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Effects of two thawing methods on the shelf life of cephalopod mollusks

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Abstract
Thawing is a critical step in cephalopod processing, as it affects physicochemical parameters, microbial stability, and overall product quality. Current European and national guidelines recommend consuming or processing thawed cephalopods within 24 hours, a restriction that often results in substantial food waste. This study evaluated the impact of two thawing methods—rapid water-immersion thawing and slow refrigerated thawing—on the quality of *Octopus Vulgaris* and *Illex Argentinus* over 48 hours of refrigerated storage. A total of 12 animals (6 per species) were thawed using either method (3 rapidly thawed and 3 slowly thawed per species), and sensory quality (organoleptic examination and electronic nose), pH, thiobarbituric acid reactive substances, total volatile basic nitrogen, trimethylamine, color, and microbiological indices (mesophilic counts, coagulase-positive staphylococci, Enterobacteriaceae, and coliforms) were analyzed at T0 (day of thawing), T1 (24 hours post-thawing), and T2 (48 hours post-thawing). The results indicate that rapid thawing more effectively preserved the chemical and sensory characteristics of *Illex Argentinus* compared to *Octopus Vulgaris* up to 48 hours post-thawing, whereas slow thawing better preserved the microbiological quality of both species over the same period. Rapid thawing also maintained microbiological parameters within acceptable limits, suggesting that the trends observed beyond the currently recommended 24 hours after thawing may offer useful information for future studies aimed at better defining post-thaw shelf-life evolution in cephalopods thawed with different methods.

Introduction
Cephalopods are currently recognized as one of the most promising fishery resources due to their abundance in marine environments and their rapid stock renewal, as their biological cycle ranges from 8 months to slightly under 2 years (Hunsicker *et al*., 2010). Among commercially relevant species, *Octopus vulgaris* and *Illex Argentinus* represent two of the most exploited cephalopods, distributed in the Mediterranean and Atlantic regions and constituting an important share of global landings as a result of their availability and established market demand (Carlini *et al*., 2006; Pérez *et al*., 2023). Captured cephalopods are subsequently stored and frozen to preserve sensory and microbiological quality because, once collected, they deteriorate rapidly as they contain a high amount of endogenous and bacterial enzymes that promote rapid protein degradation (Boiţeanu *et al*., 2024). After processing, these products may be marketed either frozen or thawed intended for immediate distribution and use (Giagkazoglou *et al*., 2024). Legislative frameworks play a role in determining shelf-life after thawing. European guidelines (European Parliament and Council of the European Union, 2004) and Italian guidelines for risk analysis in the field of food microbiology (National Guidelines for Risk Analysis in Food Microbiology, 2017) recommend that thawed seafood be stored under refrigeration and consumed or processed within 24 hours, after which it is considered unsuitable for further commercialization. Although these recommendations are designed to ensure consumer safety, they pose logistical challenges for seafood processing facilities and may lead to substantial product waste, particularly for cephalopods, whose quality deterioration is influenced by thawing dynamics (Lv and Xie, 2022). Thawing influences the physicochemical properties, drip loss, oxidative stability, and overall quality of the final product, and it represents a critical point in the processing chain (Backi, 2018). Common thawing methods include air, hydrostatic, running water, and microwave thawing, each exerting different effects on product quality. Immersion thawing is widely used in the fish-processing industry due to its speed and operational efficiency (Backi, 2018), who also evaluated additional thawing techniques, reported that this method increases the release of water-soluble components and may enhance microbial proliferation if not strictly controlled. At the present time, thawing at 4°C (low-temperature thawing) is the most effective method in preserving product quality when compared with other thawing methods (flowing water thawing, ultrasonic flowing water thawing, air thawing, and microwave thawing), based on the evaluation of thawing loss, color parameters, Total Volatile Basic Nitrogen (TVB-N), and Thiobarbituric Acid Reactive Substances (TBA) values, although this approach requires considerably longer processing times (Zhou and Xie, 2021). In this context, the present study aimed to evaluate the effects of two different

thawing methods (rapid water-immersion thawing and slow refrigerated thawing) on the physicochemical and microbiological quality of *Octopus Vulgaris* and *Illex Argentinus* over a 48-hour post-thaw storage period. The investigation focused on sensory quality (organoleptic examination and electronic nose), pH, TBARS, TVB-N, Trimethylamine (TMA), color, and microbiological parameters (Mesophilic Counts, Coagulase-positive Staphylococci, Enterobacteriaceae, and Coliforms), in order to assess the response of the two species to the two thawing techniques.

Materials and Methods

Samples collection

A total of 12 specimens of Octopus vulgaris from FAO area 51 (Western Indian Ocean) and 12 specimens of Illex argentinus from FAO area 41 (Atlantic Ocean) were caught using longline fishing, were transported in a frozen state at -18°C and subsequently stored at the same temperature at a seafood processing establishment.

A total of 24 cephalopod molluscs (12 *Octopus vulgaris* and 12 *Illex argentinus)* with an approximate weight of 200 g each, were transported in a frozen state at -18°C and subsequently stored at the same temperature at a seafood processing establishment. Two defrosting processes were carried out for cephalopods species (*Octopus vulgaris* and *Illex Argentinus)*: a slow defrosting process and a rapid defrosting process. In the slow process, frozen products were gradually brought from -18°C to 0-4°C in a refrigerated cell for 24 hours, allowing uniform temperature equilibration throughout the product. In the rapid thawing process, cephalopods were transferred into a water-based defrosting machine (Palinox DS-INM-C Immersion Defroster) and immersed in temperature-controlled water (<7°C) under continuous recirculation to ensure uniform heat transfer throughout a 30-minute defrosting cycle. At the end of the process product was defrosted and mechanically lifted using dedicated baskets and subsequently transferred into a specific cart designed for handling these items. Following the processing procedures, the samples were identified according to species and defrosting method as follows: PL (*Octopus Vulgaris* subjected to slow process), PV (*Octopus Vulgaris* subjected to rapid process), TL (*Illex Argentinus* subjected to slow process) and TV (*Illex Argentinus* subjected to rapid thawing). After identification and labeling, the samples were stored in a refrigerated chamber at a temperature between 0 and 4°C. To evaluate the impacts of the two defrosting methods (slow and rapid) on the PL, PV, TL and TV shelf-life and to compare them, two samples of *Octopus Vulgaris* and two samples of *Illex Argentinus* were taken at three different time points (T0 = 1 day of defrosting, T1 = 24 hours after defrosting, T2 = 48 hours after defrosting). At each sampling time, for each species and defrosting method, 400g of product were collected and sent to the laboratory in refrigerated boxes and immediately analyzed for the determination of physic-chemical, microbiological, and sensorial parameters.

Sensorial testing

Organoleptic Examination

The organoleptic examination was performed through the creation of specific assessment tables based on the parameters outlined in EC Regulation n. 2406/96 (Council of the European Union, 1996), which defines the quality criteria for cephalopod mollusks. The reference parameters considered at the different sampling times (T0, T1, T2) included skin appearance, odor, muscle hardness and tentacle consistency. The tables containing these parameters were presented to 5 semi-trained tasters, who were unaware of the sampling time of the evaluated products. The assessment was conducted through the visual inspection of samples subjected to the two defrosting methods, assigning each parameter a score as follows: 0 to indicate optimal product quality, 1 to indicate slight deterioration, 2 to indicate more evident alterations, and 3 to indicate complete deterioration and unsuitability for consumption. An overall score was obtained by adding the values assigned by the semi-trained testers, after which the mean of the total scores provided by the five testers was calculated.

Instrumental Odor Monitoring System (PEN3 IOMS)

The electronic nose (PEN3) was utilized to monitor and quantify variations in the volatile compound profiles of thawed cephalopod mollusks. Detection was achieved through ten integrated metal oxide semiconductor (MOS) sensors, each exhibiting selective sensitivity to different classes of volatile organic compounds. Before initiating data acquisition, samples were placed in 50 mL vials for 15 minutes to allow volatile compounds to diffuse within the container headspace. All experiments were conducted in a single laboratory room, in which the ambient air was continuously monitored and filtered using activated carbon filters connected to the instrument. The E-nose data acquisition workflow consisted of two main phases. The cleaning phase (flushing), an automatic calibration step, was performed for 300 seconds. The zero-point values recorded during this stage were used as the baseline. After baseline stabilization, volatile gases from the samples were pumped into the MOS sensor chamber, and signal acquisition was carried out for 70 seconds. Before analyzing the following sample, a cleaning phase was always performed, during which filtered air was pumped through the sensor chamber to completely remove any residual substances adsorbed onto the sensor surfaces. Two replicates were performed for each sample to generate a representative data pattern for comparative analysis.

Physical-chemical analysis

The pH measurements were carried out using a digital pH meter (Crison-Micro TT 2022, Crison Instruments, Barcelona). The Lipid oxidation (TBArs test) was monitored by determining the thiobarbituric acid ($C_4H_4N_2O_2S$) substance (TBAR) value, expressed as a malondialdehyde ($CH_2(CHO)_2$) concentration (mg/kg), which represents secondary oxidation products. Measurements were performed according to the extraction method proposed by Xiong *et al*., (2015) and modified by Di Paolo *et al*., (2023). For the determination of Protein degradation and food freshness indices, TVB-N (ABVT) and TMA were determined by Microdiffusion in a Conway cell. Measurements were performed according to the method proposed by Antonacopoulos and Vyncke (1989) and A.O.A.C. (1984), respectively. Colorimetric evaluation was determined using a Konica Minolta CR300 colorimeter (Minolta, Osaka, Japan) based on the CIEL* a* b* color scale. Measurements were performed at three random points on the mantle of the cephalopod molluscs, the mean values were calculated, and the SCI (specular component included) measurement values were considered for analysis.

Microbiological analysis

10 grams of each sample were sterilely transferred to a stomacher bag with 90 mL (1:10 (w/v)) of sterilized Peptone Water (PW, Oxoid) and homogenized for three minutes at 230 rpm using a peristaltic homogenizer (BagMixer®400 P, Interscience, Saint Nom, France). Suitable tenfold serial dilutions of each homogenate were prepared for the enumeration of the following microorganisms: total aerobic bacteria (TAB 30°C) were performed on plate count agar (PCA, Oxoid, Madrid, Spain) incubated at 30°C for 48/72 hours (ISO 4833-1:2013); total coliforms on violet red bile lactose agar (VRBL, Oxoid, Madrid, Spain) incubated at 37°C for 48 hours (ISO 4832:2006); Enterobacteriaceae on violet red bile glucose agar (VRBG, Oxoid, Madrid, Spain) incubated at 37°C for 48 hours (ISO 21528-2:2017); coagulase-positive staphylococci on BPA (Baird-Parker agar), incubated in an inverted position for 24+24 hours at 35-37°C (ISO 6888-1:2021).

Statistical analyses

Statistical analyses were performed using SPSS program, version 30 (IBM Analyt-ics, Armonk, NY, USA) and WinMuster software for electronic nose systems. data, expressed as mean ± standard error, were statistically analysed using a multivariate general linear model (GLM), considering aging time (T0, T1, T2), thawed methods (slow and rapid), and molluscs (*Octopus Vulgaris* and *Illex Argentinus*) as fixed factors. Helmert contrasts and Bonferroni correction for multiple comparisons were applied. The Tukey's test was used to calculate p-values ($p < 0.05$ and $p < 0.01$).

Results and Discussion

Sensory evaluation

Organoleptic examination of the mollusks, focusing on skin appearance, odor, muscle firmness, and tentacle consistency, was performed by five semi-trained tasters who performed sensory visual and tactile evaluation. The semi-trained tasters were prepared on the use of the scale reported in the EC Regulation n. 2406/96. All results were collected, and the mean scores for each sampling time (T0, T1, T2) were calculated. Based on these mean values, Table 1 was created to compare the outcomes. The results (Table 1) indicate that both species responded positively to the two thawed methods. Indeed, the total scores for *Illex Argentinus* and *Octopus Vulgaris* fall within the limits established EC Regulation n. 2406/96 for products of good quality. The results show that samples PV and TV were assigned lower scores than their corresponding slowly thawed samples (PL and TL, respectively) at all sampling times. It is important to note that, although TV and PV showed different trends over time, they exhibited identical scores at T1 and T2, indicating that the rapidly thawed samples were preferred by the semi-trained testers.

Instrumental Odor Monitoring System (PEN3 IOMS)

The electronic nose results obtained in this study provided data that allowed comparison with the other analyses performed. In particular, the PCA of both PV and PL revealed, as shown in Figure 1, a variation in volatile compounds over the storage. Specifically, samples at T1 and T2 showed higher variance values compared with T0 (0.854-0.774), indicating a more pronounced organoleptic variation over time in *Octopus Vulgaris*. Conversely, the PCA of *Illex Argentinus* (Figure 2) showed that both samples maintained a variance of 1% across all sampling times, suggesting a lower temporal change. This observation is consistent with the results of the Organoleptic Examination, in which the TV and TL scores show minor variation over time. Furthermore, PCA conducted on PL, PV, TL, and TV also allowed evaluation of sensors S2, S7, and S9, associated with aromatic compounds, ammonia, and nitrogen-containing volatiles, respectively. This analysis showed that PV and TV revealed lower sensor intensities than PL and TL, respectively. These results agree with *TVB-N* and TMA values, which similarly indicated lower levels of nitrogenous degradation products in the rapidly thawed samples.

Physical-chemical analysis

Effects of the two defrosting processes on the pH values of the mollusks

Figure 3 showed the pH values of the four samples examined. The results indicated a significant increase in pH values in the PV sample from T0 to T2, while in the remaining three samples the pH values slightly increased over time. It is important to note that between the two *Octopus vulgaris* samples (defrosted using the rapid and slow methods, respectively), a significant difference was discovered at T0, indicating that pH values were influenced by the defrosting method only for this species. In contrast, no significant differences were observed between the two *Illex Argentinus* samples, neither across the sampling times. These results can be explained by the possibility that rapid thawing induced more pronounced structural damage to the muscle cells, resulting in the release of a higher amount of basic nitrogenous compounds and other alkaline molecules during the short interval prior to measurement (Zhang *et al*., 2024). This would justify the higher pH observed in the rapidly thawed sample. Conversely, during slow thawing the product remained for a longer time at refrigeration temperatures, which may have promoted a different pattern of release and diffusion of soluble compounds, leading to a lower pH value at T0. Moreover, the absence of such differences in *Illex argentinus* may be related to the different protein behavior of this species. When proteins undergo denaturation, the exposure of hydrophobic residues promotes protein–protein interactions and the formation of aggregates, resulting in decreased protein solubility. Gokoglu *et al*. (2018) have reported that the protein solubility of *Illex Argentinus* muscle is lower than that of *Octopus Vulgaris*. This suggests that the muscle of *Octopus Vulgaris* is more sensitive to structural and biochemical

changes induced by the thawing process, and more inclined to exhibiting pH differences between thawing methods.

Chemical deterioration indicators

TBA, TVB-N and TMA values are reported in Table 2. According to Atrea *et al*. (2009), TBA values in the range of 1-1.5 mg MDA/kg are considered the limit beyond which fish muscle develops rancid odors and off-flavors. In the present study, all samples showed TBA levels below this limit at every sampling time, indicating that the cephalopod samples had not achieved an advanced stage of lipid oxidation. The trend was similar for all four samples, with a progressive increase in TBARS values from T0 to T2. However, the two thawing methods had different impacts on the samples. Rapid thawing was more effective in *Illex Argentinus*, which showed lower TBARS values (0.045±0.002 mg MDA/kg at T2) than the *Octopus Vulgaris* samples (0.085±0.001 mg MDA/kg at T2). On the other hand, slow thawing proved to be more effective in *Octopus Vulgaris*, in which TBARS values were 0.049±0.001 mg MDA/kg at T2, while in *Illex Argentinus*, the production of secondary oxidation compounds was significantly higher (0.130±0.002 mg MDA/kg at T2). These behaviors may be explained by the low lipid content in the mantle and arms of cephalopods and by the fact that malondialdehyde can interact with other muscle constituents such as nucleosides, nucleic acids, proteins and other aldehydes (Atayeter and Ercoşkun, 2011). In addition, according to Grim *et al*., (2010), who demonstrated that the susceptibility of muscle membranes to lipid oxidation increases with higher concentrations of polyunsaturated phospholipids and pro-oxidant species, the behavior observed in *Illex Argentinus* be explained by the loss of free water during slow thawing, which concentrated pro-oxidant molecules and enhanced lipid-oxygen interactions, thereby promoting the formation of secondary oxidation products. The effects of different thawing methods were also shown by the TVB-N and TMA index results. According to the National Guidelines for Risk Analysis in Food Microbiology, a TVB-N value 20-25 mg/100 g and a TMA value 5-20 mg/100 g for thawed cephalopod molluscs are considered acceptable. Based on these limits, *Octopus Vulgaris* and *Illex argentinus* TVB–N and TMA values analyzed in the present study correspond to the acceptable range for both indices at every sampling time (Table 2).

As shown in Table 2, the two species *Octopus Vulgaris* and *Illex argentinus* exhibited the same increasing trend in TVB-N values over the storage. It was also observed that the TVB-N values in O. vulgaris and *Illex Argentinus* slow-thawed samples were significantly higher than those of the corresponding rapidly thawed samples at all sampling times. At T2 the TL values (23.243±0.90) were significantly higher than the TV values (21.859±0.57), and a similar difference between PL and PV (14.112±0.63 and 11.345±0.89, respectively) was noted. According to Zhang *et al*., (2024), who investigated the effects of four different thawing methods on quality indicators of *Amphioctopus neglectus*, this may be because the prolonged exposure of meat to air during the thawing process leads to protein damage, with increased production of endogenous enzymes that have an impact on TVB-N values. Regarding TMA values, a similar decreasing trend was observed in *Octopus Vulgari*s (PV and PL) and *Illex Argentinus* (TV and TL) from T0 to T2. Moreover, as previously reported for the TVB-N values, a significant difference was observed between the two thawing methods, with values from T0 to T2 significantly higher in PL and TL compared to PV and TV, respectively. According to Šimat *et al*. (2009) and to the National guidelines for risk analysis in the field of food microbiology, TMA values are not a reliable indicator of quality during the first days post-thaw, as their accumulation requires longer periods of microbial and enzymatic activity. This supports the observation that TMA remained stable and below regulatory limits in all cephalopod samples throughout the 48-h monitoring period, while still providing information regarding the differences between the two thawing methods and the extent to which rapid thawing reduces the generation of alkaline substances.

Color measurements

Color parameters of the samples throughout the storage period are presented in Table 3. L* and a* parameters showed no statistically significant differences between the two thawing methods. Regarding the storage period, only the TV sample exhibited a significant decrease in lightness from T0 to T2 (48 hours post-thawing), whereas the redness values for all samples remained constant, with slight but non-significant variations over time. The absence of significant differences in L* and a* between thawing methods and during the storage, is consistent with the minimal changes and low values compared to the limits (National Guidelines for Risk Analysis in Food Microbiology) observed in pH and TVB-N and TMA respectively, indicating that protein degradation and loss of freshness remained moderate during the 48-h post-thaw period. As concerns the b* parameter, a significant difference was detected between TV and TL, and more pronounced time-related variations were observed in all samples throughout the storage period. This behavior suggests a contribution of oxidative and physical processes to yellowness development. This pattern is in line with the moderate progression of lipid oxidation detected by TBARS, as also reported by Dieterich *et al*. (2015), who demonstrated color parameters can reflect oxidative processes.

Microbiological results

Since no specific microbiological criteria are established for thawed cephalopod mollusks, the National Guidelines for Risk Analysis in the Field of Food Microbiology were used as the reference framework for interpreting the results. Figure 4 showed the results of microbiological analyses (TAB 30° and *coagulase-positive staphylococci*) and the effect of the two thawed methods on *Octopus Vulgaris* and *Illex Argentinus* over the storage. Temperature represents the primary factor limiting the proliferation of mesophilic bacteria and maintaining it under control is essential to restrain and slow down their growth (Price and Sowers, 2004). A comparison between PV and PL (Figure 4) revealed no considerable differences, as the mesophilic count showed values between 5 and 6 Log CFU/g, with only minimal variations across the three sampling times. However, 24 hours after thawing (T1), the microbial load reached 6.53 Log CFU/g, slightly superior to the acceptability limit of 6 Log CFU/g established by the National Guidelines for Risk Analysis in the Field of Food Microbiology. This value may be associated with an incorrect application of good hygienic practices during one of the handling phases following thawing (Idris Ali and Immanuel, 2017). In the comparison between TV and TL, minor differences were observed in the progression of mesophilic counts during storage. The gradual increase of approximately 1 Log CFU/g was related to the initial microbial load, as the rapidly thawed sample (TV) showed higher initial values (4.35 Log CFU/g). Similar observations have been reported in other animal matrices, where the thawing method significantly affected the microbial profile, with rapid thawing leading to higher surface temperatures and altering the initial composition of the bacterial flora (Zhou and Xie, 2021). The enumeration of *coagulase-positive staphylococci* (Figure 4) did not reveal statistical differences between samples, either in relation to the thawing method or across the different sampling times. Furthermore, in the PL sample, as previously observed for the total bacterial count, a concentration of 3.28 Log CFU/g was detected at T1. This value deviates from the trend observed at the other sampling times and supports the hypothesis of a secondary contamination occurring during a manipulation phase following thawing. It is important to note that, according to the acceptability limits outlined in the National Risk Analysis Guidelines, values equal to or lower than 3 Log CFU/g are considered acceptable. therefore, a slight exceedance was observed only for the PV. Enterobacteriaceae and Coliforms were enumerated, in both cases the measured values were not considerable. Specifically, Enterobacteriaceae reached levels on the order of 1 Log CFU/g, while Coliforms remained below 1 Log CFU/g. It is important to note that, as with the previous parameters, no specific regulatory limits are available for these microbial groups; the values reported in the National Guidelines refer to β-glucuronidase–positive *E. coli* in "molluscs or crustacean-based products," for which counts equal to or greater than 2 Log CFU/g are considered unacceptable.

Conclusions

The present study evaluated the influence of two thawing methods (slow refrigerated thawing and rapid water-immersion thawing) on the sensory, physicochemical, and microbiological quality of *Octopus Vulgaris* and *Illex Argentinus* during 48 hours of refrigerated storage after thawing. The results suggest that the response to thawing conditions may be species-dependent. *Illex Argentinus* samples showed better preservation of chemical and sensory parameters after rapid thawing, as indicated by lower TBARS values, reduced formation of nitrogenous degradation compounds, and stable volatile profiles. In contrast, *Octopus Vulgaris* appeared more susceptible to structural muscle changes associated with thawing dynamics.

From a microbiological perspective, slow thawing tended to maintain lower levels of mesophilic bacteria and coagulase-positive staphylococci during storage. Nevertheless, microbial loads observed in rapid thawed samples remained within the acceptability limits established by the National Guidelines, suggesting that rapid thawing, when carried out under hygienic and temperature-controlled conditions, may not compromise food quality. Electronic nose analysis supported these observations, highlighting temporal differentiation in volatile compounds for *Octopus Vulgaris*, whereas *Illex Argentinus* samples showed limited variations over time regardless of the thawing method.

Although color parameters showed only moderate variations, with relatively stable L* and a* values and a gradual increase in b*, these trends were consistent with the oxidative patterns indicated by TBARS measurements.

Overall, these preliminary results suggest that, even beyond the currently recommended 24 hours after thawing, the examined cephalopod species may maintain similar physicochemical, sensory, and microbiological trends when thawing and storage are carried out under controlled hygienic conditions and appropriate temperature management. The observed differences between species and thawing methods highlight the importance of considering species-specific responses when evaluating post-thaw quality. Further studies involving a larger number of samples and parameters specifically validated for cephalopods are needed to confirm these observations.

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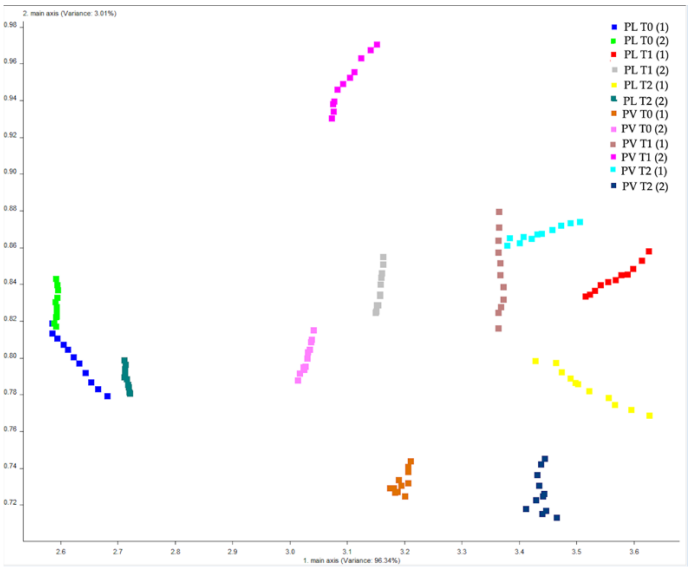

Figure 1. PCA of the samples during the storage. PV= *O. Vulgaris* thawed rapidly; PL= *O. Vulgaris* thawed slowly.

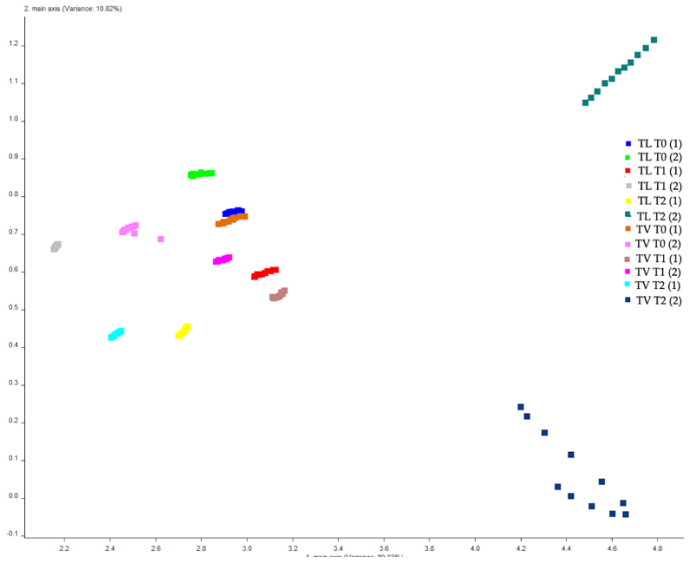

Figure 2. PCA of the samples during the storage. TV= *I. Argentinus* thawed rapidly; TL= *I. Argentinus* thawed slowly.

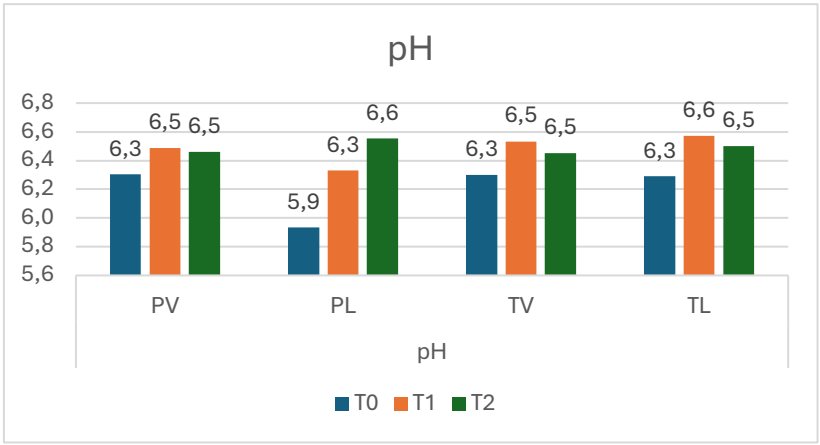

Figure 3. Trends of pH values in the four molluscs cephalopods during the storage period. PV= *O. Vulgaris* thawed rapidly; PL= *O. Vulgaris* thawed slowly; TV= *I. Argentinus* thawed rapidly; TL= *I. Argentinus* thawed slowly. The data are presented as means ± standard errors.

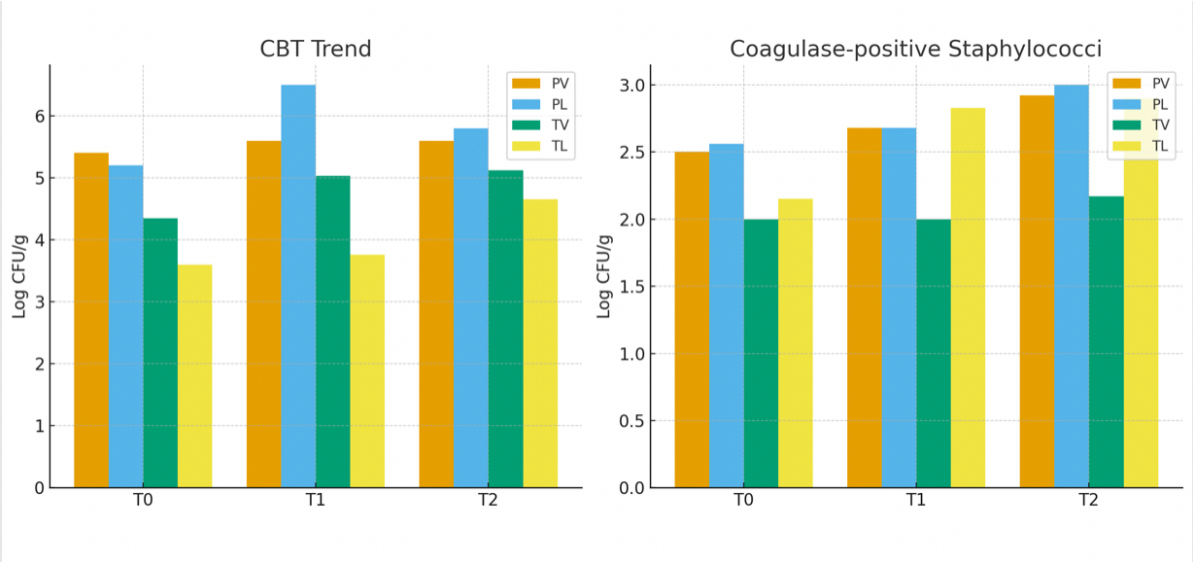

Figure 4. Results of microbiological analyses (TAB 30° and *coagulase-positive staphylococci*) of the four samples during storage after thawed. PV= *O. Vulgaris* thawed rapidly; PL= *O. Vulgaris* thawed slowly; TV= *I. Argentinus* thawed rapidly; TL= *I. Argentinus* thawed slowly.

Table 1. Organoleptic examination results of the four samples during the storage.

	T0	T1	T2
PV	6	6.5	6.8
PL	6.8	8	8.2
TV	6.5	6.8	6.9
TL	7	7.7	7.9

PV, *O. Vulgaris* thawed rapidly; PL, *O. Vulgaris* thawed slowly; TV, *I. Argentinus* thawed rapidly; TL, *I. Argentinus* thawed slowly. The data are presented as means ± standard errors.

Table 2. TVB-N, TMA and TBARs values of the four mollusks cephalopods during the storage period.

	Samples	**T0**	**T1**	**T2**
TVB-N	PV	$13.282\pm0.84^{A,X}$	$10.515\pm0.19^{B,X}$	$11.345\pm0.89^{C,X}$
	PL	$14.666\pm0.75^{A,Y}$	$13.835\pm0.26^{B,Y}$	$14.112\pm0.63^{C,Y}$
	TV	$14.155\pm0.23^{A,Z}$	$16.092\pm0.17^{B,Z}$	$21.859\pm0.57^{C,Z}$
	TL	$15.495\pm0.30^{A,W}$	$17.986\pm0.40^{B,W}$	$23.243\pm0.90^{C,W}$
TMA	PV	$3.597\pm0.07^{A,X}$	$3.320\pm0.05^{B,X}$	$2.767\pm0.03^{C,X}$
	PL	$8.301\pm0.01^{A,Y}$	$8.301\pm0.03^{A,Y}$	$3.182\pm0.06^{C,Y}$
	TV	$3.320\pm0.02^{A,Z}$	$3.735\pm0.02^{B,Z}$	$3.182\pm0.01^{C,Z}$
	TL	$5.534\pm0.09^{A,W}$	$3.320\pm0.01^{B,W}$	$3.874\pm0.01^{C,W}$
TBARs	PV	$0.069\pm0.002^{A,X}$	$0.070\pm0.004^{B,X}$	$0.085\pm0.001^{C,X}$
	PL	$0.044\pm0.001^{A,Y}$	$0.046\pm0.005^{B,Y}$	$0.049\pm0.001^{C,Y}$
	TV	$0.030\pm0.003^{A,Z}$	$0.038\pm0.002^{B,Z}$	$0.045\pm0.002^{C,Z}$
	TL	$0.055\pm0.002^{A,W}$	$0.101\pm0.001^{B,W}$	$0.130\pm0.002^{C,W}$

PV, *O. Vulgaris* thawed rapidly; PL, *O. Vulgaris* thawed slowly; TV, *I. Argentinus* thawed rapidly; TL, *I. Argentinus* thawed slowly. The data are presented as means ± standard errors. The a,b,c mean values in the same row (storage time) with different letters differ significantly for $p < 0.05$ (lowercase) or $p < 0.01$ (uppercase). The x,y,z mean values in the same column (different samples for the same storage time) differ significantly for $p<0.05$ (lowercase) or $p<0.01$ (uppercase).

Table 3. Color parameters of the samples throughout the storage period.

	Samples	T0	T1	T2
L*	PV	46.25±1.84	39.72±2.13	40.16±0.99
	PL	43.63±1.65	36.17±5.26	35.97±5.65
	TV	48.04±7.29^{A}	43.46±6.12	32.38±3.30^{B}
	TL	36.88±1.50	35.39±4.70	36.15±4.90
a*	PV	4.15±1.60	1.59±0.95	2.83±0.43
	PL	4.37±0.01	1.68±0.85	4.19±0.46
	TV	2.53±1.42	4.17±2.79	3.63±1.52
	TL	2.49±0.29	3.48±2.65	3.39±0.61
b*	PV	5.24±0.31	8.23±1.24^{a}	3.78±1.03^{b}
	PL	4.97±1.30^{A}	10.13±0.65^{B}	2.6±1.82^{A}
	TV	1.09±0.54^{A,x}	6.58±1.27^{B}	1.77±0.19^{A}
	TL	1.43±0.60^{A,y}	7.48±3.47^{B}	4.33±1.65^{A}

PV, *O. Vulgaris* thawed rapidly; PL, *O. Vulgaris* thawed slowly; TV, *I. Argentinus* thawed rapidly; TL, *I. Argentinus* thawed slowly. The data are presented as means ± standard errors. The a,b,c mean values in the same row (storage time) with different letters differ significantly for $p < 0.05$ (lowercase) or $p < 0.01$ (uppercase). The x,y,z mean values in the same column (different samples for the same storage time) differ significantly for $p<0.05$ (lowercase) or $p<0.01$ (uppercase).