Biological method based on track membranes to form controlled gaseous media for fruit cold storage

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Abstract. The article proposes a biological method based on the use of gas-selective track membranes to form a modified gas atmosphere, considering the respiration rate of fruit. The work aims to study the change in the respiration rate of apples depending on the variety and storage time to substantiate the formation of the gas medium composition depending on the respiration rate of the fruit and the area of track membranes, and to study the kinetics of oxidation reactions of biologically active substances during cold storage of apples. The research is based on autumn apple varieties: Grushovka Yudicheva, Kordonovka and Pepin Shafranny. We have obtained the kinetic curves of changes in the content of mono- and disaccharides, ascorbic and organic acids in autumn apple varieties depending on the gas composition in terms of oxygen and carbon dioxide content, as well as the duration of fruit storage using track membranes. For maximum quality preservation, nutritional and biological value of the studied apple varieties at $t = (3 \pm 1)$ °C, we have determined a recommended composition of the controlled gas atmosphere: oxygen concentration - $(5.2 \pm 0.1\%)$, carbon dioxide - $(3.6 \pm 0.1\%)$. Controlled atmosphere is created using a gas-selective composite membrane with an area of 14-22 cm$^2$/kg, depending on the variety and respiration rate.

1. Introduction

To continuously supply the population with fresh high-quality fruit and vegetables between harvests, it is important not only to increase the production, but also to introduce modern innovative storage and processing technologies.

There is a variety of apple trees in North-West region of Russia, including Leningrad region due its soil-climatic conditions [1]. Most of them are high-yielding, but not suitable for long-term storage. There are various methods for fruit preservation with high-temperature treatment being most used. However, high temperatures result in the lower content of biologically active substances in fruit. Modifying a gas medium can extend the period of fruit storage. Therefore, we propose a novel biological method, which is based on the use of gas-selective track membranes and considers the respiration rate of fruit.

The use of low positive temperatures in combination with a reduced concentration of oxygen and increased concentration of carbon dioxide promises to maintain the quality and extended storage time of fruit and vegetables [2,3]. Currently, membranes of various types made of polymer and fabric materials are recommended for storing fruit in a controlled atmosphere. They differ by gas permeability, adhesive properties, strength and operational characteristics [4].

The present study investigates the changes of respiration rate of apples depending on the variety and storage time, substantiates the formation of the gas composition depending on the respiration rate of
fruit and area of track membranes, and examines the kinetics of oxidation reactions of biologically active substances during cold storage of apples of autumn varieties.

2. Materials and methods

Apples of autumn varieties: Grushovka Yudicheva, Kordonovka and Pepin Shafranny have been chosen for the research. Apples were taken from a collection orchard located in the Pavlovsk Experimental Station of Vavilov Institute of Plant Industry (Pavlovsk, Leningrad region, Russia).

The laboratories of the Federal State Unitary Enterprise «S.V. Lebedev Institute of synthetic rubber» and Ioffe Physical-Technical Institute of the Russian Academy of Sciences developed polyethylene terephthalate track membranes with high adhesion to a gas-selective polymer. The fabrication of nanosized pores in polymer materials was performed out using ion beams.

Composite gas-selective membranes consist of a substrate based on track membranes made of polyethylene terephthalate and a selective layer based on an organosilicon block copolymer; selectivity - 5.0; pore diameter - 0.2 μm. They were used to create a controlled gas atmosphere.

A controlled atmosphere was formed and regulated by the respiration of fruits placed in hermetically sealed polymer containers with a gas-selective track membrane (GSTM). Control apple samples were stored under normal atmosphere in containers without lids. Experimental samples were stored in hermetically sealed containers, equipped with GSTM. The area of membrane varied in the range from 14 to 22 cm²/kg.

The calculation formulas of capacity and area of membranes, oxygen and carbon dioxide concentrations are as follows:

$$\mu = \left(\frac{c_{o_2}^i}{c_{o_2}^f} - 1\right) \mu_0,$$

where $c_{o_2}^i$ is the initial oxygen concentration, 21%; $c_{o_2}^f$ is the recommended storage mode, %;

$$\mu_0 = pP J^{-1},$$

where $P$ is the membrane permeability for oxygen [3,0·10⁹ m³/(s·N)]; $p$ is the oxygen partial pressure (2,1·10⁴ Pa); $J$ is the respiratory rate of fruits [2,66 m³/(kg·s)];

$$c_{CO_2} = \delta \sigma^{-1}(c_{o_2}^i - c_{o_2}^f),$$

where $c_{CO_2}$ is the carbon dioxide concentration in a stationary mode (2,0 - 0,8%); $\delta$ is the respiratory quotient (1,1); $\sigma$ is the membrane selectivity (5);

$$S = m\mu^{-1},$$

where $S$ is the membrane area, m²; $\mu$ is the membrane capacity, kg/m².

Based on the calculated values of apples respiration rate, the following membrane areas were selected: 22.0, 18.0 and 14.0 cm²/kg for Grushovka Yudicheva, Kordonovka and Pepin Shafranny, respectively.

Control and experimental apple samples were stored at a temperature of (3 ± 1) ° C for 6 months. The respiration rate of apples was determined by the titrimetric method according to the release of carbon dioxide and its absorption by a solution of potassium hydroxide.

The effectiveness of the gas medium composition was assessed by the kinetics of the oxidation reaction of the reduced form of ascorbic acid determined by the Tillmans method. The changes in the content of mono- and disaccharides were determined by the cyanide method; the amount of free organic acids was defined by the titrimetric method [5]. The data were processed using Microsoft Excel to determine the 95% confidence intervals.
3. Results and discussions

Respiration is the main physiological process that greatly affects product shelf life and quality. Respiration is main factors dependent, such as resistance of storage objects to microbiological and physiological diseases, mass changes, the intensity of post-harvest ripening, etc. The energy that is released during “breathing” is needed to support life processes in the stored products. With an increase in the intensity of respiration, the consumption of biologically active substances grows, and the quality of products decreases [6].

3.1 Respiration rate

Figure 1 shows the dependence of changes in the respiration rate in autumn varieties of apple: Kordonovka, Grushovka Yudicheva, Pepin Shafranny during cold storage.

The respiration rate depends on the variety of apples and the storage duration. At the stage of placement and initial storage, all studied varieties differed by a high respiration rate, which gradually decreased during further storage.

![Figure 1. Changes in respiration rate in autumn varieties of apples during cold storage.](image)

The slowing in the rate of intracellular reactions at low temperatures leads to a decrease in the intensity of respiration. An increase in respiration rate in the climacteric period (3-4 months) is associated with the predominance of anaerobic respiration processes without significant changes in the amount of oxygen. In the climacteric period, not only carbohydrates are used for fruit "breathing", but also organic acids, primarily malic acid. It is known that an increase in respiration rate during this period is caused by growing in the activity of decarboxylating malate dehydrogenase ("malic" enzyme), which decarboxylates malic acid to pyruvic acid, and then to acetaldehyde and ethyl alcohol. The formation of these products suppresses the activity of the "malic" enzyme, which leads to a decrease in the intensity of respiration in the postclimacteric period, changes in the structure of the cytoplasm and cell organelles, and as a consequence to senescence of fruits [7].

3.2 Mono and disaccharides

The content of mono- and disaccharides in fruits reduces during storage, as they are used for respiration of fruits and maintenance of cell structure [8].

Figure 2 demonstrates kinetic curves of changes in the total content of mono- and disaccharides in apples of autumn varieties during cold storage using track membranes. The total amount of sugars in fruits gradually decreases due to its consumption on respiration, with the exception of a first period, when, as a result of hydrolysis of starch, the amount of sugars may remain the same or increase. During storage, the fructose content increases, while the sucrose and glucose content decreases. In this regard, despite the fact that the total sugar content decreases, the taste of the fruit becomes sweeter.
3.3 Organic acids

Along with mono- and disaccharides, the taste of fruits is largely determined by the presence of organic acids. We also obtained kinetic curves of changes in the content of organic acids in apples of autumn varieties during cold storage using track membranes (Figure 3).

As follows from the data, the content of organic acids in all samples decreases, since, along with carbohydrates and other compounds, organic acids are easily involved in redox reactions during fruit respiration [8].

3.4 Ascorbic acid

Many apple varieties differ by a high content of such an important biologically active substance as ascorbic acid. However, during storage, ascorbic acid is easily oxidized to the reversibly oxidized form of dehydroascorbic acid, and then to its inert form - diketogulonic acid. The conversion rate of this acid significantly depends not only on the type, but also on temperature, gas composition and storage duration [9]. We investigated the changes in the content of ascorbic acid depending on the duration of storage in a refrigerated state of apples of autumn varieties.
Figure 4. Changes in the content of ascorbic acid in autumn varieties of apples during cold storage.

As shown in Figure 4, the amount of ascorbic acid decreases in all varieties of apples, both in control and in experimental samples. This is due to the fact that when donating protons, ascorbic acid takes part in many reduction reactions, and its reducing properties are enhanced by the action of the enzyme ascorbate oxidase. As a functional group, ascorbate oxidase contains copper (Cu), therefore it is sensitive to the action of agents that inhibit enzymes.

All the obtained dependences of changes in the content of mono- and disaccharides (Cmd), organic acids (Coa) and ascorbic acid (Caa) during cold storage of control and experimental apple samples of the studied varieties were approximated by the following equations:

- **Grushovka Yudicheva**:
  1) \(\text{Cmd} = 0.037t^3 - 0.39t^2 + 0.28t + 12.79; R^2 = 0.996\) (Control); \(\text{Cmd} = 0.027t^3 - 0.34t^2 + 0.42t + 12.79; R^2 = 0.995\) (GSTM)
  2) \(\text{Coa} = -0.016t^2 - 0.11t + 1.61; R^2 = 0.97\) (Control); \(\text{Coa} = -0.031t^2 + 0.046t + 1.45; R^2 = 0.992\) (GSTM)
  3) \(\text{Caa} = 0.085t^2 - 1.01t + 12.4; R^2 = 0.92\) (Control); \(\text{Caa} = 0.042t^2 - 0.58t + 12.4; R^2 = 0.964\) (GSTM)

- **Kordonovka**:
  1) \(\text{Cmd} = 0.062t^3 - 0.65t^2 + 0.63t + 10.98; R^2 = 0.995\) (Control); \(\text{Cmd} = 0.045t^3 - 0.53t^2 + 0.72t + 10.98; R^2 = 0.981\) (GSTM)
  2) \(\text{Coa} = -0.029t^2 - 0.023t + 1.53; R^2 = 0.996\) (Control); \(\text{Coa} = 0.029t^2 - 0.014t + 1.54; R^2 = 0.995\) (GSTM)
  3) \(\text{Caa} = -0.008t^3 + 0.12t^2 - 0.85t + 10.3; R^2 = 0.999\) (Control); \(\text{Caa} = -0.001t^3 + 0.043t^2 - 0.65t + 10.3; R^2 = 0.988\) (GSTM)

- **Pepin Shafranny**:
  1) \(\text{Cmd} = 0.041t^3 - 0.44t^2 + 0.47t + 10.99; R^2 = 0.999\) (Control); \(\text{Cmd} = 0.043t^3 - 0.44t^2 + 0.58t + 10.99; R^2 = 0.998\) (GSTM)
  2) \(\text{Coa} = 0.017t^2 - 0.22t + 1.29; R^2 = 0.997\) (Control); \(\text{Coa} = 0.01t^2 - 0.17t + 1.32; R^2 = 0.958\) (GSTM)
  3) \(\text{Caa} = 0.064t^2 - 1.26t + 11.9; R^2 = 0.976\) (Control); \(\text{Caa} = 0.035t^2 - 1.06t + 11.9; R^2 = 0.98\) (GSTM)

Table 1 shows the rate constants of the reactions for changes in the content of mono- and disaccharides (Kmd), organic acids (Koa) and ascorbic acid (Kaa) of the pseudo-first order. As seen, the reaction rates in the experimental samples are lower than in the control that allows one to preserve the biological value of the fruits.
Table 1. Rate constants of the reactions for changes in the content of mono- and disaccharides (Kmd), organic acids (Koa) and ascorbic acid (Kaa) of the pseudo-first order.

| Storage conditions | τ, Month | Kmd, 1/month | Koa, 1/month | Kaa, 1/month |
|--------------------|----------|--------------|--------------|--------------|
| Grushovka          | Control  | 6            | 0.066        | 0.121        | 0.037        |
|                    | GSTM     | 6            | 0.059        | 0.079        | 0.021        |
| Yudicheva          | Control  | 6            | 0.131        | 0.252        | 0.053        |
|                    | GSTM     | 6            | 0.101        | 0.225        | 0.045        |
| Kordonovka         | Control  | 6            | 0.075        | 0.129        | 0.064        |
|                    | GSTM     | 6            | 0.053        | 0.103        | 0.062        |
| Pepin              | Control  | 6            | 0.075        | 0.129        | 0.064        |
|                    | GSTM     | 6            | 0.053        | 0.103        | 0.062        |

4. Conclusion
We have revealed that the maximum preservation of the apples quality, nutritional and biological value is achieved with the storage at \( t = (3 \pm 1) ^\circ C \) for varieties Grushovka Yudicheva, Kordonovka and Pepin Shafranny. Based on the results obtained, we recommend the use of a membrane areas of 22.0, 18.0 and 14.0 cm\(^2\)/kg, respectively. In this case, applying a gas-selective composite membrane with a selectivity of 5.0, a subnormal gaseous medium of the following composition is formed: oxygen concentration - (5.2 \pm 0.1\%), carbon dioxide concentration - (3.6 \pm 0.1\%).

It has been proved that during storage of apples of autumn varieties, the changes in the content of ascorbic acid, mono- and disaccharides and organic acids depend on the variety of apples, storage duration, gas composition and membrane area.

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