Heliyon 9 (2023) e17740

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Preservation of black grapes by isochoric freezing

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ARTICLE INFO

Keywords:
Vitis vinifera L.
Quality
Constant volume
Isobaric
Subfreezing storage

ABSTRACT

Fruits are perishable. It's crucial to have an efficient preservation technique that may extend storage duration while maintaining the physical quality and nutritional values to avoid wastage. The majority of long-term storage solutions for fruits use refrigeration. In this study, we evaluate the potential of isochoric freezing as an alternative method of preservation for black grapes (*Vitis vinifera* L.). We compare the properties of black grapes preserved for 7 days in trehalose solution at −4 °C in isochoric conditions (average pressure 34.2 MPa) with those of fresh black grapes and with grapes preserved isobarically in four conditions (room temperature, in the fridge, in the freezer, and in a plastic bag filled with trehalose solution). The results indicate that grapes preserved by isochoric freezing at temperatures below the freezing point of water do not lose weight; on the contrary, they resulted in a very small (2%) weight gain. Freezing under isochoric conditions did not result in significant changes in terms of macroscopic appearance, colour, firmness, °Brix values, or pH. We consider that isochoric freezing has the potential to be used as a preservation method for grapes while maintaining physicochemical parameters similar to those of fresh fruits.

1. Introduction

Grapes, or *Vitis vinifera* L., are one of the oldest fruits that humans have grown and eaten. Known as the "home of grapes," the region of origin is the Middle East, south of the Caucasus, from the Black Sea to the Caspian Sea region of Iran [1], where their cultivation began 6000–8000 years ago. Today, these fruits are still very popular, and the amount they produce puts them in fourth place in the world [2]. About 75% of the world's grapes are used to make wine, according to the Food and Agricultural Organization (FAO). The other 23% are eaten fresh, and only 2% are processed. Fresh grapes have important nutrients for the body that can boost the immune system, prevent cancer, lower blood pressure, protect against heart disease, lower high cholesterol, and slow the aging process [3]. Vitamin C, vitamin K, B vitamins, minerals (potassium, calcium and magnesium), antioxidants, phytonutrients including resveratrol [4], phenols, polyphenols, and carotenoids, fibers and vitamin K [5] are the primary nutrients responsible for these effects. Without a doubt, grapes are at their healthiest when they are eaten fresh and in their natural state.

Table grapes are becoming more popular, but they are hard to store because they have a high-water content and a soft texture [6]. Under room-temperature conditions (20–25 °C), grapes have a short shelf life [7]. The most common short-term preservation methods

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https://doi.org/10.1016/j.heliyon.2023.e17740
Received 3 February 2023; Received in revised form 20 June 2023; Accepted 27 June 2023
Available online 4 July 2023

for fresh grapes are basket storage at room temperature or cold storage in the fridge. For the long term, it is impossible to preserve fresh grapes without a chemical treatment [8]. The household waste of fresh fruits and vegetables in EU countries is almost 50% [9]. The European Commission established that food waste is one of the priority areas of the Action Plan for the European Circular Economy Strategy [10], which is why we consider it important that this method of preservation offers quality for the fruit as close as possible to the natural one immediately after it is picked for the out-of-season period. The freezing point of most grapes is around −1.5 °C. According to studies, it has been shown that physiological activity decreases in grapes at the temperature of melting ice, but their metabolism remains at a normal level [11].

The results in isochoric preservation suggest that it may be practical and safe to preserve biological matter in isochoric chambers at temperatures down to −4 °C without altering the composition of the preservation medium and with limited concern over kinetic ice nucleation due to exterior perturbations [12]. We demonstrated that the energy required to preserve a certain quantity of biological matter in an isochoric freezer at −5 °C is one third of the energy required to preserve the same quantity in a conventional isobaric freezer at 5 °C. Furthermore, the quality of the food preserved in an isochoric system will be superior to that preserved in an isobaric system [13].

A different approach was made with the ice-temperature-high-humidity (ITHH) preservation technique. The temperature and humidity were maintained at −1 °C–0 °C and 95%, respectively. Thereby is avoided the destruction of the fruit's texture due to the absence of ice-crystal formation, and high humidity prevents the fruit from dehydrating [14]. The findings revealed that no notable changes occurred during the 60-day preservation period; the texture and flavor were very similar to fresh fruits [14]. The already known and used ITHH procedure is a method that has proven its effectiveness, but it has a disadvantage in terms of applicability. This type of preservation works very well in the case of grapes, which are more resistant by nature to low temperatures, even negative ones.

The technique that we are studying in this case for grapes is applicable on a large scale and can be used in the case of other foods as well, as has been demonstrated in other works [15–19]. In the following, we will compare different conventional methods for preservation with a new technique of preservation at subfreezing temperature (−4 °C), using the isochoric principle, without the tissue damage caused by the formation of ice crystals. In 2016, Năstase et al. [20] introduced a mathematical model to describe the process of freezing in isochoric thermodynamic systems. They demonstrate that the use of isochoric freezing can likely result in improved quality frozen foods as well as substantial energy savings [20]. Also, Năstase et al. [21] presented results on Tilapia muscle tissues frozen at −5 °C in an isochoric system, and the tissues appeared morphologically identical to fresh tissue with no evidence of dehydration [21]. Using the same technique, sweet cherries were preserved by isochoric freezing at −4 °C. After preservation, they had similar quality and nutritional properties to those of fresh cherries. Isochoric freezing at −4 °C preserved the texture of cherries and minimized drip loss. Also, isochoric freezing at −4 °C preserved the ascorbic acid content, phenolic compounds, and antioxidant activity in cherries since cell compartmentalization remained intact during freezing [22].

Recently, Cristina Bilbao-Sainz et al. [23] used isochoric supercooling of whole pomegranates at −2.5 °C and the technique, maintained aril quality in terms of mass, appearance, colour, and texture properties. Isochoric supercooling was also useful in maintaining characteristics that contributed to organoleptic quality, such as TSS, TA, and pH [23]. Encouraged by these previous findings, we set out to evaluate the potential of isochoric freezing in preserving black grape quality.

Therefore, the main purpose of this investigation is to identify a novel method of postharvest preservation to maintain grape berry quality characteristics for longer. If they succeed, the fruits are suitable for extended shelf-life and long-distance transportation. This would be highly helpful for improving consumer appeal in grape berries, making them suitable for export and distant markets free from quality deterioration. Most commercial requirements are one to two months of storage to reach distant markets [24]. The potential of fruit preservation by isochoric freezing has already been evaluated for cherries [22], for tomatoes [25], and for pomegranate [23], and the advantages of long-term food storage in the frozen state by the isochoric concept have been evaluated in the preliminary analysis of Năstase et al. [20].

Consequently, in this work, we evaluate the physicochemical parameters of thawed grapes frozen under isochoric circumstances in comparison to other five traditional conservation techniques in order to determine the potential of isochoric freezing for the preservation of black grapes.

2. Materials and methods

2.1. Fruit material

Fresh black table grapes (2–6 g/ripe black grape berry) were obtained from a local supermarket in Brasov, Romania. The grapes used in this study are a variety of local origin named Moldova. The clusters of the studied variety have a cylindrical or conical shape, weighing about 400 g in average. The berries are large and egg-shaped, of a violet-blue colour, and covered with a thick but tender coating. The samples were procured half an hour before being processed on the same day. The samples were kept at +5 °C pending further sample processing. All the table grapes used in these experiments were in the same condition as far as colour, maturity, size, and hardness since they were part of the same batch. Grape maturity is often expressed as a proportion of the fruit's potential sugar content. The selected grapes used in these trials fall within the range of grape ripeness, which is between 16% and 25% Brix, with greater levels indicating a higher sugar content.

The experimental units are randomly assigned to treatments, resulting in a completely randomized design. We utilized 30 berries for 1 replicate, equally distributed among all 5 treatments. Each treatment was performed in three replicates.

2.2. Isochoric system

A high-pressure reactor makes up the isochoric system, model OC-1, from High Pressure Equipment Company (Erie, PA, USA), and a pressure transducer manufactured by ESI Technology GD4200-USB in the United Kingdom for a range of 0–72,519 PSI, or 0–500 MPa, is used to measure the inside pressure. The high-pressure reactor, as can be observed in Fig. 1A, the high-pressure reactor is made of 316 stainless steel and has an internal volume of 125 ml and a one-inch inner diameter. The outside diameter of the reactor is 3″ 1/5 and the height is 6″; it is designed for a maximum working pressure of 20,000 PSI or 137.89 MPa. The reactor has a 316 stainless steel cover equipped with a Buna-N elastomer O-ring, suitable for applications with negative temperatures down to −40 °C. The O-ring provides a good seal between the reactor and the cover. Closing the reactor is done using an alloy steel cap, threaded onto the isochoric reactor.

In this experiment, we make use of a technique and method that we also used in Refs. [21,26]. For ease of understanding, the system and steps are also detailed here.

2.3. Experimental setup - equipment

In the current experiment, we used the isochoric chamber previously presented, connected to a pressure transducer, to measure the inside pressure. We connected this pressure transducer via ESI-USB Dynamic software to a laptop (ProBook 6 6570b, Hewlett Packard Enterprise, India) on which we visualize, store, and export data. The temperature is measured using two PerfectPrime TL0024 T-type thermocouples (2 m long, specific for high accuracy measurements in the refrigeration and cryogenics fields, with excellent repeatability between −200 °C and 260 °C), connected to a to a digital thermometer (MS6514 Mastech Digital Inc, China), which is connected to the same laptop to display and record temperatures. To limit the influence of ambient temperatures and cover the pressure transducer, we used polyethylene pipe insulation that was fastened with adhesive electrical tape. We advise using a safety head with a rupture disc at a pressure of 250 MPa for added security. To perform the measurements, the assembly described above is immersed in a cooling bath that uses ethylene glycol, which is recommended for temperatures down to −30 °C. The temperature is controlled by a cooling device, (RE 1225 S, LAUDA DR. R. WOBSER GMBH & CO. KG, Germany), which can lower the temperature to −25 °C within the time and temperature limits set in the experimental protocol. The cooling bath is connected to an external insulated container,

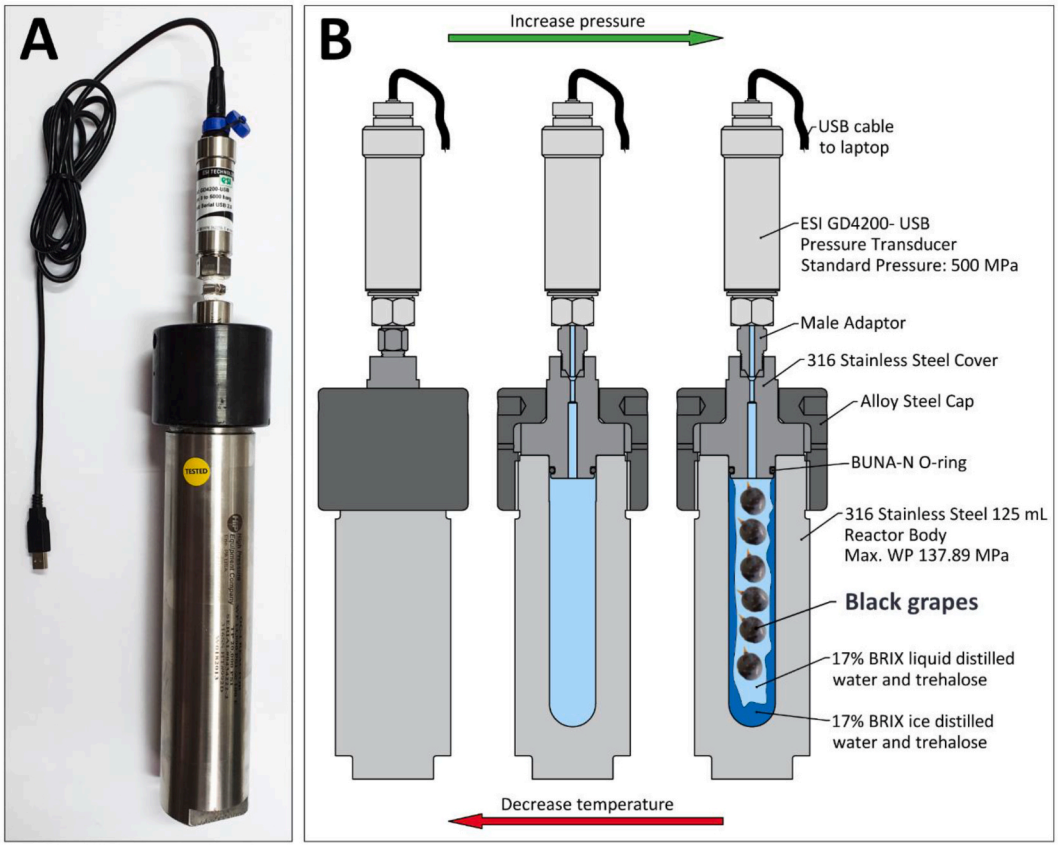

Fig. 1. (A) Photograph of the isochoric system, consisting of the high-pressure reactor, the pressure transducer, and the USB cable for connection to the laptop. (B) Illustration of the grape preservation process using the isochoric reactor and generalized freezing.

which can provide the necessary volume for the isochoric system. The externally insulated container has an internal volume of 50 L, a diameter of 190 mm, a height of 400 mm, and is made of PVC. The bath is insulated with a 9-mm elastomer self-adhesive insulation sheet. We used an external insulated container because the commercial cooling bath has an internal depth of only 200 mm and our system needs at least 350 mm. The experimental setup with all the components is presented in Fig. 2, with an open-view in the external cooling bath in Fig. 2A and a photograph of the grape's isochoric preservation experiments setup in Fig. 2B.

2.4. The experimental protocol

We measured the brix of ten fruits from the lot and found an average of 19% brix, and for the experiments, we created a 19% brix solution made of trehalose powder (SOSA Ingredients S.L., Spain) and distilled water by steam (from European Drinks). The isochoric system was filled with the trehalose solution; we added six black grape berries inside and then sealed the reactor. Grape berries were kept in the 19% Brix trehalose solution for a day at 5 °C; there was no mass change, indicating that the solution was in osmotic equilibrium with the grapes. Five different methods to preserve fresh black grape berries for up to seven days: at room temperature +21 °C and 44%RH (our laboratory conditions – isobaric); in the fridge (isobaric); at +3 °C and 65%RH; in the freezer (isobaric); at −21 °C; isobaric at −4 °C in a LDP plastic bag; in the cooling bath; and isochoric at −4 °C in the isochoric system. For all preservation methods, we used six black grape berries. For the fruits preserved in the LDP plastic bag, we used the same 19% brix trehalose solution. To keep the LDP bag immersed in the cooling bath, we used a screw inside a plastic bottle, which we tied to the bag, as can be observed in Fig. 2(A). The isochoric reactor was entirely submerged in the externally insulated container with a recirculating cooling fluid of −4 °C after being sealed and closed. The temperature difference between the cooling bath and the externally insulated container was 1 °C for the duration of the experiments. This way, for the isochoric preservation method, the temperature was −4 °C and in the cooling bath, −5 °C. The preservation temperature for this study was chosen based on preliminary literature data in Refs. [27–29], where the storage temperature recommended for table grapes is between −1 °C and +1 °C. In addition, the recommended relative humidity for the preservation of fresh grapes ranges between 90% and 95% [30]. Based on this information and considering raw grapes are 70–80% in water [31] at ripeness, we chose a temperature of −4 °C, just to be under the freezing point of water. In the isochoric reactor, lower freezing temperatures lead to higher pressure. The average pressure at the preservation temperature of −4 °C was 43.8 MPa. The cooling bath was manually adjusted to 5 °C at the end of each experiment, and the reactor was then placed in a water bath at room temperature for 10 min before being opened. Three replicates for each treatment were tested.

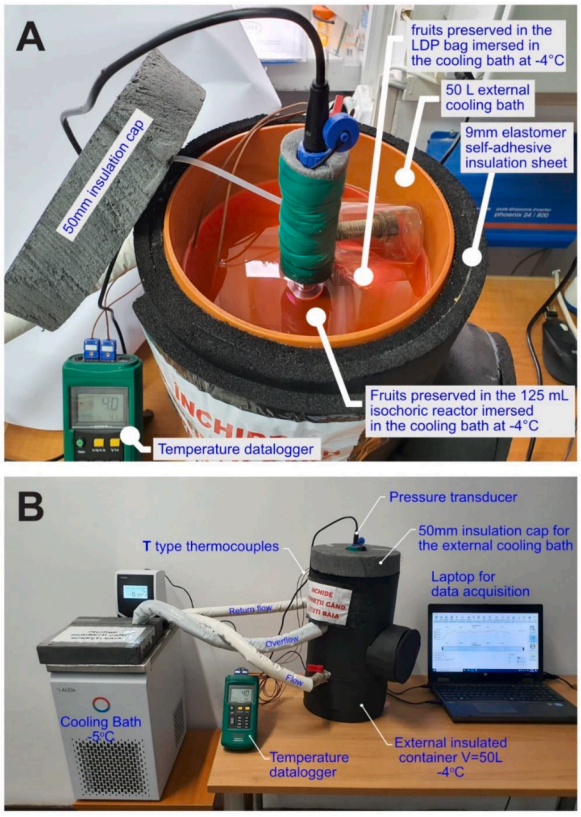

Fig. 2. Open-view in the external cooling bath (A); Photograph of the grape's isochoric preservation experiments setup (B).

2.5. Weight loss

For the preservation method used in this study weight loss were determined. The black grape berries had an initial weight of 2.23 ± 0.33 g. The weight loss of the grapes was determined gravimetrically using a digital weight MH-200 pocket scale, with a scale range from 0.01 to 200 g (MH-200, Guangzhou Juheng Electronic Co., Ltd., China).

2.6. Firmness

Some samples in these experiments were processed to observe the firmness changes. Mechanical tests were performed with a portable penetrometer model GY-1 (VTSYIQI, CN), with a range of 2–15 kg/cm^2. The grapes were squeezed using a 50-mm-diameter circular flat plate and a 3.5-mm-diameter stainless steel cylinder probe to 50% deformation in a single compression-decompression cycle at a constant speed of 0.1 mm/s. The force at which the grapes were crushed was found to be the fracture force.

2.7. Brix percentage

The sugar quantity in the processed fruits was measured with a portable refractometer (0–90%, Milton Industries Inc, China). The higher the brix value, the sweeter and tastier the fruits are. We calibrated the instrument with distilled water. The value is indicated in degrees Brix.

2.8. pH measurement

To measure the pH of the fruits, we used a portable pH meter from Testo (Testo 206-pH1, Testo, RO). The pH electrode has a measurement domain of 0–14 pH and a precision of 0.02 pH at a resolution of 0.01 pH. To measure the pH, each grape was cut in half using a scalpel, and the actual measurement was made by inserting the electrode of the pH meter directly into one of the two halves. Six grapes were tested for each group of conditions presented in this study.

2.9. Colour

The surface colour of the grapes was measured using a colorimeter with a highly sensitive photodiode as a colour sensor (PCE-XXM 20, PCE Holding GmbH, Germany). Six black grape berries were used as the test subject. The measurements were made using CIELAB colour scales, where: the L*scale ranges from no reflection (L* = 0, black) to perfect diffuse reflection (L* = 100, white); the a* scale ranges from negative values for green to positive values for red, and the b* scale ranges from negative values for blue to positive values for yellow.

The distinction between the colour of preserved fruit and its original colour of the fresh sample is measured by Delta E levels. Greater precision is shown by lower Delta E values, whereas a large mismatch is indicated by higher Delta E values.

According to the following formula, ΔE* was determined:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}\# \quad (1)$$

Before measurements, each grape has been wiped of the oleanolic acid from its outer surface.

2.10. Statistical analysis of data

All underwent an analysis of variance, and Tukey's test was used to determine the significance of any differences between treatments. For each parameter, analysis of variance (ANOVA) was carried out at a 5% threshold of significance (significance levels were tested at $P \leq 0.05$). Data is presented as mean values ± standard deviation. For all statistical analyses, Minitab version 19 Statistical software was used (Minitab Inc., State College, PA, USA). The data obtained in this study were not checked for normality and homogeneity of variants.

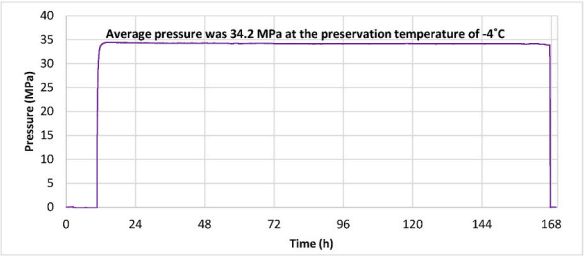

Fig. 3. This figure shows the pressure measurement over time (7 days) during the black grape preservation experiment in the isochoric system. During the experiment, the pressure transducer in Fig. 1A was used to measure the pressure in the isochoric chamber.

3. Results

In Fig. 2, the complete isochoric preservation setup is presented. Besides the isochoric reactor and the pressure transducer, it can be observed that for cooling the system we used a cooling bath, and to analyze and store the temperature and pressure data we used a laptop. The pressure transducer in Fig. 1A was used to collect data during a 7-day experiment on the preservation of black grape berries, which led to Fig. 3. It displays a typical graph that shows how the pressure changes over time as our experiments are isochronally frozen at a temperature of −4 °C. The pressure reaches a steady state and maintains that value for the full seven days of the experiment, which is an interesting feature. This shows that the thermodynamic equilibrium of the isochoric system has been achieved. Evidently, the thermal mass of the device and the heat transfer coefficient to the cooling fluid determine how long it takes to reach equilibrium. The average pressure at equilibrium was 34.2 MPa. In all of our trials, the samples attained isochoric thermodynamic equilibrium, and the states of the treated materials after achieving thermodynamic equilibrium are represented by our data.

3.1. Weight loss

In the food sector, weight loss during storage is a key concern. It happens when any food product is preserved, including grapes. The maximum allowable limit during storage is 5% mass loss; anything more than that renders the fruits ineligible for sale [32].

Isobaric freezing, which involves freezing a substance while maintaining constant pressure, can potentially affect the weight loss of black grapes. During freezing, the water inside the grapes freezes and expands, which can cause damage to the grape cells and cell walls. This can result in the release of water and other compounds from the grapes, leading to weight loss. Isochoric freezing can minimize the damage to the grape cells and cell walls, resulting in less weight loss compared to other freezing methods.

Fig. 4 compares the weight loss before and after seven days of preservation with five different methods, as described in the experimental protocol. In contrast to preservation at ambient temperature, the graph shows that none of the four preservation techniques that entailed preservation at near-negative temperatures ($<\pm 3\%$) resulted in a statistically significant change in weight. Keeping the fruits at room temperature in isobaric storage is not a good option since the weight loss was an average of 22% or more. The grape berries shrink because they lose water due to xylem backflow and transpiration [33]. The water loss in the fruits preserved at room temperature is due to the high temperature and dry air in the room. The patterns of weight loss seen in this study are in alignment with the findings of numerous other studies [19,24,29,34–36]. We believe it is a significant finding in the realm of food preservation because grapes preserved by isochoric freezing at temperatures below the freezing point of water do not lose weight. Furthermore, the isochoric preservation of the black grape berries resulted in a very small (2%) mass gain, which is most likely attributable to the trehalose solution. The same behavior we can observe also in the isobaric preservation of the black grape berries in the LDP bag, where the same solution was used, only that the gain in mass is smaller ($<1\%$). Water in grapes is a solvent and determines the concentration of all important compounds (acids, sugars, phenolics, etc.) that are essential to overall fruit quality. Therefore, it is difficult to undervalue the impact of grape water content on the quality and commercial viability of grapes.

However, the extent of weight loss during freezing of black grapes will depend on various factors such as the initial moisture content of the grapes, the freezing temperature and time, and the storage conditions after freezing. Factors such as dehydration and the loss of volatile compounds can also contribute to weight loss during frozen storage.

3.2. Firmness

Keeping grapes fresh between when they are picked and when they are eaten depends on a number of things of grapes is a quality attribute for the consumer and is mainly indicated by their firmness. A firm grape is considered a sign of longer storage times and increases the possibility that those fruits reach the market in optimal conditions. Grapes lose their firmness due to water loss or structural changes [37]. The measured firmness is the maximum force needed when penetrating a probe into the fruit to a standard

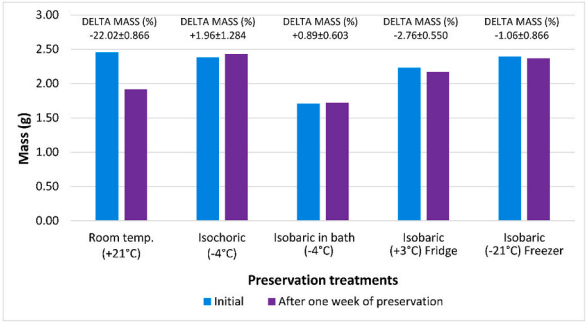

Fig. 4. Black grapes lose weight after being preserved for seven days using five different techniques. By comparing the samples' weights before and after the preservation procedure, the weight loss was calculated. Before weighing, filter sheets absorbed the surface water from the samples. Six samples were weighed after each preservation technique to determine the average weight loss. Standard deviations ± from the mean are referred to as delta mass (%).

depth. The effect of preservation using five different treatments on the black grape berries' firmness is presented in kg/cm^2 in Table 1. Because dehydration has softened the interior, grapes at room temperature have a higher firmness (type I error). When the penetration device meets the elastic skin, without the support of the dehydrated interior pulp, the outer tissue expands and increased force is applied to the penetration, resulting in the fruit being practically squashed.

Grapes are vulnerable to the drying effect of air due to their relatively high ratio between surface and volume. Without enough water, the density of the grape berries decreases, they lose their turgidity, and then wither. Contrarily, it can be observed that grapes kept at room temperature have the highest firmness among those studied. This fact is mainly caused by massive weight loss as a result of dehydration. Thus, in the firmness test, the skin of the fruit, having elasticity, breaks harder without the support of the dehydrated pulp inside, thus resulting in the highest firmness value.

3.3. Macroscopic appearance

During conventional freezing, the expansion of ice crystals can cause damage to the grape cells and cell walls, resulting in a loss of structural integrity and a change in the macroscopic appearance of the grapes. For example, the grapes may become mushy, discolored, or develop ice crystals on the surface.

In Fig. 5, the macroscopic appearance and colour of the fruits can be observed after one week of preservation with five methods, compared with the same fresh fruits before preservation. The first crucial characteristics that influence a consumer's decision about whether to accept or reject a product are appearance and colour. As a result, they are some of the fruit industry's highest-quality characteristics [38]. As can be observed, the fruits preserved at room temperature had the most advanced decaying process, with a molded and spoiled aspect (Fig. 5A′). This happens because many harmful bacteria start to grow at room temperature because of their juicy and sweet taste. The fruits preserved under isochoric conditions (Fig. 5B′) have a similar aspect if compared to those preserved in the plastic bag under isobaric conditions at −4 °C (Fig. 5C′) and with those preserved in the fridge (Fig. 5D′). The fruits from the freezer (Fig. 5E′), a few seconds after removing them from the freezer, have a dull colour or frosty look, and four of the six grape berries cracked, due to the expansion of the water in the fruit.

Isochoric freezing can help to minimize the formation of ice crystals, which can help to preserve the macroscopic appearance of the grapes. The constant volume during isochoric freezing can also help to protect the structural integrity of the grape cells and minimize the growth of ice crystals on the surface and particularly inside the grapes.

However, a number of variables, including the initial aspect of the grapes, the freezing temperature and time, and the storage conditions after freezing, will determine how well isochoric freezing can preserve the macroscopic appearance of black grapes. Factors such as dehydration, oxidation, and enzymatic reactions can also contribute to changes in the macroscopic appearance of frozen grapes during storage. Proper packaging and storage conditions can help to minimize these effects and preserve the macroscopic appearance of the grapes.

3.4. Effect of freezing on brix (%)

The brix percentage did not change significantly due to freezing, regardless of the preservation method. In fact, it seems that the presence of cold causes the sugar level to drop in the fruit. In the case of fruits left at room temperature, it can be observed that the sugar level has increased significantly, with an average of 29 ± 6.58%. This leads us to the conclusion that the presence of cold slows down the metabolic activity at the cellular level, which allows the preservation of the fruits. The percentage (% weight) of sugar in the grape juice is equivalent to a brix degree (°Bx) when measured at 20 °C.

As can be seen in Fig. 6, there are no discernible differences in terms of °Brix (related to sweetness) between the grapes maintained in isochoric conditions, if compared with the fresh fruits and with those preserved in the freezer. The °Brix value in cases of isochoric preservation had an average of 4.35% ± 6.99 lower than the fresh fruits, indicating that the 19% trehalose solution was in osmotic equilibrium with the black grape berries.

In summary, isochoric freezing can potentially help to preserve the Brix degree of black grapes by minimizing the loss of grape juice and soluble sugars during freezing, but the extent of preservation will depend on various factors and should be evaluated on a case-by-case basis.

Table 1
The firmness outcomes after subjecting black grape berries to five different preservation methods. The same letter indicates no statistically significant (not different at $p \leq 0.05$) differences between treatments at a 95% confidence level, with Tukey's test.

Treatment	Mean Firmness [kg/cm^2][a]
Fresh black grape berries	4.36 ± 0.27^{a}
Room temperature [+21 °C], after 7 days	7.65 ± 1.30^{b}
Isochoric preservation at −4 °C, after 7 days	3.71 ± 1.31^{a}
Isobaric preservation at −4 °C, after 7 days	3.99 ± 0.77^{a}
Isobaric preservation at +3 °C (in the fridge), after 7 days	3.53 ± 0.33^{a}
Isobaric preservation at −21 °C (in the freezer), after 7 days	N/A

[a] The values for firmness are mean values, at a 95% confidence level with Tukey's test.

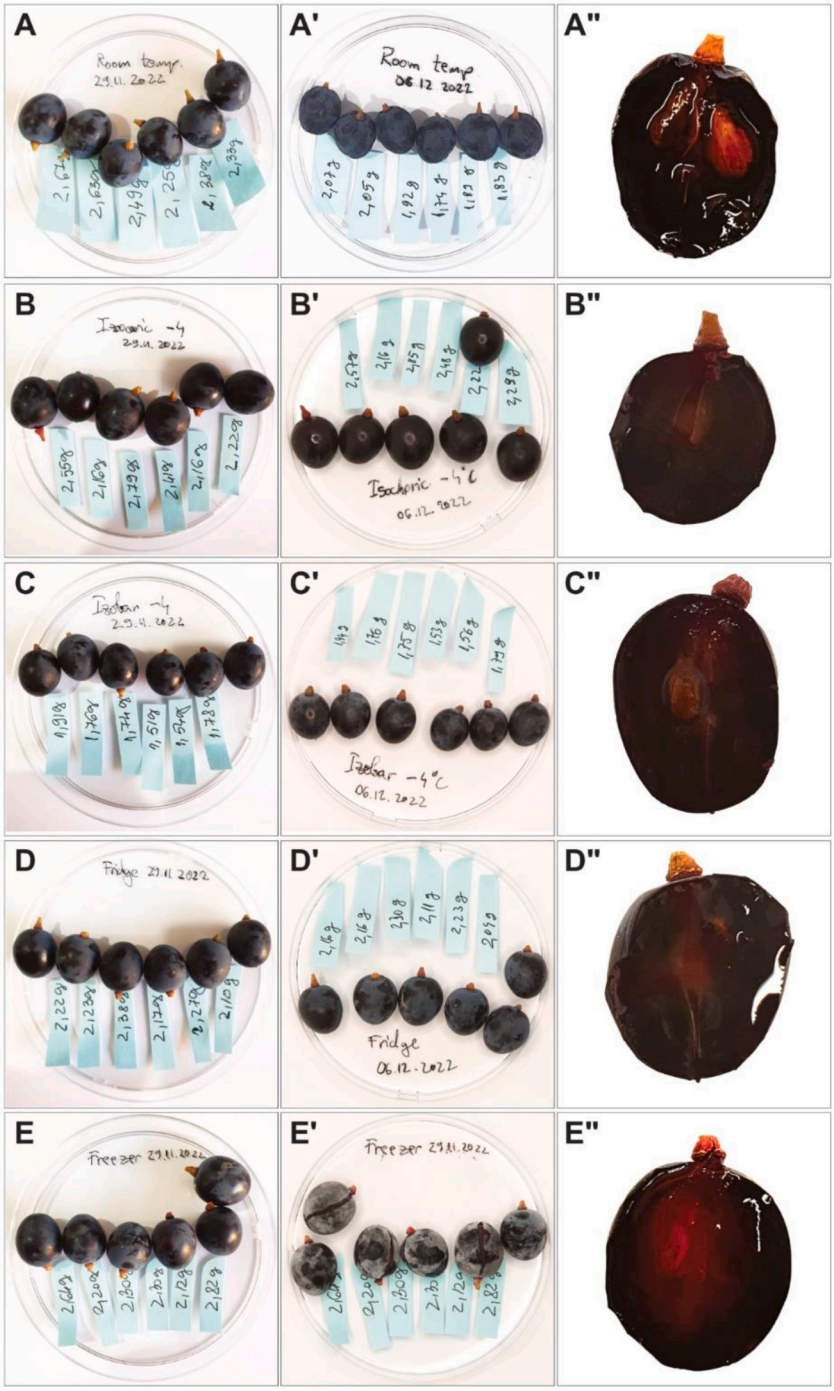

Fig. 5. Black grapes used in the experiments. Fresh fruits are on the left side, followed by the samples for preservation at room temperature (A), the samples to be preserved in the isochoric system in the same isotonic solution (B), the samples to be preserved in an isobaric condition in an isotonic trehalose solution in a plastic bag (C), the samples to be preserved in the fridge (D), and the samples to be preserved in the freezer (E). The photographs from the middle (A′–E′) present the same samples after seven days of preservation using the methods described earlier. The photographs from the right side (A″–E″) present a cut view for the same samples after seven days of preservation with the methods described earlier.

3.5. Effect of freezing on pH

Changes in pH (related to acidity or basicity) during the freezing of black grapes under isochoric and four isobaric conditions, compared with initial values and the fruits kept at room temperature for seven days, are shown in Fig. 7. The values of pH for all

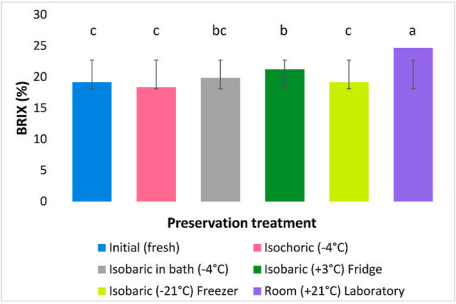

Fig. 6. Effect of freezing under five different methods on Brix value (%). The same letter indicates no statistically significant (not different at $p \leq 0.05$) differences between treatments at a 95% confidence level. Six samples were measured for each preservation technique to determine the average Brix value (%).

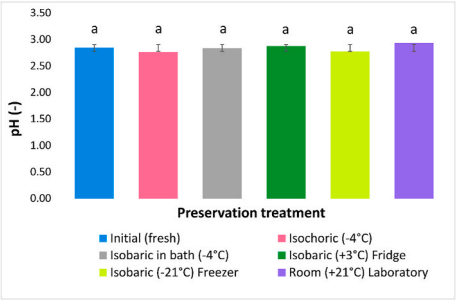

Fig. 7. Effect of freezing under five different methods on pH value. The same letter indicates no statistically significant (not different at $p \leq 0.05$) differences between treatments at a 95% confidence level. Six samples were measured for each preservation technique to determine the average pH value.

treatments did not change significantly due to freezing, but it can easily be observed that the standard deviation in the case of isochoric preservation is the smallest if compared to the fresh fruits. Juice and wine quality are greatly influenced by acidity and pH [39]. Another interesting observation is that most of the values for pH are under 3, which depresses the solubility of potassium bitartrate and results in precipitation. Further experimental information is required to establish the exact nature of the relation between pH and the psychochemical changes in the frozen and unfrozen phases of black grapes and their quality.

3.6. Colour

The colour of the skin is one of the most important biochemical characteristics of grapes for the market and consumers, along with flavor and taste composition. The colour of black grapes is determined by pigments such as anthocyanins [40] and flavonoids, which can be sensitive to changes in temperature and other environmental factors. During conventional freezing, ice crystal formation can cause damage to the grape cells and cell walls, leading to the release of pigments and changes in the colour of the grapes.

The colour attributes of fresh black grape berries compared with five treatments over seven days are presented in Table 2. The lightness (L*), greenness (a*), and yellowness (b*) of fresh black grape berries were determined to be 26.18 $\pm$ 0.26, 8.30 0.50, and 5.94 $\pm$ 0.42, respectively. For each particular parameter, analysis of variance (ANOVA) was carried out at a 5% threshold of significance. The preservation method is significant for all colorimetric characteristics, according to the statistical analysis ($P \leq 0.05$), indicating that at least one group differs from the other five groups.

To compare preservation methods at the same level of significance, Tukey's test was also run for each colorimetric coordinate. After applying the comparison approach outlined above, we can say that the fruits that were stored in the room's temperature and humidity conditions showed noticeable changes in terms of colour.

To better visualize data for all samples, Fig. 8 illustrate the box plots for L*, a*, b* and ΔE^* by showing them as a general group.

4. Discussion

Isochoric preservation is simple and requires a high-pressure resistant container, a pressure transducer, and a cooling system. Fig. 1A depicts the isochoric device's schematic with all the components that were used in our experiments. Control over the isochoric preservation is done either by pressure or temperature to completely specify the system. As can be observed in Fig. 1B, in an isochoric system at equilibrium, we have the inside solution in a two-phase state of liquid and ice in a closed, constant volume. The high-pressure reactor is a 316 stainless steel commercial pressure vessel covert with a cylinder made from the same material, and then capped by an

Table 2
The colorimetric values, CIE-L*a* b*, of black grape berries subjected to five distinct preservation techniques[a].

Treatment	L* [Lightness]	a* [red/green]	b* [blue/yellow]	ΔE^*_{ab}
Fresh black grape berries	25.92^{B}	8.82^{B}	5.90^{A}	N/A
	26.20^{B}	7.97^{B}	6.04^{A}	N/A
	25.97^{B}	8.11^{B}	6.12^{A}	N/A
	26.01^{B}	8.93^{B}	5.12^{A}	N/A
	26.47^{B}	8.32^{B}	6.18^{A}	N/A
	26.51^{B}	7.62^{B}	6.25^{A}	N/A
Average ± STDEV	26.18 ± 0.26	8.30 ± 0.50	5.94 ± 0.42	N/A
Room temperature (isobaric at +21 °C) after 7 days	30.31^{A}	3.63^{D}	3.88^{B}	6.57^{A}
	24.08^{A}	7.38^{D}	3.56^{B}	3.30^{A}
	31.60^{A}	1.15^{D}	4.13^{B}	9.15^{A}
	27.65^{A}	1.35^{D}	4.25^{B}	7.30^{A}
	29.49^{A}	1.62^{D}	4.63^{B}	7.57^{A}
	33.16^{A}	4.59^{D}	4.81^{B}	7.99^{A}
Average ± STDEV	29.38 ± 3.20	3.29 ± 2.44	4.21 ± 0.46	6.92 ± 1.99
Isochoric preservation at −4 °C, after 7 days	23.26^{B}	6.08^{CD}	5.60^{A}	3.68^{B}
	27.18^{B}	7.59^{CD}	5.77^{A}	1.24^{B}
	25.92^{B}	3.36^{CD}	6.32^{A}	4.96^{B}
	25.59^{B}	5.31^{CD}	6.31^{A}	3.07^{B}
	25.22^{B}	6.22^{CD}	6.22^{A}	2.31^{B}
	26.01^{B}	4.93^{CD}	7.17^{A}	3.59^{B}
Average ± STDEV	25.53 ± 1.29	5.58 ± 1.42	6.23 ± 0.55	3.14 ± 1.28
Isobaric preservation at −4 °C, after 7 days	28.94^{AB}	5.73^{BC}	5.41^{A}	3.81^{B}
	29.30^{AB}	6.47^{BC}	5.74^{A}	3.62^{B}
	28.57^{AB}	6.06^{BC}	6.02^{A}	3.28^{B}
	28.41^{AB}	5.98^{BC}	5.61^{A}	3.23^{B}
	25.02^{AB}	8.49^{BC}	5.60^{A}	1.22^{B}
	28.03^{AB}	8.70^{BC}	6.00^{A}	1.89^{B}
Average ± STDEV	28.05 ± 1.55	6.91 ± 1.33	5.73 ± 0.24	2.84 ± 1.04
Isobaric preservation at +3 °C (in the fridge), after 7 days	27.87^{B}	8.01^{BC}	5.83^{A}	1.72^{B}
	26.96^{B}	4.86^{BC}	6.75^{A}	3.62^{B}
	25.56^{B}	3.47^{BC}	6.67^{A}	4.92^{B}
	26.87^{B}	5.75^{BC}	5.56^{A}	2.67^{B}
	25.78^{B}	6.64^{BC}	7.92^{A}	2.61^{B}
	24.97^{B}	7.07^{BC}	6.89^{A}	1.97^{B}
Average ± STDEV	26.34 ± 1.08	5.97 ± 1.63	6.60 ± 0.84	2.92 ± 1.18
Isobaric preservation at −21 °C (in the freezer), after 7 days	25.12^{AB}	14.98^{A}	5.35^{A}	6.79^{B}
	27.74^{AB}	12.44^{A}	5.92^{A}	4.42^{B}
	27.70^{AB}	11.39^{A}	6.16^{A}	3.45^{B}
	27.70^{AB}	10.15^{A}	5.17^{A}	2.52^{B}
	27.74^{AB}	10.59^{A}	5.49^{A}	2.81^{B}
	26.92^{AB}	10.35^{A}	8.10^{A}	3.07^{B}
Average ± STDEV	27.15 ± 1.05	11.65 ± 1.84	6.03 ± 1.08	3.84 ± 1.59

[a] The values for CIE-L*, a*, b*and ΔE* parameters are mean values, at a 95% confidence level with Tukey's test.

alloy cap.

Isobaric preservation can result in ice crystal formation, which can lead to greater damage to the grape cells and cell walls [41]. This can result in a higher degree of juice loss, which can negatively impact the Brix degree, colour, and firmness of black grapes [42].

Preservation of black grapes in isochoric conditions (constant volume) involves freezing the grapes without maintaining constant pressure. Isochoric preservation can help to minimize the formation of ice crystals and the damage to the grape cells and cell walls, which can help to preserve the quality of black grapes. Additionally, isochoric preservation can help to preserve the Brix degree and colour of black grapes by minimizing the loss of grape juice and soluble sugars, and the release of pigments, respectively.

Therefore, isochoric preservation of black grapes at −4 °C can potentially be a better option for preserving the quality of black grapes compared to isobaric preservation. Close results were reported using isochoric freezing for cherries [22], for sweet cherries [43], for tomatoes [25], for pomegranate [23] and for spinach [44]. However, the extent of preservation will depend on various factors such as the initial characteristics of the grapes, the freezing temperature and time, and the storage conditions after freezing, and should be evaluated on a case-by-case basis.

Because there is no ice formation, the degradation of the cell viability can be minimized when the black grapes are preserved at subfreezing temperatures under isochoric conditions. Even after 7 days, the isochoric sample still had a solid texture. In terms of colour, no significant differences can be seen with the naked eye because of the dark shade, however the values can be seen in Table 2. It is noteworthy that the values following isochoric treatment are those that are closest to those of fresh fruit.

Consumers value these qualities because they are correlated with notions of freshness and healthiness [44]. In contrast, isobaric freezing resulted in cell injury in black grapes due to the development of ice and an increase in solute content. The cells' ability to retain water diminished as their membranes became less stable. As a result, the grapes lost a lot of weight and their firmness when water escaped the matrix during thawing. Cryoprotectants like trehalose solution and the fruit's naturally occurring sugars may have

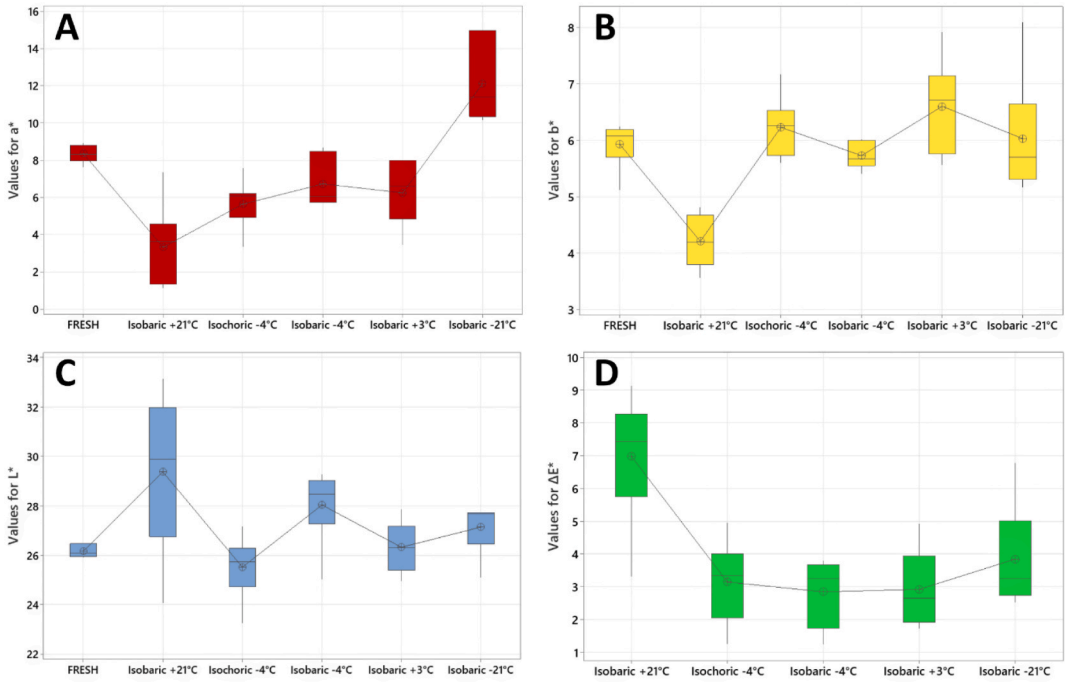

Fig. 8. Box plots of CIE Lab. The greenness a* (A), yellowness b* (B), lightness L* (C) and the total chromatic difference with respect to the fresh black grape berries ΔE* (D) are mean values, at a 95% confidence level with Tukey's test.

helped cells survive after being frozen and thawed. Hydrogen bonds between the sugar's hydroxyl groups and the polar residues in phospholipids are responsible for the membrane-stabilizing effects of sugars. This resulted in a lower freezing point for cytosol, slower ice propagation, less total water, and no membrane dehydration.

Future studies should aim to provide a more comprehensive analysis of the factors that can influence post-harvest preservation, such as plant nutrition, climatic variation during plant growth and cold management, to develop more effective and widely applicable methods for preserving black grapes. Adequate nutrient supply (nitrogen, potassium, calcium and magnesium) during plant growth and development can enhance fruit quality attributes such as colour, flavor, aroma, and texture, which can impact their post-harvest shelf life. Some key climatic factors that can impact post-harvest preservation of black grapes are temperature, humidity, rainfall, and sunlight. The use of cold storage and temperature management during transportation and storage can significantly impact the quality and shelf life of the fruit. The fresh black table grapes used in this study were obtained from a local supermarket in Brasov, Romania. It may be difficult to determine the specific agronomic management practices that were used to grow the grapes in this context. In conclusion, while the omission of key agronomic management aspects in our study, can suggest a limitation, the proposed method for post-harvest preservation of black grapes may still be valuable in certain contexts.

The results show that grapes preserved by isochoric freezing at temperatures below the freezing point of water has the potential to be used as an alternative preservation method for grapes while maintaining physicochemical characteristics similar to those of fresh fruits.

This study offers a first-in-field representation of isochoric freezing's ability to preserve black grape berries. Future research should, however, concentrate on the nutritional benefits, sensory excellence, and microbiological safety of the grapes. In conclusion, grapes can be preserved under isochoric freezing to increase their shelf life and decrease food waste.

Author contribution statement

Ștefan Ioan Câmpean: Conceived and designed the experiments; Performed the experiments; Wrote the paper. George Andrei Beșchea: Conceived and designed the experiments; Performed the experiments. Maria Bianca Tăbăcaru: Performed the experiments. Luminița Maria Scutaru, George Dragomir, Alin Ionuț Brezeanu: Analyzed and interpreted the data. Alexandru Șerban: Contributed reagents, materials, analysis tools or data. Gabriel Năstase: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at SSRN under the accession number ID 4354735.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P4-ID-PCE-2020-1706, within PNCDI III.

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