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Analysis of the contents of *Ugni molinae* Turcz fruits across the ripening stages

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ABSTRACT

Recently, a growing interest in fruits having a high content of health-promoting compounds has become a trend among consumers around the world. Wild berries have received special attention based on the high levels of healthy compounds present in them. In this work, we elaborate on the qualities of the murta (*Ugni molinae* Turcz) fruit such as the colour, the volatile organic compounds (VOCs) present, its antioxidant capacity and the phenolic compound content (including those of flavonols and anthocyanins) with respect to the different ripening stages. Four stages were established based on the skin colour parameters, which were consistent with the changes in the weight and size of the murta fruits. In addition, the total phenolic and flavonols content showed a decrease during fruit ripening, in contrast with the total anthocyanins content that increased at the final stages of ripening. Additionally, the antioxidant capacity was evaluated via two approaches: ferric reducing antioxidant power and 1,1-diphenyl-2-picrylhydrazyl discolouration assay; higher levels of antioxidants at the final stages were consistently observed. Furthermore, the VOCs profile showed terpenoids to be the main compounds, with the highest relative abundances in fully ripe fruits. These results allow us to propose a classification of the different ripening stages of the murta fruits. This would enable us to have better knowledge of this interesting fruit, which possesses highly healthy nutraceutical compounds.

Keywords: antioxidant capacity, Chilean guava, healthy compounds, phenols, volatile organic compounds

INTRODUCTION

Ugni molinae Turcz, a plant that belongs to the Myrthaceae family, is an endemic plant from Chile, where it is known as 'murta' or 'murtilla' by Spanish-speaking people, and as 'uñi' by the Mapuche people (López et al., 2018; Fredes et al., 2020; Castro et al., 2021). Such a plant is a wild evergreen shrub (height: 1–2 m) and is seen growing

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between the Maule and Aysén regions (central southern Chile), near the coastal and pre-Andean mountains. Its fruits are small globular berries (in average around 1 cm in diameter, weighing less than 1 g and containing few tens of seeds), with a pleasant smell and taste and show a high degree of variability with respect to its colour and size (Torres et al., 1999; Schreckinger et al., 2010; Augusto et al., 2015; López et al., 2018; Guenuleo et al., 2020; Neranzi et al., 2022).

Chilean indigenous people, especially Mapuches, used the leaves and fruits from *U. molinae* Turcz even before the arrival of the Spaniards during the XVI century. They were aware of the beneficial health effects of consuming the leaf infusion. The infusions have been used in traditional native medicine to treat diarrhoea and dysenteries (Suwalsky et al., 2007) and urinary and throat infections (Fredes et al., 2020). Moreover, the nutritional and sensory features of their berries allowed their fresh consumption as well as their use as preserved foods and as fermented foods. They can also be added to alcoholic beverages and spirits (Ah-Hen et al., 2013; Lorca, 2018; Schmeda-Hirschmann et al., 2019; Fredes et al., 2020). Murta can grow in the wild or be cultivated due to the domestication programs developed during the last three decades (Pastenes et al., 2003; Franck et al., 2007; Ah-Hen et al., 2012; Chacón-Fuentes et al., 2015). Its leaves and fruits have a rich and diversified composition of bioactive compounds, from phenolics to terpenoids, resulting in health benefits derived from its consumption (Aguila Chacón and Nahuelhual Muñoz, 2008; Ah-Hen et al., 2013; Augusto et al., 2015; López et al., 2018; Lorca, 2018). Moreover, the mature fruits contain volatile and non-volatile organic compounds (VOCs), which leads to the pleasant sensory attributes of not only the fresh berries but also of the processed foods made from them. Thus, although murta is not a widely known Chilean native species yet, it is an excellent candidate to be used by the food industry hereafter.

Numerous studies have been conducted on the leaves of the *U. molinae* Turcz (Retamales et al., 2014; Neranzi et al., 2022) to examine their composition of bioactive compounds. These studies have explored both aqueous (Suwalsky et al., 2007; Shene et al., 2012; Avello et al., 2013) and non-aqueous extracts (Rubilar et al., 2006; Siani et al., 2016; Peña-Cerda et al., 2017). The biological activity of murta leaf extracts and infusions has been investigated, revealing antioxidant activity (Rubilar et al., 2006, 2011; Avello et al., 2013; Peña-Cerda et al., 2017), protective effects against oxidative damage in human cells (Suwalsky et al., 2007), anti-inflammatory activity (Aguirre et al., 2006), analgesic activity (Delporte et al., 2007) and a prebiotic effect (Shene et al., 2012). In general, the bioactivity and potential applications of the murta leaf extracts have been attributed to their high content of phenolic compounds (including phenolic acids, flavonols, flavanols and tannins) and triterpenoids (Schreckinger et al., 2010; López et al., 2018). Moreover, the leaf extracts have exhibited antimicrobial properties (Shene et al., 2009; López de Dicastillo et al., 2017) and even nematicidal activity (Böhm et al., 2009). These attributes, coupled with their antioxidant capacity, make them promising candidates for applications in cosmetics (Avello et al., 2009), pharmaceuticals (López de Dicastillo et al., 2017), agriculture (Böhm et al., 2009; Chacón-Fuentes et al., 2015), packaging (Bifani et al., 2007; Gómez-Guillén et al., 2007) and other such industries.

Considering the fruits, ripe murta berries typically contain approximately 70%–85% water-soluble solids, ranging from 5°Brix to 30°Brix, and the pH values are between 3.5 and 7.0. The composition generally varies based on the origin and degree of fruit maturity (Torres et al., 1999; Lorca, 2018; Castro et al., 2021; Espinoza-Tellez et al., 2021). Moreover, the murta fruits are rich in ascorbic acid (vitamin C) (Torres et al., 1999) and also exhibit a diverse range of phenolic compounds, including flavonoids present in the leaves as well as anthocyanins, which are responsible for the berries' red colour. Consequently, phenolic extracts derived from murta fruits demonstrate significant antioxidant capacity (Alfaro et al., 2013; Augusto et al., 2015; Fuentes et al., 2019; Castro et al., 2021; Vega-Galvez et al., 2021). However, it is worth noting that the phenolic content and antioxidant activity of the berries are lower compared to those of the leaf extracts (López de Dicastillo et al., 2017). Additionally, VOCs associated with fruit scents and flavours have been identified in murta berries (Scheuermann et al., 2008), as well as soluble fibre, primarily composed of pectin found in the cell walls (Taboada et al., 2010). The thermal behaviour of murta berries has also been studied (Ah-Hen et al., 2014). Due to its biological activities, such as anti-inflammatory, antimicrobial, and antioxidant properties (Junqueira-Gonçalves et al., 2015; Cabrera-Barjas et al., 2020; Espinoza-Tellez et al., 2021), murta berries and their extracts have been proposed to act as functional foods (Scheuermann et al., 2008; Reyes-Farias et al., 2016; Ulloa-Inostroza et al., 2017; Ah-Hen et al., 2018; Garcia-Diaz et al., 2019). The health benefits associated with the consumption of murta can be attributed to its composition; There has been extensive research on the optimal conditions for drying or dehydrating the berries while preserving their antioxidant capacity and phenolic content (Puente-Díaz et al., 2013; Rodríguez et al., 2014; Scheuermann et al., 2014; Zura-Bravo et al., 2019; Pirce et al., 2021). Furthermore, murta berries have various other applications, including cosmetic (dermatological) applications, their use as bulk material for creating active films in food packaging (Augusto-Obara et al., 2017), as a food preservative (Bravo et al., 2021) and as a tool for biocontrolling herbivorous insects (Chacón-Fuentes et al., 2015). Additionally, murta berries growing in their native ecosystem possess their own microbiome, including valuable microorganisms with potential applications, such as biocontrol agents against grey mold spoilage (González-Esparza et al., 2019).

Given the numerous benefits and potential applications of murta, it is foreseeable that it will be widely used in commercial settings. However, it is important to recognise that the health advantages of consuming murta fruits, in addition to their sensory characteristics, rely on the composition of their bioactive compounds. This composition, in turn, is influenced by the degree of fruit ripeness, as demonstrated in other berry fruits (Tosun et al., 2008; Acosta-Montoya et al., 2010; Gordon et al., 2012; Giuffrè, 2013; Fu et al., 2015; Schulz et al., 2015; Seraglio et al., 2018; Li et al., 2022). Despite the growing interest in *U. molinae* Turcz fruits and their potential use in the functional food industry, the evolution of chemical and bioactive compounds throughout the development and ripening of murta berries has not yet been thoroughly described. Therefore, this study aims to provide, for the first time, a classification of four distinct developmental and ripening stages of murta berries. To achieve this, we analysed the colour, antioxidant capacity, phenolic compound contents and VOCs of wild murta fruits throughout their development. This understanding will help identify the optimal harvest stage to benefit from their nutritional and bioactive properties.

MATERIALS AND METHODS

Fruit samples

The murta fruits used in this study were harvested from wild plants that grow on small farms as a living fence in the Pelluhue town, Maule region, Chile (S 35°50′00″; W 72°38′00″). Pelluhue town has a Mediterranean climate with warm and dry summers and cold winters. The average annual temperature in Pelluhue is 20°C. For this reason, the fruits were collected at 9:00 at an atmospheric temperature ranging from 14°C to 16°C, and one or two fruits were collected per plant. The fruits were obtained from 20-year-old half sib plants of murta grown. Initially, the plants were obtained from a small group of 3–5 plants growing wild near the property that is now a commercial farm. To categorise the fruits into different developmental stages, criteria such as weight, size and fruit skin colour were considered (refer to Figure 1). The four stages are defined as follows: small green (SG) fruit, large green (LG) fruit, half ripe fruit (50%R) and fully ripe fruit (R). A total of 150 fruits were collected from each developmental stage, obtained from 20 different plants, across two consecutive growing seasons (fall of 2020 and 2021). Each fruit was rapidly frozen in liquid nitrogen and stored at –80°C until further analysis. After harvesting, the fruits were transported to the Multidisciplinary Agroindustry Research Laboratory at the Universidad Autónoma de Chile in Talca, which is approximately 150 km away from the harvest site, under cold conditions in containers having dry ice, maintaining the fruits between 4°C and 8°C.

Skin colour and physiological parameters determination

In the present study, 25 fruits from each developmental stage (free from external damage) were selected for the assessment of fruit skin colour using a Nix Pro 2 Color Sensor (Nix Sensor Ltd., Hamilton, Ontario, Canada) (https://www.nixsensor.com/free-color-converter/). The surface colour of the different fruits was characterised and represented using the Hunter scale, which includes parameters such as L^*, a^*, b^*, C^*, and $h°$. Additionally, the weight (in grams) and size (in millimetres) of the berries were measured. Values indicate the mean of 25 replicates from each season, and the standard deviations are also shown. To determine the pH, the soluble solids concentration (SSC) and titratable acidity (TA), the juice extracted from 2 g of frozen tissue was ground with liquid nitrogen, homogenised in 5 mL of distilled water and filtered using miracloth. The results for TA and SSC were expressed as the SSC/TA ratio, as described by Ramos et al. (2018) and Castro et al. (2023).

Analysis of VOCs

For the analysis of volatile compounds in murta berries during ripening, three different samples were subjected to SPME–GC–MS analysis. The stages SG and LG were combined to form a single batch representing the unripe stage, referred to as green (G) samples. Additionally, the

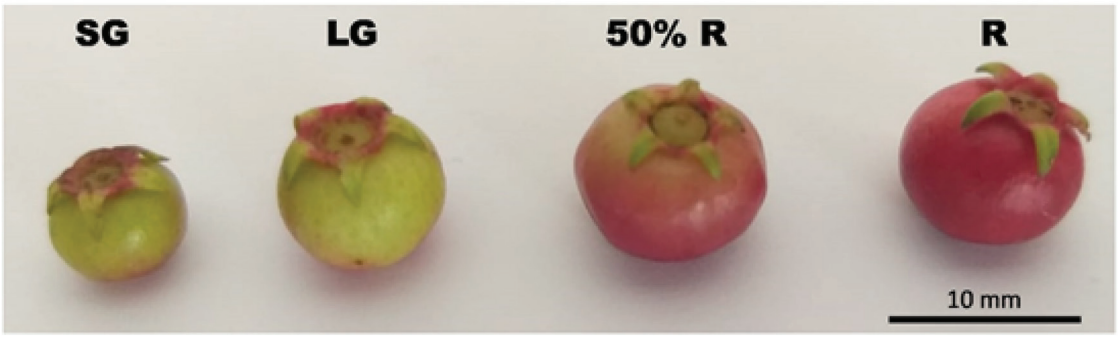


Figure 1. Different ripening stages of *Ugni molinae* (murta) fruits: SG fruit; LG fruit; 50% R fruit; and ripe fruit (R). 50%R, 50% ripe; LG, large green; SG, small green.

50% R stage was used to represent the stage before full ripening, while the R stage represented the fully ripe stage.

To perform the analysis, approximately 3 g of murta berries (consisting of 9–14 berries) were mixed with 8 mL of ultrapure water and 10 µL of a 4-methyl-2-pentanol solution (used as an internal standard) in methanol (0.1% v/v). The mixture was homogenised in a 50 mL centrifuge tube using an Ultra-Turrax™ homogeniser (Janke & Kunkel GmbH & Co. KG, Staufen, Germany). The homogenised sample was transferred to a 20 mL glass vial and incubated for 20 min at 45°C with agitation at 500 rpm using an MPS Autosampler (Gerstel, Palo Alto, CA, USA). Subsequently, a 2 cm fibre (50/30 µm carboxen/divinylbenzene/polydimethylsiloxane [Supelco, Bellefonte, PA, USA]) was exposed to the sample for 40 min.

The analysis was performed using a 7890B Agilent GC system coupled to a quadrupole mass spectrometer Agilent 5977 inert (Agilent Technologies, Palo Alto, CA, USA). The chromatographic conditions and identification criteria were consistent with those previously published in Parra-Palma et al. (2019).

Each of the three fruit stages was analysed in triplicate, and the results are expressed as relative areas (RA). The RA values were divided by the number of berries used in each replicate to obtain results on a per berry basis. Furthermore, the values were divided by the mass of berries (in grams) to obtain results on a per gram basis. The terpene compounds were categorised based on their carbon backbone, following the classification presented in Figure 2.

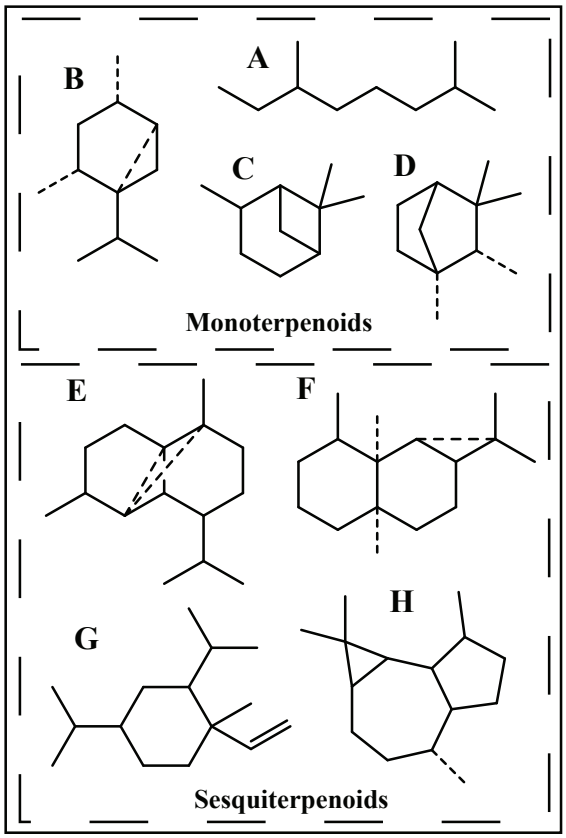


Figure 2. Chemical structures of the terpenoid volatile compounds identified in murta fruits for all developmental stages. The letters correspond to the compounds whose concentrations are the ones described in Figure 7.

Analysis of phenolic compounds content

To extract the compounds from the berries, the entire berries were homogenised using a mortar and pestle in a 1% HCl solution prepared in methanol (5 L · kg^{-1} of fruit). The homogenisation process aimed to break down the berry tissue and facilitate the extraction of the compounds. The resulting mixture was stirred for 1.5 hr at room temperature to allow for proper extraction. Subsequently, the mixture was centrifuged at 4,200 *g*, and the supernatant was collected for further analysis.

For each stage, three independent extractions were performed using 10 g of fruit tissue. This ensured the extraction of a sufficient amount of compounds for accurate analysis. Each extraction was carried out following the same procedure of homogenisation, stirring, and centrifugation.

The obtained extracts were utilised to determine the total phenolic content using the Folin–Ciocalteu reagent, a widely used method for quantifying phenolic compounds (Singleton et al., 1999). The total anthocyanin content was determined using the pH differential method described in Lee et al. (2008), which relies on the colour changes exhibited by the anthocyanins at different pH levels. The total flavonol content was determined using a colorimetric method after the addition of aluminium chloride, as reported by Chang et al. (2020). These methods allowed for the quantification of the total phenolic, anthocyanin, and flavonol contents present in the murta berry extracts, providing valuable information about the composition of these compounds at different developmental stages. The phenolic compounds were quantified based on a standard curve of gallic acid; the results are expressed as gram of gallic acid equivalents per kilogram of fruit (g · kg^{-1} GAE) and correspond to the means ± SEs of three biological replicates with two more technical replicates. Meanwhile, the flavonol results were expressed as gram of quercetin equivalents per kilogram of fruit (g · kg^{-1} quercetin). Finally, the total anthocyanin content was expressed as gram of cyanidin 3-glucoside equivalents per kilogram of fruit (g · kg^{-1} Cy3G) using the absorbance values of A = (A524–A700 nm) pH 1.0 - (A524–A700 nm) pH 4.5, with a molar extinction coefficient of 26,900 according to Parra-Palma et al. (2020).

Analysis of the antioxidant capacity

To assess the radical-scavenging ability of the methanol extracts, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) discolouration assay was conducted following the method described by Cheel et al. (2007). The procedure involved mixing an aliquot of 20 µL of the methanol extract with a DPPH solution (0.5 mM, 0.25 mL) and acetate buffer (100 mM, pH 5.5, 0.5 mL), as outlined

in Castro et al. (2019). The reaction mixtures were prepared in triplicate and then measured at 517 nm using an Infinite® 200 PRO NanoQuant spectrophotometer (Infinite M200; Tecan, Zurich, Switzerland). The extent of DPPH radical scavenging was determined by comparing the results with a negative control group consisting of DPPH with methanol.

Furthermore, the ferric reducing antioxidant power (FRAP) analysis was performed using the same methodology as described in Castro et al. (2018). This assay is commonly used to measure the antioxidant capacity of samples. The FRAP analysis involves mixing the methanol extract with a ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex, which is reduced by the antioxidants present in the extract. The reduction reaction results in the formation of a blue-coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex, which can be measured spectrophotometrically. The FRAP values obtained provide an indication of the reducing power and antioxidant capacity of the extracts.

By conducting both the DPPH discolouration assay and the FRAP analysis, the radical-scavenging and antioxidant abilities of the methanol murta berry extracts can be evaluated, providing insights into their potential health benefits and functional properties.

RESULTS AND DISSCUSION

Fruit-developmental parameters

The size of the berries at each of the four selected developmental stages was analysed, taking into consideration their weight and width. The results are presented in Figure 3. Regarding berry mass, there is an increase from SG to LG, as well as from 50%R to R stages. However, there were no significant differences in berry mass between the LG and 50%R stages (Figure 3A). These findings suggest a growth stop similar to what has been described for grape berries (*Vitis vinifera*), coinciding with the onset of colour biosynthesis known as veraison (Kennedy, 2002). On the other hand, when considering the fruit width (Figure 3B), a gradual increase in berry size was observed from SG to R, although there were no statistical differences between the 50%R and R developmental stages. This contradicts the growth stop hypothesis proposed based on berry weight since an increase in size is observed between the LG (green berries, Figure 1) and 50%R (slightly coloured, Figure 1) development stages. This aspect should be considered for yield calculations. The soluble solids concentration (SSC) of the berries increases from LG to R (Figure 3E), reaching 27°Brix for fully ripe murta berries. This increase in SSC throughout the ripening aligns with the common trend observed in other fruits (Zhao, 2007). A similar increasing trend was observed for TA (Figure 3F), which rises as ripening progresses, explaining the decrease in pH (Figure 3C) as maturity advances. Finally, the SSC/TA ratio also increases with ripening (Figure 3D), which is a common trend in fruit maturity and has been linked to the sweet–sour balance and consumer acceptance (Endrizzi et al., 2009).

Currently, it is widely accepted that fruit colour serves as an attractant for predators, particularly vertebrates, to disperse seeds away from the parent plant. When the seeds are not ready to germinate, fruits tend to be green to blend in with the leaves and often contain toxic or unappealing chemical compounds to discourage dispersers. In contrast, when the seeds are ready to germinate, fruits become colourful and nutritious, enticing dispersers (Simms, 2013). Thus, the evolution of colour during berry-ripening is an important aspect that has been associated with other sensory characteristics of fruits (Li et al., 2019a). In the conditions of this study, the colour trend of murta berries follows a common pattern (Table 1), where the colour of SG and LG stages is similar, displaying green tones, while the red component increases significantly from LG to R, as clearly demonstrated by the rise in the *a** component. The colour of fruits at harvest can influence consumer preference (López Camelo and Gómez, 2004; Lewers et al., 2020), which is significant considering the consumption of fresh murta fruits. When considering only fully ripe stage fruits (R) and disregarding postharvest changes, the berries exhibited vibrant colours, potentially offering a pleasant balance between sweetness and sourness, which are desirable traits for high consumer acceptance in terms of organoleptic attributes (Endrizzi et al., 2009).

Phenolic compounds

The total phenolic compounds, as shown in Figure 4A, were analysed using the Folin–Ciocalteu reagent, which includes compounds such as ascorbic acid, phenolic and cinnamic acids, tannins, flavanols, flavonols and anthocyanins (Singleton et al., 1999). As seen in Figure 4A, the total phenolic content of murta berries gradually decreases from the SG to the R developmental stages. This decrease in phenolic content during ripening can be related to the increase in the fruit's sensory appeal, as many phenolics have traditionally been associated with the perception of bitterness and astringency in food (Ferrer-Gallego et al., 2014; Soares et al., 2013). The decrease in total phenolic concentration can be attributed to several factors, such as dilution due to an increase in berry volume (often associated with an increase in fruit water content), degradation reactions (Tomás-Barberán and Espín, 2001) or lignification of seed tissues (Barros et al., 2015). A similar decreasing trend in total polyphenolic content during ripening has been observed in other berries such as blackberries, raspberries, blackberries and strawberries (Wang and Lin, 2000; Zhao, 2007; Li et al., 2019b).

The colour of the berry peel at each described development stage is closely related to its chemical composition. Specifically, the increase in the red component and the decrease in the green component have been associated with the accumulation of anthocyanins, which are a family of flavonoid natural pigments commonly found in fruits and flowers, as well as during the degradation of chlorophyll (Parra-Palma et al., 2019). The content of anthocyanins at

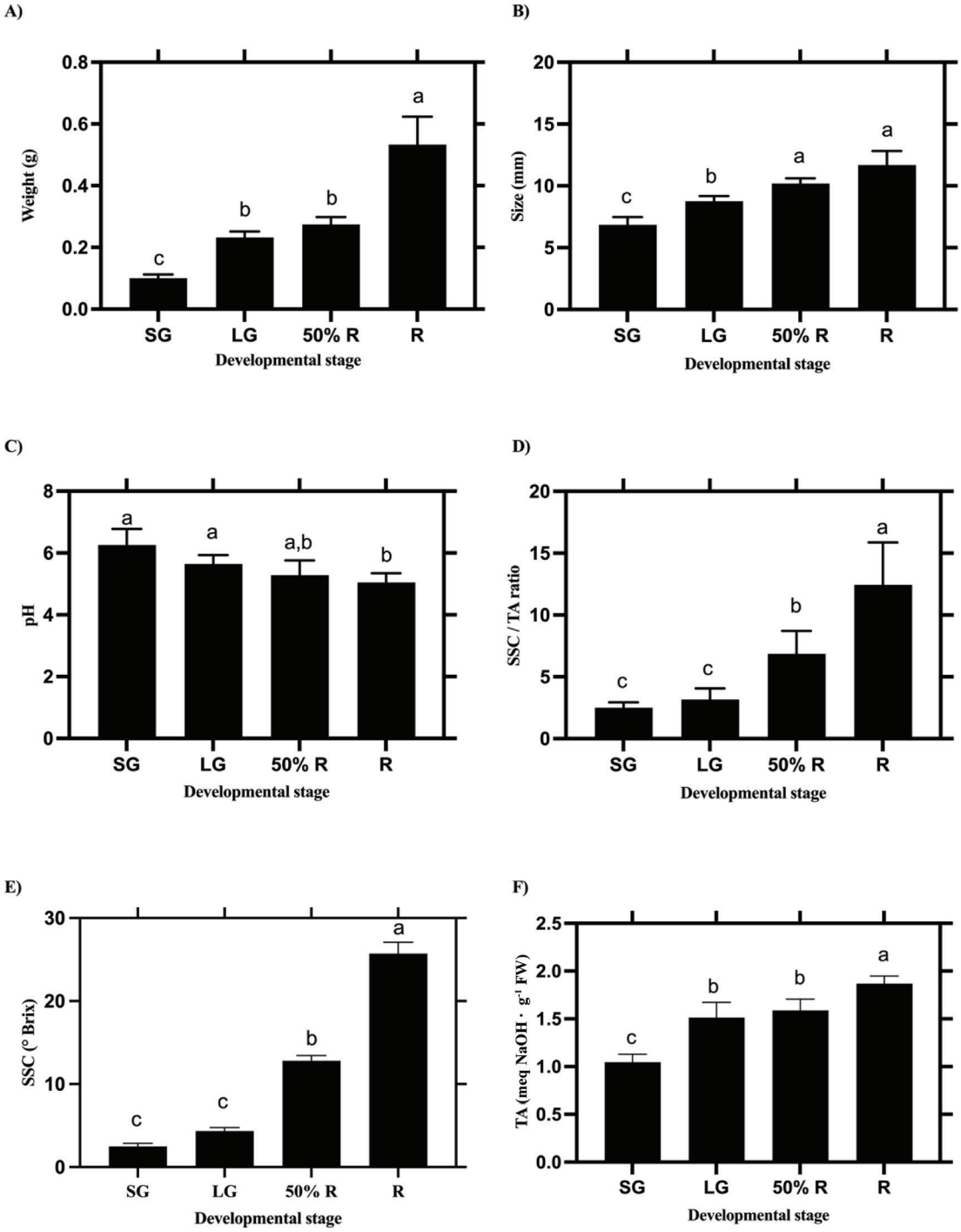


Figure 3. Physiological parameters of murta fruits in the four developmental stages: (A) Weight; (B) Size; (C) pH; (D) SSC/TA ratio; (E) SSC and (F) TA. Different letters in a chart indicate statistical differences among samples. 50%R, 50% ripe; LG, large green; SG, small green; SSC, soluble solids concentration; TA, titratable acidity.

each developmental stage is shown in Figure 4C. Anthocyanins were not detected in SG berries, indicating that the seeds were likely still immature (Wang and Lin, 2000; Zhao, 2007). Although LG berries appear green like those in the SG stage, a low content of anthocyanins was found, suggesting that this stage corresponds to the beginning of anthocyanin biosynthesis, which continues until the R stage, explaining the increasing red colour observed (Table 1). The increasing trend of anthocyanins throughout ripening has been described for other berries (Wang and Lin, 2000; Zhao, 2007) and ensures that there are sufficient colour differences between the ripe fruits and plant leaves to attract predators.

Finally, considerable amounts of flavonols have been found in ripe murta berries, which were quantified using a colorimetric method after the addition of aluminium chloride (Chang et al., 2020). According to the results shown in Figure 4B, the highest content of flavonols is

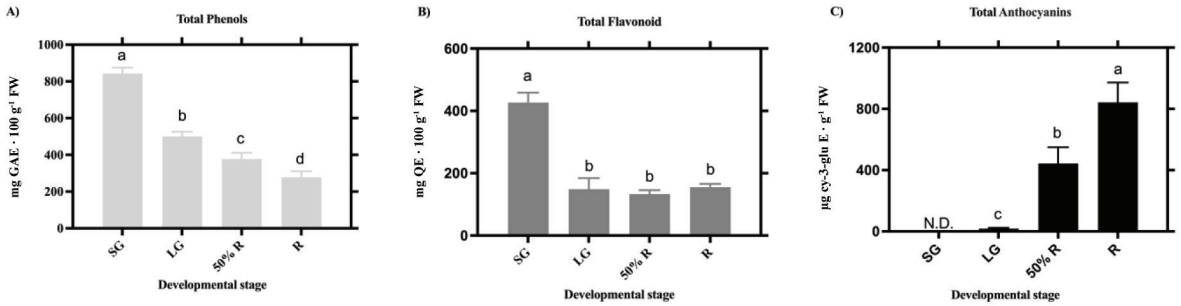


Figure 4. Chemical analyses of fruit. (A) Total phenolics, (B) total flavonoids and (C) total anthocyanins contents were analysed at different developmental stages. Data correspond to mean ± SE of three biological replicates. Different letters indicate significant differences among samples ($p \leq 0.05$; ANOVA). 50%R, 50% ripe; FW, fresh weight; GAE, gallic acid equivalents; LG, large green; SG, small green.

Table 1. Colour readings of the four stage of murta fruit peel.

Stage	*L**	*a**	*b**	*C**	*h*°	*Color*
SG	43.75 ± 7.18	–4.80 ± 1.60	27.80 ± 5.92	28.30 ± 5.63	280.42 ± 5.34	
LG	44.37 ± 10.19	–5.74 ± 5.42	22.60 ± 4.58	23.98 ± 3.93	283.96 ± 14.63	
50%R	42.70 ± 7.03	15.60 ± 10.56	25.97 ± 4.05	31.65 ± 5.96	60.95 ± 16.56	
R	33.49 ± 4.67	28.79 ± 6.63	18.02 ± 2.33	34.15 ± 5.94	32.77 ± 6.16	

50%R, 50% ripe; LG, large green; SG, small green.

observed in SG berries. Flavonol content significantly decreases between the SG and LG developmental stages, and no further variations were observed from LG to R. In our conditions, flavonols and anthocyanins exhibit inverse trends: while anthocyanin contents increase throughout maturity, the flavonol contents of ripe fruits are lower than those of unripe (SG) fruits, as also reported for raspberries (Wang et al., 2009).

Antioxidant capacity

The antioxidant capacity of murta berries gradually increases from the SG to R developmental stages, as analysed using both the FRAP and DPPH methods, as shown in Figure 5. It is logical that both methods exhibit the same trend for antioxidant capacity throughout ripening, as a strong correlation has been established between the FRAP and DPPH methodologies (Martínez et al., 2022). However, it is noteworthy that the antioxidant capacity tends to positively correlate with the total phenolic compounds, which contradicts the results of the present study. Although the total phenols decrease during the ripening of murta berries, the anthocyanin content clearly increases concurrently, which can explain the rise in antioxidant capacity as the berries mature. The progressive increase in the antioxidant capacity observed during the development of murta berries aligns with the reported data for blackberry hybrids (Siriwoharn et al., 2004), but it does not correlate with studies on other berries, which demonstrate a decrease in antioxidant capacity from red to pink stages, followed by an increase from pink to red stages (Zhao, 2007; Wang and Lin, 2000). Nevertheless, fully ripe murta berries exhibit the highest levels of antioxidant capacity. Thus, the consumption of fully ripe murta fresh fruits, which possess desirable sensory traits as mentioned earlier, could be healthier than consuming unripe berries, at least in terms of their antioxidant activity (Shahidi and Ambigaipalan, 2015). Considering the preservative properties of phenolic compounds and the higher antioxidant activity of the ripest berries, it can be expected that preserved or fermented foods made from mature murta berries would possess superior qualities.

VOCs

The profile of VOCs of murta berries was performed for three developmental stages: unripe (G, corresponding to a mixture of SG and LG berries), half-ripe (50%R) and full-ripe (R) in triplicate. The obtained VOCs profiles consists of 70 compounds found in all samples, corresponding to alcohols, aldehydes, esters, and mainly terpenoids (including monoterpenoids and sesquiterpenoids), which represent more than 90% of the identified compounds. The huge amount of identified terpenoids could be surprising when compared to previously published data (Scheuermann et al., 2008), in which few terpenoids were reported. These differences could be related not only to methodology issues, but also to the origin of the berries. It has been reported that, when eucalyptus trees are in the edges of the vineyards, the vines closer to the trees contain higher contents of 1,8-cineole (Capone et al., 2012). Thus, the environment of developing fruits could affect their VOC profile, and the wild origin of the employed murta samples could explain the high account of identified terpenoids. Nevertheless, murta plants must

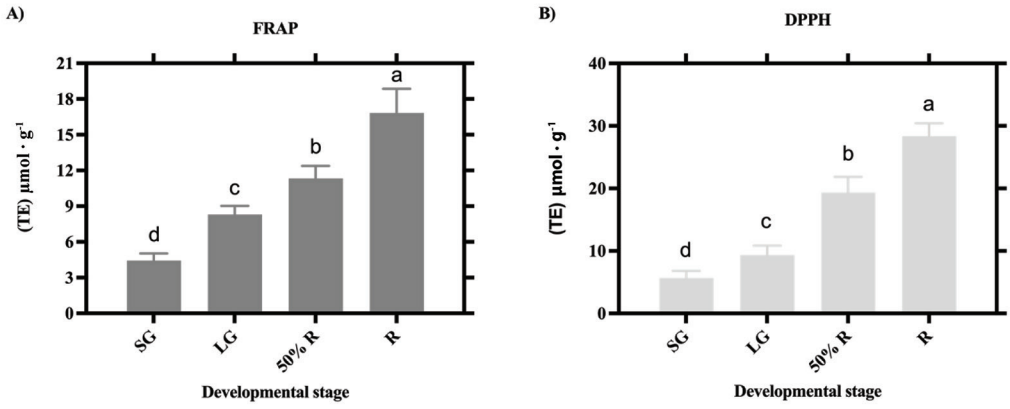


Figure 5. Antioxidant capacity. Free radical scavenging activity by DPPH (A) and FRAP (B) were estimated in different developmental stage of fruit. Data correspond to mean ± SE of three biological replicates. Different letters indicate significant differences among developmental stages [$p \leq 0.05$; ANOVA]. 50%R, 50% ripe; FRAP, ferric reducing antioxidant power; LG, large green; SG, small green.

have active biosynthetic routes for terpenoids since several works highlight its triterpenoid (Schreckinger et al., 2010) and sesquiterpenoid (Siani et al., 2016) contents.

The VOC profile of fruits is important, considering that it is related with the aromatic perception of consumers, either for fresh fruit or derivate products consumption (Xu et al., 2019). In that sense, the relative abundance of C6 compounds could be of interest, given that such compounds have been related with herbal scents (Nakamura and Hatanaka, 2002). As shown in Figure 6, the C6 compounds (including hexanal, 2-hexenal, 1-hexanol and *cis*-3-hexen-1-ol) dropped between 50%R and R berries when expressed as R.A. · g^{-1} FW; those are related with its potential impact on the aromatic perception of murta berries. However, no statistical differences were found among the ripening stages when the relative abundance of C6 compounds was expressed as R.A. · $berry^{-1}$; these are related to the biosynthesis/degradation ratio. In contrast, the results for ethyl octanoate, the ester with the highest relative abundance, remain constant among development stages when expressed in R.A. · g^{-1} FW, but gradually increase through development when expressed in R.A. · $berry^{-1}$. Esters has been related with fruity scents; specifically, the sensory descriptors for ethyl octanoate are fruity, sweet, ripe banana and pear. Thus, the results seem to indicate that the berries produce ethyl octanoate continuously throughout their development, but the increase of berry volume hides such production, maintaining constant its relative abundance. In contrast, the production of C6 compounds seems to be inactive throughout the development of fruit, decreasing its relative abundance between the 50%R and R fruits due to the dilution provoked by the increase of berry size.

The identified terpenoids correspond to monoterpenoids (C10 compounds, made up of two units of isoprene) and sesquiterpenoids (C15 compounds, made up of three units of isoprene). When expressed as RA · g^{-1} FW, even acyclic (aliphatic), monocyclic and bicyclic monoterpenoids showed the same trend, slightly decreasing from G to 50%R, to increase then significantly from 50%R to R. In contrast, when expressed as R.A. · $berry^{-1}$, the acyclic monoterpenoids just increase at the late phase of ripening, between the 50%R and R stages, while the monocyclic terpenoids gradually increase from the G to R stages. The total relative abundance of monoterpenoids (Table 2) clearly increases from the 50%R to R stages, regardless of the expression units, indicating an active biosynthesis, enough to surpass the dilution due to berry growth. In contrast, sesquiterpenoids did not show any statistical differences among the developmental stages of murta berries, probably due to the fact that they are consumed and produced simultaneously.

The identified terpenoids present in the murta berries profile were grouped according to their carbon backbone as shown in Figure 6. The four groups belonging to the monoterpenoids group showed the same trend as the total monoterpenoids, increasing its relative abundances at late ripening (from 50%R to R), either expressed as RA · g^{-1} FW or RA · $berry^{-1}$ (Figure 7). Otherwise, none of the groups belonging to the sesquiterpenoids showed any statistically significant differences among the development stages. Finally, the total relative abundance of all the terpenoids did not show statistical differences when expressed as RA · g^{-1} FW, but it showed a rise between the 50%R and R berries when expressed as RA · $berry^{-1}$, which again indicates an active biosynthesis during the last phase of ripening.

Volatile terpenoids have been related with fruity and floral scents, and they have been described as the most-important group of metabolites impacting the sensory perception and the consumers' acceptability of blueberries (Ferrão et al., 2022). Moreover, health-

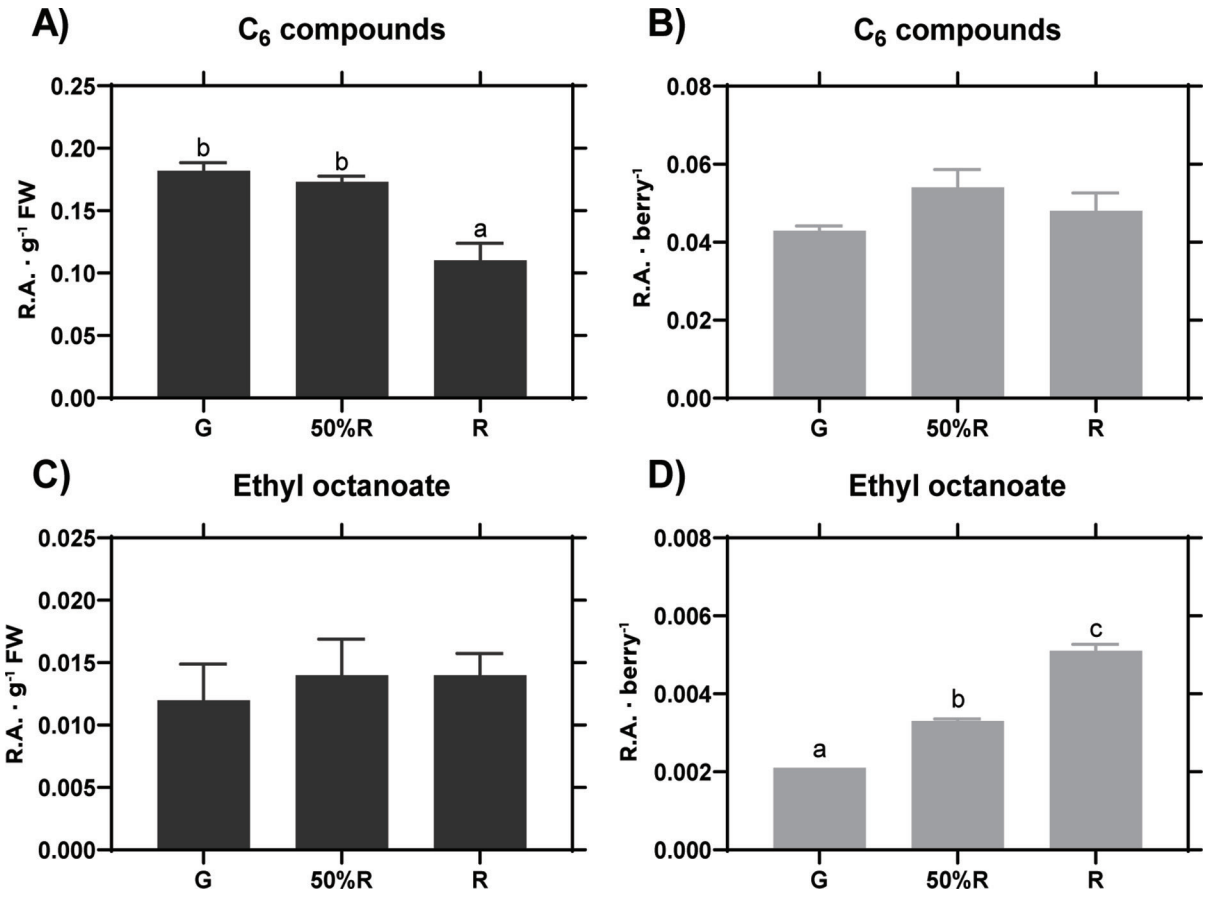


Figure 6. Volatile organic compounds profile. Relative abundance of C6 compounds relative to FW (A) and per berry basis (B). Relative abundance of ethyl octanoate relative to FW (C) and per berry basis (D). Different letters indicate significant differences among samples ($p \leq 0.05$; ANOVA). 50%R, 50% ripe; FW, fresh weight.

Table 2. Relative abundance of terpenoid volatile compounds in murta fruits according to their chemical structure, expressed as RA (RA · g^{-1} FW, and RA · $berry^{-1}$).

Developmental stage	RA · g^{-1} FW			RA · $berry^{-1}$		
	G	50%R	*R*	*G*	50%R	*R*
Acyclic monoterpenoids	4.87 ± 0.26 ab	3.63 ± 0.93 a	6.76 ± 0.38 b	1.05 ± 0.11 a	1.07 ± 0.25 a	1.94 ± 0.38 b
Monocyclic monoterpenoids	16.46 ± 0.72 ab	14.16 ± 2.99 a	24.7 ± 2.80 b	3.55 ± 0.36 a	4.17 ± 0.78 ab	7.19 ± 1.62 b
Bicyclic monoterpenoids	17.11 ± 0.49 ab	13.16 ± 2.58 a	21.11 ± 0.76 b	3.56 ± 0.53 a	3.87 ± 0.67 a	6.11 ± 1.22 a
Total monoterpenoids	38.44 ± 0.5 ab	30.95 ± 6.5 a	52.57 ± 3.18 b	8.16 ± 0.95 a	9.11 ± 1.7 ab	15.23 ± 3.16 b
Monocyclic sesquiterpenoids	1.17 ± 0.24 a	0.82 ± 0.36 a	1.47 ± 0.39 a	0.27 ± 0.03 a	0.24 ± 0.10 a	0.45 ± 0.12 a
Bicyclic sesquiterpenoids	2.32 ± 0.30 a	1.64 ± 1.30 a	2.66 ± 1.05 a	0.48 ± 0.07 a	0.48 ± 0.37 a	0.78 ± 0.22 a
Tricyclic sesquiterpenoids	2.66 ± 0.55 a	2.37 ± 1.59 a	2.87 ± 0.20 a	0.55 ± 0.11 a	0.69 ± 0.45 a	0.92 ± 0.09 a
Total sesquiterpenoids	6.15 ± 1.09 a	4.83 ± 3.25 a	7.00 ± 0.46 a	1.31 ± 0.19 a	1.41 ± 0.92 a	2.15 ± 0.14 a

Different letters in a row indicate statistical differences ($p < 0.05$) among developmental stages.
50%R, 50% ripe; FW, fresh weight; R.A. relative areas.

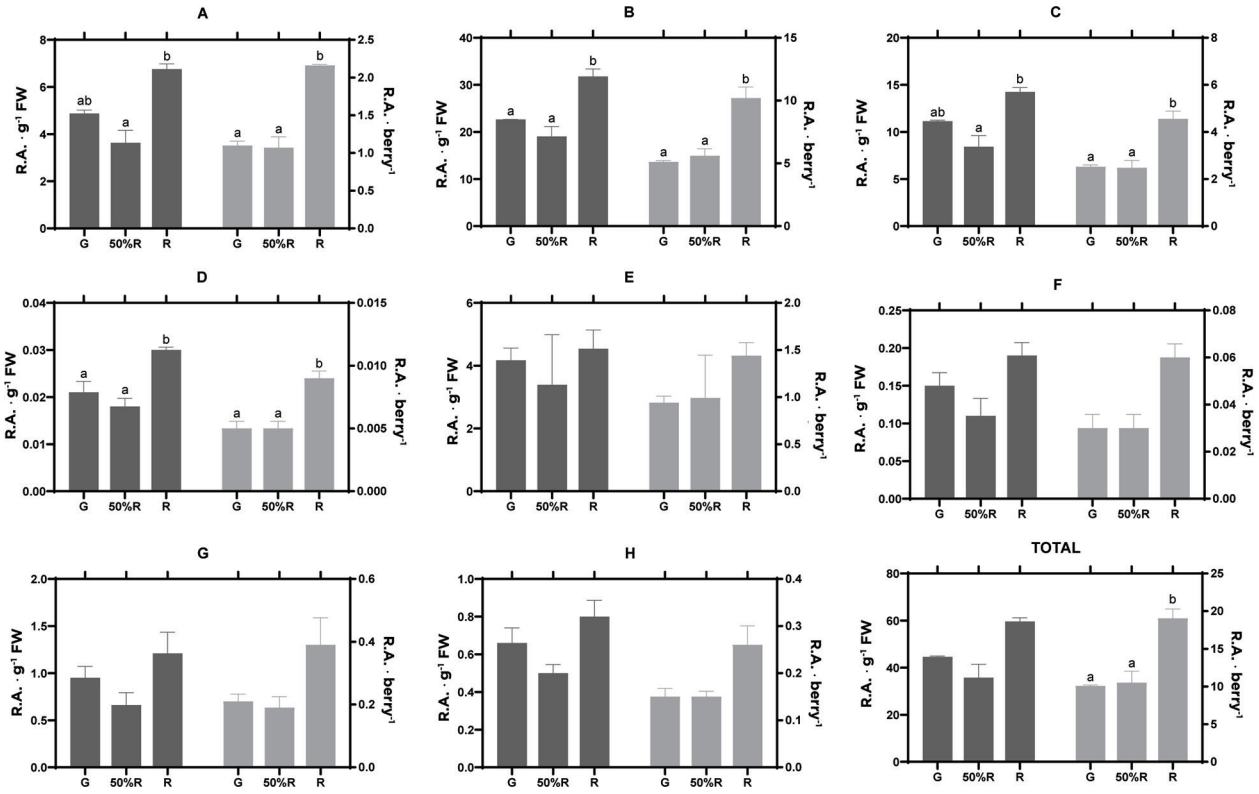


Figure 7. Terpenoid volatile compounds profile. The relative abundance of terpenoids grouped by backbone (Figure 2). (A) Pinene type, (B) terpinene type, (C) camphene type, (D) ocimene type, (E) cymene type, (F) cardinene type, (G) maaliene type, (H) elemene type, and total terpenoids. Different letters indicate statistical differences among the development stages for each concentration unit, and the graphs without letters indicate that there are no statistical differences. Dark columns correspond to the RA per gram of FW, and light columns correspond to the RA per berry. 50%R, 50% ripe; FW, fresh weight; RA relative areas.

beneficial biological activities for these compounds have been reported, including antioxidant, anti-allergenic, anti-inflammatory, antimicrobial, and anticancer activities (Feng et al., 2020; Masyita et al., 2022). Thus, considering the contents of terpenoids during the development and ripening of murta berries, a good ripening degree of harvested fruits ensures more pleasant sensory attributes and more beneficial effects on consumers' health, traits that favour their potential use as superfood.

CONCLUSIONS

The characterisation of the different developmental stages of murta (*U. molinae* Turcz.) berries provides valuable insights into the accumulation of bioactive compounds throughout ripening. This understanding allows for the identification of key traits that ensure optimal maturity at harvest. The changes in the chemical composition of the berries during maturation correspond to the evolution of their sensory attributes. Green stages are characterised by herbaceous scents and a sour/bitter taste, primarily influenced by a higher proportion of C6 phenolic compounds and a low SSC/TA ratio. In contrast, ripe berries exhibit vibrant colours, sweetness and floral/fruity flavours, attributed to their anthocyanin content, high SSC/TA ratio and increased proportion of terpenoids.

Furthermore, fully ripe berries demonstrate higher antioxidant capacity and a greater proportion of terpenoids, indicating their potential for enhanced health benefits. When considering all the reported data collectively, it becomes evident that harvesting murta berries at the optimal maturity stage is crucial to ensure desirable sensory traits and health-promoting advantages. These factors contribute to increased consumer acceptance, as they align with consumer preferences for sensory quality and potential health benefits.

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AUTHOR CONTRIBUTIONS

RIC, MG and LM-Q designed the experiments. MG, CP-P, PR. and LM-Q performed the experiments. PR, RIC, MG, AM-Y and LM-Q analysed the data. PR, AM-Y, MG and LM-Q wrote the first manuscript version. RIC, PR, MG, and LM-Q reviewed the manuscript, and all authors accepted the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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