

ABOUT THE POSSIBILITY OF DRYING AND FURTHER REHYDRATION OF MULBERRY LEAF, FOR FEEDING SILKWORM CATERPILLARS (*Bombyx mori* L.)**Mirzakhodjaev B.,***D.Sc., Scientific Secretary, Research Institute of Sericulture***Mirzakhodjaev A.,***Ph.D, Head of the Laboratory of Mechanization, Research Institute of Sericulture***Tajenova A.,***doctoral of Research Institute of Sericulture email***Radjabov I.***doctoral of Research Institute of Sericulture email*<https://doi.org/10.5281/zenodo.10137723>**Abstract**

Due to the global climate change and the spring frosts that have become more frequent in recent years, leading to the death of silkworm caterpillars of the first ages from lack of feed, there is an urgent need to find effective and cheap means to replace and preserve the natural food - mulberry leaf. One of the new and progressive drying methods for sericulture products is the method of sublimation dehydration in vacuum. In the laboratory of the Research Institute of Sericulture, an experimental spring feeding was carried out with a sublimated and restored mulberry leaf of the caterpillars of the silkworm of the Ipakchi 1 breed, in the amount of 200 pieces for each variant. For feeding, a mulberry leaf was used, collected before the fall of leaves in the autumn of 2022. For freeze drying of mulberry leaves, the following mode was selected: pre-freezing at $t = -50\text{ }^{\circ}\text{C}$, then freeze drying at condenser temperature $t = +40\text{ }^{\circ}\text{C}$, residual vacuum pressure $P = 0.3\text{ mmHg}$. Rehydration (hydration) and enrichment of the leaf was performed a day before feeding to caterpillars. The rehydration of the leaf was carried out in a high humidity mode. Further, the feed was enriched by introducing sucrose, ascorbic acid and potassium-phosphorus additive (K_2HPO_4) into the leaves.

When the leaf was rehydrated in a high humidity environment with the addition of K_2HPO_4 to the leaf, the viability of the caterpillars was 75%, and the duration of feeding was on average 12 days.

Sublimation drying of the leaf and its subsequent rehydration in a high humidity environment and enrichment of the dried leaf can be used to expand the forage base of sericulture, as well as to create artificial food recipes based on mulberry leaf.

Keywords: silkworm caterpillars (*bombyx mori* L), mulberry leaves, freeze drying, rehydration, enrichment, feeding.

INTRODUCTION

The intensive growth of cocoon production in the Republic in recent years requires the development of new technologies and high-performance equipment for processing cocoons in order to obtain raw silk that meets all the requirements of world market standards, as well as new directions in science for the use of sericulture products.

Due to the global climate change and the spring frosts that have become more frequent in recent years, leading to the death of caterpillars of the first ages from lack of feed, there is an urgent need to find effective and cheap means to replace natural food - mulberry leaf during spring frosts.

One of the promising ways to strengthen the forage base of sericulture is the preservation and enrichment of natural feeds.

In addition to the theoretical aspects, this path opens up a tempting prospect in sericulture of using the organic mass of mulberry leaves shed by plants in the autumn period.

The main purpose of canning is to protect the product from physico-chemical, autolytic and microbiological decomposition. There are several directions in the technology of food preservation.[1]

Studying them led us to the conclusion that the most promising is the preservation of the leaf in a state

of potential (possible) active life after certain effects (suspended animation) [2,3,4].

To transfer a living active organism into anabiosis, it is necessary to dry it. One of the new and progressive drying methods for sericulture products is the method of freeze-drying them in vacuum [5].

Freeze drying is the removal of moisture from frozen materials by sublimation of ice, i.e. its direct transition to a vaporous state, bypassing the liquid phase [6].

This method combines two well-known methods of canning freezing and vacuum drying. When freezing, undesirable changes in the properties of the product are minimal, and when drying in vacuum, the structure, composition and nutritional properties of food products are preserved to a greater extent than when canned by other methods. The sealed packaging of freeze-dried products allows them to be stored for a long time at an unregulated temperature.

A significant reduction in the mass of products obtained by freeze-drying (4-10 times compared to the original) significantly affects the reduction of costs during their transportation.

Currently, the sublimation dehydration method is used both in the laboratory and on an industrial scale: in medicine, biology, chemistry, enzyme and food industries.

Industrial production of freeze-dried food products is quite widely developed in a number of foreign countries. For example, in the USA, the volume of production of freeze-dried products in 2020 amounted (by raw materials) to about 200 thousand tons [7].

In England and Holland, egg white and melange for the needs of the confectionery industry are dried only by freeze-drying at specialized high-capacity enterprises. Freeze-drying of coffee has become widespread commercially [8,9,10].

A significant part of scientific works is devoted to the optimization of vacuum dewatering processes. This problem is especially widely affected in the pharmaceutical industry, where freeze drying is most common and often has no alternative. Product quality plays a key role here, and only then the duration of the dehydration cycle. Thus, in the work of scientists from Birmingham, UK [11], morphology, activity, dissolution, long-term storage, packaging and the cost of the drying object are considered as factors of economic efficiency and a high level of quality, and in the work of scientists from the University of Connecticut, USA [12], the influence of heat treatment on the qualitative characteristics of proteins during lyophilization, which they are of great importance for the development of the silkworm on the of feeding. Temperature variations during freezing and drying were studied, and the importance of molecular movements affecting protein stability was discussed. This knowledge can be used to maximize the preservation of the original properties of the mulberry leaf as the main food of the silkworm, to develop a strategy for optimizing the lyophilization process in terms of quality.

As the technique and technology of freeze-drying food production are improved, the cost of processing perishable vegetable raw materials will decrease, and in some cases it will be lower than the cost of processing it by other methods [13,14].

When determining the economic effect obtained from the introduction of the method of sublimation dehydration of sericulture products, it is necessary to take into account their advantages - a small mass is better than with other methods of canning, preservation of the original properties of the initial product, the possibility of storage without the use of cold, reducing costs when transporting these products over long distances.

Taking into account the above, in 2023, work was carried out on the selection of optimal modes of freeze-drying of mulberry leaf, work on the selection of methods of rehydration of mulberry leaf, as well as feeding silkworm caterpillars with a restored leaf.

RESEARCH METHODOLOGY

In order to test the possibility of feeding caterpillars with freeze-dried mulberry leaf, spring feeding of silkworm caterpillars was carried out in the laboratory

of the Sericulture Research Institute. To implement the tasks set, samples of the silkworm breed Ipakchi 1 in the amount of 200 pieces were selected, and laid for incubation according to the generally accepted method.

For experimental feeding, a mulberry leaf collected before leaf fall in autumn 2022 and freeze-dried under various regimes was used. Rehydration (hydration) and enrichment of the leaf was performed a day before feeding to caterpillars. The rehydration of the leaf was carried out in a high humidity mode. Further, the feed was enriched by introducing sucrose, ascorbic acid and potassium-phosphorus additive (K_2HPO_4) into the leaves [15].

Based on the analysis of processed materials for freeze drying of biological objects, the mode of freeze drying of mulberry leaves was chosen: pre-freezing at $t = -50^\circ C$, then freeze drying at condenser temperature $t = +40^\circ C$, residual vacuum pressure $P = 0.3$ mmHg.

Low-temperature sublimation preserves 7-8% of the absolute humidity in the leaf, although the leaf looks very dry, brittle. This leaf is very lyophilic and softens quickly because it has the ability to absorb vapors from the atmosphere. We observed that drying in the sun, hot air, in a vacuum at plus temperatures, in the air kills parenchyma cells, and the silkworm is not able to develop on such a dry leaf.

When the leaf was subjected to supercooling (nitricification) and then sublimation, the cells remained potentially alive.

Such a sheet can be stored for a long time at an ambient temperature in sealed plastic bags. At the same time, the nutritional properties of such a leaf are only slightly reduced. The choice of methods of rehydration (hydration) of the freeze-dried leaf is no less important than the choice of freeze-drying modes. Since the method of removing dried organisms from the state of so-called suspended animation significantly affects their survival, water during contact hydration of lyophilized cells penetrates through the microcapillaries of the shell at a pressure of almost 7 atmospheres and, as a result, separates from the shell and compresses the protoplast. The water that has penetrated into the cells displaces air through the germinal pores, which forms bubbles on the surface. Bubbles burst, when they explode, there is a violation of the structure of the germinal pore and protoplast, the release of part of the protoplast substance and the formation of a cavity in it.

Two variants of leaf rehydration were chosen by micro condensation of water vapor in a high humidity environment and contact hydration in an aqueous solution of sodium chloride (NaCl) with a mass fraction of $\omega(NaCl) \approx 0.9\%$ (saline solution). Figure 1 shows the condition of the mulberry leaf after freeze-drying, and Fig.2 after its rehydration.



Fig. 1. Mulberry leaves after freeze-drying.



Fig.2. Mulberry leaves after rehydration

RESULTS AND DISCUSSION

Determination of the effectiveness of drying a mulberry leaf by sublimation was determined by feeding silkworm caterpillars. To implement the tasks set, samples of the silkworm breed Ipakchi 1 were selected and laid for incubation according to the generally accepted methodology.

For experimental feeding, a freeze-dried mulberry leaf collected before leaf fall in autumn 2022 was used. Rehydration and enrichment of the leaf was performed immediately before feeding to the caterpillars. The rehydration of the leaf was carried out in a high humidity

mode and in a physiological mode. Further, the feed was enriched by spraying the leaf in three variants: in variant 1 - with activators of proteolytic metabolism (sucrose); in variant 2 - with an activator of redox processes (ascorbic acid); in variant 3 - with a potassium-phosphorus additive (K_2NPO_4).

Feeding was carried out until the caterpillars reached the third age. Then the caterpillars were transferred to feeding with an ordinary leaf. For each variant of the experiment, 200 caterpillars were fed. The results of feeding are shown in the table.

Table.

Results of experimental feeding of silkworm caterpillars with two variants of leaf rehydration with various additives.

№	Name of the additive	Leaf rehydration option	Number of caterpillars	The beginning of feeding	End of feeding	Number of days	Number of caterpillars aged			Percentage of food eaten
							1	2	3	
1	Sucrose	Saline solution	200	20.04.23	05.05	16	192	55	35	7
2		High humidity environment	200	21.04.23	04.05	14	189	120	118	20
3	Vitamin C	Saline solution	200	20.04.23	05.05	15	187	60	55	8
4		High humidity environment	200	21.04.23	03.05	13	190	130	120	22
5	K_2HPO_4	Saline solution	200	21.04.23	04.05	14	189	70	62	10
6		High humidity environment	200	22.04.23	03.05	12	186	155	150	25
7	Control	Fresh mulberry leaf	200	21.04.23	03.05	12	191	187	182	46

Some conclusions can be drawn from the submitted tables. In the variants, when the leaf was rehydrated in saline solution with the addition of sucrose and vitamin C, the viability of the caterpillars was 17.5-27.5%, and the duration of feeding was 15-16 days. When the leaf was rehydrated in a high humidity environment with the addition of sucrose and vitamin C, the viability of the caterpillars was 59-60%, and the duration of feeding was 13-14 days.

In the variant, when the leaf was rehydrated in saline solution with the addition of K_2HPO_4 to the leaf, the viability of the caterpillars was 31%, with a duration of feeding of 14 days, and when the leaf was rehydrated in a high humidity environment, the viability of the caterpillars was 75%, with a duration of feeding of 12 days. The Percentage of food eaten during leaf rehydration in the saline solution in variants with sucrose and vitamin C supplements was 7-8%, with the addition of K_2HPO_4 10%. When the leaf was rehydrated in a high humidity environment, the Percentage of food eaten in variants with sucrose and vitamin C was 20-22%, with the addition of K_2HPO_4 25%.

Feeding was carried out until the third age due to the determination of the possibility of caterpillars feeding with freeze-dried and then restored leaf, and also that the lack of feed in the spring mainly occurs during the first two ages of feeding. Then the caterpillars were fed with an ordinary leaf.

Chemical analysis of the leaf showed that after freeze-drying, the phosphorus content in it decreased from 0,64 to 0,42%, fat from 1,17 to 1,09%, total nitrogen – 3,65 to 3,36% per dry substance.

One of the direct proofs of the restoration of active vital functions in some cells of the parenchyma of the lyophilized mulberry leaf is the orderly movement of protoplasm. It occurs a few minutes after the plasticization of cells with a hydrator. After contact hydration in 15-20 minutes, a perfectly steady protoplasm movement was observed on the sheet. After staining the cell with vital fluorochrome, clearly fluorescent pathways of this movement were detected in the opac-porthole of an ultraviolet microscope.

However, such cells of the lyophilized mulberry leaf cannot be kept active for more than 40 minutes. Usually after 30-40 min. the soaked lyophilized leaf begins to brown in the air. Under the upper illumination with UV light under a microscope, at this moment there is a transition of the red glow of individual "living" cells to dark green. This indicates active oxidation processes in the tissue.

CONCLUSIONS

Thus, drying by sublimation of a mulberry leaf with its subsequent rehydration in a high humidity environment and enrichment of the restored leaf with a potassium-phosphorus additive can be used to expand the forage base of sericulture, as well as to create artificial food recipes based on mulberry leaf.

The introduction of improved methods of preparing mulberry leaves for drying, equipping workshops with appropriate equipment developed on the basis of scientific research, the creation of production lines for the production of high-performance freeze-drying products with mechanized and automated continuous installations will contribute to the creation of artificial

feed and increase the economic efficiency of the freeze-drying method and the sericulture industry as a whole. Mastering the method of canning and developing artificial feed will make it possible to conduct breeding work with silkworms all year round.

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