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INVESTIGATING THE EFFECTS OF STORAGE TEMPERATURE ON THE QUALITY OF FROZEN CEPHALOPODA: A MULTIMODAL APPROACH

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Abstract

Cephalopoda classes are highly valued for their culinary appeal and nutritional value. However, their perishability necessitates careful handling and optimal storage conditions to maintain their quality. This study aimed to investigate the influence of storage temperature on the quality attributes and sensory properties of five commonly consumed cephalopod types: whole squid, whole squid tubes, octopus, Loligo spp. (squid tubes), and Sepia spp. (cuttlefish tubes) classified in five different species (Loligo vulgaris (European squid), Loligo gahi (Japanese squid), Octopus vulgaris (Octopus), Sepia officinalis (Cuttlefish) and Illex argentinus (Argentine shortfin squid)). Lower temperatures (-23°C) significantly prolonged shelf life, delaying spoilage onset and preserving taste. The product named calamar had the fastest spoilage rate, followed by whole squid and tube squid, while Octopus had the slowest spoilage rate. Appropriate storage practices are vital for ensuring the freshness and palatability of frozen cephalopods.

***Key words**: frozen cephalopods, quality attributes, sensory properties, storage temperature.*

INTRODUCTION

Cephalopoda, members of the phylum Mollusca, are a diverse group of marine animals recognized for their set of tentacles, making them distinct. They are highly favoured as a seafood delicacy. This category encompasses a variety of species, including squid, octopus, cuttlefish, and nautilus (Ahne et al., 2000).

Molluscs play a significant role in meeting global seafood demand, providing consumers with high-quality protein, essential amino acids, and valuable nutrients. Their economic importance in aquaculture, coupled with their health benefits and nutritional value, underscores the importance of sustainable molluscs farming practices to support food security and healthy diets worldwide (Petcu, 2013).

Octopus, a versatile marine cephalopod, has garnered immense popularity worldwide, captivating culinary enthusiasts with its delicate flavour, satisfying texture, and nutritional richness. Its growing demand has fuelled a surge in global fish production, with the volume reaching 186.6 million metric tons in 2023, up from 184.6 million metric tons in 2022 (Statista, 2024). This unprecedented demand has placed octopus at the forefront of the global seafood market, with China and the Republic of Korea emerging as dominant players in the live, fresh, or chilled octopus trade over the past two decades.

While China and Korea have long held the reins of the octopus market, recent years have witnessed a shift in the trade dynamics. European and Mediterranean countries, including Spain, Portugal, Italy, and Morocco, have ascended to prominence, establishing themselves as key octopus importers and exporters. This transformation reflects the growing global appreciation for octopus and the adaptability of international markets.

The escalating demand for octopus necessitates a comprehensive understanding of its culinary properties, preservation techniques, and the factors influencing its quality. Previous research articles on the freshness and sensory attributes of octopus highlight the critical importance of understanding and evaluating these factors to ensure optimal quality and consumer satisfaction (Atrea et al., 2009). This article delves into the scientific realm of octopus, exploring its nutritional value, sensory attributes, and the impact of storage conditions on its shelf life. By unravelling the science behind octopus, we gain valuable insights into optimizing its preservation and ensuring its continued popularity as a culinary delicacy (Ospina-Alvarez et al., 2022).

The global seafood industry is projected to experience significant growth in the coming years, with a projected value of $155.32 billion by 2023. China, Indonesia, and India currently hold the top positions for fish production, while Asia is the world's leading aquaculture producer, with a production volume of approximately 70.5 million tons in 2020 (Figure 1). Notably, finfish was the most produced species in aquaculture, accounting for over 57.46 million metric tons. China, with its extensive fish and seafood processing industry, generated revenue of $31.6 billion in 2018. These figures underscore the immense global demand for seafood and the crucial role of aquaculture in meeting this demand (Statista, 2024).

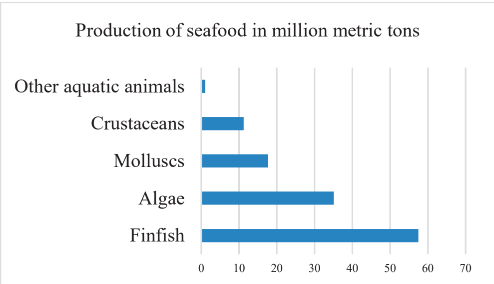

Figure 1. Global aquaculture production worldwide and major species traded in 2020 (after Statista, 2023)

Seafood consumption in Europe has exhibited a downward trend in recent times, with a projected decline of 7% in 2022, reaching 9.42 million metric tons (live weight equivalent). This decrease is attributed to various factors, including the ongoing conflict between Russia and Ukraine, which has disrupted supply chains and impacted seafood imports.

Prior to the 2022 decline, global seafood consumption had been steadily increasing. In Europe, the consumption of seafood products has predominantly been in frozen form, primarily due to the lower per capita consumption compared to other fish and mollusc species. This trend suggests that frozen cephalopod products are primarily consumed for their extended shelf life and convenience.

Over time, efforts have been made to optimize the preservation and utilization of these products, potentially affecting their quality and consumer satisfaction, resulting in millions of research articles worldwide. However, solutions are still sought to enhance their commercial and organoleptic quality, and to find the best storage and freezing conditions for edible cephalopods.

MATERIALS AND METHODS

The research was conducted at the Max Rubner Institute, Hamburg branch, Germany, using the appropriate equipment, and methodology.

Sample collection

In this study, we examined five different species of cephalopods: *Loligo vulgaris* (European squid), *Loligo gahi* (Japanese squid), *Octopus vulgaris* (Octopus), *Sepia officinalis* (Cuttlefish) and *Illex argentinus* (Argentine shortfin squid) (Figures 2 and 3).

Figure 2. *Illex* spp. (own source)

Five cephalopod marketed assortments – whole squid, whole squid tubes, octopus, *Loligo* spp. (squid tubes), *Illex* spp. and *Sepia* spp. (cuttlefish tubes) – were selected for the study. The products were purchased from local seafood markets and stored at three distinct temperatures: -15°C, -18°C, and -23°C for three months. At regular intervals, samples were collected for chemical, physical, and sensory analyses.

Thawing test: The products were stored at +4°C for three weeks, then thawed at room temperature for one hour. The pH and TVB-N content of the products were measured before and after thawing, at each storage temperature.

Chemical analysis

The samples were prepared for analysis following the guidelines outlined in BVL L

06.00-1. The pH and volatile basic nitrogen (TVB-N) content were analysed to assess microbial spoilage. pH serves as an indicator of the acidity or alkalinity of the product, according to BVL L 06.00-2 guidelines.

Figure 3. *Octopus* spp. (own source)

On the other hand, TVB-N levels are indicative of the accumulation of biogenic amines, compounds generated during protein degradation, further contributing to the assessment of spoilage.

Physical analysis

Color and texture were assessed as physical parameters of product quality. Color was measured using a Hunter Lab colorimeter, while texture was evaluated based on firmness, elasticity, and cohesiveness.

Sensory evaluation

The sensory analysis process requires a comprehensive understanding of sensory evaluation methodologies, a profound knowledge of food composition and processing, and substantial practical experience to ensure accurate and reliable results (Derndorfer, 2023).

The sensory analysis panel consisted of 10 experienced researchers who received additional training before each sensory analysis session. The trained sensory panel evaluated the overall palatability of the products using a 9-point hedonic scale ranging from 1 (dislike extremely) to 9 (like extremely). The panel assessed flavour, texture, appearance, and overall acceptability (Busch-Stockfisch, 2005).

Preliminary tests

Prior to the main tests, a series of preliminary tests were conducted to assess the freshness of the cephalopod products. The tests included:

pH measurement: the pH value was measured using a glass single-rod electrode introduced into product homogenate diluted 1:1 with distilled water. The electrode was always calibrated with pH 4.0 and pH 7.0 before the samples were measured. In order to prevent the values from changing due to long storage times, the measurement was carried out immediately after the samples had been homogenized. The homogenate, being relatively dry, was diluted with distilled water in a 1:1 ratio before measurement. Despite this dilution, the pH of the homogenate was not affected by the addition of distilled water, as the water used was within a neutral pH range. For the measurement, 20 g sample homogenate was used and a double determination was carried out in each case (Oehlenschläger et al., 2002). Fresh cephalopod products should have a pH between 6 and 7.5.

Before the TVB-N determinations were carried out, the homogenate was prepared. For this purpose, 180 ml perchloric acid solution of 6% concentration was added to a 20 g sample and then extracted for one to two minutes using an Ultraturrax. The subsequent filtration over filter paper was facilitated using Witt's pot to prevent clogging of the filter by the homogenate, which could otherwise halt the filtration process. For each investigation, two samples of the same homogenate were consistently analysed.

Volatile basic nitrogen (TVB-N) content:

The TVB-N content of the products was measured to assess the degree of microbial spoilage and protein degradation, which are indicated by the presence of various nitrogen compounds including amines and ammonia. TVB-N level, in crustaceans, shells and molluscs was determined according to the German reference method, § 64 LMBG (German Food & Feed Code, 2008) and EU recommendations (EU, 2005). However, the amount of homogenate specified in the European regulation was doubled in order to obtain a more representative sample. The volatile nitrogenous bases were extracted from the samples with perchloric acid as described above. The resulting extract (Figure 4) was subjected to steam distillation after alkalinization. The volatile basic components were absorbed in an acid reservoir. The TVB-N content was determined by titrimetric determination of the absorbed bases. The analysis was automatically carried out using a Vapodest 50 distillation device. The TVB-N content in mg/100g was calculated (Figure 4) using the following formula (EU, 2019):

$$TVB-N = \frac{(V1 - V0) \times 2 \times 100 \times 0.14}{M}$$

where:

V1 = volume of 0.01 mol standard hydrochloric acid solution in milliliters for the sample,
V0 = volume of 0.01 mol standard hydrochloric acid solution in milliliters for the blank,
M = mass of the sample in grams.

Figure 4. Samples preparation for TVB-N extraction from perchloric acid solution (own source)

To verify the results (the initial ones from day I are included in Figures 5-7), duplicate determinations of the individual extracts were performed. Given the absence of precise limit values for this type of product, values between 25 and 35 mg/100 g were considered critical. These values are derived from Commission Regulation (EC) No. 2074/2005 for certain fishery products. From this perspective, fresh cephalopod products should ideally have a TVB-N content of less than 30 mg/kg.

Sensory evaluation: The panel of 10 experienced tasters, as previously mentioned, evaluated the appearance, smell, taste, and texture of the products. Each taster was instructed to rate the products on a scale ranging from 1 to 9, with 1 representing the lowest score indicating poor quality, and 9 representing the highest score indicating excellent quality. To assess the effects of different storage temperatures on the quality of cephalopod products, two main tests were conducted. The first test investigated the effects of storage temperature on the pH, TVB-N content and sensory properties of the products. The products were stored at -15°C, -18°C and -23°C for one, two and three months.

The second test investigated the effects of thawing on the quality of the products. The pH, TVB-N content and sensory properties of the products were then measured.

RESULTS AND DISCUSSIONS

Various studies have examined the chemical and physical properties of cephalopods during storage (Sykes et al., 2009). These studies were mainly conducted on non-frozen products. The results from these studies suggest that the content of biogenic amines and volatile basic nitrogen compounds correlate with the sensory properties of cephalopods (Özogul, & Serhat, 2011; Sun et al., 2003; Vieira et al., 2008).

The present study investigated the changes in chemical and physical parameters of three cephalopod sample types (squid, cuttlefish and octopus) during storage at different temperatures. The results showed that the pH and TVB-N content of all five species: *Loligo vulgaris* (European squid), *Loligo gahi* (Japanese squid), *Octopus vulgaris* (Octopus), *Sepia officinalis* (Cuttlefish) and *Illex argentinus* (Argentine shortfin squid) increased over time, while the sensory properties of the products deteriorated. The sensory results of the profile tests did not show any significant differences, while the scalar rating test revealed that the product named calamar had the fastest spoilage rate, followed by whole squid and tube squid. Octopus had the slowest spoilage rate.

Previous research made through omission and addition tests and sensory evaluations of synthetic extracts, identified the taste-active components in the mantle muscle of oval squid (*Sepioteuthis lessoniana*) to include various amino acids, nucleotides, and ions. A simplified synthetic extract containing these key components was able to closely replicate the taste of the complete extract of *S. lessoniana* muscle. This replicated taste was consistent across other squid species tested, including *Loligo bleekeri, Loligo edulis*, and *Todarodes pacificus*, by adding specific components tailored to each species. The findings suggest

that specific combinations of taste-active components play a crucial role in determining the overall taste profile of squid species, with the synthetic extract approach offering insights into taste preferences and potential applications in food product development (Kani et al., 2008).
Innovatively, our research uncovered a compelling negative linear relationship among key biochemical indicators - pH, total volatile basic nitrogen (TVB-N) content and sensory scores - in cephalopod products. This intriguing correlation suggests a novel approach for assessing the overall freshness of cephalopods. By leveraging the levels of these biochemical compounds as indicators, we propose a groundbreaking method to precisely evaluate cephalopod freshness, providing valuable insights for both industry practitioners and consumers alike.
The storage time for cephalopods at a temperature of +4°C (39°F) without significant deterioration of sensory properties can vary depending on factors such as the species of cephalopod, initial freshness, handling practices, and storage conditions. However, it is generally true that cephalopods can be stored for a limited period at this temperature without significant adverse effects on their sensory properties.
In some cases, cephalopods can be stored for up to 14 days at +4°C without experiencing noticeable changes in sensory properties such as appearance, odor, taste, and texture. However, it's important to note that the quality of cephalopods may gradually decline over time, even at refrigeration temperatures. Therefore, while storage at +4°C may extend the shelf life compared to higher temperatures, it's still essential to monitor the quality of the product regularly and adhere to recommended storage times to ensure optimal freshness and safety.

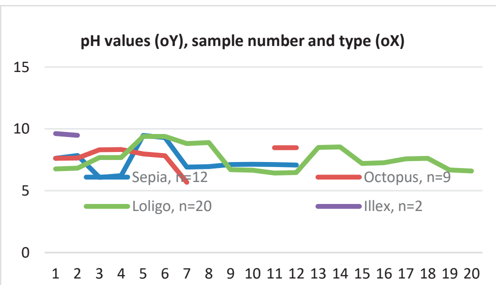

Figure 5. Results regarding initial pH values in all analyzed samples (on day 1 of storage at 4°C)

The results of the preliminary tests (Figure 5) showed that *Octopus vulgaris* and *Illex argentinus* had the highest pH values (7.2-9.6), while *Sepia officinalis* and *Loligo vulgaris, Loligo gahi,* had the lowest pH values (6.6-6.8). The results of the main tests showed that the storage temperature had a significant effect on the pH, TVB-N content (Figures 6 and 7) and sensory properties of the products.
The products stored at -23°C had the lowest pH values, TVB-N content, and the best sensory properties. The products stored at -18°C had intermediate values, while the products that were stored at -15°C had the highest pH values.
Various techniques are employed in the seafood industry, including the use of ice, chilling, super-chilling, freezing with different types of freezers and freezing mediums, freeze drying, ultra-low temperature freezing, low-temperature packaging, and additives. Each preservation method is associated with specific preservation temperatures, technologies, additives used, packaging materials, storage conditions and preferred shelf life (Siddiqui et al., 2024).

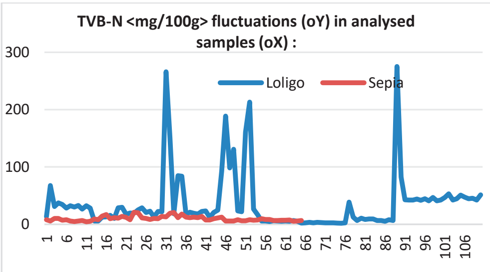

Figure 6. Results regarding the initial value of TVB-N in frozen, thawed calamar *(Loligo* spp.) and thawed squid *(Sepia* spp.) samples (on day 1 of storage at 4°C)

Chilling is a common preservation method that involves maintaining seafood at temperatures between 0-5°C. This technique utilizes vacuum packaging and modified atmosphere packaging to preserve seafood freshness by avoiding exposure to air and moisture. The preferred shelf life for chilled seafood ranges from 2-3 days for fresh products, emphasizing the importance of minimizing air and moisture contact to extend product quality.
Super-chilling extends preservation temperatures below 0°C to enhance the shelf life of seafood. With the absence of additives, super-chilling utilizes vacuum packaging and

modified atmosphere packaging to maintain product quality. Fresh seafood can be preserved for 2-3 weeks, while processed seafood can last up to 1 month, highlighting the efficacy of super-chilling in prolonging seafood freshness.
Freezing seafood at temperatures below -18°C stops bacterial growth and enzymatic activity, thereby extending the shelf life of seafood products. This preservation method commonly employs vacuum packaging, modified atmosphere packaging, or freezer-safe containers to maintain optimal storage conditions. Most seafood items can be stored for 6-12 months, with certain types lasting up to 18 months, showcasing the versatility of freezing in preserving a wide range of seafood products.
Ultra-low temperature freezing takes presservation to the next level by storing seafood below -50°C, significantly extending the shelf life of products. Similar to freezing, this method utilizes vacuum packaging, modified atmosphere packaging, or freezer-safe containers to ensure product quality. The extended shelf life of 2-3 years for most seafood items demonstrates the effectiveness of ultra-low temperature freezing in preserving seafood freshness over an extended period.
Freeze-drying is a unique preservation technique that involves removing moisture from seafood while maintaining its nutritional value and flavour. This method requires airtight packaging, often in the form of pouches or jars, to prevent exposure to air and moisture. Seafood preserved through freeze-drying can be stored at room temperature or below for 1-2 years, showcasing the longevity of this preservation method in maintaining seafood quality (Siddiqui et al., 2024).
The utilization of low-temperature preservation technologies is essential in the seafood industry to ensure the quality, safety, and shelf life of seafood products. By understanding the specific preservation temperatures, technologies, packaging materials, and storage conditions associated with each method outlined in Table 3, seafood producers and consumers can make informed decisions to enhance freshness and prolong the shelf life of seafood items. These preservation techniques not only preserve seafood quality but also contribute to reducing food waste and meeting consumer demands for high-quality seafood products.

As of the latest scientific knowledge and technological advancements, storing cephalonpods at -23°C is technically feasible using commercial freezer units and cryopreservation techniques. While storing cephalopods at -23°C is technically possible with current technology, practical considerations such as equipment availability, storage logistics, quality concerns, and cost-effectiveness need to be carefully evaluated before implementing this storage method on a large scale.
The results of the thawing test showed that the products did not regain their original freshness after thawing.

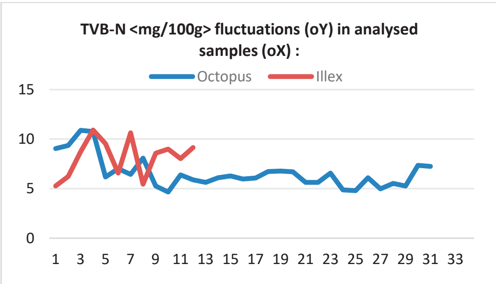

Figure 7. Results regarding final value of TVB-N in frozen, thawed Illex *(Illex argentinus)* and *Octopus (Octopus* spp.) samples

The pH values of the products were slightly higher after thawing (Figure 5); the TVB-N content and sensory properties of the products were still lower than the values of the fresh products.

CONCLUSIONS

The results of this study show that the storage temperature and thawing time have a significant effect on the quality of cephalopod products. The products that are stored at the lowest temperature (-23°C) and thawed for the shortest time have the best quality. However, even these products do not regain their original freshness after thawing. Retailers with access to commercial-grade freezers or blast freezers may indeed be able to store cephalopod products at temperatures as low as -23°C or even lower. These ultra-low temperatures are commonly used in commercial food storage facilities to extend the shelf life of perishable products, including seafood.

As for consumers, while it may be challenging to replicate such low temperatures in standard household freezers, they can still maintain the quality of cephalopod products by storing them at the lowest temperature settings available. Rapid thawing methods, such as placing the product under cold running water or in a bowl of cold water, can help preserve quality by minimizing the time spent in the thawing process. Additionally, using a microwave with a defrost function or a refrigerator for gradual thawing can also be effective methods, although they may take longer.

The study provides compelling evidence that storage temperature plays a pivotal role in preserving the quality of frozen cephalopods. Lower storage temperatures, such as -23°C, significantly delay the onset of spoilage and maintain palatability for a longer period due to several mechanisms:

1. Slowing down of biological processes: at lower temperatures, the metabolic activities of microorganisms, enzymes, and other biological agents responsible for spoilage slow down. This reduction in metabolic rate decreases the rate of food degradation and spoilage, extending the shelf life of the product.
2. Inhibition of microbial growth: many microorganisms responsible for food spoilage have optimal growth temperatures within a certain range. By storing food at temperatures well below their optimal growth range, microbial growth is inhibited or significantly slowed down, reducing the risk of contamination and spoilage.
3. Enzyme inactivation: enzymatic reactions that contribute to food deterioration are also slowed down or halted at low temperatures. This includes enzymatic browning, lipid oxidation, and other enzymatic reactions that degrade the quality of food over time.
4. Water activity reduction: lower temperatures reduce the water activity (amount of available water) in the food matrix. This decrease in water activity inhibits the growth of microorganisms and slows down chemical reactions, further extending the shelf life of the product.
5. Preservation of texture and flavour: lower temperatures help preserve the texture, flavour, and overall quality of the food by minimizing changes in the food's physical and chemical properties over time.

These findings have significant implications for consumers and retailers, emphasizing the importance of proper storage practices to ensure the freshness and enjoyment of these seafood delicacies.

Based on the results of our experiment, an innovative extension of the hypothesis could be that the exposure of cephalopods to extremely low temperatures like -23ºC not only extends their storage time but also enhances the retention of key nutritional compounds and flavour profiles. This could lead to the development of novel storage techniques that preserve the quality and sensory attributes of cephalopods for longer periods, potentially opening up new opportunities in the seafood industry and culinary applications.

An innovative approach to cephalopod conservation involves utilizing rapid freezing techniques combined with cryoprotectants at -23ºC to enhance both the quality and sustainability of cephalopod food products. This method aims to preserve the delicate texture, flavour, and nutritional profile of cephalopods while minimizing the formation of ice crystals that can degrade quality during freezing and thawing processes.

By employing this advanced freezing technology, cephalopod producers can effectively extend the shelf life of these valuable seafood items without compromising their sensory attributes. Additionally, the use of cryoprotectants can help maintain the structural integrity of cephalopods, ensuring that they retain their natural taste and appearance upon thawing. This innovative freezing method not only enhances food quality but also contributes to reducing food waste and promoting sustainable seafood practices in the industry.

Further research and development in seafood preservation technologies could lead to more efficient and sustainable methods for extending the shelf life of cephalopods in the future.

Investigations on the effects of packaging and thawing methods on the quality of frozen cephalopods are also needed. By understanding the intricate relationship between storage conditions, chemical changes, and sensory perceptions, we can optimize the preservation and consumption of these delectable marine invertebrates.

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***BVL L 06.00-2 Technische Regel, 1980-09. Messung des pH-Wertes in Fleisch und Fleischerzeugnissen.

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