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GRAPES WITH FUNGAL INFECTION DURING LONG-TERM STORAGE

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SUMMARY

Grapes (*Vitis vinifera* L.) are a seasonal fruit, and remaining fresh on the market is only up to 3–4 months. The most common way to extend the period of grape consumption is to organize its long-term storage. By storing grapes, in addition to the natural loss in weight, waste also occurs, mainly caused by fungal infections. The presented research aspired to study the composition of epiphytic microflora on the grape cultivar berries immediately after harvest and long-term storage in the refrigerator and determine the effectiveness of using sulfur dioxide to prevent grape spoilage. The Azerbaijan local table grape cultivars Gara Shaani and Ag Shaani served as the objects of microbiological studies. Determining the fungal contaminations of the berries led the experiment to inoculate the growth medium. Fumigation application with sulfur dioxide helped suppress the harmful microorganisms' activity. In fresh grape berries, the contamination microbiology differed sharply by grape cultivar. The fungi of the genus *Aspergillus* were visible in the cultivar Gara Shaani, while *Penicillium* in Ag Shaani. The results also have shown the effectiveness of using sulfur dioxide, which leads to a significant decline in fungi quantity by 87%.

Keywords: Grapes (*V. vinifera* L.), Absheron peninsula, microflora, cultivars, fungi, storage technology, fumigation

Key findings: In the stored grape (*V. vinifera* L.) berries, specific and varied microflora patterns were evident based on the biological characteristics of the studied cultivars.

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INTRODUCTION

The enhanced tendency in the world of fresh grape (*Vitis vinifera* L.) consumption (31.5 million tons in 2022, 0.9% more than in 2021) leads to higher acquaintance among grape producers, making it inevitable to adapt to market demands (OIV, 2023). Grapes with nutritional and medicinal characteristics positively affect human health and benefit nervous exhaustion, weakness, and avitaminosis treatment (Cosme *et al.*, 2018; Salimov, 2019). Each kilogram of grapes contains 150–200 g or more of sugar (glucose and fructose), up to 1.4% of organic acids (tartaric and malic), vitamins A (carotene), B2 (riboflavin), C (ascorbic acid), P (citrin), up to 1.5% of minerals (P, K, Fe, and Ca), 1% proteins, and about 1% of pectic acid (Sabra *et al*., 2021; Zhou *et al.*, 2022; Dave *et al.,* 2023).

As a seasonal fruit, the grape's consumption period ranges only from three to four months, depending on the genotypes. Prevention of loss (20%–30%) of the perishable food products is possible only by applying cold storage. In living organisms, such as fruits and vegetables, including grapes, even after harvest, the processes of respiration and transpiration persist. The intensity of these processes depends on the genetic makeup of the grape cultivars, growing location and environment, agrotechnics applied, degree of ripeness, and storage technology (Sharma *et al.,* 2018; Gorlov *et al.,* 2020). As the intensity of the biochemical processes increases, the unpreventable variations point out the enhanced aging of grape berries, decreasing the storage ability and resulting in worsened berry appearance.

Gradually, the berries become softer, lose their flavor and medicinal value, and develop various microorganisms on the fruit's surface. Grapes' resistance to microbial damage primarily depends on the berry skin properties – thickness and the presence of a vax layer. A damaged grape berry skin loses its integrity, paving the way for microbes to enter the internal layers of the tissues (Kazimova and Nabiyev, 2022; Soleimanie and Vafaee*,* 2023). The losses mainly occur due to microorganisms representing epiphytic microflora during grape storage. According to research, at different stages, grape losses can reach up to 53% (Aghayeva *et al.,* 2010).

The grapes' microflora composition mainly consisted of fungi belonging to the *Penicillium*, *Botrytis cinerea*, *Aspergillus*, *Alternaria*, *Fusarium*, *Mucor Fresen*, and *Cladosporium* species (El-Samawaty *et al.,* 2013; Kántor *et al.,* 2017; Rajput *et al.,* 2020; Salman *et al.,* 2021). Typically, decaying begins when the mold fungi develop since the acidic environment of tissue sap is favorable for them. Later, bacteria can also take part in spoilage. Spoilage occurs extremely fast at a high temperature. In an appropriate environment, the spores grow, forming reproductive tubules that infect the skin of healthy berries. However, sometimes, fungi create sclerotia - long-lasting small black, dense masses of mushroom mycelium. In high temperatures and a humid environment, sclerotia begins to develop and form conidia. A sharp increase in grape disease occurs when it rains, especially at low temperatures. The denser the bunch and thinner the skin of the berries, and the thicker the plant crown, the more susceptible to pathogens (Rajabi *et al.,* 2015; Armijo *et al.,* 2016).

Managing pathogens and preventing rotting risks requires an integrated approach. Pre-harvest control depends on preventing infection risks and is not limited only to fungicide use. Careful harvesting and packaging also minimize damages and infections; it is one of the crucial requirements for preventing rotting during storage (Szegedi and Civerolo, 2011; Rajabi *et al.,* 2015; Wei *et al.,* 2022). Storageability also depends on the structure of the berries` tissue and skin and the availability of the vax layer. The thicker the skin and tissue of the berries, the more difficult pathogens and pests to penetrate the tissue and cause harm. The prevention of losses during storage needs agrotechnical operations, permanent phytosanitary and toxicological control of the soil and the fruit yield, and developing an optimal gas environment in the storage capacity (Rajabi *et al.,* 2015; Asadullayev *et al.,* 2020; Gorlov *et al.,* 2020; Romero *et al.,* 2020).

During storage, the fruits continue to live at the expense of the plastic and energetic nutritional stuff accumulated in the vegetation period. Therefore, the main principle of fruit storage is slowing down the nutrition stuff for respiration. In such conditions, besides over-ripening of the product, suppressing the activity of the pathogen microflora also transpires. After packaging and during storage, special attention is necessary to minimize the contamination risks and prevent the spread of molds. The effectiveness of using sulfur dioxide to prevent damage to grapes during storage is often operational in past studies (Rajabi *et al.,* 2015; Ageyeva *et al.,* 2017; Sortino *et al.,* 2018; Ahmed *et al.,* 2018; Roberto *et al.,* 2019; Habib *et al.,* 2021; Li *et al.,* 2022; De-Aguiar *et al.,* 2023). The presented study sought to determine the composition of epiphytic microflora on grape berries immediately after harvest and long-term storage in the refrigerator and establish the effectiveness of using sulfur dioxide to prevent grape spoilage.

MATERIALS AND METHODS

Study object and procedure

Selecting two local table grape (*V. vinifera* L.) cultivars (Ag Shaani and Gara Shaani) became the study's object, grown in the Ampelographic Collection of the Scientific Research Institute of Viticulture and Wine-making, Azerbaijan (Figure 1, Table 1). Detailed descriptions of these cultivars are in the Vitis International Cultivar Catalogue (VIVC) database.

The Ampelographic Collection stands on the Absheron peninsula, in the Eastern part of Azerbaijan, on the coast of the Caspian Sea. The climate type of the peninsula is dry and subtropical. The mean annual air temperature is 13.5 °C–14.4 °C, the sum of active temperatures is 4192 °C–4461 °C, and the mean annual precipitation is 202–311 mm. Absheron is a traditional region of table viticulture.

The fruit yield of these two grapevine cultivars remained stored in a cold camera for

Cultivar Ag shaani | **Cultivar Gara shaani**

Figure 1. Physical appearance of the studied two cultivars of grapevine.

Table 1. Description of the two grapevine cultivars.

Cultivars	Botanical description	Agrobiological description	Technological description
Ag Shaani	Annual shoots are light brown. The leaves (19–23 cm) are green. The flower type is hermaphroditic. The clusters are medium and large (12–22 × 9–16 cm). The berries are large (20–25 × 14–19 mm), greenish-yellow. The peel is of medium thickness and elastic. The pulp is fleshy and juicy. The seeds are large (7.5 mm), one or two per berry.	The growing season is 159 days. The shoots ripen almost completely. The plant's growth vigor is medium. Productivity – 5.3–8.5 t/ha.	A table grape cultivar. The mass concentration of sugar in the juice is 16.4–22.4 g/100 cm^3; titratable acidity is 3.1–6.2 g/dm^3.
Gara Shaani	Annual shoots are light brownish. Leaves (15–20 cm) are light green. The flower type is hermaphroditic. The clusters are medium and large (12–22 × 8–12 cm). The berries are medium or large (17–21 × 15–19 mm), dark blue. The peel is durable. The pulp is juicy. The seeds are large (7.0 mm), two or three per berry.	The growing season is 146 days. The shoots ripen for 90.7%. The plant's growth vigor is high. Productivity – 7.6–9.6 t/ha.	A table grape cultivar. The mass concentration of sugar in the juice is 17.6–19.2 g/100 cm^3, titratable acidity is 4.4–5.5 g/dm^3.

four months. To suppress the activity of the pathogenic microflora during the storage period, the weekly fumigations with sulfur anhydride (2 g per each m^3 of the refrigeration camera) continued. The type of microflora on the berries' surface depended on the growth media planted. Before and during the storage period, an assessment of the number and change dynamics of the microflora and the effect of the fumigations applied ensued.

RESULTS AND DISCUSSION

Grape (*V. vinifera* L.) wastes resulted primarily from fungal infections during storage. The contagion usually occurs through damaged skin; however, the stomata and microscopic damage are not the main routes of infection. Therefore, the cultivars with thicker skin that prevent molds from penetrating inside the berry can remain stored for extended periods. Gray rot caused by the fungus *Botrytis cinerea* is considerably the most common disease during the storage of early-ripening grapevine cultivars (Kim *et al.*, 2007). However, the disease quickly spreads from rotten berries to neighboring ones (Osman *et al*, 2023; Turaeva *et al*., 2023).

Late-ripening grapevine cultivars are more susceptible to the fungus Penicillium, which causes Penicilliosis, forming soft and wet rots on grape berries. *Aspergillus* is a causative agent of black rot, bunch rot, and soft rot (Bolotyanskaya, 2020; Habib *et al.*, 2021). The results revealed that the microbiological contamination differs sharply between the two studied grapevine cultivars. Therefore, in the cultivar Ag Shaani, the fungi revealed the *Penicillium* species, while in the cultivar Gara Shaani – the *Aspergillus*. Also, significant variations concerning the quantity of microorganisms appeared on the berry surfaces of different grape cultivars (Table 2).

Fumigation proceeded immediately after putting on the stored fruits, significantly decreasing the contamination of fungal pathogens (37%–87%), depending on the grape cultivars (Ahmed *et al.,* 2018; Sortino *et al.,* 2018). Application of SO_2 led to a further decline in pathogenic microflora, and by the end of storage, the amount of fungi made was 1.6%–41.9% of the initial quantity. Weekly fumigations during the storage period lessen

Table 2. Quantity of microorganisms on grape berries surface during storage period (thousands per 1 g of berries).

Cultivars	Fungi genus	Non-fumigated before storage	Fumigated	
			Putting on storage	After storage
Ag Shaani	*Penicillium*	1.2	0.8	0.6
Gara Shaani	*Aspergillus*	2.4	0.5	0.05

Table 3. Yield of standard grapevine fruits and composition of losses after four months of storage.

Cultivars	Standard product (%)	Losses composition (%)		
		Rotten	Squashed	Detached
Ag Shaani	72.7	69	22	9
Gara Shaani	99.3	-	-	100

Table 4. Dynamics of grape weight loss during cold storage.

Cultivars	Storage period (days)	Natural loss (%)	Storage period (days)	Natural loss (%)	Storage period (days)	Natural loss (%)
Ag shaani	44	3.55	87	8.66	121	11.05
Gara shaani	44	3.34	87	6.06	121	7.67

the fungal load, with the results confirmed and supported by the findings of numerous past studies (Roberto *et al.,* 2019; De-Aguiar *et al.,* 2023; Li *et al.,* 2023).

One can see that the scale of microbiological contamination of different cultivars strictly corresponds to the size of losses after the storage period (Table 3). The Ag Shaani cultivar demonstrated considerable stem browning; the process was less intensive in the Gara Shaani cultivar. Similarly, while most of the losses in the Gara Shaani cultivar consisted mainly of detached berries, in the Ag Shaani cultivar, the microbiological rot made the general part of the losses. Moreover, the slight browning of the berries was evident in the white-colored cultivar (Ag Shaani), which corresponds with the literature data available (El-Samawati *et al.,* 2013; Rajput *et al.,* 2020; Salman *et al.,* 2021).

Water loss and consumption of organic matter through respiration are the foremost processes during grape storage. In addition, the variations interconnect with the activity of epiphytic microflora and various physiological disorders in cell metabolism. However, the amount of natural loss mostly depends upon the cultivars, quality of grapes, growing, and storage conditions (Djeneyev, 1971; Hamie *et al*., 2022). According to the presented results, in the more shelf-stable cultivar Gara Shaani, the weight loss during storage was significantly lower than in the less stable grape cultivar Ag Shaani (Table 4, Figure 2). These observations also confirm the idea that the amount of natural weight loss during storage depends on the grape cultivar and the degree of its susceptibility to the activity of pathogenic microflora (Potapenko and Ganich, 2015; Blanckenberg *et al.*, 2021).

Thus, one can conclude that the storage ability of grapes depends on the cultivar and the kind of fungi developing during the storage period. The cultivar Gara Shaani demonstrated the highest resistance to fungal diseases, while considerable losses occurred in the grape cultivar Ag Shaani caused by *Penicillium*. Moreover, the cultivar Gara Shaani showed a significantly higher yield of standard products by the end of the storage period and lower values of post-harvest weight loss. Therefore, the cultivar Gara Shaani proved quite suitable for prolonged cold storage. The relevant studies also confirmed the effectiveness of applying sulfur dioxide in suppressing the activity of epiphytic microflora during grape storage.

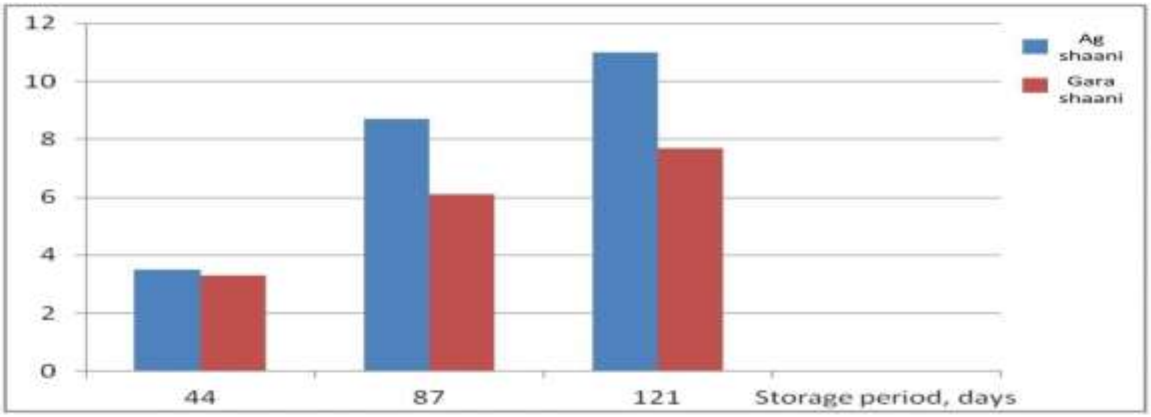

Figure 2. Grape weight loss (%) during cold storage.

CONCLUSIONS

The grape (*V. vinifera* L.) cultivar Gara Shaani emerged as more suitable for prolonged cold storage than the other cultivar, Ag Shaani. The sulfur dioxide also proved effective in suppressing the epiphytic microflora during grape storage.

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