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Enriching the Bioactive Components and Antioxidant Capacity of Concentrated Lime Juices Prepared by Cryogenic and Vacuum Processes

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Abstract: Lime juice is rich in bioactive components and exerts a wide range of therapeutic effects, especially antioxidant activity. Freeze concentration is considered an essential method to maintain the nutritional values and bioactives of fruit juices. This study aimed to compare the ability in enriching ascorbic acid, total phenolic compounds, and major flavonoids and the antioxidant activity (DPPH) of concentrated lime juices prepared by vacuum and freeze-concentrations. The ascorbic acid in the juices was analyzed using the HPLC-PDA method. The total phenolic content and DPPH inhibition ability were measured by the colorimetric method. The polyphenol profiles of two lime varieties (*C. latifolia* and *C. limonia*) were qualitatively analyzed using LC-TOF MS/MS; then, the major juices' flavonoids were analyzed by HPLC-PDA against/based on commercial standards. The results showed that *C. latifolia* was superior to *C. limonia* in ascorbic acid, TPC, major flavonoids, and antioxidants. *C. latifolia* was also more diverse than *C. limonia* in its polyphenol profile through the identified compounds (18 vs. 15). Freeze-concentrated lime juices were significantly higher than the vacuum-concentrated counterparts in ascorbic acid (mean difference from 9.41 to 22.01 mg, $p < 0.01$), and TPC (from 60.76 to 149.88 mgGAE). The quantification of major flavonoids showed that the freeze-concentrated lime juices retained high levels of hesperidin, eriocitrin, and rutin ($p < 0.01$) whereas the vacuum concentration preserved higher ones in diosmin and naringin ($p < 0.01$). The freeze-concentrated lime juice was significantly higher than vacuum-concentrated lime juices in the DPPH scavenging activity by at least 15% ($p < 0.01$). Overall, freeze concentration enriched bioactive compounds in lime juices almost threefold and improved antioxidants at least twofold. Thus, freeze concentration is promising for the industry in producing high-quality lime juice to preserve its thermal liable bioactive component.

Keywords: freeze concentration; vacuum concentration; lime juice; antioxidant; flavonoid; bioactive

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1. Introduction

World lime fruit production reached 20.529 thousand tonnes in 2019 and is still growing [1]. Vietnam is ranked as the fifth largest lime producer in Asia with a production of 366 thousand tonnes [1]. *Citrus latifolia* (*C. latifolia*) and *Citrus limonia* (*C. limonia*) are the two most common lime varieties in Me Kong Delta, Vietnam, which contribute approximately 60 thousand tonnes of fruits annually [2]. However, studies regarding the bioactive components and antioxidant capacity of these varieties are very limited. Consumption of lime and concentrated lime juices potentially offers a wide range of health benefits such as anti-oxidative, anti-inflammatory, antimicrobial, and anticancer activities [3,4]. Lime fruit juice is essential for beverage processing due to its nutritional and flavor attributes [5,6]. Regarding nutritional value, lime juice is not only rich in vitamin C but also other phytochemicals such as phenolic compounds and flavonoids [7,8]. Lime and lemon juices are particularly high in flavonoids (e.g. hesperidin, eriocitrin, rutin, and diosmin) [9,10], but flavonoid changes during juice processing have been rarely reported. Concentration not

only serves for extending the shelf-life, and saving storage and transport costs, but also for ensuring juice quality that is suitable for the food industry. For lime juice, the concentration method is required to retain the natural organoleptic properties and nutritional values of the juice. Although conventional evaporation is the most commonly used, it may cause the undesirable thermal degradation of flavors and bioactives [11,12]. Vacuum concentration using mild heat for water removal is an alternative technique for reducing phytochemical loss. Disadvantages involving this process may include thermal change of organoleptic and rheological characteristics [13,14]. However, the effects of this kind of evaporation on applying lime juice concentrate's bioactives have not been fully investigated yet. The membrane technique used for concentration is considered a nonthermal process that can protect the nutritional and bioactive components of applied food liquids. The technique has its disadvantages such as a reduction in low molecular weight aroma compounds resulting in flavor changes [15] or membrane fouling and incrustation [16]. Freeze concentration is an emerging technique of removing water from the liquid at a subzero temperature and thus effectively protecting the juices' quality from thermal degradation [17,18]. Furthermore, another advantage of freeze concentration is that it is nonselective in concentrating juice components and thus almost retains the profile of juice [15]. Freeze concentration has been proven to retain high vitamin C, polyphenols, flavonoid contents, and the antioxidant capacity of various liquid foods such as the orange, blueberry, and apple [19–21]. The concerns of this method would relate to capital cost and solid recovery [20]. However, there are very limited studies focusing on the freeze concentration of lime juice. Thus, this study aimed to investigate the ability of enrichment in the ascorbic acid, total phenolic content (TPC), flavonoid, and antioxidant capacity of lime juices from two varieties, *C. latifolia* and *C. limonia*, by freeze concentration in comparison with heat evaporation, i.e., vacuum concentration.

2. Materials and Methods

2.1. Chemicals

HPLC grade chemicals were purchased from local suppliers with brand names as follows: rutin and naringin HPLC grade were from Sigma (Saint Louis, MO, USA), eriocitrin from Toronto Research Chemical Inc. (Toronto, ON, Canada), hesperidin and diosmin from MilliporeSigma (ThermalFisher Scientific, MA, USA), and ascorbic acid and glucose from Scharlau (ExpertQ ACS, Barcelona, Spain). Folin–Ciocalteu's phenol reagent, gallic acid, acetonitrile, and ortho-phosphoric acid (Millipore, Merck, Taufkirchen Germany), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma, Saint Louis, MO, USA) were also used. Other chemicals were analytically graded.

2.2. Juice Preparation

Lime fruits were harvested at the mature stage from 6-year-old orchards at Thanh Loi Ward, Ben Luc District, Long An province, Vietnam. Lime fruits that were mature, uniform in size, and free of defects were then transported to the laboratory within 4 h. Lime fruits were washed twice with tap water, drained, and then peeled. The peeled lime fruits were equatorially cut into half and transferred into a polypropylene bag (1.2 mm thick). The bag was placed inside a perforated inox cylinder (200 mm diameter, height of 300 mm). The juice was extracted by a hydraulic press, using a piston speed at 0.4 m/min, yielding approximately 40% (w/w, based on fresh fruit weight). The juice was then passed through a 300-micron sieve to eliminate fruit pulp. The juice (original juice, OJ) was then subjected to freeze-concentration, vacuum concentration, or frozen at −18 °C for further analyses.

2.3. Freeze Concentration

Lime juices were freeze concentrated as a mode of progressive freeze concentration in combination with partial ice melting as suggested by Miyawaki, et al. [22] with some modifications. The pilot freeze concentrator (CC-V2, Khang Thinh Technical Corp., Ba Ria-Vung Tau, Vietnam) includes two freezing chambers. For each chamber, six inox

pipes (11 mm diameter, 1350 mm length) filled with R404 coolant inside were curvely organized, making a heat transfer surface area of 0.28 m^2. The inner coolant temperature was monitored at −27 °C. The liquids from two chambers were pumped using a controlled solenoid valve. For each chamber, the liquid was mixed by a circulated pump operating at the speed of 5 L/min. For freeze concentration, a process of four stages was applied to achieve a sufficiently high juice concentration. In the first stage, ten liters of precooled lime juices (9.1% soluble solid) at 5 °C were fed into one chamber and frozen for 30 min; then, the unfrozen liquid (71.8% solid yield) was pumped into another chamber. The ice on the wall of the inox pipes was partially melted at 25 °C and an initial 15% (by weight) ice melting fraction (23.5% solid yield) was collected and pooled with the unfrozen liquid, yielding a juice concentrate 1 (FCJ1) with a 17.9% soluble solid and a 95.3% solid recovery. In the second stage, the procedure was repeated with juice concentrate 1 as a feeding solution. The unfrozen liquid (69.1% yield) and 30% of the initial ice melting fraction (22.5% yield) were collected and combined as juice concentrate 2 (FCJ2), characterized by a 22.9% soluble solid and a 91.6% solid recovery. The juice concentrate 2 was used as a feed solution for stage 3 concentration. The liquid of the 65.1% yield and 40% initial ice melting fraction (25.9% yield) was mixed to obtain the freeze-concentrate 3 (FCJ3) with a 28.4% soluble solid and a 86.7% solid recovery. At the final stage, freeze concentrate 3 was fed and frozen. The unfrozen fraction accounted for a 53.4% yield and was combined with a 45% initial ice melting fraction (25.8% yield), resulting in juice concentrate 4 (FCJ4) with a 34.1% soluble solid and a 79.2% solid recovery. During the process, the soluble solid of juices was measured using a digital refractometer (PAL-BX/ACID101, Atago, Tokyo, Japan) and adjusted for the acid content according to the suggestion by Kimball [23].

2.4. Vacuum Concentration

Lime juices were vacuum-concentrated by using a pilot vacuum concentrator. The apparatus was initially operated to achieve a vacuum pressure of 0.184 bar at 60 °C. Then, 14 L of lime juice was sucked into the chamber. For comparison, the juice concentrates were collected in accordance with the freeze-concentrated juice soluble solids. The soluble solid of juices was measured and adjusted for the acid content following guidelines from Kimball (2012) as aforementioned. The vacuum juice concentrate 1 (VCJ1, 17.9% soluble solid) was collected into 50 mL centrifuge tubes, after 95 min of evaporation, and immediately placed in an ice bath. The vacuum juice concentrates 2 (VCJ2, 22.9% soluble solid), 3 (VCJ3, 28.4% soluble solid), and 4 (VCJ4, 34.1% soluble solid) were collected at 120, 128, and 134 min, respectively, of the evaporation process. Chilled juices were then kept at −18 °C for further analyses.

2.5. Ascorbic Acid Analysis

A modified protocol suggested by Uckoo, et al. [24] was used to measure the ascorbic acid. Concentrated lime juices were diluted 10 times with 3 mM ortho-phosphoric acid and then filtered into amber sample vials. The elution of ascorbic acid using 3 mM ortho-phosphoric as the mobile phase, and the Inersil C18 column (ODS 3, Tokyo, Japan) as the stationary phase were used. The Shimadzu LC-20AD system coupled with a pump, PDA detector, degassing unit, and controller (SIL-20A, SPD-20A, DGU-20A, and CBM-20Alite, respectively, Kyoto, Japan) was employed with an isocratic flow of 1 mL/min. Ascorbic acid was detected at the 254 nm wavelength.

2.6. TPC Analysis

TPC was measured based on the modified protocol described by Saikia, et al. [25]. Phenolic compounds in the 80% methanol extract from lime juices were subjected to a color reaction with the Folin–Ciocalteu 10% solution. The absorbance of the color intensity was measured at 765 nm using a spectrophotometer (Jenway 7305, Bibby Scientific, Loughborough, England). The TPC was expressed as mgGAE/100 mL of juice.

2.7. DPPH Assay

The radical (1,1-diphenyl-2-picrylhydrazyl, DPPH) scavenging capacity of lime juices was measured based on a protocol described by Xu, et al. [26] with some modifications. The 0.5 mL lime juice extracts (with methanol 80%, 1:1 v/v ratio) were mixed with 3.5 mL of DPPH (100 µM). The mixture was then incubated in the dark at a temperature of 35 °C for 30 min. The absorbance of mixtures was then measured at the wavelength of 517 nm. The DPPH inhibition (%) = $(A_c - A_j)/A_c \times 100$, where A_j was the juice sample absorbance, and A_c was the absorbance of control.

2.8. Flavonoid Analysis

For the qualification analysis, polyphenols and flavonoids in the original lime juices were analyzed using the LC-MS/MS method. Chromatography-mass spectrometry (LC-MS) ExionLCTM-X500R QTOF (AB Sciex, Framingham, MA, USA) and C18 column (LUNA 150 × 4.6; 3 µm, Phenomenex, Torrance, CA, USA) were employed. The electrospray ionization source, probe temperature of 400 °C, negative mode, and scanning m/z from 50 to 1000 were chosen for the operation. Mobile phases were acidified deionized water (A) and acetonitrile (B) with 0.1% formic acid. A flow rate of 0.5 mL/min was used with a gradient program as follows: 0–1 min: 2% B; from 1–20 min: B increased from 2 to 98%, kept for 5 min; 26–27 min: B decreased to 2%, kept for 5 min.

For quantification, a modified procedure suggested by Zhang, et al. [27] was used. The lime juice was extracted with 80% (v/v) methanol in an ultrasonic bath for 15 min, then chilled and centrifuged (5000 rpm, 10 min). The supernatant was passed through a 0.45 µm PTFE syringe filter before being subjected to a HPLC analysis. The aforementioned Shimadzu LC-20AD and reverse phase C18 (Inersil ODS 3, Tokyo, Japan) were used. Formic acid (0.1%, mobile phase A) and acidified acetonitrile (with 0.1% formic acid, mobile phase B) were used as mobile phases. A gradient mode with a total flow rate of 1 mL/min was as follows: in the first 20 min, mobile phase B was kept at 20% then increased to 30% in the next 10 min. The composition remained for 10 min, followed by an increase in mobile phase B to 50% in 5 min, and kept for 7 min. From 52–55 min: 70% B; 55–65 min: 70% B. Mobile phase B decreased to 20% in 5 min, and was kept for 10 min before starting another analysis. The chromatogram was acquired at the wavelength of 280 nm.

2.9. Statistical Analyses

The analysis of variance, Tukey's HSD comparison, and paired t-test were analyzed by using JMP 10.0.0 (SAS Institute Inc.; Cary, NC, USA) with a significant level of at least 95% ($p < 0.05$). Graphs were plotted using GraphPad Prism 8.01 (GraphPad Software Inc.; San Diego, CA, USA).

3. Results and Discussion

3.1. Bioactive Components of Original Lime Juices

3.1.1. Ascorbic Acid and Total Phenolic Content

Table 1 shows the quantitative bioactive components of lime juices from *C. latifolia* and *C. limonia* varieties. The ascorbic acid in *C. latifolia* juice was twofold higher than that of *C. limonia* juice (30.43 vs. 14.39 mg/100 mL). The ascorbic acid of *C. latifolia* juice was higher than that of the same lime variety reported in previous studies [7,28]. The ascorbic acid content of *C. limonia* in this study was comparable to that reported in the literature [29]. The ascorbic acid contents of lime fruits may be present at different levels depending on the lime variety. The ascorbic acid content was found in *Citrus aurantifolia*, *Citrus histrix*, and *Citrus microcarpa* at the level of 29.5, 21.58, and 16.78 g/100 mL, respectively [30,31]. The total phenolic content (TPC) of *C. latifolia* was also much higher than that of *Citrus limonia* which were 318.2 mgGAE/100 mL and 114.7 mgGAE/100 mL, respectively. The TPC of *C. latifolia* juice was higher than the reported level of 260 mgGAE/100 mL [32]. The TPC contents of citrus juices were different amongst varieties [8,33], possibly explaining the variation in the TPC of lime juices found in this study.

Table 1. Bioactive components of two original lime juices.

Parameters	Lime Variety	
	C. latifolia	*C. limonia*
Ascorbic acid (mg/100 mL)	30.43 ± 0.13 [a]	14.39 ± 0.83 [b]
TPC (mg GAE/100 mL)	318.20 ± 6.10 [a]	114.70 ± 4.38 [b]
DPPH inhibition (%)	44.58 ± 2.50 [a]	25.35 ± 3.20 [b]
Flavonoids (mg/100 mL)		
Hesperidin	23.72 ± 0.36 [a]	4.72 ± 0.21 [b]
Eriocitrin	12.78 ± 0.68 [a]	2.97 ± 0.16 [b]
Rutin	2.65 ± 0.07 [a]	nd
Diosmin	1.36 ± 0.08 [a]	0.69 ± 0.04 [b]
Naringin	0.91 ± 0.01 [a]	0.36 ± 0.01 [b]

Data are expressed as mean ± SD of triplicates. Different superscript letters in the same row indicate a significant difference ($p < 0.05$) between the bioactive components of *C. latifolia* and *C. limonia* juices. nd: non-detected.

3.1.2. Flavonoids of Lime Juices

The LC-MS/MS analysis of two lime juice varieties focusing on the bioactive components was conducted and the results are presented in Table 2. In the *C. latifolia* and *C. limonia* juices, six flavanones were identified based on the behavior of mass spectroscopy $[M-H]^-$ and ion fragmentation. The compound of peak 15 with a *m/z* ($[M-H]^-$) of 595.1654 and fragment of 287.0552 matched the identification of eriocitrin [34,35]. The peak 18 yielded the $[M-H]^-$ ion at 579.1709 and the MS/MS ion fragment at 271.0632 was typical for naringin, as reported in the literature [34,36]. The parent ion *m/z* of 609.1608 seen in peak 19 with product ions of 301.725 was in accordance with a loss of rhamnose and glucose residues [36,37], and thus the compound was identified as hesperidin. The *m/z* $[M-H]^-$ ion at 287.0559 with a product ion of 151.0049 observed in peak 26 was assigned for eriodictyol flavanone [38,39]. Peak 31 and 33 at *m/z* 271.0589 and *m/z* 301.0712 were dedicated as naringenin and hesperetin, respectively, as in earlier reports [34,37,40]. The MS/MS spectrum of eriocitrin, naringin, and hesperidin commercial standards matched the characteristics of ion fragments (Figures S1 and S2), confirming the authenticity.

Table 2. Spectrometric data of identified compounds in original lime juices of *C. latifolia* and *C. limonia*.

No.	Retention Time (min)	$[M-H]^-$ *m/z*	Ion Fragment	Compounds	Chemical Class	*C. latifolia*	*C. limonia*
1	1.35	191.0576	127.0422	Quinic acid	Organic acid	+	+
3	2.27	289.0703	247.0231	(+)-Catechin	Flavanol	+	+
6	2.78	353.0912	191.0579	Chlorogenic acid	Phenolic acid	+	+
11	9.04	433.0421	243.0881	Elllagic acid-*O*-arabinoside	Polyphenol	+	–
15	10.80	595.1654	287.0552	Eriocitrin	Flavanone	+	+
16	10.85	609.1426	301.0725	Rutin	Flavonol	+	–
17	10.91	593.1539	285.0401	Kaempferol-3-*O*-rutinoside	Flavonol	+	–
18	10.95	579.1709	271.0632	Naringin	Flavanone	+	+
19	11.12	609.1806	301.0725	Hesperidin	Flavanone	+	+
21	11.68	607.1643	299.0566	Diosmin	Flavone	+	+
23	12.01	461.0711	285.0428	Kaempferol-3-*O*-glucuronide	Flavonol	+	+
26	12.48	287.0559	151.0049	Eriodictyol	Flavanone	+	+
31	13.55	271.0589	161.0482	Naringenin	Flavanone	+	+
33	14.22	301.0712	164.9288	Hesperetin	Flavanone	+	+
35	14.78	285.0412	151.0056	Luteolin	Flavone	+	+
49	19.24	623.1626	315.0709	Isorhamnetin-3-*O*-rutinoside	Flavonol	+	+

(+) shows detection and (−) shows the absence in juices. Chromatograms can be found in Figure S1.

The second largest group of identified compounds in lime juices was flavanols. Peak 3 with typical ion fragmentation yielded the $[M-H]^-$ ion at *m/z* 289.0703 and the product ion at 127.0422 was recognized as the catechin flavanol [41]. Catechin, a secondary metabolite of plants, was available in both of the two lime varieties. Peak 16 showed the $[M-H]^-$ ion at 609.1426 and the ion fragmentation behavior at *m/z* 301.0725 due to the loss of $C_{12}H_{20}O_9$ as [M-H-308 (rutinose)]$^-$ and thus, the compound was identified as rutin [42]. In the present

study, this flavonol was found in *C. latifolia* but not in *C. limonia* juice. The typical ion fragment $[Y]^-$ at *m/z* 285 of kaempferol derivative of compounds at peak 17 and peak 23 was due to the loss of $C_{12}H_{20}O_9$ and $C_6H_8O_6$, respectively. Thus, the compounds were identified as kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucuronide, respectively. The identification of these compounds is in agreement with a report by Kumar, et al. [41]. Kaempferol-3-*O*-glucuronide was observed in all lime varieties whereas kaempferol-3-*O*-rutinoside was only available in *C. latifolia*. The $[M-H]^-$ ion at *m/z* 623.1626 and the fragmented ion 315.0709 were also identified as isorhamnetin-3-*O*-rutinoside based on the characteristic ion fragment reported in the literature [43,44]. Two flavones were identified as diosmin (peak 21, $[M-H]^-$ *m/z* 607.1643, product ion of 299.0566) and luteolin (peak 35, $[M-H]^-$ *m/z* 285.0412, product ion of 151.0056) because of their distinctive ion fragmentation, as reported [34,41,45]. The diosmin MS/MS spectrum of the commercial standard was also analyzed and compared to confirm its presence in lime juices (Figure S2).

In the present study, quinic acid (peak 1, $[M-H]^-$ *m/z* 191.0576, fragmented ion of 127.0422) and phenolic acids, such as chlorogenic (peak 6, $[M-H]^-$ *m/z* 353.0912, fragmented ion of 191.0579) and ellagic acid-O-arabinoside (peak 11, $[M-H]^-$ *m/z* 433.0421, fragmented ion of 243.0881), were also detected in lime juices. In general, the order of abundant flavonoids found in this study was flavanones > flavonols > flavones. The flavonoid profile of lime juices in this study is in agreement with findings reported in sweet lime (*Citrus limetta*) [9] and in lemon (*Citrus limon*) [46] juices. The qualitative analysis results also revealed that the flavonoids of *C. latifolia* juice were more diverse compared to that of *C. limonia*. Hesperidin, eriocitrin, and diosmin have been extensively reported as major flavonoids of lime juice [9,47,48]. Lime juices also contain important flavonoids such as naringin and rutin [46,49–51]. In this present study, hesperidin, eriocitrin, rutin, diosmin, and naringin of lime juices were quantitatively analyzed by HPLC-PDA (Figure S3) and the results are presented in Table 1. Hesperidin was predominantly found in two lime varieties; however, the amount in *C. latifolia* juice was more than fivefold higher than that of *C. limonia* juice (23.72 vs. 4.72 mg/100 mL). The amount of hesperidin in *C. latifolia* and *C. limonia* freshly extracted juices is rarely reported. Hesperidin content in the seedless lime (*C. latifolia*) in the present study is quite comparative with previous studies. Hesperidin in lemon (*Citrus limon*) ranged from 10.55 to 21.03 mg/100 mL [46]. In another study, hesperidin was detected in sweet lime (*Citrus limetta*) at a level from 29.05 to 31.67 mg/100 mL [9]. In the commercial lime juices of *C. latifolia*, the amount of hesperidin ranged from 2.8 to 5.6 mg/100 mL [48], much lower than the amount found in this study. Eriocitrin was the second-highest component that was quantified in lime juices. The amount of this flavanone in *C. latifolia* juice was 12.78 mg/100 mL, which was more than 4 times higher than in *C. limonia* juice (2.97 mg/100 mL). Eriocitrin was reported in lemon juices (*Citrus limon*) at the level ranging from 11 to 16.4 mg/100 mL [10]. Eriocitrin contents of lemons were diverse, depending on varieties and country origins. Eriocitrin was also reported in the range of 0–3.35 mg/100 mL for edible fruit or juice in the Key lime (*Citrus aurantifolia*) [52]. This flavanone was detected in sweet lime juice in the range of 1.15 to 1.62 mg/100 mL [9]. Rutin was found in *Citrus latifolia* juice at the level of 2.65 mg/100 mL. However, this flavonol was not detected in the juice of the *C. limonia* lime. Diosmin were quantified in *C. latifolia* and *C. limonia* juices at the levels of 1.36 and 0.69 mg/100 mL, respectively. Naringin in *C. latifolia* juice was almost three times higher than in *C. limonia* juice (0.91 vs. 0.36 mg/100 mL).

3.1.3. DPPH Scavenging Capacity of Lime Juices

The anti-oxidative capacity of original lime juices was evaluated by measuring the inhibition of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. *C. limonia* juices inhibited DPPH at the level of 25.35%, whereas the inhibition rate of *C. latifolia* juice was almost double (44.58%), indicating a stronger anti-oxidative capacity of *C. latifolia* juice. Higher ascorbic acid, TPC, and flavonoid content found in *C. latifolia* juice possibly caused the profound anti-oxidative effects. The scavenging activity of *C. laftiolia* juice was comparable

to that reported in a previous study of antioxidants of the same lime variety [51]. The DPPH inhibitory activity of *C. latifolia* juice, however, was higher than other varieties such as lemon (*Citrus limon*, 22.67–24.5%) [26,53] or kasturi lime (*Citrus microcarpa*, 14.59%) [54].

3.2. Changes in Ascorbic Acid of Lime Juices during Vacuum and Freeze Concentrations

The concentration methods strongly affected the cumulation and retention of ascorbic acid in concentrated lime juices. The vacuum evaporation increased the ascorbic acid in lime juices from 30.43 to 61.08 mg/100 mL in *C. latifolia* and from 14.25 to 28.74 mg/100 mL in *C. limonia* (Figure 1A,B). However, a significant downtrend in ascorbic acid retention along with an increase in the juice concentration was observed. At the concentration of 17.8% solid, *C. latifolia* and *C. limonia* retained 70.64 and 75.51% of ascorbic acid, respectively. The ascorbic acid retention dropped by almost half when found in both lime juices at the highest concentration of 34.1% soluble solids (Figure 1E,F). The reduction in ascorbic acid during heat evaporation has been widely reported. The ascorbic acid of orange juice was significantly reduced by thermal vacuum evaporation [55]. Other thermal processes such as conventional and microwave heating and sterilization also greatly lose the ascorbic acid content of broccoli and tomatoes [56,57]. In the present study, heat exposure of lime juices at 60 °C for more than 2 h during the vacuum evaporation process is more likely to cause a degradation in ascorbic acid and thus, reduce the retention.

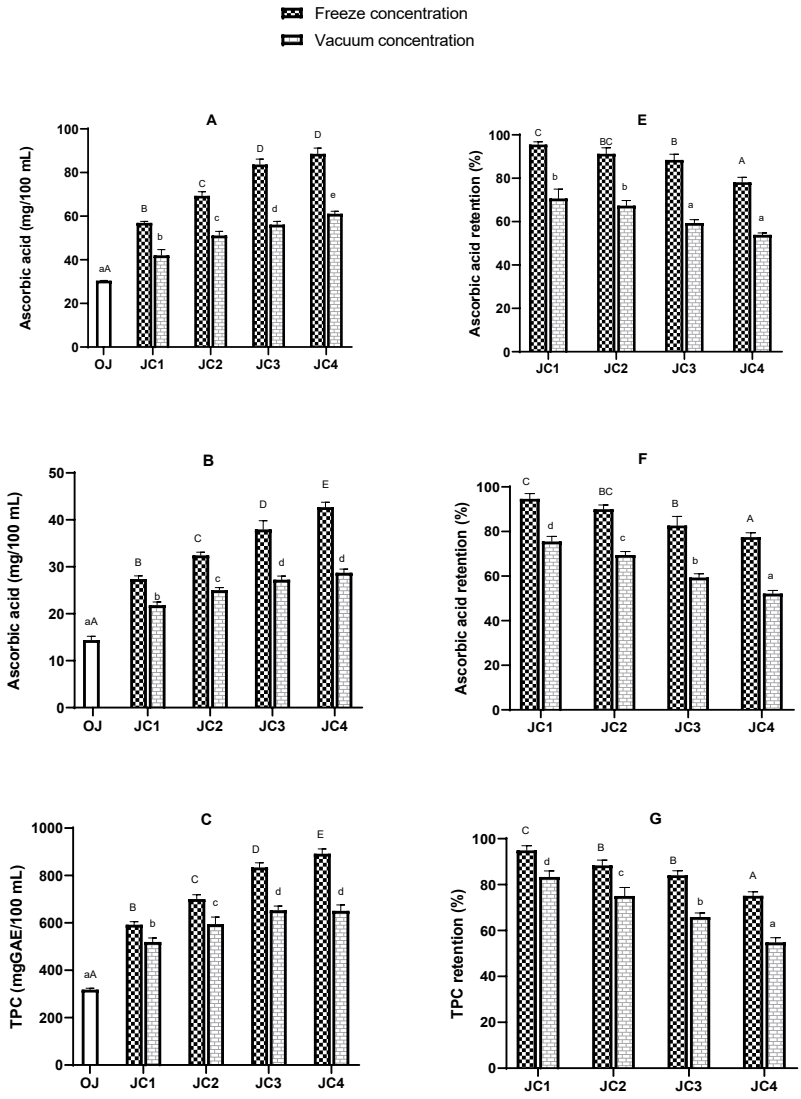

Figure 1. *Cont.*

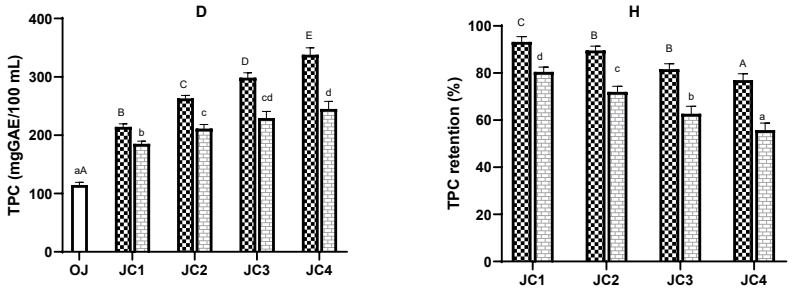

Figure 1. Changes in ascorbic acid, TPC contents, and retention (**A**,**C**,**E**,**G** in *C. latifolia*; **B**,**D**,**F**,**H** in *C. limonia*) during freeze and vacuum concentrations. The values are expressed as mean ± SD of triplicates. Different uppercase letters indicate a significant difference ($p < 0.05$) between freeze-concentrated juices. Different lowercase letters indicate a significant difference ($p < 0.05$) between vacuum-concentrated juices. OJ: original juice, JC: juice concentrate.

Freeze concentration greatly improved the ascorbic acid contents of lime juices. The ascorbic acid of concentrated juices increased almost threefold, from 30.43 to 88.61 mg/100 mL (in *C. latifolia*, Figure 1A) and from 14.25 to 42.70 mg/100 mL (in *C. limonia*, Figure 1B). On average, freeze concentration preserved about 88% of ascorbic acid in the lime juices. The ability to retain ascorbic acid in freeze-concentrated juices was reported in the literature. The freeze-concentrated orange juice had a high retention of vitamin C [17,21,58]. Ascorbic acid is heat sensitive and was protected from the low heat processing of freeze concentration. In comparison, ascorbic acid concentrations in freeze-concentrated *C. latifolia* juice (74.63 mg/100 mL) were significantly higher than in the vacuum-concentrated juice (52.63 mg/100 mL) ($t(11) = 12.0289$, $p < 0.01$) with a difference of 22.01 mg (95% CI, 17.98 to 26.03). A similar trend of higher ascorbic acid in freeze-concentrated *C. limonia* juice was also observed. The findings indicate that freeze-concentration is higher in the ability to enrich the ascorbic acids of lime juices compared to vacuum-concentration.

3.3. Changes in Total Phenolic Content of Lime Juices during Vacuum and Freeze Concentrations

Regarding the total phenolic content of the juice, vacuum evaporation caused a significant reduction in retention. *C. latifolia* lime juice lost 16.7 to 45.2% of total phenolics along with an increase in juice concentration (Figure 1G). The TPC retention of *C.limonia* juice dropped from 19.4 to 44.3% during the vacuum concentration process (Figure 1H). Though the retention was reduced, the total phenolic content of juices at 30 Brix was double that of the original juices. Thermal processing potentially caused a decrease in the phenolic content (650 mgGAE/100 mL in *C. latifolia* and 244.89 mgGAE/100 mL in *C. limonia*). The reduction in phenolic content was observed in grapefruit juice treated at 80 °C for 91 s [59]. Similarly, Darvishi, et al. [60] found that conventional vacuum heating caused a 49.6% reduction in the phenolic content of orange juice. Likewise, vacuum evaporation at 65 °C also caused a significant decrease in the phenolic content of concentrated sour cherry juice [61]. The mild heat treatment under vacuum conditions applied for the evaporation process still caused a reduction in bioactives, such as ascorbic acid and the phenolic content of lime juices, as observed in this study.

Changes in the phenolic content of lime juices during freeze concentration were investigated. The TPC of freeze-concentrated lime juices significantly increased ($p < 0.05$) in accordance with an increase in juice solids (Figure 1C,D). The level of TPC in *C. latifolia* juices was from 318.20 to 891.93 mgGAE/100 mL, causing a TPC retention range from 75.13 to 94.94% (Figure 1G). TPC in *C. limonia* juice ranged from 114.70 to 337.97 mgGAE/100 mL and the TPC retention was from 76.99 to 93.22% (Figure 1H). An improved TPC of lime juices in this study is concordant with that reported in the freeze concentration of mate extracts [62], blueberry [19,63], and orange juices [21]. The paired *t*-test analysis showed that the TPC of freeze-concentrated juice was significantly higher ($p < 0.0001$) than that of vacuum-concentrated juice (mean difference of 149.88 and 60.76 mgGAE found in *C. laftiola* and *C. limonia* juices, respectively). In freeze concentration, the thermal degradation was

bypassed and the phenolic components were protected, as previously mentioned in the literature [19,64].

3.4. Changes in Flavonoids of Lime Juices during Vacuum and Freeze Concentrations

Changes in lime juices' flavonoids during vacuum evaporation were analyzed and the results are presented in Table 3. Eriocitrin of *C. latifolia* juice initially increased to 20.12 mg/100 mL when the juice was concentrated up to 22.5%. The content of this flavonoid then gradually decreased and reached 16.30 mg/100 mL. The calculated retention based on the dried matter showed that eriocitrin in *C. latifolia* juice rapidly degraded, resulting in a low retention of 34.13%. The vacuum evaporation resulted in a slight increase in eriocitrin content ($p < 0.05$) of 34.1% *C. limonia* juice compared to that of the original juice. However, at the same juice concentration, the eriocitrin retention was found only at 45.47%. Flavonoids were reported to be unstable in the neutral solution of pH 7.0 but more stable at the low pH environment [10]. The lime juices in this study with a low pH value of approximately 2.0 are unlikely to contribute to the degradation of flavonoids. Eriocitrin degradation is possibly related to heat sensitivity. Patrón-Vázquez, et al. [65] found that eriocitrin in lemon waste was reduced from 23.17 to 11.59 mg/g when air dried at 60 °C. At a temperature higher than 90 °C, this flavanone was completely lost. The duration of heat treatment appears to affect the eriocitrin retention since the heat treatment of lemon peels at 30 min reduced the eriocitrin content almost eightfold [66]. Rutin only found in the juice of *C. latifolia* was also affected by the heat treatment of vacuum evaporation. Rutin content in this lime juice dropped from 4.08 to 3.80 mg/100 mL, though the concentration increased from 17.9 to 34.1% for the soluble solid. The retention of rutin along with changes in the juices' solid were reduced from 78.18 to 38.39%. Rutin degradation in this present study was observed when lime juice was treated at 60 °C, and rutin content in lemon peel extracts was reduced by 50% after vacuum-drying at 40 °C for 6 h [67]. In another study, rutin in lemon decreased from 158.3 to 72.4 mg/100 mL when subjected to air drying at a temperature of 50 °C [68]. Chaaban, et al. [69] reported that rutin was heat sensitive and was greatly reduced when exposed to heat treatment at a temperature higher than 70 °C. Naringin in lime juices was found to be more stable in vacuum-concentrated processing. Naringin loss was less than 10% and insignificantly differed ($p > 0.05$) along the evaporation process of two lime juice varieties. The thermal stability of naringin was reported in the literature. Naringin degradation was less than 5% when exposed to a heat treatment of 100 °C [69,70]. The mild heat in vacuum evaporation, in this study, exerted a minor impact on the degradation of naringin, resulting in high retention in the concentrated lime juices. A minor effect of vacuum evaporation on the diosmin in concentrated lime juices was also observed. Diosmin was retained in the highest concentrated *C. latifolius* and *C. limonia* juices at the level of 4.45 and 1.99 mg/100 mL and contributed to the retention of 87.67 and 85.65%, respectively. The heat stability of diosmin has been reported.

Diosmin in immature calamondin peels exposed to heat at the temperature of 150 °C for 1.5 h was not degraded [71]. The treatment mild temperature of 60 °C, in this study, was possibly inadequate to cause significant degradation of diosmin during processing, as was found. Hesperidin was predominantly found in lime juices. The hesperidin content of lime juices significantly ($p < 0.05$) increased during the vacuum concentration process. The highest hesperidin contents were found in concentrated juices of *C. latifolia* and *C. limonia* at the level of 57.94 and 11.41 mg/100 mL, respectively. During the process, hesperidin was significantly ($p < 0.05$) lost, reflected through a drop in retention. The retention of hesperidin during the vacuum evaporation process ranged from 85.08 to 65.36% and from 87.73 to 63.15% found in *C. latifolia* and *C. limonia* juices, respectively. Hesperidin seems to be susceptible to degradation during processing. Hesperidin in citrus peel extracts was retained at a level of less than 59% in vacuum-drying at 40 °C [67]. Likewise, an increase in air drying temperature, ranging from 50 to 90 °C, caused degradation from 29.5 to 47.2% [68]. Hesperidin was significantly degraded under the high heat treatment [71]. To summarize, those investigated flavonoids, eriocitrin and rutin, were less preserved under

vacuum evaporation. Hesperidin showed a significant loss during the process but at a lower rate compared to eriocitrin and rutin. Naringin and diosmin appear to be stable during the processing, showing an increase in the content and a minimal loss in concentrated juices.

Table 3. Changes in flavonoid content and retention in *C. latifolia* and *C. limonia* during freeze and vacuum concentrations.

Juice Concentrate	Flavonoid Content (mg/100 mL)				Flavonoid Retention (%)			
	C. latifolia		*C. limonia*		*C. latifolia*		*C. limonia*	
	VC	FC	VC	FC	VC	FC	VC	FC
				Eriocitrin				
JC1	19.99 ± 0.84^{cC}	23.47 ± 0.28^{aD}	4.83 ± 0.02^{aA}	5.60 ± 0.10^{aB}	79.48 ± 3.35^{dA}	93.34 ± 1.13^{dB}	80.75 ± 0.30^{dA}	93.69 ± 1.61^{dB}
JC2	20.12 ± 0.87^{cC}	28.49 ± 0.50^{bD}	4.87 ± 0.05^{aA}	6.85 ± 0.18^{bB}	63.10 ± 2.71^{cA}	89.33 ± 1.57^{cdB}	64.09 ± 0.63^{cA}	90.16 ± 2.42^{cdB}
JC3	18.63 ± 0.85^{cC}	34.14 ± 0.62^{cD}	5.06 ± 0.07^{bA}	8.23 ± 0.16^{cB}	46.69 ± 2.14^{bA}	85.57 ± 1.56^{bC}	53.32 ± 0.72^{bB}	86.77 ± 1.71^{bC}
JC4	16.30 ± 0.52^{bC}	36.39 ± 0.93^{dD}	5.17 ± 0.05^{bA}	8.85 ± 0.45^{cB}	34.13 ± 1.09^{aA}	76.19 ± 1.95^{aC}	45.47 ± 0.42^{aB}	77.84 ± 3.99^{aC}
				Rutin				
JC1	4.08 ± 0.06^{abA}	4.87 ± 0.15^{aB}	nd	nd	78.18 ± 1.23^{dA}	93.30 ± 2.90^{cB}	nd	nd
JC2	4.31 ± 0.14^{bA}	5.69 ± 0.05^{bB}	nd	nd	65.10 ± 2.14^{cA}	88.09 ± 2.96^{cB}	nd	nd
JC3	4.23 ± 0.03^{bA}	6.46 ± 0.27^{cB}	nd	nd	51.06 ± 0.42^{bA}	80.67 ± 2.01^{bB}	nd	nd
JC4	3.80 ± 0.16^{aA}	7.11 ± 0.31^{dB}	nd	nd	38.39 ± 1.64^{aA}	73.84 ± 1.65^{aB}	nd	nd
				Naringin				
JC1	1.75 ± 0.02^{aB}	1.71 ± 0.02^{aB}	0.68 ± 0.01^{aA}	0.66 ± 0.02^{aA}	97.23 ± 0.91^{aC}	95.29 ± 0.98^{cB}	95.19 ± 0.87^{bB}	92.52 ± 2.80^{cA}
JC2	2.17 ± 0.06^{bB}	2.07 ± 0.05^{bB}	0.84 ± 0.01^{bA}	0.81 ± 0.01^{bA}	94.96 ± 2.45^{aB}	90.55 ± 2.21^{cA}	92.54 ± 1.40^{abAB}	89.09 ± 1.09^{bcA}
JC3	2.69 ± 0.05^{cC}	2.42 ± 0.08^{cB}	1.04 ± 0.01^{cA}	0.97 ± 0.02^{cA}	94.11 ± 1.68^{aB}	84.61 ± 2.79^{bA}	91.85 ± 1.14^{abB}	85.33 ± 1.77^{bA}
JC4	3.21 ± 0.08^{dD}	2.64 ± 0.06^{dC}	1.21 ± 0.04^{dB}	1.03 ± 0.04^{cA}	93.94 ± 2.25^{aC}	77.34 ± 1.87^{aA}	88.63 ± 2.84^{aB}	75.75 ± 3.00^{aA}
				Hesperidin				
JC1	39.70 ± 1.16^{aC}	44.24 ± 0.75^{aD}	8.33 ± 0.17^{aA}	8.84 ± 0.12^{aB}	85.08 ± 2.48^{dA}	94.81 ± 1.62^{dB}	87.73 ± 1.83^{bA}	93.04 ± 1.24^{dB}
JC2	47.22 ± 2.47^{bC}	53.78 ± 0.99^{bD}	9.52 ± 0.09^{bA}	10.69 ± 0.31^{bB}	79.80 ± 2.43^{cA}	90.87 ± 1.67^{cB}	78.82 ± 0.72^{aA}	88.54 ± 2.54^{cdB}
JC3	52.47 ± 0.51^{cC}	62.62 ± 0.75^{cD}	10.17 ± 0.12^{cA}	12.93 ± 0.24^{cB}	70.87 ± 0.68^{bA}	84.58 ± 1.01^{bB}	67.48 ± 0.80^{aA}	85.79 ± 1.57^{bB}
JC4	57.94 ± 1.07^{dC}	68.40 ± 0.87^{dD}	11.41 ± 0.32^{dA}	13.93 ± 0.23^{dB}	65.36 ± 1.21^{aA}	77.16 ± 0.98^{aA}	63.15 ± 1.46^{aA}	77.12 ± 1.28^{aA}
				Diosmin				
JC1	2.53 ± 0.05^{aB}	2.51 ± 0.05^{aB}	1.31 ± 0.04^{aA}	1.30 ± 0.03^{aA}	94.86 ± 1.70^{bA}	94.13 ± 1.74^{cA}	93.94 ± 2.62^{bA}	92.92 ± 1.97^{cA}
JC2	3.10 ± 0.05^{bB}	3.02 ± 0.08^{bB}	1.60 ± 0.07^{bA}	1.58 ± 0.02^{bA}	91.54 ± 1.41^{abA}	89.14 ± 2.33^{bcA}	89.91 ± 3.69^{abA}	88.78 ± 1.00^{cA}
JC3	3.81 ± 0.09^{cD}	3.58 ± 0.09^{cC}	1.99 ± 0.05^{cB}	1.84 ± 0.03^{cA}	89.83 ± 2.18^{aB}	84.50 ± 2.10^{bA}	89.61 ± 2.43^{abB}	82.77 ± 1.34^{bA}
JC4	4.45 ± 0.08^{dD}	3.80 ± 0.10^{dC}	2.28 ± 0.04^{dB}	1.99 ± 0.06^{dA}	87.67 ± 1.67^{aB}	74.96 ± 1.98^{aA}	85.69 ± 1.50^{aB}	74.86 ± 2.32^{aA}

The values are expressed as mean ± SD of triplicates. Different uppercase letters of the same row of each measurement indicate a significant difference ($p < 0.05$) between juices. Different lowercase letters of the same column of each measurement indicate significant differences ($p < 0.05$) between juice concentrate. JC: juice concentrate (JC1 to JC4 in accordance with 17.9, 22.5, 28.4, and 34.1% soluble solids, respectively). VC: vacuum concentration; FC: Freeze concentration.

Flavonoids of freeze-concentrated juices were greatly enriched by freeze concentration (Table 3). Hesperidin of freeze-concentrated lime juices increased threefold, compared to fresh lime juices. At the highest juice concentration, hesperidin in *C. latifolia* and *C. limonia* reached 68.40 and 13.93 mg/100 mL. Freeze concentration improved eriocitrin in lime juices from 12.78 to 36.39 mg/100 mL (*C. latifolia*) and from 2.97 to 8.85 mg/100 mL (*C. limonia*). Rutin content improved from 2.65 to 7.71 mg/100 mL in freeze-concentrated lime juices. A significant augment in diosmin and naringin was also found since the content of this flavonoid increased around 2.8 times in freeze-concentrated lime juices. Results are consistent with the flavonoid analyses of freeze-concentrated juices in previous studies. An improvement in total flavonoid content due to the technique of freeze concentration was 2.6 times in blackberry [63] and 6.4 times in orange juice [21]. For further observation of changes in flavonoid composition during the freeze concentration, Zielinski, et al. [20] found that freeze-concentrated apple juices significantly increased at least fourfold in flavan-3-ols and threefold in flavanols (including rutin). Compared to vacuum concentration, the retention of flavonoids of freeze concentration was varied, depending on each flavonoid.

Major flavonoids such as hesperidin, eriocitrin, and rutin were effectively preserved by freeze concentration. Paired *t*-test analyses showed that freeze concentration retained approximately 11% higher in hesperidin in both juices from two lime varieties compared to vacuum concentration ($p < 0.0001$). Eriocitrin of freezed-concentrated *C. latifolia* and *C. limonia* juices was higher than that of vacuum-concentrated counterparts by 30.26% ($t(11) = 8.8115$, $p < 0.0001$) and 26.21% ($t(11) = 10.2337$, $p < 0.01$), respectively. The rutin retention of lime juices obtained from freeze concentration was also 25.79% higher than that of vacuum concentration ($p < 0.001$). In contrast, vacuum concentration resulted in a higher retention of diosmin (around 5%, $p < 0.01$) and naringin (6–8%, $p < 0.01$) lime juices compared to freeze concentration. Findings from this study suggest that freeze concentration is suitable to enrich and retain heat liable flavonoids, whereas vacuum concentration could be applied for those heat-stable flavonoids. Nevertheless, freeze concentration showed its significance in the enrichment and retention of flavonoids in lime juices, implying its potential in high-quality lime juice processing.

3.5. Changes in DPPH Scavenging Capacity of Lime Juices during Vacuum and Freeze Concentration

The DPPH scavenging ability of vacuum-concentrated lime juices was significantly increased ($p < 0.05$), compared to that of original lime juices. The vacuum concentration improved DPPH inhibition from 44.58 to 69.63% (Figure 2A) in *C. latifolia* and from 25.35 to 51.83% (Figure 2B) in *C. limonia* juices, respectively. The changes in antioxidants seem food matrix- and temperature dependent. Vacuum-concentrated beetroot juice at 50 and 55 °C increased DPPH inhibition by 18% but remained unchanged when the temperature increased to 60 °C [14]. Similarly, Li, et al. [13] found that vacuum-concentrated pear paste increased in DPPH scavenging with the applied temperature in the range of 55 and 60 °C, then gradually decreased when the temperature increased from 65 to 75 °C. The changes in antioxidant ability would involve the cumulative of potentially anti-oxidative components in the juices. Vacuum-concentrated lime juices had significantly higher cumulative ascorbic acid, TPC, and major flavonoids, which are more likely to contribute to an increase in the DPPH scavenging capacity.

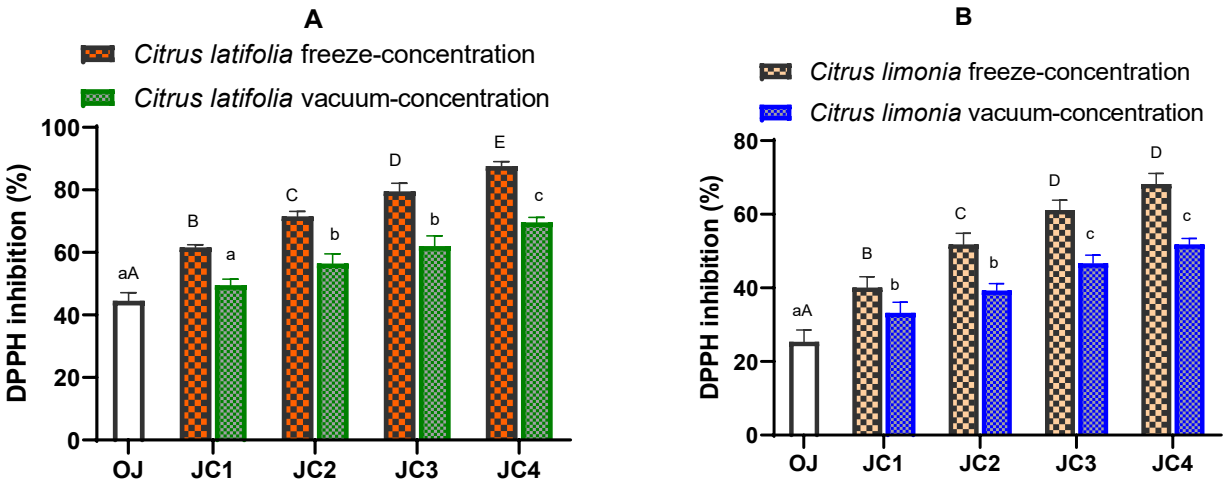

Figure 2. Changes in DPPH scavenging activity of (**A**) *C. latifolia* and (**B**) *C. limonia* lime juices during freeze and vacuum concentrations. The values are expressed as mean ± SD of triplicates. Different uppercase letters indicate a significant difference ($p < 0.05$) between freeze-concentrated juices. Different lowercase letters indicate a significant difference ($p < 0.05$) between vacuum-concentrated juices. OJ: original juice, JC: juice concentrate.

DPPH radical scavenging of lime juices remarkably increased after freeze concentrating. The improvement in DPPH inhibition was more pronounced in *C. limonia* juice

in which DPPH inhibition increased almost threefold (from 25.35 to 68.23%, Figure 2B). Freeze-concentrated *C. latifolia* juice was also nearly double in DPPH scavenging ability (from 44.58 to 87.64%). Generally, DPPH inhibition of concentrated *C. limonia* juice was lower than that of concentrated *C. latifolia* juice. Regarding concentration methods, freeze concentration resulted in higher DPPH inhibitory lime juices compared to the vacuum concentration observed at both lime varieties. Freeze-concentrated juices were 15.67% higher in DPPH inhibition compared to vacuum-concentrated juices ($t(11) = 19.3012$, $p < 0.0001$). Freeze concentration caused an increase in the anti-oxidative activity of various concentrated products such as apple [20], blueberry [63], and orange [21] juices, as well as pear paste [13]. The increase in the DPPH scavenging activity would be contributed by a great improvement in anti-oxidative components such as ascorbic acid, TPC, and flavonoids found in freeze-concentrated lime juices. Ascorbic acid has been reported as one of the major contributors to the antioxidant [55,72]. In this study, the ascorbic acid content of freeze-concentrated lime juices greatly increased and presented at a higher level compared to vacuum-concentrated juices, and thus may contribute to high DPPH scavenging activity as seen. A significantly higher TPC of freeze-concentrated lime juices is probably another factor exerting an increase in the antioxidants of freeze-concentrated lime juices. A strong correlation between TPC and the antioxidant activity of freeze-concentrated juices was documented [13,20,63], explaining the high antioxidant activity of freeze-concentrated lime juices. Rich flavonoid components of yielded juices from freeze concentration possibly induced a high antioxidant activity. Hesperidin was the most predominant flavonoid in the fresh and freeze-concentrated lime juices. Though the antioxidant activity of hesperidin was not the strongest among the observed flavonoids [10], it was proved to have potential DPPH inhibition [73] and thus, may play as an important antioxidant in freeze-concentrated lime juices. Eriocitrin, a dedicated flavanone found in lime and lemon, showed the highest antioxidant activity, followed by hesperidin, naringin, and diosmin [10]. In this study, eriocitrin was the second highest flavonoid content which was greatly enriched and retained by freeze concentration at the higher levels compared to vacuum concentration may partially explain the high DPPH inhibition of freeze-concentrated lime juices. Freeze concentration was more effective compared to vacuum concentration in the enrichment of lime juices' rutin, which was reported as a strong DPPH scavenger [74,75]. Diosmin and naringin, although available at lower contents, were significantly enriched in freeze-concentrated lime juices, perhaps as an attribute to DPPH inhibition because of their antioxidant activities [76]. The primary application of freeze-concentration is to preserve the beneficial, heat-sensitive components. In the freeze-concentration process, the recovery yields gradually reduce in accordance with an increase in the juice concentration [15,63,77]. Yet, an advantage of freeze-concentration is the ability to maintain the composition profile of the juice [18]. Further research to improve the yield may be in the quest (e.g., optimization of operation parameters); freeze concentration shows its potential in enriching bioactive components in juices. Therefore, the findings have great significance in concentrating juices which are very thermally susceptible, to avoid any undesirable changes in organoleptic and bioactive contents.

4. Conclusions

Two lime varieties, namely *C. latifolia* and *C. limonia*, were analyzed for bioactive components. *C. latifolia* juice was higher than *C. limonia* juice in its ascorbic acid, TPC, and DPPH scavenging activity. Qualitative scanning of polyphenols and flavonoids revealed that *C. latifolia* was more diverse than *C. limonia* in polyphenols and flavonoids. Juices from two lime varieties were concentrated by vacuum and freeze concentrations. Freeze concentration showed a significantly higher enrichment of ascorbic acid, TPC, and major flavonoids (e.g., hesperidin, eriocitrin, and rutin) of lime juices. Vacuum concentration was more effective in concentrating diosmin and naringin. The findings of this study highlighted a superior DPPH radical scavenging activity of freeze-concentrated lime juices compared to vacuum-concentrated juices. *C. latifolia* juice and freeze-concentrated juice

were much higher in bioactive contents and antioxidants, which is promising for juice processing. Thus, increasing the consumption of *C. latifolia* juice may bring more health benefits. The freeze concentration process bypassing heat input would be suitable to produce high-quality juices, which are not only rich in health-beneficial components but also organoleptic properties. Further studies on evaluating the sensory quality and volatile components of concentrated lime juices are recommended to elucidate the potential of freeze concentration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11071883/s1, Figure S1. Chromatograms of *C. latifolia* (A) and *C. limonia* (B) juices acquired from the quantitative analysis by LC-QTOF MS/MS; Figure S2. Chromatograms and MS spectra of standards: eriocitrin (A), rutin (B), naringin (C), hesperidin (D), and diosmin (E); Figure S3. HPLC-PDA chromatograms of *C. latifolia* (A) and *C. limonia* (B) juices at 280 nm. Elution of compounds is marked: 1. eriocitrin, 2. rutin, 3. naringin, 4. hesperidin, and 5. diosmin. Note the absence of rutin in *C. limonia* juice.

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