



# Grape pomace treatment methods and their effects on storage

Natalia M. Ageyeva\*, Anastasia N. Tikhonova, Boris V. Burtsev,  
Svetlana A. Biryukova, Ekaterina V. Globa

North-Caucasian Federal Scientific Center of Horticulture, Viticulture and Winemaking, Krasnodar, Russia

\* e-mail: [ageyeva@inbox.ru](mailto:ageyeva@inbox.ru)

Received October 28, 2020; Accepted in revised form November 20, 2020; Published online July 09, 2021

## Abstract:

**Introduction.** Grape pomace is the most important by-product of winemaking that can be used as an additional raw material. There is a need for an optimal storage technology so that pomace can be further processed to obtain new types of products. We aimed to study the effect of grape pomace treatment on its microflora.

**Study objects and methods.** We identified and quantified microflora on the fresh and one-month-stored pomace samples from white and red grape varieties. The samples were exposed to conventional drying at 60–65°C, infrared drying at 60–65°C, as well as sulfitation with sulfur dioxide and sodium metabisulfite.

**Results and discussion.** The pomace microflora can be considered a microbial community. Almost all the samples stored for one month in an open area contained *Saccharomyces cerevisiae* yeasts, higher concentrations of filmy yeasts of the *Candida*, *Pichia*, *Hansenula*, *Hanseniaspora/Kloeckera*, and *Torulaspora* genera, as well as conidia of *Mucor*, *Aspergillus niger*, and *Penicillium* molds. Prevalent bacteria included acetic acid (mainly *Acetobacter aceti*) and lactic acid (*Lactobacillus plantarum*, *Pediococcus*, *Leuconostoc*) bacteria. These microorganisms significantly changed concentrations of volatile and non-volatile components, decreasing total polysaccharides, phenolic compounds, and anthocyanins 1.7–1.9, 3.7–4.0, and 4.0–4.5 times, respectively. The contents of micromycetes and bacteria in the one-month-stored samples were significantly higher than in the fresh pomace. Pre-drying and sulfitation decreased bacterial contamination, but to a lesser extent compared to micromycetes.

**Conclusion.** Long-term storage spoiled pomace, leading to significant changes in its chemical composition. Sulfitation reduced microorganism growth during storage, but did not provide long-term preservation (over a month), while pre-drying at 60–65°C promoted longer storage.

**Keywords:** Winemaking by-products, grape pomace, yeast, bacteria, microflora, storage conditions

**Funding:** The research was financially supported by the Kuban Science Foundation as part of the scientific project No. MFI-20.1/22 “Studying the mechanisms of biotransformation of winemaking by-products for targeted management of their storage and processing”.

**Please cite this article in press as:** Ageyeva NM, Tikhonova AN, Burtsev BV, Biryukova SA, Globa EV. Grape pomace treatment methods and their effects on storage. *Foods and Raw Materials*. 2021;9(2):215–223. <https://doi.org/10.21603/2308-4057-2021-2-215-223>.

## INTRODUCTION

The accumulation of wine production waste has an adverse effect on the environmental situation in grape-growing regions. Grape pomace is a key winemaking by-product that can be used as an additional raw material [1].

Grape pomace is rich in biologically valuable components, including polyphenols, pectin substances, and microelements [2, 3]. About 10–15% of this by-product is used as a biofertilizer to improve the soil structure [4]. Grape pomace can also be a source of

dietary fiber, natural food colors, grape alcohol, tartaric acid, as well as extracts and concentrates [5–8]. Grape seeds are used to extract grape oil, which is widely used in cosmetology [9, 10]. Therefore, there is an urgent need for effective methods to process grape pomace.

Pomace can be sweet, fermented, and alcoholized, depending on the technology of grape processing.

Sweet pomace is obtained by pressing the grapes after the juice has separated. Such pomace contains microorganisms and major components of grape berries, including sugars. Sweet pomace is usually derived from white grapes during the production of table wines and

wine base for sparkling wines and champagne, as well as from red grapes processed like white grapes (without maceration or fermentation).

Fermented pomace results from pressing the fermented grapes during red table wine production. It contains ethanol (a product of natural fermentation of grape sugars), organic acids, phenolic, nitrogenous and pectin substances, aromatic components of wine base, as well as wine yeast and malolactic fermentation bacteria used for fermentation and acidity reduction.

Alcoholized pomace is produced through pressing fermented and alcoholized grapes in the production of liqueur wines, especially Kagor (fortified dessert wine) and Muscat wines. The last 15–20 years have seen a significant decrease in these wines due to a need to use grape alcohols in their production. Alcoholized pomace contains ethanol, sugars, and other components of grapes and wine base, including yeast. According to the Russian Ministry of Agriculture, alcoholized pomace accounts for 2.0–3.4% of total pomace, depending on the volume of liqueur wine production.

Various types of pomace differ in their components and microflora. Pomace can rapidly deteriorate during storage due to a combination of nutrients (sugars, nitrogenous compounds, organic acids, vitamins, etc.) and air exposed natural grape microflora (sweet pomace), as well as wine yeast and malolactic bacteria (fermented and alcoholized pomace). As a result, pomace becomes moldy, alcohol turns into acetic acid, and tartaric acid compounds get destroyed by bacteria.

Therefore, pomace needs to be processed immediately after its separation. However, sometimes it has to be stored for a certain time before processing (e.g., in the production of dietary fiber, powders, enocolorants, extracts, etc.). In this case, pomace must be stored under appropriate conditions, depending on the amount, type, and the physiological state of its microflora.

Grape pomace is usually stored on special sites, covered with tarpaulin or other material, if any. However, its surface and inside contain molds (*Aspergillus*, *Penicilium*, *Rhizopus nigricans*, *Cladosporium*, *Fusarium*, *Alternarium*, *Mucor*, *Botrytis*, and *Oospora*), yeasts (*Saccharomyces* and *Torula*), bacteria (*Bacillus stearotermophilus*, *Bacillus subtilis*, and *Staphylococcus aureus*), and many others microorganisms [11–14]. In this regard, the assessment of its microbiological state is an important part of pomace disposal, which depends on grape processing technology and storage conditions.

Our aim was to study the influence of storage conditions on the microflora of white and red grape pomace treated with different methods.

## STUDY OBJECTS AND METHODS

**Sampling and preparation for microbiological research.** We studied fresh and one-month stored pomace from *Vitis vinifera* grapes produced in Krasnodar Krai (Russia), namely: sweet (Chardonnay,

Riesling, Sauvignon Blanc, Traminer Rose, and Pinot Noir), fermented (Cabernet Sauvignon, Merlot, and Saperavi), and alcoholized (Traminer Rose, Cabernet Sauvignon, and Saperavi). The pomace came from the production of white and red table and liqueur wines. Some grape processing technologies used pectoproteolytic enzyme preparations – Trenolin Blanc and Trenolin Rouge (Erbsloeh Geisenheim AG, Germany) – in optimal manufacturer-recommended amounts. The storage temperature varied from 14°C (at night) to 26°C (at daytime). An average sample was obtained by mixing equal amounts of samples taken from the surface of the pomace mass. The samples were taken from a depth of 0.5 and 1.0 m, placed in glass flasks, filled with distilled water, and incubated at 22–25°C for two days.

**Isolation of microorganisms.** The samples were inoculated and passed on yeast-peptone agar containing 10 g yeast extract, 20 g peptone, 20 g agar-agar, and 20 g glucose per 1 L of water (Research Center for Pharmacotherapy, Russia). The elective test was performed on Lysine Medium Base (Himedia, India). Those isolates that were incapable of growing on the elective medium were considered as belonging to the genus *Saccharomyces*.

We also used solid nutrient media, such as grape juice agar (2%) alcoholized with ethanol (14% alcohol) – to identify saccharomycetes, and OFS-agar (Erbsloeh Geisenheim AG, Germany) – to quantify yeast, mold fungi, as well as lactic and acetic acid bacteria.

Chloramphenicol (50 mg/L) was introduced into the media to improve yeast growth and suppress bacterial growth. Yeast colonies were cultivated at  $24 \pm 2^\circ\text{C}$  for 6–7 days. Some of them were inoculated on selective solid nutrient media. During the cultivation, we monitored the presence of other genera yeast, including *Saccharomyces*, *Pichia*, *Hansenula*, and *Hanseniaspora*.

The colonies were microscoped to identify saccharomycetes and other microorganisms based on their cultural and morphological characteristics [13, 15]. Generic identification of the isolates was based on their morphological and biochemical characteristics.

**Physical and chemical parameters.** The pomace was extracted with hot water (65–70°C) at a hydromodule of 1:5. The extracts were analyzed to determine:

- organic acids: by capillary electrophoresis State Standard 52841-2007. Wine production. Determination of organic acids by capillary electrophoresis method. Moscow: Standartinform; 2008. 7 p.;
- ethyl alcohol: according to State Standard 32095-2013. The alcohol production and raw material for it producing. Method of ethyl alcohol determination;
- phenolic substances: by the Folin-Ciocalteu colorimetric method [16];
- anthocyanins: by the colorimetric method [16];
- polysaccharides: by the phenol sulfur method [16]; and
- volatile impurities: by gas-liquid chromatography (Crystal 5000, nitrogen carrier gas, flame ionization

detector, PEG-based HP-FFAP column, 50 m, 0.32 mm, dosing device).

**Pomace treatment before storage.** To study the effect of storage conditions on microbiological parameters, the pomace samples were treated using the following methods:

- drying at 60–65°C to constant weight in a laboratory drying oven with forced air convection (AB UMEGA-GROUP, Lithuania);
- drying at 60–65°C to constant weight in a drying oven with infrared radiation (Radiozavod, Russia);
- exposing to sulfur dioxide (sulfitation) introduced as a concentrated solution (at least 1g SO<sub>2</sub>/kg pomace); and
- treating with sodium metabisulfite introduced in tablet form into the lower part of pomace (when decomposed, it produces sulfur dioxide that evenly spreads throughout the pomace).

## RESULTS AND DISCUSSION

**Microbiological studies of fresh and stored grape pomace.** We compared the microbiological indicators for fresh and one-month stored pomace samples from various grape varieties obtained by different methods (Table 1). As we can see, fresh sweet pomace had a significantly smaller amount of micromycetes (including yeast fungi) and bacteria than fermented pomace. This was because the fermented samples contained wine yeast, which is used for alcoholic fermentation, and lactic acid-reducing bacteria, which are often introduced at the final stage of fermentation. The smallest amount of microorganisms was found in the alcoholized pomace,

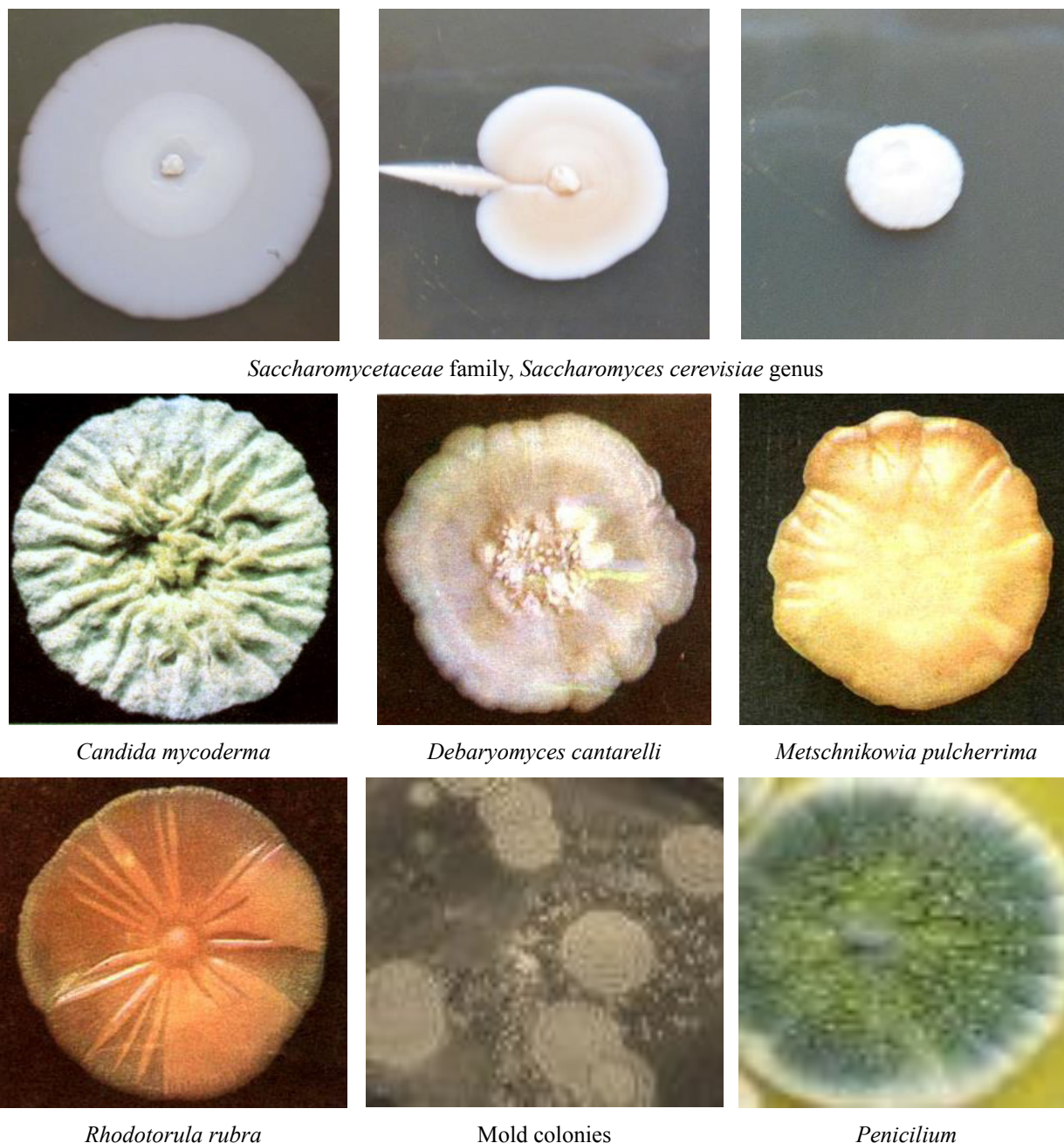
which is associated with the inhibitory effect of ethyl alcohol.

We found that the pomace microflora included microorganisms of various classes, species, and genera. Their metabolic interactions involved the transfer of metabolites between partners, a producer and a metabolizer. For example, yeast converted residual sugars to ethyl alcohol that was consumed by acetic acid bacteria to produce acetaldehyde and acetic acid. Lactic acid bacteria and yeast have a symbiotic relationship. Yeast stimulates growth in lactic acid bacteria, fortifies foods with vitamins, as well as ferments lactose and other sugars to produce antibiotic substances acting against pathogenic microorganisms.

With changes in environmental conditions, some microorganisms can suspend the processes of reproduction and fermentation of other species. Some lactic acid bacteria, mainly rod-shaped (a threat to wine production), can act antagonistically and destroy yeast cells, for example, in nitrogen-depleted media (pH < 6) [17]. Yeast and acetic acid bacteria stimulate growth in lactic acid bacteria. Thus, some biochemical processes that occur during storage can significantly change the chemical composition of grape pomace and make it unsuitable for production. In particular, pomace microorganisms destroy organic acids and polysaccharides, basic components of dietary fiber. Moreover, they consume vitamins and vitamin-like substances, leading to a significant decrease in bioactive components, so important for the production of extracts and concentrates.

**Table 1** Microbiological indicators of fresh and stored pomace obtained by pressing, CFU/g

Grape variety, type of pomace / Pressing equipment	Pomace			
	Fresh		Stored for one month	
	micromycetes, incl. yeast	bacteria	micromycetes, incl. yeast	bacteria
Chardonnay, sweet / Diemme, Italy	4.3×10 <sup>3</sup>	0.6×10 <sup>3</sup>	6.8×10 <sup>5</sup>	8.4×10 <sup>5</sup>
Chardonnay, sweet / Busher Vaslin, France	3.8×10 <sup>3</sup>	0.4×10 <sup>3</sup>	6.3×10 <sup>5</sup>	8.8×10 <sup>5</sup>
Chardonnay, sweet / Enoventa, Italy	4.6×10 <sup>3</sup>	0.4×10 <sup>3</sup>	6.6×10 <sup>5</sup>	7.8×10 <sup>5</sup>
Pinot Blanc, sweet / Enoventa, Italy	4.0×10 <sup>3</sup>	0.5×10 <sup>3</sup>	7.1×10 <sup>5</sup>	8.3×10 <sup>5</sup>
Pinot Blanc, sweet, trenolin blanc / Enoventa, Italy	4.3×10 <sup>3</sup>	0.4×10 <sup>3</sup>	6.8×10 <sup>5</sup>	7.6×10 <sup>5</sup>
Riesling, sweet / Della Toffola, Italy	3.7×10 <sup>3</sup>	0.5×10 <sup>3</sup>	7.1×10 <sup>5</sup>	7.7×10 <sup>5</sup>
Riesling, sweet + trenolin blanc / Della Toffola, Italy	3.9×10 <sup>3</sup>	0.5×10 <sup>3</sup>	9.5×10 <sup>4</sup>	5.8×10 <sup>4</sup>
Traminer Rose, sweet / Enoventa, Italy	3.1×10 <sup>3</sup>	0.4×10 <sup>3</sup>	7.4×10 <sup>5</sup>	7.0×10 <sup>5</sup>
Traminer Rose, sweet + trenolin blanc / Enoventa, Italy	3.6×10 <sup>3</sup>	0.4×10 <sup>3</sup>	7.5×10 <sup>4</sup>	6.9×10 <sup>4</sup>
Traminer Rose, alcoholized / Enoventa, Italy	2.1×10 <sup>3</sup>	0.3×10 <sup>3</sup>	3.3×10 <sup>5</sup>	2.4×10 <sup>5</sup>
Sauvignon B lanc, sweet / Enoventa, Italy	3.6×10 <sup>3</sup>	0.5×10 <sup>3</sup>	7.1×10 <sup>5</sup>	7.7×10 <sup>5</sup>
Pinot Noir, sweet / Busher Vaslin, France	3.2×10 <sup>3</sup>	0.2×10 <sup>3</sup>	4.3×10 <sup>5</sup>	1.6×10 <sup>5</sup>
Merlot, fermented / Busher Vaslin, France	7.8×10 <sup>4</sup>	0.7×10 <sup>3</sup>	8.7×10 <sup>6</sup>	6.6×10 <sup>5</sup>
Merlot, fermented + trenolin rouge / Busher Vaslin, France	8.1×10 <sup>4</sup>	0.8×10 <sup>3</sup>	6.8×10 <sup>6</sup>	6.0×10 <sup>6</sup>
Saperavi, fermented / Busher Vaslin, France	6.2×10 <sup>4</sup>	0.6×10 <sup>3</sup>	3.2×10 <sup>6</sup>	3.7×10 <sup>6</sup>
Saperavi, alcoholized / Busher Vaslin, France	3.1×10 <sup>3</sup>	0.3×10 <sup>3</sup>	1.8×10 <sup>5</sup>	2.3×10 <sup>5</sup>
Saperavi, fermented + trenolin rouge / Busher Vaslin, France	3.7×10 <sup>3</sup>	0.6×10 <sup>3</sup>	7.8×10 <sup>6</sup>	3.1×10 <sup>6</sup>
Cabernet Sauvignon, fermented/Busher Vaslin, France	6.8×10 <sup>4</sup>	0.7×10 <sup>3</sup>	5.4×10 <sup>6</sup>	2.7×10 <sup>6</sup>
Cabernet Sauvignon, alcoholized / Busher Vaslin, France	3.7×10 <sup>3</sup>	0.3×10 <sup>3</sup>	4.2×10 <sup>4</sup>	1.1×10 <sup>4</sup>
Cabernet Sauvignon, fermented + trenolin rouge / Busher Vaslin, France	7.4×10 <sup>4</sup>	0.5×10 <sup>3</sup>	5.0×10 <sup>5</sup>	3.0×10 <sup>5</sup>



*Saccharomycetaceae* family, *Saccharomyces cerevisiae* genus

*Candida mycoderma*

*Debaryomyces cantarelli*

*Metschnikowia pulcherrima*

*Rhodotorula rubra*

Mold colonies

*Penicilium*

**Figure 1** Colonies of microorganisms isolated from grape pomace samples

The Chardonnay samples can be used to show a correlation between the method of pressing and the number of microorganisms (Table 1). Different pressing equipment produces pomace that varies in moisture. The Busher Vaslin press (France) provided a higher degree of pressing and, therefore, a higher mechanical effect on grapes (fresh, fermented or alcoholized) compared to other presses, resulting in less active microorganisms and fewer colonies.

The use of enzyme preparations to produce sweet and fermented pomace led to a decomposition of many high-molecular grape components (proteins, polysaccharides, complex compounds) into low-

molecular substances easily assimilated by the microflora. The fermentation increased the concentration of sugars and nitrogenous substances, stimulating the growth of micromycetes and bacteria cells on nutrient media.

Storing the pomace samples in tarpaulin-covered cement pits with air access activated microorganism cells, leading to their significant increase, especially bacteria, in all the experiments.

Figure 1 shows colonies of microorganisms in the pomace samples stored for one month in an open area. They were isolated by inoculation on solid nutrient media. The average pomace sample contained yeast of

the *Saccharomyces cerevisiae* genus, characteristic of wine production. Its colonies varied in shape (round, with or without septa, radial or feathery, some with a well-defined inner ring), appearance (shiny or matte, dry or wet, smooth or wrinkled, with smooth or deformed edges), surface relief, and thickness. Such a variety was due to their belonging to different species [12, 14, 18–20].

Growing on the pomace surface, *Candida mycoderma* consumes extractives and releases volatile compounds that give the pomace a pungent taste and unpleasant odor, making it unsuitable for further processing [12, 14]. Moreover, its enzyme systems can break down high-molecular compounds (including pectin substances), significantly reducing the value of the pomace as a secondary raw material.

Almost all the samples contained filmy yeasts of the *Candida*, *Pichia*, *Hansenula*, *Hanseniaspora/Kloeckera*, and *Torulasporea* genera, with their greatest amount in fresh pomace of white grape varieties and the smallest

amount in alcoholized pomace. Noteworthy, yeasts of the *Brettanomyces* and *Schizosaccharomyces* genera, which are always present on grape berries, were low in our samples, under 0.7–1.0% [21]. Yeasts of the *Pichia* and *Hansenula* genera were under 1.2%, depending on the technology of pomace production. The growth of these microorganisms in our pomace samples significantly changed their aroma, giving them the tones of fermentation, ethyl acetate, and sour milk.

*Debaryomyces* yeasts, which we identified in the average pomace sample, have a poor ability to absorb sugars, metabolize tartaric, lactic, and citric acids into esters, synthesize extracellular enzymes, and decompose toxins [22, 23]. They make the pomace unsuitable for further processing.

Molds were clearly visible on the pomace surface (3.5–6.4%), namely *Mucor*, *Aspergillus niger*, and *Penicillium*. They are highly undesirable since they can produce mycotoxins and compounds with unpleasant odors and tastes [24, 25].

**Table 2** Physicochemical parameters of fresh and one-month stored pomace extracts

Parameters	Traminer Rose pomace			
	Fresh		Stored for one month	
	Sweet	Sweet fermented	Sweet	Sweet fermented
	Organic acids, g/L extract			
Tartaric	8.8 ± 0.4	9.4 ± 0.6	2.7 ± 0.3	2.5 ± 0.4
Malic	5.6 ± 0.2	6.1 ± 0.4	1.5 ± 0.2	1.3 ± 0.3
Citric	2.0 ± 0.2	2.0 ± 0.2	n.d.	n.d.
Succinic	3.2 ± 0.2	2.4 ± 0.2	n.d.	n.d.
Ascorbic	2.6 ± 0.2	3.0 ± 0.3	n.d.	n.d.
Lactic	n.d.	n.d.	2.76 ± 0.12	2.94 ± 0.08
	Volatile compounds, g/L extract			
Ethanol	8.6 ± 0.2	10.3 ± 0.2	n.d.	n.d.
Acetaldehyde	164 ± 17	182 ± 22	1210 ± 28	1421 ± 32
Acetoin	8.64 ± 0.12	4.26 ± 0.12	124.7 ± 6	88.7 ± 4
Acetone	n.d.	n.d.	44.8 ± 0.3	35.7 ± 0.2
Acetic acid	134 ± 12	146 ± 15	650 ± 22	720 ± 27
Propionic acid	n.d.	n.d.	12.6 ± 0.17	11.8 ± 0.16
Butyric acid	0.24 ± 0.07	0.28 ± 0.07	1.86 ± 0.12	2.15 ± 0.09
Ethyl acetate	246 ± 16	308 ± 22	1264 ± 23	1432 ± 31
Ethyl propionate	n.d.	n.d.	67.6 ± 2.4	65.3 ± 1.8
Methyl acetate	13.8 ± 0.6	10.4 ± 0.6	47.5 ± 1.5	52.8 ± 2.3
Propyl acetate	n.d.	n.d.	144 ± 11	165 ± 13
Butyl acetate	8.4 ± 0.6	6.7 ± 0.6	65 ± 6	78 ± 5
Isoamylacet	28.4 ± 2.2	21.6 ± 2.3	132 ± 8	118 ± 4
Ethyl lactate	44.8 ± 7	42.4 ± 6	187 ± 12	213 ± 13
1-propanol	2.8 ± 0.2	3.0 ± 0.2	43.5 ± 1.3	54.7 ± 2.0
n-propanol	14.7 ± 1.2	23.6 ± 1.6	145 ± 23	127 ± 20
n-butanol	n.d.	n.d.	87 ± 11	94 ± 15
Isoamylol	86 ± 12	78 ± 9	1254 ± 37	1152 ± 27
Glycerol	886 ± 24	935 ± 27	184 ± 11	167 ± 10
	Non-volatile compounds, mg/L extract			
Common polysaccharides	1234 ± 29	1347 ± 33	765 ± 18	684 ± 14
Common phenolic substances	886 ± 21	764 ± 17	237 ± 11	198 ± 9
Common anthocyanins	165 ± 15	144 ± 12	45 ± 6	32 ± 4

n.d. – not detected

Prevailing bacteria included acetic acid bacteria (mainly *Acetobacter aceti*) and lactic acid bacteria (including *Lactobacillus plantarum*, *Pediococcus*, and *Leuconostoc*) amounting to 6–9%, with their greatest increase in sweet pomace during storage.

The greatest growth in microorganisms was in the sweet pomace samples during storage: yeast cells converted sugars to ethanol, which was then used by acetic acid bacteria to synthesize acetic acid. Lactic acid bacteria were especially frequent in fermented pomace. We found that microorganism growth was much greater in white grape pomace compared to red grape pomace, which is rich in phenolic compounds with antiseptic and antibacterial action [26–28].

Microflora also increased in alcoholized pomace, despite 7–10% ethyl alcohol, although not as much as in the other types of samples. With acetic and lactic acid fermentation, alcoholized pomace (e.g. Cabernet-Sauvignon) still retained grape-wine tones in its aroma.

Thus, we found that red grape pomace did better during storage than white pomace due to the presence of polyphenols with antiseptic effects. Alcoholized pomace showed the smallest growth in micromycetes.

**Physicochemical parameters of fresh and one-month stored pomace extracts.** Changes in the physicochemical parameters of the Traminer Rose pomace extracts (sweet and fermented) are presented in Table 2.

The chemical composition of the extracts (Table 2) showed that microorganism growth in the stored pomace significantly decreased the concentration of tartaric, malic, and citric acids, with succinic and ascorbic acids completely oxidized. Moreover, the microorganisms consumed succinic acid and converted it into fumaric and formic acids, disrupting the pomace aroma. Tartaric acid decomposed under the action of *Debaryomyces* yeast and various lactic acid bacteria (*Lactobacillus*

*brevis*, *Lactobacillus hilgardii*, *Lactobacillus plantarum*, and heterofermentative cocci), producing diacetyl, acetic, propionic, and lactic acids [29].

The above process consumed a large amount of glycerin. The growth in lactic acid bacteria increased the concentration of lactic acid and ethyl lactate ester. Citric acid decomposed under the action of enzymes of lactic acid bacteria and molds, producing acetoin and acetone.

The growth in acetic acid bacteria and molds significantly changed concentrations of volatile components, namely:

- ethanol decomposed under the action of enzyme systems of acetic acid bacteria into acetic acid and its derivatives in the stored pomace extracts, making their further use in wine distillation impossible;
- glycerol, which is used by the pomace microflora in the biochemical processes to synthesize new components, decreased 4.4–6.0-fold;
- acetaldehyde, acetic acid, and ethyl acetate increased 7.3–7.7, 4.2–4.8, and 4.5–5.2 times respectively, all having a smell of acetic acid and thus giving the extracts an unbalanced tangy taste;
- propionic acid and its ethyl ester were identified in the stored pomace extracts, unlike the fresh extracts;
- higher alcohols, especially isoamylol and butanol, significantly increased, making the pomace unsuitable for distilling grape alcohol due to their pronounced fusel tones.

Acetic acid bacteria can oxidize mono- and polyhydric alcohols (as well as ethyl alcohol), carbohydrates and other substances in the extracts. Monohydric alcohols are oxidized to the corresponding acids (e.g., propanol to propionic acid, butyl alcohols to butyric acid), increasing their concentrations (Table 2).

Non-volatile (extractive) components, namely polysaccharides, phenolic compounds, and anthocyanins decreased 1.7–1.9, 3.7–4.0, and 4.0–4.5 times,

**Table 3** Microbiological indicators of pomace, CFU/g vs storage conditions

Grape variety, type of pomace	Pomace treatment			
	Drying at 60–65°C	Infrared drying at 60–65°C	Sulfur dioxide	Sodium metabisulfite
	Micromycetes			
Chardonnay, sweet	1.2×10 <sup>2</sup>	2.8×10 <sup>3</sup>	3.2×10 <sup>3</sup>	4.4×10 <sup>3</sup>
Riesling, sweet	0.8×10 <sup>2</sup>	1.7×10 <sup>3</sup>	2.3×10 <sup>3</sup>	3.7×10 <sup>3</sup>
Pinot Blanc, sweet	1.2×10 <sup>2</sup>	1.8×10 <sup>3</sup>	2.8×10 <sup>3</sup>	2.9×10 <sup>3</sup>
Pinot Blanc, sweet, trenolin blanc	1.3×10 <sup>2</sup>	1.4×10 <sup>3</sup>	3.0×10 <sup>3</sup>	2.8×10 <sup>3</sup>
Cabernet Sauvignon, fermented	0.8×10 <sup>2</sup>	1.5×10 <sup>3</sup>	2.7×10 <sup>3</sup>	2.6×10 <sup>3</sup>
Cabernet Sauvignon, alcoholized	0.4×10 <sup>2</sup>	0.2×10 <sup>3</sup>	1.2×10 <sup>3</sup>	1.3×10 <sup>3</sup>
Cabernet Sauvignon, fermented, trenolin rouge	0.8×10 <sup>2</sup>	0.4×10 <sup>3</sup>	2.8×10 <sup>3</sup>	2.5×10 <sup>3</sup>
	Bacteria			
Chardonnay, sweet	6.3×10 <sup>2</sup>	2.5×10 <sup>3</sup>	8.9×10 <sup>3</sup>	1.2×10 <sup>4</sup>
Riesling, sweet	6.3×10 <sup>2</sup>	1.9×10 <sup>3</sup>	8.9×10 <sup>3</sup>	2.1×10 <sup>4</sup>
Pinot Blanc, sweet	7.0×10 <sup>2</sup>	4.1×10 <sup>3</sup>	9.1×10 <sup>3</sup>	4.5×10 <sup>4</sup>
Pinot Blanc, sweet, trenolin blanc	5.8×10 <sup>2</sup>	3.8×10 <sup>3</sup>	1.2×10 <sup>4</sup>	3.1×10 <sup>4</sup>
Cabernet Sauvignon, fermented pomace	7.1×10 <sup>2</sup>	7.2×10 <sup>2</sup>	6.7×10 <sup>3</sup>	8.4×10 <sup>3</sup>
Cabernet Sauvignon, alcoholized	1.3×10 <sup>2</sup>	5.5×10 <sup>2</sup>	3.5×10 <sup>3</sup>	5.1×10 <sup>3</sup>
Cabernet Sauvignon, fermented, trenolin rouge	3.7×10 <sup>2</sup>	8.7×10 <sup>2</sup>	5.1×10 <sup>3</sup>	8.1×10 <sup>3</sup>

respectively. This reduced the production of grape dietary fiber and extracts of phenolic compounds from the pomace stored under those conditions, lowering its efficiency 4.5–6.8 times.

Thus, our experimental data showed a need to develop a pomace storage technology that would make pomace suitable for further use in production.

**Microbiological pomace parameters versus pre-storage treatment methods.** Various methods can be used to prepare pomace for storage. They include drying at various temperatures, treatment with ultraviolet and infrared rays, electromagnetic waves, regulating the gaseous environment, using chemical preservatives, alcoholization, and others [30–32].

Alcoholization is obviously the best preserver of bioactive components in grapes, but it requires large amounts of min 25% ethanol.

Our microbiological assays involved all types of the pomace samples treated by different methods: drying at 60–65°C, infrared drying at 60–65°C, adding sulfur dioxide and sodium metabisulfite (Table 3). We found that all the methods decreased contamination during storage. Drying at 60–65°C was most effective in reducing the activity of micromycetes, especially in red pomace. Infrared drying had the same effect, but to a lesser extent. It may be necessary to work out optimal processing modes, in particular, with higher temperatures.

Sulfur dioxide and its derivatives decreased the growth in micromycetes 75–100 times during one

month. Bacterial contamination also decreased, but to a lesser extent. Noteworthy, both drying methods were more efficient than sulfur dioxide and sodium metabisulfite. Most samples, including alcoholized and sulfitized ones, showed an increase in acetic and lactic acid bacteria at the end of the treatments. This indicated that these modes of sulfitation and drying did not ensure complete inhibition of the pomace microflora.

## CONCLUSION

Our experimental data led us to the following conclusions. The pomace samples were contaminated with various microorganisms, whose growth spoiled the pomace. Significant changes in its chemical composition during long-term storage can make it unsuitable for further use in food production. Available treatment methods decreased microorganism contamination, but did not ensure long-term preservation of the pomace. Sulfur dioxide or sodium metabisulfite can be used for short-term storage (up to a month). However, thermal treatment is required for longer storage to inhibit microorganism growth.

## CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## REFERENCES

1. Beres C, Costa GNS, Cabezudo I, da Silva-James NK, Teles ASC, Cruz APG, et al. Towards integral utilization of grape pomace from winemaking process: A review. *Waste Management*. 2017;68:581–594. <https://doi.org/10.1016/j.wasman.2017.07.017>.
2. Zhao X, Zhang S-S, Zhang X-K, He F, Duan C-Q. An effective method for the semi-preparative isolation of high-purity anthocyanin monomers from grape pomace. *Food Chemistry*. 2020;310. <https://doi.org/10.1016/j.foodchem.2019.125830>.
3. Minjares-Fuentes R, Femenia A, Garau MC, Meza-Velázquez JA, Simal S, Rosselló C. Ultrasound-assisted extraction of pectins from grape pomace using citric acid: A response surface methodology approach. *Carbohydrate Polymers*. 2014;106(1):179–189. <https://doi.org/10.1016/j.carbpol.2014.02.013>.
4. Cortés A, Moreira MT, Domínguez J, Lores M, Feijoo G. Unraveling the environmental impacts of bioactive compounds and organic amendment from grape marc. *Journal of Environmental Management*. 2020;272. <https://doi.org/10.1016/j.jenvman.2020.111066>.
5. Sirohi R, Tarafdar A, Singh S, Negi T, Gaur K, Gnansounou E, et al. Green processing and biotechnological potential of grape pomace: Current trends and opportunities for sustainable biorefinery. *Bioresource Technology*. 2020;314. <https://doi.org/10.1016/j.biortech.2020.123771>.
6. Tikhonova AN, Ageyeva NM. Deep processing of grapes for obtaining the grape food fibers. *Nauchnye trudy Severo-Kavkazskogo federal'nogo nauchnogo tsentra sadovodstva, vinogradarstva, vinodeliya* [Scientific works of the North-Caucasian Federal Scientific Center of Horticulture, Viticulture and Winemaking]. 2018;18:180–183. (In Russ.). <https://doi.org/10.30679/2587-9847-2018-18-180-183>.
7. Kato-Schwartz CG, Corrêa RCG, de Souza Lima D, de Sá-Nakanishi AB, de Almeida Gonçalves G, Seixas FAV, et al. Potential anti-diabetic properties of Merlot grape pomace extract: An *in vitro*, *in silico* and *in vivo* study of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition. *Food Research International*. 2020;137. <https://doi.org/10.1016/j.foodres.2020.109462>.

8. Beres C, Freitas SP, Godoy RLDO, de Oliveira DCR, Deliza R, Iacomini M, et al. Antioxidant dietary fibre from grape pomace flour or extract: Does it make any difference on the nutritional and functional value? *Journal of Functional Foods*. 2019;56:276–285. <https://doi.org/10.1016/j.jff.2019.03.014>.
9. Glampedaki P, Dutschk V. Stability studies of cosmetic emulsions prepared from natural products such as wine, grape seed oil and mastic resin. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2014;460:306–311. <https://doi.org/10.1016/j.colsurfa.2014.02.048>.
10. Dabetic NM, Todorovic VM, Djuricic ID, Antic Stankovic JA, Basic ZN, Vujovic DS, et al. Grape seed oil characterization: A novel approach for oil quality assessment. *European Journal of Lipid Science and Technology*. 2020;122(6). <https://doi.org/10.1002/ejlt.201900447>.
11. Mezzasalma V, Sandionigi A, Bruni I, Bruno A, Lovicu G, Casiraghi M, et al. Grape microbiome as a reliable and persistent signature of field origin and environmental conditions in Cannonau wine production. *PLoS ONE*. 2017;12(9). <https://doi.org/10.1371/journal.pone.0184615>.
12. Bur'yan NI. *Mikrobiologiya vinodeliya [Microbiology of winemaking]*. Simferopol': Tavriya; 2002. 433 p. (In Russ.).
13. Ageeva NM, Suprun II, Prakh AV. Variety of microorganisms groups living on berries of grapes. *Polythematic Online Scientific Journal of Kuban State Agrarian University*. 2015;(111):1586–1595. (In Russ.).
14. Viziteu G-A, Manoliu A, Andor I. Data concerning the yeasts microbiota from Cotnari vineyard. *Romanian Biotechnological Letters*. 2008;13(2).
15. Suprun II, Tokmakov SV, Ageeva NM, Prakh AV. Aprobation of genotyping method of wine yeast (genus *Saccharomyces*) by the analysis of inter-delta genomic region. *Polythematic Online Scientific Journal of Kuban State Agrarian University*. 2015;(112):484–494. (In Russ.).
16. Gerzhikova VG. *Metody tekhnokhimicheskogo kontrolya v vinodelii [Technochemical control methods in winemaking]*. Simferopol': Tavrida; 2009. 304 p. (In Russ.).
17. Dorosh AP, Gregirchak NN. Investigation of thermal resistance and antagonistic properties of the yeast *Saccharomyces cerevisiae*. *Zhivye i biokosnye sistemy*. 2015;(11).
18. Bizaj E, Cordente A, Bellon JR, Raspor P, Curtin CD, Pretorius IS. A breeding strategy to harness flavor diversity of *Saccharomyces* interspecific hybrids and minimize hydrogen sulfide production. *FEMS Yeast Research*. 2012;12(4):456–465. <https://doi.org/10.1111/j.1567-1364.2012.00797.x>.
19. Li S, Cheng C, Li Z, Chen J, Yan B, Han B, et al. Yeast species associated with wine grapes in China. *International Journal of Food Microbiology*. 2010;138(1–2):85–90. <https://doi.org/10.1016/j.ijfoodmicro.2010.01.009>.
20. Settanni L, Sannino C, Francesca N, Guarcello R, Moschetti G. Yeast ecology of vineyards within Marsala wine area (western Sicily) in two consecutive vintages and selection of autochthonous *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*. 2012;114(6):606–614. <https://doi.org/10.1016/j.jbiosc.2012.07.010>.
21. Schopp LM, Lee J, Osborne JP, Chescheir SC, Edwards CG. Metabolism of nonesterified and esterified hydroxycinnamic acids in red Wines by *Brettanomyces bruxellensis*. *Journal of Agricultural and Food Chemistry*. 2013;61(47):11610–11617. <https://doi.org/10.1021/jf403440k>.
22. Jara C, Laurie VF, Mas A, Romero J. Microbial terroir in Chilean valleys: Diversity of non-conventional yeast. *Frontiers in Microbiology*. 2016;7. <https://doi.org/10.3389/fmicb.2016.00663>.
23. Aiko V, Edamana P, Mehta A. Decomposition and detoxification of aflatoxin B<sub>1</sub> by lactic acid. *Journal of the Science of Food and Agriculture*. 2016;96(6):1959–1966. <https://doi.org/10.1002/jsfa.7304>.
24. Şen L, Nas S. Identification of ochratoxigenic fungi and contextual change on dried raisins (Sultanas). *Journal of Food, Agriculture and Environment*. 2013;11(3–4):155–161.
25. Steel CC, Blackman JW, Schmidtke LM. Grapevine bunch rots: Impacts on wine composition, quality, and potential procedures for the removal of wine faults. *Journal of Agricultural and Food Chemistry*. 2013;61(22):5189–5206. <https://doi.org/10.1021/jf400641r>.
26. Radovanović VN, Andjelković M, Arsić B, Radovanović A, Gojković-Bukarica L. Cost-effective ultrasonic extraction of bioactive polyphenols from vine and wine waste in Serbia. *South African Journal of Enology and Viticulture*. 2019;40(2):1–9. <https://doi.org/10.21548/40-2-3215>.
27. Mewa-Ngongang M, Du Plessis HW, Ntwampe SKO, Chidi BS, Hutchinson UF, Mekuto L, et al. Grape pomace extracts as fermentation medium for the production of potential biopreservation compounds. *Foods*. 2019;8(2). <https://doi.org/10.3390/foods8020051>.
28. Friedman M. Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *Journal of Agricultural and Food Chemistry*. 2014;62(26):6025–6042. <https://doi.org/10.1021/jf501266s>.



29. Kosel J, Cadež N, Schuller D, Carreto L, Franco-Duarte R, Raspor P. The influence of *Dekkera bruxellensis* on the transcriptome of *Saccharomyces cerevisiae* and on the aromatic profile of synthetic wine must. *FEMS Yeast Research*. 2017;17(4). <https://doi.org/10.1093/femsyr/fox018>.
30. Augustine S, Kudachikar VB, Vanajakshi V, Ravi R. Effect of combined preservation techniques on the stability and microbial quality and retention of anthocyanins in grape pomace stored at low temperature. *Journal of Food Science and Technology*. 2013;50(2):332–338. <https://doi.org/10.1007/s13197-011-0325-0>.
31. Tseng A, Zhao Y. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). *Journal of Food Science*. 2012;77(9):H192–H201. <https://doi.org/10.1111/j.1750-3841.2012.02840.x>.
32. Zheng Y, Lee C, Yu C, Cheng Y-S, Simmons CW, Zhang R, et al. Ensilage and bioconversion of grape pomace into fuel ethanol. *Journal of Agricultural and Food Chemistry*. 2012;60(44):11128–11134. <https://doi.org/10.1021/jf303509v>.

#### ORCID IDs

Natalia M. Ageyeva  <https://orcid.org/0000-0002-9165-6763>  
Anastasia N. Tikhonova  <https://orcid.org/0000-0002-3509-3345>  
Boris V. Burtsev  <https://orcid.org/0000-0001-9755-5384>  
Svetlana A. Biryukova  <https://orcid.org/0000-0001-8182-7429>  
Ekaterina V. Globa  <https://orcid.org/0000-0002-6890-3076>