Luke
NATURAL RESOURCES INSTITUTE FINLAND

Jukuri, open repository of the Natural Resources Institute Finland (Luke)

This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.

Author(s): Wenjia He, Oskar Laaksonen, Ye Tian, Maarit Heinonen, Lidija Bitz and Baoru Yang

Title: Phenolic compound profiles in Finnish apple (*Malus* × *domestica Borkh.)* juices and ciders fermented with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains

Year: 2022

Version: Published version

Copyright: The Author(s) 2022

Rights: CC BY 4.0

Rights url: http://creativecommons.org/licenses/by/4.0/

Please cite the original version:

He W., Laaksonen O., Tian Y., Heinonen M., Bitz L., Yang B. (2022). Phenolic compound profiles in Finnish apple (*Malus* × *domestica Borkh.*) juices and ciders fermented with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains. Food Chemistry 373, Part B, 131437. https://doi.org/10.1016/j.foodchem.2021.131437.

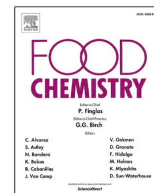
All material supplied via *Jukuri* is protected by copyright and other intellectual property rights. Duplication or sale, in electronic or print form, of any part of the repository collections is prohibited. Making electronic or print copies of the material is permitted only for your own personal use or for educational purposes. For other purposes, this article may be used in accordance with the publisher's terms. There may be differences between this version and the publisher's version. You are advised to cite the publisher's version.

Food Chemistry 373 (2022) 131437

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Phenolic compound profiles in Finnish apple (*Malus* × *domestica Borkh.*) juices and ciders fermented with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains

Wenjia He [a], Oskar Laaksonen [a], Ye Tian [a], Maarit Heinonen [b], Lidija Bitz [b], Baoru Yang [a,c,*]

[a] Food Chemistry and Food Development, Department of Life Technologies, University of Turku, FI-20014 Turku, Finland
[b] Natural Resources Institute Finland (Luke), Production systems/Horticultural technologies, Myllytie 1, FI-31600 Jokioinen, Finland
[c] Shanxi Center for Testing of Functional Agro-Products, Shanxi Agricultural University, No. 79, Longcheng Street, Taiyuan 030031, China

ARTICLE INFO

Keywords:
Apple
Cultivars
Phenolic compounds
Juice
Cider
Yeast fermentation

ABSTRACT

The phenolic compounds in juices and ciders made with *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* from eleven Finnish apple cultivars were analyzed using liquid chromatographic and mass spectrometric methods combined with multivariate data analysis. In general, the ciders contained less phenolic compounds than corresponding apple juices. In the studied apple juices and ciders, hydroxycinnamic acids were the most predominant, accounting for around 80% of total phenolic compounds. Apple juices contained more flavonol glycosides and dihydrochalcones whereas cider processing resulted in increased amount of free hydroxycinnamic acids. The contents of individual phenolic compounds were more dependent on the apple cultivars than the yeast species. Certain cultivars contained remarkably higher contents of dihydrochalcones and hydroxycinnamic acids when comparing with other cultivars. Ciders made using *S. pombe* remained higher contents of procyanidins and (+)-catechin while *S. cerevisiae* ciders contained higher individual hydroxycinnamic acids, such as 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-*p*-coumaroylquinic acid, and 4-*O*-*p*-coumaroylquinic acid.

1. Introduction

Cider is typically defined as an alcoholic beverage obtained by fermenting apple juice. During the last decades, the global cider industry has experienced staggered but steady growth, and will continue this positive trajectory in the future. Cider production also plays a promising role in the apple fruit industry and the beverage industry (Magalhães, Krogerus, Vidgren, Sandell, & Gibson, 2017). In general, cider makers prefer specific cider apple cultivars instead of using dessert apple cultivars to achieve high quality of ciders with a balance of acidity, sweetness, astringency, and bitterness. The cider industry is provided with a stable and low price supply of specialty cider apples in those countries with a long history of cider making, such as England, France, and Spain (Albert, Franck, Gilles, & Plantegenest, 2017; García, Miñarro, & Martínez-Sastre, 2018; Harper et al., 2020). The availability of cider apples is limited in Finland, and no breeding programs exist for cider apples currently. Domestic cider production can only be prepared from apple juice obtained by pressing local apple cultivars or commercial apple juice concentrate. Thus, the selection and development of Finnish local cultivars into cider apples is of highly commercially

Abbreviations: AP, Alasen Punainen; Kr, Kersti; LM, Lepaan Meloni; LK, Lohjan Kirkas; An, Aino; GB, Gustavs Bästa; Lt, Luotsi; Pk, Pieksämäki; Tr, Turso; Jr, Juuso; Hg, Hyvingiensis; 3-CaQA, 3-*O*-caffeoylquinic acid; 3-ClqA, 3-*O*-*p*-coumaroylquinic acid; 5-CaQA, 5-*O*-caffeoylquinic acid; 4-CaQA, 4-*O*-caffeoylquinic acid; ClqA I, Coumaroylquinic acid I; DiClqA, Dicaffeoylquinic acid; CA, Caffeic acid; ClqA_II, Coumaroylquinic acid II; FaH_I, Ferulic acid hexoside I; 4-ClqA, 4-*O*-*p*-coumaroylquinic acid; pA, *p*-Coumaric acid; FA, Ferulic acid; FaH_II, Ferulic acid hexoside II; FaH_III, Ferulic acid hexoside III; Di_I, PC dimer I; Cat, (+)-Catechin; Di_II, PC dimer II; E_Cat, (-)-Epicatechin; Di_III, PC dimer III; Tri_I, PC trimer I; Di_IV, PC dimer IV; Qu_gal, Quercetin 3-*O*-galactoside; Qu_glc, Quercetin 3-*O*-glucoside; Qu_pen I, Quercetin pentoside I; Kae_hex I, Kaempferol 3-*O*-hexoside I; Kae_hex II, Kaempferol 3-*O*-hexoside II; Qu_pen II, Quercetin pentoside II; Qu_rha, Quercetin 3-*O*-rhamnoside; Iso_rha, Isorhamnetin 3-*O*-rhamnoside; Qu, Quercetin; HP_mogly, Hydroxyphloretin monoglycoside; Ph_xyglu, Phloretin 2′-*O*-xyloglucoside; Ph_pthx, Phloretin-pentose-hexose; Ph_glu, Phloretin 2′-*O*-glucoside; SC1116, *Saccharomyces cerevisiae* Lalvin 1116; SP3796, *Schizosaccharomyces pombe* 3796; YPD, Yeast extract peptone-dextrose; UHPLC, Ultra-high performance liquid chromatography; DAD, Diode-array detector; ESI, Electrospray ion source; Q-TOF, Quadrupole-time-of-flight tandem mass spectrometer; PCA, Principal component analysis; PLS-DA, Partial least squares regression discrimination analysis.

* Corresponding author at: Food Chemistry and Food Development, Department of Life Technologies, University of Turku, FI-20014 Turku, Finland.
E-mail address: baoru.yang@utu.fi (B. Yang).

https://doi.org/10.1016/j.foodchem.2021.131437
Received 22 June 2021; Received in revised form 11 October 2021; Accepted 16 October 2021
Available online 20 October 2021
0308-8146/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

importance in the industry.

As a good source of the phytonutrients, apple provides a high resource of carbohydrates, vitamins, and bioactive compounds, such as phytosterols, β-carotene, and phenolic compounds (Feng et al., 2021; Wu et al., 2020). Apple polyphenols have diverse structures and can be classified into five major sub-classes: hydroxycinnamic acids and their derivatives, including caffeoyl or coumaroyl quinic acids as the most abundant compounds; flavan-3-ols, including monomeric flavan-3-ols (catechin and epicatechin) and polymeric flavan-3-ols (proanthocyanidins); dihydrochalcones, including phloridzin and its glucoside derivatives; and flavonols, including glycosylated quercetins and glycosylated isorhamnetins (Kalinowska, Bielawska, Lewandowska-Siwkiewicz, Priebe, & Lewandowski, 2014; Laaksonen, Kuldjärv, Paalme, Virkki, & Yang, 2017). The fifth group is anthocyanins, which are often accumulated on the skins of the fruit providing red color and not present in the processed apple products, such as juices and ciders (Knebel, Braun, & Dietrich, 2018). A strong correlation has been observed between apple polyphenols and their health benefits, e.g., anti-cancer, anti-inflammatory, antioxidant, and anti-inflammatory activities (Sun et al., 2017; Wang et al., 2017). Apple polyphenolic profiles play an important role in defining the sensory quality of apple ciders, as they may contribute directly to the astringency and bitterness (Laaksonen et al., 2017). In addition, the contents of procyanidins were also reported to be highly responsible for the astringency and bitterness in cider samples (Symoneaux, Chollet, Patron, Bauduin, Le Quéré, & Baron, 2015).

The polyphenolic profiles and contents of apple ciders vary among apple cultivars (Laaksonen et al., 2017; Liang et al., 2020). For example, ciders made from typical dessert apple 'Red Delicious' contained higher levels of total phenolics than the ciders made from 'Pink Lady' and 'Royal Gala' as previously reported (Girschik et al., 2017). Apple cultivars have also been reported to affect the chemical compositional changes during the fermentation process (Laaksonen et al., 2017). Moreover, the differences in the phenolic profiles of the fruit can be attributed to the maturity level of the fruit (Alberti et al., 2016). In addition to the cultivar difference, polyphenolic profiles for the final cider products are also influenced by processing methods. For example, the highest concentration of phenolic compounds were found in the skin and seeds, the loss of phenolic compounds during juice extraction can be due to the discard of peels and seeds (Laaksonen et al., 2017). Oxidative degradation of polyphenolic compounds into quinones has also been reported to be found during milling and pressing steps. For this purpose, the inhibition of oxidation can be suppressed by heat treatment and oxygen removal (De Paepe et al., 2015). Previous study showed that fermentation processing also lead to a composition change in the phenolic compounds, mainly on the hydroxycinnamic acids and flavanols (Laaksonen et al., 2017).

Apple cultivars play a significant role in the phenolic profiles of ciders (Laaksonen et al., 2017). At the same time, *S. pombe* strains in ciders lead to a decreased amount of malic acid and sour taste in comparison to the ciders produced from *S. cerevisiae* (He et al., 2021). Although the use of different yeast strains had some effects on the astringency and bitterness of the final ciders, the differences were mainly derived from different apple cultivars (He et al., 2021). Therefore, this study aimed to investigate the phenolic profiles of apple ciders together with their corresponding juices made from the selected eleven Finnish apple cultivars. The selection of suitable apple cultivars is one of the main concerns for domestic cider makers to obtain cider products with high quality and balanced sweet-tart taste. The current study is a follow-up study of our previous study (He et al., 2021), *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Schizosaccharomyces pombe* (*S. pombe*) strains were selected to produce fermented apple ciders. The fates of phenolic compounds in four major compound classes (hydroxycinnamic acids, flavan-3-ols, dihydrochalcones, and flavonols) during cider fermentation were investigated during fermentation process. Special focus was also on the effect of various apple cultivars on the phenolic compound differences and their contributions to the sensory properties, especially mouth-drying and puckering astringency as evaluated in our previous study (He et al., 2021).

2. Materials and methods

2.1. Yeast and standard compounds

S. cerevisiae Lalvin V1116 (SC1116) was purchased from Lallemand, Inc. (Montreal, Quebec, Canada) and *S. pombe* 3796 (SP3796) was provided by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inc. (DSMZ, Braunschweig, Germany). Commercial yeast extract peptone-dextrose (YPD) medium and YPD agar used for yeast inoculation was obtained from Lab M Limited (Lancashire, United Kingdom).

HPLC grade acetonitrile and LC-MS grade acetonitrile and methanol were supplied by VWR International Oy (Espoo, Finland). Ethanol (≥99.7%) was provided by Altia Oyj (Helsinki, Finland). Propan-1-ol, 3-methylbutan-1-ol, 2-methylpropan-1-ol, acetaldehyde, ethyl acetate, methanol, glycerol, and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO, United States). Reference compounds of quercetin-3-*O*-glucoside and procyanidin B2 were obtained from Extrasynthese (Genay, France). (+)-Catechin, (-)-epicatechin, 5-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, *p*-coumaric acid, and phloretin-2′-*O*-glucoside were provided by Sigma-Aldrich Co. (St. Louis, MO, United States).

2.2. Plant materials

Ten Finnish apple cultivars (*Malus* × *domestica* Borkh.) were selected, including four summer cultivars ('Alasen Punainen', 'Kersti', 'Lepaan Meloni', and 'Lohjan Kirkas'), five autumn cultivars ('Aino', 'Gustavs Bästa', 'Luotsi', 'Pieksämäki', and 'Turso'), and one winter apple cultivar ('Juuso'). These materials are Finnish local cultivars produced from seed sowing activities at the turn of the 19th and 20th century (Heinonen and Bitz, 2019). One decorative apple cultivar (*Malus* 'Hyvingiensis') was also chosen to investigate the potential of decorative apple cultivars for cider making. All the selected cultivars were cultivated in Piikkiö, Southwest Finland (60°25′N, 22°31′E), and randomly harvested in 2018 from Natural Resources Institute Finland (Luke). All the samples were stored in a fridge at 4 °C for 1 month to reach overripe stages in order to increase the flavor compounds in ciders. After that, the apples were pressed into juices for further study. The main morphological characteristics were observed in the apple collection using UPOV standards (Heinonen and Bitz, 2019). The trueness-to-type analysis has been carried out using the apple microsatellite (SSR) genotyping analysis. The information of the used apple cultivars is listed in Table 1.

2.3. Apple microsatellite (SSR) genotyping

A set of ten apple cultivars (*Malus* × *domestica* Borkh.) were selected for the cider study and additional 6 reference apple cultivars relevant for Finnish apple identification ('Antonovka', 'Astrakaani Gyllenkrok', 'Discovery', 'Grenman', 'Kaikuvuori', and 'Lantun Talvi') have been genetically analyzed in order to confirm the trueness to type of relevant apples (Bitz, Heinonen, Moisander, Tanhuanpää, and Sarvarinne, 2019). Total genomic DNA extraction, microsatellites selection (CH02c06, COL, Ch04e05, Ch01h02, Ch02c11, and Ch02c09) and PCR amplifications have been done according to the work by Bitz et al (2019). The confirmation of trueness to type was done as described in Heinonen and Bitz (2019). Beside trueness to type analyses of relevant apples cultivars, a genetic clustering has been performed by obtaining a dendrogram based on Supplementary Fig. 2 to possibly make genetic distinctions of putative cider apple clusters. For genetic clustering, nine European wild apples (*Malus sylvestris* L.) from Finland was added to the genetic analyses as wild apples have been traditionally used in preparing home-made vinegar or even cider from fermented fruits (Tardío, Arnal, &

Table 1
Description of the apple cultivars used in this study.

Name of cultivar	Seasonal category	Over color of fruit[a]	Breed	Origin of cultivar[d]	Harvest time	Abbreviations
Alasen Punainen[b]	Summer	Flushed and mottled of red	Unknown	early 1900 s in Joensuu, Finland	2018.09.03	AP
Kersti	Summer	Only stripes of orange red	Seedling of Mironchik	early 1900 s in Mikkeli, Finland	2018.09.04	Kr
Lepaan Meloni	Summer	Absent or very small red	Unknown	early 1900 s in Lepaa, Finland	2018.09.04	LM
Lohjan Kirkas	Summer	Only stripes of orange red	Transparente Blance × Gyllenkroks Astrakan	1920 s in Lohja, Finland	2018.09.04	LK
Aino	Autumn	Only solid flush of brown red	Unknown	1940 s in Suomussalmi, Finland	2018.09.03	An
Gustavs Bästa	Autumn	Only solid flush of red	Seedling of Antonovka	1980 s in Vaasa, Finland	2018.09.03	GB
Luotsi	Autumn	Solid flush with strongly defined stripes of red	Bud mutation of Simnoje polosatoje	mid 1900 s in Halikko, Finland	2018.09.04	Lt
Pieksämäki	Autumn	Absent or very small red	Unknown	mid 1800 s in Pieksämäki, Finland	2018.09.03	Pk
Turso	Autumn	Flushed, striped and mottled of red	Unknown	early 1900 s in Kangasala, Finland	2018.09.04	Tr
Juuso	Winter	Only solid flush of red	Antonovka × Lobo	1997, breed by Luke, Finland	2018.09.03	Jr
Hyvingiensis[c]	–	Flushed of red	Unknown	late 1800 s in Hyvinkää, Finland	2018.09.20	Hg

[a] The officially recognized descriptions available from 'Kersti', 'Lepaan Meloni', 'Lohjan Kirkas', 'Gustavs Bästa', 'Luotsi', 'Pieksämäki', 'Turso', and 'Juuso' in Finnish Food Authority. (Available: https://www.ruokavirasto.fi/yritykset/kasviala/Lajikkeet-ja-alkuperaiskasvit/hedelma–ja-marjakasvien-lajikeluettelo-ja–kuvaukset/virallisesti-tunnustetut-kuvaukset/omenat-malus/)

[b] Morphological characterization of 'Alasen Punainen' is unpublished. Description is available on request from Luke.

[c] Lindén and Iwarsson have identified Malus 'Hyvingiensis' as a unique Finnish local seed born cultivar of weeping decorative crap apple.(Lindén & Iwarsson, 2014)

[d] Except 'Juuso', all samples are Finnish local seed born cultivars originating from different parts of Finland. 'Juuso' is a commercial cultivar from the Finnish apple breeding program in Luke.

Lázaro, 2021). True to type wild apples from Finland have been selected from the previous study (Bitz et al, 2019) imputing that those one might give genetic contribution to the Finnish seed born cultivars used in this study. One reference apple cultivar 'Discovery' was added to the clustering analyses as well. Software DARwin version 6.0.015 was used to calculate genetic distance from dissimilarity simple matching coefficient, bootstrapping and construction and visualization of a hierarchical consensus dendrogram by neighbor-joining method.

2.4. Apple juice preparation

The apple materials were washed and sliced into small pieces before pressed into juices with a juice press (Vita Pro-Active JE810, Kenwood, United Kingdom). The obtained juice from each cultivar was pasteurized at 95 °C for 5 min and immediately cooled down to 20 °C in a water bath. All juices were stored at −20 °C till further study.

2.5. Laboratory-scale fermentations

The fermentation procedure was described in our previous study (He et al., 2021). Briefly, two yeast strains, *Saccharomyces cerevisiae* 1116 (SC1116) and *Schizosaccharomyces pombe* 3796 (SP3796), were used in the cider fermentation. Each strain was proliferated in the YPD liquid medium (50 g/L) in a shaking table (150 rpm) at 25 ℃ for 48 h, and later added into 80 mL of juices to reach the level of 10^7 CFU/mL for inoculation. The fermentation process was carried out at 25 ℃ until the end of fermentation. All the samples were performed in duplicate. After fermentation, the apple ciders were centrifuged at 3000 rpm for 5 min. The supernatants were collected and stored in −20 ℃ before further chemical analyses.

2.6. Analysis of yeast metabolites

The main by-products of yeast metabolites in apple ciders were analyzed in duplicate using a gas chromatographic method as described previously (Liu, Laaksonen, Kortesniemi, Kalpio, & Yang, 2018). A Shimadzu GC-2010plus gas chromatograph (Shimadzu Corporation, Japan) instrument was equipped with a flame ionization detector (FID) and a HP-INNOWax (30 m × 0.25 mm, i.d., 0.25 µm, Hewlett-Packard, Avondale, PA, United States) column. The column temperature started from 40 ℃ for 8 min and increased to 240 ℃ at a rate of 10 ℃/min and then held for 2 min. The injector and detector temperatures were conducted as 220 ℃ and 280 ℃, respectively. The conducted carrier gas was helium with a flow rate of 1.5 mL/min at the split ratio of 1:25. The compounds were characterized by comparing the retention time on GC chromatogram with reference compounds. An external standard method was used for quantifications. The information of external standard curves is given in Supplementary Table 2.

2.7. Analysis of phenolic compounds

The analysis of phenolic compounds in apple juices and ciders was based on the method of Mäkilä, Laaksonen, Alanne, Kortesniemi, Kallio, and Yang (2016) with a slight modification. Approximately 5 g of juice/cider sample was extracted with 15 mL of ethyl acetate for three times. The extraction was assisted with sonication for 15 min and centrifuge at 4500 g for 10 min. The combined supernatants from three-time extraction were evaporated to completely dry and dissolved in 1 mL of methanol. The re-dissolved samples were filtered with 0.2 µm filters before further analyses.

Phenolic compounds were identified by using a Bruker ultra-high performance liquid chromatography (UHPLC) system, equipped with a diode-array detector (DAD), an Apollo II electrospray ion source (ESI), and a quadrupole/time-of-flight tandem mass spectrometer (Q-TOF) (Bruker Corp., Billerica, MA, United States). The chromatographic separation was conducted at 25 ℃ using a Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 µm, Torrance, CA, United States). The flow rate was 1.0 mL/min. The mobile phase was a combination of water (A) and acetonitrile (B), both containing 0.1% (*v/v*) of formic acid. LC gradients was set as the following: 0–3 min, 0% B; 3–6 min, 0%-1% B; 6–15 min, 1%-4% B; 15–20 min, 4%-5% B; 20–25 min, 5% B; 25–27 min, 5%-6% B; 27–32 min, 6%-7% B; 32–37 min, 7%-9% B; 37–42 min, 9%-

12% B; 42–47 min, 12%-14% B; 47–52 min, 14%-18% B; 52–57 min, 18%-20% B; 57–62 min, 20%–23% B; 62–67 min, 23%-40% B; 67–68 min, 40%-70% B; 68–70 min, 70%-0% B; 70–72 min, 0% B. The chromatograms were monitored under the wavelength of 280 nm (mainly for flavan-3-ols and dihydrochalcones), 320 nm (hydroxycinnamic acids), and 360 nm (flavonols).

The eluents of 0.4 mL/min was flown into MS system. Mass dull-scan (mass range of *m/z* 20–2000) was operated under both positive and negative ionization modes. The end plate offset, nebulizer gas pressure, flow rate of drying gas, and drying gas temperature was 500 V, 2.5 bar, 11 L/min, and 280 °C, respectively. The capillary voltage was 4.5 kV (for positive mode) and 3.5 kV (for negative mode). MS^2 scan was performed using an auto MS/MS program in Q-TOF system. The ion energy of quadrupole was at 5.0 eV and the collision energy was ranged from 5.0 to 12.5 eV. A sodium formate solution (10 mM) was injected at the beginning of each of sample injection as internal calibration. MS data was processed by Compass Data analysis software 4.4.

Quantitative analysis of phenolics was performed on a Shimadzu UHPLC system equipped with a SPD-M20A diode array detector (DAD). The analytical condition of LC was same as that used in HPLC-MS analysis. The concentration of identified compounds was calculated based on the standard curves of external standards. Selection of external standards and equation of standard curves are shown in Supplementary Table 2.

2.8. Sensory evaluation

The sensory characteristics of apple ciders were evaluated by 34 untrained panelists (26 females and 8 males, age 20–65; mainly students and staff of the University). The sensory evaluation was conducted in a controlled laboratory conditions (ISO 8595). The untrained panelists were asked to first complete a check-all-that-apply (CATA) evaluation by selecting all suitable descriptors from a list of selected sensory attributes (data shown in He et al. (2021)) for apple cider samples. Then, they were asked to evaluate the samples on their appearance, odor, and flavor by 9-point scales. Panelists were asked to drink water and eat a small piece of cracker (unsalted) to clean their mouth between each samples. The data was collected and evaluated via Compusense Cloud software version 5.6 (Compusense Inc., Guelph, Canada).

2.9. Statistical analysis

Statistical difference among samples was assessed with one-way ANOVA analysis and Tukey's test by using SPSS 25.0.0.1 (IBM SPSS Statistics, Inc., Chicago, IL, United States). Multivariate models, including principal component analysis (PCA) and partial least squares regression discrimination analysis (PLS-DA), were carried out by using Unscrambler X, version 10.4 (CAMO software, Oslo, Norway), in order to explore the distribution of phenolics among juice/cider samples. PLS-DA model was also applied in the correlations between the clustering methods and phenolic profiles in current study. The correlations between phenolic profiles and sensory characteristics (data based on our previous study (He et al., 2021)) were studied with PCA models.

3. Results and discussion

3.1. Fermentation kinetics and main by-products of yeast metabolism

The fermentation kinetics of apple ciders from eleven cultivars and two yeast strains are shown in Supplementary Fig. 1. The data presented the mean value ± standard deviation of duplicate fermentations. The fermentation kinetics were mainly dependent on the yeast strains whereas the apple cultivars had a smaller effect. All the fermentations conducted with SC1116 were completed in 10–12 days, and the fermentations carried out with SP3796 completed in 14–18 days. In the current study, *S. cerevisiae* strain showed a better fermentative performance than *S. pombe* strain in all the apple cultivars, indicating a fast fermentation rate in the ciders fermented by SC1116.

The main by-products of fermentation were detected in apple ciders, primarily as ethanol, acetaldehyde, methanol, ethyl acetate, propan-1-ol, 2-methylpropan-1-ol, butan-1-ol, 3-methylbutan-1-ol, acetic acid, and glycerol (Table 2). The results suggested that ethanol was the most abundant compounds during cider fermentation, data has been reported previously (He et al., 2021). Apart from ethanol, glycerol was another abundant chemical compounds found in the cider products. Significantly higher glycerol levels were found in the cider samples produced with SP3796 strains (5.90 g/L on average) compared to those with SC1116 (3.39 g/L). This result was in agreement with previous work reporting that higher concentration of glycerol produced by *S. pombe* than fermentations with *S. cerevisiae* yeast in bilberry wines (Liu et al., 2018). Regardless of yeast strains, apple cultivars also showed an impact on the content of glycerol in ciders. Ciders made of 'Alasen Punainen' (AP) contained the highest glycerol content (5.94 and 7.75 g/L using SC1116 and SP3796, respectively) among all ciders studied. The variation of glycerol contents among different apple cultivars was ascribed to the sugar level, nitrogen, and sulfites in the apple must as reported previously (Ivit, Longo, & Kemp, 2020).

Acetaldehyde, as one of the most important sensory carbonyl compounds, were mainly derived from yeast metabolism. In the ciders fermented with SC1116 strains, the concentration of acetaldehyde was 56.20 mg/L on average. This was significantly higher than that in the ciders using SP3796 (45.43 mg/L on average). Apart from the yeast strains, the concentration of acetaldehyde in apple cider was also dependent on apple cultivars as shown in the results (Table 2). Ethyl acetate was the most abundant ester existed in the ciders, in accordance with the findings of previous study (He et al., 2021), potentially providing *fruity* and *sweet* ordors to the samples. However, a high concentration of ethyl acetate (>150 mg/L) may lead to *solvent-like* aroma (He et al., 2021). In this study, the content of ethyl acetate in all the apple ciders was under the potential spoilage threshold. Ciders produced with SP3796 contained lower ethyl acetate contents than that with SC1116 (Table 2). The content of acetic acid produced in the studied ciders ranged from 0.33 to 0.57 g/L, which was much lower than the reported detection threshold level of acetic acid (1.2 g/L) (Gamboa, Albarracin E, da Silva, da Andrade Lima, & Ferreira, 2019). The result was in accordance with the previous studies (Li et al., 2020). Moreover, fermentation with SC1116 also caused slightly lower level of acetic acid produced in ciders compared to SP3796.

In addition to ethanol, some other alcohol compounds were also detected after the fermentation of apple juices. In the current study, only trace amounts of methanol were detected in all the studied ciders. Higher alcohols have been reported as the main contributors of cider aroma. These compounds generally were present in trace amounts in apple juices, but became concentrated in ciders, due to various conversion of amino acids (Qin, Petersen, & Bredie, 2018). As shown in Table 2, 3-methylbutan-1-ol was the most abundant higher alcohol detected in the apple ciders. This was in accordance with previous reports on ciders (Qin et al., 2018; He et al., 2021). In contrast, propan-1-ol was found in most of cider samples at low levels.

3.2. Identification of phenolic compounds

UHPLC-DAD-ESI-QTOF analysis was conducted for extracts of selected apple ciders and their corresponding juices. Altogether 34 phenolic compounds were tentatively identified, including 14 hydroxycinnamic acid derivatives, 7 flavan-3-ols, 9 flavonols, and 4 dihydrochalcones. MS data and LC chromatograms are shown in Table 3 and Supplementary Fig. 3, respectively. Identification of the phenolics was carried out via the combination of the UV–Vis spectra, LC retention times, and mass fragmentation with external reference standards and previous literatures.

In the hydroxycinnamic acid group, three isomers of caffeoylquinic

Table 2
Main by-products of yeast metabolites in apple cider samples.

Samples	Ethanol (g/L)[a]	Glycerol (g/L)	Acetaldehyde (mg/L)	Ethyl acetate (mg/L)	Methanol (mg/L)	Propan-1-ol (mg/L)	2-Methylpropan-1-ol (mg/L)	Butan-1-ol (mg/L)	3-Methylbutan-1-ol (mg/L)	Sum of higher alcohols (mg/L)	Acetic acid (g/L)
Ciders made from SC1116											
AP	5.33 ± 0.57ab	5.94 ± 0.51e	45.79 ± 0.65a	41.35 ± 1.22a	26.08 ± 1.39a	8.08 ± 1.23e	43.75 ± 2.52d	15.01 ± 1.20a	198.25 ± 4.56c	265.09 ± 9.51c	0.42 ± 0.01bc
Kr	5.80 ± 0.23b	2.31 ± 0.35ab	62.51 ± 0.59b	65.95 ± 1.37c	42.01 ± 1.20c	3.09 ± 0.48b	12.37 ± 1.02a	34.15 ± 2.92bc	119.64 ± 3.11a	169.25 ± 7.53a	0.33 ± 0.02a
LM	5.21 ± 0.48ab	3.16 ± 0.42bc	78.24 ± 6.72e	82.35 ± 2.21d	33.08 ± 1.22b	5.03 ± 0.11d	22.35 ± 1.39b	28.95 ± 3.11b	175.62 ± 8.56b	237.16 ± 13.65b	0.45 ± 0.02d
LK	5.67 ± 0.68b	2.92 ± 0.25b	85.22 ± 6.86f	63.18 ± 2.11c	44.33 ± 1.78c	4.23 ± 0.25c	76.39 ± 5.35f	21.33 ± 4.63ab	181.36 ± 4.47b	283.31 ± 14.70c	0.40 ± 0.02ab
An	4.30 ± 0.68a	1.65 ± 0.16a	41.31 ± 0.22a	50.97 ± 0.25b	35.23 ± 2.97b	ND	31.12 ± 3.56c	13.22 ± 1.27a	253.12 ± 14.32d	297.46 ± 19.15c	0.44 ± 0.01c
GB	4.83 ± 0.35a	3.37 ± 0.22bc	77.26 ± 6.16d	51.50 ± 0.62b	36.02 ± 4.08b	5.33 ± 0.35d	51.23 ± 6.56e	18.01 ± 1.14a	248.19 ± 12.96d	322.76 ± 21.01d	0.38 ± 0.02b
Lt	4.66 ± 0.50a	2.89 ± 0.35b	78.14 ± 2.11c	54.63 ± 2.36b	28.09 ± 1.23ab	2.13 ± 0.37a	20.36 ± 1.02b	11.25 ± 0.89a	298.05 ± 15.06e	331.79 ± 17.34d	0.40 ± 0.03bc
Pk	5.09 ± 0.29ab	3.32 ± 0.41bc	31.06 ± 0.75a	44.58 ± 1.35a	25.52 ± 3.59a	ND	75.12 ± 2.39f	48.96 ± 1.85d	128.25 ± 10.11a	252.33 ± 14.35bc	0.40 ± 0.01b
Tr	5.51 ± 0.31b	4.15 ± 0.28d	36.64 ± 0.55a	49.67 ± 0.33b	21.03 ± 3.39a	ND	42.36 ± 2.36d	39.13 ± 2.58c	211.39 ± 8.98c	292.88 ± 13.92c	0.50 ± 0.02d
Js	5.26 ± 0.28ab	3.71 ± 0.34c	41.06 ± 0.31a	77.90 ± 0.14d	31.09 ± 5.01ab	3.25 ± 0.58b	71.36 ± 1.22f	37.12 ± 3.39c	281.23 ± 11.25e	392.96 ± 16.44e	0.39 ± 0.02b
Hg	5.76 ± 0.31b	3.88 ± 0.31c	40.96 ± 0.11a	61.05 ± 0.24c	21.34 ± 2.06a	2.34 ± 0.27a	51.52 ± 5.03e	31.65 ± 3.56b	258.35 ± 12.33d	343.86 ± 21.19d	0.44 ± 0.02c
Mean	5.22A	3.39A	56.20B	58.47B	31.27A	4.19A	45.27B	27.17B	213.95A	290.48A	0.41A
Ciders made from SP3796											
AP	4.75 ± 0.32b	7.75 ± 0.56d	38.95 ± 1.14b	33.33 ± 1.02a	19.89 ± 1.96a	14.06 ± 0.39e	23.08 ± 1.89b	20.11 ± 2.35b	200.13 ± 10.38b	257.38 ± 15.01b	0.46 ± 0.03c
Kr	5.21 ± 0.35bc	3.96 ± 0.22a	45.32 ± 0.36b	41.15 ± 0.16ab	35.93 ± 3.07bc	8.03 ± 1.05c	15.68 ± 0.59a	25.36 ± 2.88c	121.35 ± 8.29a	170.42 ± 12.81a	0.37 ± 0.02a
LM	5.12 ± 0.14bc	5.54 ± 0.49b	69.13 ± 8.25c	67.51 ± 1.23d	29.68 ± 2.95b	7.09 ± 1.01c	22.06 ± 0.60b	23.15 ± 3.74bc	231.23 ± 15.30c	283.23 ± 20.65bc	0.51 ± 0.02d
LK	5.32 ± 0.61bc	5.29 ± 0.33b	73.12 ± 4.22d	43.25 ± 2.11b	41.23 ± 5.26c	9.45 ± 1.25d	42.36 ± 1.51d	20.31 ± 0.89b	275.36 ± 14.63d	347.48 ± 18.28d	0.43 ± 0.01bc
An	4.06 ± 0.29a	5.78 ± 0.59b	41.01 ± 4.13b	41.23 ± 3.08ab	34.09 ± 2.19b	4.32 ± 0.33b	32.15 ± 4.28c	18.96 ± 1.38ab	320.12 ± 11.36e	375.55 ± 17.35e	0.46 ± 0.01c
GB	4.89 ± 0.58ab	6.59 ± 0.77b	65.19 ± 5.51c	45.62 ± 0.90b	32.08 ± 1.98b	10.38 ± 1.02d	39.16 ± 1.49d	25.36 ± 2.55c	274.13 ± 8.91d	349.03 ± 13.97d	0.57 ± 0.01e
Lt	4.63 ± 0.58ab	5.11 ± 0.24b	35.16 ± 0.66b	35.62 ± 1.35a	31.06 ± 2.66b	5.36 ± 1.02bc	25.38 ± 1.13b	17.39 ± 2.14ab	253.45 ± 9.33d	301.58 ± 13.62c	0.42 ± 0.01c
Pk	5.10 ± 0.41b	5.24 ± 0.51b	26.19 ± 1.02a	38.65 ± 2.33a	31.09 ± 1.28b	1.03 ± 0.02a	46.39 ± 1.25e	34.52 ± 5.01d	180.12 ± 7.39b	262.06 ± 13.67b	0.45 ± 0.01c
Tr	5.40 ± 0.25bc	6.27 ± 0.34b	30.95 ± 2.13ab	41.36 ± 2.39ab	27.03 ± 2.11b	ND	28.13 ± 1.17bc	33.11 ± 4.12d	259.15 ± 11.17d	320.39 ± 16.36 cd	0.41 ± 0.01b
Js	5.65 ± 0.28c	6.53 ± 0.28b	36.99 ± 1.55b	62.60 ± 3.34d	28.09 ± 3.54b	7.89 ± 0.89c	39.15 ± 2.79d	28.95 ± 5.09c	227.25 ± 8.79c	303.24 ± 17.56c	0.43 ± 0.02bc
Hg	5.35 ± 0.18bc	6.85 ± 0.36b	37.70 ± 3.15b	51.24 ± 1.22c	21.96 ± 2.85a	5.26 ± 0.66bc	32.30 ± 1.47c	13.37 ± 1.02a	198.36 ± 10.32b	249.29 ± 13.47b	0.48 ± 0.02c
Mean	5.04A	5.90B	45.43A	45.60A	30.16A	7.29B	31.44A	23.69A	230.97B	293.39A	0.45B

Results are shown as means ± standard deviations (duplicate measurements of duplicate fermentations). ND: not detected. Significant differences among the ciders of eleven apple cultivars produced with the same yeast are shown with lower case letters a-f and the differences between yeast strains are shown in upper case letters A-B (one way ANOVA with Tukey's post hoc test, $p < 0.05$). Abbreviations of apple cultivars refer to Table 1, and abbreviations of phenolic compounds refer to Table 3. [a] Data for ethanol was cited from our previous study (He et al., 2021).

acid, four isomers of caffeoylquinic acid, one dicaffeoylquinic acid, three isomers of ferulic acid hexoside were tentatively identified apart from free caffeic acid, *p*-coumaric acid, and ferulic acid (Table 3). 3-*O*-Caffeoylquinic acid (peak 1), 5-*O*-caffeoylquinic acid (peak 3), caffeic acid (peak 7), and *p*-coumaric acid (peak 11) were identified based on retention times, UV–Vis spectra, and mass spectra of the aid authentic standards. The deprotonated molecular ion of peak 4 ([M - H]- *m/z* 353.0876), corresponding to [caffeoylquinic acid - H]-, and protonated molecular ion ($[M + H]^+$ *m/z* 355.1004, corresponding to [caffeoylquinic acid + H]$^+$). Therefore, peak 4 was tentatively identified as 4-*O*-caffeoylquinic acid, which was previously reported in apples (Laaksonen et al., 2017). Peak 2, peak 5, peak 8, and peak 10 showed almost the same deprotonated ion ([M - H]-) at *m/z* 337 and protonated ion ($[M + H]^+$ at *m/z* 339, corresponding to $[C_{16}H_{16}O_8 - H]^-$ and $[C_{16}H_{16}O_8 + H]^+$, respectively. On the basis of the fragment ions detected in negative mode (*m/z* 191 to [quinic acid - H]$^-$ and *m/z* 173 to $[\text{quinic acid} - H - H_2O]^-$), those peaks were assigned as isomers of coumaroylquinic acid. Peak 2 and peak 10 were identified as 3-*O*-*p*-coumaroylquinic acid and 4-*O*-*p*-coumaroylquinic acid, respectively, according to their elution order based on previous study (Laaksonen et al., 2017). One of the peaks 5 or 8 was most likely the 5-*O*-*p*-coumaroylquinic acid, but the compounds were named coumaroylquinic acid I and coumaroylquinic acid II, respectively. Peak 6 showed a deprotonated ion ($[M - H]^-$) at *m/z* 515.1183 and protonated ion ($[M + H]^+$ at *m/z* 517.1323, corresponding to $[C_{25}H_{24}O_{12} - H]^-$ and $[C_{25}H_{24}O_{12} + H]^+$, respectively, indicated that peak 6 was an isomer of dicaffeoylquinic acid. Peak 9, peak 13, and peak 14 all showed $[M - H]^-$ at *m/z* 355 and yielded a fragment ion at *m/z* 193 owing to a loss of a hexose moiety (162 amu) and [ferulic acid - H]$^-$ (193 amu), were identified as ferulic acid derivatives, comparing their MS profiles with previous apple and pear

Table 3
Identification of phenolic compounds in apple juices and ciders.

Peak	Tentative identification	UV λ_{max} (nm)	Calculated mass (m/z) [M-H]$^-$/ [M + H]$^+$	Exact mass (m/z) [M-H]$^-$/[M + H]$^+$	Mass error (ppm) [M-H]$^-$/ [M + H]$^+$	Negative ions in MS^2 (m/z)	Positive ions in MS^2 (m/z)	Molecular formula	Identification method	Abbreviation
Hydroxycinnamic acids										
1	3-*O*-caffeoylquinic acid	327	353.0875/-	353.0877/-	0.57/-	191.0563	–	$C_{16}H_{18}O_9$	MS, standard, and literature[1]	3-CaQA
2	3-*O*-*p*-coumaroylquinic acid	311	337.0917/ 339.1073	337.0929/ 339.1074	−3.26/- 0.08	191.0553	147.0443	$C_{16}H_{16}O_8$	MS and literature[1]	3-ClqA
3	5-*O*-caffeoylquinic acid	326	353.0862/ 355.1008	353.0877/ 355.1025	−4.24/- 4.78	191.0550	163.0375	$C_{16}H_{18}O_9$	MS, standard, and literature[1,2]	5-CaQA
4	4-*O*-caffeoylquinic acid	327	353.0876/ 355.1004	353.0877/ 355.1025	−2.83/ 5.91	173.0454	163.0379	$C_{16}H_{18}O_9$	MS and literature[1,2]	4-CaQA
5	Coumaroylquinic acid I	311	337.0917/ 339.1073	337.0929/ 339.1074	−3.26/- 0.29	191.0553	147.0443	$C_{16}H_{16}O_8$	MS and literature[1]	ClqA I
6	Dicaffeoylquinic acid	–	515.1183/ 517.1323	515.1194/ 517.1342	−2.13/- 3.67	353.0551	–	$C_{25}H_{24}O_{12}$	MS and literature[2]	DiClqA
7	Caffeic acid	321	179.0332/ 181.0493	179.0349/ 181.0495	−9.49/- 1.04	135.0438	163.0385	$C_9H_8O_4$	MS, standard, and literature[2]	CA
8	Coumaroylquinic acid II	313	337.0930/-	337.0929/-	0.59/-	173.0450	–	$C_{16}H_{16}O_8$	MS and literature[1]	ClqA_II
9	Ferulic acid hexoside I	327	355.0997/ 357.1142	355.1029/ 357.1186	−9.01/- 12.21	193.0841, 175.0377	195.0405	$C_{16}H_{20}O_9$	MS and literature[1]	FaH_I
10	4-*O*-*p*-coumaroylquinic acid	310	337.0913/ 339.1070	337.0928/ 339.1074	−4.44/- 1.17	173.0397	147.0718	$C_{16}H_{16}O_8$	MS and literature[1]	4-ClqA
11	*p*-coumaric acid	310	163.0407/ 165.0546	163.0400/ 165.0546	4.29/ 0.01	–	–	$C_9H_8O_3$	MS, standard, and literature[2]	pA
12	Ferulic acid	326	193.0494/ 195.0652	193.0506/ 195.0660	−6.21/- 4.15	178.0262, 134.0363	177.0524, 145.0266	$C_{10}H_{10}O_4$	MS and literature[2]	FA
13	Ferulic acid hexoside II	327	355.1023/-	355.1029/-	−1.69/-	193.0841, 175.0378	–	$C_{16}H_{20}O_9$	MS and literature[1]	FaH_II
14	Ferulic acid hexoside III	327	355.0997/ 357.1168	355.1029/ 357.1186	−9.01/- 5.04	193.0840 175.0377	195.0405	$C_{16}H_{20}O_9$	MS and literature[1]	FaH_III
Flavan-3-ols										
15	PC dimer I	280	577.1336/ 579.1494	577.1346/ 579.1503	−2.77/- 0.52	205.0470, 289.0726, 425.0859	289.0705, 427.1206	$C_{30}H_{26}O_{12}$	MS and literature[1,3]	Di_I
16	(+)-Catechin	280	289.0760/ 291.0832	289.0781/ 291.0863	−7.26/- 10.06	245.0800, 203.0700	207.0625, 139.0370	$C_{15}H_{14}O_6$	MS, standard, and literature[1,3]	Cat
17	PC dimer II	278	577.1322/ 579.1456	577.1346/ 579.1503	−5.19/- 7.07	289.0703, 425.0835	289.0688, 427.1206	$C_{30}H_{26}O_{12}$	MS and literature[1,3]	Di_II
18	(-)-Epicatechin	279	289.0705/ 291.0832	289.0711/ 291.0825	−2.08/ 2.41	245.0800	207.0648	$C_{15}H_{14}O_6$	MS, standard, and literature[1,3]	E_Cat
19	PC dimer III	280	577.1331/ 579.1494	577.1346/ 579.1503	−2.60/- 1.56	425.0866, 289.0711	427.0987, 289.0679	$C_{30}H_{26}O_{12}$	MS and literature[1,3]	Di_III
20	PC trimer I	279	865.1991/ 867.2171	865.1980/ 867.2137	1.27/ 3.92	–	577.1326, 291.0875	$C_{45}H_{38}O_{18}$	MS and literature[1,3]	Tri_I
21	PC dimer IV	280	577.1354/ 579.1516	577.1346/ 579.1503	1.56/ 2.25	425.0804, 289.0663	301.1398	$C_{30}H_{26}O_{12}$	MS and literature[1,3]	Di_IV
Flavonols										
22	Quercetin-3-*O*-galactoside	343	463.0864/ 465.0989	463.0882/ 465.1028	−3.88/- 8.38	300.0267, 163.9528	303.0465	$C_{21}H_{20}O_{12}$	MS and literature[1,4]	Qu_gal
23	Quercetin-3-*O*-glucoside	345	463.0866/ 465.1026	463.0882/ 465.1028	−3.46/- 0.43	–	303.0409	$C_{21}H_{20}O_{12}$	MS, standard, and literature[1,4]	Qu_glc
24	Quercetin pentoside I	350	433.0756/ 435.0925	–	–	300.0264	–	$C_{20}H_{18}O_{11}$	MS and literature[5]	Qu_pen I
25	Kaempferol-3-*O*-hexoside I	352	-/449.1059	-/449.1078	-/-4.45	–	287.1240	$C_{21}H_{20}O_{11}$	MS and literature[5]	Kae_hex I
26	Kaempferol-3-*O*-hexoside II	352	447.0899/ 449.1054	447.0934/ 449.1078	−7.83/- 5.34	284.0290	287.0530	$C_{21}H_{20}O_{11}$	MS and literature[5]	Kae_hex II
27	Quercetin pentoside II	351	433.0766/ 435.0898	–	–	271.0235, 300.0264	303.0482	$C_{20}H_{18}O_{11}$	MS and literature[5]	Qu_pen II
28	Quercetin-3-*O*-rhamnoside	351	447.0932/ 449.1058	447.0933/ 449.1063	−0.23/- 1.11	301.0340	317.0486	$C_{21}H_{20}O_{11}$	MS and literature[5]	Qu_rha
29	Isorhamnetin-3-*O*-rhamnoside	368	461.1101/-	461.1084/-	3.69/-	315.0523	–	$C_{22}H_{22}O_{11}$	MS and literature[5]	Iso_rha
30	Quercetin	369	301.0356/ 303.0498	301.0354/ 303.0499	0.66/- 0.33	–	–	$C_{15}H_{10}O_7$	MS and literature[5]	Qu
Dihydrochalcones										
31	Hydroxyphloretin monoglycoside	281	451.1593/ 453.1756	451.1605/ 453.1762	−2.66/- 1.32	289.0668, 167.0317	315.0841, 453.1197	$C_{22}H_{28}O_{10}$	MS and literature[1,5]	HP_mogly
32		284				273.0754	275.0901	$C_{26}H_{32}O_{14}$		Ph_xyglu

(*continued on next page*)

Table 3 (*continued*)

Peak	Tentative identification	UV λ_{max} (nm)	Calculated mass (*m/z*) [M-H]⁻/ [M + H]⁺	Exact mass (*m/z*) [M-H]⁻/[M + H]⁺	Mass error (ppm) [M-H]⁻/ [M + H]⁺	Negative ions in MS² (*m/z*)	Positive ions in MS² (*m/z*)	Molecular formula	Identification method	Abbreviation
	Phloretin-2′-*O*-xyloglucoside		567.1699/ 569.1861	567.1714/ 569.1870	−2.65/-1.58				MS and literature[1,4]	
33	Phloretin-pentose-hexose	283	567.1693/ 569.1859	567.1714/ 569.1870	−3.70/-1.93	273.0768	–	$C_{26}H_{32}O_{14}$	MS and literature[1,4]	Ph_pthx
34	Phloretin-2′-*O*-glucoside	283	435.1287/ 437.1443	435.1292/ 437.1449	−1.15/-1.37	273.0766	–	$C_{21}H_{24}O_{10}$	MS, standard, and literature[6]	Ph_glu

Identified by [1]Laaksonen et al., 2017; [2]Liu, Marsol-Vall, Kortesiniemi, & Yang, 2020; [3]Montern, Herrero, Ibáñez, & Cifuentes, 2013; [4]Ramirez-Ambrosi et al., 2013; [5]Kolniak-Ostek, 2016; [6]Demirci, Ipek, Gul, Ozen, & Demirtas, 2018.

studies (Laaksonen et al., 2017; Kolnia-Ostek, 2016). Therefore, peak 9, peak 13, and peak 14 were all assigned as isomers of ferulic acid hexoside.

With regard to flavan-3-ols, two monomer flavan-3-ols, four dimers, and one trimer were detected in the apple juice and cider samples on the basis of MS spectra. Peak 16 and 18 were identified as (+)-catechin and (-)-epicatechin, respectively, on the basis of their retention times, UV–Vis, and MS spectra and the comparison to that of the authentic compounds. According to previous reports, only B-type procyanidins were found in apple juices, and apple juices had much lower contents of oligomeric flavan-3-ols than the corresponding apple fruits (Hellström, Törrönen, & Mattilav, 2009). Peak 15, 17, 19, and 21 presented similar UV–Vis and mass spectra, with deprotonated molecular ion at *m/z* 577 and protonated molecular ion at *m/z* 579, were tentatively identified as PC dimers (B-type) according to their MS spectra. Peak 20 was tentatively identified as an isomer of PC trimer with a protonated molecular ion at *m/z* 867.2171 and yielded fragment ions at *m/z* 577.1326 and *m/z* 291.0875.

Concerning flavonols, five quercetin derivatives, two kaempferol derivatives, one isorhamnetin derivative, and one quercetin aglycone were detected in the apple ciders and their corresponding juices. Peak 22 and peak 23 presented the identical $[M + H]^+$ at *m/z* 465 with a high energy function ion at *m/z* 303, owing to a loss of a hexose moiety (162 amu). According to the retention times and fragmentation patterns of the reference standards, peak 23 was identified as quercetin-3-*O*-glucoside and peak 22 was tentatively identified as quercetin-3-*O*-galactoside based on the previous literature data (Laaksonen et al., 2017; Ramirez-Ambrosi, Abad-Garcia, Viloria-Bernal, Garmon-Lobato, Berrueta, & Gallo, 2013). Peak 24 and peak 27 both presented protonated molecules $[M + H]^+$ at *m/z* 435 and peak 27 also yielded a fragmentation ion at *m/z* 303, owing to loss of pentosyl group (132 amu). Thus, peak 24 and peak 27 were tentatively identified as quercetin pentoside conjugates. Peak 28 was tentatively identified as quercetin-3-*O*-rhamnoside based on the protonated molecular ion $[M + H]^+$ at *m/z* 449.1058 and fragment ion at *m/z* 317.0486, which indicated the cleavage of rhamnosyl group (146 amu). Peak 25 ($\lambda_{max} = 352$ nm) and peak 26 ($\lambda_{max} = 352$ nm) both presented deprotonated molecules [M - H]- at *m/z* 447 and protonated molecules $[M + H]^+$ at *m/z* 449 and yielded fragment ions at 287 (positive mode), indicating loss of hexose moiety (162 amu), were tentatively identified as kaempferol hexoside conjugates (Ramirez-Ambrosi et al., 2013). Only four dihydrochalcones were identified in the apple juices and their corresponding ciders, as shown in Table 3. Peak 31 was identified as hydroxyphloretin monoglycoside, by comparing its MS data with reported in the literature (Laaksonen et al., 2017; Ramirez-Ambrosi et al., 2013). Peak 32 ($\lambda_{max} =$ 284 nm) and 33 ($\lambda_{max} = 283$ nm) both presented deprotonated molecules [M - H]- at *m/z* 567 and protonated molecules $[M + H]^+$ at *m/z* 569 with high energy function fragments at *m/z* 273 (negative mode) and *m/z* 275 (negative mode), indicating loss of pentosyl hexoside (294 amu). Thus, they were tentatively identified as phloretin-pentosyl-hexosides. According to the previous studies, phloretin-2′-*O*-xyloglucoside was the most abundant compound among the dihydrochalcone compounds in apple juices, and the MS data also agreed with the structure, thus, peak 32 were tentatively identified as phloretin-2′-*O*-xyloglucoside (Xiao et al., 2017). Peak 34, another major dihydrochalcone compound in apple products, was identified as phloretin-2′-*O*-glucoside based on retention times, UV–Vis spectra, and mass spectra of the aid authentic standards.

3.3. Quantification of phenolic compounds

Table 4 shows the contents of major phenolic groups in apple products made from different apple cultivars. The contents of total hydroxycinnamic acids (THA), flavonols (TAO), flavan-3-ols (TAL), and dihydrochalcones (TDC) were calculated as the sum of the individual compounds in the corresponding groups. The total content of phenolics (TPC, sum of individual phenolics) (48.74 mg/100 mL on average) in Finnish apple juices were slightly higher than the levels reported previously for the commercial apple cultivars (‘Gala’, ‘Lis Gala’, and ‘Fuji Suprema’) (Alberti et al., 2016). The variations of TPC can be ascribed to the different genetic background of the apple cultivars (Satora, Sroka, Duda-Chodak, Tarko, & Tuszyński, 2008). Among all apple cultivars, ‘Aino’ showed the highest TPC, with an amount of 100.81 mg/100 mL, whereas ‘Lepaan Meloni’ contained the lowest amount of TPC (17.91 mg/100 mL).

Moreover, the level of individual phenolic compounds also significantly depends on the different apple cultivars (Satora et al., 2008). The major polyphenolic compounds in all the studied juice samples were hydroxycinnamic acids, accounting for around 80% of total phenolic contents on average, followed by flavan-3-ols (~10%), flavonols (~5%), and dihydrochalcones (~5%) in the current study. The higher contents of hydroxycinnamic acids in Finnish apple cultivars (38.27 mg/100 mL) can be mainly ascribed to the lower temperature and better exposure to the sunlight during the maturity and harvest time (Fernández-Jalao, Sánchez-Moreno, & De Ancos, 2019). Among the hydroxycinnamic acids, 5-*O*-caffeoylquinic acid has been reported as the second highest phenolic compound only after procyanidin B in apples (Heinmaa et al., 2017). However, 5-*O*-caffeoylquinic acid were detected as the most abundant phenolic acids in the apple juices, with concentrations ranging from 5.65 to 44.17 mg/100 mL in all the studied apple juices, and it is known as the most water-soluble phenolic compound in apple (Laaksonen et al., 2017). As the most abundant group of phenolic compounds in apples (accounting for 30–70% of TPC), flavan-3-ols were present at lower concentration in the apple juices compared to the levels found in the corresponding apple fruits. This can be ascribed to the low solubility of procyanidins (Fernández-Jalao et al., 2019). The flavan-3-ol profiles can be also influenced by the apple cultivars according to Table 4. For example, ‘Aino’ contained 14.84 mg/100 mL of TFL, whereas ‘Lepaan Meloni’ only had 2.15 mg/100 mL of TFL. Extraction methods also affected the detected concentration of procyanidins. As for the dihydrochalcones, phloretin-2′-*O*-glucoside and phloretin-2′-*O*-xyloglucoside were the other two major dihydrochalcones in apple juices, and

Table 4
Concentrations of major group of phenolic compounds in apple juices and their corresponding ciders (mg/100 mL).

Samples	Free HCAs	HCA derivatives	Sum of HCAs	Monomeric flavan-3-ols	Oligomeric flavan-3-ols	Sum of flavan-3-ols	Sum of flavonols	Sum of dihydrochalcones	Sum of phenolic compounds
Juices									
AP	0.35 ± 0.02d	28.80 ± 1.34c	29.15 ± 1.36c	1.94 ± 0.07d	2.54 ± 0.07f	4.48 ± 0.11 g	6.88 ± 0.18 g	3.24 ± 0.11c	41.22 ± 1.63c
Kr	0.22 ± 0.02c	28.40 ± 0.76c	28.62 ± 0.78c	1.18 ± 0.05b	4.65 ± 0.08 g	5.83 ± 0.15f	8.46 ± 0.21 h	2.01 ± 0.02a	40.26 ± 1.45c
LM	0.03 ± 0.01a	11.70 ± 0.82a	11.73 ± 0.83a	1.38 ± 0.02c	0.95 ± 0.04d	2.33 ± 0.08b	2.15 ± 0.06c	2.65 ± 0.02b	17.91 ± 1.23a
LK	0.06 ± 0.01a	45.23 ± 0.82e	45.29 ± 0.83e	2.05 ± 0.02d	0.07 ± 0.02a	2.12 ± 0.05c	1.51 ± 0.02b	2.26 ± 0.12ab	51.10 ± 2.01d
An	0.70 ± 0.02e	68.10 ± 2.49 g	68.80 ± 2.51 h	1.96 ± 0.07d	9.20 ± 0.17i	11.16 ± 0.09 h	14.84 ± 0.11i	15.22 ± 0.32f	100.81 ± 6.69f
GB	ND	42.69 ± 1.65e	42.69 ± 1.65e	3.33 ± 0.02 g	1.88 ± 0.03e	3.82 ± 0.05e	4.89 ± 0.04f	6.85 ± 0.25e	57.76 ± 2.26e
Lt	0.04 ± 0.01a	24.69 ± 0.43b	24.73 ± 0.44b	2.37 ± 0.06f	0.49 ± 0.05c	2.40 ± 0.08b	1.57 ± 0.11b	2.81 ± 0.34b	31.47 ± 0.96b
Pk	ND	57.69 ± 2.27 g	57.69 ± 2.27 g	0.97 ± 0.00a	0.03 ± 0.01a	1.00 ± 0.06a	0.63 ± 0.05a	2.37 ± 0.02b	61.65 ± 2.59e
Tr	0.14 ± 0.02b	36.68 ± 0.99d	36.82 ± 1.01 d	1.11 ± 0.01ab	0.96 ± 0.04d	2.07 ± 0.19d	2.99 ± 0.19e	1.80 ± 0.01a	42.71 ± 1.53c
Js	0.03 ± 0.01a	25.72 ± 0.87b	25.75 ± 0.88b	1.16 ± 0.04b	0.24 ± 0.06b	1.40 ± 0.09d	2.41 ± 0.11d	3.47 ± 0.14c	32.79 ± 1.39b
Hg	0.07 ± 0.02a	49.62 ± 1.56f	49.69 ± 1.58f	2.18 ± 0.03e	0.24 ± 0.01b	2.42 ± 0.07d	2.50 ± 0.08d	4.08 ± 0.03d	58.44 ± 1.29e
Mean	0.15A	38.12B	38.27B	1.78B	1.93C	3.71C	4.44B	4.25B	48.74B
Ciders made from SC1116									
AP	0.41 ± 0.04d	26.11 ± 1.73c	26.52 ± 1.77c	0.27 ± 0.03a	0.11 ± 0.02a	0.38 ± 0.05 g	3.91 ± 0.06e	2.38 ± 0.12c	33.08 ± 2.16c
Kr	0.14 ± 0.03a	20.46 ± 0.33b	20.60 ± 0.36b	0.51 ± 0.03b	0.41 ± 0.04b	0.92 ± 0.05ef	2.74 ± 0.06c	0.90 ± 0.07a	24.74 ± 0.49b
LM	0.33 ± 0.03c	13.59 ± 0.33a	13.92 ± 0.36a	1.08 ± 0.02d	0.43 ± 0.03b	1.51 ± 0.08b	1.26 ± 0.06b	2.00 ± 0.05c	18.25 ± 0.58a
LK	0.11 ± 0.01a	42.76 ± 0.59e	42.87 ± 0.60e	1.47 ± 0.07e	0.18 ± 0.03a	1.65 ± 0.05c	1.20 ± 0.03b	2.04 ± 0.02c	47.58 ± 1.26e
An	0.72 ± 0.05e	53.44 ± 1.75 g	54.16 ± 1.80 g	1.38 ± 0.03e	4.41 ± 0.10e	5.79 ± 0.08c	6.25 ± 0.04 g	7.76 ± 0.19 g	69.54 ± 2.83 g
GB	ND	23.17 ± 1.10c	23.17 ± 1.10c	2.81 ± 0.03 h	1.20 ± 0.04d	4.01 ± 0.06ef	3.44 ± 0.09d	4.17 ± 0.18f	33.59 ± 1.39c
Lt	0.30 ± 0.03c	22.80 ± 1.43c	23.10 ± 1.46c	2.18 ± 0.01 g	1.06 ± 0.06 cd	3.24 ± 0.09d	2.51 ± 0.07c	2.39 ± 0.14c	30.17 ± 1.57c
Pk	0.43 ± 0.04d	51.99 ± 1.20 g	52.42 ± 1.24 g	0.73 ± 0.02c	0.59 ± 0.06b	1.32 ± 0.05a	0.89 ± 0.06a	1.78 ± 0.04b	55.82 ± 1.39f
Tr	0.19 ± 0.03b	33.52 ± 1.04d	33.71 ± 1.07d	0.87 ± 0.01 cd	0.81 ± 0.08c	1.68 ± 0.06f	2.48 ± 0.09c	1.21 ± 0.24ab	38.26 ± 1.51d
Js	0.09 ± 0.02a	26.61 ± 0.17c	26.70 ± 0.19c	1.00 ± 0.03d	0.40 ± 0.04b	1.40 ± 0.01e	2.49 ± 0.05c	3.01 ± 0.04d	33.20 ± 0.85c
Hg	0.29 ± 0.05c	43.38 ± 1.25f	43.67 ± 1.30f	1.68 ± 0.02f	0.50 ± 0.04b	2.18 ± 0.08 h	4.80 ± 0.07f	3.41 ± 0.08e	53.56 ± 1.63f
mean	0.27B	32.53A	32.80A	1.27A	0.92A	2.19A	2.91A	2.82A	39.80A
Ciders made from SP3796									
AP	0.54 ± 0.05d	24.73 ± 1.11c	25.27 ± 1.16c	0.64 ± 0.02a	0.16 ± 0.02a	0.80 ± 0.18f	3.79 ± 0.15f	2.03 ± 0.21b	31.73 ± 1.55b
Kr	0.14 ± 0.03a	17.73 ± 0.47b	17.87 ± 0.50b	0.80 ± 0.02b	3.11 ± 0.11f	3.91 ± 0.21f	6.84 ± 0.09 g	1.32 ± 0.12a	26.83 ± 0.57b
LM	0.30 ± 0.03b	12.04 ± 0.27a	12.34 ± 0.30a	1.13 ± 0.05c	0.52 ± 0.03bc	1.65 ± 0.06b	1.36 ± 0.05b	2.21 ± 0.07b	17.03 ± 0.82a
LK	0.12 ± 0.03a	35.53 ± 1.94de	35.65 ± 1.97de	1.81 ± 0.02e	0.16 ± 0.02a	1.97 ± 0.05c	1.28 ± 0.03b	1.92 ± 0.03b	40.66 ± 2.22d
An	0.74 ± 0.06e	49.48 ± 2.03f	50.22 ± 2.09f	1.53 ± 0.05d	3.32 ± 0.09f	4.85 ± 0.08d	5.44 ± 0.15 h	7.70 ± 0.24f	64.89 ± 4.51f
GB	ND	20.20 ± 2.38bc	20.20 ± 2.38bc	3.00 ± 0.05 g	1.36 ± 0.06e	4.36 ± 0.08d	3.17 ± 0.03e	4.22 ± 0.08e	30.59 ± 2.89b
Lt	0.30 ± 0.02b	19.93 ± 1.07bc	21.23 ± 1.09bc	2.21 ± 0.05f	1.27 ± 0.07e	3.48 ± 0.09c	2.65 ± 0.12d	2.45 ± 0.32bc	28.53 ± 1.51b
Pk	0.41 ± 0.04c	49.92 ± 2.06f	51.33 ± 2.10f	0.81 ± 0.03b	0.60 ± 0.06c	1.41 ± 0.06a	0.90 ± 0.07a	1.49 ± 0.03a	54.54 ± 2.26e
Tr	0.23 ± 0.05b	30.17 ± 2.10d	30.40 ± 2.15d	0.97 ± 0.02c	0.92 ± 0.03d	1.89 ± 0.08d	2.68 ± 0.07d	1.44 ± 0.04a	35.50 ± 2.81c
Js	0.31 ± 0.04b	24.56 ± 0.12c	24.87 ± 0.16c	1.04 ± 0.03c	0.43 ± 0.05b	1.47 ± 0.11 cd	2.08 ± 0.06c	2.42 ± 0.09c	30.41 ± 0.32b
Hg	0.27 ± 0.05b	39.96 ± 1.24e	40.23 ± 1.29e	1.78 ± 0.02e	0.41 ± 0.04b	2.19 ± 0.09e	3.46 ± 0.06f	3.68 ± 0.12d	49.15 ± 3.52e
Mean	0.31B	29.48A	29.96A	1.43A	1.11B	2.54B	3.06A	2.81A	37.26A

Results are shown as means ± standard deviations (three replicates for apple juices, duplicate measurements of duplicate fermentations for apple ciders). ND: not detected. Significant differences among juices and ciders of eleven apple cultivars produced with the same yeast are shown with lower case letters a-i and the

differences among juices and ciders are shown in upper case letters A-B (one way ANOVA with Tukey's post hoc test, $p < 0.05$). Abbreviations of apple cultivars refer to Table 1, and abbreviations of phenolic compounds refer to Table 3.

their concentrations mainly depended on the apple cultivars. For example, the contents of total dihydrochalcones in 'Aino' reached up to 15.22 mg/100 mL, remarkably higher than the content in the other apple cultivars.

Table 4 summarizes the main phenolic compound classes of apple juices and ciders, as well as the content of released free aglycones and different types of derivatives of phenolic compounds. In general, the ciders (SC1116: average 39.80 mg/100 mL; SP3796: average 37.26 mg/100 mL) showed less contents of total phenolic compounds than the apple juices (average 48.74 mg/100 mL), however, the phenolic evolution was also cultivar dependent, which was in accordance with the previous studies (Alberti et al., 2016; Laaksonen et al., 2017). For example, total contents of phenolic compounds remained stable in 'Lepaan Meloni' and 'Juuso' samples during the fermentation. Higher contents of free hydroxycinnamic acids were found in the apple ciders, which can be ascribed to cleavage of the glycosidic bonds during fermentation process (Laaksonen et al., 2017). In contrast, decreased amounts of total flavonols and dihydrochalcones were found in cider samples. Yeast strains also affected the phenolic profiles, although the impact was less significant. According to Table 4, significant higher concentrations of procyanidins were found in ciders produced with SP3796 (1.11 mg/100 mL on average) when comparing with those with SC1116 (0.92 mg/100 mL on average). Around 7–10 times of polysaccharides were released in the ciders produced with *S. pombe* strains than those with *S. cerevisiae* strains, which has been demonstrated well in previous studies (Benito, 2019; Benito, Calderón, & Benito, 2019). The formation of polysaccharide-procyanidin complexes can stabilize the reactive procyanidins, resulted in a higher amount of procyanidins in SP3796 ciders. According to Supplementary Table 1, ciders produced with SP3796 from certain apple juices contained less 5-*O*-caffeoylquinic acid (5-CaQA), 4-*O*-caffeoylquinic acid (4-CaQA), 3-*O*-*p*-coumaroylquinic acid (3-ClqA), and 4-*O*-*p*-coumaroylquinic acid (4-ClqA) when compared with ciders produced with SC1116 from the same apple juice. However, slightly higher amount of (+)-catechin (Cat) were found in ciders produced with SP3796 (0.41 mg/100 mL on average) than those with SC1116 (0.20 mg/100 mL on average).

Partial least squares discriminant analysis (PLS-DA) was used to visualize the overall effects of *S. cerevisiae* and *S. pombe* yeasts in the cider fermentations and the differences between the apple cider and juice samples. In PLS plots of Fig. 1A, 78% of chemical variables

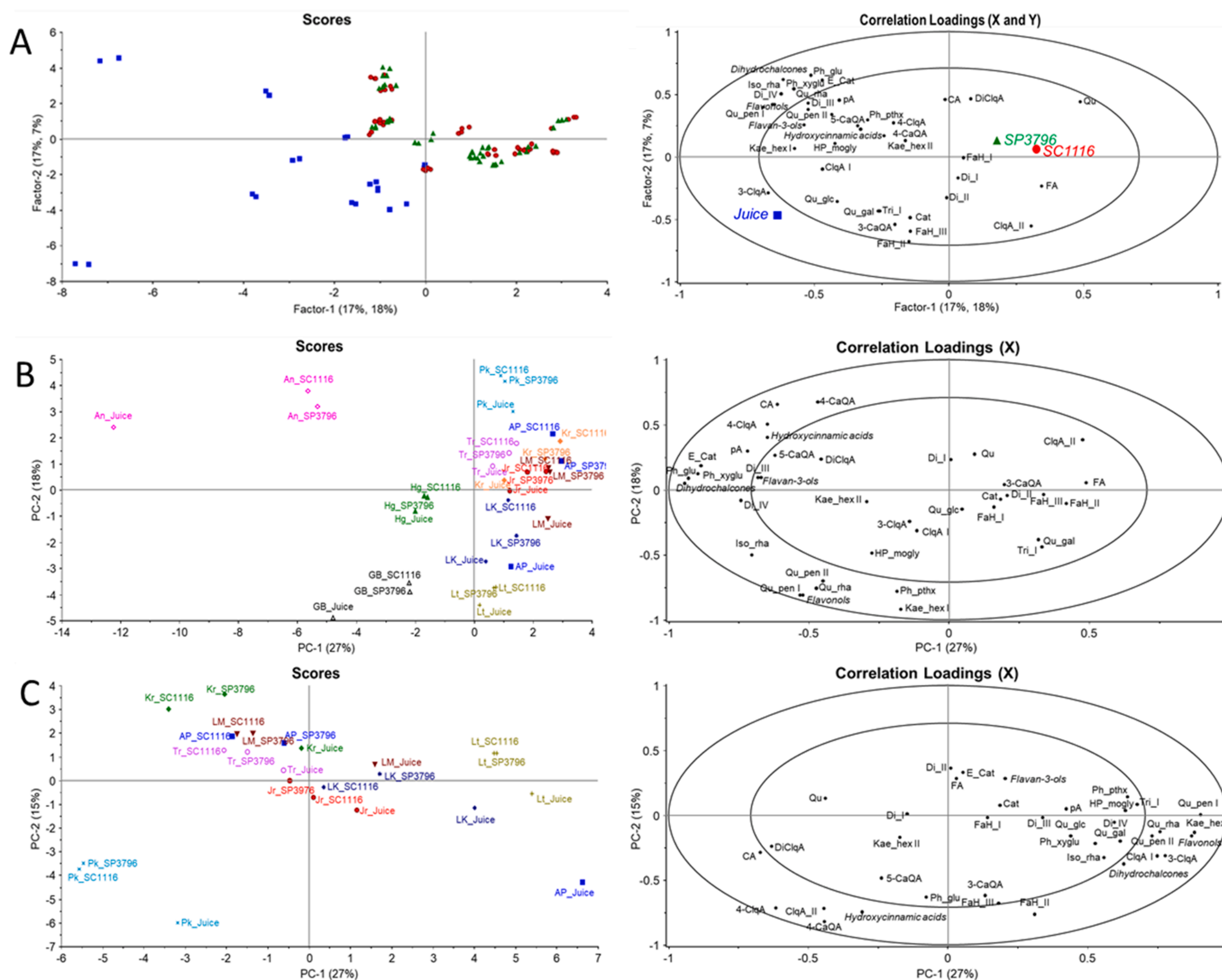


Fig. 1. PCA and PLS-DA models of phenolic compounds as X-data ($n = 34$) to elucidate the differences between apple ciders (produced from *S. cerevisiae* 1116 and *S. pombe* 3796) and their corresponding juices. Juices with blue rectangles, SP ciders with green triangles, SC ciders with red circles. A. all cultivars (PLS-DA) B. all cultivars (PCA); C. dominant cultivars ('Aino' and 'Gustavs Bästa') in B excluded (PCA). Apple cultivar shown in different colors and symbols. Abbreviations of apple cultivars refer to Table 1, and abbreviations of phenolic compounds refer to Table 3.

explained 69% of variation in seven factors (R^2 = 0.8262, validated R^2 = 0.7401). The apple juices were separated clearly from the cider samples along Factor-1. Overall, the juices are separated from the ciders by higher content of most of the phenolic compounds. Among the identified phenolic compounds, the apple juices were highly associated with flavonols and dihydrochalcones, due to higher contents of these compounds in the juices than the corresponding ciders. In the juices, the main flavonols were isorhamnetin-3-*O*-rhamnoside (Iso_rha), quercetin-3-*O*-rhamnoside (Qu_rha), and quercetin pentoside I (Qu_pen I). Dihydrochalcones were primarily as phloretin-2′-*O*-glucoside (Ph_glu) and phloretin-2′-*O*-xyloglucoside (Ph_xyglu). Other compounds, such as (-)-epicatechin (E_Cat), PC dimer IV (Di_IV), and 3-*O*-*p*-coumaroylquinic acid (3-ClqA), aslo showed positive correlation with apple juices. Yet, this model did not detect any difference in phenolic profiles of ciders produced with SC1116 from those produced with SP3796 yeast.

3.4. Variation of phenolic composition among apple cultivars

Principal Component Analysis (PCA) models were used to establish the relationships between the phenolic compositions and apple cultivars. The impact of cultivars on phenolic compositions of apple juice and cider samples are shown in Fig. 1B and Fig. 1C. Fig. 1B shows the overall effects of eleven apple cultivars on the phenolic compositions in apple juices and ciders, with PC1 and PC2 accounting for 27% and 18%, respectively. Cultivar ‘Aino’, ‘Gustavs Bästa’, and ‘Hyvingiensis’ were clearly separated with others and located in the negative side of PC1. Among which, both juice and cider of ‘Aino’ showed a strong correlation with hydroxycinnamic acids and dihydrochalcones, mainly as caffeic acid (CA), *p*-coumaric acid (pA), 4-*O*-*p*-coumaroylquinic acid (4-ClqA), phloretin-2′-*O*-glucoside (Ph_glu), and phloretin-2′-*O*-xyloglucoside (Ph_xyglu). (-)-Epicatechin (E_Cat) and PC dimer IV (DI_IV) also correlated strongly to cultivar ‘Aino’. On the contrary, ‘Gustavs Bästa’ located closely to flavonols in the loading plots, due to the abundance of quercetin pentoside I (Qu_pen I), quercetin pentoside II (Qu_pen II), quercetin-3-*O*-rhamnoside (Qu_rha), and isorhamnetin-3-*O*-rhamnoside (Iso_rha). Aside from these three cultivars, in the plots of Fig. 1C, cultivar ‘Pieksämäki’ was different from the other apple cultivars, due to the higher content of hydroxycinnamic acids, primarily as caffeic acid (CA), 4-*O*-caffeoylquinic acid (4-CaQA), coumaroylquinic acid II (ClqA_II), and 4-*O*-*p*-coumaroylquinic acid (4-ClqA). Quercetin pentoside I (Qu_pen I) also correlated strongly to cultivar ‘Luotsi’. Furthermore, Supplementary Fig. 4A-C showed similar patterns of cultivar effect on both juices and ciders. The results indicated that cultivar difference is the major factor influencing phenolic profiles of ciders, which also has been indicated by the findings of previous apple studies (Laaksonen et al., 2017).

Genetic clustering of selected apple cultivars for cider analyses, including nine Finnish wild apple cultivars and one reference apple cultivar ‘Discovery’, which is an early season dessert apple cultivar, were studied in the current study. The trueness to type was initially analyzed by comparative genetic analyses as described in our previous study (Heinonen and Bitz, 2019) and all the ten apple cultivars (excluded cultivar ‘Hyvingiensis’) for cider analyses showed the trueness to type and have identity exactly as expected (results not shown). Genetic clustering revealed the presence of three main groups of apples with subgroups as shown in Supplementary Fig. 2. ‘Juuso’, ‘Kersti’, and ‘Turso’ were clustered together with a reference apple cultivar ‘Discovery’, they could be considered as a group of putative dessert apples. ‘Luotsi’ and ‘Aino’ formed a cluster of their own. ‘Aino’ is a Finnish seed born variety and originate from Northern Finland. ‘Luotsi’ is color mutation of Syysviiru (foreign variety) and this mutation has happened in Southern Finland. Interestingly, He et al. (2021) also found out that ‘Luotsi’ and ‘Aino’ separated from other cultivars (same cultivars as in present study) because ciders from these two correlated positively with 3-methylpropan-1-ol and negatively with methyl acetate. On the other hand, ‘Alasen Punainen’, ‘Gustavs Bästa’, ‘Lepaan Meloni’, ‘Lohjan Kirkas’ and ‘Pieksämäki’ can be grouped together with the European wild apples (*M. sylvestris* L.), which have been traditionally used for cider making, thus, those apple cultivars can be designated as putative cider apples. For example, ciders made from ‘Lepaan Meloni’, ‘Lohjan Kirkas’, and ‘Gustavs Bästa’ separated from the other apple cultivars based on differential contents of acetate esters and higher alcohols (He et al., 2021). However, the separation of apple group is putative, and more genetic studies are needed to confirm if genetic clustering is correlated with cider characteristics. Fig. 2A and B shows the relationships between apple groups (by clustering methods) and phenolic compositions. As shown in the PCA model (Fig. 2A), cultivar ‘Aino’ was clearly separated from the other apple cultivars by PC1 with strong positive correlation with dihydrochalcones and hydroxycinnamic acids. PLS-DA models were further applied to visualize the impact of apple groups (by clustering method, include apple cultivars grouped together with dessert apples and wild apples as Y-data, n = 2, cultivar ‘Aino’ was excluded) on the phenolic composition (X-data, n = 34). The two apple groups are classified with a model of five validated factors (R^2 = 0.9605, validated R^2 = 0.8343). The apple cultivars grouped together with the cider apples were separated clearly from those in the putative dessert apple group along Factor-1. Among the identified phenolic compounds, the putative cider apple group were highly associated with flavonols and dihydrochalcones, primarily as kaempferol-3-*O*-hexoside I (Kae_hex I), quercetin-3-*O*-rhamnoside (Qu_rha), quercetin pentoside II (Qu_pen II), isorhamnetin-3-*O*-rhamnoside (Iso_rha), phloretin-2′-*O*-pentose-hexose (Ph_pthx), and phloretin-2′-*O*-glucoside (Ph_glu).

Moreover, the relationships between sensory properties and phenolic compositions were also studied in the current study with the sensory data from previous study for selected samples (He et al., 2021). A PCA model was applied to observe the changes in the average phenolic-sensory properties of apple ciders produced from SC1116 and SP3796 (Supplementary Fig. 5) in selected apple ciders (‘Gustavs Bästa’, ‘Turso’, ‘Juuso’, and ‘Hyvingiensis’). In this model, PC1 and PC2 accounting for 43% and 27%, respectively. Higher contents of hydroxycinnamic acids, such as caffeic acid (CA), *p*-coumaric acid (pA), and 5-*O*-caffeoylquinic acid (5-CaQA), and flavan-3-ols, such as PC dimer III (Di_III), located on the negative side of the PC1, contributed to *mouth-drying* and *puckering astringency*. The higher concentration of procyanidins resulted in a higher bitterness and astringency taste in ciders as previously reported (Symoneaux et al., 2015). No correlation was detected between phenolic composition and *bitterness* and *sourness*, which was in accordance with the previous studies (Hufnagel and Hofmann, 2008). Due to the use of untrained panelists for the sensory evaluation, more studies are needed with trained sensory panels to confirm these results concerning bitterness and astringent characteristics.

4. Conclusion

In conclusion, phenolic compounds in apple juices and ciders (fermented with *S. cerevisiae* 1116 and *S. pombe* 3796) produced from eleven Finnish local apple cultivars were identified and quantified. The phenolic compounds in ciders were mainly originated from apple juices, and the contents of these compounds generally decreased during the fermentation process. The contents of hydroxycinnamic acids, flavan-3-ols, flavonols, and dihydrochalcones in apple ciders were mainly lower than the levels in corresponding juices. However, the phenolic changes were clearly dependent on the cultivars. Certain cultivars contained remarkably higher dihydrochalcones and hydroxycinnamic acids when comparing with other cultivars. The putative cider apple group were separated from the putative dessert apple group using multivariate modes with higher flavonols and dihydrochalcones, such as kaempferol-3-*O*-hexoside, quercetin derivatives, and phloretin glucoside. Yeast fermentation also influenced the phenolic profiles in apple ciders fermented with both yeast strains. Significantly higher concentrations of procyanidins were found in SP3796 ciders in comparison to SC1116 ciders. Comparing with corresponding juices, apple ciders produced

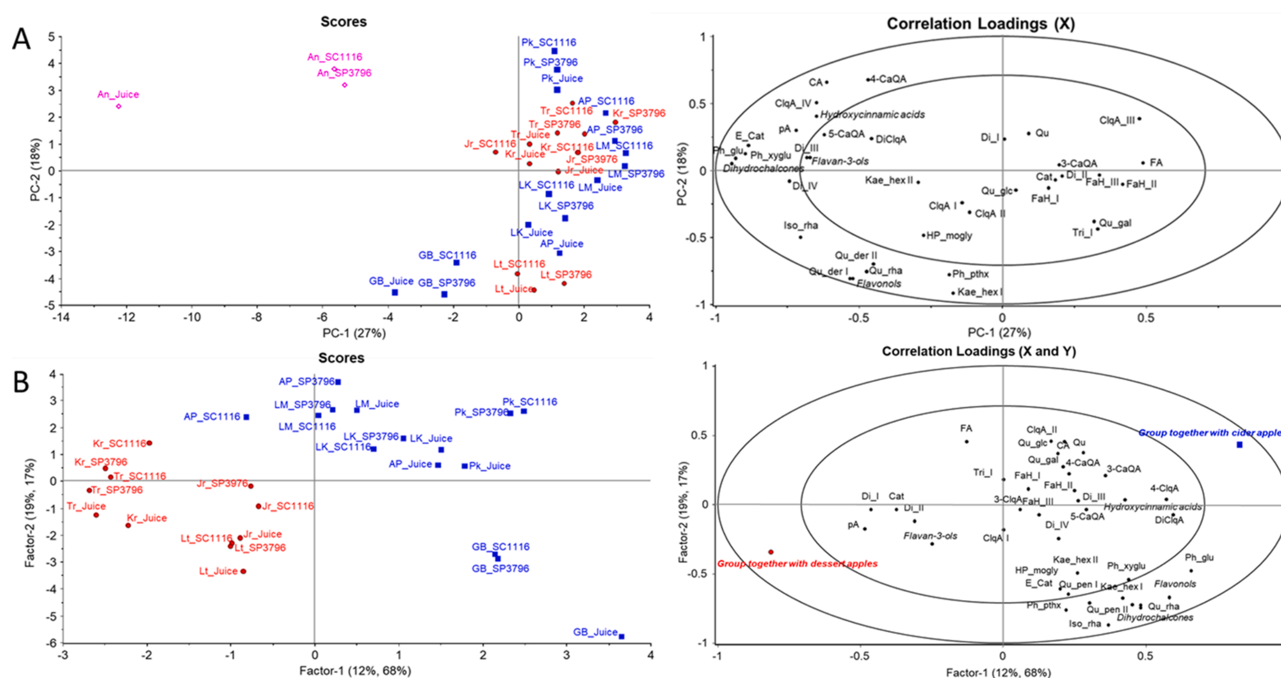


Fig. 2. PCA and PLS-DA models of phenolic compounds as X-data (n = 34) to illustrate the differences between apple grouped together with dessert and wild apple cultivars. A. all cultivars (PCA); B. dominant cultivars excluded 'Aino' (PLS-DA). 'Aino': pink diamond; Apple grouped together with wild apples: blue rectangles; Apple grouped together with dessert apples: red circles. Abbreviations of apple cultivars refer to Table 1, and abbreviations of phenolic compounds refer to Table 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with *S. pombe* retained higher concentrations of (+)-catechin and procyanidins whereas higher amounts of 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3-*O*-*p*-coumaroylquinic acid, and 4-*O*-*p*-coumaroylquinic acid were found in ciders produced with SC1116. In addition, the differences of phenolic composition, especially caffeic acid, *p*-coumaric acid, 5-*O*-caffeoylquinic acid, and PC dimer III in apple ciders may also contribute to differences in sensory quality, such as mouth-drying astringency and puckering astringency. In addition to the reduction of malic acid and sourness taste, the potential of using *S. pombe* stains in cider making and their effects on the phenolic composition have been demonstrated well in the current study. However, this needs more investigations in the future.

CRediT authorship contribution statement

Wenjia He: Methodology, Investigation, Validation, Data curation, Formal analysis, Visualization, Writing – original draft. **Oskar Laaksonen:** Methodology, Visualization, Writing – review & editing, Supervision. **Ye Tian:** Methodology, Writing – review & editing. **Maarit Heinonen:** Resources, Writing – review & editing. **Lidija Bitz:** Methodology, Writing – review & editing. **Baoru Yang:** Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wenjia HE reports financial support was provided by China Scholarship Council. Wenjia HE reports financial support was provided by Niemi-Säätiö foundation.

Acknowledgements

This study was financially supported by China Scholarship Council (China, CSC 201806790009) and Niemi-Säätiö foundation (Finland, Project 20190010).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.131437.

References

Albert, L., Franck, P., Gilles, Y., & Plantegenest, M. (2017). Impact of agroecological infrastructures on the dynamics of dysaphis plantaginea (hemiptera: Aphididae) and its natural enemies in apple orchards in Northwestern France. *Environmental Entomology, 46*(3), 528–537. https://doi.org/10.1093/ee/nvx054

Alberti, A., Machado dos Santos, T. P., Ferreira Zielinski, A. A., Eleutério dos Santos, C. M., Braga, C. M., Mottin Demiate, I., & Nogueira, A. (2016). Impact on chemical profile in apple juice and cider made from unripe, ripe, and senescent dessert varieties. *LWT - Food Science and Technology, 65*, 436–443. https://doi.org/10.1016/j.lwt.2015.08.045

Benito, Á., Calderón, F., & Benito, S. (2019). Mixed alcoholic fermentation of *Schizosaccharomyces pombe* and *Lachancea thermotolerans* and its influence on mannose-containing polysaccharides wine composition. *Amb Express, 9*(1), 1–8. https://doi.org/10.1186/s13568-019-0738-0

Benito, S. (2019). The impacts of *Schizosaccharomyces* on winemaking. *Applied microbiology and biotechnology, 103*(11), 4291–4312. https://doi.org/10.1007/s00253-019-09827-7

Bitz, L., Heinonen, M., Moisander, S., Tanhuanpää, P., & Sarvarinne, J. (2019). Differential allelic richness between *Malus sylvestris* L. and *Malus* × *domestica* Borkh. from Finland as a measure of genetic loss. *Sustainability (Switzerland), 11*(24), 1–16. https://doi.org/10.3390/SU11246949

De Paepe, D., Coudijzer, K., Noten, B., Valkenborg, D., Servaes, K., De Loose, M., … Van Droogenbroeck, B. (2015). A comparative study between spiral-filter press and belt press implemented in a cloudy apple juice production process. *Food Chemistry, 173*, 986–996. https://doi.org/10.1016/j.foodchem.2014.10.019

Demirci, M. A., Ipek, Y., Gul, F., Ozen, T., & Demirtas, I. (2018). Extraction, isolation of heat-resistance phenolic compounds, antioxidant properties, characterization and purification of 5-hydroxymaltol from Turkish apple pulps. *Food Chemistry, 269*(May), 111–117. https://doi.org/10.1016/j.foodchem.2018.06.147

Feng, S., Yi, J., Li, X., Wu, X., Zhao, Y., Ma, Y., & Bi, J. (2021). Systematic review of phenolic compounds in apple fruits: Compositions, distribution, absorption, metabolism, and processing stability. *Journal of Agricultural and Food Chemistry., 69*(1), 7–27. https://doi.org/10.1021/acs.jafc.0c05481

Fernández-Jalao, I., Sánchez-Moreno, C., & De Ancos, B. (2019). Effect of high-pressure processing on flavonoids, hydroxycinnamic acids, dihydrochalcones, and antioxidant activity of apple 'Golden Delicious' from different geographical origin. *Innovative Food Science and Emerging Technologies, 51*, 20–31. https://doi.org/10.1016/j.ifset.2018.06.002

Rodriguez Gamboa, J. C., Albarracin E, E. S., da Silva, A. J., L. de Andrade Lima, L., & E. Ferreira, T. A. (2019). Wine quality rapid detection using a compact electronic nose system: Application focused on spoilage thresholds by acetic acid. *LWT - Food Science and Technology, 108*, 377–384.

García, D., Miñarro, M., & Martínez-Sastre, R. (2018). Birds as suppliers of pest control in cider apple orchards: Avian biodiversity drivers and insectivory effect. *Agriculture, Ecosystems and Environment, 254*(November 2017), 233–243. https://doi.org/10.1016/j.agee.2017.11.034

Girschik, L., Jones, J. E., Kerslake, F. L., Robertson, M., Dambergs, R. G., & Swarts, N. D. (2017). Apple variety and maturity profiling of base ciders using UV spectroscopy. *Food Chemistry, 228*, 323–329. https://doi.org/10.1016/j.foodchem.2017.02.012

Harper, H., Winfield, M. O., Copas, L., Przewieslik-Allen, S. A., Barker, G. L. A., Burridge, A., … Edwards, K. J. (2020). The Long Ashton Legacy: Characterising United Kingdom West Country cider apples using a genotyping by targeted sequencing approach. *Plants, People, Planet, 2*(2), 167–175. https://doi.org/10.1002/ppp3.v2.210.1002/ppp3.10074

He, W., Liu, S., Heponiem, P., Heinonen, M., Marsol-vall, A., Ma, X., … Laaksonen, O. (2021). Effect of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains on chemical composition and sensory quality of ciders made from Finnish apple cultivars. *Food Chemistry, 345*, Article 128833. https://doi.org/10.1016/j.foodchem.2020.128833

Heinmaa, L., Moor, U., Põldma, P., Raudsepp, P., Kidmose, U., & Lo Scalzo, R. (2017). Content of health-beneficial compounds and sensory properties of organic apple juice as affected by processing technology. *LWT - Food Science and Technology, 85*, 372–379. https://doi.org/10.1016/j.lwt.2016.11.044

Heinonen, M., & Bitz, L. (2019). How to discover traditional varieties and shape in a national germplasm collection: The case of Finnish seed born apples (Malus × domestica Borkh.). *Sustainability (Switzerland), 11*(24), 7000. https://doi.org/10.3390/su11247000

Hellström, J. K., Törrönen, A. R., & Mattila, P. H. (2009). Proanthocyanidins in Common Food Products of Plant Origin. *Journal of Agricultural and Food Chemistry, 57*(17), 7899–7906. https://doi.org/10.1021/jf901434d

Hufnagel, J. C., & Hofmann, T. (2008). Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *Journal of Agricultural and Food Chemistry, 56*(19), 9190–9199. https://doi.org/10.1021/jf801742w

Ivit, N. N., Longo, R., & Kemp, B. (2020). The effect of non-*Saccharomyces* and *Saccharomyces* non-*cerevisiae* yeasts on ethanol and glycerol levels in wine. *Fermentation, 6*(3), 1–22. https://doi.org/10.3390/FERMENTATION6030077

Kalinowska, M., Bielawska, A., Lewandowska-Siwkiewicz, H., Priebe, W., & Lewandowski, W. (2014). Apples: Content of phenolic compounds vs. variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. *Plant Physiology and Biochemistry*, *84*, 169e188-188. doi:10.1016/j.plaphy.2014.09.006.

Knebel, T., Braun, P., & Dietrich, H. (2018). Degradation kinetics of anthocyanins and polyphenols during storage of red apple juice produced from red-fleshed apples. *European Food Research and Technology, 244*(10), 1741–1750. https://doi.org/10.1007/s00217-018-3086-4

Kolniak-Ostek, J. (2016). Identification and quantification of polyphenolic compounds in ten pear cultivars by UPLC-PDA-Q/TOF-MS. *Journal of Food Composition and Analysis, 49*, 65–77. https://doi.org/10.1016/j.jfca.2016.04.004

Laaksonen, O., Kuldjärv, R., Paalme, T., Virkki, M., & Yang, B. (2017). Impact of apple cultivar, ripening stage, fermentation type, and yeast strain on phenolic composition of apple ciders. *Food Chemistry, 233*, 29–37. https://doi.org/10.1016/J.FOODCHEM.2017.04.067

Li, C. X., Zhao, X. H., Zuo, W. F., Zhang, T. L., Zhang, Z. Y., & Chen, X. S. (2020). The effects of simultaneous and sequential inoculation of yeast and autochthonous *Oenococcus oeni* on the chemical composition of red-fleshed apple cider. *LWT - Food Science and Technology, 124*, 109184. https://doi.org/10.1016/j.lwt.2020.109184

Liang, X., Zhu, T., Yang, S., Li, X., Song, B., Wang, Y., … Cao, J. (2020). Analysis of phenolic components and related biological activities of 35 apple (*Malus pumila* Mill.) cultivars. *Molecules, 25*(18), 4153. https://doi.org/10.3390/molecules25184153

Lindén, L., & Iwarsson, M. (2014). Identification of weeping crabapple cultivars by microsatellite DNA markers and morphological traits. *Scientia Horticulturae, 179*, 221–226. https://doi.org/10.1016/j.scienta.2014.09.027

Liu, S., Laaksonen, O., Kortesniemi, M., Kalpio, M., & Yang, B. (2018). Chemical composition of bilberry wine fermented with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* and *Schizosaccharomyces pombe*) and *Saccharomyces cerevisiae* in pure, sequential, and mixed fermentations. *Food Chemistry, 266*, 262–274. https://doi.org/10.1016/j.foodchem.2018.06.003

Liu, S., Marsol-Vall, A., Laaksonen, O., Kortesniemi, M., & Yang, B. (2020). Characterization and Quantification of Nonanthocyanin Phenolic Compounds in White and Blue Bilberry (*Vaccinium myrtillus*) Juices and Wines Using UHPLC-DAD-ESI-QTOF-MS and UHPLC-DAD. *Journal of Agricultural and Food Chemistry, 68*(29), 7734–7744. https://doi.org/10.1021/acs.jafc.0c02842

Magalhães, F., Krogerus, K., Vidgren, V., Sandell, M., & Gibson, B. (2017). Improved cider fermentation performance and quality with newly generated *Saccharomyces cerevisiae* × *Saccharomyces eubayanus* hybrids. *Journal of Industrial Microbiology and Biotechnology, 44*(8), 1203–1213. https://doi.org/10.1007/s10295-017-1947-7

Mäkilä, L., Laaksonen, O., Alanne, A.-L., Kortesniemi, M., Kallio, H., & Yang, B. (2016). Stability of hydroxycinnamic acid derivatives, flavonol glycosides, and anthocyanins in black currant juice. *Journal of Agricultural and Food Chemistry, 64*(22), 4584–4598. https://doi.org/10.1021/acs.jafc.6b01005

Montero, L., Herrero, M., Ibáñez, E., & Cifuentes, A. (2013). Profiling of phenolic compounds from different apple varieties using comprehensive two-dimensional liquid chromatography. *Journal of Chromatography A, 1313*, 275–283. https://doi.org/10.1016/j.chroma.2013.06.015

Qin, Z., Petersen, M. A., & Bredie, W. L. P. (2018). Flavor profiling of apple ciders from the UK and Scandinavian region. *Food Research International, 105*, 713–723. https://doi.org/10.1016/j.foodres.2017.12.003

Ramirez-Ambrosi, M., Abad-Garcia, B., Viloria-Bernal, M., Garmon-Lobato, S., Berrueta, L. A., & Gallo, B. (2013). A new ultrahigh performance liquid chromatography with diode array detection coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry analytical strategy for fast analysis and improved characterization of phenolic compounds in apple products. *Journal of Chromatography A, 1316*, 78–91. https://doi.org/10.1016/j.chroma.2013.09.075

Satora, P., Sroka, P., Duda-Chodak, A., Tarko, T., & Tuszyński, T. (2008). The profile of volatile compounds and polyphenols in wines produced from dessert varieties of apples. *Food Chemistry, 111*(2), 513–519. https://doi.org/10.1016/j.foodchem.2008.04.007

Sun, L., Liu, D., Sun, J., Yang, X., Fu, M., & Guo, Y. (2017). Simultaneous separation and purification of chlorogenic acid, epicatechin, hyperoside, and phlorizin from thinned young *Qinguan* apples by successive use of polyethylene and polyamide resins. *Food Chemistry, 230*, 362–371. https://doi.org/10.1016/j.foodchem.2017.03.065

Symoneaux, R., Chollet, S., Patron, C., Bauduin, R., Le Quéré, J. M., & Baron, A. (2015). Prediction of sensory characteristics of cider according to their biochemical composition: Use of a central composite design and external validation by cider professionals. *LWT-Food Science and Technology, 61*(1), 63–69. https://doi.org/10.1016/j.lwt.2014.11.030

Tardío, J., Arnal, A., & Lázaro, A. (2021). Ethnobotany of the crab apple tree (*Malus sylvestris* (L.) Mill., Rosaceae) in Spain. *Genetic Resources and Crop Evolution, 68*(2), 795–808. https://doi.org/10.1007/s10722-020-01026-y

Wang, S., Li, Q., Zang, Y., Zhao, Y., Liu, N., Wang, Y., … Mei, Q. (2017). Apple polysaccharide inhibits microbial dysbiosis and chronic inflammation and modulates gut permeability in HFD-fed rats. *International Journal of Biological Macromolecules, 99*, 282–292. https://doi.org/10.1016/j.ijbiomac.2017.02.074

Wu, C., Li, T., Qi, J., Jiang, T., Xu, H., & Lei, H. (2020). Effects of lactic acid fermentation-based biotransformation on phenolic profiles, antioxidant capacity, and flavor volatiles of apple juice. *LWT - Food Science and Technology, 122*, 109064. https://doi.org/10.1016/j.lwt.2020.109064

Xiao, Z., Zhang, Y., Chen, X., Wang, Y., Chen, W., Xu, Q., Li, P., & Ma, F. (2017). Extraction, identification, and antioxidant and anticancer tests of seven dihydrochalcones from Malus 'Red Splendor' fruit. *Food Chemistry., 231*, 324–331. https://doi.org/10.1016/j.foodchem.2017.03.111