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Storage stability of berry mueslis with special focus on phenolic compounds

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ABSTRACT

To investigate stability of phenolic compounds in food products during storage, real-time (RT, at 23 °C) and accelerated shelf-life tests (ASLT, 40 °C) were conducted on modified-atmosphere-packaged strawberry, blueberry, and blackcurrant mueslis. Monitored with LC-MS and HPLC, a clear variation was observed in the phenolic profile of the berry mueslis in both tests, including 29 anthocyanins, 40 flavonols, 16 phenolic acids, and 2 flavan-3-ols. The contents of these phenolic compounds changed differently during storage. Unlike other phenolics, the contents of all identified anthocyanins were significantly decreased in the tests. The modified atmosphere package of the mueslis did not retard anthocyanin degradation at 40 °C. The largest decrease occurred in the first 56 days of ASLT, when 54–66 % of total anthocyanins were lost. The degradation was highly associated with structural features of anthocyanins, including substitution on both anthocyanidins and sugar moieties. Pelargonidin 3-*O*-glucoside, malvidin 3-*O*-arabinoside, and malvidin 3-*O*-galactoside had higher degradation rates (k = 0.0267, 0.0195, and 0.0176 day^{-1}, respectively) than others. Acylation on the sugar moieties also significantly enhanced storage stability of anthocyanins. Our results suggested that the stability of bioactive phytochemicals in food products should be considered when estimating the health-promoting function and sensorial property of the products.

1. Introduction

Berries have been widely applied in the food industry to provide products with unique flavors, delightful colors, and potential health-promoting benefits (Saarniit et al., 2023). As studied for decades, the beneficial effects of berries on human health are mainly attributed to their secondary metabolites, phenolic compounds in particular (Becker Pertuzatti et al., 2021; Ntemiri et al., 2020). Berry-derived phenolic compounds have a wild diversity in chemical structures, presenting mostly as anthocyanins, tannins, flavonols, flavan-3-ols, and hydroxycinnamic acids (Tian et al., 2017). As a major groups of berry phenolics, anthocyanins have been reported as protective effects against the oxidative stress-associated diseases, such as cancers, and cardiovascular or neurodegenerative diseases (Liang et al., 2024). The berry-derived anthocyanins can also lower the incidence of obesity by reducing fatty acid synthesis through inhibiting fatty acid synthase (Singh et al., 2020). In addition to anthocyanins, other minor phenolic groups in berries also contribute to human health as reported extensively (Saarniit et al., 2023).

Yet, berry phenolics degrade during the shelf-life of food products, resulting in a decrease in their bioactivities. Phenolic degradation is dependent on storage conditions such as temperature, light, or oxygen content (Singh et al., 2020). Our previous research suggests that the degradation rate of phenolic compounds is also highly influenced by the type of food matrix. Some food matrix provides an acidic condition that is optimal for phenolic compounds to retain their intact structures (Saarniit et al., 2023). The structural features of berry phenolics also play an essential role in their stability. For example, anthocyanidins are more stable after glycosylation and methylation on their basic structures (Zhao et al., 2017). The improved stability is attributed to the decreasing numbers of hydroxyl groups and increasing numbers of methoxy groups in the B ring of anthocyanidins (Liu et al., 2018). Moreover, the interaction among phenolic compounds or between phenolics and other components (*e.g.*, dietary fiber) is another key factor that affects phenolic degradation rate (Pico et al., 2022). Thus, instead of investigating the degradation of phenolic commercial standards, it is more

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important to study their changes within food matrices. Besides diminishing health beneficial functions, the degradation of phenolics may also cause a large shift in sensorial properties of berry-added products (Tian et al., 2017). Thus, considering the risks to food quality and consumers' acceptance, it is critical to monitor the compositional changes of berry phenolics during the shelf-life of the products.

Accelerated shelf-life testing (ASLT) is a time-efficient method for food manufacturers to assess the quality of foods with relatively long shelf-life. Compared to real-time storage tests (RT) at room temperature, ASLT speeds up the natural deterioration processes of food products without altering the sequence of reactions that occur under normal storage conditions (Calligaris et al., 2019). The acceleration can be achieved by adjusting storage conditions such as temperature, oxygen levels, light exposure, or humidity (Kilcast & Subramanian, 2000). Among which, elevating temperature is most commonly used due to their significant impact on reaction rates (Calligaris et al., 2019). As discussed in our previous study, the mechanism of ASLT relies on the Arrhenius equation, which describes the relationship between temperature and the speed of chemical reactions (Saarniit et al., 2023). The acceleration factor Q_{10} (the number of times that the reaction rate changes with a 10 °C change in temperature) is introduced to calculate the testing time points for ASLT corresponding to room-temperature storage time (ASTM International, 2021; Toledo, 2007).

Although anthocyanins are temperature-sensitive, this moderate elevating temperature (40 °C) allows for meaningful degradation analysis while avoiding unrealistic thermal stress. The temperature of 40 °C is well accepted in previous studies of anthocyanin stability as a compromise between accelerating degradation kinetics and avoiding extreme conditions that may not reflect realistic storage scenarios (De Marchi et al., 2024; Polyiam et al., 2025). To date, the ASLT is rarely used to measure the shelf-life of berry products. Most of the published results have focused only on variation in total phenolic content during ASLT period using colorimetric methods (Saarniit et al., 2023; Sadilova et al., 2006). Our research aims to systematically investigate the stability of phenolic compounds during the shelf-life of commercial food products by using both ASLT (at 40 °C) and RT (at 23 °C). Muesli products added with freeze-dried berry slices (*i.e.*, strawberries, blueberries, and blackcurrants, respectively) were chosen for their popularity as a healthy breakfast in Western countries and increasing interest gained in Asian countries (Dziki et al., 2022). Liquid chromatographic and mass spectrometric methods were applied to monitor compositional variation in anthocyanins, flavonols, flavan-3-ols, hydroxycinnamic acids, and hydroxybenzoic acids at a molecular level. Based on the changes in phenolic concentration, the degradation kinetics of major berry phenolics were determined. Statistical models of first-order kinetics, one-way analysis of variance, and calculation of Q_{10} were used to predict the influence of temperature on phenolic degradation and estimate the storage days in ASLT. As the novelties of our research, we provide new findings of the changes in phenolic profiles, degradation kinetics of anthocyanins, and statistical correlation among different phenolic compounds. These results will offer a reference for food manufacturers to ensure product quality and shelf-life.

2. Materials and methods

2.1. Chemicals

Reference standards of delphinidin, cyanidin, peonidin, petunidin, malvidin, pelargonidin 3-*O*-glucoside, quercetin, ellagic acid, *p*-coumaric acid, (+)-catechin, and *trans*-cinnamic acid were purchased from Sigma-Aldrich (St. Louis, MO, United States). The solvents of LC and MS grade, such as acetonitrile, formic acid, hydrochloric acid, ethyl acetate, and methanol were purchased from Honeywell (Espoo, Finland).

2.2. Ingredients, production and packaging of muesli samples

The muesli samples were produced using rolled oats (Balti Veski AS, Estonia), freeze-dried strawberry (*Fragaria* spp., SB), blueberry (*Vaccinium* spp., BB) and blackcurrant (*Ribes* spp., BC) slices (Freezedry OÜ, Estonia), sugar syrup (Nordic Sugar A/S, Denmark), whole milk powder (Valio OY, Finland), strawberry concentrate (Bayernwald KG, Germany), and vanilla sugar (Santa Maria AS, Estonia).

Three types of mueslis were produced. Strawberry muesli consisted of rolled oats (41.5 g/100 g), sugar syrup (21.9 g/100 g), whole milk powder (17.9 g/100 g), freeze-dried strawberry slices (14.3 g/100 g), strawberry concentrate (4.3 g/100 g), and vanilla sugar (0.2 g/100 g). Blueberry muesli contained rolled oats (43.8 g/100 g), sugar syrup (23.1 g/100 g), whole milk powder (18.9 g/100 g), freeze-dried blueberry slices (9.4 g/100 g), strawberry concentrate (4.5 g/100 g), and vanilla sugar (0.2 g/100 g). Blackcurrant muesli included rolled oats (41.5 g/100 g), sugar syrup (21.9 g/100 g), whole milk powder (17.9 g/100 g), freeze-dried blackcurrant slices (14.3 g/100 g), strawberry concentrate (4.3 g/100 g), and vanilla sugar (0.2 g/100 g). To produce the basis of the muesli, rolled oats, sugar syrup, strawberry concentrate, and vanilla sugar were mixed and baked at 130 °C for 45 min. After that, the baked basis was cooled at room temperature for 4 h. Then, the dry and cooled basis was mixed with whole milk powder and berry slices.

The muesli samples were packaged in stand-up pouches, containing 20 μm Matt-BOPP/12 μm PET/7 μm Aluminum/110 μm LDPE (Dakla-Pack Europe, Netherlands). The oxygen transmission rate of the packaging material was < 0.5 $cm^3/m^2/24$ h and the water vapor transmission rate was < 0.5 $g/m^2/24$ h. In addition, 50 mm × 57 mm of iron-based oxygen absorber (Tianhua Tech Co., Ltd, China) was added into each package.

2.3. Storage test design of muesli samples

Two storage tests were conducted. The packaged mueslis were stored at room temperature (23 °C) and in a climate chamber at 40 °C (Memmert UF110, Germany), respectively. In the test at 23 °C, the testing points of storage time were set at 6 and 12 months. For the ASLT at 40 °C, the testing time points were calculated with Q_{10} factor using Equation (1).

$$Accelerated\ aging\ time\ (AAT) = \frac{Desired\ real\ time\ (RT)}{Q_{10}^{\left(\frac{T_{AA}-T_{RT}}{10}\right)}} \quad (1)$$

where AAT is the accelerated aging time at accelerated aging temperature (T_{AA}) and RT is the real storage time at real storage time temperature (T_{RT}) (ASTM International, 2021). The Q_{10} value was set as 3 (which is a common setting for almost all food products). The time points of the ASLT were calculated to be 28, 56, 89, 120, 169, 197, and 365 days at 40 °C (Choi et al., 2017), to simulate the storage at room temperature for 0.5, 1, 1.5, 2, 3, 3.5, and 6.5 years, respectively.

2.4. Analysis of phenolic compounds

The dried berries (4 replicates for analysis of anthocyanins and 2 replicates for analysis of other phenolic compounds) were first picked from muesli samples at each time point during both storage tests and then crushed into fine powders with mortar and pestle (Supplemental Table 1). Anthocyanins and other phenolic compounds were extracted from the berries using two different methods described in our previous study with a slight modification (Tian et al., 2019). For anthocyanins, approximately 1.0 g of berry powders were mixed with acidified methanol (methanol/hydrochloric acid, 99:1, *v/v*) at a solid/solvent ratio of 1:3 (*w/v*). The extraction was assisted with ultra-sonication (at 45 kHz, for 10 min) and centrifugation (for 10 min, at 1500 × *g*). The supernatants from three-time extraction were combined and diluted to a

final volume of 10 mL with the acidic methanol. For other phenolic compounds, the berry powders (3.8 g) were mixed with 20 mL of aqueous ethyl acetate (water/ethyl acetate, 1:1, *v/v*), followed by 3 min of vortex and 15 min of centrifuge (1500 × *g*). The supernatants after centrifugation were collected and completely dried by using a rotary evaporator (at 35 °C, Heidolph, Germany). The residues were re-dissolved in 3 mL of methanol. The extracts of anthocyanins and other phenolic compounds were filtered with 0.2 mm syringe filters and stored in the freezer at −20 °C till further analyses.

The methods of identifying and quantifying phenolic compounds were described in our previous studies (Tian et al., 2017, 2019). Briefly, the identification was conducted by using a Shimadzu Ultra performance liquid chromatography (UPLC) system equipped with an SPD-M40 photo diode array detector (PDA), and a LCMS-8045 mass spectrometer (MS; Shimadzu Corp., Kyoto, Japan). LC chromatographic separation was performed with a Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 μm, Torrance, CA, United States). The reject volume was 10 μL. The total flow rate was set to 1 mL/min, and approximately 0.3 mL/min of samples were eluted into mass spectrometers. MS full scan and MS^2 product ion scan were operated in both ESI^+ and ESI^- mode. A Shimadzu LC-30AD liquid chromatograph system coupled with an SPD-M20A diode array detector (Shimadzu Corp., Kyoto, Japan) was used for quantitative analysis of phenolic compounds. All chromatograms were monitored at the wavelength of 520 nm (for anthocyanins), 360 nm (flavonols and ellagic acid derivatives), 320 nm (hydroxycinnamic acids), and 280 nm (flavan-3-ols). The identified compounds were quantified by external reference standards (Supplemental Table 2). Approximately 1 mg of reference compounds were dissolved in 10 mL ethanol and diluted into four different concentrations. The calibration curves were established between peak areas in the HPLC-DAD chromatogram and corresponding concentrations.

2.5. Degradation kinetics of anthocyanins

The degradation of anthocyanins during ASLT was analyzed following the first-order kinetics (Equation (2)).

$$C_t = C_0 \times e^{(-kt)} \quad (2)$$

where C_t and C_0 are the anthocyanin concentrations at Day t and Day 0, respectively. The value of *k* is the rate constant, and t is the storage time (day). The half-life value ($t_{1/2}$) of total anthocyanin content was calculated with Equation (3).

$$t_{1/2} = (\ln 1 / 2) / k \quad (3)$$

2.6. Statistical analyses

The concentration of each identified compound was calculated on the basis of dry weight of berries and the values are expressed as mean ± standard deviation (SD). The *k* values used in anthocyanin degradation kinetics and correlations between individual phenolic compounds in berry slices were calculated using Origin Pro 2018 (Origin Lab, Northampton, MA, United States). Cluster heatmap and correlation heatmap of Pearson's correlation coefficients were performed using MetaboAnalyst 6.0 (www.metaboanalyst.ca). Statistical differences among data were calculated based on one way-ANOVA and Tukey's post hoc test ($p < 0.05$) by IBM SPSS Statistics 28 for Windows (SPSS Inc., NY, United states).

3. Results and discussion

3.1. Phenolic profiles in berry slices

All the phenolic compounds were characterized by MS and MS^2. The identification was based on the MS fragmentation pattern by comparing molecule ions and typical fragment ions with previously reported data (Aaby et al., 2007, 2012; Ancillotti et al., 2017; Becker Pertuzatti et al., 2021; Clifford et al., 2006; Grace et al., 2019; Kelanne et al., 2020; Nie et al., 2017; Pico et al., 2022; Rothwell et al., 2013; Spínola et al., 2015; Tian et al., 2017, 2019; Álvarez-Fernández et al., 2015). As shown in Table 1, 29 anthocyanins were identified in the slices of SB, BB, and BC, presenting as delphinidin, cyanidin, pelargonidin, petunidin, peonidin, malvidin, and their glycosides. Other phenolic compounds from the groups of flavonols (40 compounds), flavan-3-ols (2), hydroxycinnamic acids (13), and hydroxybenzoic acids (3) were also detected from berry samples. Anthocyanins were the major phenolic compounds in these berry slices, the total contents of which (246.8–1086.6 mg/100 g dry weight basis, DW) were much higher than that of other phenolics (10.9–37.4 mg/100 g DW) (Supplemental Table 3-5).

In SB, six anthocyanins were tentatively identified. Pelargonidin and its glycosides accounted for 97.9 % of the total anthocyanin content (246.8 mg/100 g DW) (Fig. 1A). The results were consistent with the observation of anthocyanin contents of SB (216–385 mg/100 g DW) in the research of Wang and Lin (Wang & Lin, 2000). The flavanols found in SB included quercetin, kaempferol, isorhamnetin, and their glycosides. Ellagic acid and its glycosides (hydroxybenzoic acids and derivatives) were only identified in SB among three berry slices.

The total content of eleven anthocyanins in BB was 1086.6 mg/100 g DW, 54.9 % of which were malvidins, followed by 18.7 % delphinidins, 16.1 % petunidins, 9.7 % cyanidins, and 0.6 % peonidins (Fig. 1B). Among flavonols and flavan-3-ols, laricitrins and syringetins (including aglycones and glycosylated forms), as well as (−)-epicatechin were only found in BB. BC contained delphinidins (52.2 %) and cyanidins (47.8 %) with a total content of 355.9 mg/100 g DW (Fig. 1C). Myricetin and its glycosides (flavonols) were identified only in BB and BC. Our results of BB and BC suggested higher levels of anthocyanins in comparison with the data in previous studies (558 mg/100 g DW and 210–250 mg/100 g DW, respectively) (Dobson et al., 2017; Hosseinian & Beta, 2007). This large variation was probably caused by different berry cultivars.

3.2. Changes in major groups of phenolic compounds during storage

All dried berry samples in this study were subjected to the same standardized process of storage and sampling to ensure a reasonable comparison of the results. The changes of anthocyanin contents in SB, BB, and BC slices during ASLT and room-temperature storage are present in Fig. 2A–C. In the ASLT (40 °C), the total anthocyanin content showed a sharp decrease of 63.9 % in SB for 0–28 days but no significant degradation was observed after 89 days (Fig. 2A). Anthocyanin degradation in BB (42.8 % decrease in 28 days) was similar to that observed in SB, followed by a gradual decline after 28-day storage (Fig. 2B). In BC, a rapid degradation of anthocyanins (36.7 %) occurred from 28 to 56 days (Fig. 2C). Different degradation speeds are probably attributed to the stability of dominant anthocyanins with different structures (Dobson et al., 2017). During room-temperature storage, SB showed the largest decline (32.8 %) of total anthocyanin content in 365 days. The total anthocyanin degradation in BB and BC in 12 months at 23 °C was similar (17.4 % and 14.4 %, respectively). Anthocyanin degradations at room-temperature storage in our study were remarkably slower than that in the previous study (Piljac-Žegarac & Šamec, 2011). Piljac-Žegarac and Šamec found that no anthocyanins in SB were detected with colorimetric assay after 4-day storage at 25 °C. Thus, the modified atmosphere package in our study showed retard effect on anthocyanin degradation at room temperature. However, this package did not protect anthocyanin from the fast degradation at elevated temperatures. Most of the anthocyanins in BB (93.4 %), SB (80.0 %), and BC (75.4 %) degraded at 40 °C. For other phenolic compounds, the changes in the total contents fluctuated over 365 days. Generally, the total contents of other phenolics were increased in BB and BC but decreased in SB along with storage time (Fig. 2D–F). In SB, the contents of flavonols and hydroxycinnamic acids were decreased (49.3 % and 24.6 %, respectively), and

Table 1
Identification of phenolic compounds in strawberry (SB), blueberry (BB) and blackcurrant (BC) mueslis by HPLC-DAD-ESI-MS [a]

Tentative identification (abbreviation)	UV $\lambda_{typical}$ (*nm*)	$[M+Na]^+/[M+H]^+/[M-H]^-$ or other ions (*m/z*)	MS^2 (*m/z*)	Presence in			Literature
				BB	BC	SB	
Anthocyanins							
Delphinidin 3-*O*-rutinoside (**De-Rut**)	525	-/611.2/609.2	611.2 → 465.1, **303.1** 609.2 → **300.0**	–	+	–	1–3
Delphinidin 3-*O*-galactoside **(De-Gal)**	520	-/465.1/463.1	465.1 → **303.1** 463.1 → **300.0**	+	–	–	4–7
Delphinidin 3-*O*-glucoside (**De-Glu**)	523	-/465.1/463.1	465.1 → **303.1** 463.1 → **300.0**	+	+	–	1,2,4–7
Delphinidin 3-*O*-(6″-coumaroyl)-glucoside (**De-coGlu**)	530	-/611.2/609.1	611.2 → **303.1** 609.1 → **300.0**	–	+	–	1–3
Delphinidin 3-*O*-(6″-acetyl)-glucoside (**De-acGlu**)	527	-/507.1/505.1	507.1 → **303.1** 505.1 → **300.0**	+	–	–	4,6,7
Delphinidin 3-*O*-arabinoside (**De-Ara**)	524	-/435.1/433.1	435.1 → **303.1** 433.1 → **300.0**	+	–	–	4,6,7
Delphinidin (**De**)	525	-/303.1/301.0		+	+	–	2,7
Cyanidin 3-*O*-rutinoside (**Cy-Rut**)	518	-/595.2/593.2	595.2 → **287.1** 593.2 → **284.0**	–	+	–	1–3
Cyanidin 3-*O*-galactoside (**Cy-Gal**)	516	-/449.1/447.1	449.1 → **287.1** 447.1 → **284.0**	+	–	–	4,6,7
Cyanidin 3-*O*-glucoside (**Cy-Glu**)	515	-/449.1/447.1	449.1 → **287.1** 447.1 → **284.0**	+	+	+	1–10
Cyanidin 3-*O*-arabinoside (**Cy-Ara**)	517	-/419.1/417.1	419.1 → **287.1** 417.1 → **284.0**	+	–	–	6,7
Cyanidin (**Cy**)	523	-/287.1/285.0		+	+	–	2
Pelargonidin 3-*O*-rutinoside (**Pl-Rut**)	503	-/579.2/577.2	579.2 → 433.1, **271.1** 577.2 → **269.1**	–	–	+	9,10
Pelargonidin 3-*O*-glucoside (**Pl-Glu**)	501	-/433.1/431.1	433.1 → **271.1** 431.1 → **269.1**	–	–	+	8–10
Pelargonidin 3-*O*-(6″-malonyl)-glucoside (**Pl-maGlu**)	502	-/519.1/517.1	519.1 → **271.1** 517.1 → 473.1, **269.1**	–	–	+	9,10
Pelargonidin 3-*O*-(6″-succinyl)-glucoside (**Pl-suGlu**)	504	-/533.1/531.1	533.1 → **271.1** 531.1 → 499.1, 431.1, 337.0, **269.1**	–	–	+	phenol-explorer
Pelargonidin (**Pl**)	509	-/271.1/269.1		–	–	+	phenol-explorer
Petunidin 3-*O*-galactoside (**Pt-Gal**)	524	-/479.1/477.1	479.1 → **317.1** 477.1 → **314.0**	+	–	–	4,6,7
Petunidin 3-*O*-glucoside (**Pt-Glu**)	524	-/479.1/477.1	479.1 → **317.1** 477.1 → **314.0**	+	–	–	4–7
Petunidin 3-*O*-(6″-acetyl)-glucoside (**Pt-acGlu**)	530	-/521.1/519.1	521.1 → **317.1** 519.1 → **315.1**	+	–	–	4,6,7
Petunidin 3-*O*-arabinoside (**Pt-Ara**)	525	-/449.1/447.1	449.1 → **317.1** 447.1 → **314.0**	+	–	–	4,6,7
Petunidin (**Pt**)	533	-/317.1/315.1		+	–	–	
Peonidin 3-*O*-galactoside (**Po-Gal**)	517	-/463.1/461.1	463.1 → **301.1** 461.1 → **298.0**	+	–	–	4,6,7
Malvidin 3-*O*-galactoside (**Ma-Gal**)	526	-/493.1/491.1	493.1 → **331.1** 491.1 → **328.1**, 313.0, 299.0	+	–	–	5–7
Malvidin 3-*O*-glucoside (**Ma-Glu**)	526	-/493.1/491.1	493.1 → **331.1** 491.1 → **329.1**, 313.0, 299.0	+	–	–	5–7
Malvidin 3-*O*-(6″-acetyl)-galactoside (**Ma-acGal**)	531	-/535.2/533.1	535.2 → **331.1** 533.1 → **328.1**, 313.0, 299.0	+	–	–	4,6,7
Malvidin 3-*O*-(6″-acetyl)-glucoside (**Ma-acGlu**)	531	-/535.2/533.1	535.2 → **331.1** 533.1 → **329.1**, 313.0, 299.0	+	–	–	4,6,7
Malvidin 3-*O*-arabinoside (**Ma-Ara**)	525	-/463.1/461.1	463.1 → **331.1** 461.1 → **328.1**, 313.0, 299.0	+	–	–	6,7
Malvidin (**Ma**)	535	-/331.1/329.1		+	–	–	
Flavonols							
Myricetin 3-*O*-galactoside (**My-Gal**)	266, 355	-/481.0/479.1	481.0 → **319.0** 479.1 → **315.9**, 287.2, 271.1, 242.3, 214.2, 179.1, 151.0	+	–	–	6
Myricetin 3-*O*-glucoside (**My-Glu**)	256, 266 (sh), 353	-/481.0/479.0	481.0 → **318.7** 479.0 → **315.9**, 287.1, 270.7, 258.7, 242.2	–	+	–	1,2
Myricetin 3-*O*-(6″-*O*-malonyl)-galactoside (**My-maGal**)	257, 266 (sh), 356	-/567.0/565.0	567.0 → **319.1** 565.0 → **315.7**, 287.1, 270.7, 259.5, 242.2, 178.7	–	+	–	1,2
Myricetin 3-*O*-arabinoside (**My-Ara**)	266, 355	473.0/451.0/449.1	449.1 → 316.0, 287.0, 271.0, 214.5, 151.0	–	+	–	1,2
Myricetin-pentoside 1 (**My-Pent 1**)	270, 346	473.0/451.0/449.1	451.0 → **319.0** 449.1 → **316.0**, 287.3, 271.0, 259.4, 241.7, 214.2, 179.4, 151.2	+	–	–	6,11
Myricetin-pentoside 2 (**My-Pent 2**)	260, 350	473.0/451.0/449.1	451.0 → **319.4**	+	–	–	6,11
Myricetin (**My**)	252, 370	-/319.0/317.2	317.2 → 271.2, 179.0, 151.15	+	+	–	1,2,5
Quercetin 3-*O*-galactoside (**Qu-Gal**)	260, 350	-/465.0/463.1	465.0 → **303.3** 463.1 → **300.1**, 271.1, 255.0, 243.2, 151.2	+	+	–	1,4–6,8,11
Quercetin 3-*O*-glucoside (**Qu-Glu**)	257, 354	487.0/465.0/463.1	465.0 → **303.3** 463.1 → **300.0**, 271.1, 255.1, 243.0, 151.3	+	+	+	1,2,4,6,8,11,12

(*continued on next page*)

Table 1 (*continued*)

Tentative identification (abbreviation)	UV $\lambda_{typical}$ (*nm*)	$[M+Na]^+/[M+H]^+/[M-H]^-$ or other ions (*m/z*)	MS^2 (*m/z*)	Presence in			Literature
				BB	BC	SB	
Quercetin 3-*O*-glucuronide (**Qu-Gluc**)	260, 346	-/479.0/477.1	479.0 → **303.1** 477.1 → **301.1**, 271.2, 255.1, 151.3	+	–	+	5,9–11
Quercetin 3-*O*-(6″-*O*-malonyl)-glucoside (**Qu-maGlu**)	256, 266 (sh), 353	573.0/551.0/549.1	551.0 → **303.2** 549.1 → 505.1, **300.0**, 271.1, 255.1, 151.0	+	+	–	1,2,6
Quercetin-acetyl-hexoside 1 (**Qu-acHex 1**)	255, 351	529.0/507.1/505.1	507.1 → **303.2** 505.1 → **300.0**, 271.0, 255.0, 243.0	+	–	–	6,11
Quercetin-acetyl-hexoside 2 (**Qu-acHex 2**)	256, 355	529.0/507.0/505.1	507.0 → **303.0** 505.1 → **300.1**, 271.1, 255.2, 243.0, 151.4	+	–	–	6,11
Quercetin-coumaroyl-hexoside (**Qu-coHex**)	259, 355	-/611.1/609.1	611.1 → **303.4** 609.1 → 462.5, **300.2**, 271.1, 255.2, 150.9	+	–	–	
Quercetin 3-*O*-rhamnoside (**Qu-Rha**)	257, 346	471.0/449.0/447.1	449.0 → **303.0** 447.1 → **300.0**, 271.1, 255.1, 243.2, 151.2	+	–	–	6,11
Quercetin 3-*O*-xyloside (**Qu-Xyl**)	266, 353	457.0/435.0/433.1	435.0 → **303.1** 433.1 → **300.0**, 271.1, 255.1, 243.0, 151.2	+	–	–	11 phenol-explorer
Quercetin 3-*O*-arabinoside (**Qu-Ara**)	258, 353	457.0/435.0/433.2	435.0 → **303.2** 433.2 → **300.0**, 271.0, 255.1, 234.3	+	+	–	1,4,11
Quercetin-pentoside (**Qu-Pent**)	257, 352	457.00/435.3/433.1	435.3 → 303.0	+	–	–	6,11
Quercetin (**Qu**)	255, 366	-/303.0/301.2		+	+	–	1,2,4–6,11
Laricitrin 3-*O*-galactoside (**La-Gal**)	254, 356	517.0/495.1/493.1	495.1 → **333.1** 493.1 → **330.1**, 315.1, 286.9, 271.1, 258.7, 243.1, 151.1	+	–	–	6,11
Laricitrin 3-*O*-glucoside (**La-Glu**)	260, 346	517.0/495.1/493.1	495.1 → **333.0** 493.1 → **330.0**, 314.8, 287.1, 270.9, 259.2, 243.2, 151.5	+	–	–	6,11
Laricitrin-acetyl-hexoside (**La-acHex**)	260, 346	559.0/537.0/535.1	537.0 → **333.2** 535.1 → **330.2**, 314.6, 286.8, 270.9, 259.3, 151.20	+	–	–	6
Syringetin 3-*O*-galactoside (**Sy-Gal**)	261, 345	531.0/509.0/507.1	509.0 → **347.0** 507.1 → **344.0**, 329.1, 314.9, 300.9, 286.2, 272.9, 270.0, 258.0, 242.2, 151.5	+	–	–	6,11
Syringetin 3-*O*-glucoside (**Sy-Glu**)	260, 345	531.1/509.0/507.1	509.0 → **347.0** 507.1 → **344.0**, 329.1, 315.0, 301.0, 286.2, 273.1, 270.2, 257.9, 242.2, 151.5	+	–	–	4–6,11
Syringetin-acetyl-hexoside (**Sy-acHex**)	269, 350	573.0/551.0/549.1	551.0 → **347.1** 549.1 → **344.1**, 328.9, 315.2, 301.0, 287.4, 273.1, 269.8, 257.8, 242.1	+	–	–	11
Syringetin 3-*O*-rhamnoside (**Sy-Rha**)	260, 346	515.1/493.0/491.1	493.0 → **347.1** 491.1 → **344.0**, 329.0, 286.9, 272.7	+	–	–	11
Syringetin-pentoside (**Sy-Pent**)	265, 345	501.0/479.0/477.1	479.0 → **347.1** 477.1 → **344.1**, 329.4, 315.2, 301.1, 286.0, 273.2, 258.0, 242.2, 151.6	+	–	–	6,11
Syringetin (**Sy**)	265, 368	-/347.0/345.2		+	–	–	11
Kaempferol 3-*O*-rutinoside (**Ka-Rut**)	266, 353	-/595.1/593.1	593.1 → **284.1**	–	+	–	1,2
Kaempferol 3-*O*-galactoside (**Ka-Gal**)	265, 346	471.0/449.0/447.1	449.0 → **287.1** 447.0 → **284.1**, 255.2, 227.1	–	+	+	1,2,8,12
Kaempferol 3-*O*-glucuronide (**Ka-Gluc**)	265, 346	485.0/463.0/461.0	463.0 → **287.0** 461.0 → **285.0**, 255.0	–	–	+	8,10,12
Kaempferol-hexoside 1 (**Ka-Hex 1**)	265, 346	-/449.1/447.1	449.0 → **287.0**	–	+	–	8
Kaempferol-hexoside 2 (**Ka-Hex 2**)	263, 346	471.1/449.0/447.1	449.0 → **287.1** 447.0 → **284.0**, 255.0, 227.2	–	+	–	8
Kaempferol 3-*O*-(6″-*O*-malonyl)-glucoside (**Ka-maGlu**)	266, 346	557.0/535.0/533.1	535.0 → **287.1** 533.1 → **284.1**, 255.2, 227.0	–	+	+	1,9,10
Kaempferol-acetyl-hexoside (**Ka-acHex**)	267, 351	513.1/491.1/489.1	491.1 → **287.1** 489.1 → **284.1**, 255.2, 227.1	–	–	+	8,12
Kaempferol-pentoside (**Ka-Pent**)	265, 345	441.0/419.0/417.1	419.0 → **287.0** 417.1 → **284.0**, 255.0, 226.9	+	–	–	6
Kaempferol (**Ka**)	265, 368	-/287.1/285.1		+	–	–	5
Isorhamnetin 3-*O*-glucoside (**Is-Glu**)	265, 346	501.0/479.0/477.1	479.0 → **317.1** 477.1 → **314.1**, 299.1, 285.0, 271.1, 257.1, 243.0, 226.7	–	+	–	1
Isorhamnetin 3-*O*-glucuronide (**Is-Gluc**)	265, 346	515.1/493.1/491.1	493.1 → **317.0** 491.1 → **315.1**, 300.0, 270.6, 254.7, 243.1	–	–	+	8,12
Isorhamnetin 3-*O*-(6″-*O*-malonyl)-galactoside (**Is-maGal**)	255, 265 (sh), 345	587.0/565.0/563.1	565.0 → **317.0** 563.1 → 519.1, **314.1**, 299.2, 285.1, 271.0, 256.6, 243.1	–	+	–	1
Flavan-3-ols							
(+)-Catechin (**Cat**)	280	-/291.1/289.2		+	–	+	1,2,4–6,8–10,12
(−)-Epicatechin (**ECat**)	280	-/291.1/289.2		+	–	–	1,2,4–6
Hydroxycinnamic acid derivatives							
5-*O*-Cafferoylquinic acid (**5-CaQA**)	295 (sh), 328	377.1/355.1/353.2	355.1 → 163.3, 145.1 353.2 → 191.2, 135.3	+	+	–	1,6
3-*O*-Cafferoylquinic acid (**3-CaQA**)	295 (sh), 323	377.0/355.1/353.2	355.1 → 163.3, 145.1 353.2 → 191.2, 135.3	+	–	–	4–6,11

(*continued on next page*)

Table 1 (*continued*)

Tentative identification (abbreviation)	UV $\lambda_{typical}$ (*nm*)	$[M+Na]^+/[M+H]^+/[M-H]^-$ or other ions (*m/z*)	MS^2 (*m/z*)	Presence in			Literature
				BB	BC	SB	
Cafferoyl-glucose (**Ca-Glu**)	290 (sh), 327	365.0/-/341.2	341.2 → **179.2**, 161.3, 133.3	–	+	–	1,2,6
Caffeic acid (**CaA**)	290 (sh), 327	-/181.1/179.3		+	+	+	2,4–6
Caffeic acid derivative 1 (**Ca der1**)	290 (sh), 326	359.1/337.0/335.2	337.0 → 163.2, 144.9 335.2 → 179.2, 161.3, 135.3	+	–	–	6
Caffeic acid derivative 2 (**Ca der2**)	290 (sh), 325	391.1/369.1/367.2	369.1 → 163.25, 145.2 367.2 → 179.2, 161.3, 135.3	+	–	–	6
Caffeoyl-coumaroylquinic acid (**CaCoQA**)	290 (sh), 316	523.0/501.1/499.1	499.1 → 191.4, 173.4, 163.4, 161.1, 155.1135.2	+	–	–	13
Coumaroyloxymethylene-glucopyranosyloxy-butenenitrile (**Co-meGlu-B**)	313	444.0/422.1/420.2	444.0 → 260.1 420.2 → 163.4, 145.4, 119.4	–	+	–	1,2
p-Coumaroyl-glucose (**Co-Glu**)	290 (sh), 323	349.0/-/325.3	325.3 → **163.3**, 145.2	–	+	+	1,2,6,9,10
p-Coumaroyl-hexose (**Co-Hex**)	290 (sh), 323	349.0/-/325.3	325.3 → **163.3**, 145.2, 117.3	–	–	+	6,8–10,12
p-Coumaric acid (**CoA**)	295 (sh), 323	183.05/165.2/163.4		+	+	+	2,5,12
Cinnamoyl-glucose (**Ci-Glu**)	284	333.1/311.1/309.3	333.1 → 185.2, 171.4	–	–	+	8,10
Feruloyloxymethylene-glucopyranosyloxy-butenenitrile (**Fe-meGlu-B**)	328	474.0/452.1/450.2	474.0 → 290.1 450.2 → 193.1, 160.0, 151.6, 149.0, 134.3	–	+	–	1,2
Hydroxybenzoic acid derivatives							
Ellagic acid-deoxyhexose (**El-Deox**)	282, 371	-/449.1/447.1	449.1 → **303.0**, 286.9 447.1 → **300.0**	–	–	+	8–10,12
Ellagic acid-rhamnose (**El-Rha**)	282, 365	-/449.1/447.1	449.1 → **303.0**, 286.9 447.1 → **300.0**	–	–	+	12
Ellagic acid (**ElA**)	252, 368	-/303.0/301.2		–	–	+	9,10,12
others							
unknown phenolic acid 1 (**unknown 1**)	313	-/325.1/323.3	325.1 → 147.1, 119.2 323.3 → 145.2, 117.3	–	+	–	
unknown phenolic acid 2 (**unknown 2**)	311	-/325.1/323.2	325.1 → 147.1, 119.2 323.2 → 145.3, 117.4	–	+	–	
unknown phenolic acid 3 (**unknown 3**)	269, 300	343.0/321.0/319.2	319.2 → 183.3, 139.1, 115.5, 109.2	+	–	–	

[a] The literatures in this Table include: (1) Tian, Y., *et al.* https://doi.org/10.1021/acs.jafc.9b00033; (2) Kelanne, N., *et al.* https://doi.org/10.1021/acs.jafc.0c03354; (3) Tian, Y., *et al.* https://doi.org/10.1016/j.foodchem.2016.09.145; (4) Grace, M. H., *et al.* https://doi.org/10.1016/J.FOODCHEM.2018.10.101; (5) Pico, J., *et al.* https://doi.org/10.1016/J.JFCA.2022.104412; (6) Ancillotti, C., *et al.* https://doi.org/10.1007/s00216-016-0067-y; (7) Nie, Q., *et al.* https://doi.org/10.1002/jsfa.7885; (8) Spínola, V., *et al.* https://doi.org/10.1016/J.FOODCHEM.2014.09.163; (9) Aaby, K., *et al.* https://doi.org/10.1021/jf0702592; (10) Aaby, K., *et al.* https://doi.org/10.1016/j.foodchem.2011.10.037; (11) Becker Pertuzatti, P., *et al.* https://doi.org/10.1016/J.FOODCHEM.2020.127958; (12) Antonia, M. A., *et al.* https://doi.org/10.1021/jf506076n; (13) Clifford, M. N., *et al.* https://doi.org/10.1021/JF060536P; and Phenol-Explorer database, 2015, http://phenol-explorer.eu/.

flavan-3-ol ((+)-catechin) was not detected after 56 days. The content of hydroxybenzoic acids in SB was increased by 2 folds in ASLT. In contrast, the changing trends of other phenolic groups in BB and BC were opposite to the trends in SB. In BB, the contents of flavonols, flavan-3-ol and hydroxycinnamic acids after 365-day storage were increased by 1.5–1.9 folds compared to 0 day. An increase in both flavonols and hydroxycinnamic acids (2.2 and 2 folds, respectively) was also observed in BC.

In our study, an ASLT of 28 and 56 days was planned to simulate 6- and 12-month of RT, respectively. However, the total anthocyanin contents after 6- and 12-months of RT were 1.2–2 folds higher than that under the corresponding accelerated storage situation at 40 °C (Fig. 2A–C). The results were consistant with the previous study, in which the reductions of total anthocyanins at high temperatures of 60 °C and 80 °C (60–85 % degradation during 3-day storage) were significantly faster than that at 25 °C (3 % degradation for 14 days) (Fracassetti et al., 2013). Additionally, anthocyanins in different groups have shown varying degradation rates (46.5–90.0 %) in ASLT and room-temperature (2.7–33.4 %) during 12-month storage. This is attributed to the different number and position of hydroxyl and methoxy groups as well as sugar moieties linked to the anthocyanin aglycones (Liu et al., 2018).

For other phenolic compounds, the total contents in SB after 12-month storage at 23 °C were 1.9 folds higher than that after 56 days at 40 °C (Fig. 2D). On the contrary, other phenolic contents in BB and BC at room-temperature storage were 17.4 % and 25.4 %, respectively; which were lower than those in the ASLT (Fig. 2E and F). As the major flavan-3-ols identified in SB, (+)-catechin degraded significantly faster in 56-day storage at 40 °C than in the corresponding 12-month storage at 23 °C. Yet, the opposite degradation performance of flavan-3-ols (99.3 % (+)-catechin and 0.7 % (−)-epicatechin) was observed in BB, showing 6.1-folds higher content in 56-day storage at 40 °C than that in 12-month storage at 23 °C. The mechanism behind the different degradation pattern of flavan-3-ols is still unclear due to the complexity of phenolic compounds in the studied berry slices and the unveiled conversion pathway among them. One of the possible reasons is that the type and amount of added sugars (especially as sucrose and fructose) in food matrices could affect the degradation of polyphenols in berry products (Hanuka Katz et al., 2020). The effect of sugars on polyphenol degradation was due to a combination of several mechanisms, including decreased oxygen solubility, chelation of transition metal ions, and scavenging of reactive oxygen species (Hanuka Katz et al., 2020). Interestingly, this effect was also influenced by the berry variety. For example, by adding powdered sugar to SB, the content of catechin in the cultivar ELKAT (41.1 mg/kg, fresh weight) was higher than that in the cultivar *Senga sengana* (22.2 mg/kg, fresh weight) after 6-month frozen storage (Oszmiański et al., 2009). Therefore, the sugar syrup added to berry mueslis in this study might be one of the reasons that led to different degradation pattern of catechin.

3.3. Changes in individual phenolic compounds during storage

A heatmap was used to reveal the content changes in individual phenolic compounds in berry slices during storage (Fig. 3A–C). In SB, all the anthocyanins and most of the other phenolic compounds degraded

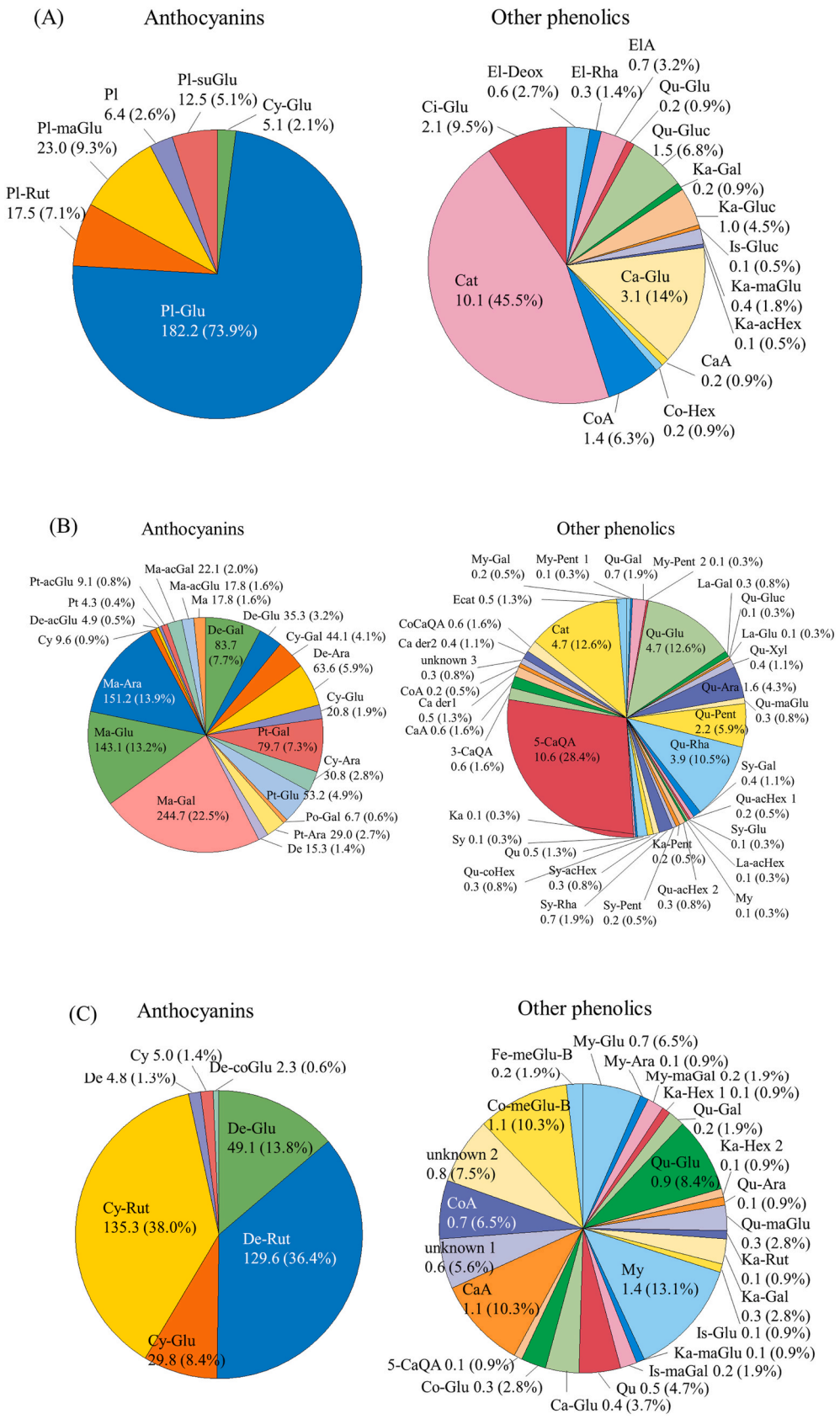

Fig. 1. Relative profiles of anthocyanins and other phenolic compounds of strawberry (A), blueberry (B) and blackcurrant (C) mueslis in Day 0. Each compound was presented as an abbreviation of compound name with compound content in the unit of mg/100 g DW (percentage of compound in total anthocyanin or total other phenolics).

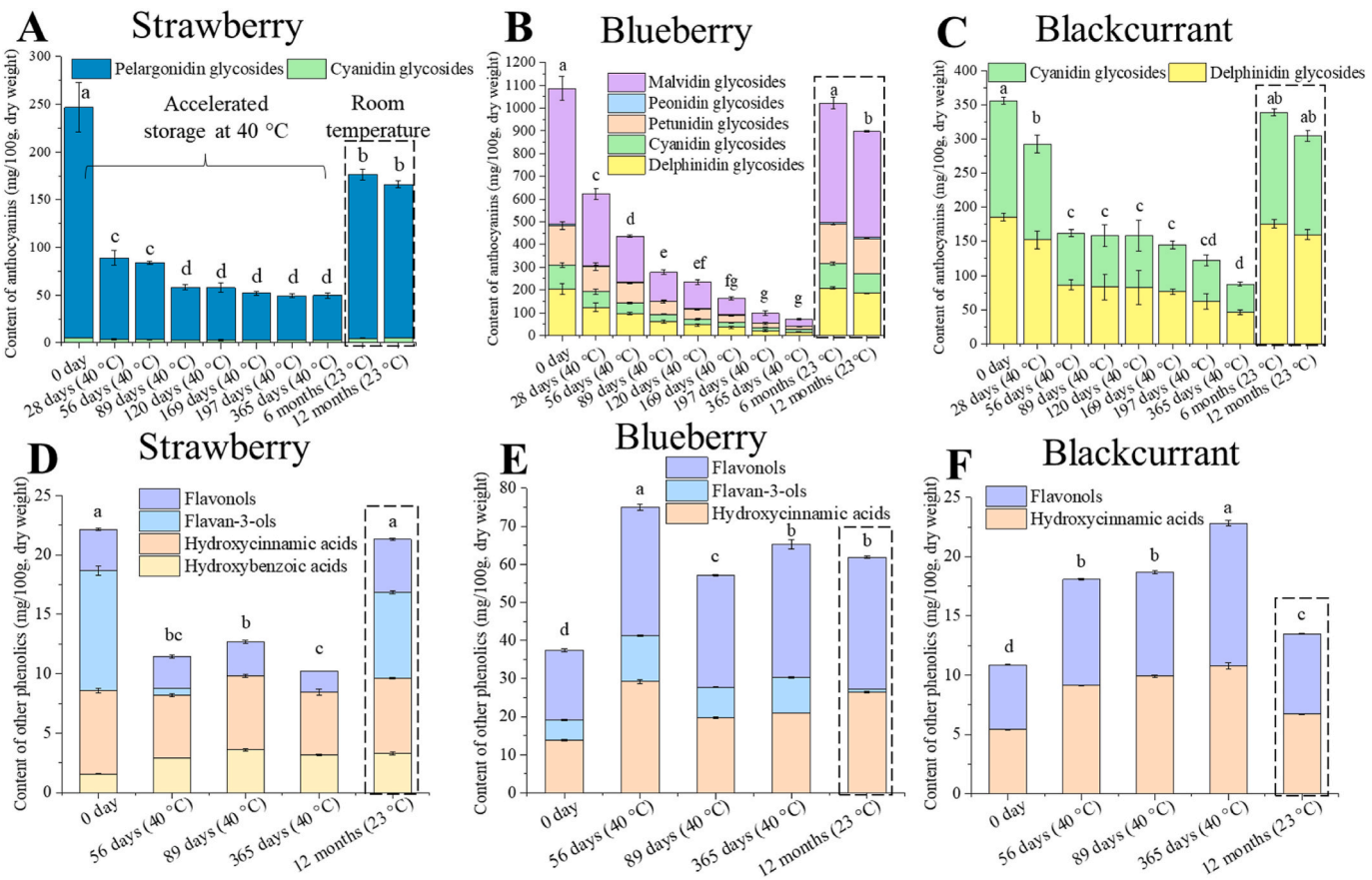

Fig. 2. Changes in the contents of anthocyanins (A–C) and other phenolic compound groups (D–F) in the studied berry slices during ASLT and room-temperature storage (room-temperature storage shown as the columns with dashed lines). Lowercase letters indicate statistical significance (Tukey' test), which was set at $p < 0.05$.

along with time in ASLT (Fig. 3A). Other phenolic compounds with significant increasing contents included isorhamnetin 3-*O*-glucuronide, kaempferol-acetyl-hexoside, ellagic acid, and *p*-coumaric acid.

In the hierarchical cluster analysis of BB, anthocyanins were separated from the categories of other phenolics. At the bottom part of the heatmap (Fig. 3B), individual anthocyanins degraded along with ASLT time. The contents of most flavonols were increased in ASLT except for quercetin 3-*O*-glucuronide, the content of which was slightly decreased. For most of the hydroxycinnamic acids, their contents were slightly increased while the contents of 3-*O*-caffeoylquinic acid and caffeic acid showed no significant difference between 0 day and 365 days.

In BC, individual anthocyanin contents were declined in the ASLT (Fig. 3C). Most flavonols and hydroxycinnamic acids were accumulated along with storage time. The exceptions included myricetin 3-*O*-(6″-*O*-malonyl)-galactoside, quercetin 3-*O*-(6″-*O*-malonyl)-glucoside, and coumaroyloxymethylene-glucopyranosyloxy-butenenitrile, the contents of which showed no significant change.

In ASLT, the degradations of the dominant anthocyanins in SB (pelargonidin 3-*O*-glucoside, 73.8 %) and BC (delphinidin 3-*O*-rutinoside, 36.4 %; and cyanidin 3-*O*-rutinoside, 38.0 %) were in proportion to the total anthocyanin content, while malvidin 3-*O*-galactoside (22.5 %) and malvidin 3-*O*-arabinoside (13.9 %) in BB had a larger decline compared to the total anthocyanins. As a result, elevating temperature to 40 °C affected individual phenolic compounds differently.

The degradation rates of anthocyanins in berry slices were also compared during 56-day storage in ASLT to the corresponding 12-month storage at 23 °C. In SB and BB, most of the individual anthocyanins and other phenolics showed higher contents in 12-month storage at 23 °C than 56-day storage at 40 °C. In BC, similar results were observed in individual anthocyanins. However, more individual flavonols and hydroxycinnamic acids in BC were detected in 56-day storage at 40 °C than in 12-month storage at 23 °C. On the other hand, some other phenolic compounds were well-fit in the accelerated storage model, showing no significant or slight difference between real time storage and corresponding simulated storage. The accelerated storage model can be applied for specific compounds such as caffeic acid and ellagic acid in SB, myricetin 3-*O*-galactoside, myricetin pentoside isomer 2 and quercetin-acetyl-hexoside isomer 2 in BB, and quercetin 3-*O*-(6″-*O*-malonyl)-glucoside, kaempferol 3-*O*-(6″-*O*-malonyl)-glucoside and kaempferol hexoside 1 in BC.

3.4. Correlation among the studied phenolic compounds

The correlation of phenolic change in berry slices in ASLT are shown in Fig. 4A–C. Generally, anthocyanins in all studied berry slices showed negative correlation to other phenolic compounds in our study. In SB, both hydroxycinnamic acids and hydroxybenzoic acids showed stronger negative correlations with anthocyanins compared to flavonols (Fig. 4A). Flavan-3-ol ((+)-catechin) positively correlated to anthocyanins and other phenolics, except ellagic acid-rhamnose, kaempferol-acetyl-hexoside, and *p*-coumaroyl-hexose. Previous study demonstrated that during degradation, sugar moieties were removed from anthocyanins (from C-ring), generating aglycones (Sadilova et al., 2006). Aglycones were further broken into smaller phenolic compounds by removing hydroxyl, methyl, and other functional groups and by aromatic rings cleavage. Phenolic acids are common degradation products from the cleavage of B-ring. Consisting with these previous findings, in our study, pelargonidin 3-*O*-glucoside was negatively correlated to two

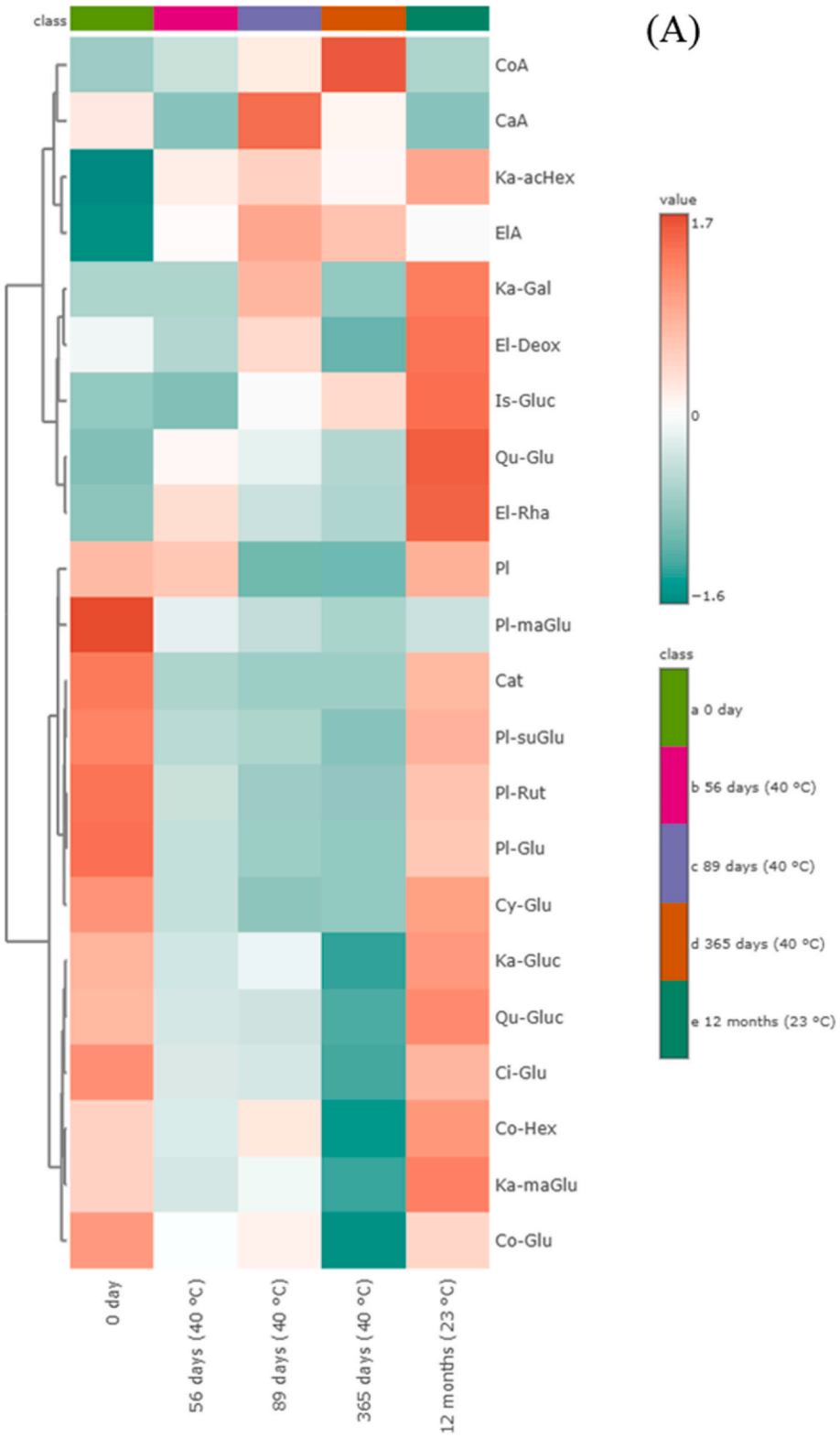

Fig. 3. Relative changes in contents of individual phenolic compounds in strawberry (A), blueberry (B) and blackcurrant (C) mueslis during ASLT and room-temperature storage. In the heatmaps, grids with a color-scale from red to white to green represent the data values from high to medium to low.

phenolic acids (*p*-coumaric acid and ellagic acid) in the studied SB, indicating the possible conversion pathway (Sadilova et al., 2006). The negative correlation between kaempferol 3-*O*-(6″-*O*-malonyl)-glucoside and kaempferol-acetyl-hexoside was probably due to the loss of carbon dioxide from flavonoid malonyl-glycosides and generating corresponding flavonoid acetyl-glycosides as mentioned in Horowitz and Asen's research (Horowitz & Asent, 1989).

In BB, flavonols showed stronger negative correlations with

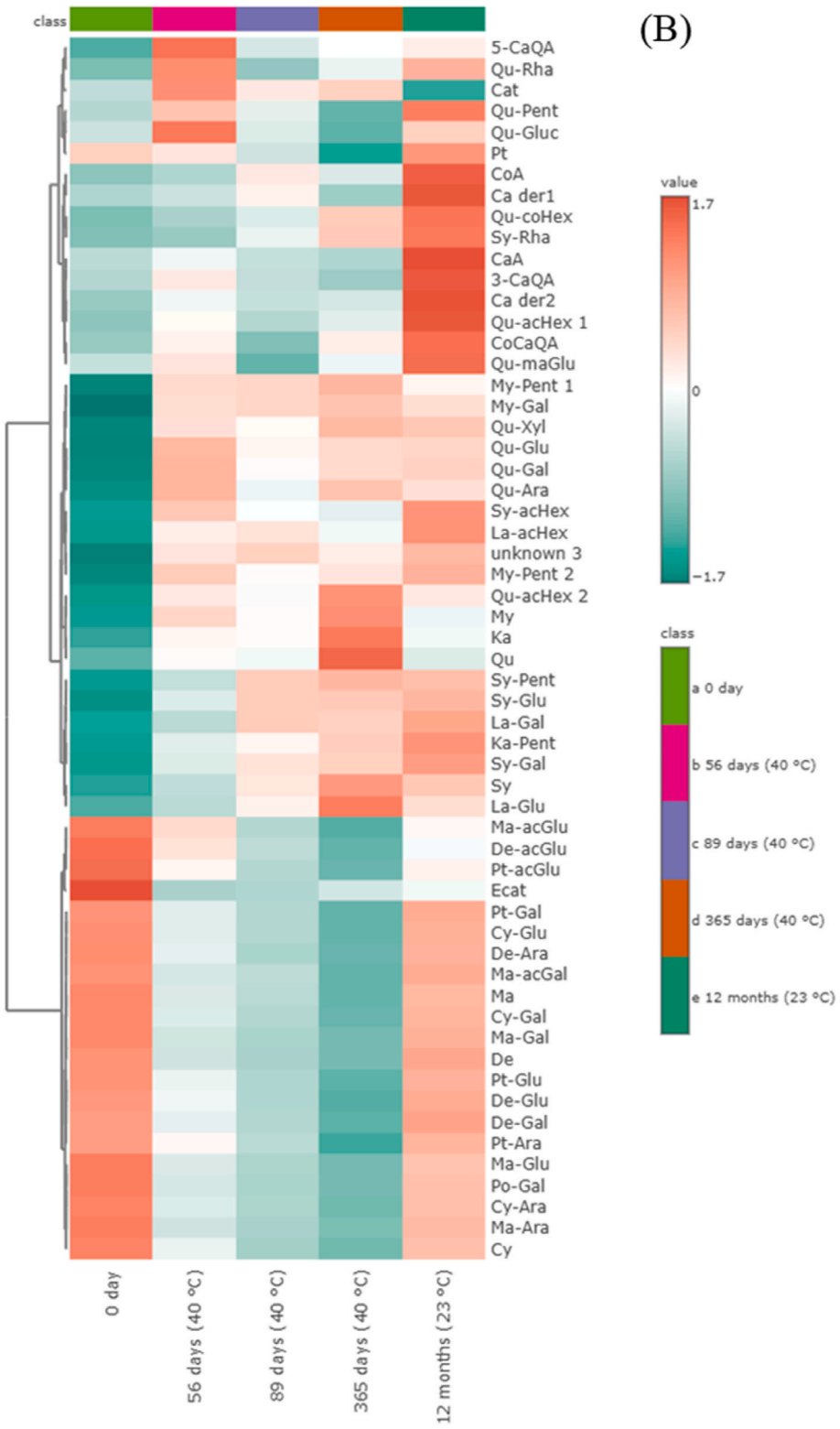

Fig. 3. (*continued*).

anthocyanins than hydroxycinnamic acids did (Fig. 4B). Consisting with the negative correlations observed in this study, quercetin glycosides were probably the degradation metabolites of cyanidin glycosides as stated in the research of Chen et al. (Chen et al., 2020). According to another study, cyanidin 3-*O*-glucoside might degrade into caffeoylquinic acid, which was consistent with the negative correlation between them in our study (Chen et al., 2020). Interestingly, (−)-epicatechin (flavan-3-ols) positively correlated to anthocyanins and negatively correlated to other phenolics. (+)-Catechin, the other flavan-3-ols in BB, correlated with anthocyanins and other phenolics in an opposite way as (−)-epicatechin did. The correlation between (+)-catechin and (−)-epicatechin was negative. The epimerization could contribute to the increase content of catechin and epicatechin degradation (Lončarić et al., 2018). In BC, anthocyanins negatively correlated to flavonols and

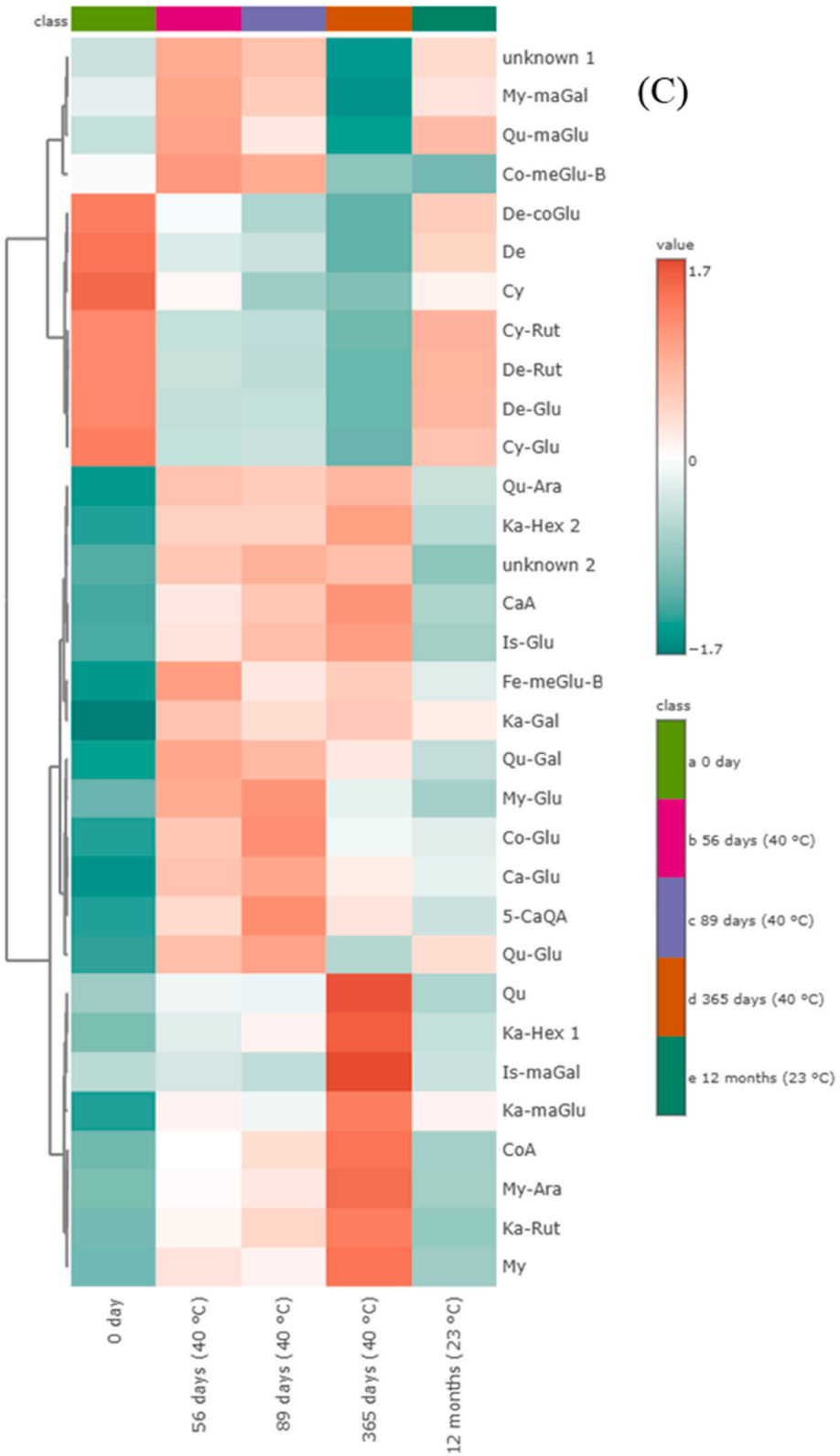

Fig. 3. (*continued*).

hydroxycinnamic acids (Fig. 4C). Flavonols positively correlated to hydroxycinnamic acids in general.

3.5. Degradation kinetics of anthocyanins in ASLT

Anthocyanins with various structures showed different degradation rates in ASLT and the rate constants were represented by the *k* values. As shown in Table 2, pelargonidin derivatives (*k* of 0.0147) degraded much faster than cyanidins (*k* of 0.0020) in SB. In BB, the degradation rate of compounds followed the descending order of malvidins (*k* of 0.0167), delphinidins (*k* of 0.0123), cyanidins (*k* of 0.0117), petunidins (*k* of 0.0115), and peonidins (*k* of 0.0115). Delphinidins (*k* of 0.0056)

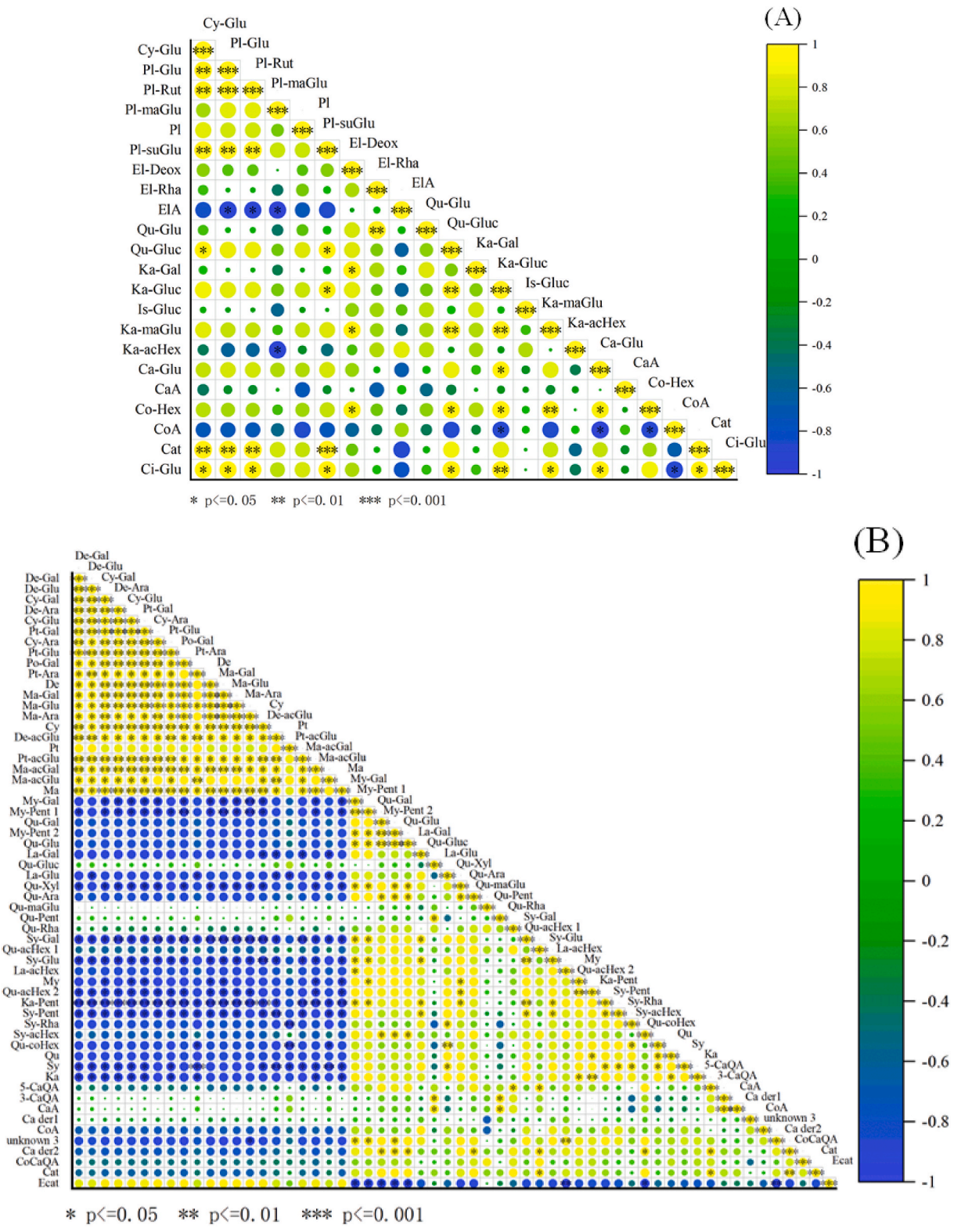

Fig. 4. Correlations between contents of individual phenolic compounds in strawberry (A), blueberry (B) and blackcurrant (C) mueslis in ASLT. In the correlation heatmaps, yellow and blue indicate positive and negative correlations, respectively. The correlation value is depicted as a size of the circle.

degraded faster than cyanidins (*k* of 0.0055) in BC. The highest *k* value of malvidins showed the highest degradation rate, indicating the substitution of hydroxyl groups of the B ring by the methoxy groups decreased the stability of anthocyanins during storage at 40 °C. This result was consisting with the study of Fleschhut et al., in which the stability of commercial standards of malvidin, cyanidin, delphinidin, pelargonidin, peonidin and their glycosides were monitored up to 5 h at 37 °C (Fleschhut et al., 2006). However, in another research of the stability of anthocyanins in red wine (pH was adjusted to 1.5), malvidin 3-*O*-glucoside was more stable than the 3-*O*-glucosides of delphinidin

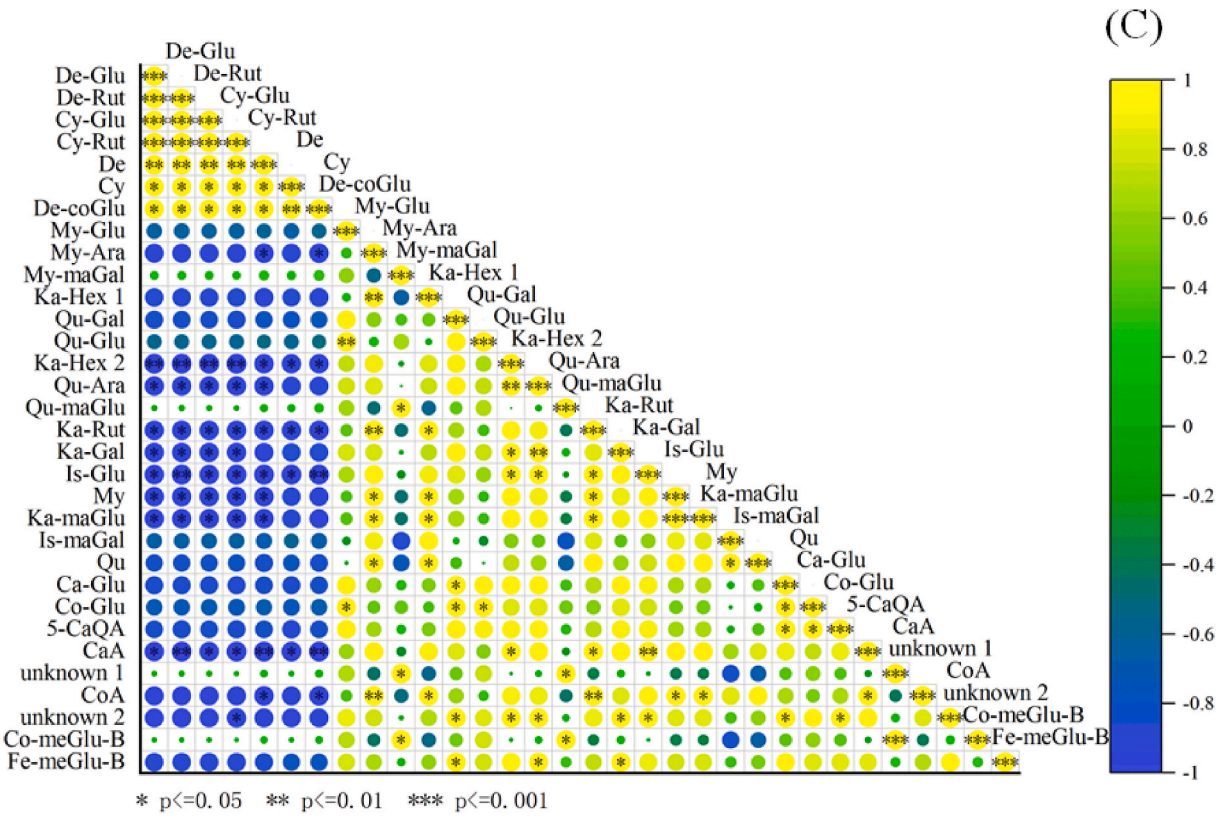

Fig. 4. (*continued*).

and petunidin during simulated digestion process (Liu et al., 2018). Since digestive enzymes were excluded in that research, the reason of different degradation rates of malvidins might be attributed to the food matrix effect and environment pH (Hanuka Katz et al., 2020). Besides, the faster degradation rate of delphinidin derivatives than cyanidin derivatives was explained as more hydroxyl groups in the B ring of delphinidins decreasing their stability (Dobson et al., 2017). In addition to different types of aglycones, the rate of degradation is highly dependent on the attached position and number of sugar moiety and acylated glycosyl groups on the anthocyanidins. In BB, the *k* values of delphinidins, cyanidins, and malvidins followed the increasing order of glucoside > galactoside > arabinoside from the most to the least stable. The results were in accordance with the findings of previous research, in which the storage stability of 15 anthocyanins in a BB product was compared (Trošt et al., 2008). Anthocyanins with hexose as sugar moiety (*e.g.*, glucose and galactose) exhibited higher stability than those attached with pentose (*e.g.*, arabinose). It is likely due to an increased steric hindrance caused by the larger structures of sugars (Fracassetti et al., 2013). In SB, pelargonidin 3-*O*-rutinoside (*k* of 0.0041) showed better stability than pelargonidin 3-*O*-glucoside (*k* of 0.0267). On the contrary, in BC, delphinidin 3-*O*-rutinoside and cyanidin 3-*O*-rutinoside had higher *k* values than their glucosides, respectively.

Acylation increased the *k* values of glycosylated anthocyanins in all our studied berries, indicating that acylation enhanced the resistance of anthocyanins to storage degradation. The stability of acylated anthocyanins is increased due to the intramolecular folding and creation of a steric hindrance by acyl groups (Zhao et al., 2017). Additionally, the stability of anthocyanins may also be influenced by the nature of acylated groups (Zhao et al., 2017). For example, in SB, pelargonidin 3-*O*-(6″-succinyl)-glucoside showed lower *k* value (0.0018) than pelargonidin 3-*O*-(6″-malonyl)-glucoside (*k* of 0.0066), indicating that the succinyl group might possess a stronger ability to stabilize pelargonidin than the malonyl group.

Moreover, the first-order kinetic model was used to describe the temperature-dependent degradation of anthocyanins in different berry samples (Table 2). The values of the determination coefficient (R^2) showed the fit between experimental values and first-order reaction. In the ASLT, most of the anthocyanins in BB had high R^2 values, ranging from 0.9064 to 0.9829. Lower values of R^2 were found among the anthocyanins in SB (0.4913–0.7146) and BC (0.8025–0.8190). As a result, compared to SB and BC, the ASLT model might be more suitable to assess the degradation rate of anthocyanins in BB.

3.6. Estimation of ASLT storage time based on Q_{10} factor

To conduct an ASLT test on berry-rich mueslis, an acceleration factor Q_{10} of 3 (a common setting for almost all food products) was selected (Choi et al., 2017). However, the comparison of test results at 23 °C and 40 °C in our study showed that the concentrations of anthocyanins declined significantly faster at higher temperatures, with equivalent changes to those observed occurring within just 8–18 days (Table 2). Therefore, to accurately reflect the changes in anthocyanin contents of berry-rich mueslis at room temperature, the acceleration factor Q_{10} must be significantly higher. Knowing the estimated storage time at 40 °C reflecting the changes at 23 °C for 365 days, corresponding Q_{10} values for total and dominant anthocyanins in each berry muesli were calculated (Table 3). Since SB showed the largest decline in anthocyanin content in RT (32.8 %) but also required the longest time for accelerated degradation (18 days), the Q_{10} value used to accelerate total anthocyanin degradation in SB was the lowest among the berries ($Q_{10} = 6$). In contrast, as BC had the smallest decline in anthocyanin content yet required the shortest time to reach equivalent degradation under accelerated conditions, the acceleration factor for conducting ASLT on BC is the highest ($Q_{10} = 9$). When assessing the quality of berry products under accelerated conditions based on the decline in specific anthocyanin concentrations, it is important to consider that different acceleration factors apply to them. For example, the accelerated degradation of total anthocyanin content in BB was described by $Q_{10} = 8$, but for

Table 2
Modelling and estimating the degradation of total and individual anthocyanins during storage at 40 °C using the first order kinetics.

Compound	k (day^{-1}) ± standard deviation	R^2	$t_{1/2}$ (days)	Estimated storage time (days) in ASLT[a]
SB				
Total anthocyanins	0.0139 ± 0.0044	0.7035	50	18
Total cyanidins (aglycones and glycosides)	0.0020 ± 0.0004	0.4913	350	–
Total pelargonidins (aglycones and glycosides)	0.0147 ± 0.0046	0.7146	47	18
Cyanidin 3-*O*-glucoside	0.0020 ± 0.0008	0.4913	350	–
Pelargonidin 3-*O*-glucoside	0.0267 ± 0.0067	0.8641	26	13
Pelargonidin 3-*O*-rutinoside	0.0041 ± 0.0018	0.4754	170	–
Pelargonidin 3-*O*-(6″-malonyl)-glucoside	0.0066 ± 0.0027	0.5220	105	112
Pelargonidin (the aglycone)	0.0001 ± 0.0000	0.4665	4951	–
Pelargonidin 3-*O*-(6″-succinyl)-glucoside	0.0018 ± 0.0007	0.5086	385	–
BB				
Total anthocyanins	0.0142 ± 0.0014	0.9732	49	10
Total delphinidins (aglycones and glycosides)	0.0123 ± 0.0011	0.9790	56	3
Total cyanidins (aglycones and glycosides)	0.0117 ± 0.0013	0.9602	59	13
Total petunidins (aglycones and glycosides)	0.0115 ± 0.0009	0.9829	60	7
Total peonidins (aglycones and glycosides)	0.0115 ± 0.0020	0.9064	60	16
Total malvidins (aglycones and glycosides)	0.0167 ± 0.0018	0.9705	41	12
Delphinidin 3-*O*-galactoside	0.0124 ± 0.0009	0.9844	56	–
Delphinidin 3-*O*-glucoside	0.0106 ± 0.0011	0.9690	65	1
Cyanidin 3-*O*-galactoside	0.0127 ± 0.0012	0.9741	55	12
Delphinidin 3-*O*-arabinoside	0.0142 ± 0.0010	0.9881	49	9
Cyanidin 3-*O*-glucoside	0.0095 ± 0.0013	0.9374	73	5
Petunidin 3-*O*-galactoside	0.0132 ± 0.0010	0.9837	52	5
Cyanidin 3-*O*-arabinoside	0.0133 ± 0.0014	0.9675	52	17
Petunidin 3-*O*-glucoside	0.0120 ± 0.0012	0.9740	58	7
Peonidin 3-*O*-galactoside	0.0115 ± 0.0020	0.9064	60	16
Petunidin 3-*O*-arabinoside	0.0099 ± 0.0006	0.9884	70	10
Delphinidin (the aglycone)	0.0130 ± 0.0020	0.9281	53	2
Malvidin 3-*O*-galactoside	0.0176 ± 0.0020	0.9706	39	8
Malvidin 3-*O*-glucoside	0.0166 ± 0.0020	0.9633	42	16
Malvidin 3-*O*-arabinoside	0.0195 ± 0.0017	0.9834	36	14
Cyanidin (the aglycone)	0.0081 ± 0.0014	0.8863	86	17
Delphinidin 3-*O*-(6″-acetyl)-glucoside	0.0044 ± 0.0010	0.8206	157	78
Petunidin (the aglycone)	0.0046 ± 0.0007	0.8994	150	–
Petunidin 3-*O*-(6″-acetyl)-glucoside	0.0087 ± 0.0018	0.8681	80	51
Malvidin 3-*O*-(6″-acetyl)-galactoside	0.0102 ± 0.0017	0.9129	68	–
Malvidin 3-*O*-(6″-acetyl)-glucoside	0.0089 ± 0.0010	0.9639	78	65
Malvidin (the aglycone)	0.0103 ± 0.0018	0.9039	67	8
BC				
Total anthocyanins	0.0056 ± 0.0013	0.8115	125	8
Total delphinidins (aglycones and glycosides)	0.0056 ± 0.0013	0.8190	124	7
Total cyanidins (aglycones and glycosides)	0.0055 ± 0.0013	0.8025	126	8
Delphinidin 3-*O*-glucoside	0.0057 ± 0.0014	0.8025	121	10
Delphinidin 3-*O*-rutinoside	0.0063 ± 0.0013	0.8402	111	7
Cyanidin 3-*O*-glucoside	0.0056 ± 0.0013	0.8142	123	21
Cyanidin 3-*O*-rutinoside	0.0060 ± 0.0014	0.8118	116	6
Delphinidin (the aglycone)	0.0006 ± 0.0003	0.3451	1155	166
Cyanidin (the aglycone)	0.0006 ± 0.0004	0.3934	1155	223
Delphinidin 3-*O*-(6″-coumaroyl)-glucoside	0.0011 ± 0.0004	0.6395	619	39

[a] The estimated time in ASLT for 1 year storage at room temperature was calculated by fitting the anthocyanin contents of 12-month in RT using $C_t = C_0 \times e^{(-kt)}$, where C_t is anthocyanin contents at t days, C_0 is anthocyanin contents at 0 day and t is the storage time.

Table 3
The acceleration factor Q_{10} values of total and dominant anthocyanins in strawberry (SB), blueberry (BB) and blackcurrant (BC) mueslis [a].

Berry compounds	Q_{10}
SB	
Total anthocyanins	6
Pelargonidin 3-*O*-glucoside	7
BB	
Total anthocyanins	8
Malvidin 3-*O*-galactoside	9
Malvidin 3-*O*-glucoside	6
Malvidin 3-*O*-arabinoside	7
BC	
Total anthocyanins	9
Delphinidin 3-*O*-rutinoside	10
Cyanidin 3-*O*-rutinoside	11

[a] Q_{10} value is the number of times that the reaction rate changes with a 10 °C change in temperature.

different dominant malvidins, this value could vary between 6 and 9. The same consideration should be applied to the quantitatively dominant anthocyanins in BC. To the author's knowledge, no comparable scientific literature exists on the anthocyanin kinetic reactions of whole freeze-dried SB, BB or BC, or on berry-rich mueslis in general. However, some comparisons can be drawn from the limited available literature of

similar berries in different matrices. For example, Moldovan and co-authors studied the effect of storage temperature on the total phenolic content of Cornelian cherry fruits extracts (Moldovan et al., 2016). In contrast to our findings, the Q_{10} value was 1.87 in their study, representing storage temperature rise from 22 to 55 °C. Similarly, Fracassetti et al. studied the effect of time and storage temperature on anthocyanin degradation in wild BB powder (Fracassetti et al., 2013). They concluded that the Q_{10} value for the degradation of anthocyanins in wild BB powder stored at 42–52 °C was 1.98. These remarkably lower indicators may be due to shorter testing times in RT, showing little changes in the content of polyphenols and anthocyanins. In more detail, as the degradation of phenolic compounds is exponential, Q_{10} value depends on the storage duration. Besides, the half-life ($t_{1/2}$) values were also compared with the results in our study and previous studies. In the research of Moldovan et al., the half-life of polyphenols at 55 °C in cherry extracts was 17.8 days (Moldovan et al., 2016), whereas the half-life of total anthocyanins for SB and BB at 40 °C in our study was 50 and 49, respectively. Comparing to the half-time of freeze-dried BB powders in the study conducted by Fracassetti et al. (39 days), our study showed higher half-time of BB (49 days) (Fracassetti et al., 2013). The difference might have been due to the slight difference of temperatures in our study (40 °C) and previous study (42 °C). Beside the temperature, the type of tested products can also affect the half-time, suggesting that anthocyanins in the freeze-dried berry powders are more susceptible to temperature than that in the freeze-dried whole berries (Fracassetti et al., 2013).

Our study offers several notable strengths that contribute to practical application in food development. Employing LC-MS allows systematic analysis of phenolic compounds in commercial food products and reveals changes in the composition of these bioactive and sensory-relevant compounds. By linking degradation dynamics with structural features of phenolics (including sugar moiety and acylation patterns), our research provides mechanistic insights into degradation behavior, which is often overlooked in food stability studies. The degradation behavior of phenolic compounds underscores the importance of molecular structure in determining the shelf-life of bioactive compounds in complex food matrices. A comprehensive evaluation of phenolic compound stability in a real food matrix will offer food industries with the guidance of product development and shelf-life prediction.

While our findings provide valuable insights, some limitations should be acknowledged. The ASLT was conducted at a single elevated temperature (40 °C) in this study, although certain phenolic compounds (*e.g.*, anthocyanins) are known to be temperature-sensitive. This study aimed to accelerate the degradation sufficiently to observe meaningful changes within a practical timeframe, while avoiding excessive thermal stress that could lead to non-specific degradation or complete breakdown. Additionally, 40 °C can reflect the storage conditions in regions with higher ambient temperatures, which impact product storage during transportation or warehousing. The complexity of the muesli matrix may also influence the changes of phenolic compounds during storage. The interactions between berry phenolics and other components (*e.g.*, proteins, fibers, lipids and phenolics from grains) should be analyzed in future research.

4. Conclusions

In summary, this study systematically revealed phenolic profiles of three berry-rich food products and their changes during both ASLT and RT. Based on 90 phenolic compounds identified by LC-MS, our results suggested that the variation in phenolic profiles was highly dependent on their molecular structures and storage temperature. The degradation rates of anthocyanins were significantly higher at 40 °C than at 23 °C. The contents of other phenolic compounds fluctuated during ASLT with either increased (in BB and BC) or decreased (in SB) total contents at 365-day storage. Although anthocyanin degradation of BB fitted better in first-order kinetics, some compound contents had significant differences between ASLT storage time points and the corresponding room-temperature storage time points. This indicates that this accelerated storage model is compound-specific. The findings provide important guidance and serve as a useful reference for designing the shelf-life and assessing the quality of berry products during storage.

CRediT authorship contribution statement

Ying Zhou: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Kärt Saarniit:** Writing – review & editing, Writing – original draft, Visualization, Resources, Funding acquisition, Formal analysis, Conceptualization. **Mahsa Sadat Jafari:** Visualization, Formal analysis, Data curation. **Sirli Rosenvald:** Writing – original draft, Supervision. **Oskar Laaksonen:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization. **Ye Tian:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **Baoru Yang:** Writing – review & editing, Supervision, Resources, Funding acquisition.

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Declaration of competing interest

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Abbreviation used

Abbreviation used in the article are: **De**, Delphinidin; **Cy**, Cyanidin; **Pl**, Pelargonidin; **Pt**, Petunidin; **Po**, Peonidin; **Ma**, Malvidin; **My**, Myricetin; **Ka**, Kaempferol, **Qu**, Quercetin; **Is**, Isorhamnetin; **La**, Laricitrin; **Sy**, Syringetin; **Cat**, (+)-Catechin; **ECat**, (−)-Epicatechin; **CaA**, Caffeic acid; **Ca der**, Caffeic acid derivative; **CaQA**, Caffeoylquinic acid; **CoA**, Coumaric acid; **CaCoQA,** Caffeoyl-coumaroylquinic acid; **Co-meGlu-B**, Coumaroyloxymethylene-glucopyranosyloxy-butenenitrile; **Fe-meGlu-B**, Feruloyloxymethylene-glucopyranosyloxy-butenenitrile; **Ci**, Cinnamoyl; **ElA**, Ellagic acid; **Rut**, rutinoside; **Gal**, galactoside; **Glu**,

glucoside; **Co-Glu**, coumaroyl-glucoside; **Ca-Glu**, caffeoyl-glucoside; **acGlu**, acetyl-glucoside; **maGlu**, malonyl-glucoside; **suGlu**, succinyl-glucoside; **Ara**, arabinoside; **Gluc**, glucuronide; **Hex**, hexoside; **maHex**, malonyl-hexoside; **acHex**, aceyl-hexoside; **Deox**, deoxyhexose; and **Pent**, pentoside.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.118119.

Data availability

Data will be made available on request.

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Five key references (indicated by an * in front of the reference in the reference section)

1 Fleschhut, J., Kratzer, F., Rechkemmer, G., & Kulling, S. E. (2006). Stability and biotransformation of various dietary anthocyanins in vitro. *European Journal of Nutrition, 45*(1), 7–18. https://doi.org/10.1007/s00394-005-0557-8This research provided possible explanations for that malvidins showed the highest degradation rate, comparing to the other anthocyanins. The reason is probably because the substitution of hydroxyl groups of the B ring by the methoxy groups decreases the stability of anthocyanins.

2 Fracassetti, D., Del Bo', C., Simonetti, P., Gardana, C., Klimis-Zacas, D., & Ciappellano, S. (2013). Effect of time and storage temperature on anthocyanin decay and antioxidant activity in wild blueberry (*Vaccinium angustifolium*) powder. *Journal of Agricultural and Food Chemistry, 61*(12), 2999–3005. https://doi.org/10.1021/jf3048884This research provided possible explanations for the different degradation rates of glucoside, galactoside, arabinoside of delphinidins, cyanidins, and malvidins.

3 Piljac-Žegarac, J., & Šamec, D. (2011). Antioxidant stability of small fruits in postharvest storage at room and refrigerator temperatures. *Food Research International, 44*(1), 345–350. https://doi.org/10.1016/j.foodres.2010.09.039In this study, total anthocyanin content decreased fast at 25 °C and no anthocyanin was detected after 4-day storage. The compared results in our study indicated that the modified atmosphere package retarded anthocyanin degradation at 23 °C.

4 Saarniit, K., Lang, H., Kuldjärv, R., Laaksonen, O., & Rosenvald, S. (2023). The stability of phenolic compounds in fruit, berry, and vegetable purees based on accelerated shelf-life testing methodology. *Foods, 12*(9). https://doi.org/10.3390/foods12091777In this study, the stability of phenolic compounds in blueberries and combinations of blueberries with other fruits were studied in RT and ASLT. The degradation of total phenolics was monitored for 14 months. The results of phenolic degradation and Q10 in this study can be compared with our results.

5 Tian, Y., Liimatainen, J., Alanne, A. L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., & Yang, B. (2017). Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry, 220*, 266–281. https://doi.org/10.1016/j.foodchem.2016.09.145This research provides the fragmentation pattern and the retention time of phenolic compounds in MS and MS2. The findings in our study were compared to this research for phenolic compound annotation.