Heliyon 10 (2024) e25982

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Changes in the antioxidant and anti-inflammatory activities of *Rosa rugosa* 'Mohong' during fermentation

Merhaba Abla [a,1], Yueyue Cai [b,1], Lu Gao [b], Jingsong Wu [a,*], Lixin Yang [a,b,c,**]

[a] *Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, China*
[b] *School of Ethnic Medicine, Yunnan Minzu University, Kunming, 650504, Yunnan, China*
[c] *Center for Biodiversity and Indigenous Knowledge, Kunming, 650034, Yunnan, China*

ARTICLE INFO

Keywords:
Rosa rugosa 'Mohong'
Fermentation
Phenolic acids
Flavonoids
Antioxidant activity
Anti-inflammatory activity

ABSTRACT

Fermented rose petals are a traditional delicacy of the Dali Bai community in Yunnan, China. Fermentation enhances the quality and nutritional value of roses, as well as their efficacy, by increasing the levels of phenolic compounds. This study aimed to investigate the significant variations in four active compounds throughout the traditional fermentation process. Four compounds in *Rosa rugosa* 'Mohong' were examined, and significant variations among polyphenols and antioxidant and anti-inflammatory activities were observed. These variations were studied during fermentation by *Saccharomyces rouxii* at varying temperatures and durations. Moreover, the results showed that gallic acid and syringic acid content significantly increased ($P < 0.05$) with a rise in temperature from 20°C-35 °C during fermentation. Simultaneously, rutin and quercetin levels significantly decreased ($P < 0.05$) at all four temperatures throughout the five periods. The antioxidant and anti-inflammatory activities of fermented *R. rugosa* 'Mohong' methanol extracts were dose-dependent. Our results provide valuable insights into optimizing the processing scale and quality control of fermented rose products.

1. Introduction

The genus *Rosa*, encompassing over 100 wild species, is widely distributed in the temperate and subtropical regions of the northern hemisphere [1]. Among these, the beach rose (*Rosa rugosa*) stands out as an important species within the genus, utilized for horticultural and edible purposes and pharmaceutical applications, owing to its abundant bioactive ingredients. The wild *R. rugosa* grows in small, scattered populations in Northeastern China, and its genetic biodiversity is lower than that of cultivated individuals, attributable to long-term asexual reproduction [2]. The cultivation and breeding of *R. rugosa,* primarily driven by its ornamental and medicinal value, began in China. It was introduced to Europe in the 18th century from the Far East and subsequently to America in 1845 [3,4]. *R. rugosa* is cultivated extensively in China, where its blossoms are used for extracting essential oils, curing chronic diseases, making tea, and other healthcare foods. The extracts from *R. rugosa* hips have great potential in the cosmetics and pharmaceutical industries

* Corresponding author.
** Corresponding author. Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, China.
E-mail addresses: jinsongwu@mail.kib.ac.cn (J. Wu), rattan@mail.kib.ac.cn (L. Yang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e25982
Received 6 October 2023; Received in revised form 25 January 2024; Accepted 6 February 2024
Available online 15 February 2024

due to their high content of bioactive compounds, such as phenols and flavonoids [5,6]. For over 500 years, *R. rugosa* has been used in the production of distinctive fermented foods in China's Yunnan Province. The traditionally fermented 'Mohong' petals of *R. rugosa*, produced in Dali Prefecture, have not only become a cultural symbol in Yunnan province but also a popular source for native food products. These include rose (flower) cakes, native rose juices, traditional snacks such as rose-flavored glutinous rice wine, and preserved fruits with rose additives, among others. As mentioned by Lang et al. [7], *R. rugosa* 'Mohong' (RM) and its derived products possess significant cultural value.

Natural antioxidants, such as phenolic acids, flavonoids, and ascorbic acid, protect human cells and organs from the harmful effects of oxidative stress and are used to preserve food [8]. Andrzej Cendrowski evaluated the effect of different harvest seasons on the total phenolic content and antioxidant activity in *R. rugosa* petals. His findings revealed a strong correlation between antioxidant properties and total phenolic content [9]. The DPPH (2,2′-diphenyl-1-picrylhydrazyl) free-radical-generating assay and the ABTS (2,2′-azino-bis [3-ethylbenzothiazoline-6-sulfonic acid]) decolorization assay have been used to evaluate the overall antioxidant capacities of many plant materials [10–12], including rose plants and extracts. These rose derivatives are known to contain high levels of potent antioxidant polyphenols [13].

Various phytochemicals contribute to the anti-inflammatory effects of *R. rugosa* [14], with phenolic compounds playing a particularly significant role [15]. Traditional fermentation techniques not only preserve the nutritional value of rose but also modify its chemical composition, thus altering its biological activity. This modification is evident through the examination of different parameters [16].

R. rugosa petals possess many important physiological activities, attributable to their rich composition of flavonoids [17], phenols [18], anthocyanins [19], polysaccharides [20], and tannins. The major active phenols identified in *R. rugosa* flowers are gallic acid, ferulic acid, syringic acid, 4-caffeic acid, vanillic acid, catechin, and *p*-coumaric acid. The principal bioactive flavonoids are rutin, quercitrin, isoquercitrin, naringenin, hesperidin, quercetin, luteolin, apigenin, and kaempferol [10,21]. The main polyphenol fraction in *R. rugosa* petals is ellagitannins, which, according to Andrzej Cendrowski, constitute approximately 69–74% of the total polyphenolic content of the petals [22]. Various phenolic acids and flavonoids are present in *R. rugosa* petals, including gallic acid, syringic acid, rutin, and quercetin, among which gallic acid and quercetin are notable for their strong antioxidant activity [17,23,24]. Gallic acid, syringic acid, rutin, and quercetin are widely used in the healthcare industry due to their antioxidant, anti-inflammatory, anti-allergic, anti-microbial, and whitening properties [25–33]. As *R. rugosa* contains the aforementioned phenolic compounds and its fermented products play a role in the treatment of stomach disorders and chronic inflammation in traditional medicine, the fermented petals are rich in biologically active ingredients with substantial commercial applications. These applications also span the pharmacological, food, and cosmetics industries, underscoring their industrial relevance and value. Consequently, optimizing the fermentation temperature and duration is crucial to maximizing the production of the major bioactive compounds in fermented *R. rugosa* 'Mohong' methanol extract (FRME).

Rose fruits exhibit diversity not only in botanical and species characteristics but also in terms of physical properties and chemical composition. The antioxidant content in rose fruits, as well as associated activities, can influenced by morphological and genetic diversity, stage of maturity, processing (under different conditions), and storage conditions [33,34]. For instance, Andrzej Cendrowski's study on L-ascorbic acid in wines made from rose fruits (*Rosa rugosa*) found that the mixing process, which introduces additional oxygen, leads to further degradation of the vitamin. His findings show that the vitamin C content in wines before fermentation (509–1009 mg GAE/100 mL) lost approximately 30–40% after aging for one month [35,36]. The optimum fermentation temperatures for achieving maximum production of gallic acid in various plants, using different microbial strains, were identified as 30 °C [35,37] and 32 °C, according to Yao et al. [38]. Similar results were obtained for syringic acid content; both compounds exhibited stronger antioxidant properties following microbiological fermentation [38,39].

During fermentation, the nutritional and sensory qualities, and functional properties of food, are enhanced due to changes in the composition and quantity of flavor compounds comprising polyphenolic acids [40–42]. Most previous studies on rose petals have focused on exploring their chemical composition, total flavonoids, total polyphenols, and corresponding biological activities. These studies have varied in their approach, encompassing different rose varieties, processing techniques, and fermentation methods [43–45]. Rose jams fermented with *Pediococcus pentosaceus* MP13 showed higher total phenol, anthocyanin, and antioxidant activity compared to spontaneously fermented rose jam [43]. Lang et al. [7] previously isolated the yeast strain TFR-1 (*Saccharomyces rouxii*) from naturally fermented rose petals. They found that fermenting the petals of *R. rugosa* 'Dianhong' with TFR-1 increased total phenolic and total flavonoid contents after 30 days at 28 °C. However, further in-depth research is needed to evaluate the anti-inflammatory and antioxidant effects, as well as to identify and quantify phenols and flavonoids in FRME during fermentation at different temperatures.

Moreover, few studies have focused on changes in bioactive components during traditional fermentation or how to improve industrial-scale production and quality standardization. The local population produces fermented rose through spontaneous fermentation, relying on personal knowledge and experience. As a result, this craft fermentation process is time-consuming (approximately half a year), and the quality characteristics of the final products vary due to factors such as the microbiological composition, temperature, and fermentation period.

R. rugosa petals, recognized for their high polyphenol content, are a valuable raw material for human dietary supplements [46]. Thus, different experimental designs have been used to find the optimal fermentation conditions to produce fermented *R. rugosa* 'Mohong'(FRM), aiming to generate a high polyphenol content and enhance its various biological effects. The HPLC-DAD method has been used to identify and quantify phenols and flavonoids over the past decades [47–49]. Therefore, in this study, in order to identify and evaluate FRM, a rapid HPLC method was developed to compare the changes in the polyphenol content of methanol extracts from FRM under various fermentation parameters. Finally, the antioxidant and anti-inflammatory activities of FRME were examined. Our

study aimed to determine the changes in polyphenol content, and antioxidant and anti-inflammatory activities of FRM during fermentation. Additionally, we sought to identify the optimum fermentation conditions for FRMP that would yield not only high polyphenol content but also various biological effects. Hence, the findings of this study can be instrumental in the certification and quality control of traditionally fermented roses in the food, pharmaceutical, and cosmetics sectors.

2. Materials and methods

2.1. Samples

R. rugosa 'Mohong' flowers were collected from Heqing County, Dali Prefecture, Yunnan, China, in May 2021. Fresh petals were handpicked and preserved at −20 °C for further study. All voucher specimens were identified by a taxonomist at Kunming Institute of Botany, Chinese Academy of Sciences.

2.2. Strains, cells, and chemicals

Yeast strain TFR-1 *(Saccharomyces rouxii*) was purchased from the China General Microbial Culture Collection Center (CGMCC) (Sr1, Beijing, China) under sample No. CGMCC19335. Agar powder was acquired from the BioFroxx company (Einhausen, Germany), sucrose from Lanjike Technology Co., Ltd. (Beijing, China), yeast extract was provided by BBI Co., Ltd. (Shanghai, China), and finally peptone sourced at Beijing Aoboxing Biotechnology Co., Ltd. (Beijing, China). Phenolic and flavonoid standards such as gallic acid (Cat. No. H20003, ≥99%), rutin (Cat. No. H355003, ≥97%), and quercetin (Cat. No. K913157, ≥98%) were obtained from D&B (Shanghai, China), and syringic acid (Cat. No. M026278, >98%) were purchased from MREDA (Beijing, China). The gallic acid (≥99.0%) standard was provided by Tianjin Zhiyuan Chemical Reagent Company (Tianjin, China). Methanol (HPLC grade), formic acid (HPLC grade), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were from MREDA (Beijing, China). 95% (v/v) methanol solution was purchased from Kunming Renke Trading Co., Ltd. (Kunming, China). Methanol (analytical grade) was from Shanghai Titan Scientific Co., Ltd. (Shanghai, China).

The mouse monocyte macrophage line RAW264.7 used for the anti-inflammatory assay was from the National Collection of Authenticated Cell Cultures (Shanghai, China). Griess Reagent, LPS, and the control drug L-NMMA were from the Sigma-Aldrich Company (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum were purchased from the Biological Industries Company (Biological Industries, Beit Haemek, Israel).

2.3. Preparation of rose tissue: fermentation and sampling

Yeast strain TFR-1 (*S. rouxii*) isolated from traditional fermented *R. rugosa* 'Dianhong' [7] was used to ferment *R. rugosa* 'Mohong' petals (RMP). Active dried TFR-1 was rehydrated and reactivated with sterile water. The diluent was inoculated into the YPD medium (1%, w/v, yeast extract; 0.4%, w/v, peptone; 1.2%, w/v, agar; 0.4%, w/v, sucrose) for culture at 28 °C for 36 h using the coating method. Prior to inoculation, the YPD medium was autoclaved at 120 °C for 45 min to kill all existing bacteria or fungi. A single colony of *S. rouxii* was transferred to 600 mL of YPD liquid culture medium above (without agar) at 28 °C for about 36h, shaking until used for rose fermentation. The populations of yeast cells were counted after coating the YPD medium with yeast cells. All fermentations were done in triplicate.

Fermented *R. rugosa* 'Mohong' (FRM) was prepared using the traditional method of fully kneading a 1:1 ratio blend of fresh *R. rugosa* 'Mohong' petals and brown sugar for approximately 1 h. The mixtures were placed on a laminar flow hood for sterilization by ultraviolet light for 2 h and transferred to 1 L fermentation bottles. The FRM was prepared by combining 1.0 kg of a mixture of petals and brown sugar and 20 mL of TFR-1 (5.0×10^6 CFU/mL) and fermenting the mixtures at 20, 25, 30, and 35 °C for 35 d. Samples were taken weekly during the fermentation process, i.e., 7, 14, 21, 28, and 35 d.

2.4. HPLC analysis of free phenolic acids and flavonoids

FRM was extracted for HPLC analysis as follows: 0.4 L of 95% (v/v) aqueous methanol was added into 100 g of FRM and extracted twice. The FRM was completely ground in methanol, and the solutions were pooled together and evaporated under reduced pressure to dryness at 55 °C to obtain the total extract (FRME). A 0.2 g sample of FRME was dissolved in 1 mL methanol (HPLC grade), and the solution was filtered the solution through a 0.22 μm filter into vials before HPLC analysis.

The peak area of the polyphenols was integrated from the HPLC chromatogram at 280 nm, using Agilent Open Lab data analysis (version 2.205.0.1344) and plotted against content. The stock solutions were prepared by dissolving 0.2 mg of each standard in 1 mL of methanol (HPLC grade). Calibration curves were prepared at five different concentrations (0.2 μg, 0.6 μg, 0.1 μg, 0.14 μg, 0.18 μg, and 2.0 μg). Calibration curves for solutions of the four standard compounds were established by plotting versus peak areas against the content solution (μg) and calculated as linear relationship: $y = ax + b$, x stands for peak area. The results showed high linearity ($r^2 = 1$) for each curve.

A new HPLC-DAD method was developed using an Agilent 1260 Infinity II HPLC connected to a diode array detector (Agilent, Munich, Germany). A ZORBAX SB-C18 reverse phase column (500 mm × 4.6 mm, 5 μm particle size) was applied at 30 °C with mobile phases consisting of 0.3% (v/v) aqueous formic acid and methanol (solvent A and B, respectively), and the flow rate set to 1 mL/min. Gradient programming applied (in v/v) time (min)/percentage solvent B: 0/10, 10/20, 25/25, 30/25, 35/50, 45/80, 48/0.

2.5. DPPH radical-scavenging assay

On the basis of the solution's discoloration, the antioxidant activity was measured by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical method with minor modifications [23]. Briefly, a 100-μM solution of DPPH in methanol was prepared, and 20 μL of this solution was added to 180 μL of different dilutions of samples (0.375, 0.25, 0.125, 0.0625 mg/mL), and total volume was 200 μL. Sample reaction time was 30 min at room temperature, not exposed to light. Then, the absorbance values of the FRME and gallic acid solutions were measured at 517 nm using an Infinite M200 microplate reader (Tecan, Austria) against a blank (without extract). 20 μL of gallic acid at a concentration of 25 μg/mL was added to 20 μL of DPPH solution and 160 μL of methanol for positive control. All rose samples were examined at least in triplicate, and all stock solutions were prepared daily. The antioxidant capacity was calculated as follows: Percentage inhibition (%) = $(A_{control} - A_{FRME}/A_{control}) \times 100$, where $A_{control}$ and A_{FRME} are the respective absorbance of samples with and without FRME.

2.6. ABTS free radical scavenging rate test

ABTS free radical scavenging ability was assessed using a method adapted from the literature with slight modifications [12]. The FRME was prepared with methanol to 0.2 mg/mL FRME sample solution to be tested, 7 mmoL/L ABTS stock solution and 1.4 mmoL/L potassium persulfate solution were mixed evenly in equal volumes and protected from light for 12–16 h. Methanol was used to adjust the absorbance value of the ABTS working solution to 0.7 ± 0.02. In brief, 160 μL of this working solution was added to 40 μL FRME sample solution to be tested in the 96-well plate. The mixture was allowed to react in the dark for 10 min. The absorbance value was then measured at a wavelength of 734 nm. Vitamin C was used as a positive control. The absorbance value obtained was used to calculate the clearance rate of antioxidant activity:

Percentage inhibition (%) = $[1 - (A_1 - A_2)]/A_0) \times 100\%$

In the formula: A_0 is the absorbance of methanol + ABTS solution; A_1 is the absorbance of FRME sample + $ABTS^+$ solution; A_2 is the absorbance of FRME sample + methanol.

2.7. Assay for the inhibition of NO production by RAW264.7 cells

RAW 264.7 cells were seeded in 96-well plates and stimulated with 1 μg/mL of LPS while being treated with samples at a final concentration of 200 μg/mL, 400 μg/mL, or 600 μg/mL. The cells were incubated for 18 h, and the culture medium was collected to detect NO production. The absorbance was measured at 570 nm. The cell viability of the residual medium was tested by adding MTS, which confirmed that the compounds had no toxic effects on the cells.

Rate of inhibition of NO production (%) = $(OD_{control} - OD_{FRME\ sample})/OD_{control} \times 100\%$.

2.8. Statistical analysis

All average values were calculated as means ± standard deviation of three replicates, and differences between groups were considered significant at $p < 0.05$. SPSS software (IBM SPSS Statistics 28.0) was used to perform two-way variance analysis (ANOVA) with Duncan's multiple range test.

3. Results

3.1. Optimization of HPLC chromatographic conditions

The methanol extraction method described above was used to quantify the four standard compounds in FRME (HPLC

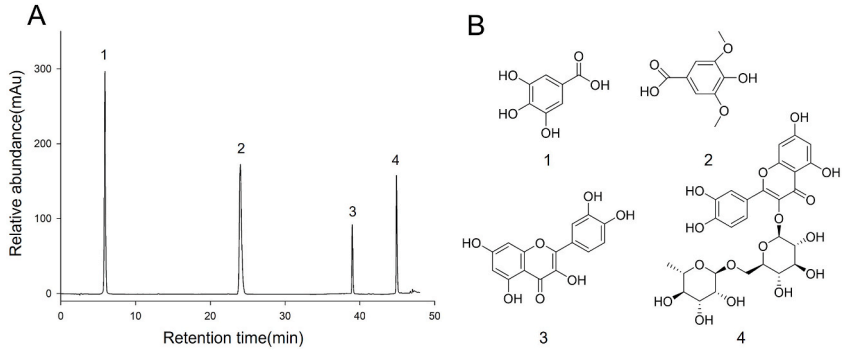

Fig. 1. Information on the four phytochemical standards: (A) HPLC chromatograms of 2 mg/mL mixed standards recorded at 280 nm; (B) Chemical structures of gallic acid (1), syringic acid (2), rutin (3), quercetin (4).

chromatographic peaks shown in Fig. 1A and structures shown in Fig. 1B). To get the best fingerprint chromatograms of FRME, the conditions for the gradient elution procedure, detection wavelength, and mobile phase composition were optimized. Two types of mobile phase were acetonitrile and methanol. To obtain good separation and shapes, formic acid (0.1%, 0.2%, or 0.3%, all v/v) was used in the water phase. 0.3% (v/v) aqueous formic acid was chosen as an optimized mobile phase to analyze FRME, as many peaks were collected in the chromatogram within 53 min. More detectable peaks were obtained, and an improved baseline at around 280 nm was noticed, resulting in better chromatographic separations for analysis. Therefore, chromatographic were collected patterns using 280 nm as the detection wavelength, yielding improved results for all target compounds in FRME, in comparison to the reference.

3.2. Quantitative determination of four components in FRME

Regression analysis was performed to determine the relationship between peak area and content in the standardized solutions for the four compounds. For each compound, the peak area (y) was plotted against the content in the solution (x, mg), resulting in the following: $y = 275.23x + 14.101$, $R^2 = 1$(for gallic acid, the linear range was 0.2–2.0 μg); $y = 259.23x + 3.106$, $R^2 = 1$ (for syringic acid, the linear range was 0.2–2.0 μg); $y = 59.672x + 4.4568$, $R^2 = 1$ (for rutin, the linear range was 0.2–2.0 μg); $y = 129.64x - 6.2028$, $R^2 = 1$ (for quercetin, the linear range was 0.2–2.0 μg). The precision of HPLC was evaluated by calculating the relative standard deviation (RSD, %) of the peak areas. The RSD values obtained were less than 2%. The HPLC method was optimized successfully, allowing for the simultaneous determination of the content of four polyphenols in FRME. For this analysis, the external standard method was applied.

3.3. Changes in contents of the four compounds during RMP fermentation

By employing HPLC-DAD, the contents of main individual phenols and flavonoids in FRME samples following fermentation at different temperatures for different periods of time were quantified (Fig. 2). Gallic acid, syringic acid, rutin, and quercetin were detected in rose petal samples that had undergone 7, 14, 21, 28, and 35 d of anaerobic fermentation. The composition of FRME was significantly affected by fermentation temperature ($P < 0.05$), with the amounts of phenolic acids and flavonoids in FRME as follows: gallic acid ranging from 112.61 to 419.58 μg/g, syringic acid from 31.86 to 62.58 μg/g, rutin from 299.40 to 705.06 μg/g, and quercetin from 80.90 to 148.29 μg/g. Throughout the entire fermentation period, the gallic acid and syringic acid contents tended to increase slowly (Fig. 2 A and B), whereas the rutin and quercetin contents tended to decline slowly (Fig. 2C and D).

Specifically, the gallic acid content in FRME significantly increased as the fermentation period increased from 7 d to 35 d ($P < 0.05$) at the temperatures of 20 °C, 30 °C, and 35 °C and reached peak levels of 148.33, 272.83 and 419.58 μg/g at 35 d of fermentation, respectively. The levels of gallic acid significantly increased ($P < 0.05$) from 7 to 28 d at 25 °C and reached their peak level (185.32 μg/g) at 21 d of fermentation. Syringic acid content (50.32–62.58 μg/g) in FRME was the highest following fermentation at 35 °C, compared to the other three temperatures. The mean levels of syringic acid significantly increased ($P < 0.05$) with a temperature increase (from 20 °C to 35 °C). However, the contents of syringic acid in FRME fermented at 20 °C or 25 °C did not exhibit a significant difference ($P > 0.05$) across the different time points (Fig. 2B).

These findings show gallic acid and syringic acid contents increased with rising temperatures, from 20 °C to 35 °C, indicating that

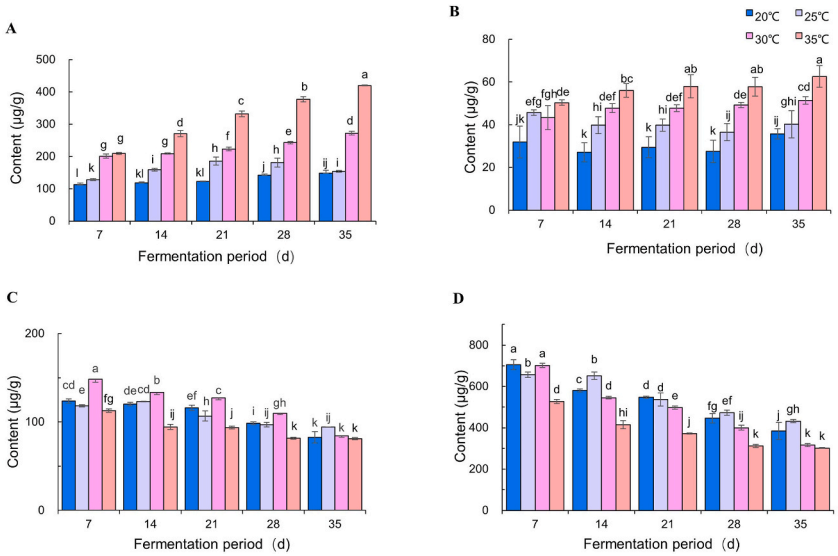

Fig. 2. Changes in gallic acid (A), syringic acid (B), rutin (C), and quercetin (D) contents of fermented *R. rugosa* 'Mohong' extracts (FRME) during the fermentation at different temperatures. Values are presented in mean ± standard deviation(n = 3) and evaluated using a two-way ANOVA test (post test: Duncan test). The different letters(a-l) in the same compounds denote significant differences at $p < 0.05$.

higher temperatures promote the accumulation of these acids. Previous studies have revealed an increase in total phenol and total flavonoid levels in fermented roses [50], leading to an increase in antioxidant activity [41]. This is confirmed by our findings, where higher levels of gallic and syringic acid contents were observed with a temperature increases from 20 °C to 35 °C (Fig. 2A and B). Therefore, we recommend that the optimum fermentation temperature to obtain high levels of these two phenolic acids is 35 °C with a fermentation period of 35 d.

Rutin and quercetin levels in FRME decreased gradually throughout the entire fermentation period at all four temperatures (Fig. 2C). After 35 d of fermentation, the rutin contents in FRME (from 20 to 35 °C) were 54.06, 65.43, 44.40, and 56.31% of their 7-d values, respectively. The rutin contents (at 7 d) were 705.06, 656.24, 701.74 and 526.63 μg/g at 20 °C, 25 °C, 30 °C and 35 °C, respectively. They significantly decreased ($P < 0.05$) by 45.94, 34.57, 55.60, and 43.69% of their 7-d values after 35 d of fermentation. The largest decline in rutin contents (55.60%) was detected in FRME fermented at 30 °C, whereas the smallest decline (34.7%) was observed in FRME fermented at 25 °C. Notably, the rutin content decreased with increasing temperature (25 °C to 35 °C) at 14, 21, 28, and 35 d of fermentation.

The quercetin content reached its highest level at 7 d of fermentation at 30 °C (148.29 μg/g). From 7d to 35d of fermentation, the quercetin content significantly decreased ($P < 0.05$), with a decrease of 34.57, 21.11, 44.62 and 29.58% at 20, 25, 30, and 35 °C, respectively, indicating that the fastest decline occurred at 30 °C and the slowest at 25 °C. In summary, the highest levels of rutin and quercetin in FRME fermented at 20 °C for 7 d, and 25 °C was the most favorable temperature for maintaining rutin and quercetin contents in FRME. We hypothesize that the significant and continuous decrease in rutin and quercetin levels ($P < 0.05$) at all four temperatures during fermentation (Fig. 3C and D) is due to degradation and structural changes [51]. Therefore, both fermentation temperature and duration have clear effects on the contents of these four phenolics and flavonoids in FRME.

3.4. Changes in the DPPH radical-scavenging activity during fermentation

The DPPH radical-scavenging activity of FRME is shown in Fig. 3. Overall, FRME exhibited notable DPPH radical-scavenging activity (16.68–86.67%). When the concentration of FRME increased from 0.0625 to 0.375 mg/mL (Fig. 3), antioxidant capacity significantly increased ($P < 0.05$). The radical-scavenging activity of FRME fermented at 20 °C showed irregular fluctuations during fermentation (Fig. 3A), whereas we noticed relatively little change in this activity in FRME during fermentation at 25 °C, 30 °C, and 35 °C (Fig. 3B–D). FRME fermented at 30 °C for 14 days exhibited the greatest radical-scavenging activity (86.67 ± 0.19%), followed by 35 °C for 14 d (86.37 ± 0.62%), 25 °C for 14 d (86.42 ± 1.33%), and 20 °C for 21 d (82.79 ± 2.70%). In addition, the DPPH radical-scavenging activity of FRME (25 °C to 35 °C) fermented for 14 d had no significant difference ($P > 0.05$) with that at 7 d.

These results indicate that both fermentation temperature and time affect the antioxidant activity of FRME. FRME had strong antioxidant capacity after fermentation at different temperatures and different time points, especially at the highest concentration (0.375 mg/mL), where the free-radical scavenging capacity ranged from 78.33% to 86.67%; even the lowest concentration (0.0625 mg/mL) of FRME had an antioxidant effect (16.68%–32.59%) (Fig. 3). The highest DPPH radical-scavenging activity (86.67%) is similar to the previously determined antioxidant activity of *R. rugosa* 'Dianhong' petals (0.625 mg/mL) fermented at 28 °C for 30 d (>85%) [7]. Therefore, the free-radical scavenging activity of FRME appears to be dose-dependent. The scavenging activity of FRME was significantly higher ($P < 0.05$) at elevated temperatures (from 25 °C to 35 °C) compared to lower temperatures (20 °C), particularly with shorter fermentation times (7-14d).

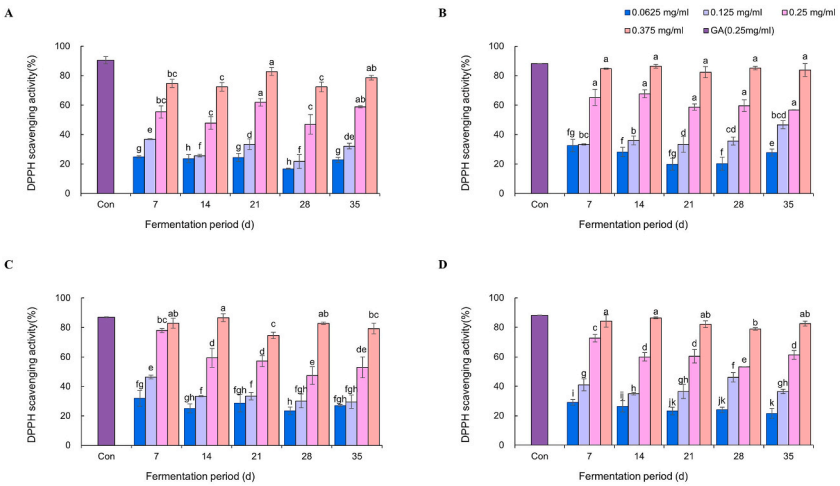

Fig. 3. Effects of different concentrations of fermented *R. rugosa* 'Mohong' extracts (FRME) on DPPH scavenging activity during fermentation (A: 20 °C, B: 25 °C, C: 30 °C, D:35 °C). Values are presented in mean ± standard deviation(n = 3) and evaluated using a two-way ANOVA test (post test: Duncan test). Different letters at the same temperature indicate that FRME is significantly different ($p < 0.05$).

3.5. Changes in the ABTS scavenging activity of FRME during fermentation

The ABTS radical-scavenging activity of FRME is shown in Fig. 4. Overall, FRME exhibited strong ABTS radical-scavenging activity (12.89–92.26%). The scavenging ability of FRME against ABTS radicals was concentration dependent. At the highest concentration (0.375 mg/mL), the free-radical scavenging capacity ranged from 89.03% to 91.52% (Fig. 4), and there was no significant difference ($P > 0.05$) in this capacity across different temperatures and fermentation periods. At the concentration of 0.25 mg/ml, FRME at 30 °C and 14 d of fermentation showed the highest ability (92.27%) to scavenge ABTS radicals (Fig. 4C), followed by 91.64% at 35 °C and 7 d of fermentation. At the concentration of 0.125 mg/ml, except at 35 °C, the ABTS radical-scavenging activity showed a decreasing trend as fermentation time increased, and the highest value (54.71%) occurred in FRME at 30 °C and 7 d of fermentation (Fig. 4C). At the lowest tested concentration (0.0625 mg/mL), the ABTS radical-scavenging activity was highest at 7d of fermentation and showed a decreasing trend thereafter. The highest value was also at 7 d of fermentation at 30 °C (Fig. 4). Therefore, as the concentration of FRME increased from 0.0625 to 0.375 mg/mL (Fig. 4), antioxidant capacity significantly increased ($P < 0.05$). In addition, the ABTS radical-scavenging activity was higher at elevated temperatures (from 30 °C to 35 °C) compared to lower temperatures (from 20 °C to 25 °C), and the highest values occurred at 7-14d of fermentation at 30 °C, across all concentrations.

3.6. Effect of FRME on RAW 264.7 cell viability

The cytotoxicity of FRME was examined using MTS assays. RAW 264.7 cells were treated with FRME (200 μg/mL, 400 μg/mL and 600 μg/mL). FRME did not affect the viability of the RAW 264.7 cells.

3.7. Effect of FRME on NO production

The ability of FRME to decrease NO accumulation in LPS-stimulated RAW264.7 cells to measure anti-inflammatory activity was evaluated (Fig. 5A–D). FRME fermented at all four temperatures tested significantly inhibited NO production at a concentration of 200–600 μg/mL and showed a concentration-dependent manner. During fermentation, the inhibitory effect on LPS-stimulated NO generation displayed erratic variations (Fig. 5). At 20 °C, the inhibition rates of NO production showed a fluctuating decreasing trend from 7 to 35 d of fermentation, and the highest value was 14.99% at the 7d of fermentation (Fig. 5A). At 25 °C, the highest inhibition rates of NO production were achieved (21.06%) at 21d of fermentation, with no significant increase ($P > 0.05$) observed after 21d (Fig. 5B). At 30 °C, the inhibition rate of NO production of FRME (16.57–17.31%) exhibited no significant changes ($P > 0.05$) from 7 to 35 d of fermentation at high concentration (600 μg/mL). However, a significant decreasing ($P < 0.05$) trend was observed during fermentation at lower concentrations (200 and 400 μg/mL) (Fig. 5C). At 35 °C, the highest inhibition rate (22.50%) occurred at 35 d of fermentation and had no significant difference with 7 d of fermentation (20.72%) (Fig. 5D). These findings indicated that FRME fermented at all four temperatures (from 20 °C to 35 °C) had anti-inflammatory activity in a dose-dependent manner. In general, the results indicated that optimum conditions were a fermentation period of 21 d at 25 °C or a fermentation period of 7 d at 20 °C, 30 °C, and 35 °C.

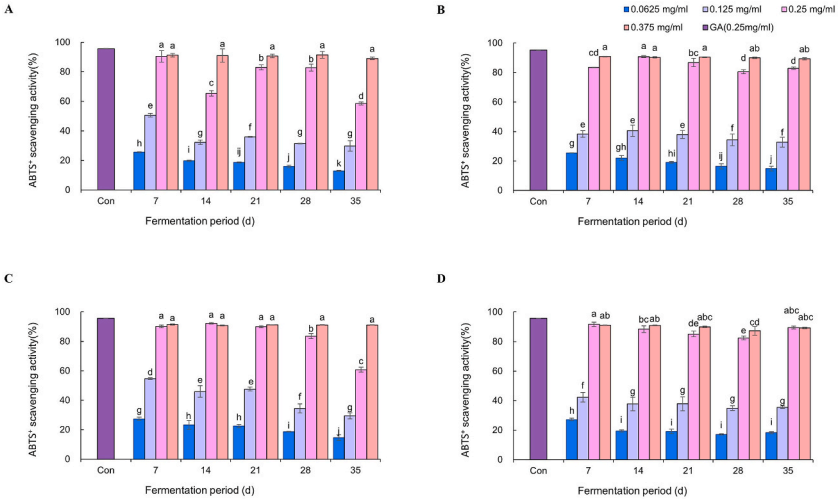

Fig. 4. Effects of different concentrations of fermented *R. rugosa* 'Mohong' extracts (FRME) on ABTS scavenging activity during fermentation at different temperatures (A: 20 °C, B: 25 °C, C: 30 °C, D: 35 °C). Values are presented in mean ± standard deviation(n = 3) and evaluated using a two-way ANOVA test (post test: Duncan test). Different letters(a-k) in the same concentration denote significant differences at $p < 0.05$.

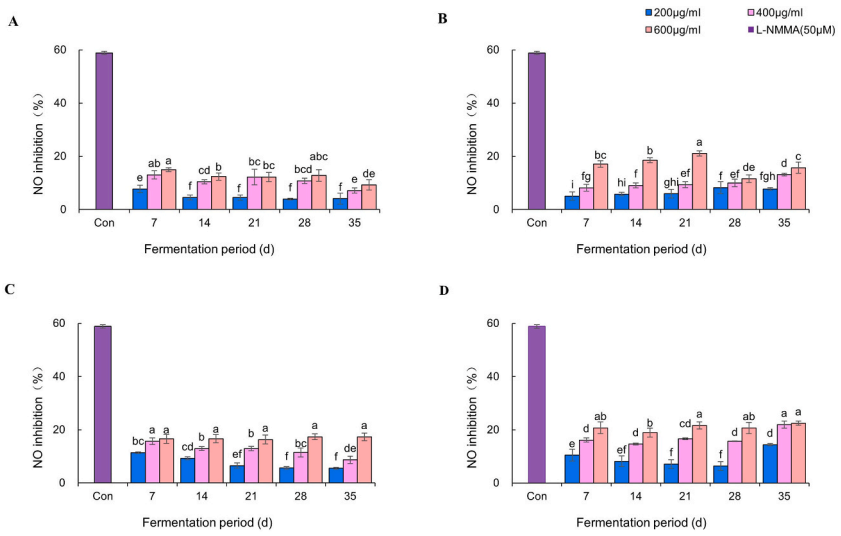

Fig. 5. Positive dose-response relationship between the concentration and anti-inflammatory activity of fermented *R. rugosa* 'Mohong' extracts (FRME) at different temperatures(A: 20 °C, B: 25 °C, C: 30 °C, D:35 °C). Values are presented in mean ± standard deviation(n = 3) and evaluated using a two-way ANOVA test (post test: Duncan test). Different letters(a-i) in the same concentration denote significant differences at $p < 0.05$.

4. Discussion

Fermented products are more nutritious, have a longer shelf life, and are more biochemically active than their unfermented counterparts [50]. Microorganisms affect the bioactivity and phenolic content of traditional fermented foods during fermentation under different parameters [52]. FRM is a popular native health food in the Bai community of Dali, Yunnan, and this traditional fermented product has numerous chemical compounds that are characterized by their strong stability and low toxicity profiles. Antioxidant and anti-inflammatory components are increasingly recognized for their crucial role in cancer research. The results of this study indicate that FRME contains high levels of bioactive substances that possess strong antioxidant and anti-inflammatory activities.

Numerous studies have shown that fermentation significantly changes the polyphenol profiles of plant-based foods [53,54]. Traditional naturally fermented rose petals are usually fermented at room temperature (approximately 20–22 °C). However, it takes more than half a year to complete this procedure. Although it is difficult to clearly determine the optimal fermentation time for producing FRM due to temperature dependencies, it is possible to ascertain the optimum duration of *R. rugosa* 'Mohong' fermentation by simultaneously measuring polyphenolic metabolites during fermentation. *R. rugosa* and fermented *R. rugosa* exhibit high antioxidant activity due to their high contents of biologically active compounds [55]. The results showed the highest levels of both phenolic acids and antioxidant activity after 35 d of fermentation at a higher temperature (35 °C) in FRME, which supports the results of many previous studies [56,57]. Ávila [58] reported that some strains of *Lactobacillus* are capable of degrading the glycosides anthocyanins, subsequently converting them into phenolic acids, such as gallic acid or syringic acid. As reported by De Noronha [59], gallic acid and some phenolic acids increased during black tea fermentation, whereas some flavonoids decreased. Silva [60] suggested that the concentrations of phenolic acids in red wine increase post-fermentation, primarily due to the breakdown of plant cell walls by yeast. He [61] reported that the addition of gallic acid during ensiling increased the preservation of the antioxidant capacity of Drumstick tree (*Moringa oleifera*) leaves in silage. Gallic acid level is considered to be a quality index of fermented tea due to its high antioxidant ability, which is known to increase with higher fermentation temperatures [57]. In this study, gallic acid content increased with time in FRME, especially at higher temperatures. Ajila [62] suggested that the contents of gallic acid and syringic acid increase with rising fermentation temperatures, probably because higher temperatures decrease the viscosity of FRM and accelerate the dissolution and diffusion of these two compounds.

The polyphenols in plant-based foods produced during fermentation exhibit structural changes and degradation during storage, thereby changing antioxidant activity [8]. For example, Chen et al. [63] fermented strawberry juice with two *Lactobacillus* species, detecting a decline in flavonoids such as rutin and (+)-catechin, while noting an increase in many phenolic acids such as gallic acid, chlorogenic acid and protocatechuic acid. Álvarez-Fernández [64] reported that the antioxidant activity of a fermented strawberry (*Fragaria* × *ananassa*) beverage reached its peak value after 60 d of storage, following which it began to decrease. The highest rutin and quercetin contents were detected after 7d of fermentation and they gradually decreased thereafter. The significant differences ($P < 0.05$) in the antioxidant content and activities of FRME underscore the vital importance of fermentation temperature and duration in the processing of RMP. Hence, FRME, when fermented under optimal conditions (temperature and time) and exhibiting high antioxidant activity, has potential applications as a preservative and antioxidant used in food and cosmetic products.

FRME was found to inhibit NO accumulation, with higher doses of FRME showing greater anti-inflammatory effects (Fig. 5). Although the anti-inflammatory activity of FRME fluctuated during fermentation, it peaked at 7d of fermentation (30 °C or 35 °C). Beyond this point, there was no significant increase in activity ($P > 0.05$), indicating that higher fermentation temperatures enable

higher anti-inflammatory activity in a shorter time. NO is important in regulating symptoms of various diseases, such as inflammation, tumor growth, and diseases of the cardiovascular system. Zhang [57] reported that excessive production of NO can induce inflammation-related tissue damage. Therefore, in this study, we focused on examining the inhibition of NO production, a direct and widely used method, to determine the anti-inflammatory activity of FRME. A recent study suggested that the polyphenolic-rich extracts of *R. rugosa* flower, stem leaf, and fruit reduce the production of inflammatory molecules (including NO) stimulated by LPS [15]. In summary, the current findings indicate that FRME reduces the production of NO, confirming the notion that FRME represents a potential anti-inflammatory agent.

The roles of the main individual components in FRME and their contributions to its antioxidant and anti-inflammatory properties may provide insights into the changes in bioactivities during fermentation. Analysis of *S. rouxii* strains isolated from chocolate syrup showed that the optimum sucrose concentration for these cells to grow in PDA medium ranges from 0 to 60%. Notably, the optimum temperature of *S. rouxii* strains increases with the sucrose content, from 28 °C to 35 °C. Additionally, it has been reported that the optimum growth temperature for *Saccharomyces* species is approximately 28 °C to 35 °C [65]. Therefore, to improve traditional fermentation processes, we recommend a temperature range of 30 °C to 35 °C for the fermentation of roses, as this range is more favorable than other temperatures. A fermentation period of 7–21 d results in FRME with a relatively balanced content of active compounds and high antioxidant and anti-inflammatory activities. This work lays the foundation for applied research on industrial scaling, quality control, and safety assurance of RMP products.

5. Conclusion

In this study, a fast, reliable HPLC-DAD method was developed for detecting major compounds in FRM. This method not only serves as a reference to improve the quality of traditional fermented rose products but also has the potential to be applied in the quality control of other rose-based products in the food and cosmetics industries. The study shows temperature and duration are vital for both RM fermentation and the quality of FRM. The results indicate that a shorter fermentation period (around 7 d), combined with higher temperatures, can yield FRM with tangible quality. This approach is more efficient compared to traditional fermentation time (approximately half a year). Given its edible and medicinal properties, this information is valuable in the development of nutraceutical products based on FRM.

However, rose fermentation differs depending on the rose variety, sugar ratio, fermentation techniques and storage conditions. Further studies are needed to shed light on the underlying mechanisms affecting the accumulation of bioactive compounds and other bioactivities in traditional fermented rose products. Such studies are crucial for enhancing the production of safe, high-quality traditional fermented rose. Nonetheless, the current results highlight the importance of traditional knowledge and techniques in rose fermentation. They demonstrate that FRM has strong antioxidant and anti-inflammatory activities, underscoring its considerable potential for application in food products, healthcare items, and cosmetics.

Ethics approval statement

Informed consent was not required for this study because this study did not involve animal or human subjects. The study protocol adhered to the guidelines established by the journal.

Data availability statement

Data associated with the study has not been deposited into a publicly available repository, and data included in the article is referenced in the article.

CRediT authorship contribution statement

Merhaba Abla: Writing – original draft, Formal analysis, Data curation. **Yueyue Cai:** Software, Data curation. **Lu Gao:** Validation, Supervision, Conceptualization. **Jingsong Wu:** Writing – review & editing, Conceptualization. **Lixin Yang:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (grant no. 31670340 and 31970357) and Postdoctoral Directional Training Foundation of Yunnan Province (China).

References

[1] V. Wissemann, C.M. Ritz, Evolutionary patterns and processes in the genus *Rosa* (*Rosaceae*) and their implications for host-parasite co-evolution, Plant Syst. Evol. 266 (2007) 79–89, https://doi.org/10.1007/s00606-007-0542-1.

[2] F. Zang, Y. Ma, Q. Wu, X. Tu, X. Xie, P. Huang, B. Tong, Y. Zheng, D. Zang, Resequencing of *Rosa rugosa* accessions revealed the history of population dynamics, breed origin, and domestication pathways, BMC Plant Biol. 23 (1) (2023) 1–2, https://doi.org/10.1186/s12870-023-04244-5.

[3] H.H. Bruun, *Rosa rugosa* thunb. Ex murray, J. Ecol. 1 93 (2) (2005) 441–470, https://doi.org/10.1111/j.1365-2745.2005.01002.x.

[4] A. Rehder, Manual of Cultivated Trees and Shrubs Hardy in North America: Exclusive of the Subtropical and Warmer Temperate Regions, Macmillan, 1927.

[5] L. Wei, J. Li, Y. Yang, M. Zhu, M. Zhao, J. Yang, Z. Yang, L. Zhou, S. Zhou, J. Gong, X. Jiang, Characterization and potential bioactivity of polyphenols of *Rosa rugosa*, Food Biosci. 1 (50) (2022) 102108, https://doi.org/10.1016/j.fbio.2022.102108.

[6] F. Zang, Y. Ma, Q. Wu, X. Tu, X. Xie, P. Huang, B. Tong, Y. Zheng, D. Zang, Resequencing of *Rosa rugosa* accessions revealed the history of population dynamics, breed origin, and domestication pathways, BMC Plant Biol. 23 (1) (2023) 1–2, https://doi.org/10.1186/s12870-023-04244-5.

[7] B. Lang, Y. Zhao, R. Yang, A. Liu, S. Ranjitkar, L. Yang, Antioxidant and tyrosinase inhibitory activities of traditional fermented *Rosa* from Dali Bai communities, Northwest Yunnan, China, Sci. Rep. 11 (1) (2021) 22700, https://doi.org/10.1038/s41598-021-02160-y.

[8] S.S. Ali, H. Ahsan, M.K. Zia, T. Siddiqui, F.H. Khan, Understanding oxidants and antioxidants: classical team with new players, J. Food Biochem. 44 (3) (2020) e13145, https://doi.org/10.1111/jfbc.13145.

[9] M. Kieliszek, A. Cendrowski, I. Ścibisz, M. Mitek, Influence of harvest seasons on the chemical composition and antioxidant activity in *Rosa rugosa* petals, Agrochimica 62 (2) (2018) 157–165, https://doi.org/10.12871/00021857201825.

[10] G.L. Chen, S.G. Chen, Y. Xiao, N.L. Fu, Antioxidant capacities and total phenolic contents of 30 flowers, Ind. Crop. Prod. 111 (2018) 430–445, https://doi.org/10.1016/j.indcrop.2017.10.051.

[11] L. Oktaviani, D.I. Astuti, M. Rosmiati, M.Y. Abduh, Fermentation of coffee pulp using indigenous lactic acid bacteria with simultaneous aeration to produce cascara with a high antioxidant activity, Heliyon 6 (7) (2020) e04462, https://doi.org/10.1016/j.heliyon.2020.e04462.

[12] Q. Ge, X. Ma, Composition and antioxidant activity of anthocyanins isolated from Yunnan edible rose (An ning), Food Sci. Hum. Wellness 2 (2) (2013) 68–74, https://doi.org/10.1016/j.fshw.2013.04.001.

[13] M.X. Li, J. Xie, X. Bai, Z.Z. Du, Anti-aging potential, anti-tyrosinase and antibacterial activities of extracts and compounds isolated from *Rosa chinensis* cv, 'JinBian.'. Ind Crop. Prod. 159 (2021) 113059, https://doi.org/10.1016/j.indcrop.2020.113059.

[14] X. Tursun, Y. Zhao, Z. Alat, X. Xin, A. Tursun, R. Abdulla, H.A. Akber Aisa, Anti- inflammatory effect of *Rosa rugosa* flower extract in lipopolysaccharide-stimulated RAW264.7 macrophages, Biomol. Ther. 24 (2) (2016) 184–190, https://doi.org/10.4062/biomolther.2015.090.

[15] E. Kim, H.K. Mok, T.K. Hyun, Variations in the antioxidant, anticancer, and anti-inflammatory properties of different *Rosa rugosa* organ extracts, Agronomy 12 (2) (2022) 238, https://doi.org/10.3390/agronomy12020238.

[16] M. Huang, X. Liu, X. Li, X. Sheng, T. Li, W. Tang, Z. Yu, Y. Wang, Effect of *Hanseniaspora uvarum–Saccharomyces cerevisiae* mixed fermentation on aroma characteristics of *Rosa roxburghii* tratt, blueberry, and plum wines, Molecules 27 (22) (2022) 8097, https://doi.org/10.3390/molecules27228097.

[17] A. Cendrowski, I. Ścibisz, M. Kieliszek, J. Kolniak-Ostek, M. Mitek, UPLC-PDA-Q/TOF-MS profile of polyphenolic compounds of liqueurs from rose petals (*Rosa rugosa*), Molecules 22 (11) (2017) 1832, https://doi.org/10.3390/molecules22111832.

[18] E. Chamani, C. Wagstaff, M. Kanani, Phenolics pattern of cut H3O rose flowers during floral development, Sci. Hortic. 271 (2020) 109460, https://doi.org/10.1016/j.scienta.2020.109460.

[19] Y. Wang, S. Li, Z. Zhu, Z. Xu, S. Qi, S. Xing, Y. Yu, Q. Wu, Q. Transcriptome and chemical analyses revealed the mechanism of flower color formation in *Rosa rugosa*, Front. Plant Sci. 13 (2022) 1021521, https://doi.org/10.3389/fpls.2022.1021521.

[20] M. Olech, N. Nowacka-Jechalke, M. Masłyk, A. Martyna, W. Pietrzak, K. Kubiński, D. Załuski, R. Nowak, Polysaccharide-rich fractions from *Rosa rugosa* Thunb. — composition and chemopreventive potential, Molecules 24 (7) (2019) 1354, https://doi.org/10.3390/molecules24071354.

[21] J. Zheng, M. Meenu, B. Xu, A systematic investigation on free phenolic acids and flavonoids profiles of commonly consumed edible flowers in China, J. Pharm. Biomed. Anal. 172 (2019) 268–277, https://doi.org/10.1016/j.jpba.2019.05.007.

[22] A. Cendrowski, I. Ścibisz, M. Mitek, M. Kieliszek, J. Kolniak-Ostek, Profile of the phenolic compounds of *Rosa rugosa* petals, J. Food Quality. (2017) 7941347, https://doi.org/10.1155/2017/7941347.

[23] A. Czyzowska, E. Klewicka, E. Pogorzelski, A. Nowak, Polyphenols, vitamin C and antioxidant activity in wines from *Rosa canina* L. and *Rosa rugosa* Thunb, J. Food Compos. Anal. 39 (2015) 62–68, https://doi.org/10.1016/j.jfca.2014.11.009.

[24] R. Nowak, M. Olech, Ł. Pecio, W. Oleszek, R. Los, A. Malm, J. Rzymowska, Cytotoxic, antioxidant, antimicrobial properties and chemical composition of rose petals, J. Sci. Food. Agr. 94 (3) (2014) 560–567, https://doi.org/10.1002/jsfa.6294.

[25] S. Choubey, S. Goyal, L.R. Varughese, V. Kumar, A.K. Sharma, V. Beniwal, Probing gallic acid for its broad-spectrum applications, Mini Rev. Med. Chem. 18 (15) (2018) 1283–1293, https://doi.org/10.2174/1389557518666180330114010.

[26] E. Ogut, K. Armagan, Z. Gül, The role of syringic acid as a neuroprotective agent for neurodegenerative disorders and future expectations, Metab. Brain Dis. 37 (4) (2022) 859–880, https://doi.org/10.1007/s11011-022-00960-3.

[27] K. Liu, S. Hu, B. Chan, E. Wat, C. Lau, K. Hon, K. Fung, P. Leung, P. Hui, C. Lam, C. Wong, Anti-inflammatory and anti-allergic activities of *pentaherb formula*, moutan cortex (danpi) and gallic acid, Molecules 18 (3) (2013) 2483–2500, https://doi.org/10.3390/molecules18032483.

[28] R. Kono, M. Nakamura, S. Nomura, N. Kitano, T. Kagiya, Y. Okuno, K. Inada, A. Tokuda, H. Utsunomiya, M. Ueno, Biological and epidemiological evidence of anti-allergic effects of traditional Japanese food ume (*Prunus mume*), Sci. Rep. 8 (1) (2018) 11638, https://doi.org/10.1038/s41598-018-30086-5.

[29] S.M. Cheemanapalli, C. Anuradha, P. Madhusudhana, M.B. Mahesh, P.S. Raghavendra, C. Kumar, Exploring the binding affinity of novel syringic acid analogues and critical determinants of selectivity as potent proteasome inhibitors, Anti-Cancer Agent Me 16 (11) (2016) 1496–1510, https://doi.org/10.2174/1871520616666160513131928.

[30] S. Sharma, A. Ali, J. Ali, J.K. Sahni, S. Baboota, Rutin: therapeutic potential and recent advances in drug delivery, Expet Opin. Invest. Drugs 22 (8) (2013) 1063–1079, https://doi.org/10.1517/13543784.2013.805744.

[31] Y.J. Jo, D.H. Yoo, I.C. Lee, J. Lee, H.S. Jeong, Antioxidant and skin whitening activities of sub- and super-critical water treated rutin, Molecules 27 (17) (2022) 5441, https://doi.org/10.3390/molecules27175441.

[32] B. Lu, Y. Huang, Z. Chen, J. Ye, H. Xu, W. Chen, X. Long, *Niosomal nanocarriers* for enhanced skin delivery of quercetin with functions of anti-tyrosinase and antioxidant, Molecules 24 (12) (2019) 2322, https://doi.org/10.3390/molecules24122322.

[33] J. Mlcek, T. Jurikova, S. Skrovankova, J. Ochor, Quercetin and its anti-allergic immune response, Molecules 21 (5) (2016) 623, https://doi.org/10.3390/molecules21050623.

[34] M.G. Lindley, The impact of food processing on antioxidants in vegetable oils, fruits and vegetables, Trends Food Sci. Technol. 9 (8–9) (1998) 336–340, https://doi.org/10.1016/s0924-2244(98)00050-8.

[35] P.M.L. Andrade, L. Baptista, J.S. Britto, A.P.T. Uetenabaro, A.M. da Costa, Co-production of tannase and gallic acid by a novel *Penicillium rolfsii* (CCMB 714), Prep. Biochem. Biotechnol. 48 (8) (2018) 700–706, https://doi.org/10.1080/10826068.2018.1487853.

[36] A. Cendrowski, M. Królak, S. Kalisz, Polyphenols, L-ascorbic acid, and antioxidant activity in wines from rose fruits (*Rosa rugosa*), Molecules 26 (9) (2021) 2561, https://doi.org/10.3390/molecules26092561.

[37] C.S. de Lima, B.T.A. Koelher, E.G.P. da Silva, A. Góes-Neto, R.P. Rezende, A.P.T. Uetanabaro, A.M. da Costa, *Pestalotiopsis mangiferae* isolated from cocoa leaves and concomitant tannase and gallic acid production, Fungal Biol. 126 (8) (2022) 471–479, https://doi.org/10.1016/j.funbio.2022.04.001.

[38] Y. Yao, M. Wu, Y. Huang, C. Li, X. Pan, W. Zhu, Y. Huang, Appropriately raising fermentation temperature beneficial to the increase of antioxidant activity and gallic acid content in *Eurotium cristatum*-fermented loose tea, LWT–Food Sci. Technol. 82 (2017) 248–254, https://doi.org/10.1016/j.lwt.2017.04.032.

[39] D. Hunaefi, N. Gruda, H. Riedel, D.N. Akumo, N.M.M.T. Saw, I. Smetanska, Improvement of antioxidant activities in red cabbage sprouts by lactic acid bacterial fermentation, Food Biotechnol. 27 (4) (2013) 279–302, https://doi.org/10.1080/08905436.2013.836709.

[40] F. Lin, F. Cai, B. Luo, R. Gu, S. Ahmed, C. Long, Variation of microbiological and biochemical profiles of Laowo dry-cured ham, an indigenous fermented food, during ripening by GC-TOF-MS and UPLC-QTOF-MS, J. Agric. Food Chem. 68 (33) (2020) 8925–8935, https://doi.org/10.1021/acs.jafc.0c03254.
[41] L. Qiu, M. Zhang, L. Chang, Effects of lactic acid bacteria fermentation on the phytochemicals content, taste and aroma of blended edible rose and shiitake beverage, Food Chem. 405 (2023) 134722, https://doi.org/10.1016/j.foodchem.2022.134722.
[42] F. Qu, W. Zeng, X. Tong, W. Feng, Y. Chen, D. Ni, The new insight into the influence of fermentation temperature on quality and bioactivities of black tea, LWT–Food Sci. Technol. 117 (2020) 108646, https://doi.org/10.1016/j.lwt.2019.108646.
[43] A.N. Xia, X.S. Meng, X.J. Tang, Y.Z. Zhang, S.M. Lei, Y.-G. Liu, Probiotic and related properties of a novel lactic acid bacteria strain isolated from fermented rose jam, LWT–Food Sci. Technol. 136 (2021) 110327, https://doi.org/10.1016/j.lwt.2020.110327.
[44] A.N. Xia, L.X. Liu, X.J. Tang, S.M. Lei, X.S. Meng, Y.G. Liu, Dynamics of microbial communities, physicochemical factors and flavor in rose jam during fermentation, LWT–Food Sci. Technol. 155 (2022) 112920, https://doi.org/10.1016/j.lwt.2021.112920.
[45] Y. Gao, X. Liu, W. Yang, X. Li, M. Li, F. Li, Dual inhibition of the renin and angiotensin converting enzyme activities of aqueous extracts of 22 edible flower petals, RSC Adv. 12 (7) (2022) 4191–4198, https://doi.org/10.1039/D1RA08978A.
[46] M.C.N.D Moreira, G.L. Almeida, E.E.N. Carvalho, J.A.D. Garcia, A.M. Nachtigall, B.M. Vilas Boas, Quality parameters, antioxidant activity, and sensory acceptability of mixed jams of rose petals and apple, J. Food Process. Preserv. 43 (12) (2019), https://doi.org/10.1111/jfpp.14272.
[47] G. Blanc, K.H. Wolfe, Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes[W], Plant Cell 16 (7) (2004) 1667–1678, https://doi.org/10.1105/tpc.021345.
[48] R.B. Martins, I. Gouvinhas, M.C. Nunes, L.M. Ferreira, J.A. Peres, A. Raymundo, A. Barros, Acorn flour from holm oak (*Quercus rotundifolia*): assessment of nutritional, phenolic, and technological profile, Curr. Res. Food Sci. 5 (2022) 2211–2218, https://doi.org/10.1016/j.crfs.2022.11.003.
[49] S. Datta, B.K. Sinha, S. Bhattacharjee, T. Seal, Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants, Heliyon 5 (3) (2019) e01431, https://doi.org/10.1016/j.heliyon.2019.e01431.
[50] R. Sharma, P. Garg, P. Kumar, S.K. Bhatia, S. Kulshrestha, Microbial fermentation and its role in quality improvement of fermented foods, Fermentation 6 (4) (2020) 106, https://doi.org/10.3390/fermentation6040106.
[51] N.B. Othman, D. Roblain, N. Chammen, P. Thonart, M. Hamdi, Antioxidant phenolic compounds loss during the fermentation of Chétoui olives, Food Chem. 116 (3) (2009) 662–669, https://doi.org/10.1016/j.foodchem.2009.02.084.
[52] W.H. Coulibaly, K.M.J.P. Bouatenin, Z.B.I.A. Boli, K.K. Alfred, Y.C.T. Bi, K.M.C. N'sa, M. Cot, C. Djameh, K.M. Djè, Influence of yeasts on bioactive compounds content of traditional sorghum beer (tchapalo) produced in Côte d'Ivoire, Curr. Res. Food Sci. 3 (2020) 195–200, https://doi.org/10.1016/j.crfs.2020.06.001.
[53] D. Cortez, L. Quispe-Sanchez, M. Mestanza, M. Oliva-Cruz, I. Yoplac, C. Torres, S.G. Chavez, Changes in bioactive compounds during fermentation of cocoa (*Theobroma cacao*) harvested in Amazonas-Peru, Curr. Res. Food Sci. 6 (2023) 100494, https://doi.org/10.1016/j.crfs.2020.06.001.
[54] C.Y. Chen, C.Y. Hu, Y.H. Chen, Y.T. Li, Y.C. Chung, Submerged fermentation with *Lactobacillus brevis* significantly improved the physiological activities of *Citrus aurantium* flower extract, H eliyon 8 (9) (2022), https://doi.org/10.1016/j.heliyon.2022.e10498.
[55] J. Lu, C. Wang, Medicinal components and pharmacological effects of *Rosa rugosa*, Record Nat. Prod. 12 (6) (2018) 535–543, https://doi.org/10.25135/rnp.60.17.12.191.
[56] X.D. Xia, Y.Q. Dai, H. Wu, X.L. Liu, Y. Wang, L.Q. Yin, Z. Wang, X.N. Li, J. Zhou, Kombucha fermentation enhances the health-promoting properties of soymilk beverage, J. Funct.Foods 62 (2019) 103549, https://doi.org/10.1016/j.jff.2019.103549.
[57] H. Zhang, Y.Z. Liu, W.C. Xu, W.J. Chen, S. Wu, Y.-Y. Huang, Metabolite and microbiome profilings of pickled tea elucidate the role of anaerobic fermentation in promoting high levels of gallic acid accumulation, J. Agric. Food Chem. 68 (47) (2020) 13751–13759, https://doi.org/10.1021/acs.jafc.0c06187.
[58] M. Ávila, M. Hidalgo, C. Sánchez-Moreno, C. Pelaez, T. Requena, S. de Pascual-Teresa, Bioconversion of anthocyanin glycosides by *bifidobacteria* and *Lactobacillus*, Food Res. Int. 42 (10) (2009) 1453–1461, https://doi.org/10.1016/j.foodres.2009.07.026.
[59] M.C. de Noronha, R.R. Cardoso, C.T.D.S. D'Almeida, M.A.V. do Carmo, L. Azevedo, V.G. Maltarollo, J.I.R. Júnior, M.R. Eller, L.C. Cameron, M.S.L. Ferreira, F.A. R. de Barros, Black tea kombucha: physicochemical, microbiological and comprehensive phenolic profile changes during fermentation, and antimalarial activity, Food Chem. 384 (2022) 132515, https://doi.org/10.1016/j.foodchem.2022.132515.
[60] L.R. Silva, P.B. Andrade, P. Valentão, R.M. Seabra, M.E. Trujillo, E. Velázquez, Analysis of non-coloured phenolics in red wine: effect of *Dekkera bruxellensis* yeast, Food Chem. 89 (2) (2005) 185–189, https://doi.org/10.1016/j.foodchem.2004.02.019.
[61] L. He, H. Lü, N. Chen, C. Wang, W. Zhou, X. Chen, Q. Zhang, Improving fermentation, protein preservation and antioxidant activity of *Moringa oleifera* leaves silage with gallic acid and tannin acid, Bioresour. Technol. 297 (2020) 122390, https://doi.org/10.1016/j.biortech.2019.122390.
[62] C.M. Ajila, S.K. Brar, M. Verma, R.D. Tyagi, J.R. Valéro, Solid-state fermentation of apple pomace using *Phanerocheate chrysosporium* – liberation and extraction of phenolic antioxidants, Food Chem. 126 (3) (2011) 1071–1080, https://doi.org/10.1016/j.foodchem.2010.11.129.
[63] W. Chen, C.Y. Xie, Q.Q. He, J.X. Sun, W.B. Bai, Improvement in color expression and antioxidant activity of strawberry juice fermented with lactic acid bacteria: a phenolic-based research, Food Chem. X 17 (2023) 100535, https://doi.org/10.1016/j.fochx.2022.100535.
[64] M.A. Álvarez-Fernández, R. Hornedo-Ortega, A.B. Cerezo, A.M. Troncoso, M.C. García-Parrilla, Determination of nonanthocyanin phenolic compounds using high-resolution mass spectrometry (UHPLC-Orbitrap-MS/MS) and impact of storage conditions in a beverage made from strawberry by fermentation, J. Agric. Food Chem. 64 (6) (2016) 1367–1376, https://doi.org/10.1021/acs.jafc.5b05617.
[65] L. Restaino, S. Bills, K. Tscherneff, L.M. Lenovich, Growth characteristics *of Saccharomyces rouxii* isolated from chocolate syrup, Appl. Environ. Microbiol. 45 (1983) 1614–1621, https://doi.org/10.1128/aem.45.5.1614-1621.1983.