



Article

Comparative Analysis of Japanese Quince Juice Concentrate as a Substitute for Lemon Juice Concentrate: Functional Applications as a Sweetener, Acidifier, Stabilizer, and Flavoring Agent

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Abstract: This research examined the viability of Japanese quince juice concentrate (JQJC) as an innovative alternative to lemon juice concentrate (LJC). Given the rising consumer demand for natural food ingredients, this study focused on a thorough analysis of the nutritional and functional characteristics of JQJC in comparison to LJC. The chemical analysis indicated that JQJC possesses a total soluble solids (TSS) content of 50.6 °Brix, with fructose and glucose, to a greater extent, being the primary contributors to its solids content. In contrast, LJC had a TSS of 39.8 °Brix and also contained glucose and fructose. Additionally, malic acid is a principal component of JQJC's acidity, determined at 20.98 g 100 g^{−1} of fresh weight (FW), while LJC mostly contained citric acid at a concentration of 30.86 g 100 g^{−1} FW. Moreover, the ascorbic acid content quantified in JQJC was eight times greater than that observed in LJC. The assessment of antioxidant activity, utilizing the DPPH• and FRAP assays, indicated that JQJC exhibits scavenging activity nearly eleven times higher than that of LJC, suggesting its superior antioxidant capacity. The total phenolic content for JQJC was quantified at 2189.59 mg 100 g^{−1} FW, significantly ($p < 0.05$) exceeding the 262.80 mg 100 g^{−1} FW found in LJC. The analysis identified 16 individual phenolic compounds in JQJC, highlighting the dominance of epicatechin, chlorogenic, and protocatechuic acids with concentrations ranging from 0.16 to 50.63 mg 100 g^{−1} FW, contributing to a total individual phenolic content of 114.07 mg 100 g^{−1} FW. Conversely, LJC is characterized by substantial contributions from hesperidin, eriocitrin, and, to a lesser extent, quercetin-3-O-rutinoside, yielding a phenolic content of 109.65 mg 100 g^{−1} FW. This study presents strong evidence supporting the utilization of JQJC as a functional substitute for LJC across a variety of product categories, including beverages, jams, and other food items. The findings indicate that JQJC has the potential to enhance product development targeted at health-conscious consumers while optimizing the utilization of a relatively underexplored fruit crop.

Keywords: antioxidants; phenolics; *Chaenomeles japonica* L. juice concentrate; nutritional value



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1. Introduction

The production of juice concentrates (JCs) is a significant commercial endeavor. By concentrating fruit juices, manufacturers effectively lower water activity (a_w), inhibiting the growth of microorganisms and significantly extending the shelf life of juice products. Fruit JCs are convenient for packaging, storage, and transportation to distant regions [1]. In the food industry, popular juice concentrates include those derived from apples, lemons, limes, oranges, grapefruits, tangerines, pomegranates, pineapples, apricots, and mangoes, among others [2]. With their unique characteristics and compositions, these concentrates

find extensive use in juice reconstitution, drink preparations, sweetening, and acidification. Notably, lemon and lime juice concentrates stand out as key acidulants, suitable for a wide range of products, including jams, jellies, drinks, snacks, baked goods, and confections. They also play a crucial role in preventing the browning of various fruit raw materials [3–6]. Consuming lemon juice concentrate (LJC) offers several health benefits, notably its ability to treat urinary tract infections [7] and support weight loss by improving digestion [8]. LJ antioxidant properties have been found to influence hematological and biochemical parameters affected by oxidative stress [9]. It is worth noting that the chemical composition of lemons can vary significantly among different cultivars and rootstock combinations, leading to marked differences in the fruit's chemical properties [10]. Various LJC are available on the market, typically within the soluble solids content (SSC) range of 30–60 °Brix. Among these, some of the more popular options include 400 GPL, with an SSC of 45–53 °Brix, and 500 GPL, with an SSC of 47–59 °Brix, both highlighting the concentration of citric acid in grams per liter (GPL) [11,12]. According to recent reports, the LJC market is projected to grow to between USD 3.79 billion and USD 12.5 billion by 2023, with an expected annual growth rate of 3.8% to 4.4% from 2030 to 2033 [13]. This growth trajectory is driven by various factors, including rising demands from the food and beverage industry, expanded industrial applications, advancements in production technology, and a consumer preference for clean labeling and natural ingredients.

As the demand for healthy and natural food ingredients continues to rise, alternative raw materials rich in acid content are making their mark in the market, presenting exciting opportunities for innovation. One notable example is the Japanese quince (JQ) (*Chaenomeles japonica* L.), which has been used in China for medicinal purposes for over 3000 years [14]. This dwarf shrub, native to East Asia and subsequently introduced to other regions, is the subject of breeding programs in several eastern and northern European countries [15,16]. The cultivation of JQ has gained traction in the Baltic States, including Latvia, Lithuania, Estonia, Finland, Sweden, and Poland [17]. This trend primarily attributed to the European Research Project “Japanese quince (*C. japonica*)—a new European fruit crop for the production of juice, flavor, and fiber (FAIR5-CT97-3894, 1998–2001)”, which led to the development of improved JQ cultivars such as ‘Darius’, ‘Rondo’, and ‘Rasa’ [18]. Moreover, recent efforts in Latvia have targeted a breeding program (Nr. 10.9.1-11/24/1543-e) aimed at introducing new JQ varieties, including ‘Jānis’, ‘Silvija’, and ‘Ada’. Notably, JQ fruits are primarily suited for processing, as their high acid content makes them unsuitable for fresh consumption [16]. An early study conducted in Spain by Hellín et al. [19] investigated the chemical composition of JQ fruits and their processed products, considering various treatment methods. The results indicated that the availability of aroma volatiles in JQ fruits makes them especially well-suited for producing various products such as ice cream, lemonade, jam, curd, and yogurt. Among the most popular offerings are juice, puree, sugared candies, syrup, jam, and marmalade [14]. Additionally, JQ fruits can be used to create less common products like alcoholic beverages (wine, liqueur), crisps, freeze-dried fruit pieces, and powders [18–22]. JQ fruits are known for their diverse chemical compositions and show high levels of bioactive compounds, including polyphenols, vitamin C, organic acids, dietary fibers, and pectins [23–27]. These components not only contribute to the unique flavors and textures of JQ fruits but also to their potential health benefits [28]. Research suggests that the polyphenols in JQ fruits may significantly help reduce oxidative stress and inflammation [29]. Vitamin C is crucial in immune function, skin health, and overall well-being [28,30]. The organic acids enhance digestion and may assist in weight management by improving metabolic processes [31]. Furthermore, dietary fibers, including the pectins found in JQ fruits, help regulate blood sugar levels and improve gut health by supporting beneficial bacteria [32]. JQ juice (JQJ) is known for its high acidity, allowing it to serve as a natural acidulant, much like LJ, making it suitable for functional foods [33]. This unique property enhances the antioxidant characteristics of various end products [18]. Studies have indicated that JQJ shows a greater bioactive compound concentration than LJ [34]. Although LJ has a higher total acid content, JQJ stands out due to its elevated

levels of specific acids, including ascorbic acid [16,35]. Furthermore, the concentration of polyphenolic compounds in JQJ is nearly double that in LJ [16,36].

Conducting a comparative analysis of JQJ as a potential substitute for LJ presents an intriguing and specialized research objective. While the nutritional value and biochemical composition of JQ have been extensively studied, the juice concentrate form (JQJC) has received limited attention. This gap represents a substantial opportunity for research into the health benefits and nutritional profile of JQJC. A comprehensive understanding of JQJC could illuminate its distinct properties and applications, particularly as a substitute in various food products or beverages. Investigating its potential benefits, such as antioxidant properties and its role in promoting overall wellness, could address a notable deficiency in the existing literature and contribute to the advancement of new, health-oriented products within the expanding market for natural ingredients. This research aimed to perform a comprehensive comparative analysis of Japanese quince juice concentrate in relation to lemon juice concentrate. This study will specifically focus on evaluating the nutritional and biochemical profiles of Japanese quince juice concentrate.

2. Materials and Methods

2.1. Chemicals, Reagents, and Standards

Commercial standards, i.e., (–)-epicatechin, (+)-catechin, caffeic acid, chlorogenic acid, gallic acid, isorhamnetin, kaempferol, kaempferol-3-*O*-rutinoside (nicotiflorin), luteolin (aglycone), luteolin-7-*O*-glucoside (cynaroside), myricetin (aglycone), myricetin-3-*O*-glucoside, myricetin-3-*O*-rhamnoside (myricitrin), neochlorogenic acid, protocatechuic acid, quercetin, quercetin-3-*O*-galactoside (hyperoside), quercetin-3-*O*-rhamnoside (quercitrin), quercetin-3-*O*-rutinoside (rutin), quercetin-3- β -glucoside (isoquercitrin), rhamnetin, glucose, fructose, sucrose, sorbitol, xylose, glycerol, 96% ethanol (EtOH), oxalic acid, tartaric acid, quinic acid, malic acid, ascorbic acid, citric acid, fumaric acid, succinic acid, Folin–Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH $\bullet$), reagent potassium chloride, potassium ferrocyanide, sodium carbonate anhydrous, and iron trichloride hexahydrate were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Acetonitrile (MeCN), methanol (MeOH), and formic acid (HCOOH) (puriss p.a., $\geq 99.9\%$) are of liquid chromatography–mass spectrometry (LC-MS) grade; they along with the analytical standard *L*-ascorbic acid, sodium hydroxide pellets, and hydrochloric acid were obtained from Merck KGaA (Darmstadt, Germany). 2,4,6-tripyridyl-*s*-triazine and tannic acid were purchased from Fluka Chemicals Ltd. (London, England). Aluminium chloride hexahydrate was obtained from Alfa Aesar, Thermo Fisher Scientific (Bremen, Germany). Sodium nitrite was purchased from VWR™ International GmbH (Darmstadt, Germany). Sodium acetate trihydrate was obtained from AppliChem Chemicals GmbH (Darmstadt, Germany). Activated carbon was obtained from Euro Plus (Dnipro, Ukraine). Ultrapure water (UPW) was obtained using the reverse osmosis “Adrona Crystal EX-1101” water purification system (Adrona, Riga, Latvia).

2.2. Sample Information

(1) Industrially produced lemon juice concentrate was generously provided for research purposes by the company Puratos Latvia. The product is manufactured in Germany, with raw materials sourced from Spain, Argentina, and Italy.

(2) Japanese quince fruits grown at the Institute of Horticulture (LatHort) (Dobele, Latvia) were used to prepare juice concentrate according to the procedure outlined in Section 2.3.

2.3. Preparation of Japanese Quince Juice and Concentrate

The process of preparing Japanese quince juice (JQJ) and concentrate (C) involved harvesting whole ripe JQ (*Chaenomeles japonica* L.) fruits at the LatHort in Dobele, Latvia (coordinates: 56°36'39.9" N 23°17'48.8" E) in late August (Figure 1). Following harvest, the fruits underwent washing, drainage of excess water, and extraction of juice using a “Voran Maschinen” GmbH basket press 60 K (Voran Maschinen GmbH, Pichl bei Wels,

Austria). Subsequently, the extracted JQJ was pasteurized at 85 ± 1 °C for 10 min, then cooled to 70 ± 1 °C, and finally packaged in low-density polyethylene (LDPE) “bag in box” bags. The pasteurized JQJ was stored for one month to facilitate the preparation of JC. For the development of the JC, the pasteurized JQJ was subjected to evaporation in wide, low glass beakers. The evaporation was conducted in the open using a laboratory water bath (JP Selecta™ Precisdig, Barcelona, Spain) with programmable temperature and time settings (60 ± 0.2 °C). The soluble solids (TSS) content in the concentrate was regularly assessed with an “Atago PAL-1” refractometer (Atago Co., Ltd., Tokyo, Japan) to monitor the evaporation until it reached 50 ± 1 °Brix. The resulting JQJC was then cooled, stored in sealed containers, and kept at 4 ± 1 °C for subsequent analyses.



Figure 1. Fruit of the Japanese quince (*Chaenomeles japonica* L.) utilized in the production of juice concentrate.

2.4. Evaluation of Nutritional Quality, Energy Value, and Microbiological Safety of Japanese Quince Juice and Lemon Juice Concentrates

The assessment of nutritional quality, energy value, and microbiological safety of JQJC and LJC was performed at the accredited laboratory of J.S. Hamilton Baltic located in Riga, Latvia, according to the following standards and regulations: PN-A-75101-04:1990/Az1:2002 (refractometrically) for total acidity as citric acid; PN-EN 12143:2000 for soluble solids content (refractometrically); PN-EN ISO 10523:2012 for pH; Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25.10.2011 for energy value; PB-116 ed. III of 11.08.2020 for protein ($N \times 6.25$); PB-286 ed. I of 26.09.2014 for fat; AOAC 991.43: 1994 for dietary fiber; PB-381 ed. II of 01.12.2021 for density at 20 °C; LVS EN ISO 4833-1:2014 for enumeration of microorganisms; LVS ISO 21527-1:2008 for enumeration of yeasts and molds; and LVS ISO 4831:2006 for enumeration of coliforms in 0.1 g.

2.5. Preparation of Extracts from Japanese Quince and Lemon Juice Concentrates for Determination of Phenolics, Flavonoids, and Antioxidant Activity Using Spectrophotometric Studies

A total of 0.5 ± 0.01 g of JQJC or LJC was placed into a 50 mL conical centrifuge tube (from Sarstedt AG & Co. KG, Nümbrecht, Germany), and 30 mL of 80% EtOH was added. The resulting mixture was vigorously vortexed for 2 min using a “Vortex REAX top” (Heidolph, Schwabach, Germany), followed by ultrasonic treatment at a frequency of 50 kHz and an output of 360 W for 30 min at 20 ± 1 °C with an “Ultrasons” ultrasonic bath (J.P. Selecta®, Barcelona, Spain). Afterward, the mixture was centrifuged at $5000 \times g$ in a “Frontier™ 5718 R centrifuge” (OHAUS Europe GmbH, Nänikon, Switzerland) for 10 min at 20 ± 1 °C. The upper organic layer was then filtered through a “Whatman®

Grade 1" filter (Cytiva, Marlborough, MA, USA). The resulting clear filtrate was utilized for spectrophotometric analysis to assess phenolic (TPC) and flavonoid (TFC) contents, as well as antioxidant activity, employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and ferric-reducing antioxidant power (FRAP) methods.

2.6. Spectrophotometric Studies

2.6.1. Determination of Phenolic Content

The total phenolic content (TPC) was measured using the colorimetric Folin–Ciocalteu method, following the protocol established by Singleton et al. [37]. In summary, a 0.1 mL sample of each extract or standard gallic acid (GA) was combined with 2.5 mL of 10-fold-diluted Folin–Ciocalteu reagent. This was followed by the addition of 2.0 mL of 7.5% Na₂CO₃ and 0.4 mL of ultra-pure water (UPW) and then incubation for 30 min at room temperature (22 ± 1 °C). The absorbance was subsequently measured at a wavelength of 760 nm using a Shimadzu "UV-1800" visible spectrophotometer (Shimadzu Corp., Kyoto, Japan). The results were reported as mg of gallic acid equivalents per 100 mL on a fresh weight basis (mg GAE 100 mL^{−1} FW).

2.6.2. Determination of Flavonoid Content

The total flavonoid content (TFC) was evaluated using a modified version of the method described by Yang et al. [38]. In summary, 1 mL of each extract was placed in a 15 mL centrifuge tube, followed by the addition of 2 mL of UPW and 0.15 mL of 5% NaNO₂. The mixture was mixed thoroughly and allowed to react for 5 min. Next, 0.15 mL of 10% AlCl₃ was added, mixed well, and allowed to react for an additional 6 min. Finally, to bring the total volume to 10 mL, 2.0 mL of 1 N NaOH and 4.7 mL of UPW were added. The absorbance was measured after 15 min at a wavelength of 510 nm, and the results were expressed as mg catechin equivalents per 100 mL on a fresh weight basis (mg CE 100 mL^{−1} FW).

2.7. Antiradical Activity of Japanese Quince and Lemon Juice Concentrates

2.7.1. DPPH• Free Radical Scavenging Activity

The DPPH• free radical scavenging activity was evaluated in accordance with the methodology established by Radenkova et al. [39], with minor modifications. Specifically, 0.1 mL aliquot of each extract was combined with 2.9 mL of a DPPH•–ethanol solution, which comprised 0.0036 g of DPPH• dissolved in 100.0 mL of ethanol. The reaction was allowed to proceed at a temperature of 22 ± 1 °C for a duration of 30 min in a dark environment. Absorbance measurements were taken at both 0 and 30 min at a wavelength of 517 nm. The DPPH• scavenging activity was expressed as moles of Trolox equivalents (TE) antioxidant capacity per 100 mL on a fresh weight basis (M TE 100 mL^{−1} FW).

2.7.2. Ferric-Reducing Antioxidant Power (FRAP)

The antioxidant capacity was evaluated using the ferric-reducing antioxidant power (FRAP) method, as outlined by Radenkova et al. [40], with minor modifications. The preparation of the fresh FRAP reagent involved the careful mixing of 200.0 mL of 0.3 M acetate buffer, 2.0 mL of 62.5 mg of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) dissolved in 40 mM HCl, and 20.0 mL of 108.1 mg FeCl₃·6H₂O dissolved in UPW (20 mM) in a volume ratio of 10:1:1 (v/v/v). Subsequently, a 0.1 mL aliquot of each extract or UPW (as the blank) was combined with 2.9 mL of the prepared FRAP reagent and incubated in the dark at ambient temperature for 10 min. The absorbance of the resulting solution was measured at a wavelength of 593 nm. The FRAP values were expressed as millimoles of Trolox equivalents (TE) antioxidant capacity per 100 mL on a fresh weight basis (mM TE 100 mL^{−1} FW).

2.8. Solid-Phase Extraction of Free Phenolics from Japanese Quince and Lemon Juice Concentrates for Analysis by LC-ESI-TQ-MS/MS

The solid-phase extraction (SPE) method utilized for the isolation and purification of bioactive compounds from JQJC and LJC was performed in accordance with the protocol established by Radenkovs et al. [41], with slight modifications. In brief, 1 ± 0.01 g of JQJC or LJC was placed in 15 mL conical centrifuge tubes in triplicate, followed by the addition of 9 mL of acidified methanol (MeOH), either at a concentration of 30% or 100% (MeOH:H₂O:HCOOH ratios of 30:69:1 or 99:0:1 v/v/v, respectively). The selection of MeOH as a solvent at these two different concentrations was based on the solubility characteristics of phenolic compounds present in the chosen products. Subsequently, the mixture was subjected to 1 min of vigorous vortex mixing, followed by ultrasonic treatment at a frequency of 50 kHz and an output power of 360 W for 30 min at 25 ± 1 °C. After extraction, the samples were centrifuged at $10,280 \times g$ for 10 min at 20 ± 1 °C. The organic layer was then carefully separated and filtered using a 0.20 µm hydrophilized polytetrafluoroethylene (H-PTFE) membrane filter (Macherey-Nagel GmbH & Co. KG, Dueren, Germany). Only those crude extracts that exhibited a higher yield of phenolic compounds in relation to the MeOH concentration (30% or 100%) as determined by the LC-ESI-TQ-MS/MS method were selected for further purification.

Phenolic compound purification was conducted through a “Supel™-Swift HLB” (57492-U) column (Supelco, Bellefonte, PA, USA), which was packed with a hydrophilic-modified, styrene-based sorbent (50–70 µm, 80–200 Å, 60 mg 3 mL). A constant flow rate of 1 ± 0.2 mL min^{−1} was maintained during the desorption of the analyte using a “Chromabond®SPE” (Düren, Germany) SPE vacuum manifold, adjusted to a pressure of 3.38×10^{-3} Pa. The SPE column was conditioned and equilibrated using 1 bed volume (3 mL) of pure MeOH, followed by 1 bed volume of UPW (H₂O:HCOOH ratio of 99:1 v/v). The loaded extract (1 mL) was subsequently washed with an additional bed volume of UPW. Flow-through fractions were collected for qualitative and quantitative chromatographic analyses of the presence of phenolic compounds and saccharides. A 1 mL solution of acidified 30% MeOH (MeOH:H₂O:HCOOH ratio of 30:69:1 v/v) was used to elute phenolic compounds from the sorbent. The resulting fractions were then collected and analyzed using an LC-ESI-TQ-MS/MS system.

2.9. The LC-ESI-TQ-MS/MS Analytical Conditions for Individual Phenolic Compounds

The analysis utilized a Shimadzu series “Nexera UC” supercritical fluid extraction–supercritical fluid chromatography–mass spectrometry (SFE-SFC-MS) system (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu triple quadrupole (TQ) mass-selective detector (TQ-MS-8050) featuring an electrospray ionization interface (ESI) (Shimadzu Corporation, Kyoto, Japan). Phenolic compounds were chromatographically separated using a reversed-phase (RP) “Shim-pack UC-RP” column (5 µm, 250 × 4.6 mm) at 45 ± 1 °C and a flow rate of 1 mL min^{−1}. The mobile phases consisted of acidified UPW (H₂O:HCOOH ratio 99:1 v/v) (A) and acidified MeOH (MeOH:HCOOH ratio 99:1 v/v) (B). The compounds were separated within 25 min using a stepwise gradient elution program for mobile phase B: T_{0min} = 5%, T_{1–5min} = 10%, T_{5–15min} = 60%, T_{15–23min} = 80%, T_{23–24min} = 10%, and T_{24–25min} = 5%. Additionally, MeOH injections were included as a blank run after each sample to mitigate the carry-over effect. The data were acquired using “LabSolutions Insight” LC-MS software version 3.7 SP3 (workstation) (Shimadzu Corporation, Kyoto, Japan). Ionization was performed in both positive and negative ion polarity modes. The operating conditions were as follows: a detector voltage of 1.8 kV, a conversion dynode voltage of 10 kV, an interface voltage of 3 kV, an interface temperature of 300 °C, a desolvation line temperature of 250 °C, and a heat block temperature of 400 °C. The nebulizing gas was argon (Ar) at a flow rate of 3 L min^{−1}, the heating gas was carbon dioxide (CO₂) at a flow rate of 10 L min^{−1}, and the drying gas was nitrogen (N) at a flow rate of 10 L min^{−1}. The phenolic compounds shown in Figure 2 were identified using authentic standards through the programmed and optimized multiple reaction monitoring (MRM) mode,

while hesperidin, eriocitrin, cryptochlorogenic acid, and quercetin rhamnoside glucoside derivatives were tentatively identified by analyzing the primary mass ions and referencing previously published data, which facilitated the comparison of spectral characteristics for accurate identification.

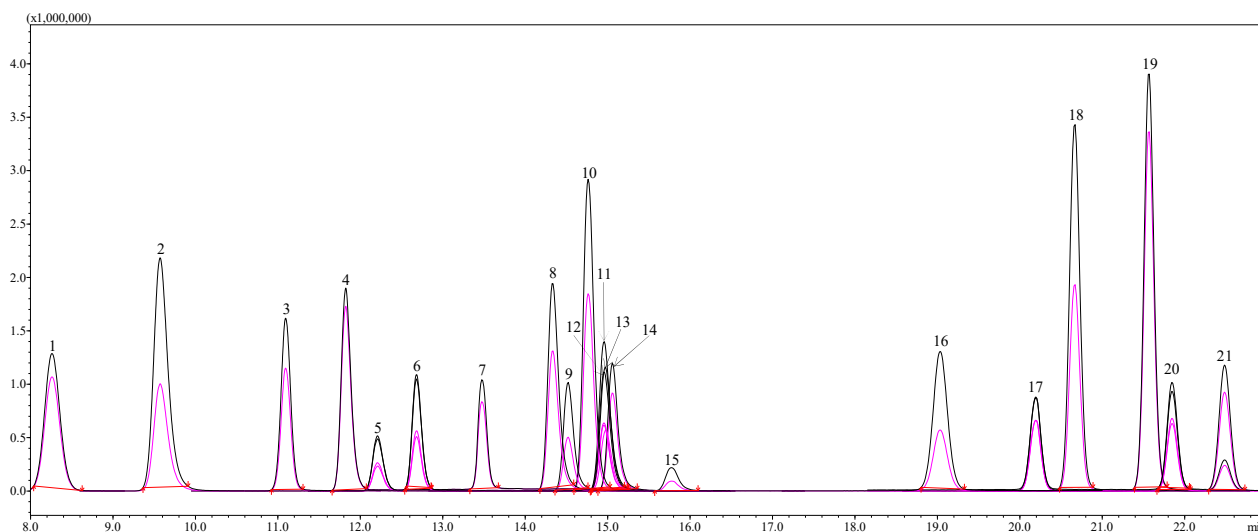


Figure 2. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) mode represents the profile of 21 phenolic standards at a concentration of $0.6 \mu\text{g mL}^{-1}$. Note: 1—Gallic acid; 2—Neochlorogenic acid; 3—Protocatechuic acid; 4—Chlorogenic acid; 5—(+)-Catechin; 6—(−)-Epicatechin; 7—Caffeic acid; 8—Myricetin-3-*O*-glucoside; 9—Quercetin-3-*O*-rutinoside (rutin); 10—Luteolin-7-*O*-glucoside (cynaroside); 11—Quercetin-3-*O*-galactoside (hyperoside); 12—Quercetin-3- β -glucoside (isoquercitrin); 13—Myricetin-3-*O*-rhamnoside (myricitrin); 14—Kaempferol-3-*O*-rutinoside (nicotiflorin); 15—Quercetin-3-*O*-rhamnoside (quercitrin); 16—Myricetin (aglycone); 17—Luteolin (aglycone); 18—Quercetin; 19—Kaempferol; 20—Rhamnetin; 21—Isorhamnetin.

2.10. Sample Preparation for Quantitative Analysis of Saccharides and Organic Acids

The extraction process for mono- and disaccharides, along with organic acids from JQC and LJC, was carried out in two stages. Initially, the samples were gently heated to a controlled temperature of $60 \pm 1^\circ\text{C}$ for 30 ± 1 min. This was subsequently followed by ultrasonic treatment at a frequency of 50 kHz and an output power of 360 W for an additional 30 ± 1 min while maintaining a temperature of $25 \pm 1^\circ\text{C}$. For the extraction of the target compounds, 1 ± 0.1 g of the sample was combined with 9 mL of 50% acetonitrile ($\text{H}_2\text{O}:\text{CH}_3\text{CN}$, *v/v*) in 15 mL conical plastic tubes (Sarstedt AG & Co. KG, Numbrecht, Germany). The mixture was then vigorously vortex-mixed for 2 min using a “ZX3” vortex mixer (Velp® Scientifica, Usmate Velate, Italy). Following this, the prepared samples were centrifuged at 4500 rpm for 10 min at a temperature of $19 \pm 1^\circ\text{C}$ using a “Sigma 2-16KC” centrifuge (Osterode near Harz, Germany) to facilitate the separation of the fractions. Prior to chromatographic analysis, the collected supernatant was filtered through a $0.45 \mu\text{m}$ H-PTFE membrane filter (Macherey-Nagel GmbH & Co. KG, Dueren, Germany).

2.11. Analytical Conditions of the HPLC-RID System for the Quantitative Analysis of Saccharides

For the quantitative analysis of mono- and disaccharides, a “Waters Alliance” HPLC system (model e2695) was employed, featuring a 2414 RI refractive index detector and a 2998 column heater (Waters Corporation, Milford, MA, USA). The chromatographic separation of saccharides was performed using an “Altima Amino” column (4.6×250 mm; $5 \mu\text{m}$; Grace™, Columbia, MD, USA), with temperatures in the column and flow cell maintained at $30 \pm 1^\circ\text{C}$. A $15 \mu\text{L}$ sample injection was carried out automatically, with the needle rinsed using a 50:50 (*v/v*) solution of acetonitrile and UPW (CH_3CN). The mobile phase was operated in isocratic mode, consisting of a mixture of CH_3CN and UPW (80:20,

v/v) at a flow rate of 1 mL min⁻¹. System control, data acquisition, analysis, and processing were managed using the “Empower3 Chromatography Data Software” version (build 3471) (Waters Corporation, Milford, MA, USA).

2.12. Analytical Conditions of the HPLC-PDA System for the Quantitative Analysis of Organic Acids

For the quantitative analysis of organic acids, including oxalic acid, tartaric acid, quinic acid, malic acid, ascorbic acid, citric acid, fumaric acid, and succinic acids, a “Shimadzu LC-20 Prominence” HPLC system (Shimadzu Corporation, Kyoto, Japan) was utilized. This system included “SPD-M20A” photodiode array detector (PDA), “LC-20A” solvent delivery system, “CBM-20A” system controller, “DGU-20A5R” degassing unit, “CTO-20AC” column oven, and “SIL-20AC” autosampler. System control, data acquisition, analysis, and processing were performed using the “LCSolution” data system software version 1.21 SP1 (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation of the organic acids was achieved using a “YMC C18” column (4.6 mm × 250 mm, 5 µm particle size; YMC Co., Ltd., Kyoto, Japan). The column temperature was maintained at 40 ± 1 °C, and a sample volume of 10.0 µL was automatically injected for analysis. The analysis was performed in isocratic elution mode with a 1.0 mL min⁻¹ flow rate. The mobile phase consisted of a 0.05 M potassium dihydrogen phosphate buffer (KH₂PO₄; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) with a pH of 2.8, adjusted using phosphoric acid (H₃PO₄; HPLC grade, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 10 mL of acetonitrile (HPLC grade, CHROMASOLV®) from Honeywell Riedel-de Haën GmbH, (Seelze, Germany). The solution was made up to a final volume of 1 L in a volumetric flask using UPW. Organic acid detection was carried out at a wavelength of 210 nm using the PDA detector. The total analysis time was up to 15 min.

2.13. Statistical Analysis

The results are presented as means ± standard deviations from duplicates ($n = 2$) for nutritional, energy, and microbiological indicators, triplicates ($n = 3$) for saccharides and organic acids, and quadruplicates ($n = 4$) for biochemical indicators. A p -value of ≤ 0.05 was employed to denote significant differences between the mean values, which were evaluated using one-way analysis of variance (ANOVA). The linear correlations between the results were evaluated by Pearson’s correlation coefficient ($p \leq 0.05$). The statistical analyses were conducted using “IBM® SPSS® Statistics” version 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. The Nutritional, Energy, Microbiological, and Biochemical Qualities of Japanese Quince Juice and Lemon Juice Concentrates

As per the data presented in Table 1, there was a significant difference ($p < 0.05$) in the total acidity of the two fruit concentrates. The total acidity of JQJC and LJC was 28.5 and 32.8 g 100 g⁻¹ citric acid equivalents FW, respectively. The high acidity levels in the examined concentrates can be attributed to the presence of specific acids, namely malic acid in JQJ [24] and citric acid in LJ [33].

Citric acid exhibits the highest acidity, with a pH level close to 2.3, while malic acid has a pH of 3.5 [42]. Research by Lorente et al. [34] revealed that pure LJ has a titratable acidity of 52.4 g L⁻¹, with citric acid as the primary organic acid. The analysis revealed a significant difference ($p < 0.05$) in the TSS content between JQJC and LJC, with measurements of 50.6 and 39.8 °Brix FW, respectively. The elevated TSS content in JQJC can be attributed to the abundance of various soluble substances, notably pectins, which contribute to this characteristic in conjunction with organic acids and saccharides [43]. Concerning the energy value, JQJC derived from *Chaenomeles japonica* L. exhibits a significantly higher ($p < 0.05$) nutritional profile than its LJC counterpart, providing 215 kcal 100 g⁻¹ FW compared to 150 kcal 100 g⁻¹ FW for the latter. Despite LJC exhibiting substantially higher contents of fats and proteins, the observed variation in energy values may be ascribed to the specific composition and types of macronutrients present in JQJC, which have not been examined

in the current study. These factors significantly influence the overall caloric contribution and result in an increased TSS content. For instance, cell-wall polysaccharides, such as hemicellulose derivatives, including oligosaccharides and starch, have the potential to enhance the TSS in JQJC, thereby affecting its overall caloric content [27].

Table 1. The nutritional, energy, and microbiological quality indicators of Japanese quince juice and lemon juice concentrates.

Item	JQJC	LJC	Percentage Difference, %
Total acidity as citric acid (g CAE 100 g ^{−1} FW)	28.5 ± 0.0 ^b	32.8 ± 0.0 ^a	14.0
Total soluble solids (°Brix)	50.6 ± 0.0 ^a	39.8 ± 0.0 ^b	23.9
pH	2.5 ± 0.0 ^a	2.3 ± 0.0 ^a	8.3
Protein (N × 6.25) (g 100 g ^{−1} FW)	0.3 ± 0.1 ^b	2.0 ± 0.1 ^a	147.8
Fat (g 100 g ^{−1} FW)	0.1 ± 0.1 ^b	0.5 ± 0.1 ^a	133.3
Dietary fiber (g 100 g ^{−1} FW)	0.5 ± 0.1 ^a	0.1 ± 0.1 ^b	133.3
Density (kg cm ³)	1.2496 ± 0.0250 ^a	1.1920 ± 0.0324 ^a	4.7
Energy value, kcal/kJ (100 g ^{−1} FW)	215/919 ^a	150/627 ^b	35.6
CFU g^{−1} FW			
Yeasts	<1.0 × 10 ²	<1.0 × 10 ²	-
Molds	<1.0 × 10 ²	<1.0 × 10 ²	-
<i>E. coli</i>	n.d.	n.d.	-
Total plate count	<1.0 × 10 ²	<1.0 × 10 ³	-

Note: values are means ± SDs of duplicates ($n = 2$). FW—value expressed on a fresh weight basis; CFU—colony forming units; n.d.—not detected. Means within the same quality indicator with different superscript letters (^{a–b}) are significantly different at $p < 0.05$.

The data presented in Table 2 indicate that the JQJC exhibits the highest total phenolic content (TPC), measured at 2189.6 mg GAE 100 g^{−1} FW. Considering that the TSS content in JQJC is five times higher than that reported by Urbanavičiūtė and Viškelis [18] for fresh JQJ, the observed TPC value in JQJC appears consistent with the TPC reported for fresh JQJ. Furthermore, Ros et al. [16] made a reasonable observation, noting a TPC range of 185 to 476 mg GAE 100 g^{−1} FW of juice across 24 genotypes of *Chaenomeles* species. The observed TPC value in commercially produced LJC was approximately 8.4 times lower, demonstrating statistical significance ($p < 0.05$), with a 262.8 mg GAE 100 g^{−1} FW value. When considering the amount of TSS in the LJ, the TPC values obtained corresponded with those reported by Hajimahmoodi et al. [36] for branded and natural LJ samples across nine evaluations.

Table 2. The concentration of biologically active compounds and antioxidant activity of Japanese quince juice and lemon juice concentrates.

Item	JQJC	LJC	Percentage Difference, %
TPC (mg GAE 100 g ^{−1} FW)	2189.6 ± 17.6 ^a	262.8 ± 5.6 ^b	157.1
TFC (mg CE 100 g ^{−1} FW)	1791.9 ± 9.1 ^a	190.5 ± 3.1 ^b	161.6
DPPH• (M TE 100 g ^{−1} FW)	3.7 ± 0.1 ^a	0.4 ± 0.0 ^b	161.0
FRAP (mM TE 100 g ^{−1} FW)	361.3 ± 8.8 ^a	37.9 ± 3.0 ^b	162.0

Note: values are means ± SDs of quadruplicates ($n = 4$). FW—value expressed on a fresh weight basis; TPC—total phenolic content; TFC—total flavonoid content; GAE—values expressed as gallic acid equivalents; CE—values expressed as catechin equivalents; mM—values expressed as millimoles; M—values expressed as moles; DPPH•—radical scavenging activity; FRAP—ferric-reducing antioxidant power; TE—values expressed as Trolox equivalents. Means within the same quality indicator with different superscript letters (^{a–b}) are significantly different at $p < 0.05$.

The analysis of total flavonoid content (TFC) reveals that JQJC exhibited a significantly elevated value ($p < 0.05$) in comparison to LJC, which demonstrated a 9.4-fold lower concentration, corresponding to 1791.9 mg CE g^{−1} FW and 190.5 mg CE g^{−1} FW, respectively.

Previous research conducted by Huang et al. [44] and Byczkiewicz et al. [31] demonstrated a notably high availability of flavonoids in *Chaenomeles* species, highlighting the presence of compounds such as quercetin, catechin, epicatechin, and specpolyphenol A. Among the flavonoids identified in LJ, the predominant compounds were hesperidin, eriocitrin, diosmin, and luteolin-7-O-rutinoside [45]. The observed TFC value aligns with those reported by Marín et al. [46] for LJ derived from the Fino and Verna varieties. Based on two methods for assessing antioxidant activity, specifically DPPH• and FRAP, it is evident that the produced JQJC exhibits significantly higher ($p < 0.05$) antioxidant activity compared to the commercially available LJC. The antioxidant activity of JQJC, as evaluated by DPPH• and FRAP assays, is approximately ten times greater than that of LJC. The observed values are 3.7 M TE 100 g⁻¹ and 361.3 mM TE 100 g⁻¹ for JQJC, compared to 0.4 M TE 100 g⁻¹ and 37.9 mM TE 100 g⁻¹ for LJC, respectively.

3.2. Saccharide Profile of Japanese Quince Juice and Lemon Juice Concentrates

The analysis of saccharides in JQJC and LJC revealed distinct profiles between the two samples (Table 3 and Figure 3A,B). In JQJC, five saccharide representatives were identified, including three monosaccharides (xylose, fructose, and glucose), one sugar alcohol, and one unidentified sugar. The observed profile is consistent with the findings of Hellín et al. [24], indicating a prevalence of fructose, followed by glucose and sorbitol in JQJ. The absence of sucrose in JQJC is attributed to its inversion into glucose and fructose caused by juice pasteurization before concentrate preparation [47]. The total sugar content in JQJC was 10.36 g 100 g⁻¹, and this value is consistent with the findings of Rehman et al. [48], indicating a range of total sugars in juice concentrates derived from mango, apple, guava, and peach, which varied between 5.38% and 9.15% (g 100 g⁻¹). In the analysis of the saccharide composition of LJC, it was observed that the amounts of glucose and fructose were nearly identical. The production of JC in industrial settings requires prolonged heat treatment to eliminate any microorganisms that may pose a health risk to consumers. However, when LJ undergoes extensive heating, the original sucrose content, typically averaging 4.5 g L⁻¹ in the juice, is converted into equivalent amounts of glucose and fructose [34]. Furthermore, this process, as well as subsequent processing or storage, can lead to the formation of 5-hydroxymethylfurfural (HMF), a compound known to be toxic at high doses [49,50].

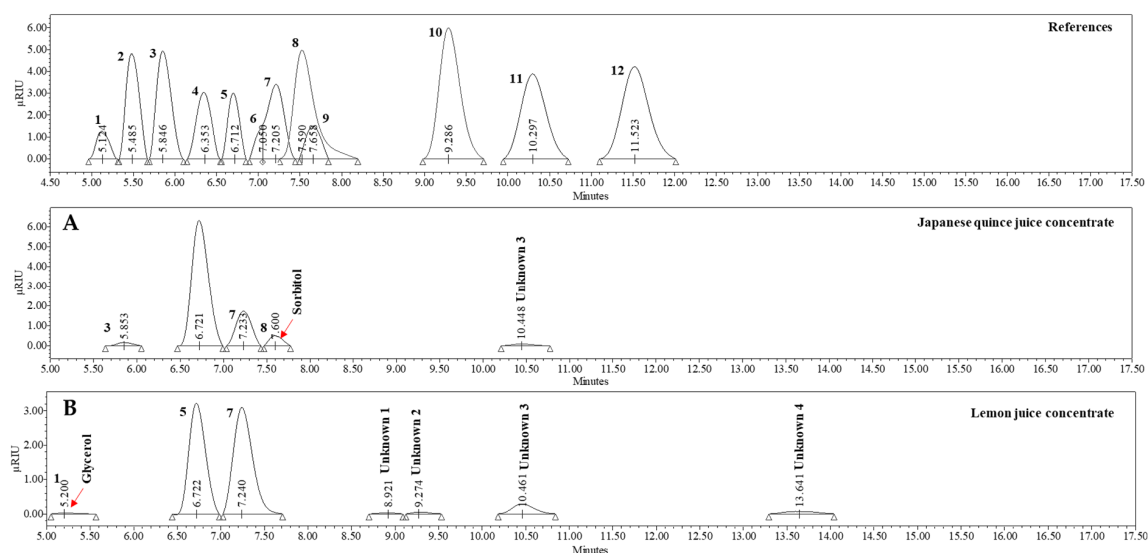


Figure 3. A representative profile of saccharides detected in Japanese quince (A) and lemon juice (B) concentrates. Sample injection volume of 15 μL, corresponding to a concentration of 0.075 μg mL⁻¹. Note: 1—Glycerol; 2—Ribose; 3—Xylose; 4—Arabinose; 5—Fructose; 6—Mannose; 7—Glucose; 8—Sorbitol; 9—Galactose; 10—Sucrose; 11—Maltose; 12—Lactose. Unknown peaks 1, 2, 3, and 4 correspond to unidentified compounds in Japanese quince and lemon juice concentrates.

Table 3. The concentrations of free mono- and disaccharides and organic acids in Japanese quince juice and lemon juice concentrates, g 100 g^{−1} FW.

Saccharide	JQJC	LJC	Percentage Difference, %
Glycerol	n.d.	1.17 ± 0.01	-
Xylose	0.31 ± 0.00	n.d.	-
Fructose	7.20 ± 0.22 ^a	3.62 ± 0.03 ^b	66.2
Glucose	1.94 ± 0.07 ^b	3.76 ± 0.00 ^a	63.9
Sorbitol	0.78 ± 0.03	n.d.	-
Unknown	n.d.	0.03 ± 0.00	-
Unknown	n.d.	0.06 ± 0.00	-
Unknown	0.14 ± 0.00 ^b	0.52 ± 0.01 ^a	115.2
Unknown	n.d.	0.19 ± 0.02	-
Total	10.36 ± 0.32 ^a	9.35 ± 0.07 ^b	10.2
Organic acid			
Oxalic acid	0.21 ± 0.00 ^a	0.12 ± 0.00 ^a	54.5
Tartaric acid	1.08 ± 0.00 ^a	0.22 ± 0.01 ^b	132.3
Quinic acid	8.50 ± 0.05 ^a	0.45 ± 0.05 ^b	179.9
Malic acid	20.98 ± 0.08 ^a	3.14 ± 0.00 ^b	147.9
Ascorbic acid	0.24 ± 0.00 ^a	0.03 ± 0.00 ^b	155.6
Citric acid	1.78 ± 0.01 ^b	30.86 ± 0.09 ^a	178.2
Fumaric acid	n.d.	0.00 ± 0.00	-
Succinic acid	0.04 ± 0.00 ^b	0.91 ± 0.40 ^a	183.2
Total	32.82 ± 0.15 ^b	35.74 ± 0.56 ^a	8.5

Note: values are means ± SDs of triplicates ($n = 3$). FW—the concentration expressed on a fresh weight basis; n.d.—not detected. Means within the same saccharide or organic acid with different superscript letters (^{a–b}) are significantly different at $p < 0.05$.

It should be noted that in the LJC, glycerol was present at a concentration of 1.17 g 100 g^{−1}. The presence of glycerol in the LJC indicates its intentional addition to the product. Glycerol prevents sugars from crystallizing and may also function as a sweetener, thickener, or preservative [51]. The total sugar content in LJC was measured at 9.35 g 100 g^{−1}, which was statistically lower ($p < 0.05$) than the total sugar content in JQJC. In the LJC sample, four unidentified saccharides were also detected, suggesting the possible presence of sugar alcohols such as myo-inositol and scyllo-inositol, as documented by Klimek-Szczykutowicz et al. [33]. Overall, JQJC, akin to LJC, offers versatile applications in food and beverage products due to its natural sweetness and preservation properties while also enhancing the flavor and nutritional profiles of various formulations.

3.3. Organic Acid Profiles of Japanese Quince Juice and Lemon Juice Concentrates

The comparison of organic acid compositions in JQJC and LJC, similar to saccharides, revealed distinct profiles between the two samples (Table 3). The analysis results indicated that malic acid was the primary organic acid in JQJC, followed by quinic acid, citric acid, and, to a lesser extent, tartaric and ascorbic acids. Rumpunen et al. [15] also emphasized the prevalence of malic acid, which was further supported by Hellín et al. [24], demonstrating a range of 2.27 to 4.82 mg 100 mL^{−1} FW in JQ juice. JQJC, with its high concentration of malic acid, presents several culinary applications that capitalize on this organic acid's unique flavor profile. As the primary organic acid in JQJC, malic acid lends a refreshing tartness that can enhance various dishes and beverages [52]. In culinary applications, JQJC can be used as a flavorful ingredient in salad dressings and marinades, where the tartness of malic acid can help cut through the richness of oils and fats, adding balance to the overall taste [53]. Additionally, JQJC can be a natural flavor enhancer in sauces and glazes, imparting a slight sourness that complements savory ingredients [23]. Moreover, using JQJC as a base for desserts is also notable, where the malic acid can elevate fruit flavors in pies, cakes, and sorbets, providing a bright contrast to sweet profiles [19]. Its presence in beverages, such as cocktails and smoothies, can create a refreshing drink with a

stimulating and satisfying complex flavor. However, only trace amounts of succinic acid were observed in JQJC, lower than those reported by Cinkmanis et al. [54] for fresh JQ juice. It is essential to emphasize that the ascorbic acid content determined in JQJC was $0.24 \text{ g } 100 \text{ g}^{-1} \text{ FW}$, which is eight times greater than that observed in LJC. Despite the careful approach employed in the preparation of JQJC, the observed reduction in ascorbic acid concentration was approximately fourfold, according to the research conducted by Bieniasz et al. [26]. Their research indicated that the vitamin C content in fresh JQ fruit varied between 0.09 and $0.15 \text{ g } 100 \text{ g}^{-1} \text{ FW}$ across nine different genotypes grown at the Experimental Station of the Agricultural University near Kraków, Poland.

Furthermore, these findings were corroborated by Urbanavičiūtė et al. [28] and by Juhnevičienė-Radenkova et al. [14] in their analyses of fresh JQ fruit grown at the Institute of Horticulture, Lithuanian Research Center for Agriculture and Forestry, Bабtai, Lithuania, and the Institute of Horticulture, Dobeles, Latvia, respectively.

The total organic acid content in JQJC was determined to be $32.82 \text{ g } 100 \text{ mL}^{-1} \text{ FW}$, a finding consistent with the results reported by Granados et al. [55] after calculating observed values in JQJ, considering TSS content. The concentration of malic acid in JQJC renders it unsuitable for consumption in its original form [24]. However, it could be an alternative to LJC in various culinary applications, adding a distinctive tangy flavor to dishes and beverages. Citric acid is the primary organic acid in LJC, contributing to its characteristic sour taste. While malic acid is also present in this product, it is significantly lower ($p < 0.05$) than in JQJC, at a concentration of $3.14 \text{ g } 100 \text{ mL}^{-1} \text{ FW}$. The total organic acid content in LJC is $35.74 \text{ g } 100 \text{ mL}^{-1} \text{ FW}$, which is statistically higher ($p < 0.05$) than that of JQJC. This observation corroborates earlier findings reported by Aguilar-Hernández et al. [10] regarding LJ. Given its elevated malic acid content, JQJC has the potential to exert therapeutic effects in preventing calcium nephrolithiasis by increasing urinary pH and citrate excretion, ultimately reducing the precipitation of calcium salts [56]. Additionally, malic acid resulting from the consumption of JQJC may enhance antioxidant capacity and promote metabolic health in offspring, thus potentially mitigating metabolic syndrome by modulating gut microbiota and improving glucose metabolism [57]. Considering the organic acid content, JQJC, despite its unsuitability for direct consumption, presents valuable culinary versatility and health benefits attributable to its unique biochemical profile, with its significant malic acid concentration enabling functional applications in salad dressings, marinades, and desserts, much like LJC, which utilizes its citric acid content to enhance flavors in similar culinary creations.

3.4. The Contents of Individual Phenolic Compounds in Japanese Quince Juice and Lemon Juice Concentrates

Based on the findings from the quantitative assessment of antioxidant-associated compounds, particularly TPC and TFC, a thorough analysis of individual phenolic compounds was initiated. This aimed to outline the primary metabolites present in the JQJC produced and those found in commercially available LJC. The data presented in Table 4 and Figure 4 reveals that before the SPE purification of JQJC extracts, a total of 16 individual, free polyphenolic compounds were extracted, and 14 of them were successfully identified.

Utilizing 100% MeOH as the sole extraction solvent, fingerprinting of the JQJC-derived extracts by LC-ESI-TQ-MS/MS revealed the presence of various hydroxybenzoic acid derivatives, including gallic acid, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, protocatechuic acid, and caffeic acid. Additionally, flavonols detected included quercetin-3-O-rutinoside (rutin), luteolin-7-O-glucoside (cynaroside), quercetin-3-O-galactoside (hyperoside), quercetin-3- β -glucoside (isoquercitrin), quercetin-3-O-rhamnoside (quercitrin), and quercetin itself. The concentration of individual phenolic compounds in the developed JQJC product ranged from 0.16 to $50.63 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$, with epicatechin being the most prevalent compound. According to clinical data, epicatechin possesses significant antioxidant properties, which assist in neutralizing free radicals within the human body [58], potentially reducing oxidative stress and minimizing associated cellular

damage [59]. This compound has been linked to cardiovascular health, as research indicates that it may enhance blood circulation, lower blood pressure, and improve cholesterol levels, all of which contribute to a healthier heart [60]. Furthermore, several studies suggest that epicatechin may support cognitive function by enhancing cerebral blood flow and offering neuroprotective effects, which could be advantageous in protecting against neurodegenerative diseases [61]. Additionally, its anti-inflammatory properties may aid in reducing inflammation, thus promoting overall health and potentially preventing chronic illnesses [62].

Table 4. The concentrations of individual phenolic compounds in Japanese quince juice and lemon juice concentrates, mg 100 g^{−1} FW.

Phenolic Compound	JQJC			LJC	
	100% MeOH	30% MeOH	30% MeOH SPE	100% MeOH	30% MeOH SPE
Gallic acid	0.60 ± 0.00 ^a	0.60 ± 0.01 ^a	0.37 ± 0.00 ^c	n.d.	n.d.
Neochlorogenic acid	1.11 ± 0.01 ^a	1.11 ± 0.04 ^a	0.88 ± 0.01 ^c	n.d.	n.d.
Protocatechuic acid	11.80 ± 0.22 ^a	12.11 ± 0.07 ^a	11.60 ± 0.26 ^a	0.15 ± 0.02 ^b	0.15 ± 0.02 ^b
Cryptochlorogenic acid	0.88 ± 0.04 ^a	0.88 ± 0.03 ^a	0.84 ± 0.01 ^a	n.d.	n.d.
Chlorogenic acid	34.75 ± 0.14 ^a	35.80 ± 0.50 ^a	35.28 ± 0.35 ^a	n.d.	n.d.
(+)-Catechin	2.96 ± 0.13 ^a	2.93 ± 0.08 ^a	3.01 ± 0.02 ^a	n.d.	n.d.
(−)-Epicatechin	50.63 ± 0.95 ^c	51.74 ± 1.68 ^b	55.07 ± 0.52 ^a	n.d.	n.d.
Caffeic acid	1.04 ± 0.08 ^a	1.06 ± 0.06 ^a	1.22 ± 0.01 ^a	n.d.	n.d.
Unknown	1.13 ± 0.02 ^a	1.16 ± 0.00 ^a	1.25 ± 0.06 ^a	n.d.	n.d.
Hesperidin	n.d.	n.d.	n.d.	81.81 ± 0.08 ^a	29.79 ± 0.04 ^b
Eriocitrin	n.d.	n.d.	n.d.	18.48 ± 0.16 ^a	6.87 ± 0.26 ^b
Unknown	0.37 ± 0.01 ^a	0.38 ± 0.00 ^a	0.39 ± 0.00 ^a	n.d.	n.d.
Myricetin-3-O-glucoside	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-O-rutinoside (rutin)	1.33 ± 0.01 ^b	1.30 ± 0.04 ^b	1.42 ± 0.02 ^b	8.96 ± 0.11 ^a	9.06 ± 0.01 ^a
Myricetin-3-O-rhamnoside (myricitrin)	n.d.	n.d.	n.d.	n.d.	n.d.
Luteolin-7-O-glucoside (cynaroside)	0.16 ± 0.01 ^a	0.15 ± 0.01 ^a	0.16 ± 0.00 ^a	n.d.	n.d.
Quercetin-3-O-galactoside (hyperoside)	0.48 ± 0.01 ^a	0.48 ± 0.03 ^a	0.53 ± 0.01 ^a	0.06 ± 0.00 ^b	0.06 ± 0.00 ^b
Kaempferol-3-O-rutinoside (nicotiflorin)	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-β-glucoside (isoquercitrin)	1.07 ± 0.00 ^a	1.09 ± 0.02 ^a	1.16 ± 0.02 ^a	0.10 ± 0.01 ^b	0.10 ± 0.00 ^b
Quercetin-3-O-rhamnoside (quercitrin)	0.39 ± 0.04 ^a	0.37 ± 0.04 ^a	0.48 ± 0.02 ^a	n.d.	n.d.
Myricetin (aglycone)	n.d.	n.d.	n.d.	n.d.	n.d.
Luteolin (aglycone)	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin	0.53 ± 0.09 ^b	0.65 ± 0.29 ^a	0.45 ± 0.01 ^b	0.11 ± 0.06 ^c	0.09 ± 0.04 ^c
Kaempferol	n.d.	n.d.	n.d.	n.d.	n.d.
Rhamnetin	n.d.	n.d.	n.d.	n.d.	n.d.
Isorhamnetin	n.d.	n.d.	n.d.	n.d.	n.d.
TOTAL	109.20 ± 0.78 ^a	111.77 ± 1.76 ^a	114.07 ± 0.49 ^a	109.65 ± 0.04 ^a	46.10 ± 0.28 ^b

Note: values are means ± SDs of duplicates ($n = 2$). FW—value expressed on a fresh weight basis; SPE—solid phase extraction; n.d.—not detected. Means within the same phenolic compound with different superscript letters (^{a–c}) are significantly different at $p < 0.05$.

Chlorogenic acid, identified as a significant component of the JQJC, possesses notable anti-inflammatory properties [63] and plays a crucial role in weight management by aiding in the reduction of fat absorption and the regulation of glucose metabolism [64]. Additionally, chlorogenic acid has demonstrated antimicrobial efficacy against various food-borne pathogens, including *Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella dysenteriae*, among others [65]. In light of these findings, JQJC may be regarded as a functional ingredient that provides health benefits and could serve as a natural preservative in food products.

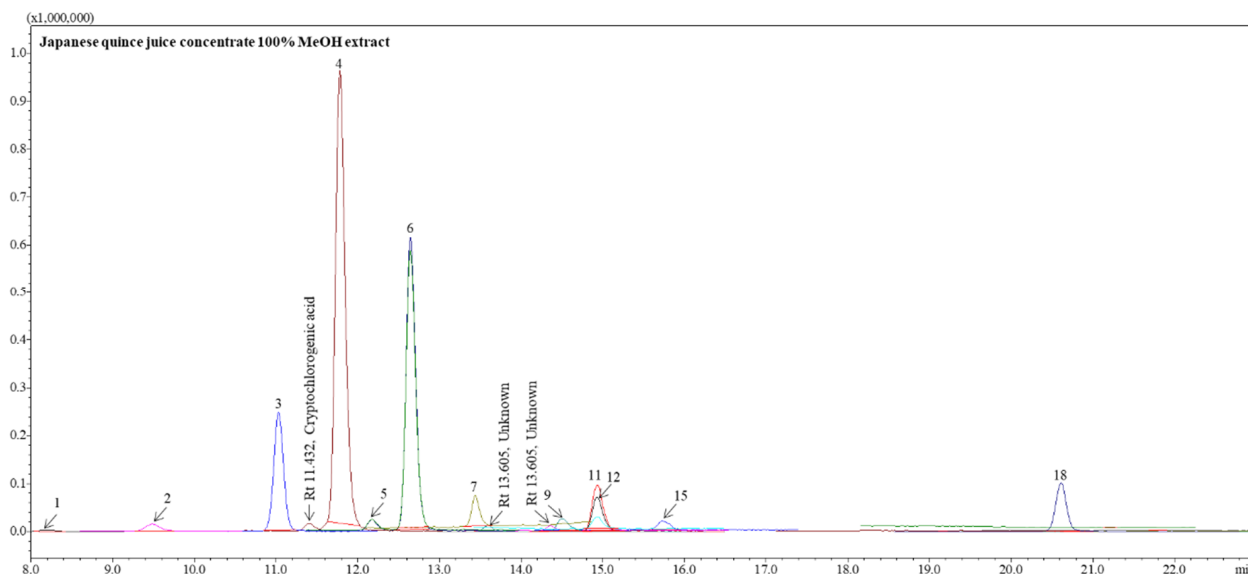


Figure 4. Extracted ion chromatogram (EIC) in multiple reaction monitoring mode represents the profile of major phenolic compounds detected in Japanese quince juice concentrate. Note: 1—Gallic acid; 2—Neochlorogenic acid; 3—Protocatechuic acid; 4—Chlorogenic acid; 5—(+)-Catechin; 6—(−)-Epicatechin; 7—Caffeic acid; 9—Quercetin-3-O-rutinoside (rutin); 11—Quercetin-3-O-galactoside (hyperoside); 12—Quercetin-3-β-glucoside (isoquercitrin); 15—Quercetin-3-O-rhamnoside (quercitrin); 18—Quercetin.

Due to the selective nature of LC-ESI-TQ-MS/MS in multiple reaction monitoring (MRM) and pre-programmed transition masses for known compounds, two peaks with retention times of 13.612 min and 14.367 min remained unidentified in the JQJC product. However, based on the main mass ions at 447, it is reasonable to speculate that these two peaks may correspond to quercetin rhamnoside glucoside I and II derivatives. The dominance of the above-mentioned phenolic compounds in JQ fruits grown at Beijing Botanical Garden, Beijing, China, was reported by Du et al. [22] and Antoniewska et al. [66]. Moreover, in the present study, cryptochlorogenic acid was tentatively identified in the JQJC sample by analyzing the primary mass ions and referencing previously published data, which facilitated the comparison of spectral characteristics for accurate identification [67,68]. The analysis demonstrated that the composition of the LJC consists of contributions of 74.6%, 16.8%, and 8.1% from hesperidin, eriocitrin, and quercetin-3-O-rutinoside, respectively (Figure 5). In the absence of reference standards, the former two compounds observed in the LJC were tentatively characterized based on the available literature and main mass ions, which correspond to m/z 301 and m/z 286, respectively [69,70]. Xi et al. [71] and Mateus et al. [72] documented a comparable profile of phenolic compounds, highlighting the predominance of hesperidin, with eriocitrin present to a lesser extent, across various morphological parts of the lemon. The presence of hesperidin and eriocitrin in LJC renders it both advantageous and essential, as these compounds are associated with numerous health benefits. Clinical research has indicated that supplementation with hesperidin may ameliorate metabolic abnormalities and inflammatory conditions in individuals diagnosed with metabolic syndrome [73]. Furthermore, it may act as a valuable adjuvant in preventing cardiovascular diseases [74]. Eriocitrin, a flavonoid derived from plants, has been identified in clinical studies for its role in promoting cognitive function and providing neuroprotective benefits [75]. Additionally, it has been shown to enhance metabolic health, facilitating improvements in insulin sensitivity and blood glucose regulation [76]. Preliminary clinical evidence also suggests that eriocitrin may contribute to digestive health by promoting a balanced gut microbiome and enhancing the functionality of digestive enzymes [77].

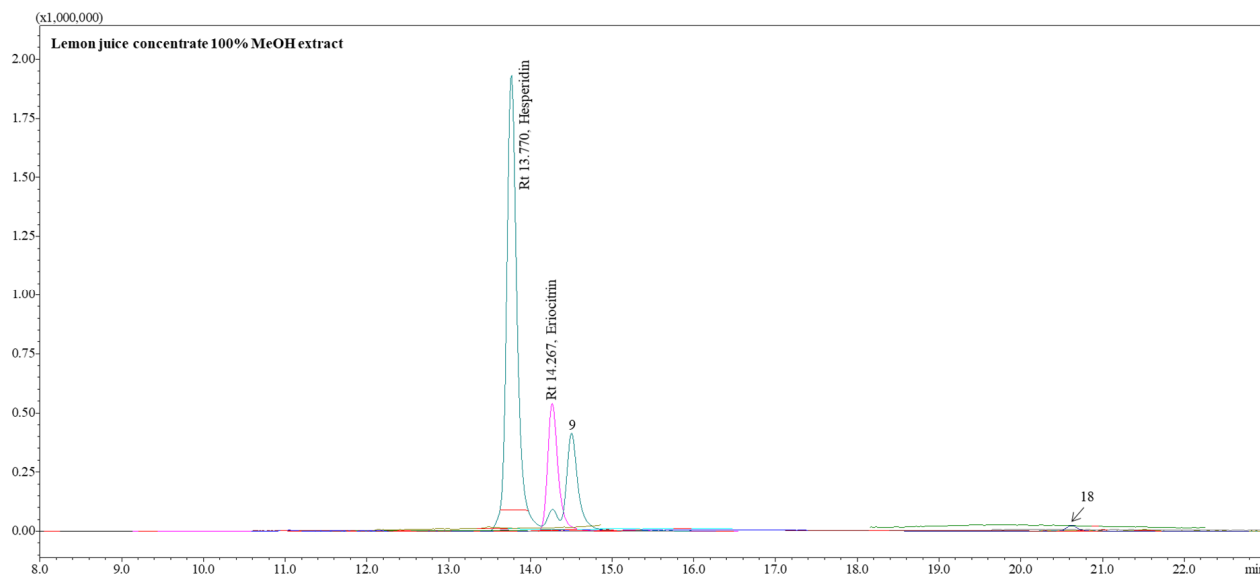


Figure 5. Extracted ion chromatogram (EIC) in multiple reaction monitoring mode represents the profile of major phenolic compounds detected in lemon juice concentrate. Note: 9—Quercetin-3-O-rutinoside (rutin); 18—Quercetin.

Although LJC exhibited a lesser diversity of phenolic compounds, the total content of individual phenolic compounds was nearly equivalent to that observed in JQJC, corresponding to $109.65 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$. The measured hesperidin amount is consistent with those reported by Marín et al. [46] for LJ derived from the Fino and Verna lemon varieties. However, it is significantly lower than the values observed by Lorente et al. [34] for Spanish LJ. In terms of eriocitrin, a value nearly two times higher has been reported by Peterson et al. [45]. The variation in the concentrations of biologically active compounds can be attributed to the additional stages involved in the production of LJ, specifically heat treatment and exposure to oxygen, which result in the degradation or modification of certain constituents. In the JQJC and LJC, the presence of side products such as organic acids, saccharides, dietary fiber, and other high-molecular-weight compounds can interfere with the accurate quantification of the compounds of interest. The 30% concentrate extracts underwent purification using SPE to address this issue. The column is packed with a hydrophilic-modified, styrene-based sorbent bearing both polar and non-polar functional groups [78]. This unique combination effectively retains and separates various analytes in diverse solvent systems. The sorbent surface properties can facilitate interactions such as hydrogen bonding and van der Waals forces, enhancing selectivity during the chromatographic process. The results indicate no significant differences ($p > 0.05$) in the concentration of phenolic compounds in the JQJC when assessing crude and purified extracts after SPE. This finding implies that the preparation technique does not impact the phenolic content in JQJC while concurrently minimizing the presence of side products, such as saccharides, in the final extract subjected to analysis by LC-ESI-TQ-MS/MS [41]. It is essential to highlight that applying a 30% MeOH solution affected the concentrations of hesperidin and eriocitrin in LJC. Due to the limited solubility of hesperidin and eriocitrin in aqueous solutions, a relatively lower recovery of these bioactives was obtained during extraction with the 30% MeOH solution [79,80]. The reduction in hesperidin and eriocitrin concentrations in LJC following SPE using 30% MeOH as the extraction and elution solvent amounted to 63.6% and 62.8%, respectively. In contrast, the concentration of rutin remained consistent regardless of the solvent employed. In our previous research, it was observed that the chemical structure of an antioxidant, rather than its concentration, played a more significant role in determining the product's ability to neutralize free radicals [81]. Oligomeric procyanidins and their monomeric counterparts, including catechin and epicatechin, are significantly responsible for the observed antioxidant activity [39,82,83]. These are the molecules previ-

ously identified in JQ fruit by Du et al. [22]. Performing a Pearson’s correlation analysis between the TPC and TFC compound groups and antioxidant activity (Table 5) revealed that all groups contribute similarly to antioxidant activity, as indicated by correlation coefficients approaching $r = 1$. However, a moderate correlation was also observed between total individual phenolic content and antioxidant activity, as determined by DPPH• and FRAP methods, corresponding to $r = 0.5576$ and $r = 0.5527$, respectively. This observation makes it possible to conclude that JQJC and LJC, in addition to already selectively determined individual phenolic compounds, are represented by other molecules associated with antioxidant activity. The spectrophotometric approach for analyzing the antioxidant activity of plant extracts rich in various micro and macromolecules is a somewhat reliable method. However, it only partially indicates constituents’ contribution to antioxidant activity. To gain a more comprehensive understanding of antioxidant activity and to clarify the relationship between individual phytochemicals and their capacities to neutralize free radicals, it would be beneficial to use complementary techniques, such as an online HPLC-DPPH• radical scavenging assay, as was demonstrated by Radenkovs et al. [39].

Table 5. Coefficients of Pearson’s correlation between total phenolics and flavonoids, and ascorbic acid and antioxidant activity, as determined by the DPPH• and FRAP methods.

Variable	TPC	TFC	DPPH•	FRAP	AA	TIP
TPC	1					
TFC	1	1				
DPPH•	0.9997	0.9996	1			
FRAP	0.9995	0.9995	0.9996	1		
AA	0.9999	0.9999	0.9994	0.9994	1	
TIP	0.5400	0.5364	0.5576	0.5527	0.5312	1

Note: TPC—total phenolics; TFC—total flavonoids; DPPH•—radical scavenging activity; FRAP—ferric reducing antioxidant power; AA ascorbic acid; TIP—total individual phenolics.

Overall, both juice concentrates demonstrated a high abundance of bioactive compounds. The comparative analysis of JQJC produced at LatHort reveals a remarkable diversity in the composition of these compounds, particularly those documented for their effectiveness in radical quenching activity and their associated health benefits [40,69,70]. The potential health advantages for consumers make JQJC a promising alternative to LJC; moreover, JQJC can improve the nutritional value of the end products it is added to, enhancing their nutritional profiles and contributing beneficial properties such as increased antioxidant activity. This feature makes JQJC a valuable ingredient for manufacturers aiming to develop healthier, more functional products for health-conscious consumers.

4. Conclusions

This study has successfully established the potential of Japanese quince juice concentrate (JQJC) as an innovative substitute for lemon juice concentrate (LJC). The comparative analysis revealed that JQJC has a higher total soluble solids (TSS) content and exhibits a remarkably enhanced nutritional profile, with greater ascorbic acid and antioxidant capacity. The antioxidant activity of JQJC is notably superior, showing nearly eleven times higher scavenging efficiency compared to LJC. Further, the total phenolic content of JQJC substantially ($p < 0.05$) exceeds that of LJC, indicating its more abundant composition of antioxidant-associated compounds, which can be leveraged for various health benefits. The presence of numerous phenolic compounds, particularly epicatechin, chlorogenic, and protocatechuic acids, proposes that JQJC could serve as a worthwhile functional ingredient in the food industry and could contribute positively to overall human health. Its advantageous properties make it suitable for beverages, jams, and other food products aimed at health-conscious consumers. The findings advocate for integrating JQJC into commercial applications as a viable alternative to LJC. This could facilitate the utilization of a relatively underexplored fruit, enhancing product innovation while catering to the

growing demand for natural and health-promoting food ingredients. Future research endeavors should examine the sensory attributes associated with consumer acceptance of JQJC and products formulated with JQJC to validate its commercial viability further. Additionally, evaluating the long-term storability of JQJC will be essential in determining its practicality and commercial potential within the food industry.

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