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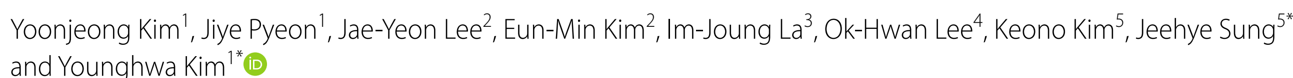

Chemical fingerprint analysis of fermented *Morinda citrifolia* L. (Noni) juice by UHPLC Q-TOF/MS combined with chemometric analysis

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Abstract
Morinda citrifolia L. (Noni) has been widely used in traditional medicine in tropical zones and has become increasingly popular globally owing to its health benefits. Most noni fruits are consumed as juice, which is traditionally produced by the natural fermentation of noni fruits. In this study, the metabolic profiles of noni fruit juice (NJ1) and fermented noni fruit juices (NJ2 and NJ3) was compared. A total of 74, 83, and 91 compounds including anthraquinones, coumarins, flavonoids, phenolic acids, phenolics, terpenoids, and miscellaneous (acids, carbohydrates, vitamins, fatty acids, etc.) were tentatively identified from NJ1, NJ2, and NJ3 in both positive and negative electrospray ionization modes. The phenolic compound composition differed significantly between noni juice and fermented noni juice. The results of the unsupervised principal component analysis and hierarchical clustering analysis showed that the non-fermented juice group clustered with the fermented juice groups. Asperulosidic acid, isoasperulosidic acid, and rutin levels were higher in the NJ1 group than those in the NJ2 group. Deacetylasperulosidic acid and monotropein contents in NJ2 were higher than those in NJ1. Similarly, NJ1 had higher asperulosidic acid and isoasperulosidic acid than those in NJ3. The findings from this study have the potential to enhance the quality of fermented noni juice.

Keywords Bioactive compounds, Fermentation, Noni juice, Untargeted metabolomics

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Introduction

Noni (*Morinda citrifolia* L) is widely cultivated in tropical and subtropical regions such as Australia, Polynesia, Hawaii, and other Pacific islands. The fruits and leaves have been used in traditional folk medicine for the treatment of several diseases, such as high blood pressure, inflammation, and diabetes [1]. Modern scientific research has shown that noni fruits possess antioxidant, anti-inflammatory, liver-protective, and immunomodulatory effects [2–4]. This fruit contains various bioactive compounds, such as flavonoids, lignans, iridoids, coumarins, anthraquinones, polysaccharides, terpenoids, sterols, fatty acids, organic acids, vitamins, and minerals [2, 5]. Phenolic compounds play a key role in the therapeutic properties of plants; for example, rutin, β-sitosterol, asperuloside, and ursolic acid are important biologically active compounds present in noni [6]. Flavonoids isolated from noni fruits, such as kaempferol, quercetin, catechin, and epicatechin have shown antidepressant and antioxidant activities [7]. Previous studies have shown that fermentation can significantly affect the composition and bioactivity of phenolic compounds. For example, the phenolic composition of *Dendrobium candidum* substantially changes after fermentation, and the contents of syringic acid, 4-hydroxybenzoic acid, and *p*-hydroxycinnamic acid increase significantly [8]. Traditionally, noni juice is produced by fermenting noni fruits in sealed jars or barrels for approximately 2 months or longer and then recovering the juice through drip extraction and/or mechanical pressure. Deng et al. reported that the phytochemical fingerprints of 13 commercial noni-fermented juices on the global market and found that they all contained scopolamine, rutin, and quercetin [9].

Fermentation is a natural process that converts sugars into products that are useful to humans using several microorganisms [10]. In addition, fermentation can lead to increased chemical changes in organic substances through the action of enzymes [11]. Lactic acid bacteria (LAB) are used in dairy and non-dairy foods such as yogurt, tea, and fruits. Recently, the demand for non-dairy probiotic products has increased because of an increase in their immune function [12]. An increasing number of investigators are focusing on the biotransformation of phytochemical substances in vegetable and fruit juices using LAB to produce functional beverages [13]. Vegetables and fruits are enriched in phenolics, organic acids, and sugars, which can be metabolized by LAB strains and therefore improve the sensory, nutritional, and functional qualities, as well as extend the shelf life of fermented products. Currently, the noni juice market is growing continuously because of its potential biological activities. Moreover, there are attempts to commercialize noni fruit through various processing methods such as fermentation and drying, and among them, fermented noni fruit juice stands out as the most popular in the market [14]. However, there is very little information on the phytochemical components of fermented noni juice in the modern scientific literature. Therefore, the objective of this study was to explore an extensive investigation of the phytochemical composition of noni juice and fermented noni juice through the application of untargeted metabolomics.

Materials and methods

Plant material

The samples (organic noni fruit juice, NJ1; fermented organic noni fruit juice I, NJ2; and fermented organic noni fruit juice II, NJ3) were provided by the R&D Center of NSTBio Co., Ltd. (Incheon, Korea) and Atomy Orot Co. (Gongju, Chungnam, Korea). Organic noni fruit, imported from Indonesia, was juiced and used as the sample (NJ1: pH 4.5, Brix 8.5). NJ1 was stored frozen at -80 °C until used in the experiment. Organic noni fruits (10 kg) were fermented with 150 mL of a mixture of probiotics (*Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus reuteri*, *Lactococcus lactis subsp. Lactis*) at 37 °C for 45 d (NJ2: pH 3.9, Brix 8.0). Initially, the inoculum size was 2.4×10^7 CFU/g. NJ3 (pH 3.9, Brix 15.0) was prepared by adding organic coconut blossom sugars, calamansi juice, and organic maltodextrin to NJ2.

Chemicals and reagents

High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) was obtained from Fisher Scientific (Seoul, Korea), and formic acid was purchased from Merck (Darmstadt, Germany). HPLC-grade methanol and isopropanol (IPA) were supplied by Honeywell Burdick & Jackson (Honeywell Burdick & Jackson, Muskegon, MI, USA) and deionized water was obtained using a Milli-Q water purification system (Millipore Ltd., Bedford, MA, USA). The standards (purity ≥ 95%) of catechin, deacetylasperulosidic acid, epicatechin, gallic acid, hesperidin, isoquercitrin, naringin, nicotinamide, nicotinic acid, *p*-coumaric acid, protocatechuic acid, quercetin, riboflavin, rosmarinic acid, rutin, scopoletin, shikimic acid, thiamine, vitamin C, and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as external standards for the identification of compounds. Isotope-labeled L-[$^{13}C_6$] phenylalanine, used as an internal standard, was purchased from Sigma-Aldrich.

Preparation of standard solutions

The individual compounds were dissolved in methanol to a concentration of 100 μg/mL. Mixed working standard solutions were prepared at concentrations of 1 μg/mL and 0.01 μg/mL in ACN/IPA/water (3:3:2, v/v/v)

from each stock solution. The stock solution of the internal standard was prepared by dissolving 0.4 mg labeled L-[$^{13}C_6$] phenylalanine in 1.0 mL of ACN/IPA/Water (3:3:2, v/v/v). All the standard solutions were stored in a refrigerator at -20 °C, and filtered through a 0.22-μm nylon syringe filter prior to analysis.

Preparation of sample solutions and quality controls

Noni juice samples (0.1 g) were accurately weighed into a 2-mL centrifuge tube and mixed with 1.5 mL of ACN/IPA/water (3:3:2, v/v/v) containing 10 μg/mL of internal standards (L-phenylalanine-$^{13}C_6$). The mixture was then vortexed for 5 min and sonicated for 1 h in an ice bath. After sonication, the mixture was centrifuged at 12,298 x *g* for 10 min at 4 °C, the supernatant was passed through a 0.22-μm nylon syringe filter (Whatman, Maidstone, UK). After that, 990 μL of supernatant was transferred to a new centrifuge tube and 10 μL of internal standard solution (0.4 mg/mL) was added before analysis. All samples were prepared in quintuplicate. Quality control (QC) samples ($n=4$) were prepared by pooling equal volumes of an aliquot of each sample extract and analyzing every five samples of the run.

UHPLC-Q-TOF/MS analysis

The ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS) system consisted of an Agilent 1260 Infinity II series (Agilent Technologies, Santa Clara, CA, USA) with a photodiode array (PDA) detector and an Agilent 6530 Q-TOF/MS (Agilent Technologies) equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed on a YMC-Pack Pro C18 (150×4.6 mm, 3 μm) column (YMC Co., Kyoto, Japan). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B) with a gradient elution: 0–30 min, 1% B; 30–31 min, 40% B; 31–35 min, 100% B; 35–36 min, 1% B; 36–45 min, 1% B, at a flow rate of 0.3 mL/min. The temperature of the column oven was maintained at 30 °C and the injection volume was 10 μL. MS analysis was performed in both positive-ion and negative-ion modes using the MS and auto-MS/MS scan modes. The MS parameters were as follows: mass range, 25–1700 m/z; collision energy, 0, 10, and 20 V; gas temperature, 300 °C; drying gas, 10 L/min; nebulizer, 45 psi; sheath gas temperature, 300 °C; sheath gas flow, 11 L/min; capillary, 4,000 V; fragmentor, 175 V; and octapole RF Vpp, 750 V.

Data processing

Data acquisition and processing were performed using Agilent MassHunter Qualitative Analysis software (Version 10.0, Agilent Technologies). Raw MS data files acquired from the UHPLC-Q-TOF/MS analysis were identified using the METLIN database B 08.00, and their authentic compounds. The amount of each metabolite obtained by UHPLC-Q-TOF/MS was determined as the relative metabolite abundance, which was calculated by dividing all data values for each sample by the chromatographic peak area of the internal standards added to the metabolite extract of each sample.

Chemometric analysis

Principal component analysis (PCA), hierarchical clustering analysis (HCA), partial least–squares discriminant analysis (PLS-DA), and orthogonal partial least-squares discriminant analysis (OPLS-DA) were performed using the SIMCA-P software package (Umetrics, Umea, Sweden). Unsupervised data analysis, including PCA and HCA, was applied as an exploratory data analysis to visualize the analytical connections among the samples. PLS-DA and OPLS-DA were used for the modeling of samples in the retention of settled classes Y. In the PLS-DA output, the variable important in projection (VIP) is an important screening index of metabolites that changed between different non-juice samples. The PLS-DA models were also validated using R^2Y and Q^2 from a random permutation test ($n=200$) in SIMCA-P. The quality of the PLS model was depicted by the cross-validation parameters R^2 and Q^2, which represent the explained variance and predictive capability of the model, respectively.

Results and discussion

Identification of bioactive compounds in noni juice and fermented noni juices

UHPLC-Q-TOF/MS was performed to analyze secondary metabolites in noni and fermented noni juice. As a result of our analysis, 130 compounds, including anthraquinones, coumarins, flavonoids, phenolic acids, phenolics, terpenoids, and miscellaneous com-pounds (acids, carbohydrates, vitamins, fatty acids, etc.) were tentatively identified from the samples (NJ1–NJ3) in both ESI+and ESI- modes (Fig. 1A and B). A total of 74, 83, and 91 compounds were detected in NJ1, NJ2, and NJ3, respectively. According to previous studies, anthraquinones, flavonoids, phenolic acids, phenolics, terpenoids, fatty acids, and carbohydrates are the major compounds in noni juice [15].

Details of the 74 compounds identified in NJ1 are listed in Table S1. NJ1 included 9 anthraquinones (rubiadin 1-methyl ether, rhabarberone, emodin, 1,3-dihydroxy-2-methoxyanthracene-9,10-dione, 8-*O*-primeverose-1,3-dihydroxy-2-methyl-anthraquinone, 1-*O*-gentiobiose-2-methylolanthraquinone, 8-*O*-primeverose-1-methoxy-3-hydroxy-2-methyl-anthraquinone, 3-*O*-primeverose-1,6,8-trihydroxy-2-methyl-anthraquinone, and 1-*O*-gentiobiose-8-methoxy-aloeemodin), 4 coumarins (4-hydroxycoumarin, esculin, esculetin, and

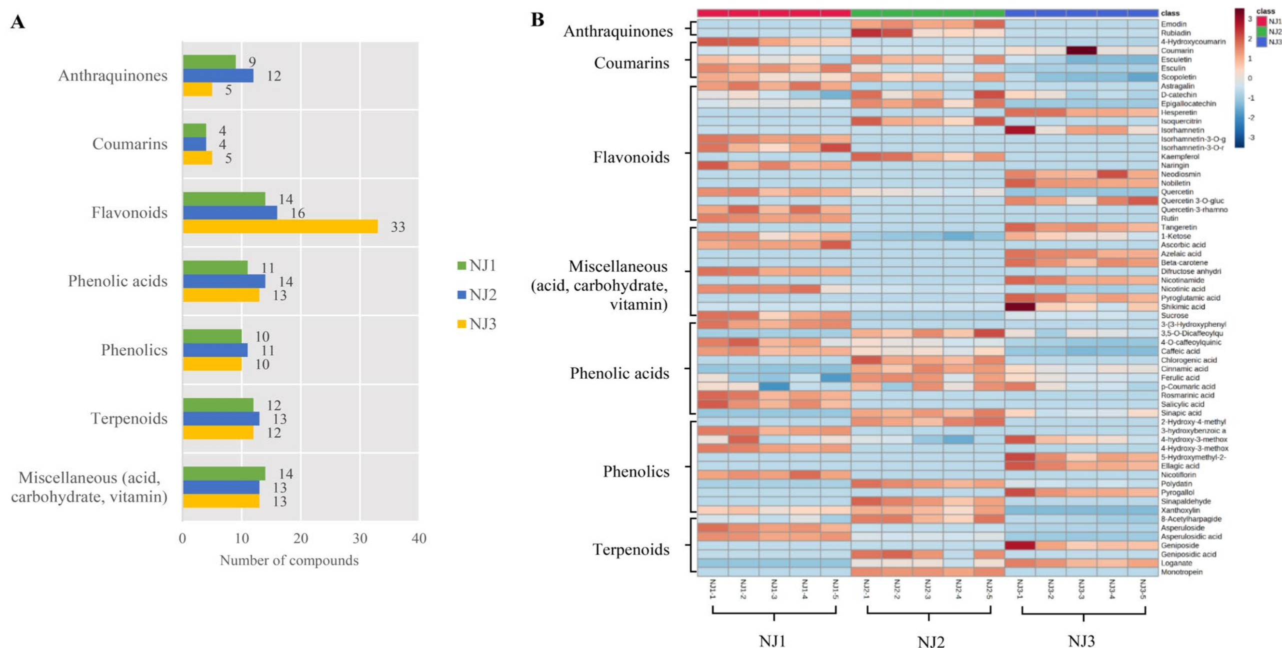

Fig. 1 Classification information of differential metabolites identified between organic noni juice (NJ1) and fermented noni juices (NJ2 and NJ3) (**A**) and heatmap visualization of significant metabolites in positive mode (**B**). The horizontal bars of green, blue, and yellow describe the number of differential metabolites identified between noni juice and fermented noni juices. The color gradient of dark blue and deep red colors in the heat map indicates low and high intensities

scopoletin), 14 flavonoids (isorhamnetin-3-*O*-galactoside, astragalin, epigallocatechin, quercetin-3-rhamnoside, D-catechin, rutin, isorhamnetin-3-*O*-rutinoside, quercetin, naringin, gallic acid, leptosin, hesperidin, kaempferol, and 2′,7-dihydroxy-4′,5′-dimethoxyisoflavone), 11 phenolic acids (3-hydroxyphenylpropionic acid, *p*-coumaric acid, 4-*O*-caffeoylquinic acid, cinnamic acid, caffeic acid, ferulic acid, salicylic acid, rosmarinic acid, hydroxybutanedioic acid, 2-hydroxy-2-phenylacetic acid, and chlorogenic acid), 10 phenolics (4-hydroxy-3-methoxystyrene, xanthoxylin, 4-hydroxy-3-methoxycinnamaldehyde, 3-hydroxybenzoic acid, nicotiflorin, protocatechuic acid, 3,4-dihydroxybenzaldehyde, 2,5-dihydroxybenzoic acid, 2-hydroxy-4-methylbenzaldehyde, and physcion), 12 terpenoids (8-acetylharpagide, asperulosidic acid, asperuloside, deacetylasperulosidic acid, monotropein, geniposidic acid, aucubin, harpagide acetate, geniposide, isoasperulosidic acid, rehmannioside A, and methoxygaertneroside), and 14 miscellaneous, such as acids, vitamins, and carbohydrates. A total of 83 compounds were detected in NJ2, including 12 anthraquinones, four coumarins, 16 flavonoids, 14 phenolic acids, 11 phenolics, 13 terpenoids, and 13 miscellaneous compounds (Table SS2). More flavonoids (16 compounds) and phenolic acids (14 compounds) were detected in NJ2 than those in NJ1 (14 flavonoids and 11 phenolic acids). In contrast, 91 compounds were putatively identified in NJ3. NJ3 contains 5 anthraquinones, 5 coumarins, 33 flavonoids, 13 phenolic acids, 10 phenolics, 12 terpenoids, and 13 miscellaneous compounds (Table SS3). The levels of the detected bioactive compounds tended to increase during fermentation. Eleven phenolic acids were detected in NJ1, whereas 14 and 13 were detected in NJ2 and NJ3, respectively. Fourteen flavonoids were identified in NJ1, and 16 and 33 flavonoids were identified in NJ2 and NJ3, respectively. In particular, astragalin, a 3-*O*-glucoside of kaempferol, was detected exclusively in NJ1, while kaempferol, the aglycone form of astragalin, was found in the post-fermentation sample (NJ2 and NJ3). It is widely acknowledged that aglycones demonstrate higher activity within the intestine compared to their glycoside counterparts, resulting in enhanced bioavailability [16]. In addition, it has been reported that LAB fermentation can cause deglycosylation from flavonoids [17]. Three more phenolic acids (3,5-*O*-dicaffeoylquinic acid, ferulic acid derivative, and sinapic acid) were found in NJ2 compared with NJ1. In the previous study, fermentation by LAB increased the total phenolic content and the antioxidant capacity in jujube-wolfberry composite juice [18]. Therefore, these results suggest that LAB fermentation could have a positive effect on the metabolite changes in noni fruit. On the other hand, the number of flavonoids was considerably higher in NJ3 than that in NJ1 or NJ2, which seemed to be derived from calamansi (*Citrus microcarpa*) juice in NJ3. Calamansi is a small citrus fruit that contains the highest amount of phenolic acids, *p*-coumaric acids, and flavonoids [19]. In this study, citrus flavonoids, such as naringenin, neodiosmin, nobiletin,

neohesperidin, and tangeretin, were exclusively found in NJ3. Noni fruits have been reported to contain large amounts of iridoid compounds, among which deacetylasperulosidic acid, asperulosidic acid, and asperuloside are the major iridoids [20]. Coumarins play important roles in regulating plant growth and metabolites [21]. They also exhibit a wide range of biological functions, including anti-inflammatory, antioxidant, antibacterial, and anticancer [22]. Among these, scopoletin is a representative coumarin derivative of noni fruit [23]. In this study, all samples contained major iridoid compounds such as deacetylasperulosidic acid, asperulosidic acid, and asperuloside.

Metabolomic profiles of different noni juice samples

Untargeted UHPLC-Q-TOF/MS chemical fingerprinting coupled with chemometric analysis was performed to determine the comprehensive metabolite profiles of the different noni juice samples. The normalized data of NJ1, NJ2, and NJ3 in both positive and negative modes for both UHPLC-Q-TOF/MS are shown in Fig. 1. After data normalization, unsupervised PCA and HCA were performed using the normalized data of different noni juice samples to reveal the same sample clustering pattern, obtain an overview of the trend, and determine putative outliers (Fig. 2A and B). The PCA score plot showed a clear separation according to the three groups of noni juice samples including 'NJ1,' 'NJ2,' and 'NJ3' with no significant sample outliers. The plot was built using the first two principal components (PCs), PC1 and PC2, of the total variance (PC1 = 40.7% and PC2 = 34.1%). HCA was performed to elucidate the similarities and dissimilarities between the metabolite profiles of different samples. The HCA dendrogram indicated a clear separation between the non-fermented juice group (Group 1) and the fermented juice group (Group 2). These results imply that the major metabolite profiles of noni juice may be affected mainly by fermentation with the mixture of probiotics used in this study.

Supervised PLS-DA was performed to calculate models that differentiated between groups and to select the metabolites responsible for the different noni juice sample-dependent clustering based on VIP values > 1. The PLS-DA score plot showed clear separation of the three clusters representing the different noni juice samples (Fig. 2C). The observed PLS-DA model had

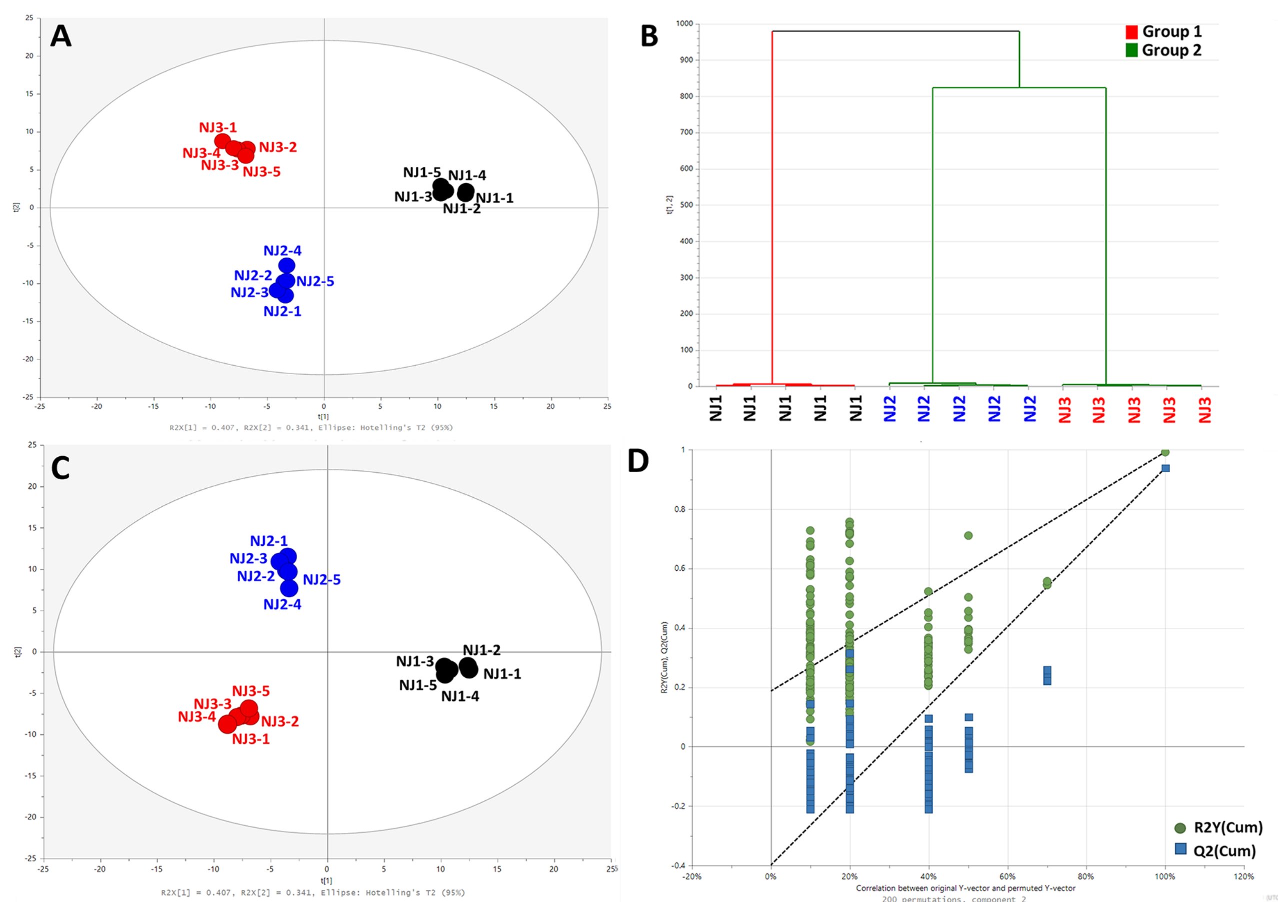

Fig. 2 Unsupervised score plots of the PCA model (**A**) and HCA dendrogram (**B**) and supervised score plot of the PLS-DA model (**C**). Two hundred permutation test (**D**) on PLS model from chemical fingerprinting of three noni juices

good coefficient fractions ($p<0.05$), with $R^2Y=0.989$ and $Q^2=0.979$ (positive predictive ability), and the variables explained 0.749 (R^2X) of the total variation. The y intercepts of R^2 and Q^2 in the permutation test were 0.187 and −0.398, respectively, indicating a valid model (Fig. 2D). The recommended values for good fitting of models have been described as R^2Y intercept < 0.3 and Q^2Y intercept < 0.05. Differential metabolites were selected according to the criteria of $p<0.05$ and VIP score > 1 from the PLS-DA model (Table 1). Hence, a combination of chromatographic fingerprints based on differential metabolite profiling with PLS-DA provides more comprehensive and insightful information regarding the dissimilarities between different noni juice samples.

Investigation of the OPLS-DA score plot and S-line plots was conducted to identify peaks that differed by sample type (Fig. 3). Within the S-line plots, iridoid compounds, flavonoids, and sucrose that showed substantial differences between each noni juice sample by matching them with an in-house library (Table SS4). In comparison between NJ1 and NJ2, the contents of asperulosidic acid (m/z 431.1202 $[M–H]^-$), isoasperulosidic acid (m/z 431.1199 $[M–H]^-$), and rutin (m/z 611.1612 $[M+H]^+$) were higher in NJ1 than NJ2, whereas the contents of deacetylasperulosidic acid (m/z 389.1099 $[M–H]^-$) and monotropein (m/z 391.1234 $[M+H]^+$) from NJ2 were higher than those from NJ1. Similarly, NJ1 had higher asperulosidic acid, and isoasperulosidic acid contents in NJ1 than those in NJ2. A previous study reported that the deacetylasperulosidic acid content was higher in fermented *Morinda citrifolia* L. extract (MCE) than that in non-fermented MCE, whereas the asperulosidic acid content was lower in fermented MCE than that in non-fermented extract [24]. These results imply that asperulosidic acid may be acetylated during noni juice fermentation. A previous study also reported that an increase in the contents of polysaccharides, free anthraquinones, rubiadin, monotropein, and fructose in noni juice fermented by *Bacillus* sp. DU-106 and *Lactobacillus plantarum* [24]. *Dendrobium officinale* fermented by *Bacillus* sp. DU-106 showed enhanced the immunostimulatory activity [26]. In addition, monotropein induced immune activation in NCM460 cells [27]. Noni juice fermented by LAB showed the enhancement of immune activities compared to non-fermented noni juice [28]. It appears that changes in metabolite in noni juice caused by LAB fermentation could affect immune activity of noni juice.

Noni fruits are typically fermented before consumption due to their unpleasant odors and taste [29]. In addition, commercially available noni products are processed in various ways by adding sugar, fruits, and condiments to reduce undesirable odor [30]. Citrus fruits, which contain linalool and limonene—very floral and strong odors—can be added as additives to noni juices to mask flavor or eliminate odor [31]. Adding fruit juices to noni juice is considered a convenient way to create value-added fruit drinks that excel in both sensory and nutritional quality. In this study, NJ3, made by adding coconut pollen sugar, calamansi juice, and maltodextrin, was used as a fermented noni juice to reflect commercial blended noni juice. NJ3 had higher contents of 3,5,7,4′-tetramethoxyflavone (m/z 341.1055 $[M–H]^-$), 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone (m/z 373.1284 $[M–H]^-$), and sucrose (m/z 341.1093 $[M–H]^-$) than those in NJ1 and NJ2 (Table SS4). Coconut blossom sugar is produced from the phloem sap of the blossom of the coconut palm tree (*Cocos nucifera* L.), which is heated until it begins to form a thick syrup [32]. Sugar contains little fructose and has a lower glycemic index than conventional refined cane or beet sugars [33]. Citrus fruits such as calamansi are rich sources of polymethoxylated flavones that could be responsible for their antioxidant, anti-inflammatory, and antiviral potentials [34–37]. The high polymethoxyflavone and sucrose contents in NJ3 could be attributed to organic coconut blossom sugar and calamansi juice, respectively. However, significant variations in metabolite profiles are expected based on the specific ingredients added to noni juice, indicating the need for further research on metabolites in commercial noni juice formulations.

In conclusion, we confirmed the metabolic profiles of noni fruit juice and fermented noni fruit juices by using UHPLC-Q-TOF/MS. A total of 74, 83, and 91 compounds were identified in NJ1, NJ2, and NJ3, respectively. Untargeted UHPLC-Q-TOF/MS chemical fingerprinting coupled with chemometric analysis showed the comprehensive metabolite profiles of the different noni juice samples. These results can be used as basic information for developing products with fermented noni juice. Finally, further research is ongoing to improve the quality of noni juice according to fermentation conditions.

Table 1 Variable importance in projection (VIP) scores of partial least squares-discriminant analysis (PLS-DA)

No.	Compound name	Mode	Molecular Formula	RT (min)	m/z	Mass error (ppm)	CV	VIP Score
1	Monotropein	(+)	$C_{16}H_{22}O_{11}$	1.744	413.1052	-0.484	0.325	1.205
2	2,3-Dihydroxy-1-guaiacylpropanone	(-)	$C_{10}H_{12}O_5$	2.531	211.0613	0.474	0.154	1.203
3	Deacetylasperulosidic acid	(-)	$C_{16}H_{22}O_{11}$	1.641	389.1081	-2.056	0.039	1.203
4	3,4-Dihydroxybenzaldehyde	(-)	$C_7H_6O_3$	4.885	137.0247	2.189	0.129	1.201
5	2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone	(-)	$C_{17}H_{14}O_6$	27.969	313.0713	-1.597	0.196	1.198
6	Ferulic acid derivative	(-)	$C_{19}H_{24}O_{12}$	6.223	443.1177	-4.062	0.324	1.196
7	Emodin	(+)	$C_{15}H_{10}O_5$	9.810	271.0617	5.903	0.177	1.196
8	2-Hydroxy-4-methylbenzaldehyde	(+)	$C_8H_8O_2$	1.051	137.0595	-1.460	0.358	1.189
9	Chlorogenic acid	(+)	$C_{16}H_{18}O_9$	1.752	355.1023	-0.282	0.139	1.183
10	Loganate	(-)	$C_{16}H_{24}O_{10}$	6.186	375.1298	0.267	0.252	1.183
11	Sinapaldehyde	(+)	$C_{11}H_{12}O_4$	17.881	209.0814	2.870	0.732	1.181
12	Polydatin	(+)	$C_{20}H_{22}O_8$	12.566	391.1358	-7.414	0.168	1.173
13	4-Hydroxy-3-methoxy-styrene	(-)	$C_9H_{10}O_2$	15.711	149.0607	-0.671	0.113	1.172
14	Xanthoxylin	(+)	$C_{10}H_{12}O_4$	1.274	197.0806	-1.015	0.113	1.167
15	Epigallocatechin	(+)	$C_{15}H_{14}O_7$	3.737	307.0845	10.746	0.309	1.164
16	Kaempferol	(+)	$C_{15}H_{10}O_6$	12.503	287.0555	1.742	0.356	1.164
17	Didymin	(-)	$C_{28}H_{34}O_{14}$	0.756	593.1935	9.946	0.177	1.162
18	Azelaic acid	(+)	$C_9H_{16}O_4$	34.241	189.1137	8.461	1.094	1.161
19	Nobiletin	(+)	$C_{21}H_{22}O_8$	27.218	403.1385	-0.496	0.106	1.159
20	Tangeretin	(+)	$C_{20}H_{20}O_7$	29.618	373.1281	-0.268	0.036	1.157
21	beta-Carotene	(+)	$C_{40}H_{56}$	32.644	536.4370	-1.305	0.118	1.155
22	Nicotinamide	(+)	$C_6H_6N_2O$	0.644	123.0543	-8.126	0.334	1.154
23	Hesperetin	(+)	$C_{16}H_{14}O_6$	15.222	303.0845	-5.939	0.229	1.153
24	Citric acid	(-)	$C_6H_8O_7$	0.648	191.0206	4.712	0.238	1.152
25	1-Hydroxy-2,3-dimethoxyanthracene-9,10-dione	(-)	$C_{16}H_{12}O_5$	23.723	283.0610	-0.707	0.475	1.152
26	Pyroglutamic acid	(+)	$C_5H_7NO_3$	1.050	130.0497	-1.538	0.234	1.150
27	Protocatechuic acid (3,4-dihydroxybenzoic acid)	(-)	$C_7H_6O_4$	3.230	153.0191	-1.307	0.107	1.149
28	Ellagic acid	(+)	$C_{14}H_6O_8$	3.352	303.0146	3.630	0.250	1.146
29	Pyrogallol	(+)	$C_6H_6O_3$	3.412	127.0388	-1.574	0.053	1.143
30	Isoquercitrin	(+)	$C_{21}H_{20}O_{12}$	5.812	465.1029	0.215	0.139	1.143
31	8-Acetylharpagide	(+)	$C_{17}H_{26}O_{11}$	3.046	407.1525	-6.140	0.094	1.140
32	Neodiosmin	(+)	$C_{28}H_{32}O_{15}$	13.193	609.1814	0.000	0.250	1.140
33	8-O-Primeverose-1,3-dihydroxy-2- methyl -anthraquinone	(-)	$C_{26}H_{28}O_{14}$	6.967	563.1448	7.458	0.255	1.134
34	Luteolin 7-neohesperidoside	(-)	$C_{27}H_{30}O_{15}$	9.507	593.1504	-1.349	0.169	1.134
35	Cinnamic acid	(+)	$C_9H_8O_2$	2.492	149.0592	-5.367	0.099	1.130
36	1-Kestose	(-)	$C_{18}H_{32}O_{16}$	3.783	503.1619	0.199	0.122	1.129
37	2-Hydroxy-2-phenylacetic acid	(-)	$C_8H_8O_3$	3.192	151.0398	-1.986	0.130	1.124
38	1-O-Gentiobiose-2-methylolanthraquinone	(-)	$C_{27}H_{30}O_{14}$	9.829	577.1522	-7.104	0.122	1.124
39	Neohesperidin	(-)	$C_{28}H_{34}O_{15}$	0.683	609.1887	10.178	1.435	1.123
40	5-Hydroxymethyl-2-furaldehyde	(+)	$C_6H_6O_3$	3.412	127.0388	-1.574	0.017	1.122
41	Sinapic acid	(+)	$C_{11}H_{12}O_5$	5.259	225.0754	-1.333	0.636	1.121
42	Cinnamic acid	(-)	$C_9H_8O_2$	2.310	147.0450	-1.360	0.169	1.118
43	Salicylic acid	(-)	$C_7H_6O_3$	5.629	137.0246	1.460	0.187	1.114
44	Emodin	(-)	$C_{15}H_{10}O_5$	3.228	269.0424	-11.522	0.412	1.112
45	Rutin	(+)	$C_{27}H_{30}O_{16}$	12.273	611.1600	-1.145	0.195	1.112
46	Disaccharide (formate adduct)	(-)	$C_{13}H_{24}O_{13}$	13.423	387.1107	-9.558	0.310	1.111
47	Succinic acid	(-)	$C_4H_6O_4$	1.202	117.0191	-1.709	0.186	1.111
48	Asperulosidic acid	(+)	$C_{18}H_{24}O_{12}$	6.185	455.1162	0.439	1.398	1.110
49	Isorhamnetin-3-O-galactoside	(+)	$C_9H_8O_2$	2.490	149.0596	-0.671	0.114	1.109
50	Nicotiflorin	(+)	$C_{27}H_{30}O_{15}$	13.753	595.1648	0.336	0.212	1.109
51	Quercetin	(-)	$C_{15}H_{10}O_7$	18.183	301.0354	0.000	0.151	1.109
52	4-Hydroxy-3-methoxystyrene	(+)	$C_9H_{10}O_2$	1.014	151.0749	-3.310	0.239	1.108

Table 1 (continued)

No.	Compound name	Mode	Molecular Formula	RT (min)	m/z	Mass error (ppm)	CV	VIP Score
53	3-(3-Hydroxyphenyl)propionic acid	(+)	$C_9H_{10}O_3$	1.200	167.0698	-2.993	0.194	1.108
54	Caffeic acid	(+)	$C_9H_8O_4$	3.044	181.0498	1.657	0.029	1.108
55	3-Hydroxybenzoic acid	(+)	$C_9H_{10}O_3$	14.669	165.0532	-9.088	0.328	1.107
56	Narirutin	(-)	$C_{27}H_{32}O_{14}$	0.683	579.1769	8.633	0.095	1.107
57	Methoxygaertneroside	(-)	$C_{27}H_{30}O_{14}$	0.683	577.1622	10.223	0.365	1.105
58	Xanthoxylin	(-)	$C_{10}H_{12}O_4$	5.814	195.0660	-1.538	0.078	1.105
59	Feralolide	(-)	$C_{18}H_{16}O_7$	2.236	343.3000	1.232	0.212	1.105
60	Difructose anhydride	(+)	$C_{12}H_{20}O_{10}$	3.784	325.1131	0.615	0.197	1.105
61	Quercetin	(+)	$C_{15}H_{10}O_7$	18.325	303.0481	-5.940	0.161	1.104
62	Sucrose	(-)	$C_{12}H_{22}O_{11}$	0.595	341.1093	1.173	0.521	1.103
63	Astragalin	(+)	$C_{21}H_{20}O_{11}$	3.636	449.1066	-2.672	0.145	1.103
64	Caffeic acid	(-)	$C_9H_8O_4$	1.015	179.0350	0.000	0.084	1.103
65	Ascorbic acid	(+)	$C_6H_8O_6$	12.016	177.0392	1.130	0.235	1.102
66	Chlorogenic acid	(-)	$C_{16}H_{18}O_9$	6.002	353.0835	-12.178	0.165	1.101
67	Difructose anhydride	(-)	$C_{12}H_{20}O_{10}$	3.783	323.0986	0.619	0.016	1.100
68	Asperuloside	(+)	$C_{18}H_{22}O_{11}$	6.186	415.1235	-1.204	0.056	1.099
69	2-Hydroxy-4-methylbenzaldehyde	(-)	$C_8H_8O_2$	6.884	135.0452	0.000	0.200	1.096
70	Loganate	(+)	$C_{16}H_{24}O_{10}$	2.121	377.1433	-2.387	0.162	1.096
71	1-Borneol-beta-apisyl-beta-glucopyranoside	(-)	$C_{21}H_{36}O_{10}$	18.552	447.2240	0.894	0.154	1.096
72	Naringin	(+)	$C_{27}H_{32}O_{14}$	18.733	581.1866	-0.688	0.049	1.095
73	Kaempferol	(-)	$C_{15}H_{10}O_6$	21.211	285.0404	-0.351	0.344	1.095
74	tianshic acid	(-)	$C_{18}H_{34}O_5$	23.352	329.2337	1.215	0.196	1.095
75	3-Hydroxyphenylpropionic acid	(-)	$C_9H_{10}O_3$	14.654	165.0559	1.212	0.163	1.095
76	geniposide	(-)	$C_{17}H_{24}O_{10}$	5.494	387.1285	-3.100	0.132	1.094
77	Quercetin 3-O-glucosyl-xyloside	(+)	$C_{26}H_{28}O_{16}$	1.087	597.1449	-0.167	0.407	1.091
78	Esculin	(+)	$C_{15}H_{16}O_9$	6.367	341.0866	-1.173	0.326	1.091
79	Salicylic acid	(+)	$C_7H_6O_3$	3.782	139.0392	1.438	0.248	1.091
80	Rosmarinic acid	(+)	$C_{18}H_{16}O_8$	16.479	361.0948	7.754	0.148	1.090
81	quercetin-3-rhamnoside	(+)	$C_{21}H_{20}O_{11}$	3.857	449.1065	-3.340	0.145	1.088
82	Sucrose	(+)	$C_{12}H_{22}O_{11}$	1.016	365.1049	-1.369	0.183	1.088
83	ibericin	(-)	$C_{17}H_{14}O_5$	11.909	297.0775	2.356	0.235	1.088
84	Isorhamnetin-3-O-galactoside	(-)	$C_{22}H_{22}O_{12}$	7.184	477.1068	6.288	0.169	1.086
85	Sativanone	(-)	$C_{17}H_{16}O_5$	12.980	299.0922	-1.003	0.137	1.086
86	Isoscutellarein 7-xyloside	(-)	$C_{20}H_{18}O_{10}$	15.416	417.0818	-2.158	0.022	1.086
87	Monotropein	(-)	$C_{16}H_{22}O_{11}$	2.601	389.1082	-1.799	0.074	1.085
88	Esculin	(-)	$C_{15}H_{16}O_9$	5.448	339.0724	0.590	0.261	1.081
89	3,5-O-Dicaffeoylquinic acid	(+)	$C_{25}H_{24}O_{12}$	7.472	517.1332	-1.740	1.066	1.080
90	Asperuloside	(-)	$C_{18}H_{22}O_{11}$	2.121	413.1032	-13.798	0.146	1.080
91	1-Kestose	(+)	$C_{18}H_{32}O_{16}$	0.647	527.1581	-0.379	0.047	1.079
92	3-O-Primeverose-1,6,8-trihydroxy-2-methyl-anthraquinone	(-)	$C_{26}H_{28}O_{15}$	9.875	579.1351	-0.691	0.203	1.076
93	Aucubin	(-)	$C_{15}H_{22}O_9$	4.927	345.1188	-0.869	0.282	1.068
94	Rutin	(-)	$C_{27}H_{30}O_{16}$	12.275	609.1460	-0.164	0.405	1.066
95	4-O-Caffeoylquinic acid	(-)	$C_{16}H_{18}O_9$	5.998	353.0855	-6.514	0.242	1.066
96	Geniposidic acid	(-)	$C_{16}H_{22}O_{10}$	3.968	373.1137	-0.804	0.247	1.062
97	Hesperetin	(-)	$C_{16}H_{14}O_6$	17.815	301.0715	-0.996	0.076	1.061
98	Geniposide	(+)	$C_{17}H_{24}O_{10}$	14.854	389.1452	2.570	0.565	1.059
99	3,5,7,4′-Tetramethoxyflavone	(-)	$C_{19}H_{18}O_6$	0.756	341.1055	7.036	0.315	1.057
100	Isorhamnetin-3-O-rutinoside	(+)	$C_{28}H_{32}O_{16}$	14.118	625.1754	-0.960	0.309	1.053
101	Scopoletin	(+)	$C_{10}H_8O_4$	10.243	193.0495	0.000	0.071	1.048
102	Ricinoleic acid	(-)	$C_{18}H_{34}O_3$	31.846	297.2429	-2.019	0.159	1.047
103	1,5-Anhydro-2,6-dideoxy-3-O-α-D-glucopyranosyl-D-arabino-hexitol	(-)	$C_{12}H_{22}O_8$	5.924	293.1237	-1.706	0.172	1.046
104	4-Hydroxycoumarin	(+)	$C_9H_6O_3$	3.049	163.0390	0.000	0.035	1.043

Table 1 (continued)

No.	Compound name	Mode	Molecular Formula	RT (min)	m/z	Mass error (ppm)	CV	VIP Score
105	Rubiadin 1-methyl ether	(-)	$C_{16}H_{12}O_4$	0.643	267.0681	6.740	0.347	1.041
106	Nicotinic acid	(+)	$C_6H_5NO_2$	0.645	124.0392	-0.806	0.241	1.041
107	4-Hydroxycoumarin	(-)	$C_9H_6O_3$	9.506	161.0248	2.484	0.134	1.040
108	Esculetin	(+)	$C_9H_6O_4$	6.552	179.0339	-0.559	0.052	1.033
109	Geniposidic acid	(+)	$C_{16}H_{22}O_{10}$	1.105	375.1284	-0.533	0.042	1.025
110	Rubiadin	(+)	$C_{15}H_{10}O_4$	4.171	255.0647	-1.960	0.473	1.015
111	Gluconic acid	(-)	$C_6H_{12}O_7$	0.941	195.0535	12.817	0.053	1.007
112	Scopoletin	(-)	$C_{10}H_8O_4$	10.245	191.0349	-0.523	0.107	1.006
113	1-O-Primeverose-rubiadin	(-)	$C_{26}H_{28}O_{13}$	10.800	547.1450	-1.279	0.163	1.004
114	1,8-Dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione	(-)	$C_{15}H_{10}O_5$	25.753	269.0458	1.115	0.152	1.004

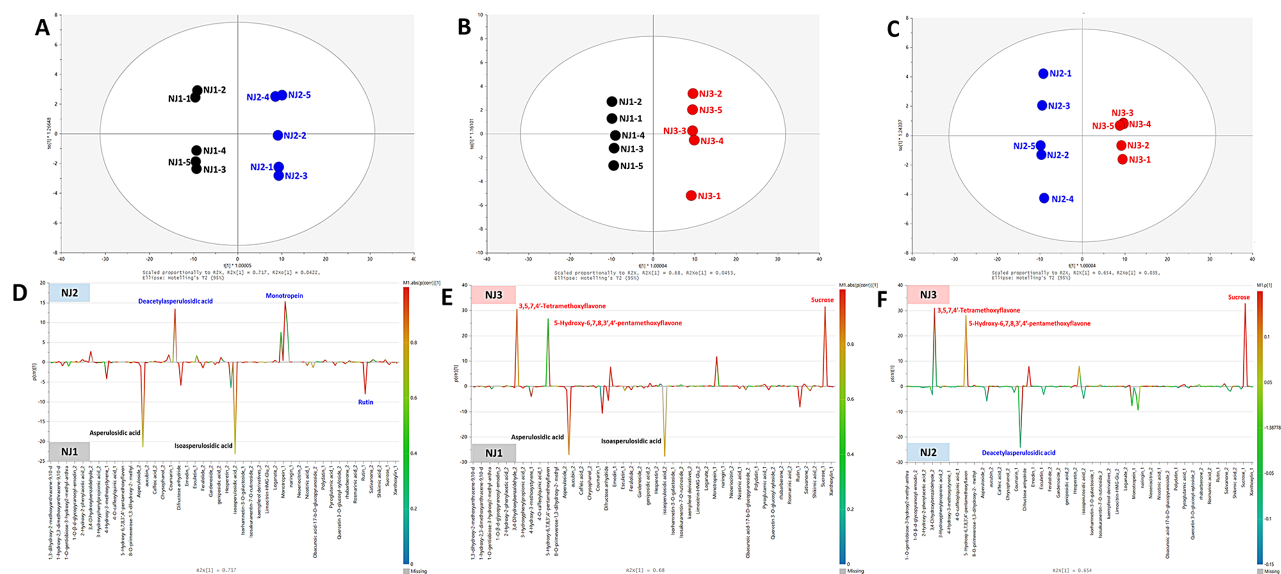

Fig. 3 OPLS-DA score plot and S-line plots of the non-fermented noni juice (NJ1) and the fermented noni juice (NJ2) (**A**, **D**; $R^2X = 0.723$, $R^2Y = 0.992$, $Q^2 = 0.985$), the non-fermented noni juice (NJ1) and the fermented noni juice added with organic coconut blossom sugar, calamansi juice and organic maltodextrin (NJ3) (**B**, **E**; $R^2X = 0.686$, $R^2Y = 0.991$, $Q^2 = 0.987$), and the the only fermented noni juice (NJ2) and the fermented noni juice added with the supplementary materials (NJ3) (**C**, **F**; $R^2X = 0.689$, $R^2Y = 0.999$, $Q^2 = 0.976$)

Abbreviations

LAB	Lactic acid bacteria
NJ1	Organic noni fruit juice
NJ2	Fermented organic noni fruit juice I
NJ3	Fermented organic noni fruit juice II
ACN	Acetonitrile
IPA	Isopropanol
QC	Quality control
UHPLC-Q-TOF/MS	Ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry
PCA	Principal component analysis
HCA	Hierarchical clustering analysis
PLS-DA	Partial least-squares discriminant analysis
OPLS-DA	Orthogonal partial least-squares discriminant analysis

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions
Yoonjeong Kim contributed to the data acquisition, data analysis and paper writing. JP and KK contributed to the data acquisition. JYL, EMK, IJL, and OHL contributed to conception and design of study. JS and Younghwa Kim contributed to the analysis of data and paper revision. All authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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