




Impact of microencapsulated watermelon (*Citrullus lanatus*) and beetroot (*Beta vulgaris L*) on storage stability of l-citrulline and dietary nitrate

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Abstract The aim the current study was to developed a watermelon rind powder (WRP), watermelon pulp powder (WPP), and beetroot powder (BP), in order to compare the stability of L-citrulline and nitrate with that of watermelon rind juice (WRJ), watermelon pulp juice (WPJ), and beetroot juice (BJ), respectively. The stability was evaluated during 32 days at 25, 4 and – 20 °C. L-arginine and L-ornithine content were also evaluated. At day 0, a significantly higher L-citrulline and L-ornithine content in WRP was observed when compared to WPP. However, a significantly lower L-arginine content in WRP was observed when compared to WPP. L-citrulline content in WRP and WRJ was stable over 32 days in all temperatures evaluated, whereas it reduced in WPP in 32 days at 25 °C and it is reduced in in WPJ in day 16 and day 32 at 25 °C.

L-arginine content in WRP and WPP was stable over 32 days in all temperatures evaluated. A reduction was observed in WRJ at day 2, 4 and 32 at 25 °C and in WPJ at day 2, 4, 8, 16 and 32 days at 25 °C. L-ornithine content in WRP and WPP was stable over 32 days in all temperatures evaluated. An increase was observed in WRJ at day 2, 4 and 32 at 25 °C and in WPJ in day 2, 4, 8, 16 and 32 at 25 °C. Nitrate content in BP was stable over 32 days in all temperatures evaluated, while nitrate content in beetroot juice was reduced in day 2 at 25 °C and day 8 at 4 °C. In conclusion, L-citrulline of the microencapsulated watermelon rind and nitrate of the microencapsulated beetroot were stable throughout storage.

Keywords Microencapsulation · Bioactive compounds · L-citrulline · Nitrate · Watermelon · Beetroot

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Abbreviations

NO	Nitric oxide
NOS	Nitric oxide synthase
WRJ	Watermelon rind juice
WPJ	Watermelon pulp juice
WRP	Watermelon rind powder
WPP	Watermelon pulp powder
BJ	Beetroot juice
BP	Beetroot powder
HPLC	High performance liquid chromatography
HClO ₄	Perchloric acid
K ₂ CO ₃	Potassium carbonate

Introduction

The consumption of beetroot and watermelon juice has been proposed as a nutritional strategy to promote cardiovascular health due to the possible effect of the nitrate and L-citrulline present in these foods, respectively, in nitric oxide (NO) bioconversion (Volino-Souza et al. 2018; Oliveira et al. 2017; Figueroa et al. 2013). Beetroot is considered a vegetable with very high nitrate content (> 250 mg/100 g fresh weight) (Santamaria 2006), while watermelon is the richest known source of L-citrulline, where it is present in both the pulp and rinds of the fruit (Akashi et al. 2017; Tarazona-Díaz et al. 2011).

NO is an important molecule involved in many physiological processes in the body, especially in the cardiovascular system. NO regulates blood flow; platelet aggregation; maintenance and regulation of vascular tone; and blood pressure. Reduction in NO bioavailability is associated with endothelial dysfunction and cardiovascular diseases (Lundberg et al. 2015). NO is generated by nitric oxide synthase (NOS) enzymes by using L-arginine as a substrate (Lundberg et al. 2015), formatting L-citrulline as a subproduct. However, L-citrulline can be recycled to L-arginine and generates more substrate for NOS (Lundberg et al. 2009). Dietary nitrate can be reduced to nitrite in the oral cavity through commensal facultative anaerobic bacteria that express nitrate reductase enzymes. The salivary-derived nitrite can be reduced to NO in the acidic environment of the stomach or enter the systemic circulation, where in conditions of low oxygen availability and low pH, nitrite can also be reduced to NO (Lundberg et al. 2015; Lundberg et al. 2009).

Due to the importance of nitrate and L-citrulline to enhance NO production and bioavailability, its use as a therapeutic agent has been a prominent area of research. Currently, the food industry is conducting research on natural foods rich in bioactive compounds that can substitute pharmacologic products. Since watermelon is a natural source of L-citrulline and beetroot is a natural source of nitrate, they may indeed provide alternatives to this end. However, the stability of nitrate and L-citrulline present in beetroot and watermelon, as well as their food product derivatives (juice, gel), is important when considering their functional properties. Corleto et al. (2018) showed a decrease in nitrate content in beetroot juice during 32 days of storage at room temperature (25 °C) and refrigerated (4 °C) conditions. In beetroot juice, the nitrate content was reduced by 56% after 1-day storage in room temperature and 15% after 8 days storage in refrigerated environment (4 °C). In watermelon juice enriched with ~ 330% of L-citrulline, Tarazona-Díaz et al. (2017)

evaluated the stability and showed that citrulline content was reduced 3% after 30 days of storage at 4 °C.

Microencapsulation techniques have been widely used by the food industry to protect bioactive compounds against degradation (Paulo and Santos 2017). In this process, biologically active compounds are immobilized and incorporated inside solid particles (microspheres), providing stability to the compound structure and its protection. Therefore, spray drying has been chosen by the food industry due to its several benefits, including its potential for large-scale production, the simplicity and efficiency of the equipment, the stability of the final product, and low processing cost (Speranza et al. 2017; Madena et al. 2006). Furthermore, food in powder form has the technological advantages of being easy to store at room temperature and easy to carry (Verma and Singh 2015). Although the microencapsulation technique is widely used in food industry for preservation of the bioactive compounds, each compound has specific characteristics that may induce different levels of stability. For this reason, investigating the stability of nutrients from microencapsulated foods is warranted (Dias et al. 2015). Moreover, to the best of our knowledge, the stability of L-citrulline and nitrate in microencapsulated watermelon and beetroot by spray dryer has not been evaluated.

Based on these considerations, the aim of this study was to investigate the stability of the L-citrulline and nitrate present in microencapsulated watermelon and beetroot by spray drying with them with maltodextrin over a storage period of 32 days at three different storage temperatures (25, 4 and – 20 °C). Proximate composition and sugar content of the microencapsulated food products were also evaluated. It was the hypothesis of the present paper that the L-citrulline and nitrate in microencapsulated watermelon and beetroot, respectively, could be more stable than juice. Additionally, it was expected that watermelon rind would have a lower sugar content than pulp.

Material and methods

Materials

Amino acids (L-arginine monohydrochloride, L-citrulline and L-ornithine monohydrochloride), sugars (D-glucose, D-sucrose and D-fructose), 2,5-Dimethyl-1H-pyrrole-3,4-dicarbaldehyde, sodium nitrate, tetrabutylammonium hydroxide, triethylamine and carboxymethyl cellulose were purchased from Sigma-Aldrich (Brazil). Methanol and acetonitrile were purchased from Tedia (Brazil). Maltodextrin was purchased from Athletica Nutrition (Brazil).

Watermelon and beetroot juice preparation

All the watermelon used in the present study belongs to the Cucurbitaceae family and the *Citrullus lanatus* species and the beetroot used belongs to the Chenopodiaceae family and the *Beta vulgaris* L. species. Beetroot and watermelon were acquired in the city of Macaé, Rio de Janeiro, Brazil (22° 22' 15" S, 41° 47' 13" W) and were thoroughly washed in tap water and sanitized with a chlorine solution. The rind and pulp of the watermelon were separated and put into a centrifuge blender (Model CE700, Black & Decker, Baltimore, MD) for watermelon rind juice (WRJ) and watermelon pulp juice (WPJ) production. The beetroot juice (BJ) was also produced in a centrifuge blender. The juices were first filtered through organza for elimination of suspended solids.

Microencapsulation

The process of spray-drying was carried out using a mini spray dryer (Model B-290, Büchi) having 1.0 mm standard diameter nozzle, and evaporation capacity of 1.0 L/h. The experiments were performed by using an inlet temperature of 160 °C, feed rate of 30%, and air flow of 80%.

For watermelon rind powder (WRP) production, maltodextrin (dextrose equivalent around 10) (9.5%) and carboxymethylcellulose (0.5%) were added to the WRJ. For watermelon pulp powder (WPP) production, maltodextrin (5%) and carboxymethylcellulose (0.5%) were added to the WPJ. For beetroot powder (BP), maltodextrin (5%) and carboxymethylcellulose (0.5%) were added to BJ. The solutions were homogenized and were used as feed in the spray drying process. The yields were 5.89%, 5.23% and 5.58% respectively.

Maltodextrin and carboxymethyl cellulose were chosen as carrier agents since they have been widely used for obtaining dried food products due to their low hygroscopicity (Verma and Singh 2015). The proportion used was defined from preliminary tests and previous study (Quek et al. 2007).

Storage stability procedures

The resulting powder was divided in several 15 mL conical tubes (polypropylene) for every day of the analysis to avoid possible contamination of the samples. The samples were stored for 32 days in different conditions: room temperature (25 °C), refrigerated (4 °C) and in freezer (− 20 °C). Samples from various temperatures were taken for analysis at 0, 2, 4, 8, 16 and 32 days. First analysis after production of the samples was considered the initial day (day 0). The storage period (0–32 days) was chosen based on a previous study that evaluated the stability of nitrate present in

beetroot (Corleto et al. 2017). Since no evidence on the stability of L-citrulline from watermelon has been previously published and in order to maintain the standardization of the experimental procedures, the same period was chosen to assess L-citrulline and nitrate stability in order to allow for comparison.

Amino acids analysis

The amino acids L-citrulline, L-arginine and L-ornithine content in WRP, WPP, WRJ and WPJ were analyzed as previously described by Gatti et al. (2010), but with some modification. Since L-arginine and L-ornithine are involved in L-citrulline metabolism, these amino acids were analyzed in the current study (Arena et al. 1999). Briefly, the WRP and WPP were solubilized and diluted in deionized water and 100 µL of each sample were mixed with 100 µL of the 1.5 M HClO₄. Afterwards, 750 µL of the deionized water and 50 µL of the 2 M K₂CO₃ were added following centrifugation at 10,000 r.p.m. for 1 min. After centrifugation, 50 µL of the supernatant was collected, and 50 µL of the deionized water and 40 µL of the 2,5-dimethyl-1H-pyrrole-3,4-dicarbaldehyde were added before allowing it to rest for 10 min. Finally, 360 µL of the 0.05 M triethylammonium phosphate buffer (pH 2.5) was added into the samples and 20 µL was injected into the HPLC system.

The HPLC system (Shimadzu, Kyoto, Japan) was fit with C18 column (250 × 4.6 mm ACE), guard column (5-mm, 50 × 4.6 mm) and photodiode array detector monitoring absorbance at 320 nm. The isocratic elution was performed by using a mobile phase solution consisting of 0.05 M triethylammonium phosphate (pH 2.5) and methanol (92:8; v/v), at a flow of 0.42 mL/min.

Nitrate analysis

The nitrate content in BJ and BP was analyzed as previously described by Croitoru (2012). Briefly, 125 µL of the sample was mixed with 500 µL of the deionized water and 625 µL of the acetonitrile and centrifuged at 14,000 r.p.m. for 10 min. After centrifugation, 150 µL of the supernatant was collected and 150 µL of the 5 mM tetrabutylammonium hydroxide (pH 2.5) was added and 150 µL of the samples were injected into the HPLC system.

The HPLC system (Shimadzu, Kyoto, Japan) was fit with C18 column (100 × 4.6 mm Kromasil), guard column (5-mm, 50 × 4.6 mm) and photodiode array detector monitoring absorbance at 222 nm. The isocratic elution was performed by using a mobile phase solution, consisting of 5 mM tetrabutylammonium hydroxide (pH 2.5) and acetonitrile (90:10; v/v), at flow 1.0 mL/min.

Table 1 Amino acids content in WRP and WPP

	WRP	WPP
L-citrulline (mM)	72.31 ± 9.26	35.95 ± 3.15***
L-arginine (mM)	19.75 ± 3.36	22.97 ± 2.60*
L-ornithine (mM)	1.06 ± 0.09	0.14 ± 0.05***

WRP, watermelon rind powder; WPP, watermelon pulp powder. Data are expressed as means of 8 replicates (± standard deviation). *($p < 0.05$) and ***($p < 0.001$) indicate significant difference from WRP. Degree of freedom = $t(14)$

Proximate composition and sugar analysis

The moisture, total lipid, crude protein and ash content were determined for WRP, WPP and BP samples as described by AOAC (2012). Sucrose, fructose and glucose content in WRP, WPP and BP were analyzed as previously describe by Duarte-Delgado et al. (2015). Firstly, 1 g of the WRP, WPP and BP were solubilized in 45 mL of the 10 mM sulfuric acid, homogenized and centrifuged at 10,000 r.p.m. for 15 min. After, 150 µL of the supernatant was injected in the HPLC system.

The HPLC system (Shimadzu, Kyoto, Japan) was equipped with Aminex HPX 87H column (300 × 7.8 mm, Bio-Rad), guard column (30 × 4.6 mm) and refraction index detector. The isocratic elution was performed by using mobile phase solution, consisting of 10 mM sulfuric acid, at a flow of 0.60 mL/min and a column temperature of 18 °C.

Statistical analysis

To identify differences in L-citrulline, L-arginine and L-ornithine between WRP and WPP, an independent sample t test was performed. To identify differences in the

proximate composition and sugar content between WRP, WPP and BP a one-way ANOVA was performed. To identify differences in the levels of amino acids and nitrate during storage a two-way ANOVA was performed. Additional post-hoc tests with Bonferroni adjustment were performed. Statistical significance was set at the 0.05 level of confidence. All analyses were performed using a commercially available statistical package (IBM SPSS Statistics version 23 for Mac) and the results were expressed as means and standard deviations.

Results and discussion

Amino acids and sugar contents of the microencapsulated L-citrulline and nitrate

WRP presented a significantly higher level of L-citrulline and L-ornithine at initial day when compared to WPP. However, a significant lower initial level of L-arginine was observed compared to WPP (Table 1). These results are in line with previous studies (Akashi et al. 2017; Rimando and Perkins-Veazie 2005). Akashi et al. (2017) showed higher L-citrulline concentration in peripheral portion compared to pulp. Rimando and Perkins-Veazie (2005) also showed that watermelon rind presented higher L-citrulline concentration on a dry weight basis when compared to pulp. In contrast, Joshi et al. (2019) concluded that there was no difference in L-citrulline content between rind and pulp. These adverse results might be explained by differences in genotypic and environmental factors. Fish (2014) suggests that growing conditions can influence the L-citrulline content in watermelon rind and pulp.

WRP showed significantly lower levels of the sucrose, glucose and fructose compared to WRP (Table 2). Akashi et al. (2017) also showed that sucrose, fructose and glucose

Table 2 Proximate composition and sugar content of the WRP, WPP and BP

	WRP	WPP	BP
<i>Proximate composition</i>			
Moisture (%)	4.07 ± 0.03	3.91 ± 0.05 ^a	3.67 ± 0.04 ^{ab}
Protein (%)	8.77 ± 0.05	14.00 ± 0.06 ^a	11.68 ± 0.05 ^{ab}
Lipid (%)	0.19 ± 0.03	1.78 ± 0.06 ^a	0.71 ± 0.0 ^{3ab}
Ash (%)	7.93 ± 0.04	7.09 ± 0.02 ^a	6.83 ± 0.04 ^{ab}
<i>Sugar</i>			
Sucrose (mg/mL)	16.69 ± 0.78	45.51 ± 6.78 ^a	253.31 ± 33.05 ^{ab}
Glucose (mg/mL)	25.15 ± 3.65	46.77 ± 10.41 ^a	2.78 ± 0.97 ^{ab}
Fructose (mg/mL)	57.32 ± 5.73	144.25 ± 24.21 ^a	41.35 ± 8.97 ^b

BP beetroot powder, WRP watermelon rind powder, WPP watermelon pulp powder. Data are expressed as means of 8 replicates (± standard deviation)

^adenotes significant difference from WRP; ^bdenotes significant difference from WPP. Degree of freedom = $F(2, 15)$

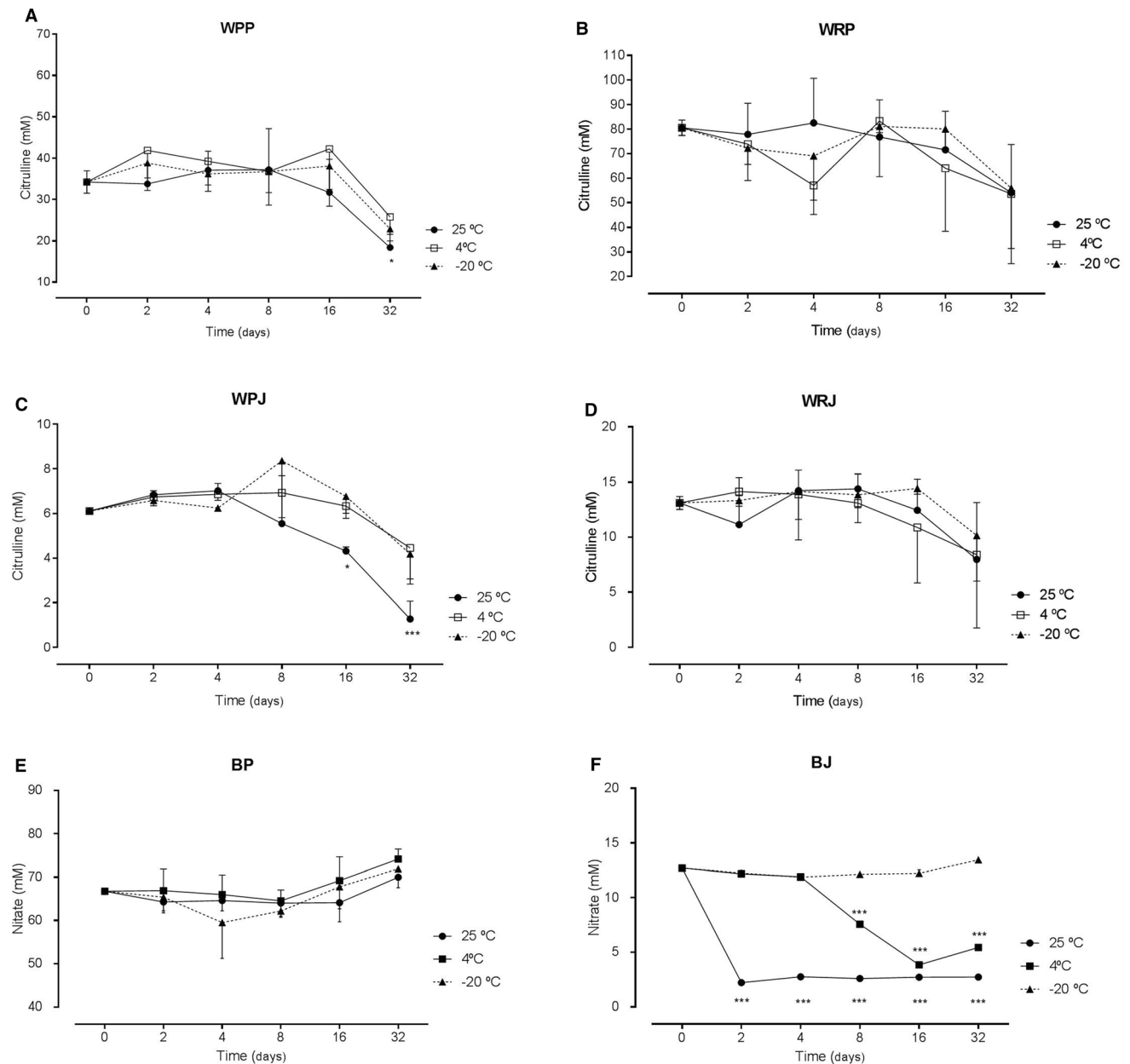


Fig. 1 Levels of L-citrulline in **a** watermelon rind powder, **b** watermelon rind juice, **c** watermelon pulp powder and **d** watermelon pulp juice, and nitrate in **e** beetroot powder and **f** beetroot juice during storage at 25 °C, 4 °C and -20 °C for 32 days. Differences were

analyzed for different temperatures and time points. Data are expressed as means of 4 replicates (\pm standard deviation). * ($p < 0.05$) and *** ($p < 0.001$) denote significant difference from day 0. Degree of freedom = F(2, 9)

were low in the peripheral portion of the watermelon when compared to pulp. Knowledge regarding the sugar content present in foods expected to induce change in the vascular endothelial function (due to its bioactive components, such as L-citrulline) is crucial, since ingestion of a high sugar content per se might negatively modulate the vascular response (Soares et al. 2019).

Stability of microencapsulated L-citrulline and nitrate

L-citrulline in WRP and WRJ were stable during 32 days at 25 °C, 4 °C and -20 °C. WPP was stable during 32 days at 4 °C and -20 °C; however, there was a significant reduction in WPP detected on day 32 at 25 °C, compared to day 0. L-citrulline in WPJ was stable at 4 °C and -20 °C; however, there was a significant reduction

Table 3 L-arginine stability of the WRP, WPP, WRJ and WPJ during 32 days storage at 25, 4 and –20°C

		Day 0	Day 2	Day 4	Day 8	Day 16	Day 32
WRP	25 °C	19.84 ± 4.38	19.90 ± 3.68	19.78 ± 2.75	18.95 ± 2.63	16.79 ± 1.73	12.90 ± 1.31
	4 °C		19.71 ± 2.39	16.94 ± 7.45	23.62 ± 7.30	19.23 ± 4.46	12.98 ± 2.67
	– 20 °C		20.20 ± 5.44	18.54 ± 4.70	22.45 ± 2.49	21.15 ± 5.72	14.00 ± 2.12
WPP	25 °C	21.52 ± 2.58	23.74 ± 3.76	23.59 ± 1.83	27.13 ± 0.78	22.16 ± 1.63	15.22 ± 4.22
	4 °C		26.72 ± 4.15	24.63 ± 3.49	24.34 ± 5.02	27.01 ± 3.79	18.85 ± 7.28
	– 20 °C		27.36 ± 2.22	27.36 ± 2.22	27.28 ± 3.03	29.79 ± 2.66	18.26 ± 4.77
WRJ	25 °C	3.47 ± 0.93	1.31 ± 0.56***	0.71 ± 0.35*	0.49 ± 0.01	0.66 ± 0.43	0.11 ± 0.04*
	4 °C		3.90 ± 1.34	3.14 ± 1.70	1.60 ± 1.53	1.48 ± 1.40	1.46 ± 1.53
	– 20 °C		3.90 ± 1.36	3.90 ± 1.36	4.06 ± 1.09	4.00 ± 1.40	2.58 ± 0.23
WPJ	25 °C	4.15 ± 0.06	1.99 ± 1.33*	1.80 ± 1.58*	0.13 ± 0.04*	0.15 ± 0.04*	0.09 ± 0.04*
	4 °C		4.37 ± 0.19	4.63 ± 0.16	4.85 ± 0.66	2.48 ± 2.71	2.48 ± 2.85
	– 20 °C		4.37 ± 0.20	4.16 ± 0.10	5.87 ± 2.93	4.67 ± 0.21	3.08 ± 1.10

WRP, watermelon rind powder; WPP, watermelon pulp powder; WRJ, watermelon rind juice; WPJ, watermelon pulp juice. Data are expressed as means of 4 replicates (± standard deviation).*(*p* < 0.05) and ***(*p* < 0.001) denote significant difference from day 0. Degree of freedom = *F*(2, 9)

Table 4 L-ornithine stability of the WRP, WPP, WRJ and WPJ during 32 days storage at 25, 4 and –20°C

		Day 0	Day 2	Day 4	Day 8	Day 16	Day 32
WRP	25 °C	1.06 ± 0.13	1.17 ± 0.28	1.43 ± 0.18	1.11 ± 0.16	0.99 ± 0.15	0.44 ± 0.18
	4 °C		1.07 ± 0.19	0.88 ± 0.57	1.18 ± 0.38	1.05 ± 0.28	0.49 ± 0.39
	– 20 °C		0.93 ± 0.21	0.94 ± 0.23	0.99 ± 0.11	1.20 ± 0.41	0.47 ± 0.28
WPP	25 °C	0.13 ± 0.06	0.16 ± 0.02	0.17 ± 0.01	0.17 ± 0.06	0.17 ± 0.00	0.37 ± 0.29
	4 °C		0.20 ± 0.03	0.18 ± 0.00	0.14 ± 0.03	0.14 ± 0.03	0.51 ± 0.38
	– 20 °C		0.15 ± 0.03	0.13 ± 0.03	0.14 ± 0.04	0.14 ± 0.04	0.52 ± 0.42
WRJ	25 °C	0.19 ± 0.04	4.08 ± 2.19*	3.93 ± 1.75***	2.26 ± 0.38	0.84 ± 0.78	1.82 ± 0.77*
	4 °C		0.24 ± 0.09	0.20 ± 0.13	3.04 ± 3.21	2.59 ± 2.55	0.67 ± 0.49
	– 20 °C		0.21 ± 0.09	0.25 ± 0.03	0.20 ± 0.07	0.24 ± 0.12	0.09 ± 0.05
WPJ	25 °C	0.02 ± 0.00	1.83 ± 0.60***	2.21 ± 0.73*	2.50 ± 1.62*	3.28 ± 2.18*	3.02 ± 2.36*
	4 °C		0.03 ± 0.00	0.02 ± 0.00	0.08 ± 0.03	0.75 ± 0.25	0.33 ± 0.32
	– 20 °C		0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.00	0.08 ± 0.06

WRP, watermelon rind powder; WPP, watermelon pulp powder; WRJ, watermelon rind juice; WPJ, watermelon pulp juice. Data are expressed as means of 4 replicates (± standard deviation).*(*p* < 0.05) and ***(*p* < 0.001) denote significant difference from day 0. Degree of freedom = *F* (2, 9)

detected on day 16 and day 32 at 25 °C, compared to day 0 (Fig. 1). Tarazona-Diaz et al. (2017) also showed a reduction in L-citrulline present in WPJ after storage during 30 days at 4 °C. In plants, L-citrulline can be converted to arginine by enzymatic activity. Argininosuccinate synthase (ASS) can convert L-citrulline to L-argininosuccinate before converting it to arginine by argininosuccinate lyase (ASL) (Joshi et al. 2019). Based on the RNAseq profiles of watermelon fruits, Gou et al. (2013) suggested that L-citrulline content in watermelon pulp can be regulated by ASS and ASL-like genes, but it is unlikely to occur in

watermelon rind, thereby supporting the results of the present study that demonstrated a reduction of L-citrulline in WPP and WPJ, but not in WRP and WRJ.

The L-arginine contents in WRP and WPP were stable for 32 days at 25, 4 and – 20 °C; however, L-arginine content in WRJ significantly reduced on day 2, day 4 and day 32 at 25°C compared to day 0. L-arginine content in WPJ significantly reduced on day 2, day 4, day 8, day 16 and day 32 compared to day 0 (3). L-ornithine contents in WRP and WPP was s during 32 days at 25, 4 and – 20 °C; however, L-ornithine content in WRJ

significantly increased on day 2, day 4 and day 32. L-ornithine content in WPJ presented a significant increase on day 2, day 4, day 8, day 16 and day 32 compared to day 0 (4). The reduction of the L-arginine content observed in WRJ (days 2, 4 and 32) and WPJ (days 2, 4, 8, 16 and 32) was accompanied by increased L-ornithine content. L-arginine present in plants can be reduced to L-ornithine by arginase activity (Ibrahim et al. 2018), which may explain the results in the current study. In plants, the degradation of L-arginine to L-ornithine is catalyzed by arginase, a binuclear manganese metalloenzyme present in bacteria, fungi and yeast (Ibrahim et al. 2018). Our data demonstrates that L-arginine decreased when stored at 25 °C; whereas it was stable at 4 and –20 °C, suggesting that temperature might have influenced this process. It has been reported that optimal temperature for the activity of the arginase derived from *Vigna catjang cotyledon* is around 30 °C (Dabir et al. 2005), reinforcing the role of temperature in arginine metabolism.

There was a significant reduction in nitrate content in BJ 25 °C on day 2, day 4, day 8, day 16 and day 32 compared to day 0. BJ also presented a significant reduction at 4 °C in day 8, day 16 and day 32 compared to day 0. At –20 °C, BJ was stable for 32 days of storage (Fig. 1). In contrast with BJ, nitrate content in BP had not changed during storage at 25 °C. These results are in agreement with previous studies demonstrating reduction in nitrate content in BJ during storage at room temperature (Corleto et al. 2018; Tamme et al. 2010; Chung et al. 2004). In microencapsulation by spray dryer, the water content in the food is removed by rapid evaporation on spray droplet under high temperature exposure (Verma and Singh 2015). For decrease water content and water activity this technique reduces the risk of chemical and biological degradation (Drosou et al. 2016). A previous study that evaluated the stability of nitrate in beetroot juice showed a reduction at 25 °C over 32 days, for which the authors have suggested that this reduction may have occurred due to bacteria that express nitrate reductase (Corleto et al. 2017; Ji et al. 2015). However, the current study did not evaluate bacterial activity; therefore, future studies evaluating the effect of bacteria in nitrate reduction among the different forms of beetroot is necessary.

In the current study, it has been concluded that the nitrate present in beetroot juice can be preserved only when stored at –20 °C; whereas beetroot powder maintains unchanged throughout 32 days of storage, suggesting that microencapsulated products would be more suitable in terms of nitrate content. Furthermore, microencapsulated products are not only easy to store, they require no equipment to maintain stability during storage and they are easy to carry.

Conclusion

In conclusion, these results demonstrate that L-citrulline and nitrate from microencapsulated watermelon and beetroot are more stable than they are in juice form over a 32-day period. It was also demonstrated that microencapsulated watermelon rind has a higher L-citrulline content and lower soluble sugar content as compared to the pulp of the fruit. Therefore, considering that watermelon and beetroot are foods widely consumed in human diet, the data of the current study suggest that the consumption of nitrate and L-citrulline from microencapsulated watermelon rind and beetroot may be a suitable technological strategy to preserve and/or increase the stability and biological properties of these nutrients in commercial products while diminishing sugar ingestion.

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Author contributions MVS and OCV contributed substantially to the development of the WPP, WRP and BP. MVS contributed to chromatographic analysis, data acquisition, statistical analysis and interpretation. MVS, GVO, TSA and CACJ wrote the article. All authors read and approved the final article.

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

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest. This article does not contain any studies with human or animal subjects.

References

- Akashi K, Mifune Y, Morita K, Ishitsuka S, Tsujimoto H, Ishihara T (2017) Spatial accumulation pattern of citrulline and other nutrients in immature and mature watermelon fruits. *J Sci Food Agric* 97:479–487. <https://doi.org/10.1002/jsfa.7749>
- AOAC International (2012) Official methods of analysis of AOAC. Association of Official Analytical Chemists International, 19th edn. AOAC International, Arlington, TX
- Arena ME, Saguira FM, Manca de Nadra MC (1999) Arginine, citrulline and ornithine metabolism by lactic acid bacteria from wine. *Food Chem* 86:105–111. [https://doi.org/10.1016/S0168-1605\(99\)00133-6](https://doi.org/10.1016/S0168-1605(99)00133-6)
- Chung JC, Chou SS, Hwang DF (2004) Changes in nitrate and nitrite content of four vegetables during storage at refrigerated and ambient temperatures. *Food Addit Contam* 21:317–322. <https://doi.org/10.1080/02652030410001668763>

- Corleto KA, Singh J, Jayaprakasha GK, Patil BS (2018) Storage stability of dietary nitrate and phenolic compounds in beetroot (*Beta vulgaris*) and arugula (*Eruca sativa*) juices. *J Food Sci* 83:1237–1248. <https://doi.org/10.1111/1750-3841.14129>
- Croituru MD (2012) Nitrite and nitrate can be accurately measured in samples of vegetal and animal origin using an HPLC-UV/VIS technique. *J Chromatogr B Analyt Technol Biomed Life Sci* 911:154–161. <https://doi.org/10.1016/j.jchromb.2012.11.006>
- Dabir S, Dabir P, Somvanshi B (2005) Purification, properties and alternate substrate specificities of arginase from two different sources: *Vigna catjang* cotyledon and buffalo liver. *Int J Biol Sci* 1:114–122. <https://doi.org/10.7150/ijbs.1.114>
- Dias MI, Ferreira ICFR, Barreiro MF (2015) Microencapsulation of bioactives for food Applications. *Food Funct* 6:1035. <https://doi.org/10.1039/c4fo01175a>
- Drosou CG, Krokida MK, Biliaderis CG (2016) Encapsulation of bioactive compounds through electrospinning/electrospraying and spray drying: a comparative assessment of food-related applications. *Drying Technol* 35:139–162. <https://doi.org/10.1080/07373937.2016.1162797>
- Duarte-Delgado D, Narváez-Cuenca CE, Restrepo-Sánchez LP, Kushalappa A, Mosquera-Vásquez T (2015) Development and validation of a liquid chromatographic method to quantify sucrose, glucose, and fructose in tubers of *Solanum tuberosum* Group Phureja. *J Chromatogr B Analyt Technol Biomed Life Sci* 975:18–23. <https://doi.org/10.1016/j.jchromb.2014.10.039>
- Figuerola A, Wong A, Hooshmand S, Sanchez-Gonzalez MA (2013) Effects of watermelon supplementation on arterial stiffness and wave reflection amplitude in postmenopausal women. *Menopause* 20:573–577. <https://doi.org/10.1097/GME.0b013e3182733794>
- Fish WW (2014) The expression of citrulline and other members of the arginine metabolic family in developing watermelon fruit. *Int J Agri Innov Res* 2:2319–1473
- Gatti R, Gioia MG, Leoni A, Andreani A (2010) 2,5-Dimethyl-1H-pyrrole-3,4-dicarbaldehyde as a precolumn derivatization reagent for HPLC/UV detection of amino acids. *J Pharm Biomed Anal* 53:207–211. <https://doi.org/10.1016/j.jpba.2009.12.031>
- Guo S, Zhang J, Sun H et al (2013) The draft genome of watermelon (*Citrullus lanatus*) and resequencing of 20 diverse accessions. *Nat Genet* 45:51–58. <https://doi.org/10.1038/ng.2470>
- Ibrahim HO, Agbaje AB, Afolabi HA, Usman H (2018) Effects of temperature, pH, and agitation rate on the production of microbial L-arginase. *J Pharm Biol Sci* 13:01–12. <https://doi.org/10.9790/3008-1305050112>
- Ji B, Yang K, Zhu L, Jiang Y, Wang H, Zhou J, Zhang H (2015) Aerobic denitrification: a review of important advances of the last 30 years. *Biotechnol Bioprocess Eng* 20:643–651. <https://doi.org/10.1007/s12257-015-0009-0>
- Joshi V, Joshi M, Silwal D, Noonan K, Rodriguez S, Penalosa A (2019) Systematized biosynthesis and catabolism regulate citrulline accumulation in watermelon. *Phytochemistry* 162:129–140. <https://doi.org/10.1016/j.phytochem.2019.03.003>
- Kmiecik W, Lisiewska Z, Słupski J (2004) Effects of freezing and storing of frozen products on the content of nitrates, nitrites, and oxalates in dill (*Anethum graveolens* L.). *Food Chem* 86:105–111. <https://doi.org/10.1016/j.foodchem.2003.08.015>
- Lundberg JO, Gladwin MT, Ahluwalia A et al (2009) Nitrate and nitrite in biology, nutrition and therapeutics. *Nat Chem Biol* 5:865–869. <https://doi.org/10.1038/nchembio.260>
- Lundberg JO, Gladwin MT, Weitzberg E (2015) Strategiestoincreaseningnitricoxide signaling in cardiovascular disease. *Nat Rev Drug Discov* 14:623–641. <https://doi.org/10.1038/nrd4623>
- Madene A, Jacquot M, Scher J, Desobry S (2006) Flavour encapsulation and controlled release—a review. *Int J Food Sci Technol* 41:1–21. <https://doi.org/10.1111/j.1365-2621.2005.00980.x>
- Morris SM (2016) Arginine metabolism revisited. *J Nutr* 146:2579S–2586S. <https://doi.org/10.3945/jn.115.226621>
- Oliveira GV, Morgado M, Conte-Junior CA, Alvares TS (2017) Acute effect of dietary nitrate on forearm muscle oxygenation, blood volume and strength in older adults: a randomized clinical trial. *PLoS ONE* 12:e0188893. <https://doi.org/10.1371/journal.pone.0188893>
- Paulo F, Santos L (2017) Design of experiments for microencapsulation applications: a review. *Mater Sci Eng C Mater Biol Appl* 77:1327–1340. <https://doi.org/10.1016/j.msec.2017.03.219>
- Quek SY, Chok NK, Swedlund P (2007) The physicochemical properties of spray-dried watermelon powders. *Chem Eng Process* 46:386–392. <https://doi.org/10.1016/j.ccep.2006.06.020>
- Ray S, Raychaudhuri U, Chakraborty R (2016) An overview of encapsulation of active compounds used in food products by drying technology. *Food Biosci* 13:76–83. <https://doi.org/10.1016/j.fbio.2015.12.009>
- Rimando AM, Perkins-Veazie PM (2005) Determination of citrulline in watermelon rind. *J Chromatogr A* 1078:196–200. <https://doi.org/10.1016/j.chroma.2005.05.009>
- Sampaio GLA, Pacheco S, Ribeiro APO, Galdeanob MC, Gomes FS, Tonon RV (2019) Encapsulation of a lycopene-rich watermelon concentrate in alginate and pectin beads: characterization and stability. *LWT—Food Science and Technology* 1116. <https://doi.org/10.1016/j.lwt.2019.108589>
- Santamaria P (2006) Nitrate in vegetables: toxicity content intake and EC regulation. *J Sci Food Agric*. 86:10–17. <https://doi.org/10.1002/jsfa.2351>
- Soares R, Colosio A, Murias JM, Pogliaghi S (2019) Non-invasive and in vivo assessment of upper and lower limb skeletal muscle oxidative metabolism activity and microvascular responses to glucose ingestion in humans. *Appl Physiol Nutr Metab*. <https://doi.org/10.1139/apnm-2018-0866>
- Speranza B, Petrucci L, Bevilacqua A et al (2017) Encapsulation of active compounds in fruit and vegetable juice processing: current state and perspectives. *J Food Sci*. 82:1291–1301. <https://doi.org/10.1111/1750-3841.13727>
- Sugino T, Shirai T, Kajimoto Y, Kajimito O (2008) L-Ornithine supplementation attenuates physical fatigue in healthy volunteers by modulating lipid and amino acid metabolism. *Nutr Res* 28:738–743. <https://doi.org/10.1016/j.nutres.2008.08.008>
- Tamme T, Reinik M, Püssa T, Roasto M, Meremäe K, Kiis A (2010) Dynamics of nitrate and nitrite content during storage of homemade and small-scale industrially produced raw vegetable juices and their dietary intake. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 27:487–495. <https://doi.org/10.1080/19440040903439796>
- Tarazona-Díaz MP, Martínez-Sánchez A, Aguayo E (2017) Preservation of bioactive compounds and quality parameters of watermelon juice enriched with L-citrulline through short thermal treatment. *J Food Qual* 1–10. <https://doi.org/10.1155/2017/3283054>
- Tarazona-Díaz MP, Viegas J, Moldao-Martins M, Aguayo E (2011) Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. *J Sci Food Agric* 91:805–812. <https://doi.org/10.1002/jsfa.4250>
- Verma A, Singh SV (2015) Spray drying of fruit and vegetable juices—a review. *Crit Rev Food Sci Nutr* 55:701–719. <https://doi.org/10.1080/10408398.2012.672939>
- Volino-Souza M, Oliveira GV, Alvares TS (2018) A single dose of beetroot juice improves endothelial function but not tissue oxygenation in pregnant women: a randomised clinical trial. *Br J Nutr* 120:1006–1013. <https://doi.org/10.1017/S0007114518002441>