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CARACTERIZAÇÃO DE UVAS DE MESA E ESTRATÉGIAS PARA MANUTENÇÃO DA QUALIDADE DURANTE O ARMAZENAMENTO DE CACHOS E DE BAGAS DESTACADAS

Santa Maria, RS
2022

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Dissertação de Mestrado apresentada ao Curso de Pós-Graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de Mestre em Agronomia.

Orientador: Prof. Dr. Vanderlei Both

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Santa Maria, RS
2022

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Dedico essa dissertação aos meus amados pais, Carlos Soldateli e Ivaneli Bassani Soldateli, e aos meus irmãos, Ivan Carlos Soldateli e Bruno João Soldateli, maiores incentivadores e fontes inesgotáveis de apoio, amor e compreensão, bem como as minhas sobrinhas Débora Soldateli e Maria Eduarda Soldateli, as quais tenho muito amor e carinho.

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RESUMO

CARACTERIZAÇÃO DE UVAS DE MESA E ESTRATÉGIAS PARA MANUTENÇÃO DA QUALIDADE DURANTE O ARMAZENAMENTO DE CACHOS E DE BAGAS DESTACADAS

AUTOR: Francis Júnior Soldateli
ORIENTADOR: Vanderlei Both

A partir do lançamento de novas cultivares de uvas híbridas de mesa com maior adaptabilidade e tolerância às pragas em relação ao grupo 'Itália', está havendo um interesse crescente no cultivo de uvas destinadas ao consumo *in natura* na região Sul. No entanto, a produção é concentrada em um curto período do ano, elevando a dependência de uvas de mesa provenientes de outras regiões produtoras durante a entressafra. Nesse sentido, a adoção de um sistema de armazenamento que mantenha a qualidade por longos períodos, pode beneficiar a produção local e ampliar a oferta das uvas. Além disso, a comercialização na forma de bagas pode aumentar o aproveitamento de uvas com baixo valor comercial e suprimir alguns inconvenientes encontrados na comercialização na forma de cachos. O objetivo desse trabalho foi caracterizar a qualidade das novas cultivares de uvas de mesa 'BRS Núbia' e 'BRS Isis', além da tradicional 'Itália' produzidas em clima temperado e avaliar diferentes técnicas durante o armazenamento refrigerado, sobre a manutenção da qualidade das uvas na forma de bagas destacadas e de cachos. O artigo 1 caracterizou a qualidade das uvas 'BRS Núbia', 'BRS Isis' e 'Itália', provenientes da Serra Gaúcha. O artigo 2 contemplou o armazenamento dessas cultivares na forma de bagas destacadas durante 30 e 60 dias (0,5 °C), nas seguintes condições: [1] controle; [2] dióxido de enxofre (SO_2) (1,5 g $Na_2S_2O_5$ kg^{-1} uva); [3] etanol (30% por 5 min); [4] água quente (50 °C por 10 min) e [5] 1-metilciclopropeno (1-MCP) (2,0 μL L^{-1}). O artigo 3 destacou o armazenamento de uvas 'BRS Isis' durante 60 e 90 dias (0,5 °C) nas seguintes condições: [1] controle; [2] SO_2; [3] 1.125 ppm de etanol; [4] 2.250 ppm de etanol; [5] 10 kPa de CO_2 e [6] 15 kPa de CO_2. Uvas 'BRS Núbia' e 'BRS Isis' apresentaram concentrações de compostos bioativos superiores às uvas 'Itália', especialmente a 'BRS Núbia', com elevado conteúdo de antocianinas na casca. A 'Itália' apresentou bagas com altas concentrações de compostos voláteis, especialmente terpenos que conferem aroma moscato. As cultivares apresentaram bom potencial de armazenamento na forma de bagas destacadas quando associado a aplicação de SO_2, etanol ou água quente. A água quente controlou a incidência de podridões e suprimiu a degradação das uvas até os 30 dias. O SO_2 e o etanol permitiram a manutenção da qualidade das uvas até os 60 dias. O etanol apresentou resultados semelhantes ao SO_2 no controle de podridões. Entretanto, a aplicação de etanol e água quente aumentaram a incidência de rachaduras em uvas 'BRS Isis'. O etanol também aumentou as rachaduras nessa cultivar armazenada na forma de cacho. Aplicação de 15 kPa de CO_2 e 2.250 ppm de etanol apresentaram eficiência semelhante ao SO_2 no controle de podridões durante o armazenamento por até 90 dias. Entretanto, a aplicação de etanol resultou no acúmulo de acetaldeído, que pode conferir aroma desagradável às uvas. Altas concentrações de CO_2 mantiveram maiores concentrações de compostos fenólicos e dos compostos voláteis hexanal e (E)-2-hexenal, indicando uma melhor qualidade das uvas.

Palavras-chave: Compostos fenólicos. BRS Isis. BRS Núbia. Itália. Qualidade pós-colheita.

ABSTRACT

CHARACTERIZATION OF TABLE GRAPES AND STRATEGIES FOR QUALITY MAINTENANCE DURING STORAGE OF BUNCHES AND DETACHED BERRIES

AUTHOR: Francis Júnior Soldateli
ADVISOR: Vanderlei Both

With the release of new table grapes hybrid cultivars, with greater adaptability and tolerance to pests in relation to the 'Italia' group, there is a growing interest in the cultivation of grapes intended for fresh consumption in the South region. However, the harvest is concentrated in a short period of the year, increasing dependence on table grapes from other growing regions during the off-season. In this sense, the adoption of storage systems that maintain the quality for long periods can benefit local production and expand the supply of grapes. In addition, marketing in the form of detached berries can increase the use of grapes with low commercial value and eliminate some inconveniences found in bunches marketing. The objective of this study was to characterize the quality of the new table grape cultivars 'BRS Nubia' and 'BRS Isis', and the traditional 'Italia' produced in temperate climates, and to evaluate the effects of different techniques during cold storage, on the quality maintenance of the grapes in the form of detached berries and bunches. The first manuscript characterized 'BRS Nubia', 'BRS Isis', and 'Italia' table grapes quality, produced on Serra Gaúcha region. The second manuscript evaluated the storage of these cultivars in the form of detached berries for 30 and 60 days (0.5 °C), in the following conditions: [1] control; [2] sulfur dioxide (SO_2) (1.5 g $Na_2S_2O_5$ per kg^{-1} of grape); [3] ethanol solution (30% for 5 min); [4] hot water (50 °C for 10 min) and [5] 1-methylcyclopropene (1-MCP) (2.0 μL L^{-1}). The third manuscript highlighted the storage of 'BRS Isis' table grapes for 60 and 90 days (0.5 °C) under the following conditions: [1] control; [2] SO_2; [3] 1,125 ppm ethanol; [4] 2,250 ppm ethanol; [5] 10 kPa of CO_2 and [6] 15 kPa of CO_2. 'BRS Nubia' and 'BRS Isis' grapes showed higher concentrations of bioactive compounds than 'Italia', especially 'BRS Nubia' with higher content of anthocyanins in the skin. 'Italia' presented berries with high concentrations of volatile compounds, especially terpenes that give the moscato aroma. The cultivars showed a good storage potential in the form of detached berries when associated to SO_2, ethanol or hot water treatment. Hot water was effective on decay incidence control and suppressed the degradation of grapes up to 30 days. SO_2 and ethanol allowed the table grapes quality maintenance for up to 60 days. Ethanol showed similar results to SO_2 in the decay control. However, ethanol and hot water treatments increased the cracking incidence in 'BRS Isis' grapes. Ethanol also increased the cracking on the berries of this cultivar stored in the bunch form. The storage with 15 kPa of CO_2 and 2,250 ppm of ethanol presented similar efficiency to SO_2 in the decay control during storage for up to 90 days. However, the ethanol treatment resulted in acetaldehyde accumulation, which can result in off-flavors to the grapes. High CO_2 concentrations maintained higher concentrations of phenolic compounds and volatile compounds hexanal and (E)-2-hexenal, indicating a better quality of the grapes.

Keywords: Phenolic compounds. BRS Isis. BRS Nubia. Italia. Postharvest quality.

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1 INTRODUÇÃO E JUSTIFICATIVA

A produção brasileira de uvas em 2019 ocupou uma área de 74.202 hectares, com produção de 1.445.705 toneladas. Esta produção é destinada a elaboração de vinhos, sucos e derivados, ou mesmo para o consumo *in natura*, que em 2019 representou 49% do destino da uva brasileira. A região Sul corresponde a 53,5% da produção nacional, na qual o Rio Grande do Sul destaca-se como o maior estado produtor, com 46.361 hectares cultivados, produzindo 666.423 toneladas, especialmente de uvas americanas (*Vitis labrusca*) (IBGE, 2020). As condições climáticas temperadas na Serra Gaúcha, principal região produtora, são desfavoráveis ao cultivo de uvas de mesa (*Vitis vinifera*) (ECHEVERRIGARAY et al., 2020), a exemplo do grupo 'Itália', que é altamente suscetível a doenças. Entretanto, nos últimos anos houve um aumento do cultivo de uvas de mesa nessa região (LATTUADA et al., 2020), devido, sobretudo, ao lançamento de cultivares híbridas, com maior adaptabilidade e tolerantes ao míldio (*Plasmopara viticola*) (MAIA et al., 2018), viabilizando o cultivo em regiões com ambiente mais ameno e úmido.

Dentre as cultivares de uva de mesa mais difundidas recentemente, destacam-se a 'BRS Núbia' e a 'BRS Isis' (MAIA et al., 2018). A 'BRS Núbia' é uma cultivar de ciclo médio, pirênica que apresenta cachos cônicos (Figura 1), relativamente compactos com bagas preta-azuladas de tamanho grande (MAIA et al., 2013). A 'BRS Isis' é uma cultivar de ciclo longo, com elevada fecundidade de cachos, tolerante ao míldio e que apresenta cachos predominantemente cilíndrico-alados (Figura 1), compactos e com bagas vermelhas (RITSCHEL et al., 2013). A produtividade destas cultivares pode chegar a 50 t ha^{-1} em regiões com dois ciclos anuais, entretanto, para a região Sul, onde é possível apenas um ciclo produtivo, obtém-se produtividade de 30 t ha^{-1} (MAIA et al., 2018). Esta produção concentrada de janeiro a março resulta em elevada oferta seguida por períodos de escassez e valorização dos preços. Desta forma, maior disponibilidade de uvas para o consumidor durante um maior período do ano, depende do fornecimento de outras regiões ou países. No entanto, a adoção de um sistema de armazenamento que mantenha a qualidade por longos períodos, poderia beneficiar a produção local e ampliar a oferta das uvas.

Figura 1 - Uvas de mesa produzidas na Serra Gaúcha.

Fonte: (Autor).

O armazenamento refrigerado, onde ocorre somente a redução da temperatura e controle da umidade relativa, é um dos principais métodos de armazenamento das frutas (ROMERO et al., 2021). A redução da temperatura até um determinado limite, reduz o metabolismo e retarda alterações fisiológicas e bioquímicas atreladas à degradação das frutas (PEREIRA et al., 2018). Entretanto, este tipo de armazenamento apresenta limitações no armazenamento de uvas pela suscetibilidade a patógenos, escurecimento da ráquis e degrana (PINTO et al., 2015), o que compromete a aceitação pelos consumidores (PEREIRA et al., 2018). Portanto, novas técnicas de armazenamento para reduzir as perdas e manter a conservação e fornecer uvas durante grande parte do ano em volume e qualidade satisfatória aos consumidores necessitam ser avaliadas.

Em sistemas de armazenamento ou para o transporte de uvas a longas distâncias, está sendo comumente utilizado o dióxido de enxofre (SO_2), principalmente pela sua eficiência no controle de patógenos (DOMINGUES et al., 2018). O SO_2 aumenta a atividade de enzimas e a expressão de genes envolvidos no metabolismo do enxofre, principalmente relacionadas a mecanismos de defesa das uvas (ZHANG et al., 2022). Além disso, o SO_2 pode suprimir a atividade de enzimas relacionadas ao amolecimento das bagas e a degradação da membrana celular, suprimindo a degradação dos tecidos e a senescência das uvas (XUE et al., 2018). Por outro lado, o uso de SO_2 pode aumentar o degrane (CHEN et al., 2019), causar injúrias nas bagas (YUAN et al., 2022; ZOFFOLI et al., 2008), alterar o sabor (LURIE et al., 2006) e estar relacionado a possíveis impactos negativos à saúde humana (USTUN et al., 2012).

A utilização de tratamentos químicos, como o etanol, e físicos como a água quente e altas concentrações de CO_2 estão sendo avaliados como técnicas alternativas ao uso do SO_2 no controle de patógenos durante o armazenamento das uvas (CHIABRANDO; GIACALONE, 2020; ROMERO et al., 2021; SHAHKOOMAHALLY et al., 2021). Além de atuarem como agentes antimicrobianos, esses tratamentos podem afetar diversos processos metabólicos que estão relacionados a manutenção da qualidade das uvas. Recentemente foi proposto que o etileno pode mediar a ocorrência de alguns processos relacionados ao amadurecimento e a senescência das uvas, especialmente na ráquis (LI et al., 2015). Assim, a aplicação de inibidores da síntese do etileno, como o etanol e o CO_2, ou ainda inibidores da ação do etileno, como o 1-metilciclopropeno (1-MCP), poderiam reduzir o efeito do etileno no amadurecimento e senescência das uvas após a colheita (ZHU et al., 2020). Desta forma, a adoção de técnicas alternativas ao uso do SO_2 não está somente atrelada ao controle de patógenos, mas a aspectos fisiológicos e bioquímicos que suprimam a degradação das uvas.

Outra alternativa para reduzir as perdas e aumentar o aproveitamento das uvas, especialmente de cachos fora do padrão comercial, é a comercialização de bagas degranadas. Nesta forma de comercialização, as bagas são destacadas da ráquis e comercializadas em pequenas porções, ao invés de embalagens convencionais de cachos intactos (SABIR et al., 2021). Essa estratégia de comercialização reduz os inconvenientes encontrados em uvas comercializados na forma de cachos, como o escurecimento da ráquis e o degrane natural das bagas. Além disso, bagas provenientes de cachos pequenos e com baixa fertilização, que apresentam baixo valor comercial, poderiam ser aproveitadas. Por outro lado, o desprendimento da baga pode aumentar a suscetibilidade a incidência de patógenos e acelerar processos fisiológicos e bioquímicos, limitando a conservação das bagas a poucos dias (TYAGI et al., 2020). Assim, o estudo de técnicas de armazenamento é necessário para reduzir as perdas pós-colheita e viabilizar a comercialização das uvas por períodos prolongados.

1.1 HIPÓTESES

a) As novas cultivares de uvas de mesa ‘BRS Núbia’ e ‘BRS Isis’ apresentam bagas com epiderme de coloração escura, assim, podem apresentar uma maior concentração de compostos bioativos em relação à tradicional ‘Itália’ cultivadas em condições temperadas.
b) A água quente, etanol e o 1-MCP podem apresentar eficiência semelhante ou superior ao SO_2 no controle de patógenos durante o armazenamento de bagas degranadas.

c) A aplicação de etanol e o armazenamento com alto CO_2 apresentam efeito antimicrobiano e agem sobre a rota de síntese do etileno e no metabolismo respiratório, assim, atuam na manutenção da qualidade dos cachos de uvas de mesa.

1.2 OBJETIVOS

a) Caracterizar os parâmetros físico-químicos, compostos fenólicos e volátil de uvas de mesa 'BRS Núbia', 'BRS Isis' e 'Itália' produzidas na Serra Gaúcha.
b) Avaliar diferentes condições de armazenamento que possam substituir o uso de SO_2, a fim de prolongar a vida pós-colheita de uvas de mesa na forma de bagas degranadas.
c) Avaliar a utilização de etanol e de alto CO_2 como agentes antimicrobianos e seus efeitos na manutenção da qualidade de uvas de mesa durante o armazenamento prolongado de uvas na forma de cachos.

2 CAPÍTULO 1

2.1 CHARACTERIZATION OF TABLE GRAPES GROWN IN TEMPERATE CONDITIONS REGARDING PHYSICOCHEMICAL PROPERTIES, PHENOLIC AND VOLATILE COMPOUNDS[1]

ABSTRACT

This study aimed to characterize the physicochemical parameters and phenolic and volatile compounds of table grapes produced in temperate climates. 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes were analyzed according to their dimensions, colors, flavors, phenolic compositions, antioxidant activities, and volatile compounds in two crop seasons. 'BRS Nubia' grapes presented larger dimensions and mass, and, like the 'Italia', a more rounded shape, while the 'BRS Isis' presented berries with a more elongated shape and firmer, regardless of the maturation index. 'BRS Nubia' grapes have the highest concentrations of phenolic compounds in the skin, resulting from the higher concentration of anthocyanins. Interestingly, there was a low relationship between phenolic concentration in the skin and the antioxidant activity in the berries, with higher antioxidant activity being found in grapes with higher levels of soluble solids. 'Italia' grapes were more aromatic, especially because of the terpenes, found in higher concentrations in muscat-scented grapes. This study showed that the new cultivars, 'BRS Isis' and 'BRS Nubia', are rich in bioactive compounds, especially 'BRS Nubia', with large berries and dark color, while 'BRS Isis' stands out for the absence of seeds and crispy texture. On the other hand, the traditional 'Italia' was more aromatic in relation to the other cultivars, which presented an aroma characterized as neutral. This study reported relevant information on table grapes produced in temperate climates, especially for the new cultivars 'BRS Nubia' and 'BRS Isis', which are recent alternatives for production and consumption to the traditional 'Italia'.

Keywords: Berry quality, BRS Isis, BRS Nubia, Hybrid grapes, Italia

[1]Artigo formatado de acordo com as normas da revista Food Research International.

2.1.1 Introduction

Grape production is a traditional segment of fruit growing in Brazil. It is aimed at the production of wines, juices, and derivatives, and for fresh consumption, which in 2019 represented 49% of the destination of Brazilian grapes (IBGE, 2020). The growing interest in the consumption of fresh grapes is related to the diversity of cultivars that meet the demands of the consumers, such as grapes with large berries, seedless and dark color, with a crunchy, juicy texture and adequate relationship between sugars and organic acids (Maia et al. al., 2018; Zhou et al., 2015). Recently, other characteristics such as aroma (Rahman et al., 2021) and the concentration of phenolic compounds (Pinto et al., 2022) are being considered by consumers as parameters of grape quality. The latter promotes several health benefits due to their antioxidant, cardioprotective, anticancer, anti-inflammatory, and antimicrobial properties (Nassiri-Asl and Hosseinzadeh, 2016).

Brazilian viticulture is widespread from the southern (30°S) to the northern region (05°N) of the country, with considerable environmental diversity over the production zones, including temperate, subtropical, and tropical regions (Maia et al., 2018). The South region corresponds to 53.5% of the domestic production, in which Rio Grande do Sul stands out as the largest producer state, mainly of American grapes type (*Vitis labrusca*) (IBGE, 2020). The temperate climatic conditions (Cfb) in the Serra Gaúcha (Kuinchtner and Buriol, 2001), the leading producing region in the State of Rio Grande do Sul, are unfavorable for the cultivation of table grapes (*Vitis vinifera*), due to low insolation, high humidity, and frequent rainfall, which results in a higher incidence of pathogens and poor-quality grapes compared to other producing regions (Alderete, 2014; Echeverrigaray et al., 2020; Würz et al., 2020). However, in recent years, the cultivation of table grapes has increased in the southern region of the country (Lattuada et al., 2020). This expansion is the result of the advances in the techniques and management of vines and, above all, in the improvement and launch of new cultivars, mainly hybrids, with greater adaptability and resistance to pests, emerging as an alternative to the traditional cultivation of grapes of the ‘Italia’ group (Maia et al., 2018), enabling the cultivation of table grapes for fresh consumption in regions with a milder and more humid climate.

Among the most popular table grape cultivars recently widespread, ‘BRS Isis’ and ‘BRS Nubia’ stand out (Maia et al., 2018). The ‘BRS Isis’ table grapes, resulting from the cross between ‘CNPUV 681-29’ [Arkansas 1976 X CNPUV 147-3 (Niagara X Venus)] x ‘BRS Linda’, is a seedless cultivar, tolerant to downy mildew, which presents predominantly cylindrical-winged, compact clusters, with red berries, firm and colorless pulp with neutral flavor (Ritschel et al., 2013). The ‘BRS Nubia’ table grapes, resulting from the cross between

‘Michele Palieri’ x ‘Arkansas 2095’, is a seeded cultivar that presents relatively compact conical clusters, with large-sized bluish-black berries, firm and colorless pulp with a neutral flavor (Maia et al., 2013). Experiments carried out in subtropical (Cfa) and semi-arid (BSwh) regions characterized some organoleptic properties of these cultivars (Ahmed et al., 2019; Leão et al., 2020; Silvestre et al., 2017).

However, the sensory properties of the grapes are altered by several factors, especially the climatic conditions of the growing region (Oliveira et al., 2019; Padilha et al., 2019; Sun et al., 2015). Some works have reported the effects of climate on the quality of grapes, particularly on mass, size, color, sugar, organic acids, phenolic and volatile compounds (Mattar et al., 2019; Oliveira et al., 2019; Padilha et al., 2019; Sun et al., 2015; Urvieta et al., 2018; Xu et al., 2015). These properties related to the physiological aspects of ripening are crucial for various cultural practices, for setting the harvest (Leão et al., 2020). In addition, they are commonly associated with the quality aspect by consumers (Ahmed et al., 2019; Maia et al., 2018; Zhou et al., 2015) which are changed according to weather conditions during the productive period (Padilha et al., 2019).

Although some works studies report the characteristics of new table grape cultivars (Ahmed et al., 2019; Leão et al., 2020; Maia et al., 2013; Ritschel et al., 2013; Silvestre et al., 2017), there are no works in the literature that evaluate the organoleptic characteristics of cultivars in temperate conditions. Furthermore, the results obtained in these experiments cannot always be extrapolated from one region to another or from one cropping system to another. In this context, the objective of this study was to characterize the physicochemical parameters, phenolic and volatile compounds of table grape cultivars produced in a temperate climate, comparing them with the traditional cultivar of table grape ‘Italia’.

2.1.2 Material and methods

2.1.2.1 Plant material and sample preparation

‘BRS Isis’, ‘BRS Nubia’, and ‘Italia’ table grapes (Supplementary figure 1) were harvested in two commercial vineyards located in Serafina Corrêa, Rio Grande do Sul (RS), (28°42’49’’ S, 51°55’30’’ W, 509 m) and in São Domingos do Sul, RS, Brazil (28°30’20’’ S, 51°53'50" W, 660 m), in the Serra Gaúcha region, in March 2021 and 2022, respectively. The soil of the Serafina Corrêa-RS vineyard is classified as Argiluvic Chernosol and the soil of São Domingos do Sul-RS is classified as Haplic Nitosol (Santos et al., 2018). The climate is of the Cfb type (Kuinchtner and Buriol, 2001), according to the Köppen climate classification, with a mean annual temperature ranging from 16.1 to 17 °C and mean annual rainfall between 1,600

and 1,700 mm (Wrege et al., 2012). The vines, aged between three and seven years old, were grafted onto Paulsen 1103 rootstock, with a spacing of 2.0 x 2.8 m between plants and rows, respectively. The vines were conducted under a plastic cover, in a trellis system, with an irrigation system through drip tapes, and the addition of fertilizers, pest control, and other vine operations (pruning, thinning of berries, defoliation, and thinning of shoots) were carried out according to with the technical recommendations. The grapes were harvested at full ripening, with a soluble solids content above 15 °Brix. In each year, twelve clusters (~450 g) of each cultivar were collected, randomly selected from six vines, with three replications of four clusters each (Rahman et al., 2021). After harvesting, the clusters were transported to the Post-Harvest Research Center (NPP), at the Federal University of Santa Maria, RS, where the analyses were carried out.

2.1.2.2 Physical-chemical analyses

2.1.2.2.1 Resistance to abscission

Resistance to abscission was determined using the average force required to separate 30 berries from their respective clusters, measured with the aid of a precision balance (Marconi, AL500C), with results expressed in gram-force (gf) (Pinto et al., 2015).

2.1.2.2.2 Mass, length, diameter, mean geometric diameter, sphericity, surface area, and shape of the berries

To determine the mass, length, and diameter, 30 berries were analyzed per sample collected from the basal, central, and apical regions of the clusters. The mass of the berries was determined by weighing them on a precision scale (Marconi AL500C), and the results were expressed in grams (g). The length (L) and diameter (D) were determined in the central region of the berries with the aid of a manual caliper, the values being expressed in mm. The mean geometric diameter (Gd), sphericity (Sb), surface area (Sa), and the shape of the berries (Sb) were calculated using equations proposed by Mohsenin (1986):

$$Gd = (L \times D^2)^{\frac{1}{3}} \quad (1)$$

$$Sb = 100(Gd/L) \quad (2)$$

$$Sa = \pi \times Gd^2 \quad (3)$$

$$Fb = D/L \quad (4)$$

Where:

Dg and *Sb* are expressed in mm;

Sa is expressed in mm^2.

2.1.2.2.3 Firmness

Firmness is set from the insertion of an 8 mm tip with the aid of a manual penetrometer (Effegi, model FT 327, Milan, Italy) in the equatorial region of 20 berries per sample, collected randomly from each cluster. The values were expressed in Newton.

2.1.2.2.4 Soluble solids, acidity, and maturation index

From the crushing of berries randomly collected from each cluster in a centrifuge (Philips Walita®, RI1858, Barueri, Brazil), the extracted juice was placed on the prism of a refractometer, where the soluble solids content was determined, and the results were expressed in °Brix. A 10 mL aliquot of the same juice was diluted in 100 mL of distilled water, and titration was carried out using 0.1 N NaOH solution until reaching pH 8.1. Results were expressed as % tartaric acid. The maturation index was obtained through the ratio between soluble solids and acidity (Ahmed et al., 2019).

2.1.2.2.5 Color

The color of the berries was determined from the allocation of two berries placed side by side in a black-bottomed container where the color reading was performed with an electronic colorimeter (Konica Minolta CR-310, Osaka, Japan), previously calibrated with a white standard plate (Thewes et al., 2021). The container was fitted to the colorimeter reading area, providing the same distance for each reading, to reproduce each determination as faithfully as possible. Ten determinations were performed for each repetition, and six color parameters were determined: luminosity (L*), parameter a*, parameter b*, chroma (C*), hue angle (h°), and color index of red grapes (CIRG). L* varies between 0 (black) and 100 (white), a* represents the color between red and green and varies between 60 and -60, b* represents the color between yellow and blue and varies between 60 and -60, C* indicates the purity or intensity of the color, h° varies between 0 and 360°, with values close to 30, 90, 180 and 270° corresponding to the colors red, yellow, green and blue, respectively. Equation 5 was used to determine the CIRG, and the values close to 1, 3, and 6 correspond to the colors green, red, and purple, respectively (Carreño et al., 1995).

$$CIRG = (180 - h°)/(L^* + C^*) \quad (5)$$

2.1.2.2.6 Anthocyanins and yellow flavonoids

For the determination of anthocyanins and yellow flavonoids of the grapes, small portions of fresh skins were used, taken from the central region of 30 berries per sample and carefully detached from the pulp with a sterilized lamina and washed with distilled and deionized water, using samples of 1.5 g of the skin. The skins were added to amber flasks containing 15 mL of acidified methanol (1% HCl + 99% methyl alcohol) and left in the dark at room temperature for 48 h (Peppi et al., 2006). The samples were evaluated with the aid of a spectrophotometer (Femto 600 S, São Paulo, Brazil) at 520 nm for the determination of anthocyanins, and at 374 nm for the determination of yellow flavonoids, the results being expressed in mg 100 g^{-1} of fresh skin.

2.1.2.2.7 Flavonoids and total phenolic compounds

For quantification of the flavonoids and total phenolic compounds, small portions of fresh pulp and skins were removed from the central region of 30 berries, totaling 2 g per sample, being carefully separated with a sterilized lamina and washed with distilled and deionized water. The samples were homogenized in 20 mL of 50% methyl alcohol in a mixer (Philips Walita®, RI1602, Barueri, Brazil) for 2 min and kept for 2 h in the dark for extraction. After the extraction period, centrifugation was performed at 3,500 rpm for 15 min and the extract was filtered with filter paper (Ahmed et al., 2019). Total flavonoids were determined according to the methodology proposed by Woisky and Salino (1998). A 2 mL aliquot of the filtered extract was placed in test tubes wrapped in aluminum foil with 2 mL of 2% $AlCl_3$, which was stirred for 10 s with the aid of a vortex and kept in the dark for 1 h. Absorbance was obtained using a spectrophotometer with a reading of 415 nm. From the values obtained and discounting the value found in the blank sample, where extracting solution was used instead of the filtered extract, the flavonoid content was calculated from the calibration of a curve with quercetin. Values were expressed in mg of quercetin equivalent (QE) per 100 g^{-1} of fresh pulp or skin. The determination of phenolic compounds was based on the Folin-Ciocauteau method, as performed by Ahmed et al. (2019). A 0.2 mL aliquot of the extract was mixed in aluminum foil-wrapped test tubes with 1.8 mL of distilled water and 10 mL of Folin-Ciocauteau reagent diluted 10-fold. Next, 8 mL of 7.5% Na_2CO_3 solution were added. All test tubes with the mixture were vortexed for 10 s and kept in the dark for 2 h. The absorbance of each sample was obtained with the aid of a spectrophotometer at 765 nm, the value obtained in the blank sample, which was prepared with water instead of the extract, being discounted in the calculation. The determination of total phenolic compounds was calculated from the calibration of a curve with

gallic acid. Values were expressed in mg of gallic acid equivalent (GAE) per 100 g^{-1} of fresh pulp or skin.

2.1.2.2.8 Antioxidant activity

Juice samples from 50 berries were stored at -30 °C until analysis, when they were removed from the cold and thawed at room temperature. Afterward, they were centrifuged at 3,500 rpm for 15 min and the extract was filtered with filter paper. The determination of the antioxidant activity of the grapes was carried out using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. Determination using the ABTS method was performed according to Re et al. (1999). Briefly, the ABTS radical was formed by adding 88 µL of 140 mM potassium persulfate solution to 5 mL of 7 mM ABTS stock solution, followed by a 16 h incubation period in the dark. Then, the mixture was diluted in ethyl alcohol until obtaining an absorbance of 0.7 nm ± 0.05 nm with the aid of a spectrophotometer at 734 nm. Next, 100 µL of the sample was added to 3 mL of the ABTS radical, and after 6 min in the dark, the readings were performed in a spectrophotometer at 734 nm. The antioxidant activity by the DPPH method was obtained according to Sharma and Bhat (2009). Briefly, 100 µL of the sample was added to 2.9 mL of 60 µM DPPH solution dissolved in methyl alcohol. The samples were kept for 30 min in the dark and then readings were taken in a spectrophotometer at 515 nm. For both methods, results were obtained after plotting the reading values into a linear regression equation obtained from different concentrations of Trolox. Results were expressed as µM of Trolox equivalent (TEAC) per mL of juice.

2.1.2.2.9 Volatile organic compounds (VOCs)

Juice samples from 50 berries per sample were stored at -30 °C until analysis when they were thawed at room temperature. Therefore, 2 mL of juice were used and mixed with 0.6 g of NaCl and 10 µL of 3-octanol standard solution (16 µg mL^{-1}), placed in a 5 mL glass bottle, sealed with a screw cap, and kept for 5 min in a water bath at 35 °C. After stabilization of the sample temperature, the adsorption of VOCs was performed using solid phase microextraction (HS-SPME), by exposing a fiber covered with divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) (Supelco, 50/30 µm × 20 mm) in the internal and free space of the bottle for 60 min. The fiber was thermally desorbed in the injector of a gas chromatograph (DANI Instruments SpA., Viale Brianza, Cologno Monzese, Italy) at 250 °C for 10 min for desorption of VOCs. The compounds were separated on a DN-5 fused silica apolar capillary column

(Chrompack, USA; 30 m x 0.25 mm x 0.25 µm). The initial column temperature was adjusted to 35 °C and maintained for 3 min, followed by a temperature ramp from 1 °C min^{-1} to 45 °C, after 2 °C min^{-1} to 80 °C, then it was increased by 6 °C min^{-1} to 230 °C, remaining like this for 5 min. The flame ionization detector (FID) was maintained at 230 °C. VOCs were identified using a Shimadzu QP2010 Plus gas chromatograph coupled to a mass spectrometer (GC/MS; Shimadzu Corporation, Kyoto, Japan). The same extraction, desorption, and injection procedures were adopted to identify the VOCs described above, using helium as carrier gas. The MS detector was run in electronic ionization mode, with ionization energy of +70 eV, a scanning range of m/z 35–350, at 250 °C. The identification was performed based on the comparison with the library of mass spectra available at the National Institute of Standards and Technology (NIST) and with the calculated Kovats indices with those available in the scientific literature, according to the methodology proposed by Both et al. (2014). Each identified compound was semi-quantified in relation to the chromatographic peak and the concentration of the 3-octanol standard injected into each sample.

2.1.2.3 Statistical analysis

Statistical analyses were performed in order to compare the cultivars within each harvest. Data were submitted to normality analysis using the Shapiro-Wilk test and the analysis of variance homogeneity was done using the Bartlett test. Next, the results were submitted for analysis of variance, and the data that showed significant differences ($p \leq 0.05$) were submitted to the Tukey test at 5% probability, using the statistical program SISVAR version 5.6 (Lavras, MG, Brazil). The data were subjected to Pearson's correlation analysis, and for an overview of the results, the data were also subjected to principal components analysis using the MetaboAnalyst program. Before the multivariate analysis of the data, the data matrix for each variable was automatically scaled to obtain the same weight for all variables (mean = 0 and variance = 1).

2.1.3 Results and discussion

2.1.3.1 Principal components analysis

The analysis of the principal components presents an overview of the results obtained for the three assessed cultivars (Figure 1). For the first crop season (2021), the principal components (PC I and PC II) explained 92.1% of the total variation, where 57.6% were attributed to PC I and 34.5% to PC II (Figure 1a and b). PC I related the ‘Italia’ grapes to the highest maturation index, antioxidants, and, above all, the highest concentration of volatile

compounds, particularly terpenes, such as linalool, geraniol, nerol, citronelool, and α-terpineol, which confer a floral, sweet and citrus aroma to the grapes (Piazzolla et al., 2016), much appreciated by consumers (Ruiz-García et al., 2014). PC I was efficient to sort cultivars according to color, as color parameters such as luminosity, chroma, and b* were related to 'Italia' grapes, indicating light, intense and yellowish berries, while positive values of the color parameter a* indicates reddish hues, the 'BRS Isis' and 'BRS Nubia' were assigned. On the other hand, PC II related the 'BRS Nubia' to the size of the berries, and to the attributes of color, hue angle, and color index for red grapes (CIRG), which characterize a dark violet coloration of the epidermis, related to a higher concentration of anthocyanins, flavonoids and total phenolic compounds in the skin. These results corroborate other studies that obtained a higher concentration of bioactive compounds in dark-skinned grapes compared to light-skinned grapes (Aubert and Chalot, 2018; Mikulic-Petkovsek et al., 2018). Also, the 'BRS Isis' grapes was related to the shape and firmness of the berries, and, like 'Italia', to a higher concentration of phenolics in the pulp, because of the high ratio between pulp and skin in grapes (Zhu et al., 2019), may justify a higher antioxidant activity in these cultivars in relation to 'BRS Nubia'.

For the second crop season (2022), PC I and PC II explained 88.6% of the total variation found in the work, with 52.7 and 35.9% explained by PC I and PC II, respectively (Figure 1c and d). Through the separation attributed to PC I, the 'Italia' grapes were related to a light-shaded epidermis, according to a greater luminosity, chroma, and b* parameter, in addition to a greater concentration of volatile compounds, particularly terpenes and some aldehydes important to the aroma, such as hexanal and (E)-2-hexenal, which gives herbal notes to the aroma (Wu et al., 2019). PC I related the 'BRS Nubia' grapes to a higher concentration of phenolic compounds, flavonoids, and anthocyanins in the skin, which contributes to a more intense and dark coloration of the epidermis as indicated by the CIRG and the hue angle. On the other hand, PC II was efficient in separating cultivars according to the size of the berry, relating the 'BRS Nubia' to a greater mass, width, geometric diameter, and surface area, while 'BRS Isis' was related to shape, due to a higher length and width ratio. 'BRS Isis', is also related to greater firmness, soluble solids, and possibly due to the higher content of sugars, which contribute to the sequestration of free radicals (Bolouri-Moghaddam et al., 2010; Price et al., 2004; Van den Ende and Valluru, 2009), was related to greater antioxidant activity.

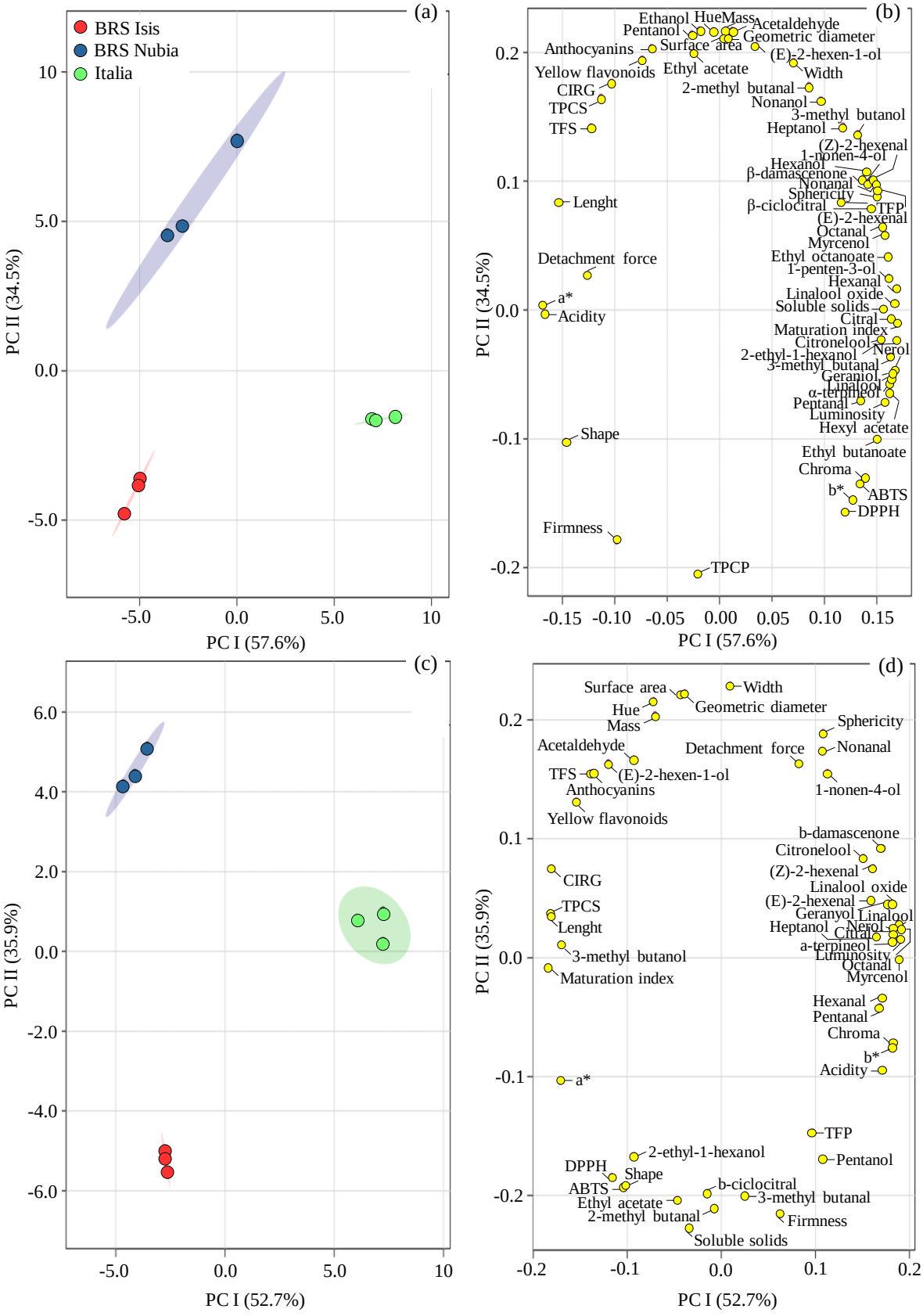

Figure 1. Principal components analysis of ‘BRS Isis’, ‘BRS Nubia’, and ‘Italia’ table grapes harvested in 2021 (a and b) and 2022 (c and d).

2.1.3.2 Flavor, texture, and dimensional parameters

The table grape cultivars differed significantly ($p \leq 0.05$) for all the assessed dimensional, texture, and flavor parameters (Figure 2). The berry detachment force, which is an index of adhesion force, is determined by the linking forces between the 'brush' and the berry pulp, and traction, in the abscission zone at the junction between the pedicel and the berry (Li et al., 2020). A greater detachment force was necessary to detach the berries of the cultivars 'BRS Isis' in the 2021 and for 'Italia' in 2022, not significantly differing from 'BRS Nubia' in both years (Figure 2a). The detachment force, commonly associated with the berry detachment potential (Li et al., 2020), may be related to the degree of maturation of the grapes (Supplementary figures 3 and 4) as the grapes that had a lower detachment force ('Italia' in 2021 and 'BRS Isis' in 2022) showed a higher maturation index, as a result of the higher concentration of soluble solids and lower acidity (Figure 2j, k, and l). During the ripening of grapes, the synthesis of auxins is reduced and there is an imbalance in the relationship between auxin and ethylene, which may result in several physiological and morphological changes (Li et al., 2020), such as tissue death and formation of intercellular cavities in the abscission zone (Xiao et al., 2021), promoting the detachment of berries.

The size of the berries is an important characteristic considered by consumers when purchasing table grapes (Maia et al., 2018). 'BRS Nubia' grapes presented the highest masses, not significantly differing from the 'Italia' grapes in 2021 (Figure 2b). This result may be related to a larger dimension of the 'BRS Nubia' grapes, according to the high correlations found for these parameters (Supplementary figures 3 and 4), as they presented berries with greater width, geometric diameter, and surface area, in addition to a greater length, a parameter that did not differ from 'BRS Isis' in both crop seasons (Figure 2c, d, e, and g). The dimensions of the berry are determined according to the genetic characteristics of the cultivars (Zhang et al., 2021; Zhang et al., 2022), rootstock, berry density, vine nutrition, hormonal balance, and the climate (Ahmed et al., 2019; Gatti et al., 2020; Leão et al., 2020; Mattar et al., 2019). In addition to being an important quality parameter for consumers of fresh grapes, the dimensions of the berry are considered by breeders for the development and selection of new cultivars, particularly when the goal is the clusters with large berries (Maia et al., 2018), and also by raisin producers, from the adoption of strategies to optimize the drying of fresh grapes, as the drying time is changed according to the surface area of the berries (Patidar et al., 2021).

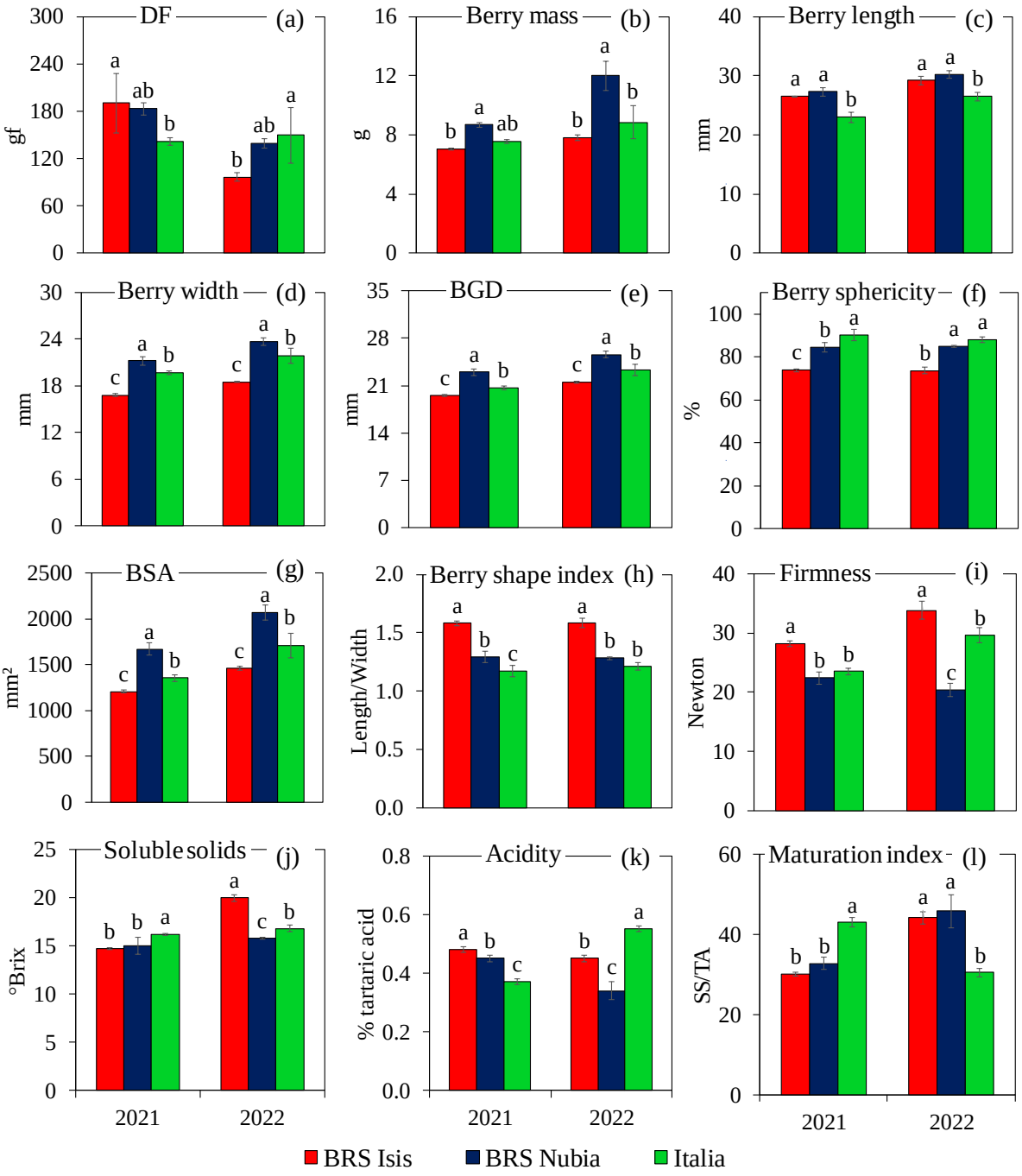

Figure 2. Dimensional, texture, and flavor parameters of 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes harvested in 2021 and 2022. DF: detachment force; BGD: Berry geometric diameter; BSA: surface area of berries. Mean value ± standard deviation (n = 3). Means followed by the same letter in the same year do not differ by Tukey's test ($p \leq 0.05$).

Regarding the shape of the berries, the 'Italia' grapes presented berries with greater sphericity, not differing from 'BRS Nubia' in 2022, while berries of 'BRS Isis' presented the lowest values (Figure 2f). This result is related to a lower ratio between length and width (Figure

2h) which is found in ‘Italia’ and ‘BRS Nubia’ grapes, giving a more rounded shape to the berries, while the ‘BRS Isis’ grapes presented berries with a more elongated shape. Berry length and width are determined by cell proliferation, which includes the rate and duration of cell division, and cell expansion, affecting the size of the organ (Zhang et al., 2021). Therefore, cell division and expansion lead to the formation of berries in different shapes, and genes related to transcription factors, cell wall metabolism, plant hormones, ubiquitin ligases, and serine/threonine protein kinases have been reported as potential characterizing agents of the shapes of the berries (Zhang et al., 2022).

Besides the visual aspect, the texture and flavor parameters are important quality characteristics considered by consumers (Maia et al., 2018). Concerning texture, determined according to the firmness of the berries, ‘BRS Isis’ grapes showed the highest values, in both crop seasons (Figure 2i), regardless of the maturation index. This is a relevant result since crunchier grapes are more appreciated by consumers (Zhou et al., 2015). Regarding flavor, values of soluble solids were found in 2021 ranging from 14.7 to 16.2 °Brix for the cultivars ‘BRS Isis’ and ‘Italia’ respectively, while in 2022, the cultivars presented values between 16.8 and 20 °Brix, for ‘BRS Nubia’ and ‘BRS Isis’, respectively (Figure 2j). Regarding acidity, the highest concentrations of acids were found in ‘BRS Isis’ and ‘Italia’ grapes in the 2021 and 2022 crop seasons, respectively, while the lowest was found in ‘Italia’ and ‘BRS Nubia’ grapes for these crops seasons (Figure 2k). These results are similar to those found in the literature for ‘BRS Isis’ (14.2 to 21.3 °Brix and 0.3 to 0.8% tartaric acid) (Ahmed et al., 2019; Leão et al., 2020; Ritschel et al., 2013), ‘BRS Nubia’ (11.9 to 19.0 °Brix and 0.5 to 0.8% tartaric acid) (Maia et al., 2013; Silvestre et al., 2017) and ‘Italia’ grapes (14.8 to 21.2 °Brix and 0.3 to 1.0% tartaric acid) (Auber and Chalot, 2018; Mikulic-Petkovsek et al., 2018; Piazzolla et al., 2016), where several factors related to cultural practices, climatic conditions (Supplementary figure 2), and ripening stage affect the concentrations of sugars and acids in grapes (Ahmed et al., 2019; Leão et al., 2020; Padilha et al., 2019; Piazzolla et al., 2016; Silvestre et al., 2017). The maturation index, determined from the ratio between soluble solids and acidity, was higher in ‘Italia’ grapes in 2021, while in 2022, the highest rates were found in ‘BRS Isis’ and ‘BRS Nubia’ grapes. However, all cultivars showed a high TSS/TA ratio (>18) (Ahmed et al., 2019), indicating that the cultivars presented sugar and acid ratios that meet the consumers’ requirements.

2.1.3.3 Color, phenolic compounds, and antioxidant activity

The table grape cultivars differed significantly ($p \leq 0.05$) for all the assessed parameters of color, phenolic compounds, and antioxidants (Figure 3). Color is one of the most important visual characteristics of table grapes, and this attribute is commonly associated with cluster quality and directly related to consumer acceptance (Ahmed et al., 2019). The epidermis color of the grapes was well characterized according to luminosity, a*, b*, chroma, hue, and CIRG (Figures 3a to f). Luminosity is a parameter that indicates the brightness of colors, thus berries with more intense and darker pigmentation, are indicated by positive values in the a* color parameter, such as 'BRS Isis' and negative values for the b* parameter, found in 'BRS Nubia' grapes, characterized the new cultivars with less brightness and greater color opacity in relation to 'Italia' grapes.

The color of the berries can also be characterized by the hue angle and the CIRG (Figures 3e and f), as proposed by Carreño et al. (1995). For both color scales, the cultivars 'BRS Isis', 'BRS Nubia', and 'Italia' displayed red, dark violet, and yellowish-green coloration, respectively. Hue values close to 90, 30, and 300° and CIRG values close to 1, 3, and 6 correspond to the colors yellow, red, and violet, respectively (Carreño et al., 1995). These results corroborate those found in other studies for the cultivar 'Italia' (Mikulic-Petkovsek et al., 2018; Piazzola et al., 2016). However, Ahmed et al. (2019) reported a lower color intensity (h° = ~56) for 'BRS Isis' grapes grown in a subtropical climate compared to the results of this study (h° = ~45). These results may be related to climatic conditions during grape maturation, which has a high influence on the color of the epidermis (Ahmed et al., 2019), especially in colored cultivars (Oliveira et al., 2019). Among the main climatic factors, the temperature influences the pigmentation of grapes (Urvieta et al., 2018), and temperatures between 17 and 26 °C are ideal for the accumulation of anthocyanins (Oliveira et al., 2019). This is because the color of the berry epidermis is largely controlled by a single locus on chromosome 2, where in unfavorable conditions, a retrotransposon insertion into the *MYBA1* gene results in a loss of pigmentation interrupting anthocyanin biosynthesis (Kobayashi et al., 2004). Thus, unlike the climatic conditions of the present study (Supplementary figure 2), cultivation in warmer climates, where there are high average temperatures and low daily thermal amplitude (Oliveira et al., 2019), commonly requires the application of products such as abscisic acid and ethephon, to intensify and standardize the color, and their applications are not always effective (Roberto et al., 2013).

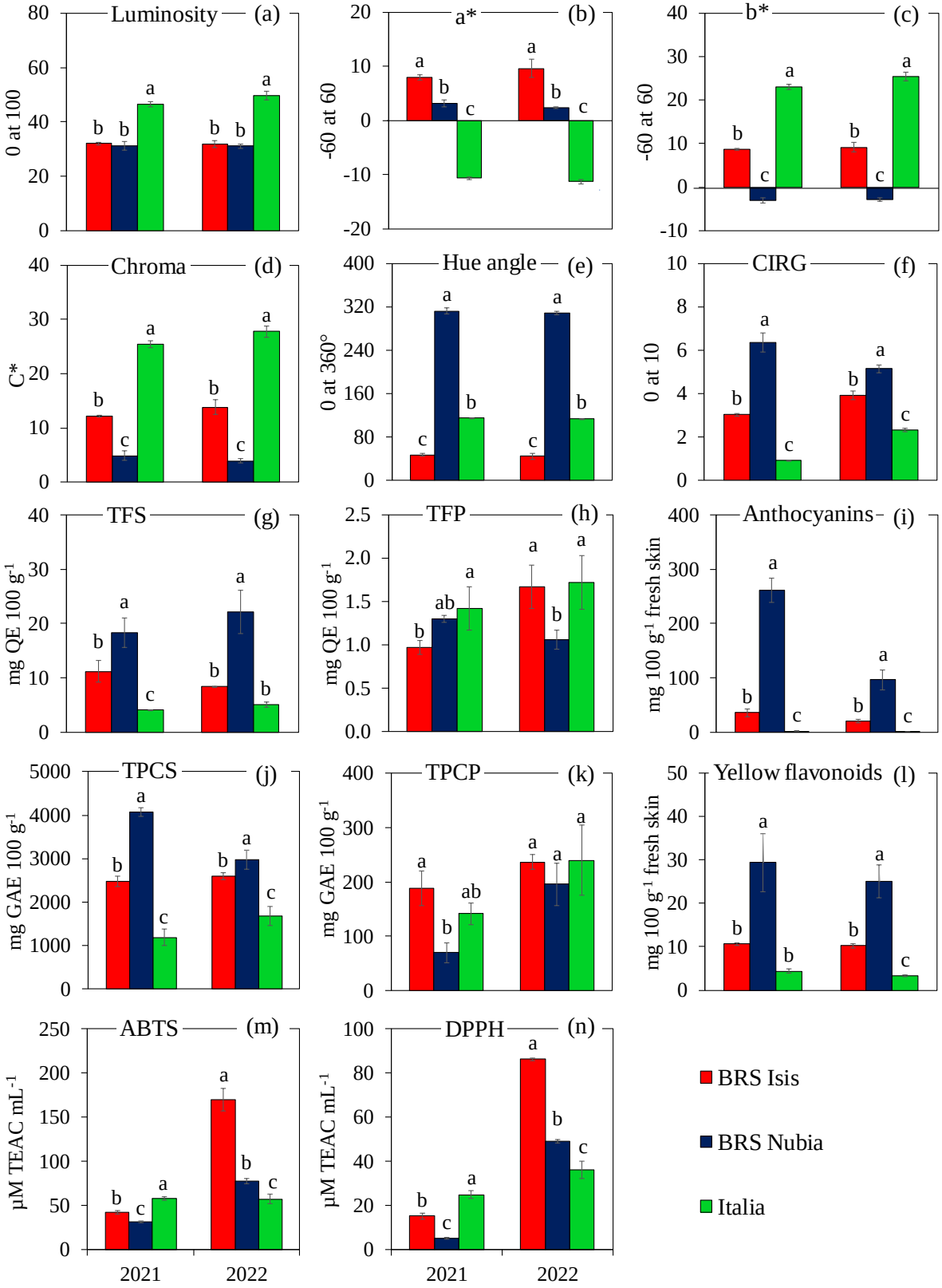

Figure 3. Color parameters, phenolic compounds, and antioxidant activity of 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes harvested in 2021 and 2022. CIRG: color index for red grapes; TFS: total flavonoids in the skin; TFP: total flavonoids in the pulp; TPCS: total phenolic compounds in the skin; TPCP: total phenolic compounds in the pulp. Mean value ± standard

deviation (n = 3). Means followed by the same letter in the same year do not differ by Tukey's test ($p \leq 0.05$).

The ‘BRS Nubia’ grapes showed the highest concentration of bioactive compounds in the skin (Figure 3g, i, j, and l), especially anthocyanins, with concentrations on average of 6 and 108 times higher than the cultivars ‘BRS Isis’ and ‘Italia’, respectively. These results corroborate those obtained by Aubert and Chalot (2018), who found up to 162 times more anthocyanins in dark-skin cultivars compared to light-skin cultivars, such as ‘Italia’ grapes. A higher concentration of anthocyanins found in ‘BRS Nubia’ grapes contributed to a greater contraction of phenolics in the skin of these grapes (Figure 3j), as anthocyanins are the primary phenolic compounds found in the skin of the grapes (Pastrana-Bonilla et al., 2003). In addition, the cultivars had about 14 times more phenolic compounds in the skin than in the pulp (Figure 3j and k). However, due to the high pulp-to-skin ratio found in grapes (Zhu et al., 2019), the concentration of phenolics in the pulp has a greater contribution to the total phenolic concentration of the berries (Pastrana-Bonilla et al., 2003). In the present study, ‘BRS Isis’ grapes showed the highest concentrations of phenolics in the pulp, not differing from ‘Italia’ in 2021, while in 2022, there was no difference between cultivars (Figure 3k). Although factors related to cultural practices (Ahmed et al., 2019; Pinto et al., 2022) and climate (Oliveira et al., 2019; Padilha et al., 2019; Urvieta et al., 2018), may alter the phenolic composition of grapes, the phenolics are particularly dependent on the genetic characteristics of the cultivars (Mikulic-Petkovsek et al., 2018). Aubert and Chalot (2018) reported that red or violet cultivars have high concentrations of phenolics from the anthocyanin group, while yellow-green cultivars have especially high levels of the hydroxycinnamic acids and flavanols group, which are related to the maintenance of physiological and biochemicals processes such as antioxidants (Cheng et al., 2017).

In addition to playing an important role in the quality of grapes due to their contribution to flavor and color (Aubert and Chalot, 2018), polyphenols also have antioxidant activities (Cheng et al., 2017). Higher antioxidant activity was found in ‘Italia’ grapes in the 2021 harvest and ‘BRS Isis’ in 2022, for both assessed methods (Figures 3m and n). These results may be related to the maturation index, especially to the soluble solids content (Supplementary figures 3 and 4), as a higher concentration of sugars can increase their availability for the oxidative pathway of pentose phosphate, creating a greater reducing power to produce glutathione (Bolouri-Moghaddam et al., 2010). In addition, some oligo and polysaccharide sugars, such as raffinose and fructans, appear to be effective free radical scavengers (Van den Ende and

Valluru, 2009), in addition to modulating the gene expression of enzymes related to oxidative stress, such as chalcone synthase, glucose-6-phosphate dehydrogenase and glutathione-S-transferase (Price et al., 2004), positively affecting the antioxidant activity. Another well-known aspect is the antioxidant capacity of phenolic compounds in grapes (Cheng et al., 2017). Thus, antioxidant activity is also regulated, in part, by the concentration of phenolic compounds, especially flavones, isoflavones, flavonones, flavonols, anthocyanins, and catechins (Pastrana-Bonilla et al., 2003). Thus, although 'BRS Nubia' grapes showed a higher concentration of phenolics in the skin due to the high ratio between pulp and skin (Zhu et al., 2019), the antioxidant activity was related to the concentration of phenolic compounds in the pulp (Supplementary figures 3 and 4), justifying a lower antioxidant activity in 'BRS Nubia' grapes compared to other cultivars, especially in 2021 crop season.

2.1.3.4 Volatile organic compounds (VOCs)

Based on the concentration of VOCs, grapes are commonly sorted into three groups: Foxy aroma, attributed to *V. labrusca* cultivars and its hybrids, which present an abundance of esters; Muscat aroma, attributed to *V. vinifera* cultivars and their hybrids, which present high concentrations of terpenes and; Neutral aroma, mainly attributed to *V. vinifera* cultivars, which have less volatiles other than C6 compounds (Yang et al., 2009). In the literature, some studies have reported the volatile profile of 'Italia' grapes, being classified as having a muscat aroma (Aubert and Chalot, 2018; Piazzolla et al., 2016). However, there are no works that describe the volatile profile of the new hybrid cultivars 'BRS Isis' and 'BRS Nubia' grapes, even though they are classified by their developers as having neutral aroma (Maia et al., 2013; Ritschel et al., 2013). In this work, thirty-eight VOCs were identified, where 11 were aldehydes, 10 alcohols, 10 terpenes, 5 esters, and 2 ketones (Figures 4, 5, and 6).

2.1.3.4.1 Aldehydes

The 'Italia' grapes showed the highest concentrations of aldehydes in both evaluated years. The lowest values were found in 'BRS Isis' grapes, not differing in 2022 from the 'BRS Nubia' (Figure 4a). The C6 aldehyde compounds, which give green notes to the aroma, are the basic background of volatiles in grapes (Wu et al., 2019) as well as the products of the enzymatic degradation of unsaturated fatty acids, which are converted into other compounds during ripening, reducing its concentration according to the increase in enzymatic activity caused by the gradual disintegration of the cellular structure (Yang et al., 2011). The hexanal, which is produced by the oxidation of linoleic and linolenic acids catalyzed by the enzyme

lipoxygenase (Noguerol-Pato et al., 2012), and (E)-2-hexenal, which is produced by the isomerization of (Z)-3-hexenal (Zhu et al., 2012) from linolenic fatty acid, were the predominant aldehydes in all cultivars (Figure 4f and h), both above their sensory thresholds (15 and 17 µg L^{-1}, respectively) (Yao et al., 2021). Similar results were found for 'Centennial Seedless', 'Chasselas', 'Italia', 'Italia Rubi', 'Alphonse Lavallée', and 'Muscat Hambourg' table grapes (Auber and Chalot, 2018). In the present study, 'Italia' grapes showed the highest concentrations of hexanal and (E)-2-hexenal, not differing for the latter compound from 'BRS Nubia' in both years. Furthermore, the variation found between the years may be due to climatic conditions, as the abundance of lipoxygenase is increased in conditions of lower water availability after veraison (Deluc et al., 2009; Xu et al., 2015), as seen after January (veraison) in 2022 (Supplementary figure 2), thereby higher levels of C6 aldehydes could be produced.

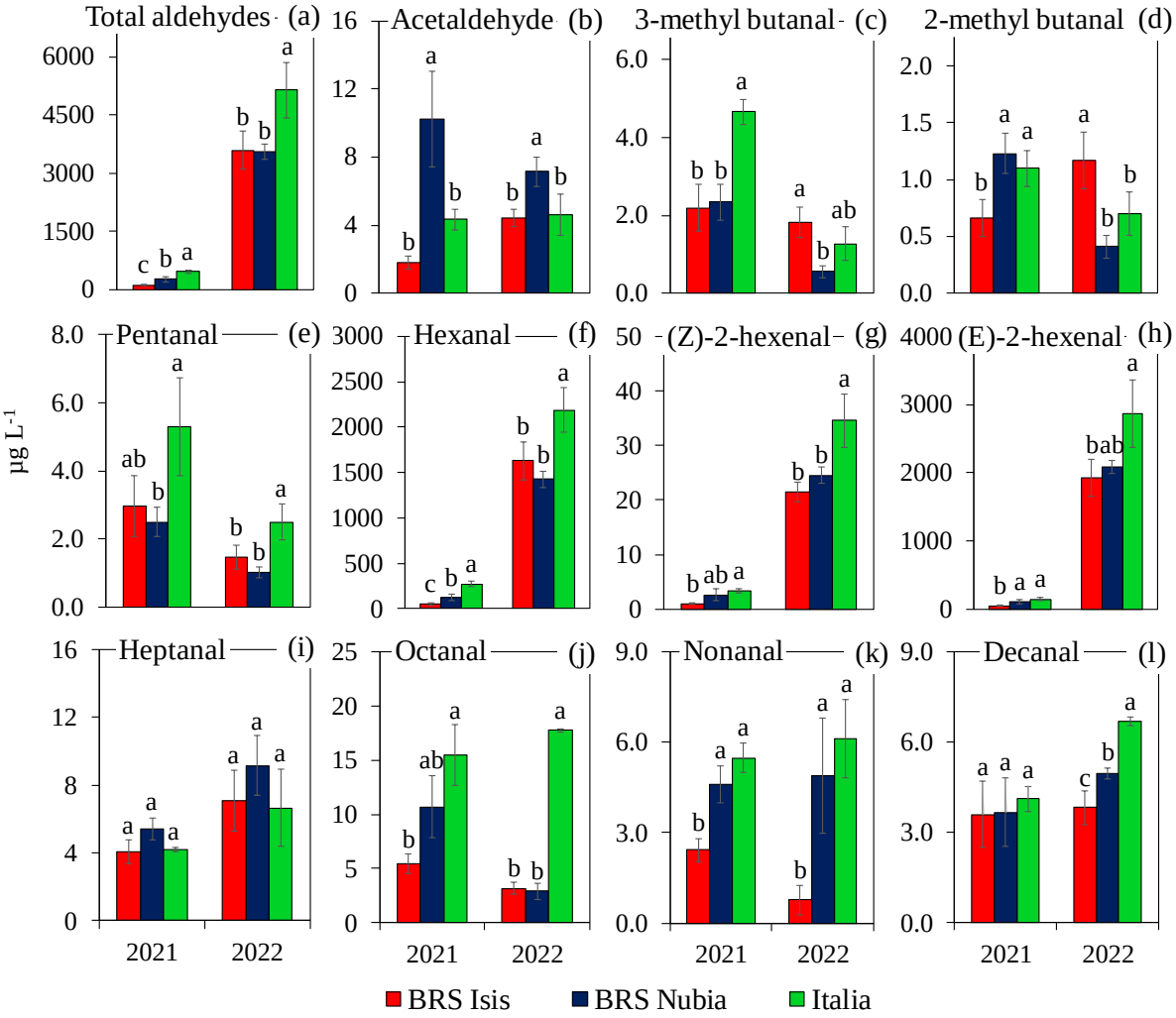

Figure 4. Aldehydes found in 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes harvested in 2021 and 2022. Mean value ± standard deviation (n = 3). Means followed by the same letter in the same year do not differ by Tukey's test ($p \leq 0.05$).

2.1.3.4.2 Alcohols

Alcohols are known for imparting green notes to the aroma of grapes (Piazzolla et al., 2016). The highest concentrations of alcohols were found in 'BRS Núbia' grapes at both assessed harvests (Figure 5a). Hexanol and (E)-2-hexen-1-ol, formed by the reduction of their respective aldehydes by the enzyme alcohol dehydrogenase (Yao et al., 2021), were the alcohols found at the highest concentrations in the cultivars (Figures 5f and g). These compounds have also been reported as the major alcohols in other cultivars, such as 'Brancellao', 'Hutai-8', 'Moravia Agria', 'Jingxi', 'Bimeijia', and 'Jingya' (García-Carpintero et al., 2011; Noguerol-Pato et al., 2012; Yang et al., 2011; Yao et al., 2021). Auber and Chalot (2018) also reported high concentrations of hexanol in 'Italia', 'Italia Rubi', 'Alphonse Lavallée', and 'Muscat

Hambourg' grapes. In the present work, 'BRS Nubia' grapes showed the highest concentrations of (E)-2-hexen-1-ol in both years, not differing from the 'Italia' cultivar in 2021, which also showed the highest concentrations of hexanol in 2021, while in 2022, there were no differences among cultivars. Ethanol is formed from acetaldehyde (Figure 5b) through the action of the enzyme alcohol dehydrogenase. The presence of this alcohol may be related to cellular senescence during ripening (Yao et al., 2021). Interestingly, its concentration was higher in 'BRS Nubia' grapes in 2021, although its maturation index was lower than the 'Italia' grapes. However, due to their high sensory thresholds (Wu et al., 2016), the contributions of alcohols to the overall aroma are small, as reported by Yao et al. (2021).

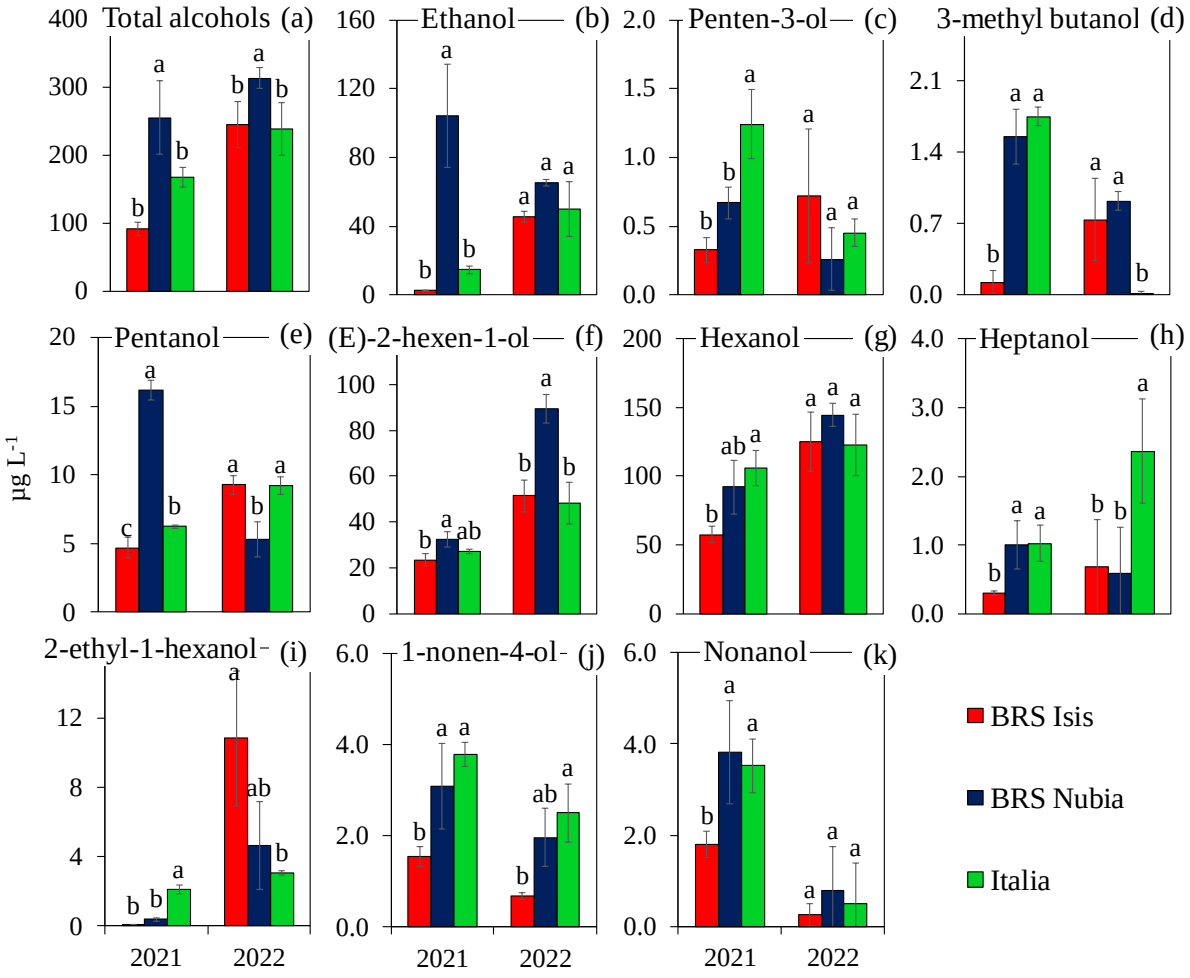

Figure 5. Alcohols found in 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes harvested in 2021 and 2022. Mean value ± standard deviation (n = 3). Means followed by the same letter in the same year do not differ by Tukey's test ($p \leq 0.05$).

2.1.3.4.3 Terpenes, esters, and ketones

Terpenes are compounds that impart a floral aroma to grapes (Piazzolla et al., 2016), where the main aromatic compound in grapes classified as having a muscat aroma (Rahman et al., 2021), the most attractive and known aroma by consumers (Ruiz- Garcia et al., 2014). 'Italia' grapes showed the highest terpene concentrations, with values ranging from 7.6 to 54.9 times higher than the concentrations found in the other two cultivars (Figure 6a). Terpenes are synthesized from the precursors of isopentenyl diphosphate and its isomer, dimethylallyl diphosphate. In grapes, these five-carbon precursors are synthesized mainly from the pathway plastid 2-methyl-D-erythritol-4-phosphate, which provides substrates for the synthesis of monoterpenes (C10) (Yue et al., 2020), being catalyzed by terpene synthases (TPS) and monoterpenol β-D-glycosyltransferases (GTs) (Luo et al., 2019). The biosynthesis and accumulation of monoterpenes are affected by several factors, such as the ripening stage of the grapes (Luo et al., 2019; Yang et al., 2011) and the position of the berry in the cluster (Noguerol-Pato et al., 2012), but particularly by the climatic conditions (Wen et al., 2015) and genetic differences among cultivars (Yue et al., 2020; Wu et al., 2016; Wu et al., 2019). In some cultivars, the concentration of terpenes can correspond between 0.16 and 55.31% of the total concentration of volatiles (Rahman et al., 2021), and the expression of *VviTPSs* and *VviGTs* genes, such as *VviGT14*, *VviUGT88A1L1*, and *VviPNLinNer1* in 'Riesling' grapes and *VviPNLGl1*, *VviPNLGl2* and *VviPNLGl4* in 'Muscat Hambourg' grapes appear to regulate the biosynthesis and accumulation of terpenes (Yue et al., 2020).

Monoterpenes, such as linalool, geraniol, nerol, and citronelool are the major terpenes found in grapes characterized by a muscat aroma (Yang et al., 2009). Linalool which is produced directly from geranyl diphosphate (Luo et al., 2019), imparts citrus and sweet notes (Piazzolla et al., 2016) and it is found mainly in the pulp of grapes (Wu et al., 2016). The concentration of this terpene was higher in 'Italia' grapes in both crop seasons, while the lowest concentrations were found in 'BRS Isis' grapes, which did not differ from 'BRS Nubia' in 2022 (Figure 6e). These results correspond to concentrations in 'Italia' that are 163 and 215 times higher than 'BRS Isis' and 25 and 67 times higher than 'BRS Nubia' in 2021 and 2022, respectively. Other studies that evaluated the volatile profile of 'Italia' grapes also characterized linalool as the terpene found in the highest concentrations (Piazzolla et al., 2016; Ruiz-García et al., 2014). The variation between years may be due to solar radiation (Supplementary figure 2), as the transcription of the *VviPNLinNer1* gene involved in the linalool synthesis pathway increases with increasing sun exposure (Zhang et al., 2017), which may explain a higher concentration of linalool in 2022.

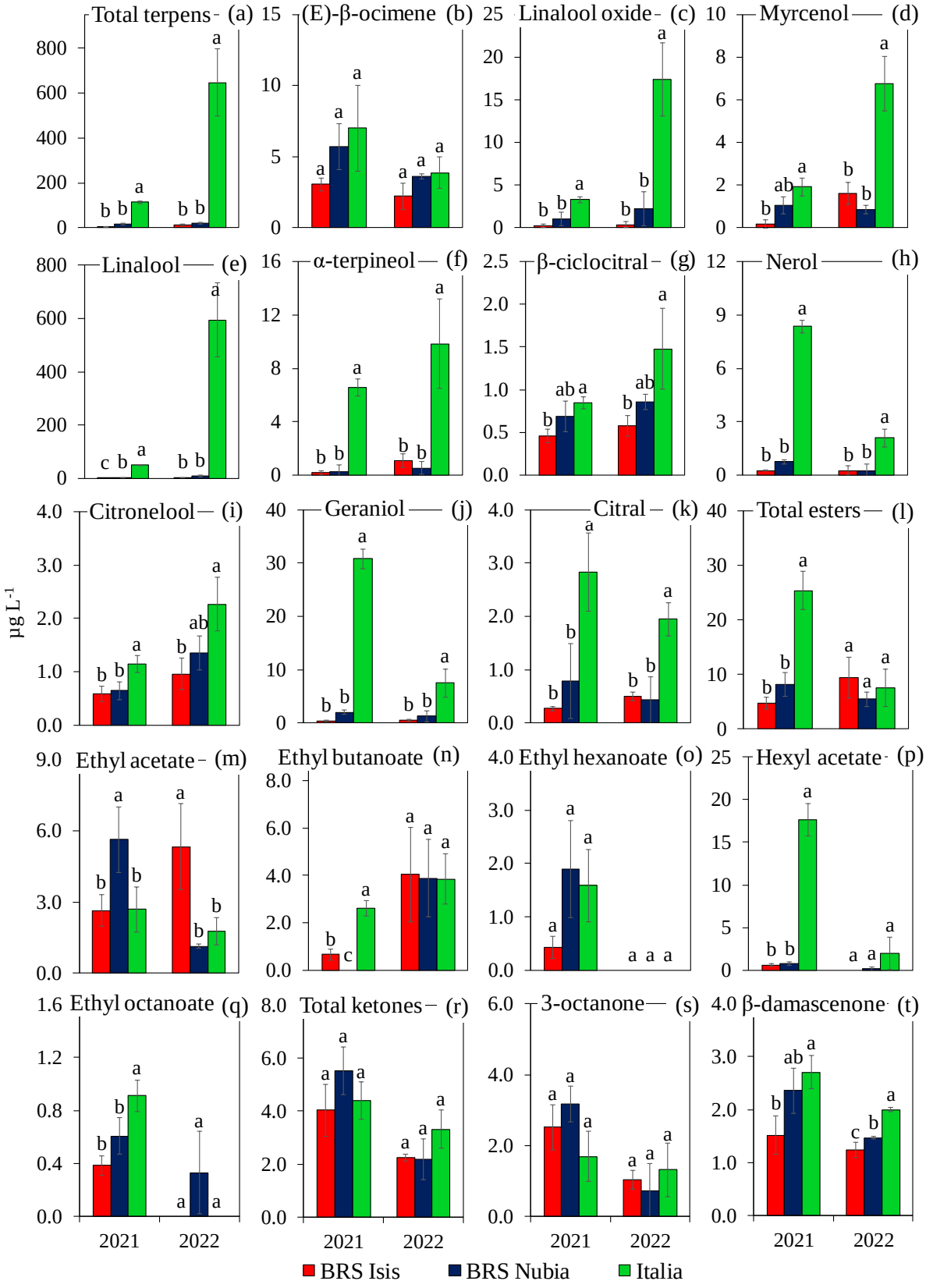

Figure 6. Terpenes, esters, and ketones, found in 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes harvested in 2021 and 2022. Mean value ± standard deviation (n = 3). Means followed by the same letter in the same year do not differ by Tukey's test ($p \leq 0.05$).

Geraniol, unlike linalool and despite its origin from the same precursor (geranyl diphosphate) (Luo et al., 2019), confers floral notes (Piazzolla et al., 2016) besides being commonly found in the skin of the grapes (Wu et al., 2016). In addition, it is less sensitive to solar radiation, and may even reduce the concentration as the solar radiation increases (Zhang et al., 2017), as found in the present study (Supplementary figure 2). 'Italia' grapes had the highest concentrations of geraniol in both years (Figure 6j). Geraniol was the second main terpene found in 'Italia' grapes, corroborating the results of some studies on cultivars with muscat aroma (Yang et al., 2009; Yang et al., 2011; Yue et al., 2020). 'Italia' grapes showed the highest concentrations of geraniol derivatives, such as nerol (Figure 6h), which is formed from geraniol by an unknown isomerase, and citronelool (Figure 6i), which is reduced from geraniol by an unknown reductase enzyme (Lin et al., 2019). This last compound does not differ from 'BRS Nubia'. On the other hand, lower concentrations of nerol and citronelool were found in 'BRS Isis' grapes, where the latter did not differ from 'BRS Nubia' in the 2022 crop season. The new cultivars 'BRS Isis' and 'BRS Nubia', as well as those found for linalool, showed low concentrations of geraniol and its derivatives. Such results are commonly seen in cultivars with neutral aroma (Aubert and Chalot, 2018; Wu et al., 2016; Yang et al., 2009; Yang et al., 2011).

Esters, which are synthesized according to enzymatic β-oxidation and specificity in the fatty acid metabolism pathway (Dudareva et al., 2004), are mostly found in *V. labrusca* grapes (Rahman et al., 2021). Also, they are related to the foxy aroma, although some esters such as ethyl acetate, ethyl butanoate, and ethyl hexanoate are the major compounds related to the strawberry aroma in grapes (Yang et al., 2009). In this experiment, 'Italia' grapes had the highest concentrations of total esters in 2021, while no differences were found among the cultivars in 2022 (Figure 6l). However, all esters found in this work had concentrations below their sensory thresholds (Yao et al., 2021), therefore, they did not contribute much to the aroma of the cultivars. Yang et al. (2011) found higher concentrations of esters in hybrid grapes ('Jingya') with strawberry aroma, while in *V. vinifera* grapes with neutral ('Jingxiu') and muscat ('Bimeijia') aroma, the esters provided small contributions to it. Wang et al. (2016) found a higher concentration of esters in *V. labrusca* grapes, while the concentration of esters varied significantly among the hybrid cultivars. According to the authors, this variation between hybrid cultivars is the result of the genetic characteristics inherited from their parents. These results reinforce the hypothesis that the new cultivars 'BRS Isis' and 'BRS Nubia', due to their low concentrations of compounds in addition to C6 aldehydes, have a neutral aroma.

Regarding total ketones, no significant differences were found among the cultivars (Figure 6r), which showed concentrations in 2021 and 2022, between 4.03 and 5.53 μg L^{-1} and

2.18 and 3.31 µg L^{-1}, respectively. Although ketone concentrations are lower than other aromatic groups (Figure 4a, 5a, 6a, and l), their sensory thresholds are generally low (Luo et al., 2019), and are commonly associated with a greater presence of fruity, floral, and sweet notes, linked to honey and roasted apple (García-Carpintero et al., 2011; Wu et al., 2016). Among the identified ketones, only the concentrations of β-damascenone, which are probably produced through direct TPS biosynthesis activities or enzymatic hydrolysis of norisoprenoid glycosides in the berries (Luo et al., 2019), differed significantly among cultivars (Figure 6t), being above its sensory threshold (0.05 µg L^{-1}) (Yao et al., 2021). 'Italia' grapes showed the highest concentrations of β-damascenone in both years, not differing from 'BRS Nubia' in 2021, while the lowest concentrations were found in 'BRS Isis', not differing from 'BRS Nubia' in the first year. Like some esters, high concentrations of β-damascenone (~200 µg L^{-1}) are commonly found in strawberry-aroma scented grape cultivars (Wu et al., 2016; Yao et al., 2021).

2.1.4 Conclusion

The three table grape cultivars evaluated in this study showed different characteristics of color, size, texture, and flavor, in addition to phenolic composition, antioxidant and volatile profiles, which are mainly genetic characteristics of each cultivar. 'BRS Nubia' grapes presented the largest berries, rounded shape, dark violet color, and especially because of the anthocyanins, presented the highest concentrations of phenolic compounds in the skin. On the other hand, the antioxidant activity was related to the concentration of soluble solids and the concentration of polyphenols in the pulp of the grapes. 'BRS Isis' grapes presented elongated berries, reddish color, and more firmness, regardless of the maturation index, being a relevant characteristic for consumers who appreciate more crunchy grapes. 'Italia' grapes presented berries with a rounded shape, and yellowish-green color, with low concentrations of polyphenols in the skin and a high concentration of volatile organic compounds.

This study reported the volatile profile of the new cultivars 'BRS Isis' and 'BRS Nubia', which showed only high concentrations of hexanal and (E)-2-hexenal, which characterizes them as having a neutral aroma, while the cultivar 'Italia' showed generous concentrations of terpenes, especially linalool, being found mainly in muscat-scented grapes. Finally, relevant information was reported on table grapes produced in temperate climates, especially regarding the new cultivars 'BRS Nubia' and 'BRS Isis', which are recent alternatives for production and consumption to the traditional 'Italia' grapes.

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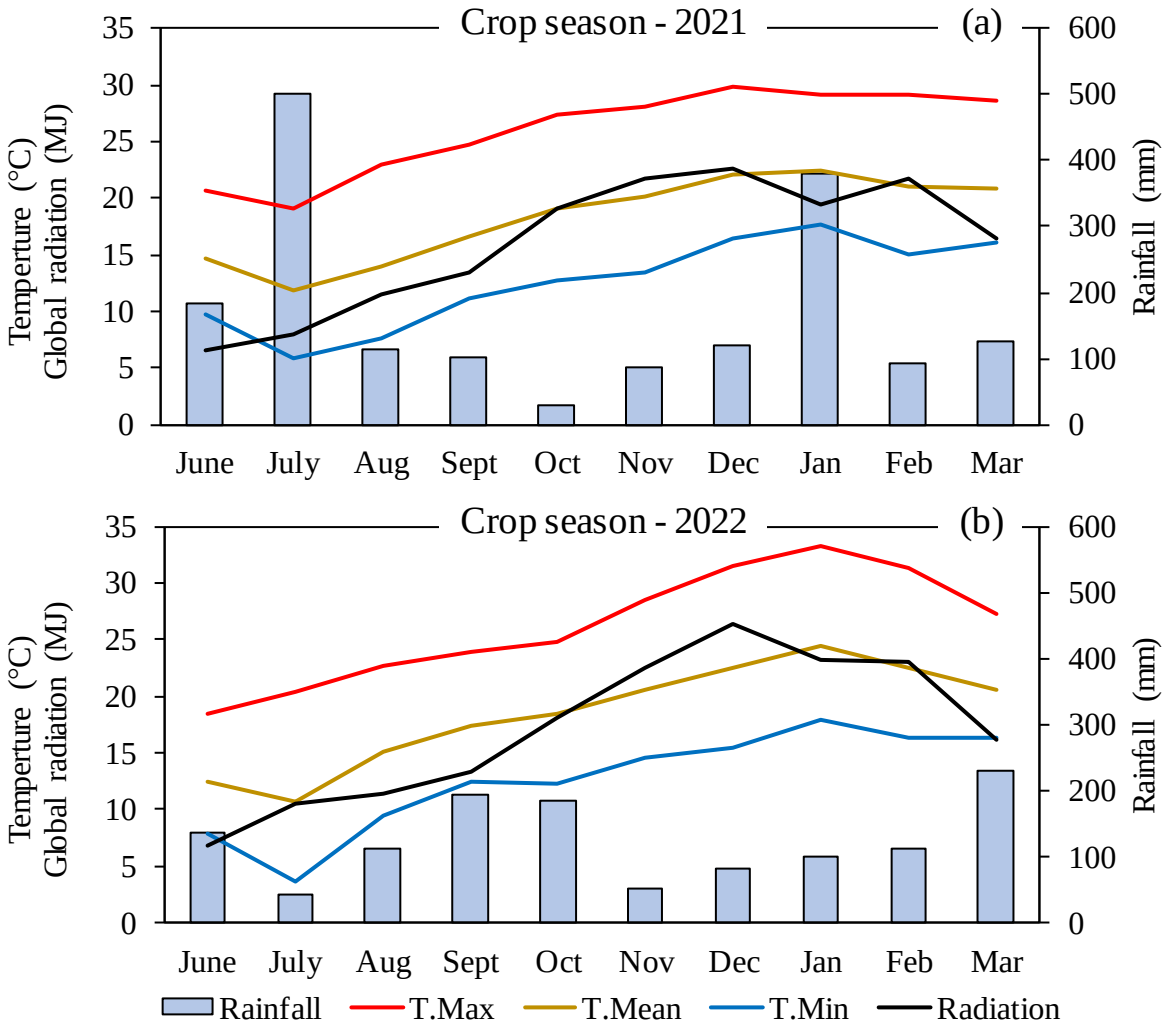
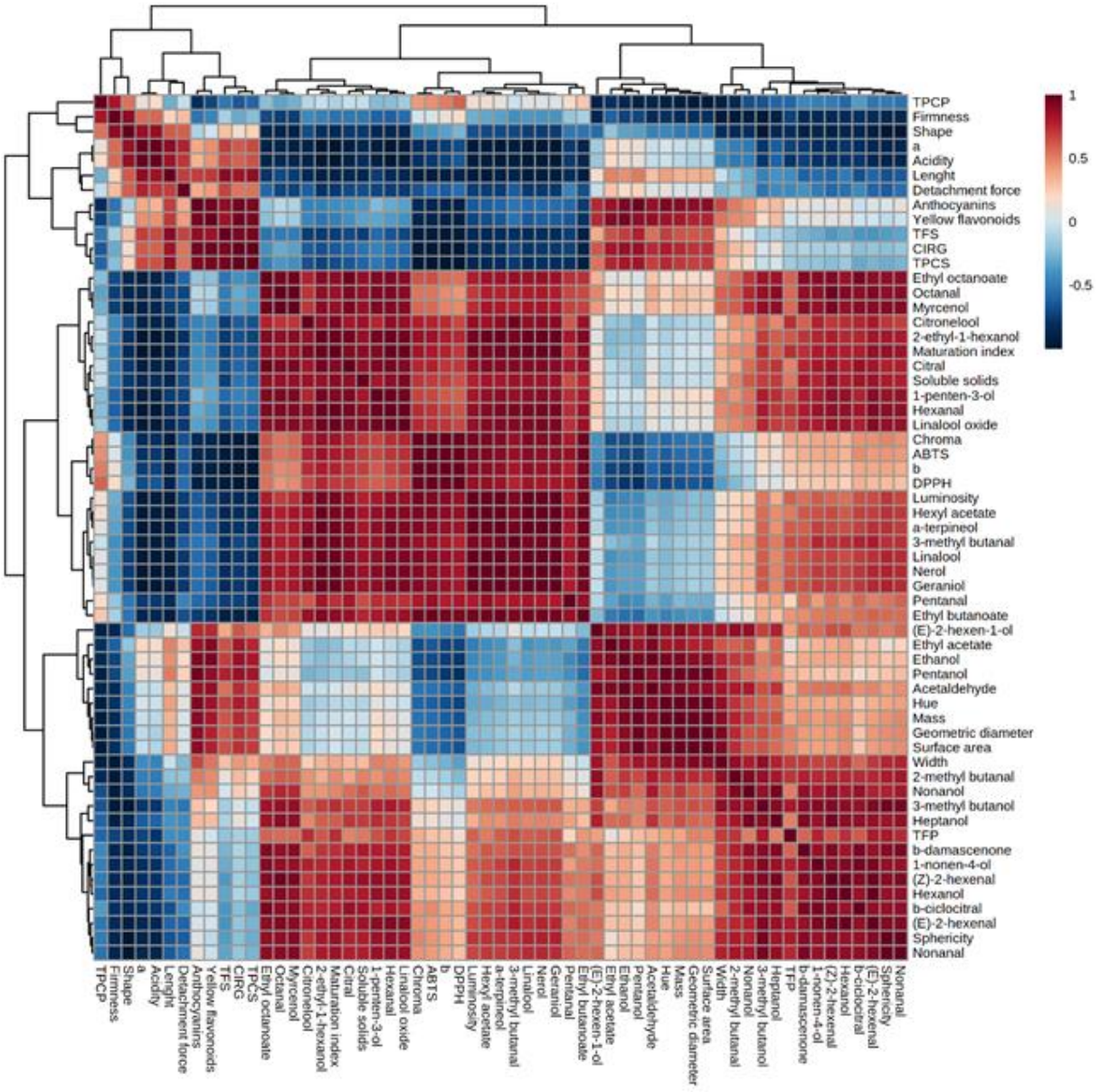
2.1.6 Supplementary material

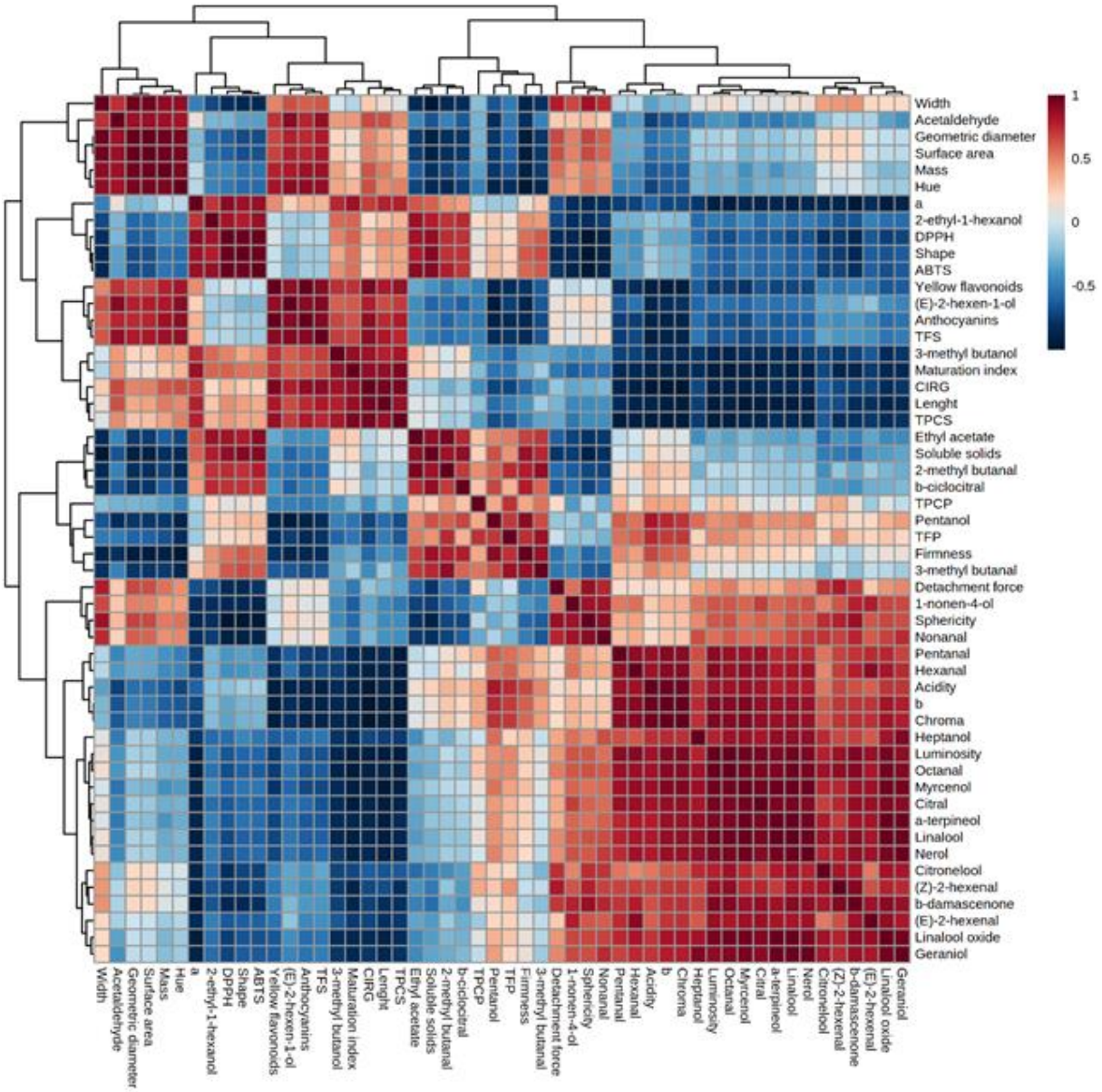
Supplementary figure 1. Table grapes cultivars evaluated.



Supplementary figure 2. Maximum, mean and minimum temperature, global radiation and rainfall accumulated monthly between the 2020/2021 (a) and 2021/2022 (b) crop seasons (INMET, 2022).



Supplementary figure 3. Pearson's correlation analysis among physical-chemical parameters of ‘BRS Isis’, ‘BRS Nubia’ and ‘Italia’ table grapes harvested in 2021. CIRG: color index for red grapes; TFS; total flavonoids in the skin; TFP: total flavonoids in the pulp; TPCS: total phenolic compounds in the skin; TPCP: total phenolic compounds in the pulp.



Supplementary figure 4. Pearson's correlation analysis among physical-chemical parameters of ‘BRS Isis’, ‘BRS Nubia’ and ‘Italia’ table grapes harvested in 2022. CIRG: color index for red grapes; TFS; total flavonoids in the skin; TFP: total flavonoids in the pulp; TPCS: total phenolic compounds in the skin; TPCP: total phenolic compounds in the pulp.

3 CAPÍTULO 2

3.1 ALTERNATIVES FOR THE QUALITY MAINTENANCE OF READY-TO-EAT TABLE GRAPES DURING COLD STORAGE[2]

ABSTRACT

The objective of this study was to evaluate the effects of different techniques on quality maintenance of ready-to-eat 'BRS Nubia', 'BRS Isis' and 'Italia' table grapes during 30 and 60 days of storage (0.5 °C) plus four days of shelf-life at 20 °C. The treatments evaluated were, as follows: [1] control (only detached berries); [2] sulfur dioxide (SO_2) (1.5 g $Na_2S_2O_5$ for kg^{-1} of grape); [3] ethanol solution (30% for 5 min); [4] hot water (50 °C for 10 min) and [5] 1-methylcyclopropene (1-MCP) (2.0 μL L^{-1} for 24 h). The 1-MCP did not reduces the senescence of table grape berries during storage. Immersion in hot water and ethanol showed similar or superior results to SO_2 in decay control until 30 days, but after 60 days of storage, only ethanol and SO_2 controlled the decay incidence. However, the application of hot water and ethanol abruptly increased the incidence of cracks in 'BRS Isis' grapes. The phenolic content did not change between storage periods in 'Italia' grapes, while it decreased in 'BRS Nubia' and 'BRS Isis'. The SO_2 treatment caused berry bleaching but did not reduce the phenolic concentration of the grapes. SO_2 and ethanol are efficient on quality maintenance of ready-to-eat table grapes during storage at 0.5 °C. In addition, hot water treatment, and especially ethanol, can be used as alternatives to chemical treatments to extend the shelf-life of ready-to-eat table grapes stored for long periods.

Keywords: 1-MCP, BRS Isis, BRS Nubia, ethanol, Italia

[2] Artigo formatado de acordo com as normas da revista Scientia Horticulturae.

3.1.1 Introduction

With the release of new table grape cultivars linked to the improvement in the living standards of the consumers has resulted in an interest in the trade of ready-to-eat table grapes (Sabir et al., 2021; Tyagi et al., 2020). In this form of trade, the berries are detached from the bunches and sold in small portions, instead of the conventional packaging of intact bunches (Sabir et al., 2021), making them more attractive to consumers (Chiabrando and Giacalone, 2020; Tyagi et al., 2020). In addition, it does not present the inconvenient found in the grape commercialized in the bunches form, caused by the rachis darkening and the natural berries abscission (Blanckenberg et al., 2021; Nicolosi et al., 2018). It also allows, through the classification of berries, to increase the use of berries from small bunches and insufficient and uneven color. However, the detachment of berries intensifies several physiological and biochemical processes that limit their post-harvest conservation, in addition to increasing the incidence of pathogens in the berries during storage (Kou et al., 2007; Kou, 2009).

Cold storage, characterized by a reduction in the temperature (~0 °C) and relative humidity control (~95%), is one of the main grape storage methods (Romero et al., 2021). However, this storage type is still limited by dehydration and susceptibility to pathogens (Pinto et al., 2015), being one of the main causes of post-harvest deterioration of grapes (Lurie et al., 2006), especially the minimally processed ones (Kou et al., 2007; Kou, 2009), limiting conservation to only 15 to 30 days (Nicolosi et al., 2018; Sabir et al., 2021; Tyagi et al., 2020). For post-harvest pathogen control, sulfur dioxide (SO_2) can be used (Yuan et al., 2022). However, its use is restricted as it alters the flavor (Lurie et al., 2006) and is related to the presence of cracks (Zoffoli et al., 2008) and berries bleaching (Yuan et al., 2022), in addition to of causing possible negative impacts on human health (Sabir and Sabir, 2013). Since SO_2 had been introduced for pathogen control, few alternative technologies have been developed to preserve the commercial quality of grapes (Yuan et al., 2022). Thus, there is a growing interest in implementing innovative techniques that avoid post-harvest quality losses using sustainable technologies.

Among some technologies, the use of hot water is a simple and promising alternative to delay post-harvest deterioration and reduce the incidence of pathogens in ready-to-eat table grapes (Chiabrando and Giacalone, 2020). The effectiveness of the use of heat treatment through immersion in hot water in the decay incidence control has already been well reported in several minimally processed fruits such as apple (Aguayo et al., 2015), melon (Morgado et al., 2016), kiwi (Chiabrando et al., 2017) and in some grape cultivars (Chiabrando and Giacalone, 2020; Sabir and Sabir, 2013). Furthermore, the thermal treatment causes several

physiological changes and is related to increased activity of defense mechanisms in berries and oxidative stress (Wu et al., 2015). Another alternative is the application of ethanol, which sterilizes the berry surface and reduces the incidence of decay during storage (Lurie et al., 2006). In grapes, ethanol has similar or superior efficiency to SO_2 in decay control (Lurie et al., 2006) in addition to increases the intensity of skin color and reduces mass loss (Candir et al., 2012). Furthermore, ethanol inhibits the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and oxidase enzymes, reducing the ethylene production (Asoda et al., 2009), which is involved in grape senescence (Xu et al., 2022). Recently, some studies have reported the effects of ethylene on physiological and biochemical aspects in non-climacteric fruits, such as grapes (Li et al., 2015; Zhang et al., 2022).

The use of products that inhibit the synthesis (ethanol) or the action of ethylene, such as 1-methylcyclopropene (1-MCP) is being evaluated in order to reduce the effect of ethylene on the grapes ripening and senescence after harvest (Zhu et al., 2020). 1-MCP is a gaseous molecule that binds to ethylene receptors on the cell membrane, inhibiting their action (Watkins, 2006). Although grape berries are less responsive to ethylene than rachis (Li et al., 2015), the application of 1-MCP maintains greater firmness and anthocyanin concentration (Silva et al., 2013) and reduces the mass loss and decay incidence in grapes (Zhu et al., 2020). In addition, in ready-to-eat table grapes, part of the pedicel, which has a climacteric response (Li et al., 2015), remains attached to the berry, which can affect the maintenance of berry quality during storage. However, few works in the literature evaluate the application of inhibitors of ethylene synthesis and action in the conservation of ready-to-eat grapes.

Although some works have evaluated alternative techniques to SO_2 in ready-to-eat table grapes, especially in traditional cultivars such as ‘Alphonse Lavallée’ (Sabir et al., 2021), ‘Red Globe’ (Sabir and Sabir, 2013), ‘Crimson Seedless’ (Chiabrando and Giacalone, 2020) and ‘Flame Seedless’ (Tyagi et al., 2020), there are no works in the literature on the new table grape cultivars ‘BRS Nubia’ and ‘BRS Isis’ in their minimally processed forms. Some characteristics of these cultivars, such as large berries (‘BRS Nubia’) and seedless (‘BRS Isis’), in addition to the intense and dark color of the skin, are widely appreciated by consumers (Maia et al., 2018), can benefit from their commercialization in the berries form. In this context, the objective of this study was to evaluate the effect of different techniques on quality maintenance of ready-to-eat ‘BRS Nubia’, ‘BRS Isis’, and ‘Italia’ table grapes during cold storage.

3.1.2 Material and methods

3.1.2.1 Plant material and sample preparation

'BRS Nubia', 'BRS Isis', and 'Italia' table grapes were acquired during the commercial harvest period (Table 1) from a vineyard in Serafina Corrêa, State of Rio Grande do Sul (RS), Brazil (28°41'07'' S, 51°56'24'' W, 509 m), in the Serra Gaúcha region, in the 2021 crop season. After harvesting, the bunches were transported to the Post-Harvest Research Center (NPP), at the Federal University of Santa Maria, RS, where the selection of berries was carried out, eliminating those that presented mechanical damage, injuries caused by pathogens, or with the presence of physiological disorders. Then, the remaining berries in the bunches were detached from a cut in the pedicel region (~2 mm), with the aid of sterilized scissors, and washed with distilled and deionized water (Sabir et al., 2021). After detaching the berries, a sample of the grapes was analyzed to characterize the initial quality (Table 1). The grapes were submitted to the following conditions: control; immersion in ethanol; immersion in hot water; SO_2 application and; 1-MCP application described below. Each treatment consisted of three repetitions of 300 g of berries, arranged in polyethylene clamshell.

Table 1. Initial analysis of 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes berries. Mean ± standard deviation (n = 3).

Quality parameters	BRS Isis	BRS Nubia	Italia
Soluble solids (°Brix)	14.7 ± 0.12	15.0 ± 0.87	16.2 ± 0.12
Acidity (% tartaric acid)	0.48 ± 0.01	0.45 ± 0.01	0.37 ± 0.01
Maturation index (SS/TA)	30.1 ± 0.40	32.8 ± 1.50	43.0 ± 1.18
Firmness (N)	28.2 ± 0.48	22.4 ± 1.06	23.5 ± 0.53
Ethylene (ng C_2H_4 kg^{-1} s^{-1})	0.006 ± 0.001	0.01 ± 0.001	0.005 ± 0.001
Respiration rate (µg CO_2 kg^{-1} s^{-1})	3.89 ± 0.15	6.01 ± 1.02	4.25 ± 0.27
Luminosity	32.0 ± 0.40	31.2 ± 1.58	46.6 ± 0.98
a*	7.98 ± 0.47	3.12 ± 0.65	-10.6 ± 0.28
b*	8.69 ± 0.20	-3.41 ± 0.57	23.1 ± 0.54
Chroma	12.2 ± 0.16	4.89 ± 0.82	25.4 ± 0.61
Hue angle	47.0 ± 2.09	312.3 ± 6.04	114.6 ± 0.01
Anthocyanins (mg 100 g^{-1})	36.0 ± 7.26	261.2 ± 21.8	1.66 ± 0.74
Total flavonoids (mg QE 100 g^{-1})	11.2 ± 2.01	18.3 ± 2.72	4.05 ± 0.12
Phenolic compounds (mg GAE 100 g^{-1})	2477 ± 121	4076 ± 97	1188 ± 184

3.1.2.1.1 Ethanol application

Ethanol (ethyl alcohol, 92.5%) was applied as proposed by Lurie et al. (2006). In short, the berries were immersed in ethanol solution (30%) for 5 min. Then, the berries were dried at room temperature for 30 min.

3.1.2.1.2 Hot water application

The berries were immersed in hot water (50 °C) for 10 min as performed by Chiabrando and Giacalone (2020). Next, the samples were kept for 30 min at room temperature for drying.

3.1.2.1.3 Sulfur dioxide (SO_2) application

The SO_2 application was performed fortnightly in an 0.08 m^3 experimental chamber, at a dose of 1.5 g kg^{-1} of grapes (Choudhury, 2001), in the form of sodium metabisulfite ($Na_2S_2O_5$). The $Na_2S_2O_5$ was wrapped in sheets of parchment paper in a sachet shape (15 cm x 25 cm) and placed on the polyethylene clamshell inside the chamber. When in contact with air humidity, $Na_2S_2O_5$ volatilizes, releasing SO_2.

3.1.2.1.4 1-methylcyclopropene (1-MCP) application

The 1-MCP (Smartfresh® at 0.14% active ingredient) was applied at a concentration of 2 μL L^{-1} as performed by Li et al. (2015). Briefly, the product was solubilized in 20 mL of distilled water in an airtight container and the solution was transferred to a petri dish, inside an experimental chamber (0.23 m^3) at 0.5 °C. After application, the chamber was immediately sealed, remaining so for 24 h with constant internal air circulation. At the end of this period, the berries were removed and placed in experimental chambers according to storage conditions.

3.1.2.2 Storage conditions

After sample preparation, the grapes were placed in 0.18 m^3 metallic chambers and stored for 30 and 60 d at 0.5 ± 0.1 °C and 95 ± 3% relative humidity. The chambers remained inside a cold chamber, where the temperature was controlled by a thermostat and monitored daily by Hg bulb thermometers. Relative humidity was adjusted inside the chambers and monitored with the aid of a psychrometer.

3.1.2.3 Physical-chemical analysis

After the storage period, the grapes were kept for four days of shelf-life at 20 ± 1 ºC and 80 ± 5% relative humidity, when the analyses described below were performed. The mass loss,

bleaching, cracking and decay incidence were also determined soon after removal from the chambers.

3.1.2.3.1 Mass loss

Mass loss was quantified by weighing the berries using an analytical precision balance at the beginning and end of each storage period (Pinto et al., 2015). Based on the difference in mass between the periods considered, the mass loss was quantified, expressed as a percentage (%).

3.1.2.3.2 Berry cracking and bleaching

Berry cracking was determined based on the ratio between the number of crack berries and the total number of berries (Pinto et al., 2015). Results were expressed as % of cracked berries. Bleaching is characterized by the discoloration of the berries and was determined using the scale proposed by Franck et al. (2005), according to the surface area of the berries where, (1) undamaged berries, (2) light damage (<10%), (3) moderate damage (11-30%) and (4) severe damage (> 30%). The level of injury caused by bleaching was calculated using the following equation:

$$Berry\ bleaching = \frac{\Sigma(berries\ number\ in\ each\ level \times level)}{total\ berries} \times 100 \quad (1)$$

3.1.2.3.3 Decay

Decay incidence was quantified using the ratio between the number of berries that showed wounds caused by fungi and the total number of berries, and the results were expressed in % of spoiled berries (Pinto et al., 2015).

3.1.2.3.4 Ethylene production and the respiratory rate

In order to determine the ethylene production and the respiratory rate, the berries were previously inspected in order to avoid those with some type of rot that could affect the analysis. The samples were kept for three hours in hermetically sealed containers with a volume of 600 mL. Ethylene production was obtained from two 1 mL samples taken from the headspace of the containers and injected into a gas chromatograph (Varian®, model Star 3400CX) equipped with a flame ionization detector (FID) and a Porapak N80/100. The temperatures in the column, injector, and detector were 90, 140, and 200 °C, respectively. Using the volume of the container, the mass of the berries and the concentration of ethylene produced during the period enabled to

calculate the ethylene production, and the results were expressed in ng kg^{-1} s^{-1}. After the determination of ethylene, the respiratory rate was determined using the accumulation of CO_2 in the same containers used for the previous analysis, obtained with the aid of a continuous flow gas analyzer (Schele®, model KB7). The results were expressed in μg kg^{-1} s^{-1}.

3.1.2.3.5 Total soluble solids, acidity, and maturation index

The soluble solids content was measured using refractometry from the juice of the berries randomly collected from each sample and ground in a centrifuge (Philips Walita®, Barueri, Brazil), and the results were expressed in °Brix. To determine acidity, a 10 mL aliquot of the juice was diluted in 100 mL of distilled water and titrated with 0.1 N NaOH solution to pH 8.1. The results were expressed as % tartaric acid. The maturation index was obtained using the ratio between soluble solids and acidity (Ahmed et al., 2019).

3.1.2.3.6 Firmness

Firmness was quantified with the aid of a penetrometer with a tip of 8 mm in diameter (Effegi, model FT 327, Milan, Italy), inserted in the equatorial region of 15 berries randomly selected from each sample, with values expressed in Newton.

3.1.2.3.7 Color

The color of the berries was determined with an electronic colorimeter (Konica Minolta CR-310, Osaka, Japan), previously calibrated with a white standard plate. Briefly, two berries were placed side by side in a container with a black bottom, where the color reading was performed (Thewes et al., 2021), in order to provide the same distance for each reading, reproducing each determination as faithfully as possible by fitting the colorimeter reading area into the container. Ten determinations were carried out for each repetition, where the following color parameter was simultaneously determined: luminosity, parameter a*, parameter b*, chroma (C*), and the hue angle. Luminosity ranges from 0 (black) to 100 (white); a* represents the color between red (60) and green (-60); b* represents the color between yellow (60) and blue (-60), C* indicates the saturation and the hue angle, which ranges from 0 to 360°, indicates the shade of the color, with values close to 30, 90, 180 and 270° corresponding to the colors red, yellow, green and blue, respectively.

3.1.2.3.8 Anthocyanins

Portions of fresh skins were removed from the central region of the berries and detached from the pulp, with the aid of a sterilized blade, and washed with distilled and deionized water. Anthocyanin content was determined in 1.5 g samples of the skin. To extract the anthocyanin compounds, the skins were placed in amber flasks with 15 mL of acidified methanol (1% HCl + 99% methanol) and kept in the dark at room temperature for 48 h (Peppi et al., 2006). After the extraction period, absorbance readings of the samples were taken with the aid of a spectrophotometer (Femto 600 S, São Paulo, Brazil) at 520 nm, and the results were expressed in mg of anthocyanins in 100 g^{-1} of fresh skin.

3.1.2.3.9 Flavonoids and total phenolic compounds

The flavonoids and total phenolic compounds were quantified from samples of small portions of fresh skins taken from the central region of the berries. Samples of 2 g of fresh skin were homogenized in a mixer (Philips Walita, Barueri, Brazil) with 20 mL of 50% methanol for 2 min, followed by a 2 h extraction period in the dark. Afterward, centrifugation was performed at 3,500 rpm for 10 min and the extract was filtered with filter paper (Ahmed et al., 2019). To determine the total flavonoids, the methodology proposed by Woisky and Salino (1998) was used. Briefly, a 4 mL aliquot of the filtered extract was homogenized with 4 mL of 2% $AlCl_3$ in a vortex for 10 s and kept in the dark for 1 h. Next, absorbance readings were taken with the aid of a spectrophotometer at 415 nm. Using the values obtained, the concentration of total flavonoids was calculated from the calibration of a curve with quercetin. The values were expressed in mg quercetin equivalent (QE) 100 g^{-1} of fresh skin. To determine the phenolic compounds, the Folin-Ciocauteau method was used (Ahmed et al., 2019). Briefly, a 0.2 mL aliquot of the extract was mixed with 1.8 mL of distilled water and 10 mL of Folin-Ciocalteau reagent 10-fold diluted in test tubes wrapped in aluminum foil. Afterward, 8 mL of 7.5% Na_2CO_3 solution was added. The samples were vortexed for 10 s and kept in the dark for 2 h. Absorbance readings were performed using a spectrophotometer at 765 nm. From the values obtained, the concentration of phenolic compounds was calculated from a calibration curve with gallic acid. Values were expressed in mg of gallic acid equivalent (GAE) per 100 g^{-1} of fresh skin.

3.1.2.4 Statistical analysis

Data were subjected to analysis of normality of errors and homogeneity of variances, using the Shapiro-Wilk and Bartlett tests, respectively. Then, the results were submitted for

analysis of variance, and those that showed significant differences ($p \leq 0.05$) were submitted to the test of Tukey at 5% probability, with the statistical program SISVAR version 5.6 (Lavras, MG, Brazil). For an overview of the results, the data were submitted to the principal component analysis using the MetaboAnalyst software. To obtain the same weight for all variables, the data matrix was automatically scaled for each variable (mean = 0 and variance = 1).

3.1.3 Results and discussion

3.1.3.1 Principal component analysis

The principal component analysis (PC) provides an overview of the results obtained between conditions and storage period for the three ready-to-eat table grape cultivars (Figure 1). PC I and II explained 62.8, 63.9, and 75.2% of the total variation obtained in 'BRS Isis', 'BRS Nubia' and 'Italia' grapes at 30 days of storage, respectively (Figure 1a to f). The application of 1-MCP failed in maintaining the quality of the grapes during storage, as it was related to a greater synthesis of ethylene, respiratory rate, mass loss, and decay incidence, especially during the shelf-life, like the control treatment. These results corroborate those obtained by Li et al. (2015), who did not observe the effect of 1-MCP on the quality of the berries. The application of SO_2 was related to the presence of bleaching in the berries, however, the damage was considered light (Yuan et al., 2022), as it was not related to significant physicochemical changes. Ethanol was related to higher anthocyanin content, especially in 'BRS Nubia' grapes, which have high phenolic content (Maia et al., 2018), and to firmness and color parameters in 'Italia' grapes, such as luminosity, b*, and chroma, indicating light, intense and yellowish-green berries, corresponding to a less advanced stage of maturation (Piazzola et al., 2015). In addition, the application of ethanol, as well as the thermal treatment with hot water, was also related to firmness, total flavonoids, and anthocyanins in 'BRS Isis' grapes. Nevertheless, for this cultivar, immersions in ethanol and hot water were related to a higher incidence of cracking possibly due to the morphological characteristics of the berries (Zhang et al., 2020), which make them prone to cracking under high water content conditions as observed in immersion treatments.

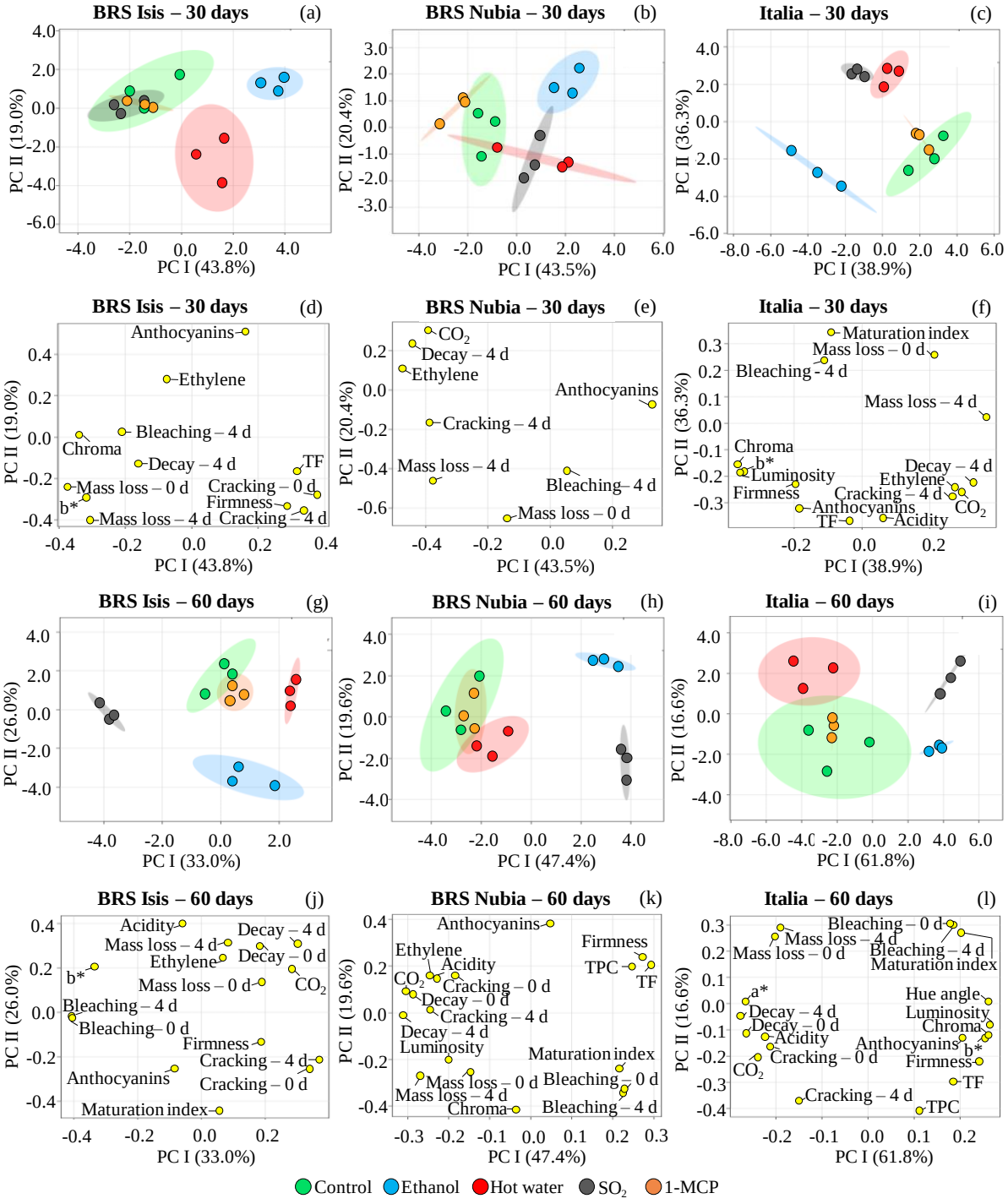


Figure 1. Principal component analysis of ready-to-eat ‘BRS Isis’, ‘BRS Nubia’, and ‘Italia’ table grapes, stored for 30 and 60 days at 0.5 °C and after four days of shelf-life at 20 °C.

After 60 days of storage, the PC I and II explained 59.0, 67.0, and 78.4% of the results obtained in ‘BRS Isis’, ‘BRS Nubia’, and ‘Italia’ grapes, respectively (Figure 1g to l). According to the separation performed by the PCs, the 1-MCP and the thermal treatment with hot water were related to higher ethylene synthesis, respiratory rate, mass loss, the incidence of

decay and cracking (‘BRS Nubia’ and ‘Italia’), like the control treatment, indicating advanced ripening of the berries. Thus, although the thermal treatment suppresses the expression and activity of several enzymes related to cell degradation (Lurie, 2006; Zheng et al., 2012; Zhu et al., 2020) and induces the activity of defense mechanisms of the berry (Wu et al., 2015), this does not prevent the occurrence of pathogenic reinfections (Kou, 2009), limiting the quality of grapes stored for long periods. The SO_2 was related to bleaching (Yuan et al., 2022), and, like ethanol, to a higher maturation index, the concentration of phenolic compounds, and firmness, in addition to higher luminosity, chroma, and hue angle, especially in ‘Italia’ grapes. However, in ‘BRS Isis’ grapes, ethanol and the thermal treatment were related to a higher incidence of cracking, as observed at 30 days. Thus, the application of hot water and ethanol by immersion in grapes with thinner and firmer skin, such as ‘BRS Isis’ grapes (Ritschel et al., 2013), even though they maintain better quality, especially ethanol, which has a longer-lasting effect in relation to heat treatment, increase the incidence of cracks, which impairs the visual aspect and may limit the shelf-life of the grapes.

3.1.3.2 Mass loss, disturbs, and decay incidence

Fresh products lose water continuously after harvest, which is the main factor responsible for mass loss. This loss is highly affected by the shape, period, and storage conditions, in addition to the cultivar's characteristics (Karabulut et al., 2004; Romero et al., 2021; Sabir and Sabir, 2013; Sabir et al., 2021). In this work, an increase was observed in mass loss of the berries under all conditions evaluated during the storage periods in ‘BRS Nubia’ and ‘Italia’ grapes, while in ‘BRS Isis’ grapes, the increases in the mass loss were observed between the periods (30 to 60 days) on berries treated with ethanol and hot water (Figure 2a and b). These results corroborate other studies that found an increase in mass loss during the storage of ready-to-eat grapes (Sabir and Sabir, 2013; Sabir et al., 2021).

Among the treatments evaluated, only ethanol was efficient in reducing the mass loss of grapes in relation to untreated grapes at 30 days of storage and in both shelf-life, while at 60 days of storage no treatment was efficient in controlling mass loss (Figure 2a and b). Some studies have reported the ability of ethanol to retain water in grapes (Karabulut et al., 2004; Romero et al., 2021). This result may have been caused by the ability of ethanol to maintain cell membrane integrity, as ethanol suppresses the activity of lipoxygenase, one of the key enzymes in membrane lipid peroxidation (Xue et al., 2018), in addition to suppressing the expression of aquaporin genes *VvPIP1.2*, *VvPIP1.3* and *VvPIP2.1* which are related to water loss from berries during storage (Romero et al., 2021). On the other hand, hot water treatment

increased berry mass loss after 60 days of storage, especially in ‘BRS Isis’ and ‘Italia’ grapes. Heat treatment can cause microcracks in the epicuticular layer of the skin, which can increase transpiration-induced water loss (Pangavhane et al., 1999). Furthermore, the presence of microcracks can act as an entrance site for pathogen infection.

Decay incidence is one of the main causes of post-harvest deterioration of table grapes (Lurie et al., 2006), particularly in minimally processed ones (Kou et al., 2007; Kou, 2009), requiring the use of products for the control of pathogens during storage (Karabulut et al., 2004). In this work, only ethanol and SO_2 controlled decay incidence during storage, where values below 10.5% were found during shelf-life after 60 days of storage (Figure 2c and d). These values are higher than those found by Sabir et al. (2010), who found a decay incidence of 5.7% in ready-to-eat grapes treated with ethanol. However, the results of those authors are from grapes stored for only 28 days. The applied products did not affect the incidence of pathogens at 30 days of storage (Figure 2c). During the shelf-life, berries treated with antimicrobial agents (ethanol, hot water, and SO_2) showed a lower incidence of pathogens, especially in ‘Italia’ grapes, while in ‘BRS Nubia’ and ‘BRS Isis’ grapes, the products differed only from berries treated with 1-MCP (Figure 2d). However, only ethanol and SO_2 were effective in controlling pathogens in all cultivars after 60 days of storage, especially during shelf-life.

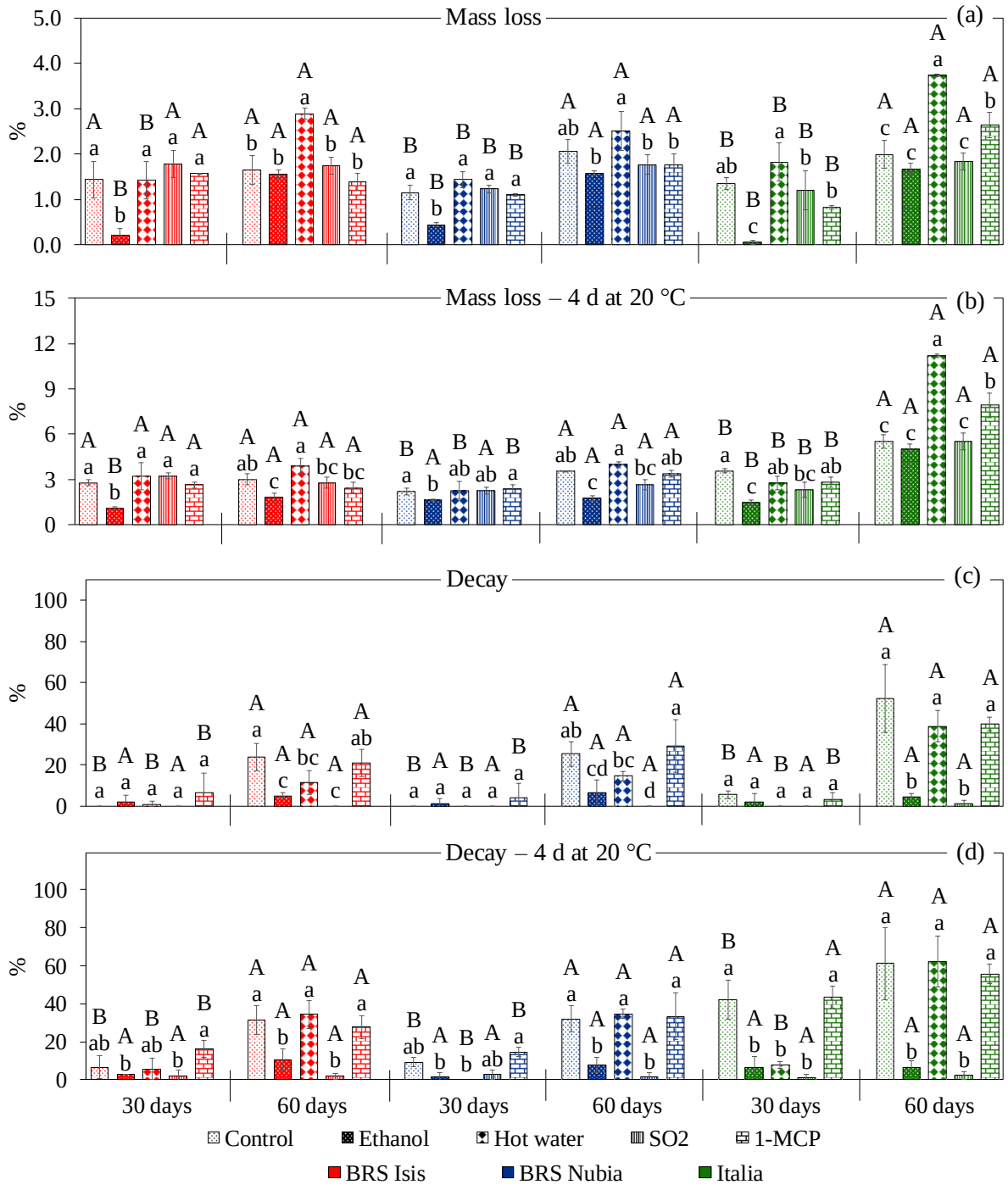


Figure 2. Mass loss (a and b) and decay incidence (c and d) in ready-to-eat 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes during storage and after four days of shelf-life at 20 °C. Mean value ± standard deviation (n = 3). Means followed by the same lowercase letter in the same storage period and uppercase letters between storage periods do not differ by the test of Tukey ($p \leq 0.05$).

Besides SO_2, which had been demonstrated in controlling post-harvest decay in grapes (Yuan et al., 2022), the effectiveness of ethanol and hot water in controlling pathogens has

already been reported in some studies (Chiabrando and Giacalone, 2020; Lurie et al., 2006; Karabulut et al., 2004; Sabir and Sabir, 2013). These antimicrobial agents sterilize the berry surface, killing the inoculum that is responsible for much of the further deterioration (Lurie et al., 2006). In addition, ethanol seems to delay mycelial development and control inoculum in inner layers due to lower surface tension compared to water, which favors greater contact and penetration into the berry (Gabler et al., 2005), while the heat treatment induces several defense mechanisms in the berries, mainly in the accumulation of phytoalexins, which is involved in cellular homeostasis (Wu et al., 2015), but it does not prevent pathogenic reinfections (Kou, 2009). Thus, unlike ethanol, the thermal treatment with hot water does not control latent infections that arise during storage (Karabulut et al., 2004), as found in this experiment.

The SO_2 is the main product applied in the conservation of table grapes after harvest (Yuan et al., 2022), and its application may cause physicochemical changes, especially bleaching. This discoloration of the epidermis (Supplementary figure 1), commonly seen near the pedicel insertion region (Yuan et al., 2022), is caused by the change in the structure of the skin pigments, the anthocyanins particularly, due to the formation of colorless structures derived from sulfur (Cavalcanti et al., 2011). As expected, only SO_2-treated berries showed bleaching (Figures 3a and b). However, although the effects have progressed over storage and shelf-life, the damage was considered mild in all cultivars. These results indicate that the dosage and application method were adequate, as they succeeded in controlling pathogens, without, however, causing significant damage to the berries. Another relevant aspect is that the main regions of SO_2 input and accumulation in grapes are the rachis and pedicel (Yuan et al., 2022). Thus, in ready-to-eat grapes, where the rachis is discarded, and the pedicel is reduced (~2 mm), the input and accumulation of SO_2 in the berries are limited, reducing the incidence of damage caused by SO_2, such as the bleaching of the berries.

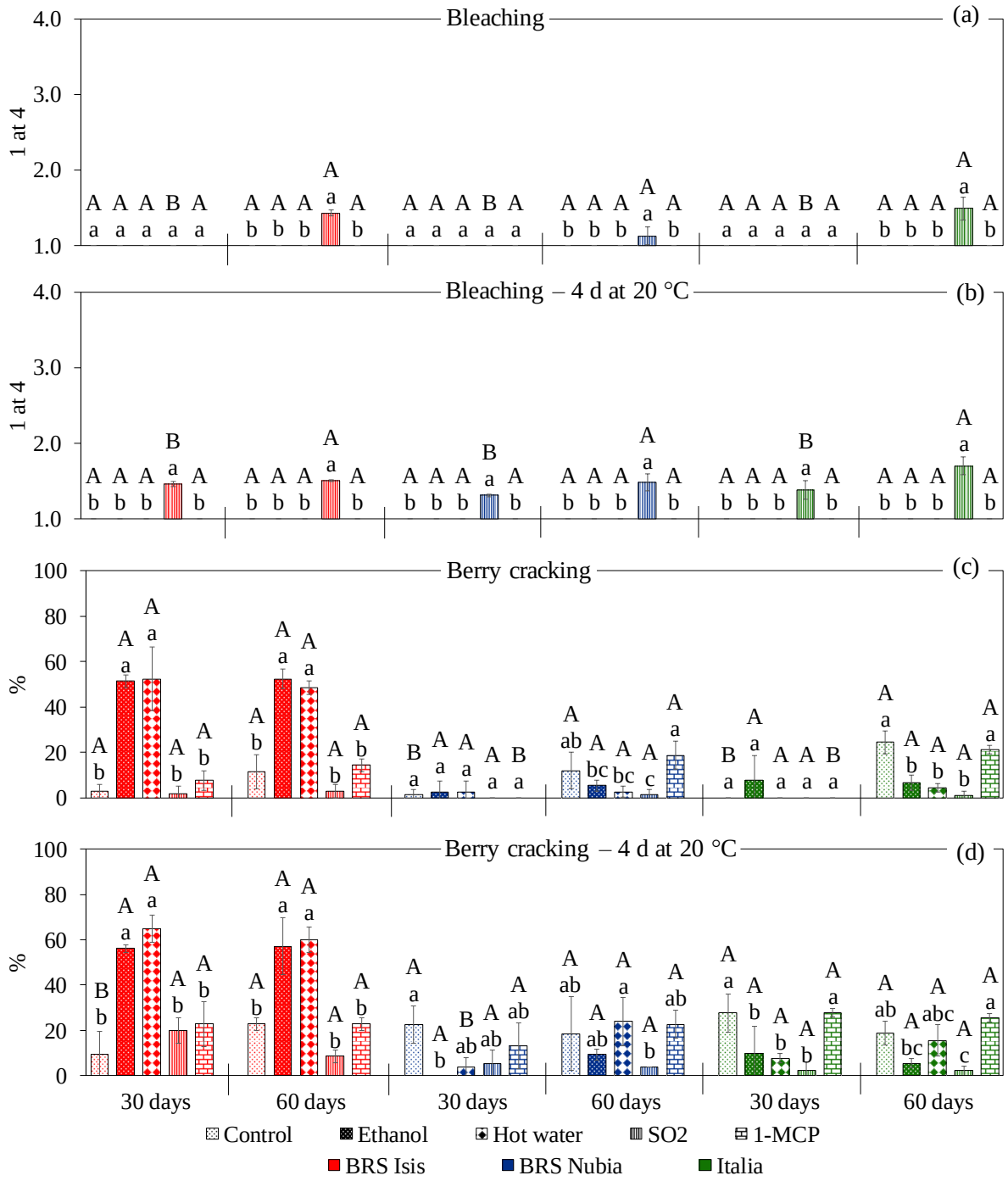


Figure 3. Bleaching (a and b) and berry cracking (c and d) in ready-to-eat 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes during storage and after four days of shelf-life at 20 °C. Mean value ± standard deviation (n = 3). Means followed by the same lowercase letter in the same storage period and uppercase letters between storage periods do not differ by the test of Tukey ($p \leq 0.05$).

Another relevant and commonly found disorder in grapes is berry cracking (Supplementary figure 1). In the present work, storage conditions affected the cultivars

differently (Figures 3c and d). In ‘BRS Nubia’ and ‘Italia’ grapes, no differences were found between the conditions at 30 days of storage, and berries from the control treatment showed a higher incidence of cracks during the shelf-life, differing only from berries treated with ethanol in ‘BRS Nubia ’grapes, and the other antimicrobial agents in ‘Italia’ grapes, which presented the lowest values. The efficiency of antimicrobial agents in the control of cracks was also observed at 60 days of storage, although only SO_2 maintained its efficiency in the control of cracks in relation to the control treatment during the shelf-life. These results may have been caused by the effects of ethanol, hot water, and SO_2 in delaying ripening and reducing the incidence of pathogens in grapes (Chiabrando and Giacalone, 2020; Sabir and Sabir, 2013; Romero et al., 2021; Xue et al., 2018). During grape senescence, increases in the expression and activity of enzymes such as peroxidase, endoglycosidase, pectinmethylesterase, and polygalacturonase are observed, which cause the degradation of cell wall polysaccharides (Zhu et al., 2020), thus, altering the mechanical properties of the pericarp and makes it more prone to cracking.

However, the incidence of berry cracking is also defined by the level of susceptibility of the cultivar (Zhang et al., 2020). Thus, while in ‘BRS Nubia’ and ‘Italia’ grapes, the application of ethanol and hot water reduced the incidence of cracks, especially during storage, these conditions favored the incidence of cracks in ‘BRS Isis’ grapes, regardless of the storage period, with values significantly higher than the other conditions after 30 days of storage (Figure 3c and d). Karabulut et al. (2004) reported the possibility of ethanol and hot water applied by immersion in causing berries to crack. This expressive incidence of cracks in ‘BRS Isis’ grapes may be due to the morphological characteristics of the berries, such as lower elasticity and thickness of the cuticle and compact arrangement of epidermal and subepidermal cells (Zhang et al., 2020), which limit cell expansion with the increase in cell turgor caused by the absorption of water during the immersion of the berries (Zoffoli et al., 2008). Consequently, grapes with thinner and firmer skin, such as ‘BRS Isis’ grapes (Maia et al., 2018), are less resistant to the occurrence of cracks, especially in conditions of high-water content, such as those found in treatments with immersion in ethanol and hot water. Although being efficient in some cultivars, such as ‘BRS Nubia’ and ‘Italia’, these treatments should be carried out carefully and evaluated for each cultivar.

3.1.3.3 Physical-chemical parameters

Grapes are characterized as non-climacteric fruits (Li et al., 2015), as there are no significant increases in ethylene production and respiratory rate during ripening, as found in the

present study (Figures 4a and b). However, ethylene is related to several metabolic changes that affect the integrity of cells, and consequently, the quality of grapes (Li et al., 2015; Zhang et al., 2022). Ethylene production was well characterized in ready-to-eat table grapes in both storage periods (Figure 4a). In 'BRS Nubia' grapes, the application of ethanol, hot water, and SO_2 suppressed ethylene production, although only SO_2 differed from the control treatment at 60 days. The application of these antimicrobial agents (ethanol, hot water, and SO_2) also suppressed the production of ethylene in 'BRS Isis' grapes, especially at 60 days, and in 'Italia' grapes at 30 days, and 60 days no differences between storage conditions were found.

These results may be related to the inhibitory effects of these treatments on gene expression and activity of ethylene-precursor enzymes, such as 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and oxidase (Asoda et al., 2009; Lurie, 2006; Xue et al., 2018). On the other hand, the application of 1-MCP, an important inhibitor of the ethylene action (Watkins, 2006), did not suppress the production of ethylene regardless of cultivar and storage period, corroborating the results found in 'Shine Muscat' grapes by Zhang et al. (2022). In contrast, Bellincontro et al. (2006) reported lower production of ethylene in 'Aleatico' grapes treated with 1-MCP. However, unlike this work, where only the berries adhered to a small portion of the pedicel were evaluated, in that study, the effect of 1-MCP on the entire bunch was considered, where ethylene production is about 58 times lower in the berries in relation to rachis, which presents a climacteric response (Li et al., 2015). In this sense, the blocking of ethylene receptors by 1-MCP does not seem to be enough to reduce the basal synthesis of ethylene in the berries, which shows that the effect of this product is related to the receptors found in the rachis.

The application of ethanol, hot water, and SO_2 reduced the respiration of the berries, compared to the control in 'Italia' and 'BRS Nubia' grapes, although in the latter only immersion in hot water differed from the control treatment at 30 days (Figure 4b). These results may be the overcome of the lower ethylene production found in berries treated with ethanol, hot water, and SO_2 (Figure 4a). These treatments may have inhibited the response of the grapes to the low basal levels of ethylene that are naturally produced by non-climacteric fruits, and that may affect in at least some aspects the ripening of the grapes (Li et al., 2015). On the other hand, an increase occurred in the respiratory rate of 'Italia' grapes between 30 and 60 days of storage, in berries of the control treatment, treated with hot water and 1-MCP. The increase in the respiration during storage was also observed in 1-MCP-treated berries in 'BRS Isis' grapes.

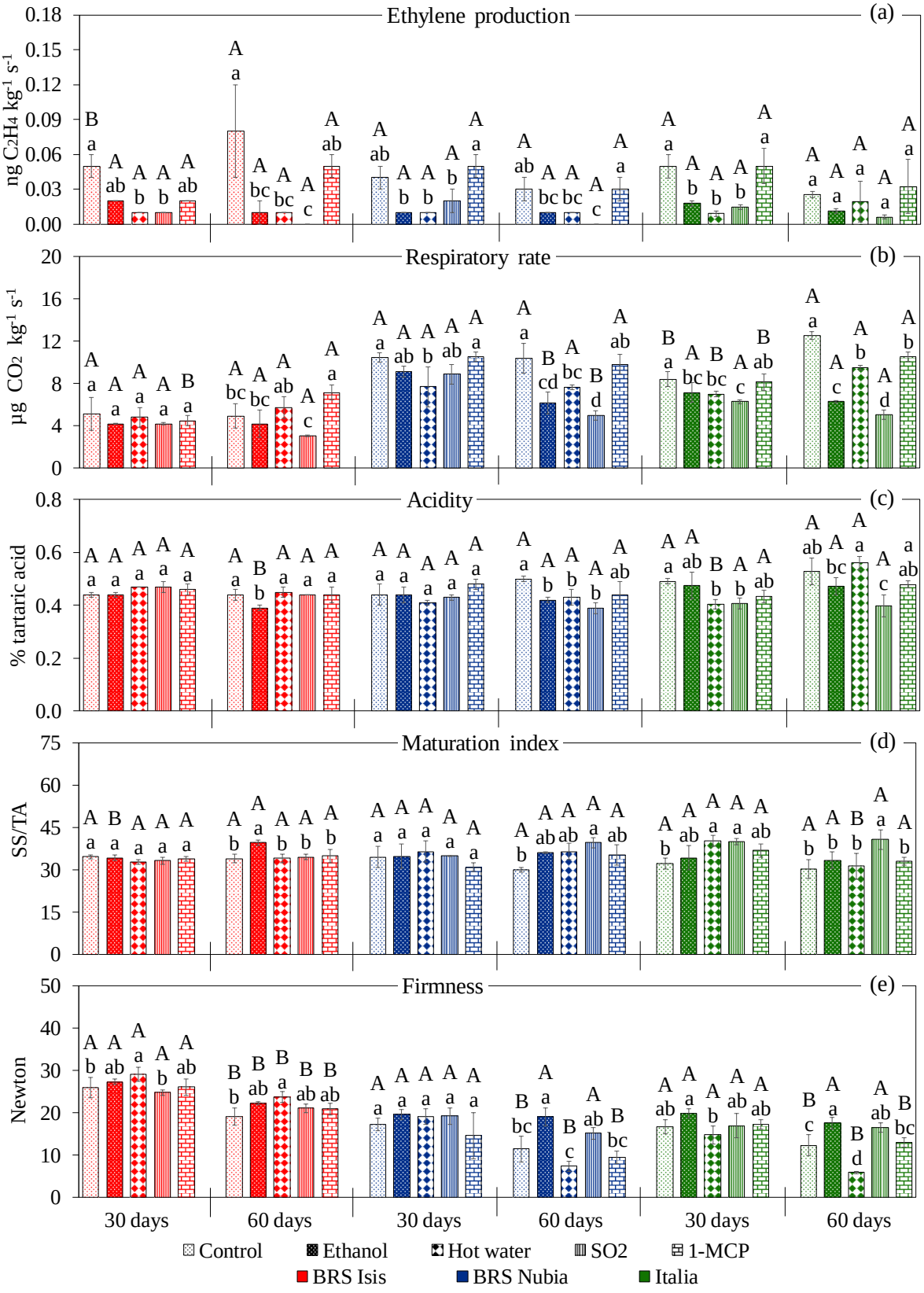


Figure 4. Ethylene production (a), respiratory rate (b), acidity (c), maturation index (d), and berry firmness (e) of ready to eat 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes after 30 and 60 days of storage plus four days of shelf-life at 20 °C. Mean value ± standard deviation (n =

3). Means followed by the same lowercase letter in the same storage period and uppercase letters between storage periods do not differ by the test of Tukey ($p \leq 0.05$).

The increase in the respiratory rate in these treatments may be related to the increase in infections caused by pathogens during storage (Tyagi et al., 2020) (Figure 2c and d), as damaged tissues stimulate respiration (Kou et al., 2007). In addition, the increase in respiratory rate is an indication of an addition in the metabolic activity, related to cellular degradation and tissue senescence, indicating that the application of 1-MCP does not have a positive effect on the maintenance of grape quality.

One of the most important parameters that determine the consumer's acceptability of table grapes is the relationship between sugar and acid content (Sabir et al., 2010). The content of soluble solids was not altered by storage conditions and periods in the three ready-to-eat table grape cultivars (data not shown). Regarding acidity, only at 60 days of storage differences were observed for 'BRS Nubia' grapes, with higher acidity in the control, while grapes treated with antimicrobial agents showed the lowest values. In 'BRS Isis' grapes, the lowest values were found in berries treated with ethanol (Figure 4c). In relation to 'Italia' grapes treated with SO_2, they had lower acidity, differing from the control treatment in both periods. These results corroborate with Candir et al. (2012), who observed lower acidity in 'Red Globe' grapes stored for four months treated with ethanol and SO_2. The lower acidity found in berries treated with antimicrobial agents may be caused by the lower incidence of pathogens, which results in the accumulation of organic acids (Ustun et al., 2012). On the other hand, 'BRS Nubia' and 'Italia' grapes treated with SO_2 and 'BRS Isis' grapes treated with ethanol showed the highest maturation rates, especially at 60 days of storage (Figure 4d). However, all grapes had a high maturation index (>18) (Ahmed et al., 2019), indicating that the grapes had satisfactory sugar and acid ratios for consumption.

Berry firmness is a quality parameter, which is related to the market value and shelf-life of the grapes (Ma et al., 2020; Xu et al., 2022). The application of hot water maintained greater firmness compared to untreated berries in 'BRS Isis' grapes, despite the decrease found in firmness during storage in all treatments (Figure 4e). Nicolosi et al. (2018) also reported decreases in firmness in ready-to-eat grapes during storage, with the level of reduction varying by cultivar. On the other hand, ethanol and SO_2 were efficient in maintaining the firmness of 'BRS Nubia' and 'Italia' grapes, and only the berries treated with ethanol and SO_2 did not show a significant reduction in firmness after 60 days of storage. These results may have been caused by the efficiency of these treatments in suppressing the activity of the enzymes peroxidase,

cellulase, pectinmethylesterase, and polygalacturonase (Zhu et al., 2020), which are directly related to the softening of the berries (Ma et al., 2020). The synergistic action of these cell-wall degrading enzymes is manifested as pectin dissolution, loss of neutral sugar, xylan depolymerization, alteration of cell cohesion, and, finally, reduction of the berry firmness (Balic et al., 2022; Ma et al., 2020). However, the thermal treatment can increase the activity and expression of mannan endo-1,4-beta-mannosidase, an enzyme that catalyzes the hydrolysis of hemicellulose (Wu et al., 2015), reducing berry firmness as observed in 'BRS Nubia' and 'Italia' grapes after 60 days of storage.

3.1.3.4 Color

Color is one of the most important visual characteristics of grapes and is commonly regarded by consumers as a quality parameter (Ahmed et al., 2019). Regarding luminosity, only the application of ethanol and SO_2 affected the color of the berries in relation to the control treatment (Figure 5a). While ethanol-treated 'BRS Nubia' grapes showed less brightness at 60 days, ethanol enhanced brightness in 'Italia' grapes in all periods, not differing only from SO_2 at 60 days. These results are certainly related to the effect of ethanol on the activity of enzymes that degrade skin pigments, such as polyphenol oxidase (Valero et al., 1990). This enzyme catalyzes the hydroxylation of monophenols and the oxidation of diphenols to quinones (Lamikanra et al., 1992), therefore degrading the color in addition to being able to cause the formation of dark pigments in the skin, particularly shown in light-skinned cultivars such as 'Italia', 'Regal Seedless', 'Victoria', 'Princess' and 'Majestic' (Zoffoli and Latorre, 2011). Thus, ethanol may have reduced the degradation of phenolic compounds, especially anthocyanins in 'BRS Nubia' grapes (Figure 6), and the formation of dark pigments in 'Italia' grapes. These results are relevant, as ethanol maintains lower brightness in dark-colored grapes such as 'BRS Nubia' while intensifying the brightness in light-skinned grapes, such as 'Italia'.

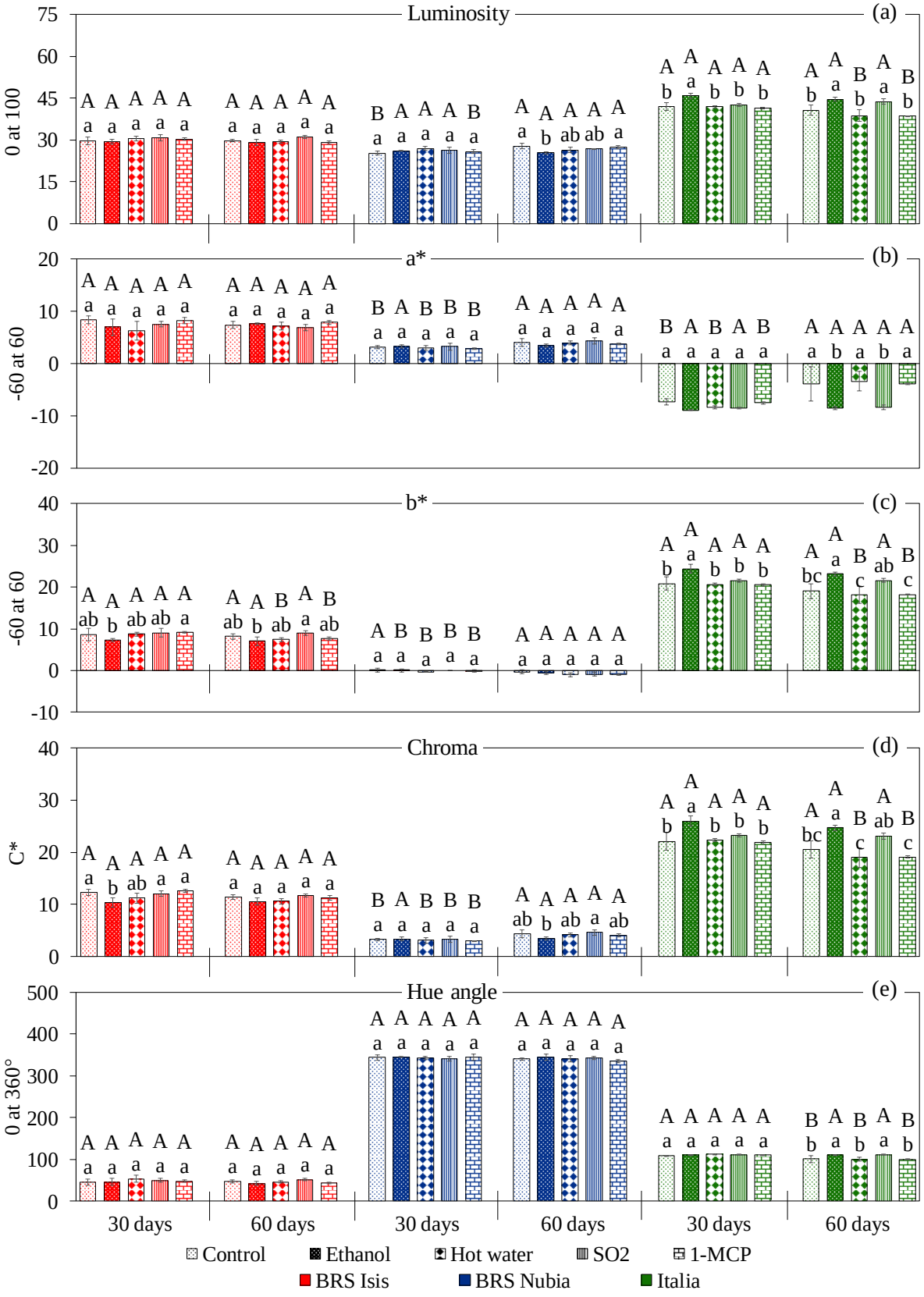


Figure 5. Luminosity (a), parameter a* (b), parameter b* (c), chroma (d), and hue angle (e) of ready-to-eat 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes after 30 and 60 days of storage plus four days of shelf-life a 20 °C. Mean value ± standard deviation (n = 3). Means followed

by the same lowercase letter in the same storage period and uppercase letters between storage periods do not differ by the test of Tukey ($p \leq 0.05$).

The treatments did not affect the parameters a* and b* and the hue angle in 'BRS Nubia' and 'BRS Isis' grapes in relation to untreated grapes (Figure 5b, c and e). However, in 'Italia' grapes, berries treated with ethanol and SO_2 presented the lowest values of the a* parameter and the highest values of the b* parameter and chroma, especially those treated with ethanol, indicating berries with intense color and yellowish-green hue, according to the larger hue angle (Figure 5e), indicating that ethanol and SO_2 suppressed the expression and activity of enzymes related to pigment degradation and skin darkening (Lamikanra et al., 1992). Furthermore, according to the hue angle values, 'Italia' grapes treated with ethanol and SO_2 maintained their typical color during storage, while the other treatments failed in delaying the ripening of the berries, indicated by the more yellowish color, commonly associated with a more advanced stage of maturation in light-skinned grapes (Piazzola et al., 2015).

3.1.3.5 Phenolic compounds

The concentration of total phenolics in this work was reduced from 30 to 60 days of storage in 'BRS Nubia' and 'BRS Isis' grapes, while in 'Italia' grapes, the reduction was observed only in berries treated with hot water (Figure 6a). These results corroborate other works that reported a decrease in phenolic content during grape storage (Bellincontro et al., 2006; Nicolosi et al., 2018; Xu et al., 2022). Nicolosi et al. (2018) observed a reduction in phenolic compounds in ready-to-eat 'Crimson Seedless', 'Red Globe' and 'Black Pearl' grapes after seven days of storage. This reduction may be caused by the decrease in the activity of phenylalanine ammonia-lyase, a key enzyme in the accumulation of phenolic compounds (Xue et al, 2018), or even by the rise in the activity of polyphenol oxidase and peroxidase, which are related to the degradation of phenolic compounds (Lamikanra et al., 1992). Regarding storage conditions, the products applied did not affect the concentration of phenolic compounds in 'BRS Isis' grapes. In 'BRS Nubia' grapes, at 60 days of storage, berries immersed in ethanol had a higher concentration of phenolic compounds, not differing from berries treated with SO_2, while the other conditions did not differ from the control treatment. In 'Italia' grapes, immersion in hot water reduced the concentration of phenolic compounds, compared to control and ethanol, also only at 60 days. The thermal treatment with hot water also causes a reduction in the enzymatic activity, however polyphenol oxidase was only completely inactive at 70 °C in 'Riesling' grapes (Zheng et al., 2012), a temperature higher than that used in this experiment

(50 °C). Furthermore, Maghoumi et al. (2013) reported that the heat treatment suppresses polyphenol oxidase activity, but it enhances peroxidase activity in minimally processed pomegranate. So, the thermal treatment may not have been efficient in reducing or even increasing the activity of enzymes related to the degradation of phenolic compounds in the skin of 'Italia' grapes.

Flavonoids are one of the main classes of phenolics found in grapes. In this work, the application of ethanol in 'BRS Nubia' and 'Italia' grapes prevented the decrease of flavonoids from 30 to 60 days of storage, in addition to SO_2 in 'Italia' grapes. In the other cultivars and treatments, a reduction was observed as the storage progresses (Figure 6b). In the 'BRS Nubia' grapes, the conditions did not affect the concentration of flavonoids at 30 days, but at 60 days, the grapes treated with ethanol had the highest concentrations of flavonoids, not differing from grapes treated with SO_2. In 'BRS Isis' grapes, differences between the evaluated conditions were found only at 30 days, and berries immersed in ethanol and hot water had the highest values, not differing from the control treatment. In the 'Italia' grapes, the application of ethanol in berries with a higher concentration of flavonoids in both periods, not differing from the control berries treated with 1-MCP at 30 days. The higher concentration of flavonoids in berries treated with ethanol and SO_2 may be the result of the increased expression and activity of phenylalanine ammonia-lyase caused by these treatments as reported by some studies (Romero et al., 2021; Xue et al., 2018).

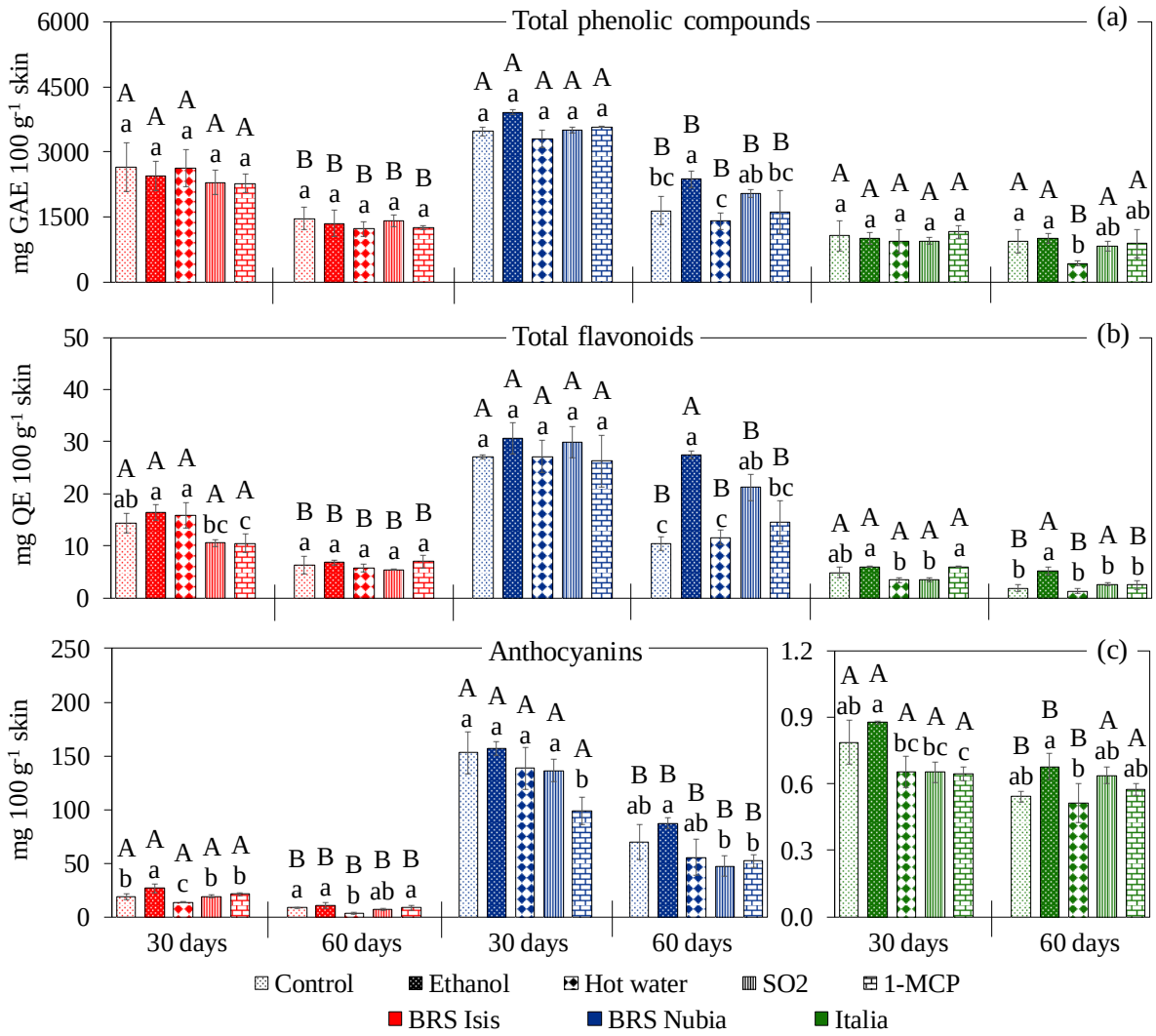


Figure 6. Total phenolic compounds (a), flavonoids (b), and anthocyanins (c) present in the skin of ready-to-eat 'BRS Isis', 'BRS Nubia', and 'Italia' table grapes after 30 and 60 days of storage plus four days of shelf-life at 20 °C. Mean value ± standard deviation (n = 3). Means followed by the same lowercase letter in the same storage period and uppercase letters between storage periods do not differ by the test of Tukey ($p \leq 0.05$).

Regarding the anthocyanin content, a great difference was found between the evaluated cultivars, with high values for 'BRS Nubia' and extremely low for 'Italia' (Figure 6c). In general, the extension of storage from 30 to 60 days resulted in a reduction of anthocyanins in the skins of all cultivars (Figure 6c). Ethanol maintained a higher concentration of anthocyanins in 'BRS Isis' and 'Italia' grapes at 30 days in relation to the other products applied, while at 60 days it was higher only for berries treated with hot water. In 'BRS Nubia' grapes, the application of 1-MCP reduced the anthocyanin content at 30 days, while at 60 days, ethanol maintained a higher anthocyanin content, significantly differing from the berries treated with SO_2 and 1-

MCP. These results corroborate with Ustun et al. (2012), who reported that ethanol applied in the form of Antimold®80 and Antimold®60 sachets maintained a higher concentration of anthocyanins in relation to SO_2 in 'Red Globe' grapes during storage. Romero et al. (2021) reported that ethanol increases the expression of the *VviMYB137* gene which is involved in the anthocyanin synthesis pathway. In this sense, among the alternatives for maintaining the quality of ready-to-eat table grapes during storage, the application of ethanol maintains the better quality of the berries, as the visual appearance and nutritional value of the grapes decrease as the phenolic content decreases (Xu et al., 2022).

3.1.4 Conclusion

The application of 1-MCP does not reduce the senescence of ready-to-eat 'BRS Nubia', 'BRS Isis' and 'Italia' grape berries during storage at 0.5 °C. The application of hot water and ethanol presented similar or superior results to SO_2 in the decay control until 30 days, while only ethanol and SO_2 were efficient in decay control after 60 days of storage. One inconvenience of SO_2 is the disadvantage of causing the blanching of the berries, however, it is efficient in maintaining the quality of the grapes. Ethanol and hot water are promising treatments for preserving the quality of 'BRS Nubia' and 'Italia' grapes, however, they cause cracks in 'BRS Isis' grapes.

Therefore, the application of ethanol and SO_2 maintains the quality of ready-to-eat table grapes after 60 days of storage, which is longer than the works found in the literature. Treatment with hot water, and especially ethanol, can be considered as having a low impact on the health of the consumers and with good potential to extend the shelf-life of ready-to-eat grapes for long periods. The new table grape cultivars 'BRS Nubia' and 'BRS Isis', in addition to the traditional 'Italia', present organoleptic characteristics that may trigger the commercialization and consumption in the form of berries, being a promising alternative to increase the use and reduce the post-harvest losses of grapes.

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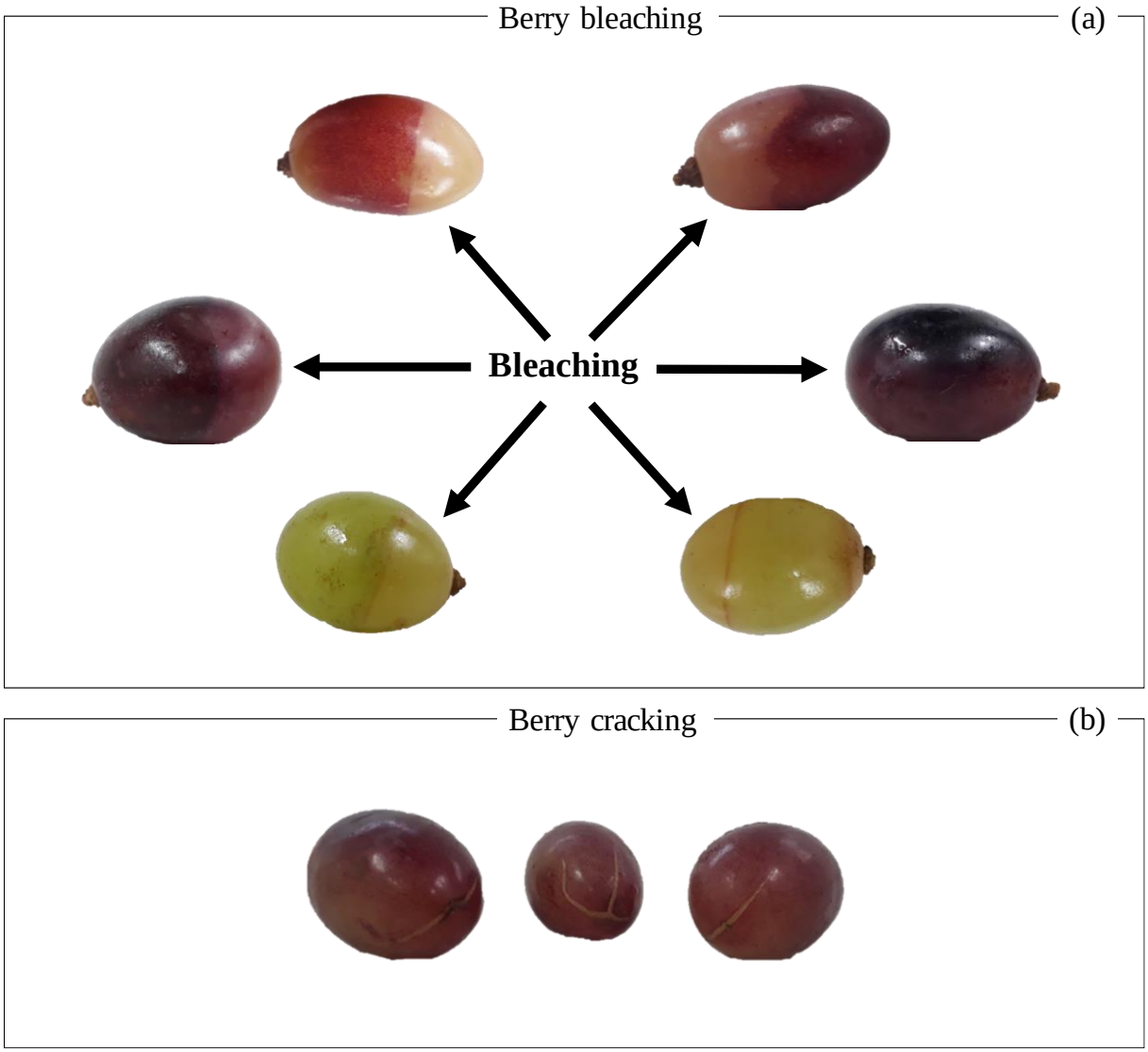
3.1.6 Supplementary material



Supplementary figure 1. Identification of bleaching (a) and berry cracking (b) in ‘BRS Isis’, ‘BRS Nubia’, and ‘Italia’ table grapes during cold storage (0.5 °C).

4 CAPÍTULO 3

4.1 ETANOL E ALTO CO_2 COMO ESTRATÉGIAS DE ARMAZENAMENTO PARA A MANUTENÇÃO DA QUALIDADE, COMPOSIÇÃO FENÓLICA E VOLÁTIL DE UVAS DE MESA 'BRS ISIS'

RESUMO

O objetivo do presente trabalho foi avaliar o efeito de diferentes concentrações de etanol e CO_2 na qualidade de uvas de mesa 'BRS Isis', como estratégias alternativas ao uso de dióxido de enxofre durante 60 e 90 dias de armazenamento (0,5 °C) mais quatro dias de vida de prateleira (20 °C). Foram avaliadas as seguintes condições de armazenamento: [1] controle; [2] dióxido de enxofre (SO_2); [3] 1.125 ppm de etanol; [4] 2.250 ppm de etanol; [5] 10 kPa de CO_2 e [6] 15 kPa de CO_2. O armazenamento com 2.250 ppm de etanol e 15 kPa de CO_2 apresentaram eficiências semelhantes ao SO_2 no controle do degrane das bagas e de podridões. O SO_2 evitou o escurecimento da ráquis, porém causou o branqueamento nas bagas. Ambas as concentrações do alto CO_2 e a aplicação de 2.250 ppm de etanol mantiveram maior firmeza das bagas, entretanto o etanol aumentou a incidência de rachaduras. Além disso, o etanol aumentou a concentração de álcoois, aldeídos e, especialmente ésteres, no entanto, causou o acúmulo de acetaldeído e acetato de etila que podem estar relacionados à aromas desagradáveis nas uvas. O alto CO_2 foi eficiente na manutenção da qualidade das uvas, mantendo maior concentração de compostos fenólicos, flavonoides e antocianinas, além dos compostos voláteis hexanal e (E)-2-hexenal, que estão relacionados ao frescor das uvas. Portanto, o tratamento com etanol e, especialmente, o alto CO_2, são alternativas promissoras ao uso do SO_2 na manutenção da qualidade de uvas de mesa 'BRS Isis' durante o armazenamento prolongado.

Palavras-chave: Compostos voláteis. Podridão. Qualidade pós-colheita. SO_2. Uvas apirênicas.

4.1.1 Introdução

Uvas de mesa (*Vitis spp*.) são frutas perecíveis, altamente suscetíveis a perdas de qualidade após a colheita, ocasionadas pelo escurecimento da ráquis, degrane natural das bagas e a incidência de patógenos (CHEN et al., 2019; LI et al., 2022; ROMERO et al., 2021). Além disso, diversos processos fisiológicos e bioquímicos são intensificados (MAOZ et al., 2019), alterando propriedades organolépticas, como o aroma e a concentração de compostos fenólicos, que recentemente estão sendo considerados como importantes parâmetros de qualidade das uvas (CEFOLA et al., 2018; SANCHEZ-BALLESTA et al., 2020; WU et al., 2019). Grande parte da uva de mesa não é consumida imediatamente após a colheita (XU et al., 2022), tornando necessário o desenvolvimento de estratégias para prolongar a qualidade, a fim de ampliar a oferta da fruta por longos períodos.

O armazenamento refrigerado é um dos principais métodos de conservação das uvas de mesa (ROMERO et al., 2021). No entanto, este tipo de armazenamento é limitado pela suscetibilidade a patógenos, desidratação, escurecimento da ráquis e degrana (PINTO et al., 2015; SHAHKOOMAHALLY et al., 2021), além da degradação de compostos fenólicos e voláteis (CEFOLA et al., 2018; MAOZ et al., 2019; XU et al., 2022). Uma das estratégias comumente utilizadas durante o armazenamento é a aplicação do dióxido de enxofre (SO_2) (YUAN et al., 2022), devido a sua comprovada eficácia no controle de podridões (XIAO et al., 2019; ZHANG et al., 2022). No entanto, deve ser utilizado com cautela em função de aumentar o degrane (CHEN et al., 2019), ocasionar rachaduras (ZOFFOLI et al., 2008) e branqueamento nas bagas (YUAN et al., 2022), além de alterar o sabor (LURIE et al., 2006; YUAN et al., 2022) e causar possíveis impactos negativos à saúde humana (USTUN et al., 2012). Entretanto, poucas tecnologias alternativas ao SO_2 foram desenvolvidas para a conservação das uvas de mesa (YUAN et al., 2022). Assim, há um interesse crescente em implementar técnicas que mantenham a qualidade das uvas durante a pós-colheita usando tecnologias sustentáveis.

A utilização de etanol é uma estratégia promissora para retardar a deterioração pós-colheita e reduzir a incidência de patógenos em uvas de mesa (ROMERO et al., 2021). O etanol, aplicado comumente na forma de vapor (USTUN et al., 2012), apresenta efeito antimicrobiano e induz diversos mecanismos de defesa nas bagas envolvidos na homeostase celular (ROMERO et al., 2021), com eficácia semelhante ao SO_2 no controle de podridões (LURIE et al., 2006). O etanol preserva o estado hídrico (ROMERO et al., 2021) e suprime a síntese de etileno (ASODA et al., 2009; THEWES et al., 2021), que está relacionado ao acréscimo da atividade metabólica, degradação celular e senescência das uvas (LI et al., 2015; LI et al., 2022; XU et al., 2022). Por outro lado, a aplicação de etanol pode contribuir para desidratação da ráquis e aumentar a

atividade da enzima fenilalina amônia-liase, envolvida na síntese de compostos fenólicos (ROMERO et al., 2021), podendo ocasionar o escurecimento da ráquis e das bagas (LURIE et al. 2006) conforme a concentração e período de exposição (LICHTER et al., 2002; USTUN et al., 2012). Além disso, são escassos os estudos que tenham avaliado os efeitos do etanol sobre os compostos voláteis em uvas de mesa, enquanto que em outras frutas, como melão (LIU et al., 2012), amora e morango (BLANCH et al., 2011) seus efeitos já são conhecidos.

Outra estratégia já bem reportada em diversas frutas, como mirtilo, framboesa e morango (ROMERO et al., 2022) e, que recentemente tem sido avaliada na manutenção da qualidade de uvas de mesa (SANCHEZ-BALLESTA et al., 2020; SHAHKOOMAHALLY et al., 2021; VAZQUEZ-HERNANDEZ et al., 2020), é o aumento da concentração de CO_2 na atmosfera de armazenamento. A aplicação de altas concentrações de CO_2 ($\geq$10 kPa) controla a incidência de podridões, reduz a perda de massa, retarda o amolecimento das bagas e a degradação de compostos fenólicos e voláteis, mantendo melhor a qualidade das uvas (CEFOLA et al., 2018; MAOZ et al., 2019; SANCHEZ-BALLESTA et al., 2020). Entretanto, o alto CO_2 pode causar o escurecimento da ráquis (CRISOSTO et al., 2002), reduzir a concentração de compostos fenólicos (ROMERO et al., 2008) e desenvolver aroma desagradável às uvas (CEFOLA et al., 2018) conforme a sensibilidade da cultivar ao alto CO_2 (CRISOSTO et al., 2002; SANCHEZ-BALLESTA et al., 2020). Assim, a concentração ideal de CO_2 para uma determinada cultivar pode não ser a mesma para outras cultivares (SHAHKOOMAHALLY et al., 2021).

Embora alguns trabalhos tenham reportado os efeitos do CO_2 e do etanol sobre a manutenção da qualidade de cultivares de uvas de mesa tradicionais, como ‘Autumn Royal’ (VAZQUEZ-HERNANDEZ et al., 2020), ‘Cardinal’ (VAZQUEZ-HERNANDEZ et al., 2018), ‘Itália’ (CEFOLA et al., 2018), ‘Red Globe’ (USTUN et al., 2012) e ‘Thompson Seedless’ (LURIE et al., 2006), não há na literatura estudos que tenham avaliado essas técnicas no armazenamento em uvas de mesa ‘BRS Isis’. Essa recente cultivar apirênica, de cor vermelha e sabor neutro, apresenta textura firme e ciclo produtivo tardio (RITSCHEL et al., 2013), o que a torna uma possibilidade interessante visando o armazenamento por longos períodos, a fim de aumentar a oferta na entressafra, especialmente em regiões onde é realizada apenas uma safra ao ano. Nesse contexto, o objetivo deste trabalho foi avaliar o efeito de diferentes concentrações de etanol e CO_2 na qualidade geral, composição fenólica e volátil de uvas de mesa ‘BRS Isis’, como estratégias alternativas ao uso de SO_2 durante 60 e 90 dias de armazenamento.

4.1.2 Material e métodos

4.1.2.1 Material vegetal e preparo das amostras

Uvas de mesa 'BRS Isis' foram colhidas em um parreiral comercial em Serafina Corrêa (28°41'07'' S, 51°56'24'' W, 509 m), Rio Grande do Sul (RS), Brasil, na safra de 2021. Após a colheita, os cachos foram transportados ao Núcleo de Pesquisa em Pós-Colheita (NPP), da Universidade Federal de Santa Maria, RS. Logo, os cachos foram selecionados, eliminando-se bagas pequenas, com danos mecânicos, injúrias causadas por patógenos ou com presença de distúrbios fisiológicos. As uvas apresentavam 14,7 ± 0,12 °Brix de sólidos solúveis, 0,48 ± 0,01% de ácido tartárico, 28,2 ± 0,48 N de firmeza e concentração fenólica de 36 ± 7,26 mg de antocianinas, 11,2 ± 2,01 mg de flavonoides e 2477 ± 121 mg de compostos fenólicos por 100 g de casca, no momento da colheita. As amostras experimentais foram compostas por três cachos, sendo cada tratamento composto por três repetições. Os cachos (~400 g) foram dispostos em cumbucas de polietileno e submetidos as seguintes condições de armazenamento: [1] controle; [2] SO_2; [3] 1.125 ppm de etanol; [4] 2.250 ppm de etanol; [5] 10 kPa de CO_2 e; [6] 15 kPa de CO_2.

4.1.2.1.1 Aplicação de dióxido de enxofre (SO_2)

O SO_2 foi aplicado quinzenalmente (1,5 g kg^{-1} de uva) na forma de metabissulfito de sódio ($Na_2S_2O_5$) conforme proposto por Choudhury (2001). O $Na_2S_2O_5$ foi embrulhado previamente com uma folha de papel manteiga em forma de sachê (15 cm x 25 cm) e colocado sobre as cumbucas no interior da minicâmara. O $Na_2S_2O_5$, quando em contato com a umidade do ar, volatiliza, liberando o SO_2.

4.1.2.1.2 Aplicação de etanol

O etanol (álcool etílico, 92,5%) foi aplicado conforme realizado por Lurie et al. (2006). O etanol foi colocado em um tubo de polipropileno com volume de 15 mL contendo um pavio de papel toalha, sendo disposto no interior das minicâmaras, para que ocorresse a liberação lenta do produto. Foram realizadas aplicações quinzenais de 1 e 2 mL de etanol por kg^{-1} de uvas, obtendo concentrações de 1.125 e 2.250 ppm de etanol, respectivamente.

4.1.2.1.3 Altas concentrações de CO_2

O CO_2 foi injetado em minicâmaras hermeticamente vedadas a partir de cilindro de alta pressão, até atingir os níveis desejados (10 e 15 kPa). Semanalmente foram realizadas aferições dos níveis dos gases no interior das minicâmaras. Quando constatado variação de ± 0,50 kPa

de CO_2, as concentrações foram corrigidas pela adição de mais CO_2 ou remoção do excesso pela absorção com um absorvedor de cal hidratada. A concentração de O_2 foi mantida em condições atmosféricas normais (20,8 kPa O_2), sendo que em condições de variação de ± 4 kPa as concentrações foram corrigidas pela adição de ar atmosférico na minicâmara.

4.1.2.2 Condições de armazenagem

As uvas, dispostas em cumbucas de polietileno, foram colocadas em minicâmaras experimentais metálicas com volume de 0,18 m^3 para avaliar as condições de armazenamento descritas anteriormente. As uvas foram armazenadas por 60 e 90 dias a 0,5 °C ± 0,1 °C e 95 ± 3% de umidade relativa. As minicâmaras permaneceram no interior de uma câmara fria, onde a temperatura foi controlada por termostato e monitorada diariamente por termômetros de bulbo de Hg. A umidade relativa foi monitorada com o auxílio de um psicrômetro e ajustada dentro das minicâmaras.

4.1.2.3 Análises físico-químicas

Após os períodos de armazenamento mais quatro dias a 20 ± 1 ºC e 80 ± 5% de umidade relativa, foram realizadas as análises descritas a seguir, sendo que os compostos voláteis foram determinados apenas aos 60 dias.

4.1.2.3.1 Perda de massa

A perda de massa dos cachos foi obtida a partir da pesagem com balança digital. Pela diferença de massa entre o início e o término do armazenamento obteve-se a perda de massa (PINTO et al., 2015), sendo expressa em percentagem (%).

4.1.2.3.2 Branqueamento, rachadura e degrane das bagas

O branqueamento foi determinado conforme escala visual desenvolvida por Frank et al. (2005), conforme a área superficial das bagas onde, (1) bagas sem danos, (2) danos leves (<10%), (3) danos moderados (11-30%) e (4) danos severos (>30%). O nível de injúria causado pelo branqueamento foi calculado pela equação 1. A rachadura nas bagas foi determinada pela razão entre o número de bagas rachadas e o número total de bagas nos cachos, sendo os resultados expressos em % de bagas rachadas (PINTO et al., 2015). Após uma breve agitação dos cachos, determinou-se o degrane das bagas, conforme a razão entre o número de bagas degranadas e o número total de bagas dos cachos, sendo os resultados expressos em % de bagas degranadas (CHEN et al., 2019).

$$Branqueamento\ das\ bagas = \frac{\Sigma(número\ de\ bagas\ em\ cada\ nível \times nível)}{bagas\ totais} \times 100 \quad (1)$$

4.1.2.3.3 Escurecimento da ráquis

Determinada conforme escala visual proposta por Pinto et al. (2015). Foram considerados cinco níveis de escurecimento: (1) ausência de escurecimento da ráquis; (2) início do escurecimento da região do pedicelo (até 50% atingido); (3) escurecimento do pedicelo e de até 10% do eixo principal da ráquis; (4) escurecimento do pedicelo e de até 50% do eixo principal da ráquis e; (5) escurecimento do pedicelo e de mais de 50% do eixo principal da ráquis. O nível de escurecimento da ráquis foi calculado pela seguinte equação:

$$Escurecimento\ da\ ráquis = \frac{\Sigma(número\ de\ ráquis\ em\ cada\ nível \times nível)}{número\ de\ ráquis\ por\ amostra} \times 100 \quad (2)$$

4.1.2.3.4 Podridão

Determinada a partir da razão entre o número de bagas que apresentavam lesões ocasionadas por fungos e o número total de bagas nos cachos, sendo os resultados expressos em % de bagas deterioradas (PINTO et al., 2015).

4.1.2.3.5 Firmeza

Determinada a partir da inserção de uma ponteira de 8 mm com auxílio de um penetrômetro (Effegi, modelo FT 327, Milão, Itália) na região equatorial de 20 bagas por amostra, coletadas aleatoriamente de cada cacho. Os valores foram expressos em Newton.

4.1.2.3.6 Sólidos solúveis, acidez e índice de maturação das bagas

A partir da trituração de bagas coletadas aleatoriamente de cada cacho em centrífuga (Philips Walita®, Barueri, Brasil), determinou-se o ter de sólidos solúveis por refratometria, sendo os resultados expressos em °Brix. A acidez foi determinada a partir de uma alíquota de 10 mL do suco diluída em 100 mL de água destilada e titulado com solução de NaOH 0,1 N até pH 8,1. Os resultados foram expressos em % de ácido tartárico. O índice de maturação foi obtido por meio da relação entre sólidos solúveis e acidez (AHMED et al., 2019).

4.1.2.3.7 Produção de etileno e taxa respiratória

A produção de etileno e a taxa respiratória foram determinadas em cachos previamente inspecionados, a fim de retirar bagas que apresentavam algum tipo de podridão ou distúrbio que pudesse afetar a análise. Após a remoção das bagas danificadas, os cachos foram mantidos

por quatro horas em recipientes hermeticamente vedados com volume de 5 L. A produção de etileno foi obtida a partir de duas amostras de 1 mL por repetição, retiradas do espaço livre dos recipientes e injetadas em um cromatógrafo a gás (Varian®, modelo Star 3400CX) equipado com detector de ionização por chama (FID) e coluna Porapak N80/100. As temperaturas da coluna, do injetor e do detector foram de 90, 140 e 200 °C, respectivamente. A partir do volume do recipiente, massa dos cachos, tempo de fechamento dos frascos e a concentração de etileno produzida durante o período, calculou-se a produção de etileno, sendo os resultados expressos em ng kg^{-1} s^{-1}. A taxa respiratória foi obtida pela produção de CO_2 nos mesmos recipientes utilizados para análise anterior, determinada com auxílio de um analisador de gás de fluxo contínuo (Schele®, modelo KB7), sendo os resultados expressos em μg kg^{-1} s^{-1}.

4.1.2.3.8 Cor

A coloração das bagas foi determinada conforme Thewes et al. (2021). A partir da alocação de duas bagas postas lado a lado em um recipiente de fundo preto, foi realizada a leitura da cor com um colorímetro eletrônico (Konica Minolta CR-310, Osaka, Japão), previamente calibrado com uma placa padrão branca. A partir de dez análises por repetição, determinou-se simultaneamente a luminosidade, parâmetro a*, parâmetro b*, croma e ângulo hue das bagas. A luminosidade varia entre 0 (preto) e 100 (branco), a* representa a coloração entre vermelho (60) e verde (-60), b* representa a coloração entre amarelo (60) e azul (-60), C* indica a intensidade e o ângulo hue, que varia entre 0 e 360°, indica a tonalidade da cor.

4.1.2.3.9 Antocianinas, flavonoides e compostos fenólicos totais

Para estas determinações foram utilizadas cascas frescas retiradas da porção central de 30 bagas por amostra e separadas da polpa com uma lâmina esterilizada e lavada com água destilada e deionizada. O conteúdo de antocianinas foi determinado em amostras de 1,5 g de cascas frescas conforme descrito por Peppi et al. (2006). As amostras foram avaliadas com o auxílio de um espectrofotômetro (Femto 600 S, São Paulo, Brasil) a 520 nm. Os resultados foram expressos miligramas de antocianinas por cem gramas de cascas frescas (mg 100 g^{-1}). Para a determinação dos flavonoides e compostos fenólicos totais, foram utilizadas amostras de 2 g de cascas frescas conforme realizado por Ahmed et al. (2019). Os flavonoides totais foram determinados conforme metodologia proposta por Woisky e Salino (1998). A absorbância foi obtida com auxílio de um espectrofotômetro com leitura a 415 nm. Os valores foram expressos em mg de equivalente quercetina (EQ) por 100 g^{-1} de cascas frescas (mg EQ 100 g^{-1}). A determinação dos compostos fenólicos foi baseada no método de Folin-Ciocauteau conforme

realizado por Ahmed et al. (2019). A absorbância de cada amostra foi obtida com auxílio do espectrofotômetro a 765 nm. Os valores foram expressos em mg de equivalente ácido gálico (EAG) por 100 g^{-1} de cascas frescas (mg EAG 100 g^{-1}).

4.1.2.3.10 Compostos orgânicos voláteis

As amostras de suco extraídas de 50 bagas por amostra foram armazenadas a -30 °C até o dia da análise, quando foram descongeladas em temperatura ambiente. Foram utilizados 2 mL de suco e misturados com 0,6 g de NaCl e 10 µL de solução padrão de 3-octanol (16 µg mL^{-1}), sendo colocados em um frasco de vidro de 5 mL, selado com tampa rosca e mantidos por 5 min em banho-maria a 35 °C. Após a estabilização da temperatura, foi realizada a adsorção dos compostos voláteis por microextração em fase sólida (HS-SPME), pela exposição de uma fibra coberta por divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) (Supelco, 50/30 µm × 20 mm) no espaço interno e livre do frasco durante 60 min. Para a dessorção dos compostos voláteis, a fibra foi dessorvida termicamente no injetor de um cromatógrafo gasoso (DANI Instruments SpA., Viale Brianza, Cologno Monzese, Italy) a uma temperatura de 250 °C durante 10 min. Os compostos foram separados em uma coluna capilar apolar de sílica fundida DN-5 (Chrompack, EUA; 30 m x 0,25 mm x 0,25 µm), com temperatura inicial de 35 °C, mantida por 3 min, seguida de uma rampa de temperatura de 1 °C min^{-1} até 45 °C, após 2 °C min^{-1} até 80 °C, em seguida elevou-se 6 °C min^{-1} até 230 °C, permanecendo assim por 5 min. O detector por ionização de chama (FID) foi mantido a 230 °C. Os compostos voláteis foram identificados a partir de um cromatógrafo a gás (Shimadzu QP2010 Plus) acoplado a um espectrômetro de massa (GC/MS; Shimadzu Corporation, Kyoto, Japão). Foram utilizados os mesmos procedimentos de extração, dessorção e de injeção para identificação dos compostos voláteis descritos acima, com hélio como gás de arraste. O detector MS foi operado no modo de ionização eletrônica, com energia de ionização de +70 eV, uma faixa de varredura de m/z 35–350 e uma temperatura de 250 °C. A identificação dos compostos foi realizada com base na comparação com a biblioteca de espectros de massa disponível no Instituto Nacional de Padrões e Tecnologia (NIST) e com os índices calculados de Kovats com aqueles disponíveis na literatura científica, de acordo com metodologia proposta por Both et al. (2014). Os compostos foram semi-quantificados em relação ao pico cromatográfico e a concentração do padrão 3-octanol, injetado em cada amostra.

4.1.2.4 Análises estatísticas

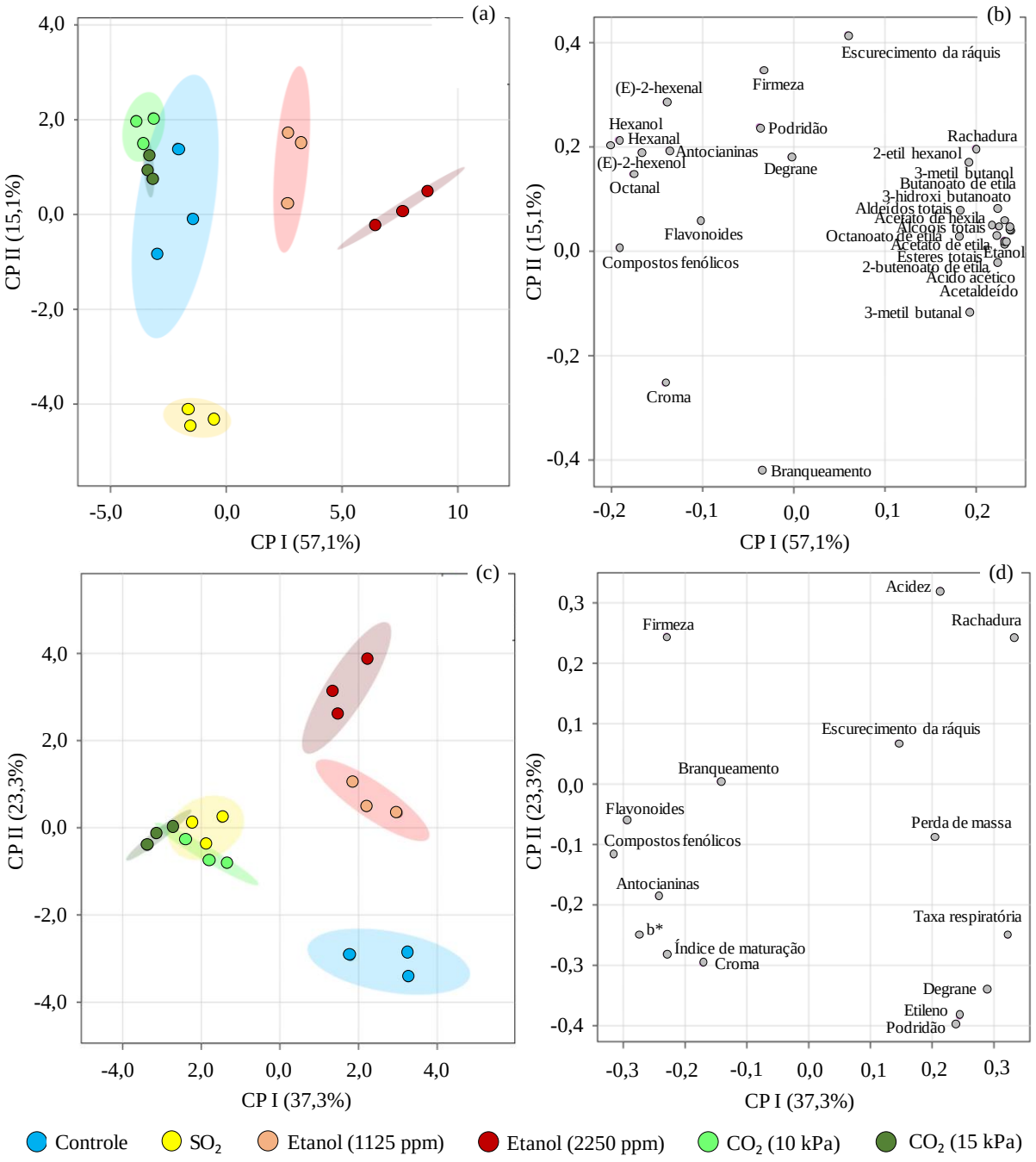
Os dados foram submetidos ao teste de Shapiro-Wilk e Bartlett para avaliar a normalidade dos erros e a homogeneidade das variâncias, respectivamente. Em seguida, realizou-se a análise de variância e os resultados que apresentaram diferenças significativas ($p \leq 0,05$) foram submetidos ao teste de Scott-Knott a 5% de probabilidade, com o programa estatístico SISVAR versão 5.6 (Lavras, MG, Brasil). Os dados foram submetidos a análise de correlação de Pearson e, para uma visão dos resultados, à análise dos componentes principais, utilizando o programa MetaboAnalyst. Previamente, a matriz de dados foi escalada automaticamente para cada variável (média = 0 e variância = 1) visando obter o mesmo peso para todas as variáveis.

4.1.3. Resultados e discussão

4.1.3.1 Análise dos componentes principais

A análise dos componentes principais (CPs) possibilita uma visão geral dos resultados obtidos entre as condições e período de armazenamento de uvas de mesa 'BRS Isis' (Figura 1). Para os 60 dias de armazenamento, os componentes principais (CP I e CP II) explicaram 72,2% da variação total, sendo que 57,1% foi atribuído ao CP I e 15,1% ao CP II (Figura 1a e b). Através da separação atribuída ao CP I, o etanol foi relacionado a maior incidência de rachaduras e concentração de compostos voláteis, especialmente ésteres, como o butanoato de etila, corroborando com os obtidos em melão (LIU et al., 2012), amora e morango (BLANCH et al., 2011), e a uma maior concentração de compostos da fermentação, especialmente acetaldeído e acetato de etila, que podem conferir aromas desagradáveis às uvas quando em altas concentrações (CEFOLA et al., 2018; MAOZ et al., 2019). O alto CO_2 foi relacionado a uma maior concentração de compostos C6, especialmente hexanal e (E)-2-hexenal, que conferem notas verdes ao aroma e estão relacionados ao frescor das uvas (WU et al., 2019). O alto CO_2 também foi relacionado ao conteúdo fenólico, flavonoides e antocianinas, assim como reportado por MAOZ et al. (2019). Por outro lado, o CP II relacionou o SO_2 à presença de branqueamento nas bagas, ainda que os danos tenham sido considerados leves (YUAN et al., 2022), e separou as condições de armazenamento conforme o escurecimento da ráquis, indicando que apenas o SO_2 manteve uma melhor qualidade da ráquis, como previamente reportado por alguns estudos (LICHTER et al., 2011; XUE et al., 2018; ZHANG et al., 2022).

Figura 1 - Análise dos componentes principais de uvas de mesa ‘BRS Isis’ armazenadas durante 60 (a e b) e 90 dias (c e d) a 0,5 °C mais quatro dias de vida de prateleira a 20 °C.



Fonte: (Autor).

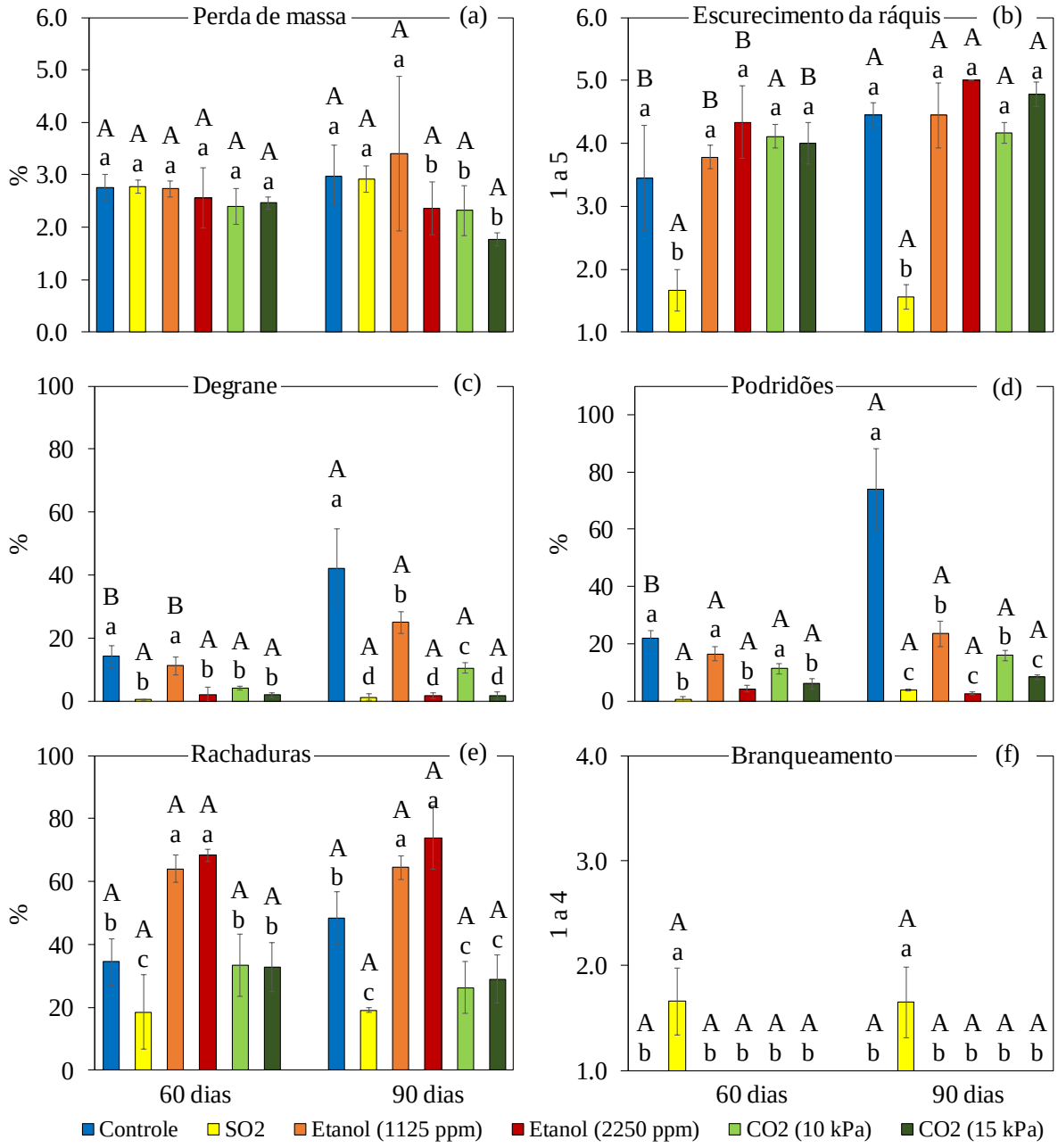
Após 90 dias de armazenamento, os CPs explicaram 60,6% da variação total, sendo 37,3 e 23,3% explicados pelo CP I e CP II, respectivamente (Figure 2c e d). Através da separação realizada pelos CPs, o tratamento controle foi relacionado a produção de etileno, taxa respiratória e especialmente, a uma maior incidência de degrane e de podridões, indicando que assim como o SO_2, o etanol e principalmente, o alto CO_2, reduzem a degradação celular e a

senescência das uvas, corroborando com diversos estudos (CEFOLA et al., 2018; MAOZ et al., 2019; ROMERO et al., 2021; SANCHEZ-BALLESTA et al., 2020; USTUN et al., 2012). Entretanto, o etanol foi relacionado a uma maior acidez e a incidência de rachaduras, possivelmente devido a alteração e degradação de compostos nas células epidérmicas e subepidérmicas (PARSONS et al., 2013; WANG et al., 2018; ZHU et al., 2020), o que pode prejudicar o sabor e o aspecto visual, além de limitar a vida útil das uvas. Por outro lado, o alto CO_2 foi relacionado a uma maior concentração de compostos fenólicos, assim como encontrado aos 60 dias, indicando uma melhor qualidade das uvas, pois o aspecto visual e o valor nutricional decrescem conforme o decréscimo do conteúdo fenólico (XU et al., 2022).

4.1.3.2 Perda de massa, distúrbios e incidência de podridões

As condições avaliadas não reduziram a perda de massa entre os períodos de armazenamento (Figura 2a). No entanto, a concentração de 2.250 ppm de etanol e o alto CO_2 (10 e 15 kPa) reduziram a desidratação das uvas após 90 dias de armazenamento. Alguns estudos reportaram a capacidade do etanol e de altas concentrações de CO_2 em reduzir a desidratação das uvas de mesa (ROMERO et al., 2021; SANCHEZ-BALLESTA et al., 2020; SHAHKOOMAHALLY et al., 2021; VAZQUEZ-HERNANDEZ et al., 2020). A desidratação é uma das principais causas do escurecimento da ráquis (LI et al., 2015), sendo comumente considerado pelos consumidores como um importante parâmetro de qualidade (LI et al., 2022). Entretanto, apenas o SO_2 reduziu o escurecimento da ráquis (Figura 2b), corroborando com diversos estudos que reportaram a capacidade do SO_2 em manter a qualidade da ráquis durante o armazenamento (XUE et al., 2018; ZHANG et al., 2022). Lichter et al. (2011) reportaram que a desidratação afetou o escurecimento da ráquis em uvas ‘Thompson Seedless’, mas não em uvas ‘Superior Seedless’, indicando que outros aspectos, como a produção de etileno (LI et al., 2015; LI et al., 2022) estão relacionados ao escurecimento. No entanto, ainda que o etanol e o CO_2 possam suprimir a produção de etileno (Figura 4a), em altas concentrações podem acumular acetaldeído e reduzir a integridade da membrana celular (LURIE et al., 2006), contribuindo para o escurecimento da ráquis. Assim, o SO_2 por suprimir a síntese de etileno e a atividade de enzimas relacionadas a degradação da clorofila (XUE et al., 2018), mantém a ráquis com tonalidade mais verde ou amarela caqui, sendo mais atrativa aos consumidores em relação à ráquis escura (LICHTER et al., 2011).

Figura 2 - Perda de massa (a), escurecimento da ráquis (b), degrane (c), incidência de podridões (d), rachaduras (e) e branqueamento (f) em uvas de mesa 'BRS Isis' após 60 e 90 dias de armazenamento a 0,5 °C mais quatro dias de vida de prateleira a 20 °C. Valor médio ± desvio padrão (n = 3). As médias seguidas pela mesma letra minúsculas no mesmo período de armazenamento e maiúsculas entre os períodos de armazenamento, não diferem pelo teste de Scott-Knott ($p \leq 0,05$).



Fonte: (Autor).

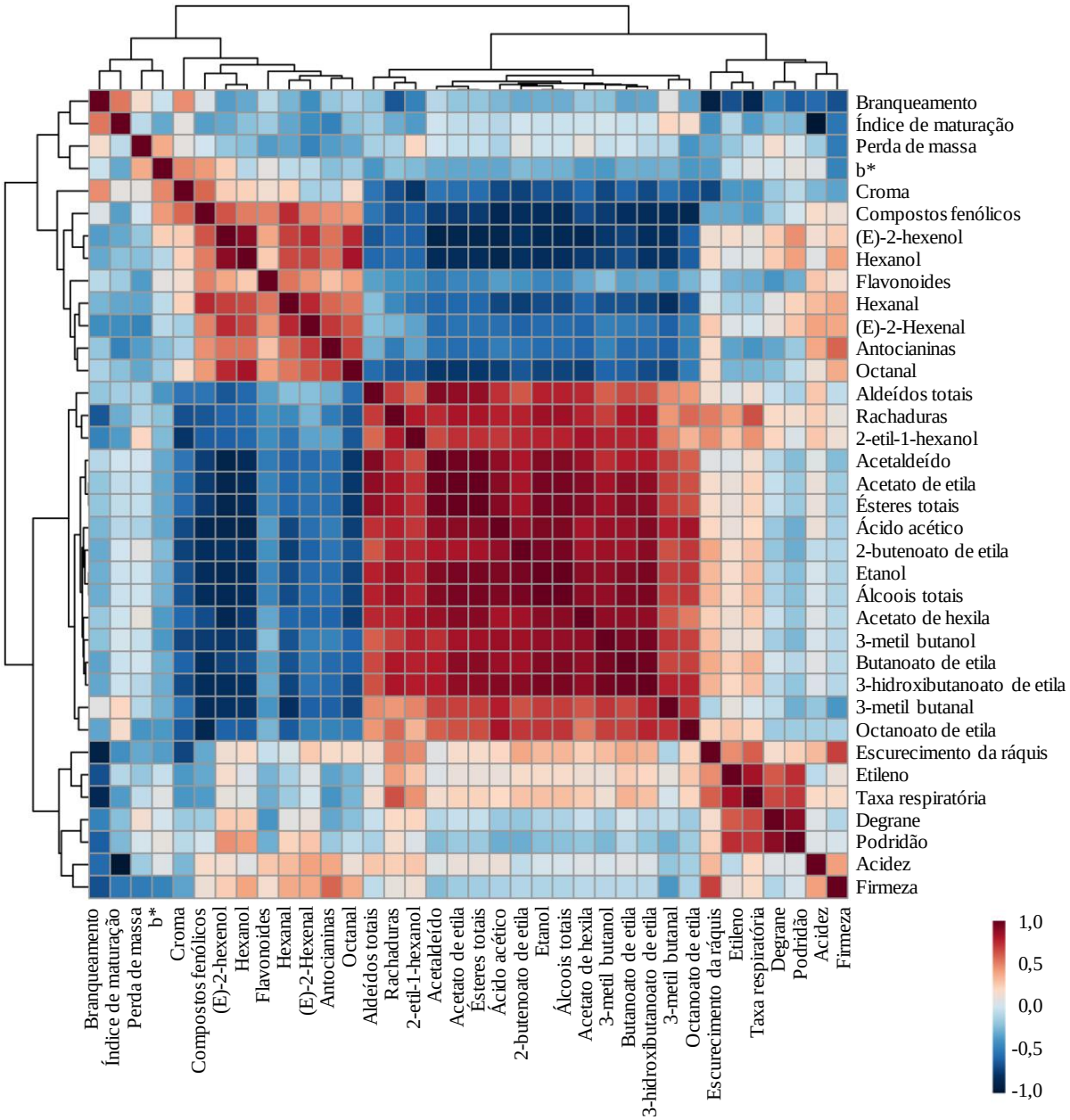
Em relação ao degrane das bagas, com exceção da menor concentração de etanol (1.125 ppm) aos 60 dias, todos os tratamentos aplicados foram eficientes no controle do degrane durante o armazenamento, especialmente o SO_2 e as maiores concentrações de etanol (2.250

ppm) e CO_2 (15 kPa) (Figura 2c). Esses resultados podem estar relacionados à supressão do etileno (Figura 4a) envolvido na sinalização de enzimas, como a poligalacturonase, celulase e pectinametilesterase, que atuam na degradação da parede celular e a formação de cavidades intercelulares na zona de abscisão (CHEN et al., 2019). O desprendimento das bagas também é favorecido pela incidência de patógenos, que estimulam a síntese de etileno e aumentam a senescência das uvas (SHAHKOOMAHALLY et al., 2021), indicado pela alta correlação positiva entre esses parâmetros (Figura 3). Além disso, bagas degranadas são mais suscetíveis a infecções patogênicas em relação a bagas aderidas ao cacho (KOU et al., 2007). Assim, além de controlar o degrane, a aplicação de SO_2 e as maiores concentrações de etanol (2.250 ppm) e CO_2 (15 kPa) foram eficientes também no controle de podridões em ambos os períodos de armazenamento (Figura 2d), corroborando com alguns estudos (CRISOSTO et al., 2002; LURIE et al., 2006; PRETEL et al., 2006). Esses agentes antimicrobianos esterilizam (LURIE et al., 2006; SHAHKOOMAHALLY et al., 2021) e induzem diversos mecanismos de defesa nas bagas envolvidos na homeostase celular (MAOZ et al., 2019; ROMERO et al., 2021; ZHANG et al., 2022). Esses resultados indicam que o armazenamento em alto CO_2 e a aplicação de etanol, podem ser alternativas eficazes ao uso de SO_2 no controle de podridões durante o armazenamento de uvas de mesa.

Uvas de mesa são altamente suscetíveis a rachaduras (CHANG; KELLER, 2021). A rachadura das bagas pode ser causada por diversos fatores relacionados a anatomia da casca, conteúdo de sólidos solúveis, turgor celular e firmeza das bagas (CHANG; KELLER, 2021; PARSONS et al., 2013; ZHU et al., 2020). No presente estudo, interessantemente, o etanol aumentou a incidência de rachaduras independentemente do período de armazenamento (Figura 2e). Entretanto, não foi relacionada a firmeza, perda de massa (Figura 3) e ao conteúdo de sólidos solúveis (Figura 4c), indicando que o etanol pode ter alterado a estrutura celular. Zhu et al. (2020) reportaram que aspectos relacionados ao metabolismo lipídico atuam na rachadura das uvas. O etanol pode alterar a composição da membrana celular, possivelmente aumentando a concentração de monômeros C16, que reduzem a elasticidade da cutícula (MARGA et al., 2001) e aumentam a suscetibilidade às rachaduras (PARSONS et al., 2013). Além disso, o etanol pode ter alterado a composição das pectinas nas células epidérmicas e subepidérmicas, que estão mais expostas ao vapor de etanol, através da esterificação (WANG et al., 2018), o que reduz a coesão celular e contribui para o aumento da sensibilidade dos tecidos às rachaduras. Por outro lado, o SO_2 e altas concentrações de CO_2 (10 e 15 kPa) foram eficientes no controle da incidência de rachaduras, especialmente após 90 dias de armazenamento (Figura 2e). Esses resultados podem ser devidos aos efeitos do SO_2 e alto CO_2 em retardar a senescência

e reduzir a incidência de patógenos nas uvas (SHAHKOOMAHALLY et al., 2021; VAZQUEZ-HERNANDEZ et al., 2020; XUE et al., 2018; ZHANG et al., 2022), suprimindo a ocorrência de alterações nas propriedades mecânicas do pericarpo (ZHU et al., 2020) que as tornam mais propensas à rachaduras.

Figura 3 - Análise de correlação de Pearson entre parâmetros físico-químicos, compostos fenólicos e voláteis em uvas de mesa 'BRS Isis' após 60 dias de armazenamento.



Fonte: (Autor).

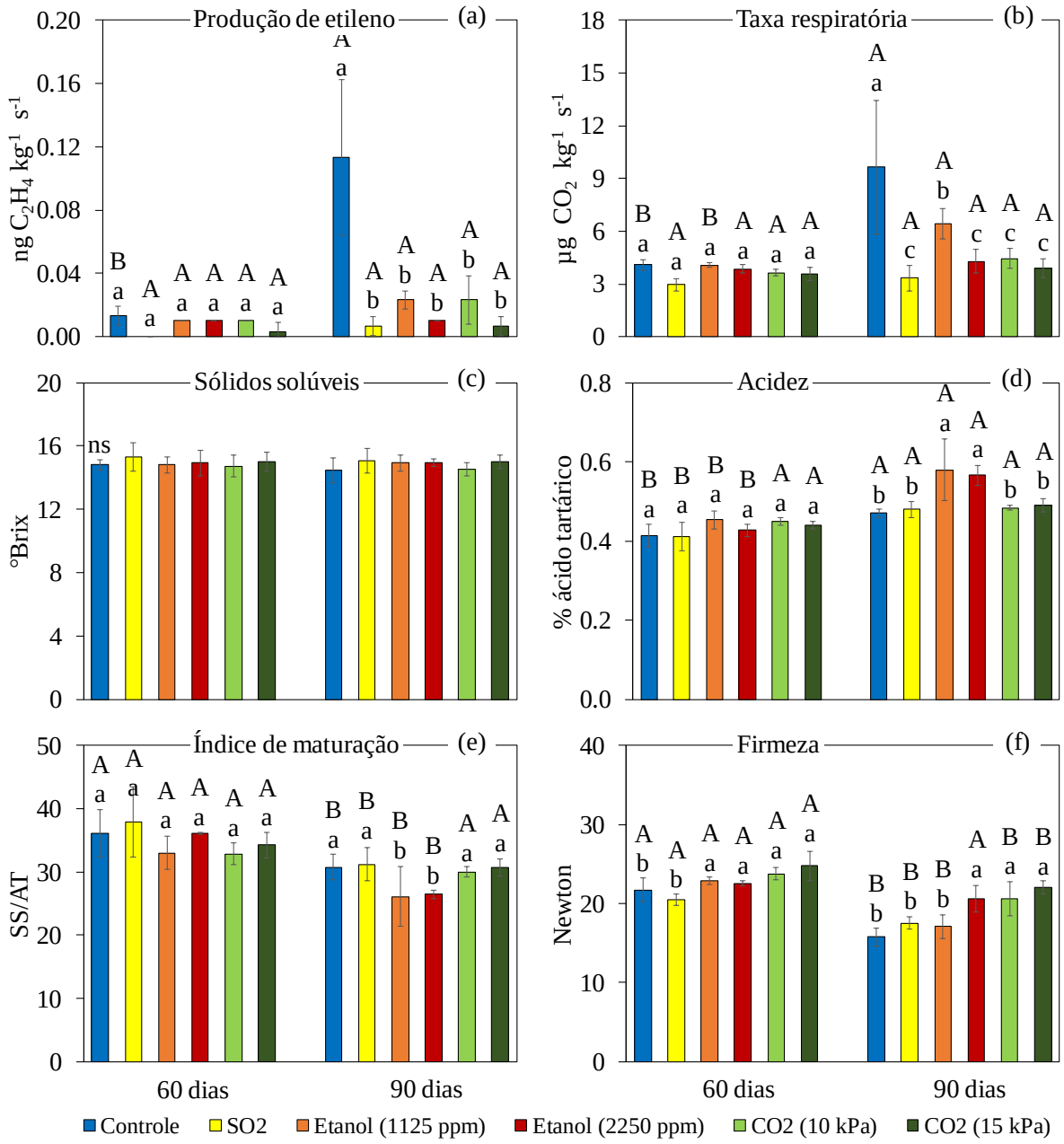
A aplicação de SO_2 é a principal estratégia usada para a manutenção da qualidade de uvas de mesa durante o armazenamento (YUAN et al., 2022). Entretanto, a sua utilização pode

ocasionar alterações físico-químicas, como o branqueamento da epiderme, devido a alteração da estrutura dos pigmentos da casca pela formação de estruturas incolores derivadas de enxofre (CAVALCANTI et al., 2011). Como o esperado, somente bagas tratadas com SO_2 apresentaram branqueamento (Figura 2f). Entretanto, os danos foram considerados leves (YUAN et al., 2022) e não progrediram conforme o aumento do período de armazenamento como reportado por Zoffoli et al. (2008). A gravidade dos danos ocasionados pelo SO_2 é alterada conforme a forma de aplicação, concentração e tempo de exposição (XIAO et al., 2019). Assim, os resultados encontrados no presente estudo indicam que a concentração e o método de aplicação foram adequados, pois foram eficientes no controle do escurecimento da ráquis (Figura 2b), rachaduras (Figura 2e) e de podridões (Figura 2d), sem, no entanto, aumentar o degrane (Figura 2c) e causar danos significativos às bagas.

4.1.3.3 Produção de etileno, taxa respiratória e qualidade físico-química

Uvas são frutas não climatéricas, entretanto, alguns estudos recentes relacionaram o etileno a alguns aspectos fisiológicos e bioquímicos envolvidos na senescência das uvas (LI et al., 2015; LI et al., 2022; XU et al., 2022). No presente estudo, todas as condições de armazenamento avaliadas reduziram a produção de etileno após 90 dias de armazenamento em relação ao tratamento controle (Figura 4a), estando possivelmente relacionados a supressão da expressão gênica e atividade das enzimas precursoras do etileno, como o ácido 1-aminociclopropano-1-carboxílico (ACC) sintase e oxidase (ASODA et al., 2009; ROMERO et al., 2019; XUE et al., 2018). Por outro lado, a produção de etileno aumentou entre os períodos de armazenamento somente em uvas do controle (Figura 4a). Este acréscimo pode ser devido ao aumento das infecções patogênicas constatadas durante o armazenamento (Figura 2d), pois tecidos danificados e o próprio metabolismo fúngico estimulam a síntese de etileno e a taxa respiratória (SHAHKOOMAHALLY et al. 2021), como observado no presente estudo (Figura 4b). Essa constatação pode ser reforçada pela alta correlação positiva encontrada entre esses parâmetros (Figura 3). Li et al. (2015) reportaram uma taxa respiratória 11,4 vezes superior na ráquis em relação as bagas. Como apenas o SO_2 manteve a qualidade da ráquis (Figura 2b), possivelmente outros aspectos, como o degrane das bagas (Figura 2c) e a incidência de podridões (Figura 2d) tenham contribuído para o acréscimo na taxa respiratória no tratamento controle. O aumento da taxa respiratória é um indicativo de acréscimo da atividade metabólica, relacionado a degradação celular e senescência dos tecidos.

Figura 4 - Produção de etileno (a), taxa respiratória (b), sólidos solúveis (c), acidez (d), índice de maturação (e) e firmeza (f) em uvas de mesa 'BRS Isis' após 60 e 90 dias de armazenamento a 0,5 °C mais quatro dias de vida de prateleira a 20 °C. Valor médio ± desvio padrão (n = 3). As médias seguidas pela mesma letra minúsculas no mesmo período de armazenamento e maiúsculas entre os períodos de armazenamento, não diferem pelo teste de Scott-Knott ($p \leq 0,05$).



Fonte: (Autor).

Enquanto o conteúdo de sólidos solúveis não foi alterado durante o armazenamento (Figura 4c), somente bagas armazenadas em alto CO_2 (10 e 15 kPa) mantiveram a concentração de ácidos semelhantes entre os períodos de armazenamento, enquanto nas demais condições ocorreram acréscimos da acidez (Figura 4d). Pretel et al. (2006) observaram que o ácido

tartárico, o ácido mais abundante encontrado nas uvas (USTUN et al., 2012), permanece constante ou mesmo aumenta durante o armazenamento. Além disso, a aplicação de etanol, em ambas as concentrações, aumentou a acidez das bagas após 90 dias, possivelmente devido a degradação das células relacionas a ocorrência de rachaduras (Figura 2e). Devido a maior acidez, bagas tratadas com etanol apresentaram os menores índices de maturação ao término do segundo período de armazenamento (Figura 4e), indicado também pela alta correlação negativa entre esses parâmetros (Figura 3). Entretanto, todas as condições mantiveram uvas com alto índice de maturação (>18) (AHMED et al., 2019), indicando relação entre açucares e ácidos satisfatórias aos consumidores.

Em relação a firmeza, somente uvas tratadas com 2.250 ppm de etanol mantiveram a firmeza semelhante entre os períodos de armazenamento (Figura 4f). Em ambos os períodos, maior firmeza das bagas foram encontradas em uvas armazenadas em alto CO_2 (10 e 15 kPa) e tratadas com 2.250 ppm de etanol, enquanto a menor concentração de etanol (1.125 ppm) foi eficiente na manutenção da firmeza somente até os 60 dias. O efeito positivo do alto CO_2 na manutenção da firmeza das bagas foi reportado em diversas cultivares de uvas de mesa, como ‘Triumph’, ‘Supreme’ (SHAHKOOMAHALLY et al., 2021), ‘Aledo’ (PRETEL et al., 2006) e ‘Cardinal’ (VAZQUEZ-HERNANDEZ et al., 2018). Por outro lado, a aplicação de etanol não afetou a firmeza das bagas durante o armazenamento de uvas ‘Superior Seedless’ (LICHTER et al., 2002) e ‘El-Bayadi’ (AL-QURASHI; AWAD, 2013). No entanto, aqueles estudos realizaram a aplicação do etanol por imersão, em que a eficiência do etanol é limitada por apresentar um menor efeito residual nos cachos em relação a aplicação por vapor (LICHTER et al., 2002), como realizado no presente estudo. Assim, os resultados encontrados podem ser devidos ao etanol e do alto CO_2 suprimirem a atividade de enzimas, como a peroxidase, celulase, pectinametilesterase e poligalacturonase, que estão relacionadas ao amolecimento das bagas (ZHU et al., 2020), reduzindo o processo de senescência das uvas.

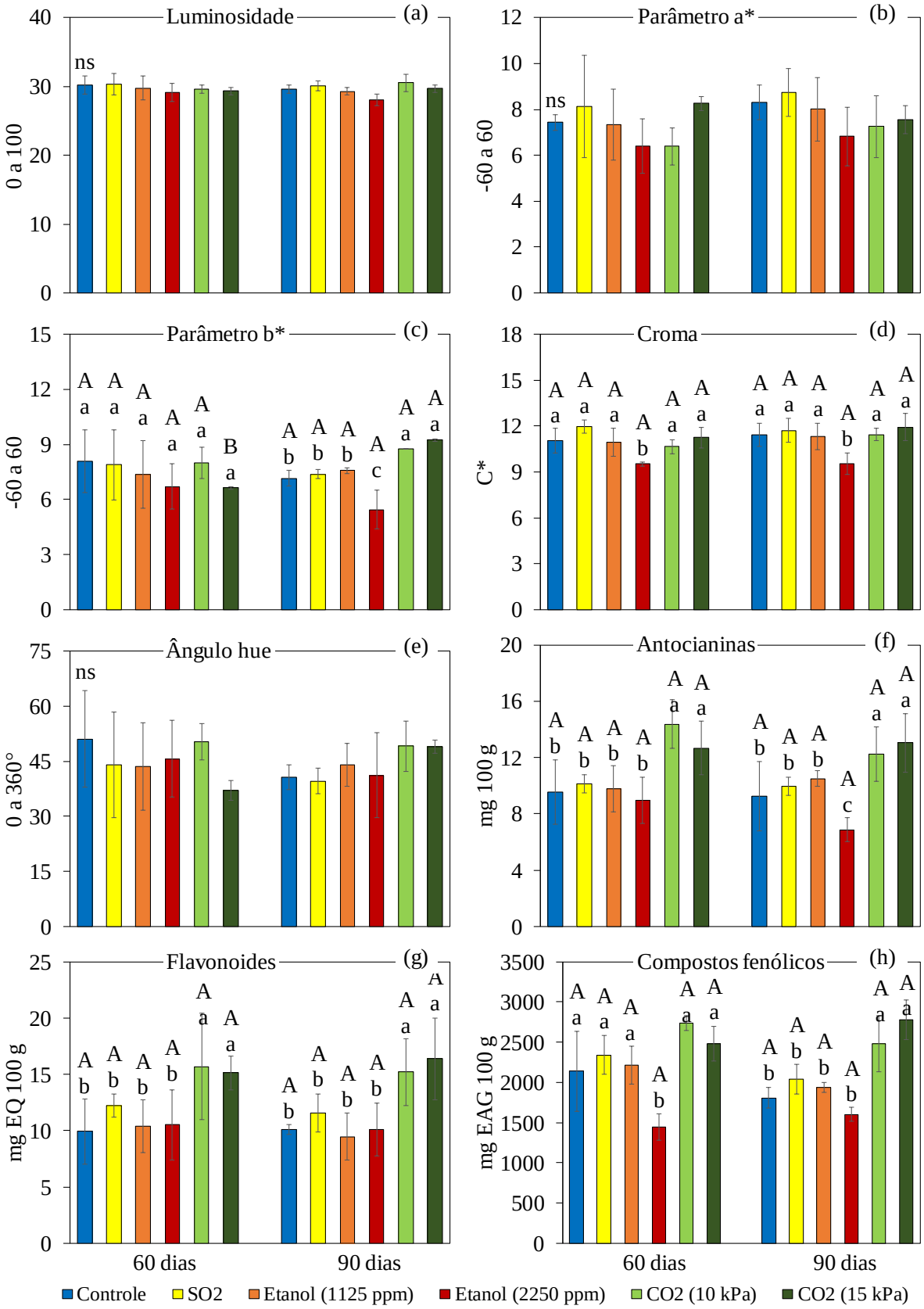
4.1.3.4 Coloração e compostos fenólicos

A coloração das uvas foi bem caracterizada a partir da luminosidade, parâmetro a*, parâmetro b*, croma e ângulo hue (Figura 5a à e). No entanto, apenas o parâmetro b* e o croma foram afetados pelas condições de armazenamento, havendo poucas alterações na cor durante o armazenamento (Figura 5c e d). Uvas armazenadas com alto CO_2 (10 e 15 kPa) apresentaram os maiores valores do parâmetro b*, enquanto os menores valores foram encontrados aos 90 dias em uvas tratadas com 2.250 ppm de etanol (Figura 5c). A maior concentração de etanol também resultou em bagas com o menor croma em ambos os períodos, indicando bagas com

coloração menos intensa (Figura 5d). Lurie et al. (2006) reportaram que a aplicação de etanol em doses superiores a 5 mL kg^{-1} causou o escurecimento de uvas 'Thompson Seedless' após 45 dias de armazenamento. Em altas concentrações, o etanol pode inibir a atividade da fenilanina amônia-liase, envolvida na rota de síntese dos compostos fenólicos (YAN et al., 2015) e aumentar a atividade da polifenol oxidase (JI et al., 2021), que causa a degradação de compostos que pigmentam a casca das uvas, especialmente as antocianinas. Este resultado pode ser confirmado pela menor concentração de antocianinas encontrada após 90 dias de armazenamento em bagas tratadas com 2.250 ppm de etanol (Figura 5h).

Maior concentração de compostos fenólicos totais, flavonoides e antocianinas foram encontrados em uvas armazenadas em alto CO_2 (10 e 15 kPa) em ambos os períodos de armazenamento (Figura 5f, g e h). O alto CO_2 pode inibir a oxidação de monofenóis pela polifenol oxidase (MURR; MORRIS, 1974), reduzindo a degradação dos compostos fenólicos durante o armazenamento. Além disso, Maoz et al. (2019) reportaram acréscimo da atividade da fenilanina amônia-liase e concentração de compostos fenólicos em uvas 'Superior Seedless' armazenadas a 15 kPa em relação a 5 kPa de CO_2. Por outro lado, Romero et al. (2008) constataram que a aplicação de 20 kPa de CO_2 por três dias reduziu o conteúdo de antocianinas durante o armazenamento em uvas 'Cardinal'. Sanchez-Ballesta et al. (2019) reportaram que o armazenamento em 20 kPa de CO_2 aumentou a expressão de alguns genes envolvidos na síntese de estilbenos, como *VviSTS6*, *VviSTS7*, *VviSTS16* e *VviSTS46* em uvas 'Dominga', enquanto suprimiu a expressão em uvas 'Red Globe'. Assim, o efeito do CO_2 sobre o conteúdo fenólico é dependente do período de exposição e concentração do CO_2, além das características genéticas da cultivar. Nesse sentido, o armazenamento em alto CO_2 no presente trabalho manteve melhor qualidade das uvas, pois o aspecto visual e o valor nutricional decrescem conforme o decréscimo do conteúdo fenólico (XU et al., 2022).

Figura 5 – Cor e compostos fenólicos de uvas ‘BRS Isis’ após 60 e 90 dias de armazenamento. Valor médio ± desvio padrão (n = 3). As médias seguidas pela mesma letra minúsculas no mesmo período e maiúsculas entre os períodos de armazenamento, não diferem pelo teste de Scott-Knott ($p \leq 0,05$).

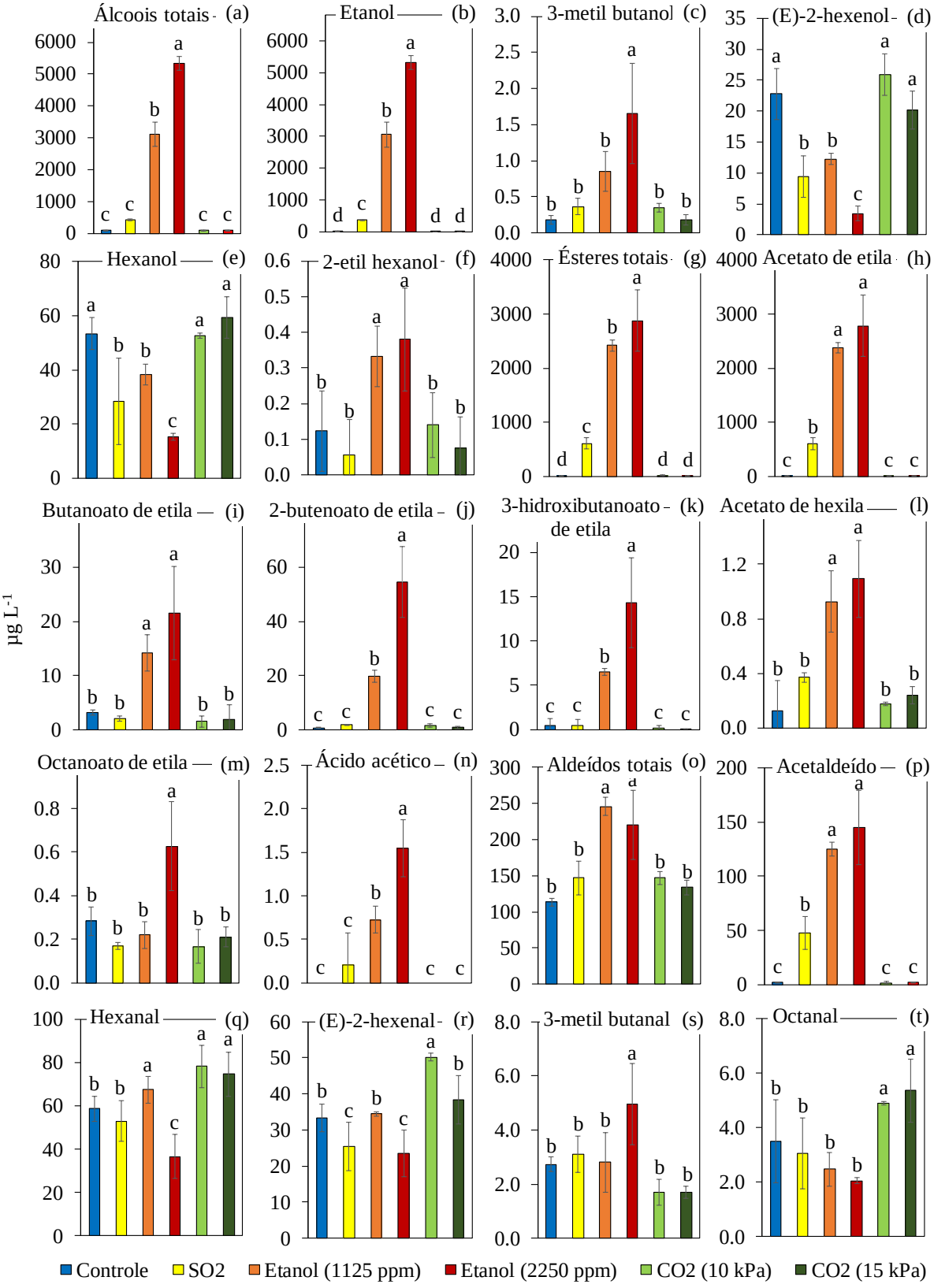


4.1.3.5 Compostos orgânicos voláteis

No presente estudo, foram encontrados 32 compostos orgânicos voláteis nas uvas após 60 dias de armazenamento, incluindo aldeídos, álcoois, ésteres, terpenos, cetonas e ácidos (Figura 6). Dentre estes, cinco aldeídos (acetaldeído, 3-metil butanal, hexanal, (E)-2-hexenal e octanal), cinco álcoois (etanol, 3-metil butanol, hexanol, (E)-2-hexenol e 2-etil hexanol), seis ésteres (acetato de etila, butanoato de etila, 2-butenoato de etila, 3-hidroxibutanoato de etila, acetato de hexila e octanoato de etila) e um ácido (ácido acético) foram significativamente afetados pelas condições de armazenamento.

Como o esperado, as aplicações das diferentes doses de etanol resultaram em maior acúmulo de etanol nas uvas, sendo as maiores concentrações encontradas em bagas tratadas com 2.250 ppm (Figura 6b). Interessantemente, o SO_2 também aumentou a concentração de etanol, ainda que tenha ocorrido em menor proporção. Aparentemente, o SO_2 pode aumentar a peroxidação lipídica (KIMMERER; KOZLOWSKI, 1982), envolvida em uma das rotas de síntese do etanol (PESIS, 2005). No entanto, ainda que a concentração do etanol tenha sido inferior ao seu limiar sensorial (100.000 µg L^{-1}) (MAOZ et al., 2019), este é um importante precursor para a síntese de outros compostos voláteis. O etanol aplicado na fruta pode ser metabolizado pela esterificação, formando ésteres de etila ou oxidado, formando acetaldeído (PESIS, 2005). No presente estudo, foi observada uma maior conversão de etanol em acetato de etila em relação ao acetaldeído (Figura 6h e p). Este resultado é devido as frutas metabolizarem preferencialmente o etanol em ésteres etila, devido ao acetaldeído ser altamente tóxico às frutas (PESIS, 2005), estando relacionado ao escurecimento da ráquis e das bagas em uvas (LURIE et al., 2006). As maiores concentrações de acetato de etila e acetaldeído foram encontrados em uvas tratadas com SO_2 e especialmente, com etanol (Figura 6h e p), porém apenas o acetaldeído foi encontrado em concentração superior ao seu limiar sensorial (15 µg L^{-1}) (MAOZ et al., 2019). No entanto, enquanto em baixas concentrações este composto confere notas adocicadas e frutadas, em alta concentração está relacionado a ocorrência de sabores desagradáveis nas uvas (MAOZ et al., 2019). Cefola et al. (2018) constataram a ocorrência de *off-flavours* em uvas ‘Itália’ a partir de 10 µg L^{-1} de acetaldeído. Esses resultados indicam que as aplicações de SO_2 e especialmente de etanol, reduziram a qualidade aromática das uvas, devido ao acúmulo de compostos desagradáveis, o que pode limitar a aceitação dos consumidores. Por outro lado, o armazenamento em alto CO_2 não provocou aumento na concentração deste composto *off-flavour* (Figura 6h e p).

Figura 6 - Compostos voláteis de uvas de mesa ‘BRS Isis’ após 60 dias de armazenamento (0,5 °C) mais quatro dias de vida de prateleira a 20 °C. Valor médio ± desvio padrão (n = 3). Médias seguidas pela mesma letra não diferem pelo teste de Scott-Knott ($p \leq 0,05$).



Fonte: (Autor).

A aplicação de etanol resultou em uvas com concentração significativamente superior de ésteres (Figura 6g), corroborando com resultados encontrados em outras frutas, como em melão (LIU et al., 2012), amora e morango (BLANCH et al., 2011). Entretanto, apenas as concentrações de butanoato de etila foram superiores ao seu limiar sensorial (1 µg L^{-1}) (WU et al., 2019), especialmente em uvas tratadas com etanol (Figura 6i). O acréscimo na concentração de butanoato de etila indica uma maior presença de notas de morango ao aroma das uvas (WU et al., 2019). Blanch et al. (2011) observaram que o etanol aumentou a concentração de butanoato de etila em amora e morango, mas não em framboesa, indicando que fatores genéticos podem estar relacionados a sensibilidade das frutas ao etanol na síntese de ésteres. Assim, os resultados encontrados no presente estudo indicam que o etanol aumentou a concentração de ésteres, porém, com exceção do butanoato de etila, em concentrações que pouco contribuíram para o aroma das uvas 'BRS Isis', assim como reportado em cultivares de aroma neutro (WU et al., 2019).

Os aldeídos C6, especialmente hexanal e (E)-2-hexenal, conferem notas verdes ao aroma e são o fundo básico dos voláteis em uvas (WU et al., 2019). No presente estudo, foram observadas as maiores concentrações destes compostos em uvas armazenadas em 10 kPa de CO_2, não diferindo a concentração de hexanal de uvas armazenadas com 1.125 ppm de etanol e 15 kPa de CO_2, enquanto o armazenamento em 2.250 ppm de etanol reduziu significativamente a concentração de ambos os compostos (Figura 6q e r). Entretanto, todas as condições mantiverem concentrações de hexanal e (E)-2-hexenal acima de seus limiares sensoriais (4,5 e 17 µg L^{-1}, respectivamente) (WU et al., 2019). Esses resultados certamente estão relacionados ao metabolismo dos lipídios da membrana celular, pois enquanto etanol reduz a atividade da enzima lipoxigenase (LIU et al., 2012), o alto CO_2 resulta em acréscimos na concentração de ácidos graxos insaturados, principalmente dos ácidos linoleico e linolênico (VAZQUEZ-HERNANDEZ et al., 2020), que são oxidados pela enzima lipoxigenase em aldeídos, como o hexanal e (E)-2-hexenal (NOGUEROL-PATO et al., 2012). Cefola et al. (2018), associaram o hexanal e (E)-2-hexenal a uma maior qualidade das uvas, sendo correlacionados negativamente com os compostos da fermentação (etanol, acetaldeído e acetato de etila), a exemplo do presente estudo (Figura 3). Esses resultados indicam que o alto CO_2 foi eficiente na manutenção da qualidade das uvas, pois esses compostos aromáticos que estão relacionados ao aspecto de frescor (CEFOLA et al., 2018; WU et al., 2019), são rapidamente convertidos em outros conforme o aumento da atividade enzimática causada pela desintegração da estrutura celular durante o amadurecimento (YANG et al., 2011).

4.1.4 Conclusões

O armazenamento com 2.250 ppm de etanol e 15 kPa de CO_2 apresentam eficiências semelhantes ao SO_2 no controle do degrane e de podridões. O SO_2 reduz o escurecimento da ráquis, porém causa branqueamento nas bagas. O etanol apresenta o inconveniente de aumentar a incidência de rachaduras. Além disso, o etanol aumenta a contração de álcoois, aldeídos e, especialmente ésteres, no entanto causa o acúmulo de acetaldeído que pode causar aroma desagradável às uvas. Altas concentrações de CO_2 são eficientes na manutenção da qualidade das uvas, mantendo maior concentração de compostos fenólicos e voláteis, especialmente hexanal e (E)-2-hexenal, que estão relacionados ao frescor das uvas.

A exposição ao etanol durante o armazenamento, e especialmente, o uso de alto CO_2, são alternativas promissoras ao SO_2 na manutenção da qualidade de uvas de mesa 'BRS Isis' durante o armazenamento prolongado (90 dias). Uvas 'BRS Isis' apresentam um bom potencial de armazenamento, contribuindo para o aumento da oferta em épocas de escassez de uvas de mesa no mercado. Estudos futuros que avaliem a interação entre concentrações de etanol e de CO_2 são recomendados, a fim de avaliar o efeito sinérgico das técnicas na redução da senescência das uvas, reduzir a ocorrência de rachaduras pelo etanol e obter uvas mais aromáticas em condições de armazenamento em alto CO_2.

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5 DISCUSSÃO GERAL

A produção de uvas é um segmento tradicional da fruticultura do Estado do Rio Grande do Sul, sendo que cerca de 80% da produção é proveniente da região da Serra Gaúcha. Entretanto, essa produção consiste, em sua grande maioria, de uvas do tipo americana (*Vitis labrusca*), destinadas para o processamento, como para elaboração de vinhos e sucos. Uma pequena parcela destinada ao consumo *in natura*, é proveniente de uvas de mesa (*Vitis vinifera*), especialmente da tradicional 'Itália' e de suas mutações, 'Benitaka', 'Rubi' e 'Brasil'. Ainda que sejam amplamente apreciadas pelos consumidores, devido a elevada suscetibilidade a doenças e requerer mão de obra intensiva, a expansão da produção dessas cultivares é limitada em condições de clima mais úmido como o da Serra Gaúcha.

Recentemente, a partir do lançamento de novas cultivares híbridas pelo programa de melhoramento da Embrapa 'Uvas do Brasil', está havendo um interesse crescente na produção de uvas de mesa destinadas ao consumo *in natura*, seja pela instalação de novas áreas produtoras ou substituição das cultivares tradicionais (MAIA et al., 2018). Essas novas cultivares se caracterizam por uma maior adaptabilidade e tolerância a pragas, o que favorece o cultivo em diferentes condições edafoclimáticas. A exemplo disso, cultivares de uvas de mesa como a 'BRS Núbia' e a 'BRS Isis' que foram lançadas inicialmente para a produção no Vale do São Francisco, estão sendo produzidas também na Serra Gaúcha (MAIA et al., 2013; MAIA et al., 2018; RITSCHEL et al., 2013).

No entanto, a qualidade das uvas pode ser amplamente afetada pelas condições climáticas (OLIVEIRA et al., 2019). Além disso, diferentemente do Vale do São Francisco, é possível apenas um ciclo produtivo na região Sul, concentrando a produção de uvas em um curto período do ano, gerando demanda de uvas provenientes de outras regiões ou países no período de entressafra. Assim, devido à falta de relatos sobre a qualidade de uvas de mesa produzidas em condições temperadas na Serra Gaúcha, foi realizado um estudo para caracterizar a qualidade das novas cultivares 'BRS Núbia' e 'BRS Isis', além da tradicional 'Itália'. Além disso, foram realizados dois estudos para avaliar técnicas de armazenamento a fim de prolongar a qualidade pós-colheita das uvas na forma de cachos e de bagas degranadas, o que poderia beneficiar a produção local e ampliar a oferta das uvas. Resultados destes estudos também podem ser aplicados visando à conservação durante o transporte marítimo de uvas exportadas para outros países.

As novas cultivares de uvas de mesa 'BRS Núbia' e 'BRS Isis' possuem altas concentrações de compostos bioativos, especialmente a 'BRS Núbia', devido a elevada

concentração de antocianinas na casca, que atribui uma coloração mais escura à epiderme (Artigo 1). A ‘BRS Núbia’ apresenta bagas de tamanho grande, significativamente superior a grande maioria das uvas de mesa encontradas nas gôndolas dos mercados (LEÃO et al., 2020; MASCARENHAS et al., 2012). Devido as uvas com bagas grandes serem amplamente apreciadas pelos consumidores (MAIA et al., 2018) e o crescente interesse em alimentos com maior concentração de compostos bioativos (PINTO et al., 2022), a ‘BRS Núbia’ apresenta potencial para se tornar a principal cultivar de uvas de mesa com epiderme escura no mercado nacional, considerando que o mercado de uvas de epiderme escura atualmente é pouco diversificado e é suprido em quase sua totalidade pelas uvas ‘Brasil’ (MAIA et al., 2018).

As uvas ‘BRS Isis’ apresentam bagas médias, de coloração vermelha, porém com maior saturação de cor (h° = ~45) em relação a uvas produzidas no Vale do São Francisco (h° = ~56) (AHMED et al., 2019). Assim, o cultivo em clima mais ameno pode favorecer o acumulo de antocianinas e uma maior intensidade e cobertura de cor vermelha nas uvas. Esse resultado apresenta uma utilização prática relevante, pois enquanto o cultivo em climas mais quentes, onde há altas temperaturas médias e baixa amplitude térmica diária (OLIVEIRA et al., 2019), comumente são necessárias aplicações de produtos, como ácido abscísico e etefon, para intensificar e uniformizar a cor, em condições de temperaturas mais amenas a aplicação desses produtos pode ser suprimida. A aplicação destes fitorreguladores, além de incrementarem o custo de produção, nem sempre são eficazes (ROBERTO et al., 2013). Além da ausência de sementes, outra característica importante da ‘BRS Isis’, é a sua maior firmeza das bagas, o que pode contribuir para uma maior resistência a injúrias ocasionadas durante a colheita, após o processo de embalagem e transporte, ou ainda prolongar o período de armazenamento.

A tradicional ‘Itália’ apresenta bagas relativamente grandes de coloração verde-amarela, sendo a principal cultivar de coloração clara no mercado brasileiro (MAIA et al., 2018). A uva ‘Itália’, ainda que apresente baixa concentração de compostos fenólicos, assim como a maioria das cultivares de epiderme claras (AUBERT; CHALOT, 2018; MIKULIC-PETKOVSEK et al., 2018), apresenta elevado conteúdo de compostos voláteis, especialmente terpenos, como linalool, geraniol, nerol, citronelool e α-terpineol, que caracterizam o aroma moscato, amplamente apreciado pelos consumidores (RUIZ-GARCÍA et al., 2014). Por outro lado, as uvas ‘BRS Núbia’ e ‘BRS Isis’, podem ser caracterizadas como de aroma neutro, por não apresentarem altas concentrações de compostos voláteis, exceto dos aldeídos hexanal e (E)-2-hexenal, que são compostos de fundo do aroma das uvas (WU et al., 2019). Inicialmente, possivelmente a partir de análise sensorial, foi proposto que estas cultivares apresentam aroma

neutro (MAIA et al., 2013; RITSCHEL et al., 2013), porém este é o primeiro estudo que caracteriza o perfil volátil das uvas 'BRS Núbia' e 'BRS Isis', validando esta hipótese.

Algumas características encontradas nessas cultivares, como bagas de tamanho grande ('BRS Núbia'), boa firmeza ('BRS Isis') e aroma moscato ('Itália') pode favorecer a comercialização destas uvas na forma de bagas degranadas. Entretanto, essa forma de comercialização é limitada por alterações fisiológicas e bioquímicas causadas durante o destacamento das bagas da ráquis, que acelera o processo de senescência das uvas e aumenta a suscetibilidade a incidência de patógenos, limitando o potencial de armazenamento entre 15 e 30 dias (NICOLOSI et al., 2018; SABIR et al., 2021; TYAGI et al., 2020). No presente estudo (Artigo 2), todas as cultivares mantiveram boa qualidade após 60 dias de armazenamento a 0,5 °C associada a aplicação quinzenal de SO_2 ou a imersão inicial em etanol, sendo um período de armazenamento superior ao encontrado na literatura. A presença de uma pequena porção do pedicelo (~2 mm) aderido a baga pode ter contribuído para uma menor incidência de patógenos e suprimido a degradação das uvas.

Como parte do pedicelo, que apresenta resposta climatérica (LI et al., 2015), permanece aderida a baga, era esperado que o 1-MCP poderia manter a qualidade das uvas. No entanto, o 1-MCP não suprime a produção de etileno e reduz a senescência das bagas durante o armazenamento, assim como encontrado em uvas 'Shine Muscat' (ZHANG et al., 2022). O SO_2 é eficiente na manutenção da qualidade das uvas, sendo que os danos causados foram considerados leves, possivelmente devido ao método e concentração do produto aplicado terem sido adequados ou mesmo a uma menor sensibilidade das cultivares ao SO_2. Outro aspecto relevante é que em uvas degranadas, onde o pedicelo é reduzido, sendo a principal entrada para o SO_2 nas uvas (YUAN et al., 2022), o acúmulo do SO_2 nas bagas pode ter sido limitado. O tratamento térmico com água quente e o etanol são técnicas simples e promissoras que podem ser utilizadas como alternativa ao uso do SO_2, especialmente o etanol, que mantem a qualidade das bagas por até 60 dias, enquanto a imersão em água quente perde eficiência na manutenção da qualidade das uvas após 30 dias. Essa menor eficiência do tratamento térmico pode ser devida a não impedir reinfecções patogênicas (KOU, 2009), mesmo que induza diversos mecanismos de defesa nas bagas (WU et al., 2015). Entretanto, a aplicação de água quente e etanol em uvas 'BRS Isis' deve ser melhor avaliado, pois promove a ocorrência de rachaduras nas bagas desta cultivar apenas.

O etanol aplicado na forma de vapor também aumenta a ocorrência de rachaduras em uvas 'BRS Isis' armazenadas na forma de cachos (Artigo 3). Esse resultado indica que possivelmente algumas características anatômicas relacionadas ao arranjo das células

epidérmicas e subepidérmicas, além de uma menor elasticidade e espessura da cutícula (ZHANG et al., 2020), acrescido de possíveis alterações causadas pelo etanol na membrana celular e na lamela média das células epidérmicas (PARSONS et al., 2013; WANG et al., 2018), possam favorecer a ocorrência de rachaduras nessa cultivar. No entanto, recomenda-se a realização de estudos para melhor elucidar estes resultados. O armazenamento com 2.250 ppm de etanol e 15 kPa de CO_2 apresenta eficiência semelhante ao SO_2 no controle do degrane e de podridões nas uvas 'BRS Isis' armazenadas por 90 dias. Esses resultados são relevantes, pois o etanol e o CO_2 suprimem a ocorrência dos principais problemas que ocorrem durante o armazenamento de uvas de mesa.

Entretanto, apenas o SO_2 reduz o escurecimento da ráquis, mantendo a ráquis com tonalidade mais verde ou amarela caqui, sendo mais atrativa aos consumidores em relação às tonalidades escuras (LICHTER et al., 2011). Alguns estudos reportaram que o etanol e o CO_2 em altas concentrações não mantem a qualidade da ráquis (CRISOSTO et al., 2002; LURIE et al., 2006), porém no presente estudo não houve diferença em relação ao controle. Além disso, a aplicação de etanol causa o acúmulo de acetaldeído que pode conferir aroma desagradável às uvas (CEFOLA et al., 2018). O SO_2 foi relacionado a ocorrência de branqueamento das bagas, devido a alteração da estrutura das antocianinas pela formação de estruturas incolores derivadas de enxofre (CAVALCANTI et al., 2011), entretanto, não foi suficiente para reduzir a concentração de compostos fenólicos e antocianinas da casca.

As maiores concentrações de compostos fenólicos, flavonoides e antocianinas, foram encontrados em bagas armazenadas em alto CO_2, indicando uma menor sensibilidade ao alto CO_2 como observado em uvas 'Superior Seedless' (MAOZ et al., 2019), enquanto em algumas cultivares o CO_2 parece conferir um efeito negativo na concentração de compostos fenólicos (SANCHEZ-BALLESTA et al., 2020). O armazenamento em alto CO_2 manteve maior concentração dos compostos voláteis hexanal e (E)-2-hexenal, que são compostos de fundo do aroma (WU et al., 2019) e conferem um aspecto de frescor, indicando uma melhor qualidade das uvas. Devido a isso, o armazenamento em alto CO_2 pode ser uma alternativa promissora ao uso do SO_2 no controle de patógenos e na manutenção da qualidade das uvas 'BRS Isis' durante 90 dias de armazenamento.

6 CONSIDERAÇÕES FINAIS

As cultivares de uvas de mesa ‘BRS Núbia’ e ‘BRS Isis’ produzidas em condições temperadas da Serra Gaúcha são ricas em compostos bioativos, especialmente a ‘BRS Núbia’, que apresenta bagas grandes de coloração preta-azuladas e elevada concentração de antocianinas. A ‘BRS Isis’ apresenta bagas apirênicas de tamanho médio, coloração vermelha e destaca-se por ter boa firmeza, o que pode contribuir para uma maior resistência a danos mecânicos causados na colheita e durante o transporte a longas distâncias, ou mesmo favorecer o armazenamento por longos períodos. A tradicional uva ‘Itália’, apresenta bagas grandes de cor verde-amarela com altas concentrações de compostos orgânicos voláteis, especialmente terpenos, que conferem o aroma moscato e que é amplamente apreciado pelos consumidores. Assim, características como bagas grandes, boa firmeza e com aroma moscato encontradas nestas cultivares podem favorecer as suas comercializações na forma de bagas.

Essas uvas podem ser conservadas na forma de bagas degranadas por até 60 dias em armazenamento refrigerado associado a aplicação quinzenal de SO_2 ou imersão inicial em etanol. O tratamento térmico com água quente é uma técnica simples que pode ser utilizada na conservação das uvas por até 30 dias. O etanol apresentou eficiência no controle de patógenos semelhante ao SO_2 durante o armazenamento. O SO_2 apresenta o inconveniente de causar o branqueamento das bagas. A aplicação de 1-MCP não é recomendada por não reduzir a senescência das bagas. A aplicação de etanol e água quente em uvas ‘BRS Isis’ deve ser melhor avaliada, devido a ocorrência de rachaduras nas bagas, o que prejudica o aspecto visual e consequentemente, pode reduzir a aceitação do consumidor.

Na forma de cachos, uvas ‘BRS Isis’ podem ser armazenadas por até 90 dias quando aplicado SO_2, etanol ou altas concentrações de CO_2 na atmosfera de armazenamento. O armazenamento com 2.250 ppm de etanol e 15 kPa de CO_2 apresenta eficiência semelhante ao SO_2 no controle do degrane e de podridões. Apenas o SO_2 mantém uma melhor qualidade da ráquis, porém causa branqueamento nas bagas. A aplicação de etanol não é recomendada em uvas ‘BRS Isis’, pois ocasiona rachaduras nas bagas e o acúmulo de acetaldeído, que pode causar aroma desagradável às uvas. Altas concentrações de CO_2 mantem as uvas com maior concentração de compostos fenólicos e voláteis, especialmente hexanal e (E)-2-hexenal, que remetem ao aspecto de uvas recém colhidas. Assim, levando em consideração a qualidade das uvas, a melhor alternativa ao uso do SO_2 no armazenamento de uvas ‘BRS Isis’ é a utilização do alto CO_2, especialmente 15 kPa, pois controla a incidência de patógenos e o degrane semelhante ao SO_2, além de preservar a qualidade fenólica e volátil das uvas.

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