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Investigation of carotenoids and C_{13}-norisoprenoids dynamics in developing grapes of *Vitis vinifera* cv. Blaufränkisch under varying sunlight exposure

Sebastian Scharf[1,†], Nikolaus Schlögl[2,†], Christian Philipp[2], Recep Gök[1,*]

[1] Institute of Food Chemistry, Technische Universität Braunschweig, 38106 Braunschweig, Germany
[2] Federal College and Research Institute for Oenology and Pomology, 3400 Klosterneuburg, Austria
[†] These authors contributed equally to this work.

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**correspondence:*
r.goek@tu-braunschweig.de

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ABSTRACT

This study investigates the evolution of carotenoids and C_{13}-norisoprenoids in whole berries of Blaufränkisch grapes during ripening. The concentrations of these compounds were compared between the east- and west-facing sides of a north-south oriented vineyard canopy, corresponding to morning and afternoon sun exposure. Grapes were sampled at nine developmental stages, including three potential harvest times: preripeness, ripeness, and overripeness.

Results demonstrated significant interactions between ripening stage and canopy side (morning *vs* afternoon sun exposure), indicating that sunlight exposure dynamically modulated carotenoid accumulation and degradation patterns as well as C_{13}-norisoprenoid formation during berry development. In overripe grapes, higher β-damascenone concentrations were found on the morning sun side, whereas higher β-ionone concentrations occurred on the afternoon sun side. At ripeness, grapes exposed to the morning sun have a significantly higher carotenoid content than grapes exposed to the afternoon sun. From ripeness to overripeness, carotenoid levels declined substantially in berries exposed to the morning sun, while they remained stable in berries exposed to the afternoon sun. Regarding C_{13}-norisoprenoids, a rapid accumulation was observed across the first five developmental stages, followed by a plateau during ripening. Interestingly, the substantial late-stage degradation of carotenoids on the morning side did not yield an increase in C_{13}-norisoprenoids. Conversely, afternoon-exposed grapes showed a sharp terminal spike in specific volatiles, such as vitispiranes and TDN, at overripeness, despite stable precursor levels. These results highlight the impact of canopy orientation on grape secondary metabolism and suggest that, under dry autumn conditions and moderate ripening temperatures, delayed harvest could enhance the aromatic profile of Blaufränkisch by accumulating specific volatile norisoprenoids.

KEYWORDS: Kékfrankos, Frankovka modrá, Lemberger, β-carotene, xanthophylls, TDN, β-damascenone

INTRODUCTION

The grape variety Blaufränkisch (Austria), also known as Lemberger (Germany), Kékfrankos (Hungary), or Frankovka modrá (Slovakia), has increasingly established itself due to its adaptability to changing climatic conditions, particularly in Hungary (7279 ha; Hungarian Ministry of Agriculture, 2024), Austria (2550 ha; Austrian Wine Marketing Board (AWMB), 2025), Germany (1888 ha; Deutsches Weininstitut (DWI), 2025), as well as in other European and transatlantic countries. Blaufränkisch is presumed to originate from Central Europe and has been identified as a cross between *Vitis vinifera* L. cv. Gouais Blanc and *Vitis vinifera* L. cv. Sbulzina (Regner, 2000; Maul *et al*., 2016).

Blaufränkisch grapes exhibit a complex aroma potential defined by polyfunctional thiols, C6 alcohols, and monoterpenes, while C_{13}-norisoprenoids, derived from carotenoid degradation, contribute significantly to the varietal and ripeness-related aroma profile (Vas *et al*., 1998; Philipp *et al*., 2023; Schlögl *et al*., 2025). Concentrations of carotenoids and norisoprenoids can be influenced by agronomic practices such as leaf removal, artificial shading, and cluster thinning (Kwasniewski *et al*., 2010; Alessandrini *et al*., 2018; Winterhalter & Gök, 2013). Hence, precise determination of carotenoid and norisoprenoid profiles in Blaufränkisch is highly worthwhile (Lee *et al*., 2007; Philipp *et al*., 2023).

Carotenoids are photoprotective tetraterpenes synthesised mainly between berry set and veraison, regulated by phytochromes (Cazzonelli & Pogson, 2010). Their total content depends on many factors, such as grape variety, climatic conditions, and plant water status, and is generally higher in warmer than in cooler climates (Marais *et al*., 1991; During & Davtyan, 2002; Joubert *et al*., 2016). During the berry ripening process, the total content of carotenoids decreases (Razungles *et al*., 1988; Fraser *et al*., 1994; Razungles *et al*., 1996; Joubert *et al*., 2016). The most abundant are β-carotene and lutein, accounting for up to 85 % of the total content, followed by neoxanthin and violaxanthin (Joubert *et al*., 2016; Young *et al*., 2016; Asproudi *et al*., 2020). Modulation of light in the grape zone influences carotenoid levels and their degradation products, particularly C_{13}-norisoprenoids (Bureau *et al*., 2000; Kwasniewski *et al*., 2010; Winterhalter & Ebeler, 2013; Grebneva *et al*., 2022; Asproudi *et al*., 2016). During early berry development, light exposure promotes carotenoid biosynthesis and the activation of photoprotective processes, including energy dissipation and protection against photooxidative stress (Cazzonelli & Pogson, 2010; Young *et al*., 2016; Anić *et al*., 2024). As berry ripening progresses, increased radiation exposure and elevated temperatures enhance oxidative carotenoid degradation, such that degradative processes increasingly predominate, resulting in the overall decline of carotenoid concentrations after veraison (Joubert *et al*., 2016).

In addition to developmental changes during berry ripening, microclimatic conditions within the vineyard canopy strongly affect carotenoid degradation and norisoprenoid formation. Sunlight exposure, row orientation, and canopy side determine the light and temperature regime of grape clusters, creating distinct microclimates between berries exposed to the morning (east) and afternoon (west) sun. East-facing clusters generally receive milder irradiation during cooler morning hours, which tends to preserve heat-sensitive carotenoids, whereas west-facing clusters experience more intense afternoon radiation and higher berry surface temperatures, accelerating carotenoid degradation and norisoprenoid accumulation (Yuan & Qian, 2016; Hickey & Wolf, 2019; Gao *et al*., 2021; Lu *et al*., 2024). Field observations in north-south-oriented vineyards have shown that west-exposed berries can exceed east-side berry temperatures by several degrees, reaching thresholds that promote pigment loss and increase the risk of sunburn, while morning-sun exposure supports colour stability and retention of labile compounds (Hickey & Wolf, 2019). Such microclimatic gradients translate into measurable compositional differences: in ripe berries, exterior, sun-exposed clusters typically show lower carotenoid but higher C_{13}-norisoprenoid contents compared with shaded or interior clusters (Gao *et al*., 2021; Lu *et al*., 2024). These orientation-dependent effects persist into the wine, as grapes from cooler canopy sides have been reported to yield higher β-damascenone concentrations than those from hotter canopy sides (Lu *et al*., 2024).

As mentioned above, microclimate-driven shifts in carotenoid content are directly linked to the biosynthesis of C_{13}-norisoprenoids, which originate from carotenoid cleavage and represent crucial aroma precursors in grapes and wines (Winterhalter & Gök, 2013). These norisoprenoids are chemically diverse compounds (*e.g*., ketones, aldehydes, alcohols) with a 13-carbon skeleton, which gives them their designation. Important representatives include β-damascenone, β-ionone, TPB (4-(2,3,6-trimethylphenyl)buta-1,3-diene), TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), Riesling acetal (2,2,6,8-tetramethyl-7,11-dioxatricyclo[6.2.1.0] undec-4-ene), isomeric vitispiranes and actinidols. Among these, β-damascenone (apple, rose, dried fruits, honey, exotic fruits, tropical flowers) and β-ionone (raspberry, floral, fruity) are particularly important, as they contribute strongly to the fruity wine aroma and can mask vegetal or green-grassy notes from methoxypyrazines or C6 alcohols (Pineau *et al*., 2007; Escudero *et al*., 2007; Sefton *et al*., 2011; Robinson *et al*., 2014). In red wines, C_{13}-norisoprenoids are regarded as important contributors to varietal aroma expression and aroma complexity due to their association with floral, fruity, berry-like, and dried-fruit sensory attributes (Mendes-Pinto, 2009; Robinson *et al*., 2014). Variations in their concentration during grape ripening may therefore influence the sensory properties and aromatic potential of the resulting wines (Escudero *et al*., 2007; Pineau *et al*., 2007). In general, C_{13}-norisoprenoids are characterised by their low odour molecular weight, poor water solubility, and low perception thresholds, which contribute to their high sensory relevance in grapes and wines (Schwab *et al*., 2008).

The biodegradation of carotenoids to C_{13}-norisoprenoids occurs either directly or via glycosylated intermediates (Baumes *et al*., 2002; Hjelmeland & Ebeler, 2015). They are considered significant aroma precursors in grapes and wines (Razungles *et al*., 1988; Razungles *et al*., 1996; Baumes *et al*., 2002). Direct biodegradation yields the free form of the respective C_{13}-norisoprenoid, while degradation via intermediates results in the corresponding glycoside (Caffrey *et al*., 2020). Several studies have shown that norisoprenoid glycosides accumulate during berry ripening in a cultivar-specific manner, with significant variation in concentration among grape varieties (Gambetta *et al*., 2014; Wei *et al*., 2021). An example is the *VvCCD1* gene, which encodes a dioxygenase responsible for the cleavage of zeaxanthin into β-ionone precursors; its expression occurs in the early developmental stages of the grape (Mathieu *et al*., 2005). β-Ionone can also be formed through photooxidation via the intermediate step dihydroactinidol (Mendes-Pinto, 2009). Environmental conditions further modulate norisoprenoid biosynthesis: targeted UV attenuation lowers the concentration of free and bound norisoprenoids in grapes at harvest (Bureau *et al*., 2000). Temperature also plays a critical role: both low and high temperatures promote the accumulation of norisoprenoids, while temperatures in the range of 20 to 30 °C can have adverse effects (Wang *et al*., 2020a). During grape processing, the loss of berry integrity leads to the release of norisoprenoid aglycones by chemical or enzymatic hydrolysis, which are associated with floral and fruity attributes (Winterhalter & Schreier, 1994; Mathieu *et al*., 2005; Dunlevy *et al*., 2009; Robinson *et al*., 2014).

The carotenoid profile of grape varieties and their degradation products is known to influence the wine aroma significantly (Razungles *et al*., 1996; Baumes *et al*., 2002). However, comprehensive data on the dynamics of carotenoids and C_{13}-norisoprenoids in Blaufränkisch are still scarce (Philipp *et al*., 2023). This study therefore aimed to characterise the carotenoid and norisoprenoid composition of Blaufränkisch grapes during ripening, from early berry development to overripeness, and to monitor the evolution of key compounds. In addition, grapes were sampled separately from the morning and afternoon sun-exposed sides of a north-south-oriented vineyard to assess the influence of cluster position. Furthermore, acid hydrolysis was applied to enable the determination of total norisoprenoids by releasing glycosidically bound, non-volatile precursor compounds present in the grape matrix, analogous to acid-catalysed release processes occurring during wine ageing. Following hydrolytic cleavage, the liberated aglycons can be determined by gas chromatographic analysis, thereby allowing the assessment of the overall norisoprenoid potential of the grapes. By linking carotenoid profiles to their norisoprenoid derivatives, this work contributes to a better understanding of the aroma formation potential in Blaufränkisch and may support future viticultural and oenological practices targeting flavour optimisation.

MATERIALS AND METHODS

1. Sampling

1.1. Vineyard site

The vineyard used for the experiment is situated in the Austrian federal state of Burgenland, specifically in the Mittelburgenland region, which is internationally known as "Blaufränkischland". The selected vineyard site is called "Altsatz" and is recognised for its exceptional quality in the winemaking village of Lutzmannsburg. The vineyard, located at 47°27′02″ N, 16°38′45″ E at an elevation of 261 m a.s.l., is characterised by a brown loam soil derived from calcareous fine sediments of Tertiary loess material. It was planted in 2003 with the Blaufränkisch variety (clone: IBY selection; rootstock: Kober 5BB) and was in its 20th year after planting at the time of the sampling. The grapevines were planted at a spacing of 0.8 m, with row spacing of approximately 2.65 m. The total area of the vineyard is 0.24 ha. The vines were trained in a high culture system with standard commercial practices. No leaf removal was applied in the fruit zone, and no grape thinning was carried out, allowing the grapes to develop under natural crop load and shading profiles. Additionally, a conventional plant protection and an alternating soil management system were implemented. The vineyard has a north-south orientation, enabling separate sampling of grapes exposed to morning (east) and afternoon (west) sun.

1.2 Grape sampling

Sampling was performed from pea size to overripeness. The experimental setup is schematically illustrated in Figure 1. Initially, weekly; later, biweekly intervals were used. To avoid a thinning effect, previously sampled vines were marked and not resampled. Sampling was restricted to healthy Blaufränkisch vines without symptoms of trunk diseases (*e.g.*, ESCA, Bois noir). Border rows and the first vine section were excluded, and only phenologically representative clusters were selected. To maintain proportionality, basal and distal grapes were sampled in a 1:1 ratio. As indicated earlier, samples were taken separately from the east and west sides. On each sampling date, six clusters from the east and six from the west canopy side were collected and pooled to obtain one representative composite sample per canopy side. Samples were then flash-frozen with solid CO_2(s) at –70 °C (FA Linde, Vienna, Austria), vacuum-sealed, and stored at –26 °C until chromatographic analysis. The rapid freezing caused pedicels to become brittle, facilitating berry separation and thus improving sample representativeness.

Canopy temperatures were recorded throughout the experiment with iButton loggers (model MF1921G/H/Z, Moritz Fuchs, Weingarten, Germany) placed at three vineyard positions. Details regarding exposure to morning and afternoon sun are presented in Table 1. The supplementary data in Figure S1 show the measured average temperatures.

2. Chemicals

Methanol (≥ 99.8 % HPLC grade), ethanol (≥ 99.8 % HPLC grade), methyl *tert*-butyl ether (tBME, ≥ 99.5 % HPLC grade), and dichloromethane (HPLC grade) were purchased from Fisher Scientific (Loughborough, UK). *Trans*-β-Apo-8′-carotenal (≥ 96 % (UV)), (all-*E*)-β-carotene (≥ 97 % (UV)), aceton (≥ 99.8 % HPLC grade), and citric acid (≥ 99.5 %, water-free) were purchased from Sigma-Aldrich (Steinheim, Germany). Butylated hydroxytoluene (BHT, ≥ 99.8 %) was obtained from Fluka (Neu-Ulm, Germany). An authentic standard of (all-*E*)-lutein was purchased from Biomol (Hamburg, Germany). Butylhydroxyanisole (BHA, ≥ 98 %) were purchased from Fluka Chemie. Disodium hydrogen phosphate dihydrate (≥ 99.5 %) and sodium chloride (99.8 %) were purchased from Carl Roth (Karlsruhe, Germany). Deionised water (Nanopure Werner GmbH, Leverkusen, Germany) was used for all experiments. All samples for chromatographic analysis were filtered through 0.2 µm polytetrafluoroethylene (PTFE) filters from Agilent Technologies (Waldbronn, Germany).

3. Analysis of carotenoids

3.1 Extraction of carotenoids

Precautions were taken to minimise artefact formation and carotenoid degradation due to isomerisation and oxidation (Oliveira *et al.*, 2003; Oliveira *et al.*, 2004; Oliveira *et al.*, 2006). All extraction steps were conducted under red light in a nitrogen atmosphere; freeze-drying was avoided (Lashbrooke *et al.*, 2010). Frozen grapes were de-stemmed, embrittled with liquid nitrogen (two cycles), and supplemented with 20 µL BHA solution (120 mg/10 mL ethanol) prior to pulverisation in a laboratory mill (IKA-Werke, Staufen, Germany). Approximately 5 g of berry powder was weighed into 25 mL Eppendorf tubes, and 50 µL of the internal standard *trans*-β-apo-8′-carotenal (313 µg/mL in ethanol) was added. Extraction was carried out with 5 mL dichloromethane containing 0.1 % BHT. Samples were vortexed for 1 min, shaken for 30 min, and centrifuged (5000 rpm, 5 min, 4 °C). The extraction was repeated twice, and the combined organic phases were evaporated to dryness under nitrogen. Extracts were stored at −24 °C and reconstituted in 300 µL tBME/MeOH (90:10; *v/v*) and filtered through a 0.2 µm PTFE syringe filter (Agilent Technologies, Waldbronn, Germany) into amber vials prior to analysis by UHPLC-DAD or LC-APCI-MS/MS^n. All samples were extracted in triplicate.

3.2 UHPLC-DAD analysis of carotenoids

The quantitative analysis of carotenoids was performed on an Agilent 1290 Infinity II System (Agilent Technologies, Waldbronn, Germany) equipped with a binary solvent manager, an autosampler, a column heater, and a diode array detector. The column was an Accucore C30 column (150 × 3.0 mm; 2.6 µm particles) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) protected by a C_8 guard column (10 × 3.0 mm, 2.6 µm particles) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a column temperature of 14 °C. The mobile phases consisted of

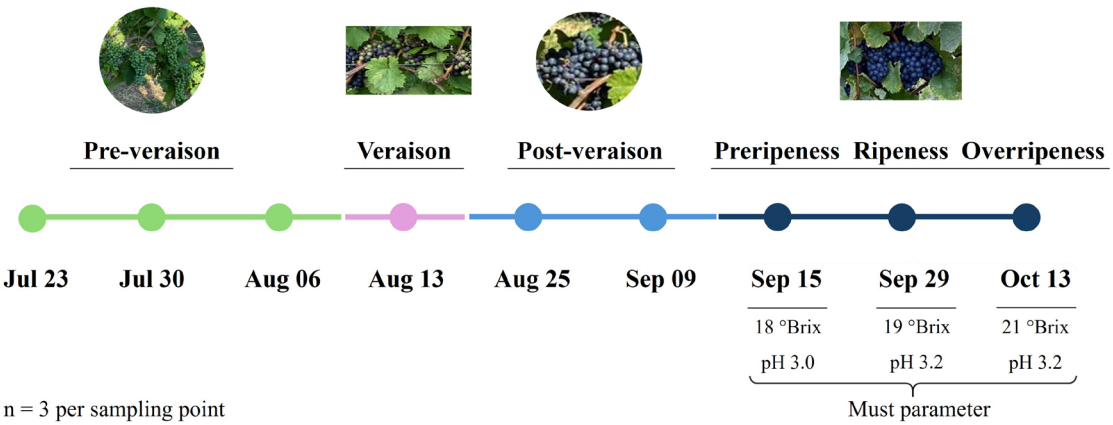


FIGURE 1. Experimental setup showing the sampling of Blaufränkisch grapes across nine developmental stages during ripening in 2023.

TABLE 1. Information on the morning and afternoon sun during the 2023 growing period at the vineyard in Lutzmannsburg.

Sunrise and sunset Lutzmannsburg	Morning sun	Afternoon sun
July 2023	06:00 – 12:00 (6 h)	13:00 – 20:00 (7 h)
August 2023	06:00 – 12:00 (6 h)	13:00 – 19:30 (6.5 h)
September 2023	07:00 – 12:00 (5 h)	13:00 – 18:00 (5 h)
October 2023	07:00 – 12:00 (5 h)	13:00 – 18:00 (5 h)

Calculated based on sunrise and sunset data from timeanddate.com (2023).

methanol/water (87:13, *v*/*v*; eluent A) and methanol/tBME/water (7:90:3, *v*/*v*/*v*; eluent B), using a gradient program as follows: 0 min, 2 % B; 1.8 min 14.5 % B; 2 min, 14.5 % B; 12 min, 21 % B; 12.5 min, 21 % B; 17 min, 25 % B; 32 min, 69 % B; 33 min 95 % B; 36 min 95 % B; 37 min 2 % and isocratic at 2 % B for 5 min. The flow rate was 0.4 mL/min, and the injection volume was 5 µL. The UV−vis spectra were acquired in the range of λ 200–600 nm, while the chromatograms were analysed at λ 450 nm. Quantitation was performed using external calibration with (all-*E*)-lutein recorded with 7 points from 1–90 mg/L, each measured 2-fold at λ 450 nm ($R^2 = 0.997$). Carotenoids were quantified as (all-*E*)-lutein equivalents. Data analysis was performed using OpenLab Chromatography Data System (CDS), ChemStation Edition, Version 3.4 (3.4.0) (Agilent Technologies, Waldbronn, Germany).

3.3 HPLC-MS analysis of carotenoids

For identification, samples were analysed on an Agilent 1100/1200 series (Waldbronn, Germany) HPLC system, consisted of a binary pump (G1312A), autosampler (G1329A), column oven (G1316A), and diode array detector (G1315B), and was coupled with an ion-trap mass spectrometer (HCT Ultra ETD II, Bruker Daltonics, Bremen, Germany) using an APCI ionization source. The column temperature was set to 22 °C. The mobile phases consisted of methanol/water (85:15, *v*/*v*; eluent A) and methanol/tBME (10:90, *v*/*v*; eluent B), using a gradient program as follows: 0 min, 2.0 % B; 1.8 min 14.5 % B; 2 min, 14.5 % B; 12 min, 21 % B; 12.5 min, 21 % B; 17 min, 25 % B; 32 min, 69 % B; 33 min 95 % B; 36 min 95 % B; 37 min 2 % and isocratic at 2 % B for 5.0 min. The flow rate was 0.25 mL/min, and the injection volume was 5 µL. The diode array detector was operated in an acquisition range of λ 200–700 nm. The HPLC-MS runs were additionally monitored at λ 450 nm. The APCI source was operated in positive mode using nitrogen as nebuliser gas at a pressure of 45 psi, with a vaporiser temperature of 350 °C. The scan range was set between *m*/*z* 125 and 1250 using the "ultra-scan" mode with a mass scanning range of 26,000 *m*/*z* per second. Ionisation voltage at high-voltage (HV) capillary −3500 V, HV end plate offset −500 V, trap drive 64.0, octopole radiofrequency (RF) amplitude 187.1 Vpp, lens 2 −60.0 V, capillary exit −115.0 V, target mass 500 *m*/*z*, max. accumulation time 200 ms, ion charge control (ICC) target 100,000 and average of four spectra. Data were processed and evaluated with Data Analysis 4.0 software (Bruker Daltonics, Bremen, Germany).

4. Analysis of C_{13}-norisoprenoids

4.1 Extraction of C_{13}-norisoprenoids and acid hydrolysis

Approximately 5 g of berry powder, from the same extraction batch as used for carotenoid analysis (Section 3.1), was weighed into a centrifuge tube. Subsequently, 5 mL of methanol/water (80:20, *v*/*v*) was added, and the mixture was shaken for 1 min. Extraction was carried out for 4 h on the laboratory shaker, followed by centrifugation for 5 min (5000 rpm, 4 °C). An aliquot of 4 mL of the supernatant was collected and stored at –20 °C until analysis by HS-SPME-GC-MS/MS.

For acid hydrolysis, 100 µL of the methanolic extract was mixed with 4.9 mL of McIlvaine buffer (pH 3.2) and 2 g of sodium chloride. Hydrolysis was carried out at 100 °C for 4 hours in a drying oven. Upon completion of the hydrolysis, the samples were rapidly cooled to –24 °C. Prior to analysis, deuterated C_{13}-norisoprenoids were added as internal standards.

4.2 HS-SPME-GC-MS/MS analysis of norisoprenoids

Post-hydrolysis (total) norisoprenoids in grape samples were analysed by headspace solid-phase microextraction coupled to gas chromatography–tandem mass spectrometry (HS-SPME-GC-MS/MS) using a Thermo Trace 1300 gas chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) coupled to a TSQ Duo triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), following the method described by Scharf *et al*. (2026), with modifications regarding the SPME fibre type and extraction conditions to account for differences in sample matrix compared to the original method. HS-SPME extraction was performed using a divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fibre (50/30 µm coating thickness; 2 cm length; Supelco, Bellefonte, PA, USA). Samples were pre-incubated for 6 min at 40 °C. Adsorption lasted 45 min at the same temperature. Then desorption took place in the injector in splitless mode for 2 min at 200 °C. The fibre was then reconditioned for 2 min at 260 °C.

Quantification was performed using an internal standard-based assay as described by Scharf *et al*. (2026). Briefly, TDN, vitispiranes, β-damascenone, and β-ionone were used as representative standards, and their isotopically labelled analogues (d6-TDN, d5-vitispiranes, d4-β-damascenone, and d3-β-ionone) served as internal standards. Calibration curves were established for each of the 4 standard compounds. For norisoprenoids lacking authentic reference standards, concentrations were estimated using the internal standard and the calibration curve of the chemically most similar reference compound. All analyses were carried out as triplicates. Instrument control, data acquisition, and data analysis were carried out using Xcalibur (version 3.0.63).

Compounds present as isomers (actinidols and vitispiranes) were quantitated as one and referred to as one.

5. Statistical analysis

Statistical differences among samples were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. In addition, two-way ANOVA was performed to evaluate the effects of ripening stage, canopy side, and their interaction on carotenoid and C_{13}-norisoprenoid concentrations. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using OriginPro 2022b (Academic) (OriginLab Corporation, Northampton, MA, USA) software. Basic data visualisation was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

1. Characterisation of the pigment profile

A combination of chromatographic and spectroscopic information based on online DAD spectra was used to characterise the carotenoids by comparison with standard spectra and literature-reported values. Spectroscopic characteristics, such as the position of absorption maxima λ_{max} and the spectral fine structure (ratio of the peak sizes between the highest absorption bands, (III/II [%]), provided information about the chromophores. Mass spectrometric analysis (LC-MS^n) enabled tentative identification of carotenoids for which no standards were available.

As illustrated in Figure 2, the qualitative carotenoid profile of Blaufränkisch grapes included a total of 21 carotenoids. Among them, lutein-5,6-epoxide, luteoxanthin, flavoxanthin, neochrome a and b, (all-*E*)-neoxanthin, and auroxanthin were tentatively identified (Crupi *et al*., 2010b; van Breemen *et al*., 2012; Rivera *et al*., 2014). (all-*E*)-β-carotene, (all-*E*)-lutein, and (all-*E*)-zeaxanthin were unequivocally identified using authentic standards. The presence of these compounds is consistent with previous work on other grape varieties (Mendes-Pinto *et al*., 2004; Fariña *et al*., 2010; Crupi *et al*., 2010a). No carotenoid esters were detected in the Blaufränkisch grapes. In addition, chlorophyll b (peak 19), pheophytin a (peak 21), and pheophytin b (peak 20) were tentatively identified by comparison with literature data (see also Table 2; Guedes de Pinho *et al*., 2001; Mendes-Pinto *et al*., 2005; Kamffer *et al*., 2010; Yuan & Qian, 2016).

1.1. Xanthophylls

(all-*E*)-lutein (peak 14) and (all-*E*)-zeaxanthin (peak 15) are the quantitatively most important xanthophylls in grapes across a wide range of red and white cultivars (Guedes de Pinho *et al*., 2001; Oliveira *et al*., 2003; Oliveira *et al*., 2004; Mendes-Pinto *et al*., 2004; Lashbrooke *et al*., 2010; Kwasniewski *et al*., 2010; Kamffer *et al*., 2010; Bunea *et al*., 2012). These xanthophylls were also detected in Blaufränkisch grapes. Lutein is an asymmetric dihydroxyl xanthophyll with one β- and one ε-ring. Consequently, it will undergo retro-Diels−Alder (RDA) fragmentation of the ε-ring to produce $[M+H-56]^+$ or eliminate the ε-ring to present $[M+H-122]^+$, which can be used to distinguish lutein from zeaxanthin, in addition to their slightly distinct UV–vis absorption spectra. *Cis*-isomers of lutein were observed, supported by the small hypsochromic shift (λ 4–6 nm) and the presence of a characteristic cis-peak in the near-UV region. Based on retention times and spectral features, the 9*Z*- and 9′*Z*-lutein isomers were tentatively assigned (Aman *et al*., 2005).

Neoxanthin isomers were detected, with (all-*E*)-neoxanthin identified based on λ_{max} values (398, 422, 450 nm) and MS values [M+H] 601.4 (Crupi *et al*., 2010b; Rivera *et al*., 2014). Both neochrome a and b were also tentatively identified using absorption maxima and mass spectrometric data (Kamffer *et al*., 2010; Mendes-Pinto *et al*., 2004). Luteoxanthin, auroxanthin, lutein-5,6-epoxide, flavoxanthin, and violaxanthin were tentatively assigned based on characteristic λ_{max} and retention times, as reported in previous studies (Crupi *et al*., 2010a; Fariña *et al*., 2010; Guedes de Pinho *et al*., 2001; Lashbrooke *et al*., 2010;

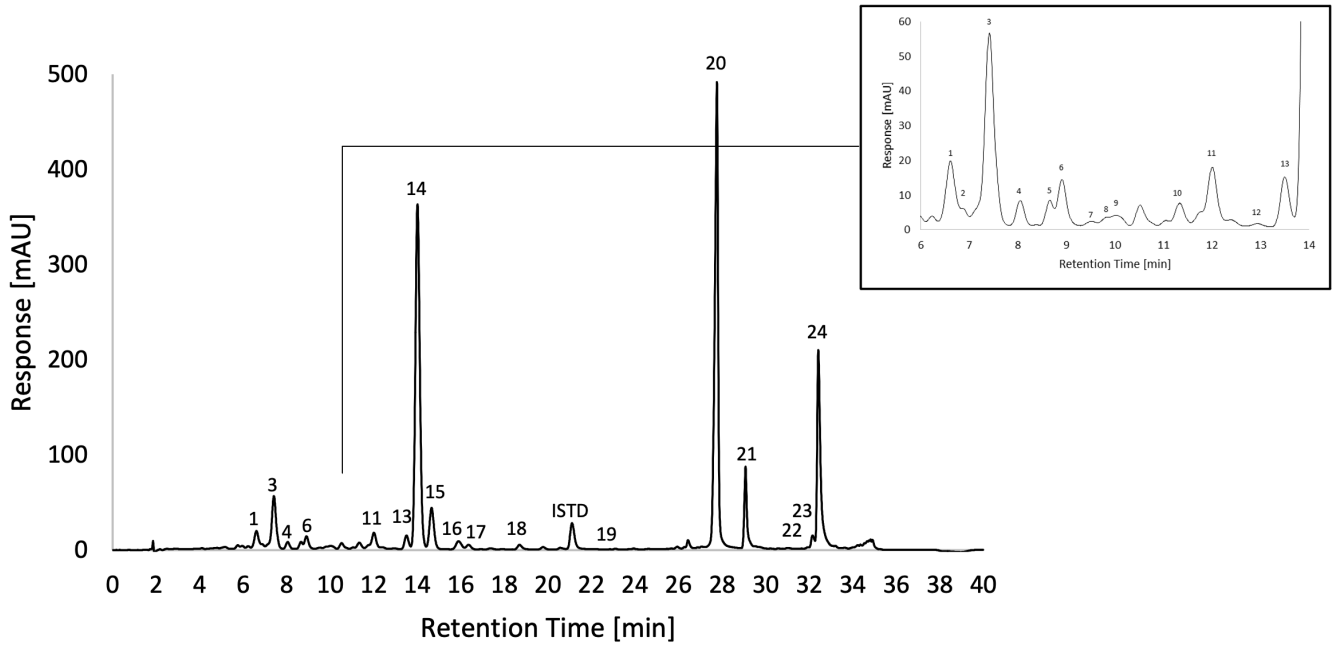


FIGURE 2. UHPLC chromatogram (recorded at λ 450 nm) of the carotenoid extract from a representative grape sample. For peak assignment, see Table 2.

TABLE 2. UV–Vis and MS information and identification of carotenoids and chlorophyll derivatives in Blaufränkisch grape berries.

				λ_{max} [nm][c]				APCI(+)MS^n	
peak[a]	compound	Rt [min][b]	cis	I	II	III	III/II [%]	*m/z* $[M+H]^+$	*m/z* fragment
1	neochrome a	6.43	-	402	422	448	102.0	601.4	583.4, 565.4
2	unknown 1	6.58	-	-	-	-	-	-	-
3	(all-*E*)-neoxanthin	7.17	-	414	438	466	83.9	601.4	583.4, 565.4
4	neochrome b	7.76	-	398	422	448	88.0	601.4	583.4, 565.4
5	luteoxanthin	8.36	-	398	422	448	109.1	-	-
6	unknown 2	8.66	-	400	422	450	84.6	-	-
7	auroxanthin	9.22	-	382	402	426	91.2	-	-
8	lutein-5,6-epoxid	9.51	-	418	440	470	85.7	585.4	567.1, 493.1
9	unknown 3	9.78	-	420	446	474	66.7	585.4	567.1, 549, 493.1
10	unknown 4	10.98	-	400	422	448	70.6	-	-
11	flavoxanthin	11.59	-	402	424	450	87.2	-	-
12	unknown 5	12.56	-	-	426	452	-	-	-
13	unknown 6	12.98	-	404	428	456	57.9	569.4	551.4
14	(all-*E*)-lutein	13.58	-	422	446	474	69.2	569.4	551.4
15	(all-*E*)-zeaxanthin	14.14	-	428	452	480	40.3	569.4	551.4
16	(9*Z*)-lutein	15.57	332	420	440	468	28.6	569.4	551.4
17	(9´*Z*)-lutein	15.97	332	420	440	468	47.4	569.4	551.4
18	unknown 7	19.19	-	420	452	482		-	-
IS	β-Apo-8´-carotenal (IS)	20.77			466			417	-
19	chlorophyll b	22.84			316, 344, 466, 598, 648			907.5	-
20	pheophytin b	27.53			436, 528			885.5	-
21	pheophytin a	28.87			410, 506, 536			871.5	-
22	unknown 8	31.91	-					-	-
23	(13-*Z*)-β-carotene	32.10	338	422	448	474		-	-
24	(all-*E*)-β-carotene	32.31	-	428	454	480		-	-

[a] Peak number [b] Retention time on the C_{30}-column. [c] Identification by comparison with UV-vis spectrum of the standard compound. [d] Identification by comparison with the MS spectrum of the standard compound (Rosso & Mercadante, 2007; Crupi *et al.*, 2010b; Zepka & Mercadante, 2009; Aman *et al.*, 2005; van Breemen *et al.*, 2012; Kamffer *et al.*, 2010).

Kamffer *et al*., 2010). Other minor xanthophylls such as antheraxanthin, cryptoxanthin, chrysanthemaxanthin, and echinenone were not detected (Guedes de Pinho *et al*., 2001; Lashbrooke *et al*., 2010; Zepka & Mercadante, 2009).

1.2. Carotenes

In contrast to the diverse xanthophyll profile described above, (all-*E*)-β-carotene (peak 24) was the only carotene detected in quantitatively relevant amounts, consistent with literature reports (Guedes de Pinho *et al*., 2001; Oliveira *et al*., 2003; Oliveira *et al*., 2004; Lashbrooke *et al*., 2010; Kwasniewski *et al*., 2010). Two additional minor peaks potentially corresponding to β-carotene isomers were observed, but their concentrations were below the limit of quantification (LOQ) of the UHPLC-DAD method (Kwasniewski *et al*., 2010; Zepka & Mercadante, 2009).

2. Evolution of carotenoid levels during berry ripening

The temporal evolution of individual carotenoids and total carotenoid concentrations throughout the nine developmental stages is illustrated in Figure 3, whereas a heatmap representation summarising the relative concentration changes across ripening stages and canopy sides is provided in the Table S1. The evolution of carotenoid levels in Blaufränkisch berries followed a pattern comparable to that described for other *Vitis vinifera* cultivars. Across all developmental stages, β-carotene and lutein represented the predominant carotenoids, accounting for the major proportion of total carotenoids throughout ripening (Razungles *et al*., 1996; Kwasniewski *et al*., 2010; Aubert & Chalot, 2018; Asproudi *et al*., 2020). Other carotenoids, including zeaxanthin, neoxanthin, and neochromes a/b, contributed to a lesser extent but displayed distinct temporal variations.

In the literature, a clear change in the carotenoid profile has already been shown in berries during the ripening process (Asproudi *et al*., 2020). Previous findings showed β-carotene and some xanthophylls (neochrome, neoxanthin, flavoxanthin, and lutein) are already abundant before veraison, although their concentrations decreased sharply thereafter (Razungles *et al*., 1988; Marais *et al*., 1991; Razungles *et al*., 1996). Other xanthophylls, including violaxanthin, luteoxanthin, and lutein-5,6-epoxide, accumulated later in the ripening process. This observation also applies to the Blaufränkisch grape variety, with the highest levels of nearly all carotenoids detected at the initial sampling date (Jul 23), which then decreased until maturity and some carotenoids (*e.g.*, neochrome b) are no longer detectable at ripeness.

For both canopy sides, total carotenoid concentration decreased significantly during ripening, although the magnitude and timing of these changes differed between the two sun-exposed sides. Two-way ANOVA confirmed ripening stage as the dominant factor affecting carotenoid concentrations, with significant effects observed for nearly all quantified compounds ($p < 0.0001$; Table 3). In addition, canopy side significantly influenced the concentrations of most major carotenoids, including (all-*E*)-β-carotene, lutein, zeaxanthin, neoxanthin, flavoxanthin, and lutein-5,6-epoxide, indicating a strong microclimatic effect of cluster exposure within the canopy. Significant interactions of ripening stage and canopy side further demonstrated that carotenoid degradation and accumulation patterns differed between morning- and afternoon-exposed berries throughout ripening, suggesting that canopy microclimate modulates carotenoid accumulation and degradation dynamics in a stage-dependent manner. On the afternoon sun (west) side, the most pronounced decline occurred within two weeks after veraison, likely associated with increased expression of carotenoid cleavage dioxygenases (CCDs) *VvCCD1* and *VvCCD4* from the time of veraison, as previously reported (Mathieu *et al*., 2005; Young *et al*., 2012). In contrast, berries from the morning sun (east) side exhibited only a slight short-term decrease after veraison, followed by a significant increase approximately 4 weeks after veraison. Although carotenoid concentrations generally decline after veraison due to plastid transition processes and enhanced carotenoid cleavage activity (Razungles *et al*., 1988; Young *et al*., 2012), delayed declines or temporary increases of individual carotenoids during ripening have also been reported in grape berries under specific environmental conditions (Asproudi *et al*., 2020). In the present study, this behaviour was predominantly observed in berries from the morning-sun side and coincided with significant ripening time × canopy side interaction effects identified by two-way ANOVA, suggesting that canopy microclimate influenced carotenoid degradation dynamics during ripening. Reduced thermal and photooxidative stress on the morning-sun side may therefore have delayed carotenoid degradation and cleavage processes, resulting in temporarily elevated concentrations during mid-ripening. A further decline was observed as berries approached normal ripeness. Between ripeness (Sep 29) and overripeness (Oct 13), carotenoid concentration remained stable on the west side, whereas a significant decrease was observed on the east side, and finally the lowest concentration was reached.

Considering individual carotenoids, a consistent pattern emerged: most carotenoids peaked prior to veraison and declined thereafter, though at compound-specific rates. β-carotene decreased by 51 % at the morning-sun side and by 63 % at the west side until veraison. Thereafter, concentrations at the east side increased again, while they remained nearly constant on the west side. Initial β-carotene levels (Jul 23) were comparable between both sides (2765 µg/kg FW for the morning-sun and 2357 µg/kg FW for the west side). At maturity (Sep 29), however, the east-side berries contained substantially higher levels (1400 µg/kg FW) than those from the west side (839 µg/kg FW). Lutein showed a similar trend, with a pronounced increase about four weeks after veraison at the east side, whereas concentrations continuously declined on the west side, a pattern that agrees with findings in *Vitis vinifera* cv. Nebbiolo (Asproudi *et al*., 2020). Among the xanthophylls, zeaxanthin was initially higher at the morning-sun side (1418 µg/kg FW) than at the west side (838 µg/kg FW), but levels dropped sharply at both

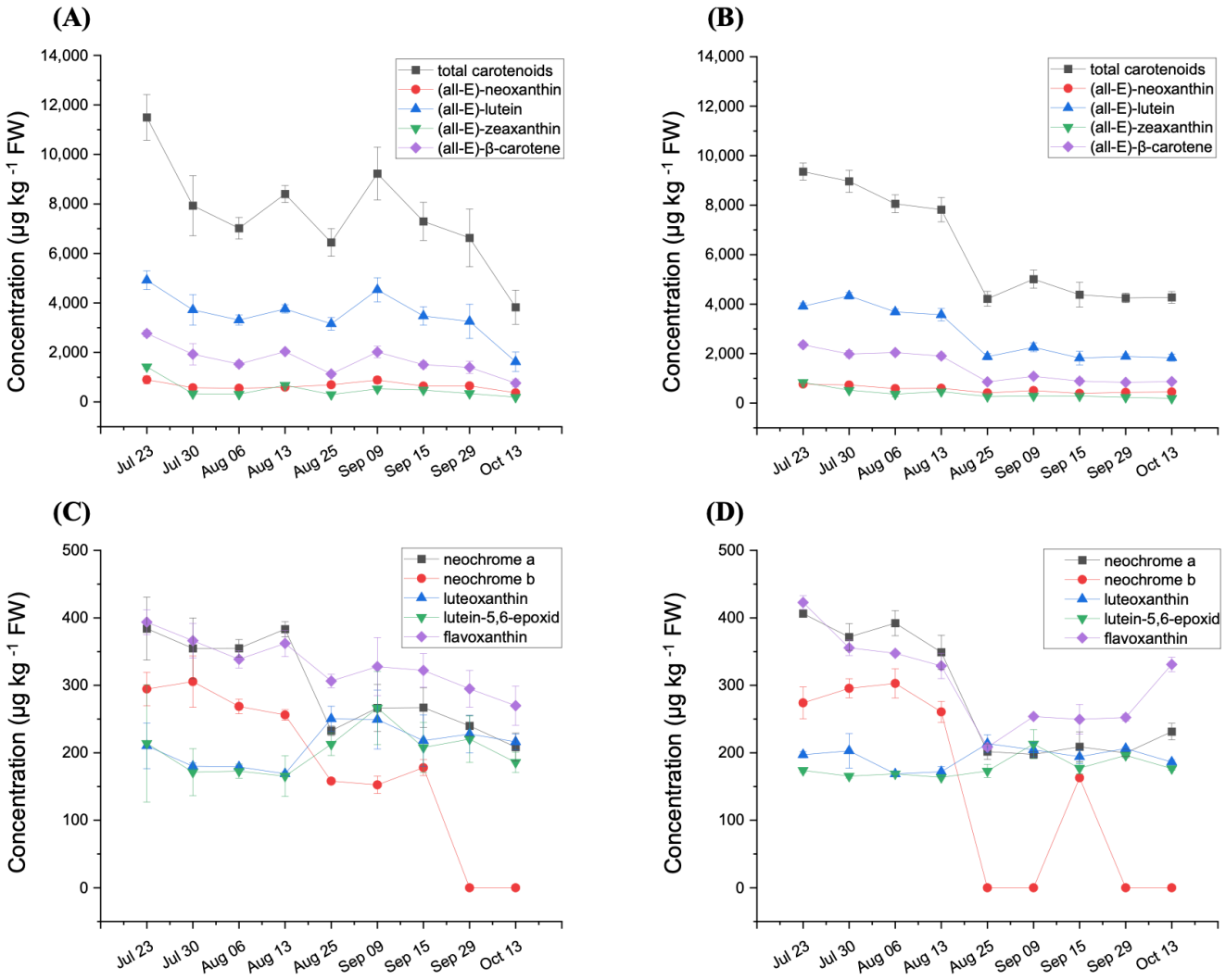


FIGURE 3. Evolution of total and individual carotenoid concentrations during berry ripening of *Vitis vinifera* L. cv. Blaufränkisch grown under morning sun (A, C) and afternoon sun (B, D) exposure. Panels A and B show the major carotenoids together with the total carotenoid concentration, whereas panels C and D display the minor carotenoids. Values are expressed as mean ± standard deviation (n = 3).

TABLE 3. Effects of ripening stage, canopy side, and their interaction on carotenoid concentrations in Blaufränkisch grapes determined by two-way ANOVA.

Compound	Ripening stage	Canopy side	Ripening stage × Canopy side
neochrome a	**<0.0001**	**0.02349**	**0.00079**
(all-*E*)-neoxanthin	**<0.0001**	**<0.0001**	**<0.0001**
neochrome b	**<0.0001**	**<0.0001**	**<0.0001**
luteoxanthin	0.15534	0.43226	0.76468
lutein-5,6-epoxid	**<0.0001**	**<0.0001**	**<0.0001**
flavoxanthin	**<0.0001**	**<0.0001**	**<0.0001**
(all-*E*)-lutein	**<0.0001**	**<0.0001**	**<0.0001**
(all-*E*)-zeaxanthin	**<0.0001**	**<0.0001**	**<0.0001**
(all-*E*)-β-carotene	**<0.0001**	**<0.0001**	**<0.0001**

p-values obtained from two-way ANOVA evaluating the effects of ripening stage, canopy side, and their interaction on carotenoid concentrations in Blaufränkisch grapes. Significant effects ($p < 0.05$) are indicated in bold.

locations, with a minor transient rise around veraison. The comparatively high initial zeaxanthin concentrations observed on the morning-sun side may reflect transient microclimatic differences within the canopy. Zeaxanthin is part of the dynamic xanthophyll cycle, which enables rapid photoprotective adjustment to fluctuating irradiance and temperature conditions through reversible interconversion with violaxanthin and antheraxanthin. Under the warm and dry conditions prevailing prior to sampling, short-term differences in light exposure and thermal stress may therefore have contributed to the pronounced positional differences observed for zeaxanthin. At maturity, zeaxanthin contents were reduced by 76 % and 72 % at the east and west sides, respectively, relative to initial values. Neoxanthin exhibited the highest concentrations at the onset of sampling, followed by a temporary increase several weeks after veraison and a continuous decline thereafter. Neochrome b remained nearly constant until veraison but decreased sharply afterwards and was no longer detectable at full ripeness in berries from either side.

Overall, carotenoid concentrations were consistently higher in berries from the east (morning sun) side compared to those from the west (afternoon sun) side. This observation agrees with previous studies in different grape varieties showing that berries exposed to lower light and temperature accumulate higher carotenoid levels (Marais *et al*., 1991; Oliveira *et al*., 2004; Asproudi *et al*., 2020). However, the significant interaction of ripening stage and canopy side observed for most major carotenoids indicates that the effect of canopy exposure was not constant throughout ripening, but dynamically changed during berry development. In particular, berries from the east side showed distinct temporal patterns of carotenoid evolution, including delayed declines and transient increases after veraison, whereas west-exposed berries generally exhibited a more continuous decrease. These findings suggest that reduced irradiance and thermal exposure not only promote carotenoid accumulation, but also modulate carotenoid turnover kinetics and degradation dynamics associated with photooxidative stress and CCD activity. In summary, the ripening-dependent decline in carotenoid concentrations in Blaufränkisch grapes appears to be strongly modulated by the interaction between developmental stage and microclimatic conditions within the canopy, jointly determining the balance between carotenoid biosynthesis, photoprotection, and enzymatic cleavage into aroma-active C_{13}-norisoprenoid precursors.

3. Changes in the C_{13}-norisoprenoid profile in the course of maturation

In this study, twelve C_{13}-norisoprenoids were quantified in Blaufränkisch grapes using HS-SPME-GC-MS/MS, including, β-damascenone, β-ionone, TPB (4-(2,3,6-trimethylphenyl) buta-1,3-diene), 4-(2,3,6-trimethylphenyl)-butan-2-one, 3,4-didehydro-β-ionone, edulan, TDN, Riesling acetal, isomeric vitispiranes and actinidols. Additionally, two further compounds (unknown_3 MW_172 and unknown_4 MW_172), previously described in the method development study by Scharf *et al*. (2026) (see Appendix Table A1 for mass spectral data), were included. These compounds exhibit mass spectral characteristics, suggesting a structural and biogenetic relationship to known carotenoid degradation products and pathways, particularly TDN (Winterhalter & Gök, 2013). It should be noted that norisoprenoids were determined after acid hydrolysis of the extracts, thus representing the total (free and glycosidically bound) fraction rather than the native volatile composition of the grapes. Therefore, the observed trends should be interpreted in terms of relative changes in norisoprenoid potential rather than absolute changes in aroma-active compounds. The temporal evolution of individual C_{13}-norisoprenoids and total C_{13}-norisoprenoid concentrations throughout the nine developmental stages is illustrated in Figure 4, whereas a heatmap representation summarising the relative concentration changes across ripening stages and canopy sides is provided in Table S2.

Two-way ANOVA identified ripening stage as the dominant factor influencing the concentrations of all quantified C_{13}-norisoprenoids ($p < 0.0001$; Table 4), confirming a strong developmental regulation of norisoprenoid formation during berry maturation. In contrast, canopy side showed compound-specific effects and significantly influenced only selected norisoprenoids, including β-damascenone, actinidols, and 3,4-didehydro-β-ionone. Significant interactions of ripening stage and canopy side were observed for most compounds, indicating that the temporal evolution of norisoprenoid concentrations differed between morning- and afternoon-exposed berries. These findings suggest that microclimatic conditions within the canopy modulate not only the extent, but also the timing and patterns of norisoprenoid formation during ripening.

The concentration of vitispiranes showed a strong overall increase during ripening, reaching the highest levels in the late and overripe stages. This increase was statistically significant across ripening stages for both MS and AS grapes. In MS grapes, vitispirane concentrations increased from 6.70 µg/kg FW at the first sampling date to the highest value of 22.30 µg/kg FW on Aug 25, after which concentrations remained at a comparably high level until the final sampling date. In AS grapes, concentrations also increased significantly during ripening, although the pattern was less linear, reaching the highest value of 28.99 µg/kg FW at the overripe stage on Oct 13.

Similarly, TDN showed an overall increase during the ripening period, with minor fluctuations in the mid-stages but a pronounced rise towards overripe stages, suggesting enhanced formation during late ripening phases. Significant differences among ripening stages were observed for both MS and AS grapes. In MS grapes, TDN increased from 5.92 µg/kg FW on Jul 23 to the highest value of 18.41 µg/kg FW on Sep 09, followed by slightly lower but still elevated concentrations during the later sampling dates. In AS grapes, TDN showed a more fluctuating pattern, including a temporary decrease on Aug 13, but reached its highest concentration at the final sampling date on Oct 13 with 20.55 µg/kg FW.

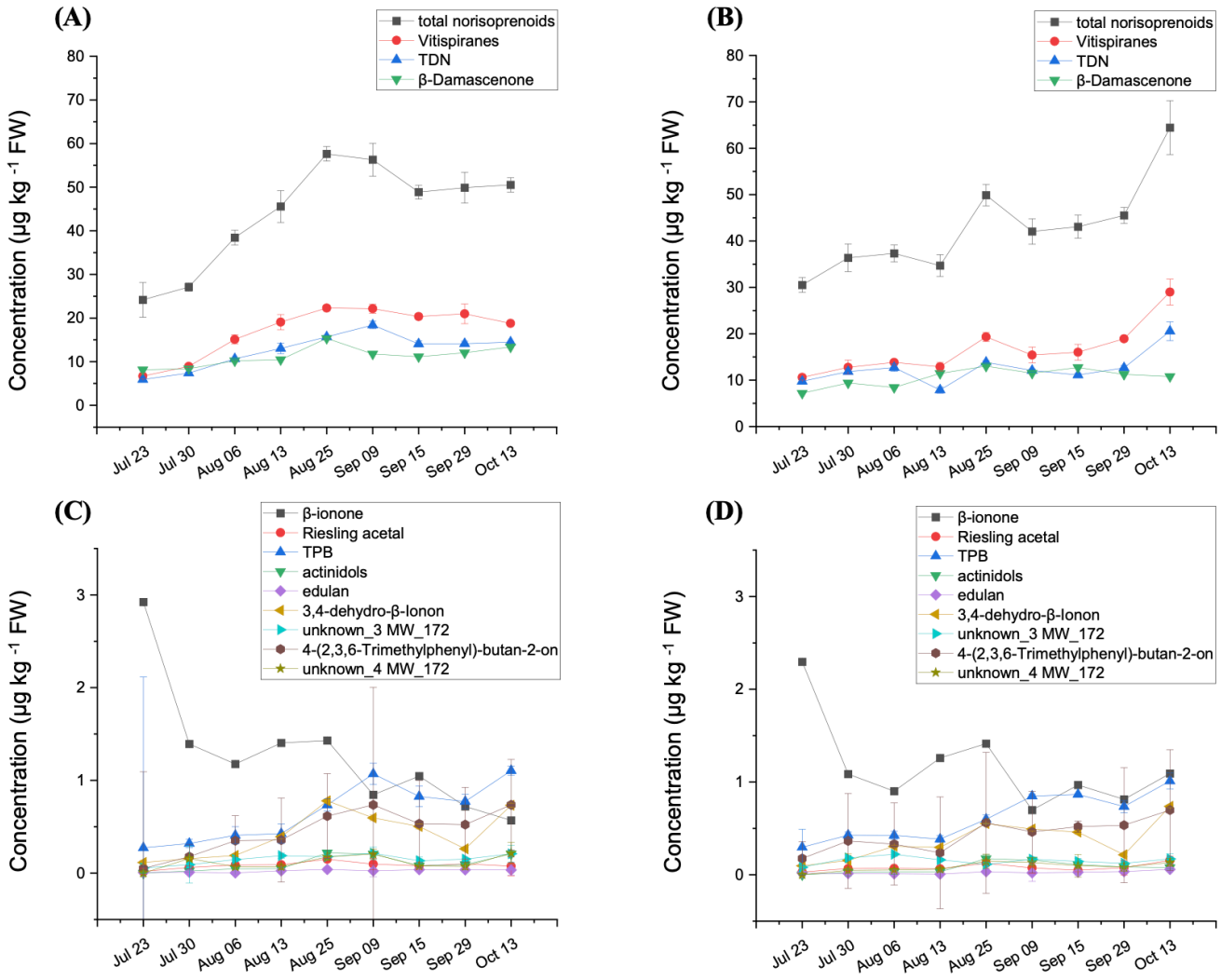


FIGURE 4. Evolution of norisoprenoid concentrations during berry ripening of *Vitis vinifera* L. cv. Blaufränkisch under morning sun (A, C) and afternoon sun (B, D) exposure. Panels A and B show the major norisoprenoids together with the total norisoprenoid concentration, whereas panels C and D display the minor norisoprenoids. Values are expressed as mean ± standard deviation (n = 3).

TABLE 4. Effects of ripening stage, canopy side, and their interaction on norisoprenoid concentrations in Blaufränkisch grapes determined by two-way ANOVA.

Compound	Ripening stage	Canopy side	Ripening stage × Canopy side
Vitispiranes	**<0.0001**	0.4537	**<0.0001**
Riesling acetal	**<0.0001**	0.3847	**0.00060**
TDN	**<0.0001**	0.54862	**<0.0001**
β-damascenone	**<0.0001**	**0.001**	**<0.0001**
TPB	**<0.0001**	0.08926	**0.03514**
β-ionone	**<0.0001**	0.37053	0.63995
actinidols	**<0.0001**	**0.00649**	**0.01127**
edulan	**<0.0001**	0.99305	**0.02037**
3,4-didehydro-β-ionone	**<0.0001**	**0.00313**	**0.00063**
unknown_3 MW_172	**<0.0001**	0.99186	**<0.0001**
4-(2,3,6-Trimethylphenyl)-butane-2-one	**<0.0001**	0.08223	**<0.0001**
unknown_4 MW_172	**<0.0001**	0.0507	0.50553

p-values obtained from two-way ANOVA evaluating the effects of ripening stage, canopy side, and their interaction on carotenoid concentrations in Blaufränkisch grapes. Significant effects ($p < 0.05$) are indicated in bold.

The concentration of β-damascenone also increased, though less linearly; it rose during the mid-ripening phase, followed by a slight decline, and then increased again at the overripe stage. Significant changes during ripening were observed for both MS and AS grapes. In MS grapes, β-damascenone increased from 8.12 µg/kg FW at the first sampling date to the highest value of 15.34 µg/kg FW on Aug 25, followed by lower concentrations during subsequent ripening stages. In AS grapes, the highest value was likewise observed on Aug 25, with 13.02 µg/kg FW, whereas later stages remained at intermediate levels. Compared with vitispiranes and TDN, β-damascenone therefore showed an earlier maximum and a less pronounced late-ripening increase, indicating a compound-specific accumulation pattern.

In contrast, the concentration of β-ionone remained relatively low throughout ripening and generally decreased, indicating a less pronounced tendency for accumulation, a trend that has also been reported in other grape cultivars (Asproudi *et al*., 2018). Significant differences among ripening stages were nevertheless observed for both MS and AS grapes. The highest β-ionone concentrations occurred at the first sampling date, with 2.92 µg/kg FW in MS grapes and 2.30 µg/kg FW in AS grapes. Thereafter, concentrations declined, particularly in the later ripening stages, reaching 0.57 µg/kg FW in MS grapes at the final sampling date. In AS grapes, β-ionone followed a similar decreasing trend during ripening, although a slight increase was observed again at the overripe stage. Its initially higher concentrations are consistent with the role of *VvCCD1*, a dioxygenase gene induced at early berry development and implicated in zeaxanthin cleavage (Mathieu *et al*., 2005).

Notably, the accumulation of vitispiranes, TDN and β-damascenone coincided with the increase in berry weight and sugar concentration, whereas β-ionone followed the opposite trend. The observed trends align with findings in Pinot noir, where a similar accumulation dynamic for carotenoids, norisoprenoids, and other volatile compounds was found (Yuan & Qian, 2016). Similarly, Strauss *et al*. (1987) documented increases in vitispirane, TDN, and β-damascenone during grape ripening in Riesling. Yuan & Qian (2016) further noted that the accumulation of these three norisoprenoids begins approximately five days before veraison. In Blaufränkisch, however, this process appears to commence significantly earlier, with substantial amounts of these norisoprenoids detected as early as 21 days before veraison. This discrepancy may reflect varietal differences or abiotic factors (Gerdes *et al*., 2002; Oliveira *et al*., 2003; Oliveira *et al*., 2004; Joubert *et al*., 2016). Overall, the statistically significant differences among ripening stages confirm that C_{13}-norisoprenoid accumulation in Blaufränkisch is strongly ripening-dependent but follows distinct compound-specific accumulation patterns.

The norisoprenoid TPB exhibited a general increasing trend during ripening, peaking at overripeness. This ripening-related increase was statistically significant for both MS and AS grapes. In MS grapes, TPB remained at comparatively low levels from Jul 23 to Aug 13, ranging from 0.27 to 0.43 µg/kg FW, before increasing significantly from Aug 25 onwards and reaching the highest concentrations on Sep 09 and Oct 13. A similar pattern was observed in AS grapes, where TPB remained low until Aug 13 and then increased significantly during the later ripening stages, with the highest concentration observed at the overripe stage on Oct 13. This sensorially potent norisoprenoid, reported in various grape varieties, such as Sémillon, Chardonnay, and Riesling, contributes vegetal and grassy notes (Janusz *et al*., 2003; Cox *et al*., 2005). Reported concentrations in wines range from 0.05 to 2.1 µg/L, with a sensory detection threshold of 0.04 µg/L in white wine (Janusz *et al*., 2003). This compound may contribute to the aroma potential of Blaufränkisch due to its relatively high content at the overripe stage. No data are currently available on the precise ripening dynamics or agronomic factors influencing the concentration of this norisoprenoid. A structurally related ketone, 4-(2,3,6-trimethylphenyl)-butane-2-one, also exhibited an increasing concentration trend, particularly from the mid-ripening phase onwards. Significant differences among ripening stages were observed for both MS and AS grapes, with the highest concentrations occurring during the later stages, particularly on Sep 09 and Oct 13 in MS grapes and on Oct 13 in AS grapes. However, little is known beyond its first identification in Riesling (Winterhalter, 1991).

The formation of 3,4-didehydro-*β*-ionone increased substantially in samples until mid-ripening, likely driven by increasing concentrations of specific precursor compounds, followed by fluctuations and no consistent further increase towards overripeness. This temporal pattern was statistically significant for both MS and AS grapes. In MS grapes, concentrations increased from 0.11 µg/kg FW at the first sampling date to a maximum of 0.78 µg/kg FW on Aug 25, followed by a decline on Sep 29 and a renewed increase at the overripe stage. In AS grapes, concentrations also increased during ripening, reaching elevated values from Aug 25 onwards and the highest value at the final sampling date on Oct 13. This compound is known to be mechanistically linked to TDN formation but does not occur as a main precursor in grapes. It can be formed via thermal carotenoid degradation or released from glycosidically bound precursors and subsequently rearranges to TDN under acidic conditions (Gueldner & Winterhalter, 1991). In the context of viticulture, no insights are currently available regarding its sensory properties, behaviour during berry ripening, or potential agronomic influencing factors. Similarly limited is the knowledge about the norisoprenoid edulan, whose concentration in this study remained largely constant until the mid-ripening phase and only showed a slight increase at the overripe stage. Significant differences among ripening stages were nevertheless observed for both MS and AS grapes. In MS grapes, edulan reached its highest values from Aug 25 onwards, although concentrations remained very low overall. In AS grapes, the most pronounced increase occurred at the overripe stage, where the highest concentration was observed on Oct 13. Edulan was first identified and described in *Vitis vinifera* by Strauss *et al*. (1986).

The concentration of Riesling acetal in Blaufränkisch grapes increases during the early and mid-ripening stages, followed by a slight decline around Sep 09 and a subsequent rise in the overripe stage. Significant differences among ripening stages were observed for both MS and AS grapes. In MS grapes, Riesling acetal increased from 0.01 µg/kg FW at the first sampling date to the highest concentration of 0.15 µg/kg FW on Aug 25, followed by lower but still elevated concentrations during the later ripening stages. In AS grapes, concentrations were generally low during early and mid-ripening, but the highest value was reached at the final sampling date on Oct 13. This norisoprenoid is associated with fruity sensory attributes, and elevated concentrations have been observed in aged Riesling wines (Winterhalter, 1991; Winterhalter & Rouseff, 2002). Riesling acetal has been proposed as a precursor of TDN (Daniel *et al*., 2009). However, no data are currently available on the behaviour of this compound during berry ripening or on viticultural practices that might influence its concentration.

In the Blaufränkisch grape variety, actinidols exhibit low concentrations during the early ripening stages but show an increase from the mid-ripening phase onwards, reaching their highest levels during later ripening stages. This increase was statistically significant for both MS and AS grapes. In MS grapes, actinidol concentrations were lowest during the first sampling dates and increased markedly from Aug 25 onwards, with the highest concentrations observed on Aug 25, Sep 09, and Oct 13. In AS grapes, concentrations also increased significantly from the mid-ripening phase onwards, with the highest values observed on Aug 25 and Sep 09, followed by slightly lower concentrations during the later stages. Strauss *et al*. (1986) demonstrated that *trans*-actinidols can be formed as major products via acid-catalysed degradation of megastigma-4,7-diene-3,6,9-triol, with smaller amounts of TDN being generated under these conditions. Olfactorily, actinidols are described as camphor-like or woody-resinous, similar to the norisoprenoid vitispirane. However, due to their typically very low concentrations, often below the detection threshold, their contribution to wine aroma is considered negligible (Ghiglieno *et al*., 2023).

The two unidentified compounds (unknown_3 MW_172 and unknown_4 MW_172) exhibited moderate changes over the course of ripening rather than a clear accumulation pattern. Both compounds showed an initial increase towards mid-ripening stages, followed by fluctuations and no consistent further rise towards overripeness. Hydrolysis experiments reported by Scharf *et al*. (2026) demonstrated that unknown_4 MW_172 exhibits formation trends comparable to TDN. A similar behaviour is observed in Blaufränkisch grapes, where the evolution of its concentration during ripening follows a pattern comparable to that of TDN.

4. Effects of morning and afternoon sun exposure across ripening stages

During early ripening, vitispirane concentrations tended to be higher on the afternoon sun (AS) side, whereas during mid- and full ripening, higher concentrations were generally observed on the morning sun (MS) side. Towards overripeness, however, vitispirane concentrations increased markedly on the AS side, reaching 28.99 µg/kg FW compared with 18.79 µg/kg FW on the MS side at the final sampling date. This suggests that the influence of canopy side on vitispirane accumulation was not constant throughout ripening, but shifted during the later ripening phase.

A broadly similar pattern was observed for TDN. At the early sampling dates, TDN concentrations were higher on the AS side, whereas from Aug 13 to Sep 29, MS grapes generally showed higher or comparable concentrations. At overripeness, however, TDN increased considerably on the AS side, reaching 20.55 µg/kg FW compared with 14.48 µg/kg FW on the MS side. These results suggest a partly parallel late-ripening response of vitispiranes and TDN, which is consistent with their interconvertible precursors (Gök, 2015). While Gao *et al*. (2021) reported higher concentrations of vitispirane, TDN, and TPB on the east-facing (morning sun) side in Chardonnay and Cabernet franc, the present study indicates a shift towards higher vitispiranes and TDN levels on the AS side at overripeness. This discrepancy may be related to cooler late-season conditions (< 20 °C on average) combined with afternoon sun exposure from late September onwards, conditions that have been shown to favour norisoprenoid accumulation (Wang *et al*., 2020b).

β-damascenone showed a different pattern. Concentrations were slightly higher on the AS side at some early and mid-ripening stages, particularly on Aug 13 and Sep 15, whereas MS grapes showed higher values at Aug 25, Sep 29, and at the final overripe stage. At overripeness, β-damascenone reached 13.39 µg/kg FW on the MS side compared with 10.77 µg/kg FW on the AS side. These observations suggest that β-damascenone did not follow the same late-stage increase on the AS side as observed for vitispiranes and TDN. This is in line with reports of reduced β-damascenone in white wines from grapes exposed to high temperatures and intense sunlight (Marais *et al*., 1992; Kwasniewski *et al*., 2010). Similarly, Gao *et al*. (2021) found no clear differences in β-damascenone concentration between grapes sampled from different vineyard positions. A recent study by Ghiglieno *et al*. (2023) also confirmed that practices such as shading or defoliation did not result in consistent differences in β-damascenone concentration. In the present study, the higher β-damascenone levels in overripe MS grapes may therefore reflect a compound-specific response to the interaction between canopy side, ripening stage, and late-season temperature conditions.

β-ionone concentrations were generally lower than those of β-damascenone, and canopy side differences were comparatively small throughout most of ripening.

During early ripening, β-ionone tended to be higher on the MS side. In the later stages, however, concentrations remained low on both canopy sides, with a slight increase on the AS side at overripeness. At the final sampling date, β-ionone reached 1.09 µg/kg FW on the AS side compared with 0.57 µg/kg FW on the MS side. Among the investigated C_{13}-norisoprenoids, β-ionone was the only compound that did not exhibit a continuous increase during berry development. Instead, the highest concentrations were observed during the early ripening stages, followed by a gradual decline towards overripeness. Since β-ionone is formed through the oxidative cleavage of β-carotene by carotenoid cleavage dioxygenases (CCDs), its comparatively high concentrations during early ripening may reflect enhanced β-carotene degradation at this stage. The subsequent decline in β-ionone concentration suggests that its accumulation is not solely governed by precursor degradation. Instead, β-ionone may undergo further metabolic transformation, volatilisation, or reduced net formation during ripening due to changes in enzymatic activity. However, the slightly higher β-ionone concentrations observed on the AS side at overripeness may indicate that extended sunlight exposure under cooler late-season conditions favoured β-ionone formation or retention. This interpretation is consistent with reports that extended sunlight can enhance β-ionone formation (Song *et al*., 2015), and with the observation that lower temperatures during full ripening and overripeness may contribute to norisoprenoid accumulation (Wang *et al*., 2020a). Gao *et al*. (2021) also documented significant differences in β-ionone concentrations in grapes sampled from various vineyard positions. The combination of lower temperatures and extended sunlight exposure seems to have a decisive influence on the increase in β-ionone concentrations. Since no glycosylated precursors have been identified, β-ionone is present and accumulates exclusively in its free form, and is, therefore, considered an important contributor to the aroma potential of Blaufränkisch (Pedroza *et al*., 2010).

When considering the overall changes during berry development, carotenoid concentrations were generally higher in berries exposed to morning sunlight than in those exposed to afternoon sunlight. In contrast, the response of C_{13}-norisoprenoids was more compound- and stage-dependent. While several norisoprenoids showed higher concentrations on the morning sun side during large parts of berry development, vitispiranes, and TDN increased markedly on the afternoon sun side at overripeness. Notably, the pronounced reduction in carotenoid concentrations on the afternoon sun side during late ripening was not immediately accompanied by a corresponding increase in total C_{13}-norisoprenoids, although specific compounds such as TDN and vitispiranes increased markedly at overripeness. This observation suggests that canopy-side differences in norisoprenoid accumulation cannot be explained solely by precursor availability.

CONCLUSION

This study demonstrates that berry exposure to sunlight (morning *vs* afternoon side of the canopy) exerts a decisive influence on carotenoid accumulation and degradation, and consequently on the formation of C_{13}-norisoprenoids in Blaufränkisch grapes. Lutein and β-carotene were identified as the predominant carotenoids, accounting for the majority of the total carotenoid content. A significant decrease in carotenoid content was observed for berries from both canopy sides during ripening. However, on the morning sun-exposed side, a transient increase in carotenoid content, particularly in lutein and β-carotene, was detected after veraison. Between ripeness and overripeness, carotenoid concentrations decreased substantially on the morning sun side, while remaining relatively stable on the afternoon sun side. Overall, berries exposed to morning sun contained significantly higher carotenoid levels compared to those from the afternoon sun side. The more pronounced decrease is likely attributable to higher average temperatures and greater sunlight exposure, which accelerated degradation. These results highlight the critical role of canopy microclimate in modulating carotenoid turnover and norisoprenoid formation during berry ripening. Significant interactions between ripening stage and canopy side were observed for most carotenoids and several norisoprenoids, indicating that the effects of sunlight exposure were not constant throughout development, but dynamically changed during ripening. In particular, berries exposed to morning sun exhibited delayed carotenoid degradation and transient post-veraison increases in lutein and β-carotene, whereas afternoon-exposed berries generally showed a more continuous decline associated with enhanced carotenoid turnover. These findings further demonstrate that microclimatic conditions generated by differential sun exposure within the canopy strongly influence carotenoid stability and norisoprenoid formation dynamics during grape ripening. Interestingly, the generally lower carotenoid concentrations observed in afternoon-exposed berries were not consistently associated with higher concentrations of C_{13}-norisoprenoids. This finding suggests that carotenoid degradation alone is insufficient to account for the observed differences in norisoprenoid accumulation. Instead, the formation of individual C_{13}-norisoprenoids appears to be regulated by a complex interplay of metabolic and environmental factors that vary with canopy position and ripening stage. It was observed that overripe Blaufränkisch grapes in a north-south oriented vineyard exhibited higher β-damascenone concentrations on the morning sun side and higher β-ionone concentrations on the afternoon sun side. Accordingly, harvest timing and canopy orientation may represent effective tools for modulating the aromatic profile of Blaufränkisch. A fractional harvest at overripeness, followed by separate vinification of grapes from the two canopy sides, may enhance distinct aroma attributes, namely β-damascenone-driven fruity and floral notes and β-ionone-driven violet-like nuances. Future work should aim to characterise additional, partly unidentified

norisoprenoids and to evaluate their sensory contributions as well as their role in known pathways, thereby providing a more comprehensive understanding of agronomic and microclimatic factors shaping Blaufränkisch grape and wine aroma.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualisation: C.P.; R.G.; S.S.; N.S.; data curation: S.S.; R.G.; formal analysis: S.S.; N.S.; investigation: S.S.; N.S.; methodology: S.S.; R.G.; project administration: R.G.; C.P.; resources: R.G.; C.P.; supervision: R.G.; validation: S.S.; R.G.; visualisation: S.S.; N.S. R.G. writing: - Original Draft: S.S.; N.S. Writing - Review & Editing: S.S.; N.S. R.G.; C.P.

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