The Resurrection Experiment of Liquid Nitrogen Quick-Frozen Fish

Haoyuan Xu

Shenzhen Senior High School International Division, Shenzhen, 518000, China

Keywords: Goldfish, Liquid nitrogen, Resurrection

Abstract: In order to confirm the phenomenon of quick-frozen fish resurrection, the conditions and mechanism of quick-frozen fish thawing and resurrection were further explored. In this experiment, goldfish were used as material for quick freezing in liquid nitrogen, the quick freezing time and thawing temperature were changed, and the degree of damage to individuals and cells was observed under a microscope. The experiment confirmed the existence of quick-frozen fish resurrection phenomenon. By precisely controlling the quick-freezing time and thawing at 20°C, small fish with a body length of 10 cm and below can be resurrected. The death of quick-frozen fish is due to the dehydration and rupture of tissue cells. This experiment provides a theoretical basis for the frozen preservation of aquatic food.

1. Introduction

Fungal spores can be resurrected at room temperature. Chipmunk's body temperature is close to 0℃ during hibernation, but they will return to normal life as soon as the spring breeze arrives.[1] Scientists have done such an experiment. The goldfish was taken out of the water, and after the surface was slightly dry, it was immediately put into liquid nitrogen at -269℃(close to absolute zero - 273℃) and frozen. After 10 seconds, the quick-frozen goldfish was placed in warm water, and the goldfish miraculously “resurrected from the dead”, swimming peacefully in the water as usual.

Fish is rich in protein and fatty acids, and the meat is delicious, and it has always been an important ingredient on people's table. [2]Liquid nitrogen is a colorless, odorless, low-viscosity transparent liquid with stable chemical properties and no chemical reaction with food ingredients, which meets the requirements of food hygiene and is an ideal freezing preservative. The extremely fast freezing rate can quickly pass through the maximum ice crystal formation zone, so that the ice crystals in the food are smaller and evenly distributed, and the damage and loss of nutrients are less, and the quality of the food is better maintained. [3][4]In recent years, liquid nitrogen freezing has gradually become a fish storage method that has been widely concerned by researchers at home and abroad.

The target and significance on study of freezing revival of fish is that live fish are placed in liquid nitrogen 196 degrees below zero celsius for tens of seconds and immediately covered with ice. And then put the freezing fish back into 20℃ degrees water .The result is that the small size fish are able to revive. The phenomena indicates that fish are able to maintain biological organism activity in liquid nitrogen.

2. Materials and Methods

2.1 Materials

fresh goldfish, fresh crucian carps ,fresh loaches.

liquid nitrogen, knives, stopwatch, tweezers, water, vacuum cups , thermometers, microscope.

2.2 Methods

1) Investigate and Study the Main Components and Biological Structures of Organisms through the Internet and Related Books to Understand Fish Characteristics.

2) Verify the reaction of fish in liquid nitrogen 196 degrees below zero celsius and record the phenomenon and meanwhile replace the existing fish for other species for research and study to

understand other species' characteristics.

3) Record the Survival Time of Freezing Fish after Revival and Discuss and Analyze the Experimental Results.

4) Change the Time of Fish Placed in Liquid Nitrogen. Take Some Samples of Fish Organism. Observe the Damage under a Microscope . Record and Analyze the Causes.

2.3 Research Schedule

1) Preliminary Study on Freezing Revival of Fish

First, put goldfish into liquid nitrogen and then into 20℃ degrees water respectively for ten seconds. Observe whether the goldfish are revived and meanwhile measure the time that is needed for revival. Take down the survival time after the goldfish are revived. Finally, take samples of the revived fish for microscope observation. The target is to determine the best time for freezing revival of fish through the experiment.

2) Explore the Influence of Temperature of Water for Thawing on Freezing Revival of Fish.

First, put the goldfish into liquid nitrogen. And then respectively place the freezing fish into 10℃,20℃, 30℃degrees of water for the same time. Observe whether the goldfish will revival or not . Measure the time that is needed for revival. Take down the survival time after revival. Take samples of freezing fish for microscope observation.Through the experiment to determine the most proper water temperature for freezing revival of fish.

3) Explore the Influence of the Sizes of Fish on Freezing Revival of Fish.

First, freeze the sizes of big, medium and small goldfish in liquid nitrogen for the same time. And then, put the freezing fish into 20℃, degrees of water respectively. Observe whether the goldfish will revival or not . Measure the time that is needed for revival. Take down the survival time after revival. Take samples of revived fish for microscope observation.

4) Explore the Influence of Fish Species on Freezing Revival of Fish

First, take fish of different species, preferably of similar size. And then, freeze them in liquid nitrogen for the same time. Last, put them in 20℃ degrees of water respectively . Observe whether the goldfish will revival or not . Measure the time that is needed for revival. Take down the survival time after revival. Take samples of revived fish for microscope observation.

5) Observe the Influence on the Cells of Freezing Revival of Fish.

To study the influence of freezing revival on fish cells by microscopic observation.

2.4 Experimental Procedures

1) First, we put the liquid nitrogen jar on the horizontal table and opened the lid , white gas coming out. And then, we took out 100ml liquid nitrogen with an iron spoon and put it into a breaker. Immediately, the liquid nitrogen boiled a large amount of white gas coming out. The sides of beaker frosted and surrounding temperature decreased.

2) We wearing liquid nitrogen protective suit put the selected fish into the beaker. For fear that the fish would freeze on the sides of the beaker ,we rinsed the fish into the liquid nitrogen with plastic test tubes.

3) When the Fish Was Put into Liquid Nitrogen , the Liquid Nitrogen Boiled More Violently with a Large Amount of White Gas Coming out and the Surrounding Temperature Dropped by 10 Degrees.

4) We froze the fish in liquid nitrogen and then took it out after the specified time according to the requirement of the experiment and quickly put the freezing fish into the water with the specified temperature. A small amount of white solid floating on the water swam fast. After a while , the fish moved slowly to the water wave. Then, some fish swam normally. Some fish died.

3. Results

By the research of the project, we have a comprehensive understanding of liquid nitrogen and biological structures of fish. We verify the reaction of freezing fish in liquid nitrogen. Replace fish with other biological organisms in the experiment. Record the survival time and analyze the causes.

Change the time of biological organisms in liquid nitrogen. Record the survival time and analyze the causes.

Under the proper conditions, the larger the fish species are, the more difficult to revive. The smaller of the same fish species are more likely to revive.(table 1)

20℃ degrees of water is fit for freezing revival of fish. The higher temperature water leads to severe expansion and contraction, which leads to cell rupture. (Table 1)

The longer the freezing time is , the harder for fish to revive. (Table 1)

Through the observation of the dead fish tissue, all the causes of death of freezing fish are due to basic cellular tissues' dehydration, necrosis, rupture after freezing, which cause irreversible damage to the final death. A positive correlation is found that there lateral cell necrosis and the inside of the cell is still alive for the large size fish. We should take it into consideration that if the dead cells play an important role for living, the fish are not able to revive. Overall, these results indicate that the size of fish around 10cm are frozen for 15 seconds and returned to 20℃ degrees of water , which is the limit value of universal revival. Beyond the value, it is difficult to revive because once the cells are damaged beyond repair; fish are not able to revive. But for some vigorous fish, their cells can be replaced faster, so they can revive beyond the limit value. Obviously, this freezing revival technique is not applicable to large mammals. (Table 1)

Table 1 Effects of Liquid Nitrogen Quick-Freezing and Resurrection Time on Fish Resurrection Status.

	Freezing time	Fish Sizes	resurrection temperature	Species	Whether to resurrect	Time to live
1	15s	5cm	20℃	goldfish	positive	Normal span
2	20s	5cm	20℃	goldfish	positive	Normal span
3	25s	5cm	20℃	goldfish	negative	
4	15s	10cm	20℃	goldfish	positive	Normal span
5	15s	15cm	20℃	goldfish	negative	
6	15s	10cm	10℃	goldfish	negative	
7	20s	30cm	20℃	crucian carp	negative	
8	20s	40cm	20℃	loaches	positive	Died after four minutes
9	15s	10cm	30℃	goldfish	negative	

4. Discussion

A major contribution of this experiment is to verify the freezing revival of fish. The condition of the revival of fish is that the accurate control of the freezing time in liquid nitrogen and thawing in 20℃ degrees of water. In order to control accurate time, we use plastic test tubes with a small hole at the bottom to contain fish. The advantage is that plastic tubes can be directly put into the water for thawing. The experiment is hard to conduct. The success rate of freezing revival of fish is not very high. For one thing, the main obstacle is the control of time. For another , it is the fact that the experiment requires scientific research group members to crowd together to observe the experimental results, which cause difficulty for accurate water temperature.

Acknowledgments

Thanks for the close cooperation among the team members, thanks to the teachers for their careful guidance, and thanks to the parents and classmates for their support and encouragement.

References

[1] He Jinchao. Cryopreservation of human body is not a dream [J].Friends of Science, 2001(10). Influence[J]. Agricultural Products Industry, 2019(15):18-21.

[2] Zhang Longyi, Zhang Wei, Zhang Pengcheng, Chen Tingting, Ke Huan, He Wenqian, Ye

Haolong. Effects of liquid nitrogen quick-freezing on the resurrection state of crucian carp [J]. Agricultural Products Industry, 2019(15): 18-21.

[3] Xie A, Sun DW, Zhu Z, et al. Nondestructive measurements of freezing parameters of frozen porcine meat by NIR hyperspectral imaging [J].Food and Bioprocess Technology, 2016 (9): 1444-1454.

[4] Lu Jun. Effects of liquid nitrogen deep-freezing and quick freezing on the quality and muscle tissue of hairtail and silver pomfret [D]. Hangzhou: Zhejiang University, 2015.