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Isabelle Fan Yang * and Changhong Liu *

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Combined Microbial Fermentation Converts Bioactive Compounds in *Nitraria tangutorum* Bobrov Fruit and Displays Its Antidiabetic Potential

Isabelle Fan Yang [1,*] and Changhong Liu [2]

[1] Nanjing Vinson Biotech Co. Ltd, Nanjing China
[2] Life science college, Nanjing University, Nanjing China
* Correspondence: isabelle_f_young@126.com

Abstract: *Nitraria tangutorum* Bobrov is a berry shrub with white flowers and red fruits, which grows in the deserts in the Tibetan Plateau, Mongolia, and western China. Its fruit *N. tangutorum* fruit (NTF) contains various bioactive compounds, with anti-fatigue, anti-inflammatory, and neuroprotective functions. However, the high saccharide content of NTF makes it unsuitable for diabetic patients. In this study, we fermented NTF to obtain *N. tangutorum* fermented juice (NTJ), and *N. tangutorum* fermented residue (NTR), which are suitable for diabetics to consume. We characterized the bioactive compounds in NTF, NTR, and NTJ, and found that fermentation increased the diversity of bioactive compounds, and greatly reduced sucrose, glucose, and fructose content while generating trehalose, which has the potential to manage blood glucose levels. Further, NTJ displays anti-diabetic potential due to various compounds anti-diabetic properties. This study provides a basis for further clinical research on NTJ's anti-diabetic function in humans.

Keywords: *Nitraria tangutorum* Bobrov; fermentation; biotransformation; lactic acid bacteria; high performance liquid chromatography mass spectrometry; phenolic compounds; anti-diabetic

1. Introduction

The *Nitraria tangutorum* Bobrov is a resilient desert shrub found in the arid regions of western China, Mongolia, and the Tibetan Plateau. This plant blooms from May to June, yielding clusters of fragrant white flowers and red cherry-like fruit known as "desert cherry," which has a delightful sweet and sour flavor. The shrub's ability to thrive in saline soil and endure drought makes it an essential component in preventing soil erosion and facilitating vegetation restoration in desert areas.

In addition to its environmental benefits, the fruit of *Nitraria tangutorum* is rich in nutrients, containing approximately 6.43% fat, 13.06% protein, and 23.17% saccharides (dry weight). Notably, the seeds of NTF are particularly rich in linoleic acid, constituting around 65% of the fat content [1]. This compound has been linked to reducing levels of low-density lipoprotein cholesterol, thereby conferring cardioprotective properties to NTF oil [2]. Moreover, NTF is a source of essential vitamins such as vitamin C, vitamin B1, vitamin B2, vitamin E, and vitamin K1 [1].

NTF offers various health-promoting properties, including anti-fatigue, antioxidant, anti-mutagenic, anti-hypotensive, and hepatoprotective effects. Over thousands of years, local communities have utilized NTF to address conditions such as dizziness, dyspepsia, stomach syndrome, spleen weakness, and neurasthenia [3]. Studies on mouse models have shown that NTF polysaccharides can lower blood glucose levels, decrease superoxide dismutase and glutathione peroxidase activities, and enhance creatine phosphokinase activities [4]. Furthermore, anthocyanins derived from NTF exhibit neuroprotective and cardioprotective effects [3,5], with potential applications in the treatment of type 2 diabetes [6,7]. Additionally, NTF contains multiple phenolic compounds known for their potent antioxidant and anti-inflammatory properties, contributing to the plant's overall health-promoting characteristics.

Despite its numerous health benefits, the high saccharide content, including sucrose, fructose, and glucose, makes NTF unsuitable for individuals with diabetes. This limitation hinders the

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utilization of NTF's health benefits for this demographic. Fermentation presents an opportunity to mitigate the presence of undesirable saccharides in NTF while potentially introducing new flavors and functions [8]. Therefore, the aim of our study is to explore the fermentation process applied to NTF, with the goal of reducing blood glucose-elevating saccharides while preserving or enhancing the existing health benefits of NTF products.

2. Materials and Methods

2.1. Materials and Regents

Dried NTF was obtained from Gansu Province China in August 2023. *Lactobacillus acidophilus* CICC 20244, *Lactobacillus plantarum* subsp. *plantarum* CICC 20022, *Lactobacillus paracasei* CICC 20241, and *Lactobacillus reuteri* CICC 6121 were obtained from the China Center of Industrial Culture Collection, CICC. Supeclean LC-18 SPE tube was purchased from Sigma-Aldrich (St. Louis, USA). Ethanol, methanol, ethyl acetate, and alloxan monohydrate were purchased from Sigma-Aldrich (Shanghai, China). Kunming mice were purchased from Hunter Biotech (Hangzhou, China).

2.2. Fermentation of NTF

The dried NTF (1kg) was thoroughly washed and soaked in 4L of water at a temperature of 40-60℃ for 24 hours. The seeds were carefully removed from the fruit, and the pulp was blended and boiled for 30 min to deactivate enzymes and eliminate unwanted bacteria, resulting in the original NTF pulp. To the NTF pulp, 50g of whey powder was added. The original NTF juice was then cooled to approximately 25 °C and inoculated with 20g of lyophilized probiotic culture, which contained 5g of lyophilized *L. acidophilus* ($1x10^9$cfu/g), 5g of lyophilized *L.plantarum* subsp. *plantarum* ($1x10^9$cfu/g), 5g of lyophilized *L. paracasei* ($1x10^9$cfu/g), and 5g of lyophilized *L. reuteri* ($1x10^9$cfu/g). The mixture was fermented anaerobically at 35 °C for 36 hours. After the 36-hour anaerobic fermentation, the mixture was filtered using a Buchner funnel-equipped filter. The filtered *N. tangutorum* fermented juice (NTJ) was bottled, pasteurized, and stored at room temperature for further use. The fermented residue of *N. tangutorum* (NTR) was collected, dried at 65℃, and ground to powder for further experiments.

2.3. Sample Preparation

Extraction of bioactive compounds from lyophilized NTF and fermented NTR:

Lyophilized NTF and NTR were ground to powder and used for bioactive compound extraction. 10g sample was weighed, added into 200ml 70% ethanol (v/v), and homogenized at 12000 rpm for 5 min. The homogenized mixture was sonicated at 65℃ for 40 min. After sonication, the sample was ice-bathed for 15 min, and centrifuged at 12000 rpm for 15 min. The supernatant was carefully collected and filtered through a 0.45 μm filter membrane. The filtered supernatant was condensed under vacuum to approximately 40 ml. 150 ml ethyl acetate was added into the condensed supernatant and fully mixed and then settled for 5 min to allow the ethyl acetate layer and aqueous layer to fully separate from each other. The ethyl acetate phase was collected and evaporated under vacuum to fully dry, then dissolved with 5 ml 95% ethanol, filtered through 0.45 μm cellulose filter membrane for further analysis.

A 100 ml NTJ sample was taken to mix with 200 ml ethyl acetate. After mixing the fermented juice and ethyl acetate fully, the mixture was settled until the ethyl acetate phase was separated from the aqueous phase. The ethyl acetate phase was collected and evaporated under the vacuum to fully dry, then dissolved with 5 ml methanol, and filtered through a 0.45 μm cellulose filter membrane. 200 μl methanol dissolved sample was mixed with 200 μl methanol and 400 μl nano-pure water, vortexed, and filtered through Supeclean LC-18 SPE tube. The filtered extraction sample was lyophilized and dissolved with 2 ml methanol and filtered through a 0.45 μm cellulose filter membrane for further analysis.

2.4. UHPLC-Q-TOF/MS/MS Analysis

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The analysis was carried out by a UHPLC-Q-TOF/MS/MS system (AB Sciex Pte. Ltd. Singapore) equipped with an autosampler, a mass spectrometer, a column compartment, a PDA detector, and a binary pump. UHPLC column (100 mm × 2.1 mm, 3μm, Thermo-Scientific, AQ RP-C18) was used for chromatographic separation. The flow rate was controlled at 0.4ml/min, and the column temperature was set at 40℃. The injection volume was 1μl. The detection wavelength was 280nm. Mobile phase A is 0.1% formic acid in water, mobile phase B was acetonitrile. The chromatographic separation was carried out by gradient elution procedure as follows: 0-2.0 min, 5% phase B, 2.0-19.0 min, 5%-30% phase B, 19.0- 23.0 min, 30%-70% phase B, 23.0 -25.0 min, 70%- 95% phase B, 25.0- 25.1 min, 95%- 5% phase B, 30.0 min stop.

MS detection was conducted by a Triple TOF 4600-1 system (AB Sciex Pte. Ltd. Singapore), equipped with an electrospray ionization (ESI) source in the positive ESI mode [ESI(+)]. High-purity helium is used as collision gas, collision energy was 26 V collision-activated dissociation (CAD) was 6 units, ion spray voltage was 5500 V, and curtain gas pressure was 25 psi. High-purity nitrogen is used as nebulizing gas at 40 psi and drying gas at 550℃. The heater gas pressure was 40 psi. The period cycle time was 760ms, and the pulser frequency was 12.891kHz with an accumulation time of 150.0ms. The sampling frequency was 10 Hz, and the cell temperature was 40℃. The LC-MS/MS data analysis was performed by Analyst MD software (Version 1.6.3, AB Sciex Pte Ltd. Singapore).

For identified compounds, their relative content of bioactive compounds were calculated as below:

Relative content = A_i/A_0 x 100%

A_i is the peak area of the specific compound, and A_0 is the sum of the area of all the compounds in the tested entity.

2.5. Determination of Fructose, Sucrose, Maltose and Glucose

The quantitative analysis of fructose, sucrose, lactose, maltose, and glucose was conducted by Ti Testing and Certification Group, according to the standardized method in GB 5009.8. In brief, 21.9g $Zn(CH_3COO)_2{\cdot}2H_2O$ was weighed, and mixed with 3 ml acetic acid, placed in a 100 ml volumetric flask, and distilled water was added into the volumetric flask until reached 100ml in total volume.10.6g $K_4[Fe(CN)_6]{\cdot}3H_2O$ was weighed and dissolved with distilled water to reach 100 ml. Standard solutions of fructose, glucose, maltose, and sucrose were prepared with 20mg/ml concentration. Chromatographs of standard solution for fructose, glucose, maltose, and sucrose were obtained via HPLC, with mobile phase 70% acetonitrile and 30% water (v/v) running for 30 min. The flow rate was set at 1.0ml/min, column temperature was 40℃. The injection sample volume was 20 μl. Peak areas of the standard solution were measured according to the pre-described method. Then 2 g of dried NTF, 2g of NTR, and 50 ml NTJ were taken for the analysis of fructose, sucrose, lactose, maltose, and glucose. Samples were placed in 100ml volumetric flasks with 5ml $Zn(CH_3COO)_2$ solution and $K_4[Fe(CN)_6]$ solution, respectively, and distilled water was added into the volumetric flask to reach 100ml in total volume. Samples were fully dissolved in the water with the assistance of 30min sonication. The sample solution was filtered through 0.45 μm cellulose filter membrane, for further HPLC analysis. Fructose, sucrose, glucose, maltose, and lactose were determined in the filtered sample solutions via the pre-described HPLC method same as the method for standard solutions. Peak areas of fructose, sucrose, glucose, and maltose in sample solutions were obtained. The concentration of fructose, sucrose, glucose, and maltose in the samples was determined via comparison of peak area with standard solutions.

3. Result and Discussion

3.1. Fermentation Reduced Saccharides Content in NTF

The quantitative analysis presented in Table 1 delineates the variations in fructose, glucose, sucrose, and maltose concentrations among dried NTF, NTR, and NTJ. Evidently, fermentation exerted a profound impact on the levels of these saccharides within NTF. Initially, dried NTF exhibited substantial quantities of fructose (94 mg/g), glucose (73 mg/g), sucrose (11 mg/g), and

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maltose (14 mg/g). Following fermentation, the fructose content decreased to 6.3 mg/g, glucose to 11 mg/g, sucrose to 8.5 mg/g, and maltose to 5.5 mg/g in NTR (fermented residue). This represents significant reductions of 93.3%, 84.9%, 22.7%, and 60.7% for fructose, glucose, sucrose, and maltose, respectively. Notably, NTJ fermented juice displayed absence of detectable fructose, glucose, sucrose, or maltose.

The altered saccharide profiles following fermentation suggest microbial metabolism, particularly by *Lactobacillus acidophilus, Lactobacillus plantarum subsp. plantarum, Lactobacillus paracasei*, and *Lactobacillus reuteri*, with a notable consumption of fructose and glucose. These sugars are likely metabolized via the phosphoketolase pathway by heterolactic acid bacteria during fermentation [9]. Maltose, another fermentable sugar, was presumably utilized by *L. reuteri* to produce glucose [10], subsequently consumed by all four lactic acid bacteria. Table 1 shows that the content of sucrose also declined after fermentation, but not as great as the reduction of fructose and glucose. Sucrose content in NTF reduced from 11mg/g to 8.5 mg/g through the fermentation process. Unfermented NTF does not contain as much sucrose (11mg/g) as fructose (94 mg/g) and glucose (73 mg/g), and sucrose was not consumed a lot by the fermentation process. The decrease in sucrose content, though less pronounced compared to fructose and glucose, indicates metabolic activity within NTF. *Lactobacillus paracasei* and *Lactobacillus reuteri* might exhibit limited reactions to sucrose[11], impacting its utilization during fermentation. Conversely, *L. plantarum* can metabolize sucrose in an anaerobic condition [12], and *L. acidophilus* also uses sucrose as an energy source, but not as much as fructose and glucose [13], *L. plantarum* and *L. acidophilus* likely played significant roles in sucrose metabolism within NTF.

The absence of fructose, glucose, sucrose, and maltose in NTJ suggests potential benefits for individuals with type 2 diabetes. Furthermore, fermentation facilitated the conversion of sucrose into trehalose, a compound with potential implications for blood glucose management. Trehalose was detected in NTJ, contrasting with the absence of sucrose, indicating its potential presence. Trehalose, less sweet than sucrose [14]. There are reports regarding the presence of glucosyltransferase A and inulosucrase in *L. reuteri*, and the existence of β-fructofuranosidase in *L. plantarum* [15] [16]. In the fermentation system, it is possible that the fructosyl moiety of sucrose was cleaved by fructofuranosidase and transferred to the acceptor molecule to form fruoctooligosaccharides, while the glucosyl moiety of sucrose formed trehalose with the assistance of glucosyltransferase [17] [18] [19]. Trehalose has demonstrated efficacy in modulating blood sugar and associated metabolic processes. Trehalose can improve insulin sensitivity through PAI-1 down-regulation [20], increasing adiponectin release [21]. Trehalose is also able to bind with glucose transporter (GLUT) receptors and inhibit their activity, consequently preventing excessive glucose absorption in the gastric intestinal tract [22] [23]. NTJ, enriched with trehalose and devoid of sucrose, holds promise for contributing to glucose homeostasis and insulin sensitivity, thereby potentially ameliorating blood sugar management in diabetic populations.

3.2. Fermentation Modified Bioactive Compounds in NTF and Altered Their Relative Content

The bioactive compounds present in NTF, NTR, and NTJ are outlined in Table 2, along with their relative concentrations. Detailed information on these compounds is provided in Supplementary Tables S1, S2, S3, and S4. Table 2 indicates the identification of 114 bioactive compounds in NTF, 91 in NTR, and 73 in NFJ. Previous research has identified anthocyanins in *Nitraria tangutorum*, such as cyanidin 3-O-sophoroside, cyanidin 3-O-hexose, petunidin 3-O-rutinoside-glucose, peonidin 3-O-sophoroside, petunidin 3-O-rhamnoside, malvidin 3-O-arabinose, peonidin 3-O-hexose, cyanidin 3-O-(cis-p-coumaroyl)-diglucosdie, cyanidin 3-O-(trans-p-coumaroyl)-diglucoside, pelargonidin 3-O-(p-coumaroyl)-diglucoside, peonidin 3-O-malonyl-glucoside, petunidin 3-O-(p-coumaroyl)-glucoside. Additionally, it has been reported that Nitraria tangutorum fruit contains multiple phenolic compounds, including quercetin 3-O-hexoserhamnosyl-glucosdie, kaempferol glucoside-rutinoside, isorhamnetin 3-(rham-galactosyl-robinobioside), isorhamnetine 3-(rham-glucosyl-robinobioside), quercetin-rutinose, isorhamnetin 3-O-robinobiosdie, isorhamnetin 3-O-rutinoside [24]. Only isorhamnetin 3-O-rutinoside is identified in this investigation, anthocyanins, quercetin

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derivatives, kaempferol derivatives we identified in NTF are different from the previous report, which may due to different cultivar. Additionally, various phenolic compounds have been reported in *N. tangutorum* fruit, such as quercetin derivatives, kaempferol derivatives, and isorhamnetin derivatives.

According to Table 2, NTF, NTR and NTJ all contain some amino acids and their derivatives, alkaloids, anthocyanins, indole derivatives, phenolic compounds, and some other compounds. Regarding amino acids and their derivatives, arginine and N-acetylphenylalanine remained stable across fermentation but exhibited changes in relative concentrations. . In NTF, arginine's relative content was 3.65%, in NTR, arginine's relative content was 1.73%, in NTJ, arginine's relative content was 2.47%. Arginine's relative content was highest in NTF, suggesting its partial consumption by lactic acid bacteria during fermentation via the arginine deiminase (ADI) pathway. N-acetylphenylalanine exhibited increased relative content in NTJ, its relative content was 0.30% in NTF, 0.23% in NTR, and 0.79% in NTJ possibly due to decreased compound numbers. Other compounds like tyrosine and tryptamine were consumed during fermentation, while new compounds like L-Pyroglutamic acid and 3-hydroxyanthranilic acid were generated in NTJ. It has been reported that some thermophilic lactic acid bacteria are able to generate L-Pyroglutamic acid during fermentation. [25]

In the alkaloid category, some compounds remained unchanged but showed varying relative contents, indicating complex metabolic activities during fermentation. Quinoline 4-carboxylic acid and lupine have their highest relative contents in NTJ, which are 0.87% and 3.26% respectively. Norharman and harmane have the highest relative content in NTR, which might indicate that norhaman and harmane were generated during fermentation, but did not release into the fermented juice, thus accumulated in NTR. Theophylline, sparteine, vinpocetine and 2-(hydroxymethyl)-4(3H)-quinazolinone only presented in NTF, not in NTR or NTJ, which suggests that these compounds were consumed by the microorganisms during the fermentation. Deoxyvasicinone presented in NTF and NJR not in NTJ. Harmine, harmol hydrochloride, ephedrine, hordenine and tabersonine existed only in NTJ, which indicated that they are newly generated through fermentation. Harmol hydrochloride was found in NTJ might be due to the reason that hydrochloride form of harmol increased its solubility, thus it existed in NTJ. It has been reported that under the catalysis of tyrosine decarboxylase from *Lactobacillus brevis*, tyrosine can be converted to hordenine. [26] Tyrosine was detected in NJF prior to fermentation, and vanished after fermenation, (see Table 2), and lactic acid bacteria were employed in this fermentation process, therefore, it is very likely that during the fermentation tyrosine was converted to hordenine.

The only amine in the NTR and NTJ is etilefrine, which is absent in NTF. Hence etilefrine was generated during fermentation.

Anthocyanins exhibited diverse distributions across NTF, NTR, and NTJ, suggesting conversion and accumulation processes during fermentation. Peonidin-3-o-glucoside and peonidin-3-o-galacoside exist in all the three entities: NTF, NTR and NTJ, and they both have their highest relative content in NTR and lowest relative content in NTJ. It can be speculated that fermentation process might have generated some peonidin-3-o-glucoside and peonidin-3-o-galacoside and they were trapped in the fruit or microorganism tissue and was not able to be released in the fermented juice. Petunidin 3-O-glucoside existed in NJF and NJR but not in NTJ, the relative content of petunidin 3-O-glucoside was higher in NTR than NTF while petunidin 3-galactoside presented only in NTJ and not in NTF or NTR, which suggests that during the fermentation process, new petunidin 3-O-glucoside was generated, but and some might have converted to petunidin 3-galactoside and released into NTJ. Peonidin 3-O-D-glucopyranoside presented in NTF and NTR, its relative contents were similar in these two entities, which suggests that peonidin 3-O-D-glucopyranoside did not change during fermentation and remained in NTR after fermentation. Cyanidin 3-O-rhamnoside was detected in NTF and not in NTR or NTJ. Cyanidin 3-O-glucoside existed in both NTF and NTR. Cyanidin 3-O-galactoside presented in NTJ only, and cyanidin presented in NTR and NTJ not in NTF. The distribution of cyanidin and its derivatives indicated that cyanidin 3-O-rhamnoside might have

 doi:10.20944/preprints202403.1251.v1

been converted to cyanidin and cyanidin 3-O-glucoside might have been partly converted to cyanidin 3-O-galactoside.

Indole derivatives displayed distinct patterns across the constituents, with some newly generated compounds detected only in NTJ, indicating microbial activity during fermentation. Indole 3-carboxaldehyde and 3-formylindole exist in both NTF and NTR not NTJ, with their relative content significantly higher in NTR. Six indole derivatives were newly generated during fermentation, including serotonin, alpha-oxo-1h-indole-3-propanoic acid, 2-(5-methoxy-1H-indol-3-yl) acetic acid, indole 3-acetic acid, 5-hydroxyindole-3-acetic acid, and beta-oxo-1h-indole-3-propanoic acid, among which beta-oxo-1h-indole-3-propanoic acid presented in NTR, all the other compounds presented in NTJ. Serotonin, a neurotransmitter with potential health benefits, was detected in NTJ, potentially synthesized from tryptamine or tryptophan. NTF. Mora-Villablobos' previously reported that Escherichia coli could produce tryptophan with culture media containing glucose, and converts tryptophan to 5-hydroxytryptophan (5HTP) and subsequently serotonin through decarboxylase [27]. In plants, tryptamine is converted to serotonin by tryptamine 5-hydroxylase (T5H) [28]. It has been reported that NTF contains tryptophan [29], and our research also revealed the existence of tryptamine in NTF (see Table 2), therefore, under systemic fermentation with *L. acidophilus, L. plantarum, L. paracasei,* and *L. reuteri* , the serotonin in NTJ could be converted from tryptamine or tryptophan.

In the organic acid category, hippuric acid exists in NTF and not in NTR or NTJ, kojic acid were newly generated in NTJ. Kojic acid is present in NTJ, which may be the product of fermentation. Kojic acid is the typical product of aerobic fermentation. Since the employed fermentation process was facultative anaerobic, in the initial stage, there was oxygen in the container, L. acidophilus, L. plantarum, L. paracasei, and L. reuteri, grew in aerobic condition, as their aerobic respiration continued, the volume of oxygen in the container reduced, yet the container was not completely sealed, small amount of oxygen could still enter the container, but the main fermentation process was still facultative anaerobic. Thus, in the aerobic phase, sucrose, glucose, maltose, and fructose all could be used as carbon sauce to produce kojic acid [30]. Although in industrialized production, kojic acid is usually produced via fermentation of rice by *Aspergillus oryzae*, *Aspergillus parasiticus*, and *Aspergillus candidus* [30], it is possible that during the fermentation of NTF by *L. acidophilus, L. plantarum, L. paracasei, and L. reuteri*, kojic acid can also be generated.

Phenolic compounds showed diverse distributions and transformations during fermentation, with various compounds exhibiting higher relative contents in NTJ, possibly due to reduced compound numbers or microbial activity. Biotransformations and compound conversions were evident, impacting the final composition of the fermented product. caffeic acid, trans-4 coumaric acid, esculetin, chlorogenic acid, daphnetin, vicenin 2, isorhamnetine 3,7-diglucoside, isohamnetin 3-O-galactoside-6''-rhamnoside, isorhamnetin 3-O-rutinoside, isorhamnetin 3-galactoside, quercetin 3-O-beta-glucopyranosyl-7-O-alpha-rhamnopyranoside, rutin, isoquercetin, ferulic acid, sinapic acid, kaempferol 3-O-glucoside-2''-rhamnoside-7-rhamnoside, kaempferol 3-O-robinoside-7-O-rhamnoside, kaempferol 3-O-galactoside-7-O-rhamnoside, kaempferol 3-O-rutinoside, kaempferol 3-O-glucoside-7-O-rhamnoside, vitexin, isovitexin, diosmetin 7-O-rutinoside, and licoflavanone exist in all three entities (NTF, NTR and NTJ). Among these compounds, caffeic acid, esculetin, vicenin 2, quercetin 3-O-beta-glucopyranosyl-7-O-alpha-rhamnopyranoside, ferulic acid, sinapic acid and vitexin have the highest relative content in NTJ, which may be because fewer compounds were detected in NTJ (totally 73 compounds were identified in NFJ, while 114 compounds were detected in NTF), and elevated the relative content of these compounds. Additionally, the increased relative content of these compounds in NTJ might be because microorganisms consumed fibers in NTF and released some secondary metabolites into the fermented juice (NTJ), thus elevating their relative contents in NTJ. Esculetin's relative contents in NTJ is around ten times that in NTF, sinapic acid's relative contents in NTJ is around nine times that in NTF, ferulic acid's content in NTJ is around 7 times that in NTF. The reason behind this may be bacteria's consumption of fiber and cell walls during fermentation, and in turn releasing some phenolic compounds that were trapped by the fiber, biotransformation from other compounds could be another possible reason for the compounds with

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high relative content. Trans-4-courmaric acid, daphnetin, isorhamnetin 3-O-galactoside-6''-rhamnoside, diosmetin 7-O-rutinoside, and licoflavanone have highest relative contents in NTR, which suggests that these compounds have been accumulating in the residue during fermentation and not tend to release in the fermented juice. The possible reason behind this phenomenon may be these compounds are more attached to the flesh of NTF, may bond with the fruit tissue, and cannot be released into fermented juice easily. 36 hours' fermentation may not be able to consume all the fiber in NTJ completely. Some other compounds, including arginine, chlorogenic acid, isorhamnetin 3,7-di-O-glucoside, isorhamnetin 3-O-rutinoside, isorhamnetin 3-galactoside, rutin, isoquercetin, kaempferol 3-O-glucoside-2''-rhamnoside-7-rhamnoside, kaempferol 3-O-galactoside-7-O-rhamnoside, kaempferol 3-O-rutinoside, kaempferol 3-O-glucosdie-7-O-rhamnoside, and isovitexin have their highest relative content in NTF, which may indicate that these compounds may be partly converted into some other forms or consumed by the microorganisms to some extent during fermentation. For instance, vitexin (apigenin-8-C-glucoside) shows the highest relative content in NTJ, while isovitexin (apigenin-6-C-glucoside) has its highest relative content in NTF, which may suggest that isovitexin in NTF might have been converted to vitexin in part via fermentation, and thus increased the relative content of vitexin in NTJ. Additionally, apigenin-7-O-glucoside is only present in NTF and NTR, which might indicate the biotransformation between vitexin, isovetixn, and apigenin-7-O-glucoside during fermentation. It is possible that isovitexin in NTF was biotransformed to both vitexin and apigenin-7-O-glucoside under lactic acid bacteria fermentation, vitexin was released into the fermented juice (NTJ), and apigenin-7-O-glucoside remained in the fermented residue (NTR).

According to Table 2, some compounds are newly generated during fermentation process, including osthol, protocatechuic acid, xanthurenic acid, syringic acid, fraxetin, syringaldehyde, feruloyl quinic acid, homooreintin, sinapic acid, kaempferol 3-Orhamnoside, lonicerin, coumarin, nepetin 7-glucoside, 3,5-dimethoxycinnamic acid, diosmetin, cirsimarin, oenin and tricin. Some of them exist in only NTJ, others exist only in NTR, as it is shown in Table 2. Five kaempferol derivatives have their highest relative contents in NTF, but kaempferol 3-O-rhamnoside only present in NTJ, kaempferol 7-O-glucoside present in NTR and NTJ, not NTF. Therefore it is reasonable to speculate that these five kaempferol derivatives, namely kaempferol 3-O-glucoside-2''-rhamnoside-7-rhamnoside, kaempferol 3-O-robinoside-7-O-rhamnoside, kaempferol 3-O-galactoside-7-O-rhamnoside, kaempferol 3-O-rutinoside, and kaempferol 3-O-glucoside-7-O-rhamnosdie, can be utilized by fermentation bacteria and convert to some other forms, such as kaempferol 3-O-rhamnoside and kaempferol 7-O-glucoside. Syringic acid is one of the compounds that exists in fermented NTJ, but not NTF or NTR. It has been reported that under certain conditions, such as in the presence of Paecilomyces variotii, sinapic acid could be converted to syringic acid, and the concentration of reduced sinapic acid is not in a linear correlation with the increased concentration of syringic acid in the cultural media [31]. Therefore, it is possible for the biotransformation from sinapic acid to syringic acid. As it is shown in Table 2, sinapic acid is present in all three constituents: NTJ, NTR, and NTF. Thus, it is likely that under systemic fermentation with L. acidophilus, L. plantarum, L. paracasei, and L. reuteri sinapic acid could be converted into syringic acid. Although sinapic acid's relative content is higher in NTJ, it might due to the reduced total compounds in NTJ, or some other compounds were converted to sinapic acid, such as phenylalanine [32]. There is also syringaldehyde present in fermented NTJ, which does not exist in NTR or NTF. Under certain circumstances, syringaldehyde can be converted to syringate under aldehyde dehydrogenase, which is an enzyme in *Escherichia coli* and could also exist in *L. acidophilus, L. plantarum, L. paracasei*, and *L. reuteri*, as well [33]. Therefore, the syringaldehyde in NTJ was probably converted to syringic acid by aldehyde dehydrogenase, which is present in the microorganisms. There might be a dynamic balance between syringaldehyde and syringic acid in NTJ. Xanthurenic acid was newly generated through fermentation and existed in NJR only. It has been reported that NTF contains tryptophan [29], and tryptophan can be converted to kynurenine [34], then kynurenine can be further hydroxylated by kynurenine monooxygenase to form xanthurenic acid [35]. Fraxetin was newly generated from the fermentation process and was found only in NTJ. It has been reported that fraxetine can be

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synthesized from esculetin and ferulic acid by E.coli, and esculetine and ferulic acid both exist in NTF. Esculetin can be converted to scopoletin by an O-methyltransferase, and then scopoletin can be synthesized to fraxetin by scopoletin 8-hydroxylase. Ferulic acid can also be synthesized to scopoletin by *E.coli*., and then further synthesized to fraxetin [36]. Coumarin was found only in NTJ, not in NTF or NTR, which indicated that coumarin was newly generated through fermentation process. Previous research has found that phenylalanine can be first converted to trans-cinnamic acid by phenylalanine aminolyase, then trans-cinnamic acid can form coumarin via ortho-hydroxylation, UDP-glycosidation, trans/cis isomerization of the side chain, and lactonization [37].Diosmetin is newly generated by the fermentation process and present in NTR, while diosmetin-7-O-rutinoside (diosmin) exist in all three entities (NTF, NTR and NTJ). It has been reported that when cultured with gut microorganisms, diosmin can be converted to diosmetin [38], it is likely that the fermentation process with L. acidophilus, L. plantarum, L. paracasei, and L. reuteri may transform diosmin to diosmetin. Tricin was found only in NTR, which indicated that tricin was formed during the fermentation process. Previous study has found that chrysoeriol can be transformed by CYP75B4 to selgin, and subsequently to tricin by 3′,5′-OMT [39]. Chrysoeriol was found in NTF and NTR, thus it is possible that chrysoeriol was converted to tricin during fermentation.

Furthermore, newly generated compounds during fermentation included vitamins, and other compounds, suggesting dynamic metabolic processes. pyridoxine was found in NTF and NTJ, D-pantothenic acid was found only in NTJ but not NTF or NTR. Kynurenic acid present in all three entities (NTF, NTR, and NTJ). 2-phenylacetamide, tryptoline, 2-ureidopentanedioic acid, and ophiopogonoside A present in NTR only, phytosphingosine was found in NTJ only, which indicated that they were newly generated during the fermentation process. Kynurenic acid's relative content was 1.55% in NTF, 2.14% in NTR, and 3.42% in NTJ, which suggests that kynurenic acid concentration might be elevated during fermentation, and the fermentation process might have generated some new kynurenic acid. As it was mentioned before that Nitraria tangutorum Bobrov fruit contains tryptophan [29] and tryptophan can be converted to kynurenine [34], and kynurenine can be further converted to kynurenic acid [40], thus tryptophan conversion by L. acidophilus, L. plantarum, L. paracasei, and L. reuteri might be the reason of elevated kynurenic acid NTJ and NTR. Phytosphingosine also exists in NTJ, which is not in NTF or NTR. Phytosphingosine is a component of sphingolipids, which exist in prokaryotes and are involve in cell differentiation. Phytosphingosine in NTJ is probably from the cells of lactic acid bacteria, which were utilized in the fermentation process. Phytosphingosine is usually found in animal foods, plants and fungi, potatoes and sweet potatoes. Therefore, it is generally considered safe to consume phytosphingosine. Since phytosphingosine is the only compound from the fermentation microorganism, which has been detected in our research, therefore, the fermented Nitraria tangutorum Bobrov products could be considered safe as foods. These findings shed light on the complex biochemical transformations occurring during the fermentation of NTF, NTR, and NTJ, influencing their bioactive compound profiles and potential health benefits. Further research is warranted to elucidate these processes comprehensively and their implications for product quality and functionality

3.3. Fermented NTF Product Displays Anti-Diabetic Potential

The presence of various bioactive compounds in NTJ, such as L-arginine, pyridoxine, chlorogenic acid, daphnetin, caffeic acid, ferulic acid, sinapic acid, and quercetin derivatives, among others, likely contributes to its anti-diabetic properties. L-arginine, as a free amino acid in NTB fruit and remains unchanged through fermentation, has demonstrated a beneficial effect in supporting nitric oxide (NO) production, and has the ability to lower blood pressure [41]. Diabetes patients are more likely to experience hypertension, because hyperinsulinemia is caused by insulin resistance, and hyperglycemia promotes vascular remodeling in their body, which leads to peripheral vascular resistance and elevated circulatory fluid volume [42]. Thus, if the arginine molecule in NTF and its fermented products is confirmed as L-arginine in further research, then NTR and NTJ may be able to display health functions in blood pressure management, especially in diabetes patients. Pyridoxine is vitamin B6, which is essential in red blood cell metabolism, converting iron to hemoglobin.

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Pyridoxine also possesses functions of quenching reactive oxygen species and serves as a neurotransmitter [43]. Chlorogenic acid has demonstrated the ability to lower fasting plasma glucose, triglycerides, and total cholesterol levels in impaired glucose tolerance patients. Clinical trials revealed that oral administration of 1200 mg chlorogenic acid per day significantly reduced fasting plasma glucose, triglycerides, low-density lipoprotein cholesterol, body weight, and waist circumference, as well as increased insulin sensitivity in impaired glucose tolerance patients [44]. Daphnetin may have the potential to treat diabetes via its protective properties to the pancreas. In the in vitro experiment, daphnetin showed a protective effect on insulinoma (INS-1) cells and promoted their glucose-stimulated insulin secretion [45]. Therefore, daphnetin-containing NTJ may have the potential to assist in blood sugar management. Caffeic acid has an antihyperglycemic effect and revealed properties of lowering blood glucose and glycosylated hemoglobin levels, and simultaneously elevating plasma insulin, and leptin levels in db/db mice [46]. Additionally, caffeic acid exhibited the capacity to protect the liver and kidney from oxidative damage, and reduced atherogenic indices in type 1 diabetic mice, which indicated that caffeic acid may be able to mitigate diabetic symptoms [47]. Ferulic acid is a potent antioxidant and can protect the liver and pancreas from oxidative damage. It can also increase the activity of glucokinase enzymes and decrease glucose production in the liver, thus promoting glucose homeostasis [48]. Sinapic acid showed a dose-dependent capacity to attenuate hyperglycemia in diabetic rat models [49]. Harmane is found to inhibit triglyceride accumulation in cell-cultured studies, which may indicate its capacity to mitigate obesity [50]. The quercetin derivatives in NTJ are quercetin 3-O-rhamnopyranosyle(1-2)-D-glucopyranoside-7-O-rhamnopyranoside, quercetin 3-O-beta-glucopyranosyl-7-O-alpha-rhamnopyranoside, quercetin 4'-O-glucoside, rutin, and isoquercetin. Animal studies have reported that isoquercetin has the effect on decreasing cholesterol and triglyceride levels and improving the function of pancreatic islets, thus alleviating diabetes [51]. Rutin exhibits antidiabetic properties by inhibiting small intestine carbohydrate absorption, increasing tissue glucose consumption, and stimulating insulin secretion [52]. Some isorhamnetin glycosides exhibit antioxidant, anti-inflammatory, anti-cancer, and antidiabetic activities [53]. Diosmetin 7-rutinoside is also named diosmin, which has demonstrated its ability to lower plasma glucose levels and increase plasma insulin levels in diabetic rats. Additionally, diosmin exhibits anti-cancer, anti-oxidant, and anti-inflammation properties [54]. Vicenin-2 displayed the capacity to attenuate high-glucose-induced elevated reactive oxygen species, increased vascular permeability, and activation of nuclear factor (NF)-κB in human cell culture study and mice experiments, which suggests that vicenin-2 has the potential to manage diabetic complications [55]. It has been reported that vitexin and isovitexin have the effect of reducing postprandial blood glucose levels in rodents. Besides, in a rat model study, vitexin demonstrated the ability to attenuate lipopolysaccharide-induced damage in islet tissue [56]. Oral administration of syringic acid has shown effects in lowering glucose levels, increasing insulin levels, and increasing glycogen levels in diabetic rats model. Syringic acid displays antihyperglycemic function by inhibiting the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase [57]. The presence of syringic acid in NTJ shows the potential of NTJ in managing diabetes. Petunidin 3-galactoside shows antioxidant activity and has the ability to inhibit α-glucosidase, thus inhibiting carbohydrates from breakdown and in turn alleviating hyperglycemia. Petunidin 3-galactoside also demonstrates a recovery effect on hepatocytes, which have impaired glucose uptake capacity due to exposure to high glucose. This finding indicates that petunidin 3-galactoside might have the ability to promote restoration to hepatocytes and alleviate hyperglycemia via improving glucose uptake in the liver [50]. With so many compounds possessing anti-diabetic properties in the fermented juice, it is reasonable that NTJ demonstrated anti-diabetic effects.

The abundance of compounds with anti-diabetic properties in NTJ underscores its potential as a therapeutic agent for managing diabetes. Further research is warranted to elucidate the specific mechanisms underlying its efficacy and optimize its therapeutic use.

4. Conclusion

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NTF harbors a plethora of bioactive compounds with diverse health-promoting attributes. Through microbial fermentation, the content of blood glucose-elevating saccharides in NTF is diminished, while the array of bioactive compounds is augmented, resulting in alterations in their constituent and relative content within NTF. Fermented products derived from *Nitraria tangutorum* Bobrov fruit, namely NTJ, displays anti-diabetic potential. These findings suggest that post-fermentation, *Nitraria tangutorum* Bobrov fruit has the potential to be used as a viable option for diabetic individuals, offering potential benefits for blood glucose management. However, further clinical investigations are warranted to validate their anti-diabetic efficacy in human subjects. Moreover, additional studies are needed to elucidate the specific anti-diabetic compounds present in fermented NTF products and unravel the underlying mechanisms responsible for their anti-diabetic effects.

Table 1. Fructose, glucose, sucrose, and maltose content in NTF, NTJ, and NTR.

Samples	Fructose (mg/g)	Glucose (mg/g)	Sucrose (mg/g)	Maltose (mg/g)
NTF	94	73	11	14
NTJ	Not detected	Not detected	Not detected	Not detected
NTR	6.3	11	8.5	5.5

Table 2. Bioactive compounds in NTF, NTR and NTJ.

Identified compounds	**Molecular formula**	**Theoretical Mass**	**relative content in NTF**	**relative content in NTR**	**relative content in NTJ**
Amino acid and its derivatives					
Arginine	C6H14N4O2	175.1195	3.65%	1.73%	2.47%
Tyrosine	C9H11NO3	182.0817	1.03%	ND	ND
Tryptamine	C10H12N2	161.1079	0.07%	ND	ND
L-Phenylalanine	C9H11NO2	166.0868	0.83%	ND	0.19%
N-Acetylphenylalanine	C11H13NO3	208.0974	0.30%	0.23%	0.79%
L-Pyroglutamic acid	C5H7NO3	130.0504	ND	ND	3.18%
3-Hydroxyanthranilic acid	C7H7NO3	154.0504	ND	ND	0.69%
N-Acetylleucine	C8H15NO3	174.113	ND	ND	0.74%
Alkaloid					
Theophylline	C7H8N4O2	203.0545	3.61%	ND	ND
Quinoline 4-carboxylic acid	C10H7NO2	174.0555	0.34%	0.20%	0.87%
Lupinine	C10H19NO	170.1545	2.10%	2.00%	3.26%
Harmol	C12H10N2O	199.0871	0.12%	0.39%	0.73%

 doi:10.20944/preprints202403.1251.v1

Norharman	C11H8N2	169.0766	0.34%	0.79%	0.63%
Sparteine	C15H26N2	235.2174	0.58%	ND	ND
Harmane	C12H10N2	183.0922	1.11%	2.21%	1.98%
Deoxyvasicinone	C11H10N2O	187.0871	0.07%	0.13%	ND
Vinpocetine	C22H26N2O2	351.2073	0.08%	ND	ND
2-(hydroxymethyl)-4(3H)-quinazolinone	C9H8N2O2	177.0664	0.23%	ND	ND
Harmine	C13H12N2O	213.1028	ND	ND	0.09%
Ephedrine	C10H15NO	166.1232	ND	ND	0.67%
L-Oxonoreleagnine	C11H10N2O	187.0871	ND	0.97%	ND
Hordenine	C10H15NO	166.1232	ND	ND	0.06%
Tabersonine	C21H24N2O2	337.1916	ND	ND	0.09%
Amine					
Etilefrine	C10H15NO2	182.1181	ND	0.12%	0.13%
Anthocyanin					
Cyanidin-3-O-rhamnoside	C21H21O10	433.1135	0.07%	ND	ND
Cyanidin-3-O-glucoside	C21H20O11	449.1084	0.43%	0.58%	ND
Cyanidin 3-O-galactoside	C21H21O11	449.1084	ND		0.06%
Cyanidin	C15H10O6	287.0556	ND	0.21%	0.13%
Peonidin-3-O-D-glucopyranoside	C22H23O11	463.1240	0.13%	0.15%	ND
Peonidin 3-O-glucoside	C22H22O11	463.124	0.97%	1.09%	0.06%
Peonidin 3-O-galactoside	C22H23O11	463.124	0.52%	1.12%	0.11%
Petunidin-3-O-glucoside	C22H22O12	479.1190	0.24%	0.51%	ND
Petunidin 3-galactoside	C22H23O12	479.119	ND	ND	0.09%
Indole derivatives					
Indole-3-carboxaldehyde	C9H7NO	146.0606	0.48%	1.29%	ND
3-Formylindole	C9H7NO	146.0606	0.43%	1.13%	ND
Serotonin	C10H12N2O	177.1027	ND	ND	0.09%
Alpha-oxo-1h-indole 3-propanoic acid	C11H9NO3	204.0661	ND	ND	0.13%

 doi:10.20944/preprints202403.1251.v1

2-(5-methoxy-1H-indol-3-yl)acetic acid	C11H11NO3	206.0817	ND	ND	0.24%
Indole 3-acetic acid	C10H9NO2	176.0712	ND	ND	0.10%
5-Hydroxyindole-3-acetic acid	C10H9NO3	192.0661	ND		0.46%
Beta-oxo-1h-indole-3-propanoic acid	C11H9NO3	204.0661	ND	0.24%	ND
Organic acid					
Hippuric acid	C9H9NO3	180.0661	0.13%	ND	ND
Kojic Acid	C6H6O4	143.0344	ND	ND	18.91%
Phenolic compounds					
Neochlorogenic acid	C16H18O9	355.1029	0.39%	0.12%	ND
Gentisinic acid	C7H6O4	155.0344	0.38%	ND	ND
trans-Caffeic acid	C9H8O4	181.0486	0.11%	ND	0.46%
Caffeic acid	C9H8O4	181.0501	0.50%	0.61%	1.08%
4-Coumaric acid	C9H8O3	165.0552	0.39%	0.09%	
Trans-4-Coumaric acid	C9H8O3	165.0552	1.62%	3.66%	3.35%
Esculetin	C9H6O4	179.0341	0.15%	0.14%	1.40%
Chlorogenic acid	C16H18O9	355.103	9.95%	3.16%	9.20%
Daphnetin	C9H6O4	179.0344	0.62%	0.75%	0.27%
3-Acetylphenanthrene	C16H12O	221.0966	0.64%	ND	ND
gerberinside	C16H18O8	339.1080	0.29%	ND	ND
Vicenin 2	C27H30O15	595.1663	0.31%	0.36%	0.42%
Isorhamnetin 3,7-di-O-glucoside	C28H32O17	641.1718	0.10%	0.06%	0.09%
Isorhamnetin 3-O-galactoside 6''-rhamnoside	C28H32O16	625.1769	1.13%	1.40%	1.11%
Isorhamnetin 3-glucoside-7-rhamnoside	C28H32O16	625.1769	1.62%	0.56%	ND
Isorhamnetin	C16H12O7	317.0661	2.82%	ND	1.08%
Isorhamnetin 3-O-rutinoside	C28H32O16	625.1769	8.10%	3.12%	0.60%
Isorhamnetin 3-galactoside	C22H22O12	479.119	1.94%	1.82%	0.68%
Isorhamnetin 3-O-glucoside	C22H22O12	479.1190	2.92%	5.21%	ND
Isorhamnetin 3-O-neohesperoside	C28H32O16	625.1769	0.07%	ND	ND
4-methoxy-6-prop-2-enyl-1,3-benzodioxole	C11H12O3	193.0865	0.95%	ND	ND
Quercetin-3-O-rhamnopyranosyl(1-2)-D-glucopyranoside-7-O-rhamnopyranoside	C33H40O20	757.2191	0.15%	ND	ND

 doi:10.20944/preprints202403.1251.v1

Quercetin 3-O-beta-glucopyranosyl-7-O-alpha-rhamnopyranoside	C27H30O16	611.1612	0.19%	0.17%	0.23%
Quercetin-3-O-robinobioside	C27H30O16	611.1612	0.12%	ND	ND
Quercetin 4'-O-glucoside	C21H21O12	465.1033	0.48%	ND	0.11%
Rutin	C27H30O16	611.1612	0.47%	0.16%	0.30%
7-O-Methylquercetin-3-O-galactoside-6''-rhamnoside	C34H42O20	771.2348	2.26%	ND	ND
Isoquercetin	C21H20O12	465.1033	1.27%	0.50%	0.46%
Quercetin-3-Rhamnoside	C21H20O11	449.1084	0.95%	3.87%	ND
Quercetin	C15H10O7	303.0505	0.38%	0.58%	ND
Quercetin 3-O-alpha-L-rhamnopyranosyl(1-2)-beta-D-glucopyranoside 7-O-alpha-L-rhamnopyranoside	C33H40O20	757.2191	ND	0.13%	0.16%
Ferulic acid	C10H10O4	195.0657	0.64%	1.15%	4.36%
Sinapic acid	C11H12O5	225.0763	0.21%	0.15%	1.81%
Kaempferol 3-O-glucoside-2''-rhamnoside-7-Rhamnoside	C33H40O19	741.2242	0.10%	0.06%	0.09%
Kaempferol 3-O-robinoside-7-O-rhamnoside	C33H40O19	741.2242	0.08%	0.07%	0.07%
Kaempferol 3-O-galactoside-7-O-rhamnoside	C27H30O15	595.1663	0.17%	0.16%	0.16%
Kaempferol 3-O-rutinoside	C27H30O15	595.1663	0.25%	0.24%	0.16%
Kaempferol 3-O-glucoside-7-O-rhamnoside	C27H30O15	595.1663	0.40%	0.11%	0.18%
Kaempferol	C15H10O6	287.0557	0.39%	0.30%	ND
Kaempferol-3-O-glucoside	C21H20O11	449.1084	0.99%	0.99%	ND
Kaempferol-3-rhamnoside	C21H20O10	433.1135	1.31%	3.78%	ND
Kaempferol-3-O-glucoside-6''-p-coumaroyl	C30H26O13	595.1452	0.04%	ND	ND
Kaempferol 3-O-rhamnoside	C21H20O10	433.1135	ND	ND	0.06%
Kaempferol 7-O-glucoside	C21H20O11	449.1084	ND	0.20%	0.12%
Vitexin	C21H20O10	433.1135	0.43%	0.50%	0.66%
Isovitexin	C21H20O10	433.1135	0.44%	0.28%	0.27%
Apigenin 6-C-glucoside-8-C-arabinoside	C26H28O14	565.1557	0.19%	ND	0.31%
Apigenin-7-O-glucoside	C21H20O10	433.1135	0.26%	0.38%	ND
Hispiduloside	C22H22O11	463.1240	0.18%	ND	ND
Datiscetin-3-O-rutinoside	C27H30O15	595.1663	1.13%	0.37%	ND
Naringenin-7-O-glucoside	C21H22O10	435.1291	0.09%	ND	ND
Apigenin-7-O-neohesperidoside	C27H30O14	579.1714	0.07%	ND	ND

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Diosmetin-7-O-neohesperidoside	C28H32O15	609.1820	0.16%	0.14%	ND
Diosmetin 7-O-rutinoside	C28H32O15	609.182	5.40%	7.75%	1.51%
Diosmetin	C16H12O6	301.0712	ND	0.15%	ND
Luteolin-7-O-glucoside	C21H20O11	449.1084	0.14%	ND	ND
Luteolin	C15H10O6	287.0557	0.24%	0.37%	ND
Pectolinarin	C29H34O15	623.1976	0.27%	0.30%	ND
Acacetin-7-O-rutinoside	C28H32O14	593.1873	1.29%	1.48%	ND
Acacetin-7-glucoside	C22H22O10	447.1291	0.11%	0.23%	ND
Acacetin	C16H12O5	285.0763	0.19%	0.25%	ND
Demethoxycentaureidin 7-O-rutinoside	C29H34O16	639.1925	0.35%	0.20%	ND
Licoflavanone	C20H20O5	341.1389	1.00%	1.63%	1.16%
Citreorosein	C15H10O6	287.0556	0.12%	ND	ND
Fisten	C15H10O6	287.0557	0.26%	0.42%	ND
Chrysoeriol	C16H12O6	301.0712	1.93%	4.84%	ND
Eupafolin	C16H12O7	317.0661	2.25%	5.89%	ND
Aurantioobtusin	C17H14O7	331.0818	0.06%	ND	ND
cirsimaritin	C17H14O6	315.0869	0.14%	0.29%	ND
Wogonin	C16H12O5	285.0763	0.04%	ND	ND
Daidzein	C15H10O4	255.0657	0.07%	0.06%	ND
Jaceosidin	C17H14O7	331.0818	0.20%	ND	ND
Xanthotoxol	C11H6O4	203.0344	0.30%	ND	ND
Osthol	C15H16O3	267.0997	ND	ND	0.31%
Protocatechuic acid	C7H6O4	155.0344	ND	ND	0.19%
Xanthurenic Acid	C10H7NO4	206.0453	ND	0.26%	ND
7,8-Dihydroxy-4-methylcoumarin	C10H8O4	193.0481	ND	0.18%	ND
Syringic acid	C9H10O5	199.0607	ND	ND	2.57%
Vanillin	C8H8O3	153.0552	ND	2.48%	ND
Syringaldehyde	C9H10O4	183.0657	ND	ND	1.49%
Scopoletin	C10H8O4	193.0501	ND	0.15%	ND
Feruloyl quinic acid	C17H20O9	369.1186	ND		0.86%
Hispiduloside	C22H22O11	463.1240	ND	0.08%	ND
Homoorientin	C21H20O11	449.1084	ND	0.31%	ND
Fraxetin	C10H8O5	209.045	ND	ND	0.20%
Lonicerin	C27H30O15	595.1663	ND	0.09%	ND
Coumarin	C9H6O2	147.0446	ND	0.42%	ND
Nepetin 7-glucoside	C22H22O12	479.119	ND	ND	0.75%
3,5-Dimethoxycinnamic acid	C11H12O4	209.0814	ND	0.11%	ND
Cirsimarin	C23H24O11	477.1397	ND	0.16%	ND
Oenin	C23H25O12	493.1346	ND	0.37%	ND
Tricin	C17H14O7	331.0818	ND	0.07%	ND

 doi:10.20944/preprints202403.1251.v1

Saccharide					
Trehalose	C12H22O11	365.106	ND	ND	9.12%
Sucrose	C12H22O11	365.1060	5.59%	10.05%	ND
Sesquiterpene					
Atractylenolide III	C15H20O3	271.1310	0.14%	ND	ND
Tetratepnoid derivative					
Abscisic acid	C15H20O4	265.144	0.16%	ND	4.49%
Vitamins					
Pyridoxine	C8H11NO3	170.0817	1.00%	ND	1.56%
D-Pantothenic acid	C9H17NO5	220.1185	ND	ND	1.87%
Others					
Uridine	C9H12N2O6	245.0774	0.13%	ND	ND
Guanosine	C10H13N5O5	284.0995	0.27%	ND	ND
2-O-Methyladenosine	C11H15N5O4	282.1202	0.29%	ND	ND
6-methoxyquinoline	C10H9NO	160.0762	0.27%	ND	ND
Kynurenic acid	C10H7NO3	190.0504	1.55%	2.14%	3.42%
4-oxo-5-phenylpentanoic acid	C11H12O3	193.0865	0.62%	ND	0.77%
Loliolide	C11H16O3	197.1178	2.27%	ND	ND
Lumichrome	C12H10N4O2	243.0882	0.23%	ND	ND
Aloe-emodin	C15H10O5	271.0607	0.17%	0.21%	ND
Octadecanedioic acid	C18H34O4	315.2535	0.95%	ND	ND
Anileridine	C22H28N2O2	353.2229	0.13%	ND	ND
Isopimpinellin	C13H10O5	247.0607	0.06%	0.15%	ND
Lauramidopropyl betaine	C19H39N2O3	343.2961	0.09%	ND	ND
Imperatorin	C16H14O4	271.0903	0.24%	ND	ND
Senegenin	C30H45ClO6	537.2983	0.20%	0.08%	ND
isoimperatorin	C16H14O4	271.0903	0.10%	ND	ND
Schisandrin A	C24H32O6	417.2277	4.54%	ND	ND
Maltol	C6H6O3	127.0395	ND	7.17%	ND
2-Phenylacetamide	C8H9NO	136.0762	ND	0.70%	ND

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Tryptoline	C11H12N2	173.1089	ND	0.21%	ND
2-ureidopentanedioic acid	C6H10N2O5	191.0668	ND	0.12%	ND
Ophiopogonoside A	C21H38O8	441.2464	ND	0.19%	ND
Phytosphingosine	C18H39NO3	318.3008	ND	ND	0.16%

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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