu-ray treatment during the storage of SV squid by inhibiting microbial growth and reducing fat oxidation. And the end of shelf-life (control group and irradiated group) was 504, 240, and 120 h. Although Blu-ray irradiation affected the color of the squid, especially yellowing, it did not affect the sensory characteristics of the squid. Moreover, our results showed that the quality of squid decreases as the storage time increases.

10. Industrialized food is from raw material control and supply chain control to hazard analysis of each processing/storage process, key points control, detailed food safety records, traceability systems, and recall mechanisms. Develop a squid product with new consumer properties based on the results of our research. All production processes comply with ISO standards (ISO 22000:2018 Food safety management systems — Requirements for any organization in the food chain and ISO/TS 22002-1:2009 Prerequisite programmes on food safety — Part 1: Food manufacturing).

11. According to "Methods and Parameters of Economic Evaluation of Construction Projects" (Second Edition) prepared by China Development and Reform Commission and China's relevant fiscal and taxation systems. We performed an economic benefit analysis that the total investment of this project is RMB 6,800,000 (US dollar 1,046,153), the company finances itself RMB 1,000,000 (US dollar 153,846), and the financing amount is RMB 5,800,000 (US dollar 892,307). The return on investment of the project is 44.12%, with an annual return of 3,000,000 yuan, a break-even point of 100 tons per year, and a return period of 2.27 years.

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APPENDICES

Patent: Refrigeration type blue light sterilization aquatic product display cabinet

证书号第13522528号

实用新型专利证书

实用新型名称：一种冷藏式蓝光杀菌水产展示柜

发　明　人：崔震昆;闫寒;张浩;刘本国;赵岩岩;周威;毕继才;莫海珍
胡梁斌;李红波;张泽华

专　利　号：ZL 2020 2 2638458.3

专利申请日：2020年11月16日

专利权人：河南科技学院

地　　址：453000 河南省新乡市红旗区五一路东段

授权公告日：2021年06月25日　　授权公告号：CN 213542923 U

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2021年06月25日

第1页(共2页)

其他事项参见背面

Patent: Blue light sterilization box for experiment

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郑州中科鼎佳专利代理事务所（特殊普通合伙） 蔡佳宁
(0379-60225664)

发文日：

2021 年 03 月 11 日

申请号或专利号：**202120510074.5**　　发文序号：**2021031100724110**

专 利 申 请 受 理 通 知 书

根据专利法第 28 条及其实施细则第 38 条、第 39 条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：202120510074.5
申请日：2021 年 03 月 10 日
申请人：河南科技学院
发明创造名称：一种实验用蓝光杀菌工作箱

经核实，国家知识产权局确认收到文件如下：
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Certificate of product application of sous vide cooking squid

CERTIFICATE OF PRODUCT APPLICATION

Henan Sanjia Food Co., Ltd. has conducted pilot tests on the sous vide cooking squid products with consumer characteristics developed by Cui Zhenkun from 2020 to 2021. Furthermore, the company puts the product on the market in 2021 for sale, which is deeply loved by consumers and has brought direct economic benefits to our company more than 350,000 US dollars.

Henan Sanjia Food Co., Ltd.

December 2021