Storage stability of L-citrulline in cucumber (Cucumis sativus) and watermelon (Citrullus lanatus) juices

Estabilidade em armazenamento de L-citrulina em sucos de pepino (Cucumis sativus) e melancia (Citrullus lanatus)

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ABSTRACT
Since foods rich in L-citrulline are important for vascular health, it is important to determine the amount of this compounds in foods present in the human diet. Therefore, the present study evaluated storage stability of L-citrulline in cucumber rind and pulp juices and to compare them with watermelon rind and pulp juices. The L-citrulline stability was evaluated for 12 days at refrigerated condition (4 ºC). Since L-arginine is an amino acid involved in L-citrulline metabolism, L-arginine content was also evaluated.

On the initial day, L-citrulline content in cucumber rind and pulp was lower when compared to watermelon rind and pulp juices, respectively. A significative reduction in L-citrulline content in cucumber rind and pulp and watermelon pulp juices was observed in day 6 and day 12 when compared to the first day. However, L-citrulline content was stable for 12 days in watermelon rind juice. A significative reduction in L-arginine content was also observed in cucumber pulp and watermelon pulp juices, but not in cucumber rind and watermelon rind juices in day 6 and day 12 when compared to the first day. The results suggested that L-citrulline in cucumber rind and pulp and watermelon pulp juices were unstable at refrigerated conditions for 12 days.

Keywords: cucumber, watermelon, L-citrulline, storage stability, bioactive compounds.

RESUMO
Visto que a ingestão de alimentos ricos em L-citrulina é importante para a saúde vascular, é relevante determinar o teor desses compostos nos alimentos presentes na dieta humana. Portanto, o presente estudo avaliou o teor e a estabilidade de armazenamento da L-citrulina nos sucos da casca e polpa do pepino e da melancia. A estabilidade da L-citrulina foi avaliada por 12 dias sob refrigeração (4 ºC). Como a L-arginina é um aminoácido envolvido no metabolismo da L-citrulina, o conteúdo de L-arginina também foi avaliado. No dia inicial, o teor de L-citrulina na casca e na polpa do pepino foi menor comparado à casca e polpa da melancia, respectivamente. Foi observado uma redução significativa do teor de L-citrulina na casca e polpa do pepino e da polpa da melancia no dia 6 e no dia 12 em relação ao dia inicial. No entanto, o teor de L-citrulina foi estável por 12 dias no suco de casca de melancia. Uma redução significativa no teor de L-arginina também foi observada na polpa do pepino e da melancia, mas não na casca de pepino e da melancia no dia 6 e dia 12 quando comparados ao primeiro dia. Os resultados sugeriram que a L-citrulina na casca e na polpa do pepino e na polpa da melancia foram instáveis em refrigeração por 12 dias.

Palavras-chave: pepino, melancia, L-citrulina, estabilidade em armazenamento, compostos bioativos

1 INTRODUCTION

Cardiovascular diseases (CVD) is a group of cardiac and vascular disorders that constitute the principal cause of mortality worldwide (Mensah et al, 2019). Due to impact of CVD on health, studies have been evaluating new alternatives to prevent this condition through diet. Several bioactive compounds have been investigated, mainly compounds that act on endothelial function, such as arginine, citrulline and nitrate (Oliveira et al, 2017; Volino-Souza et al, 2018; Figueroa et al. 2013).

Citrulline is a non-essential amino acid that has been investigated on cardiovascular health due to its possible role as an essential intermediate in the biosynthesis of nitric oxide (NO) from L-arginine (Allerton et al. 2018). NO is an important molecule involved in vascular tone regulation (Lundberg et al, 2008). Watermelon fruit (*Citrullus lanatus*) is one of main citrulline source from diet and studies have shown improvement in vascular function after watermelon ingestion (Massa et al. 2016; Figueroa et al. 2016; 2013). Besides watermelon, citrulline has been found in other foods, such as cucumber (Cucumis sativus) (Fish 2012).

Although watermelon represent an important source of citrulline from diet, evaluating other food sources of this compound is important since ingestion of variety of vegetables and fruits is necessary to improve diet quality and, hence, cardiovascular health (Keim et al, 2014). To promote healthy eating patterns, the Dietary Guidelines for Americans 2015-2020 recommends ingestion of 2½ cup-equivalents of vegetables per day (Dietary Guidelines for Americans, 2015). Furthermore, vegetables and fruits choices should be varied to provide all of the nutrients and potential health benefits.

Previous studies have investigated the effect of ingestion of watermelon and cucumber in juice on health (Furtado et al., 2021; Figueroa et al., 2013; Mukherjee et al., 2013). Fruits and vegetables in juice form have many benefits, including their ease of transport and consumption, as well as their attractive taste (Benton and Young, 2019). However, evaluating the stability of bioactive compounds presented in juice is important when considering their functional propriety. Based on this consideration, the present study evaluated L-citrulline content in cucumber and watermelon, as well as the stability of L-citrulline after having been stored for 12 days under refrigerated conditions.

2 MATERIALS AND METHODS

2.1 MATERIALS

Amino acids (L-arginine monohydrochloride and L-citrulline monohydrochloride), 2,5-Dimethyl-1H-pyrrole-3,4-dicarbaldehyde and triethylamine were purchased from Sigma-Aldrich (Brazil). Methanol and acetonitrile were purchased from Tedia (Brazil).

2.2 CUCUMBER AND WATERMELON RIND AND PULP JUICE PRODUCTION

All the cucumber used in the present study belongs to the Cucurbitaceae family and *C. sativus* species and the watermelon used belongs to the Cucurbitaceae family and the C. *lanatus* species. Cucumber 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. The rind and pulp of the cucumber and watermelon were separated and put into a centrifuge blender (Model CE700, Black & Decker, Baltimore, MD) for cucumber rind juice (CR), cucumber pulp juice (CP), watermelon rind juice (WR) and watermelon pulp juice (WP) production. The juices were filtered first in organza for elimination of suspended solids. The citrulline content and stability of watermelon was evaluated to allow comparison with cucumber since watermelon is a main source of L-citrulline (Fish 2012).

2.3 STORAGE STABILITY PROCEDURES

The juices were stored in 1.5 mL microtubes for every day of the analysis to avoid possible contamination of the samples. The samples were stored for 12 days at refrigerated (4 °C) conditions. Samples were taken for analysis at 0, 6, and 12 days. Initial analysis after production of the samples was considered the initial day (day 0). Refrigeration was chosen to mimic the usual conditions of juice storage. Two cucumbers and three watermelons were analyzed in duplicate.

2.4 CITRULINE AND ARGININE ANALYSIS

The amino acids L-citruline and L-arginine content in CP, CR, WP and WR were analyzed as previously described by Gati et al. (2010), but with some modification. Since L-arginine is involved in L-citrulline metabolism, this amino acid was also analyzed in the current study (Arena et al. 1999). Briefly, 100 µL of each sample were mixed with 100 µL of 1.5 M perchloric acid. Afterwards, 750 µL of deionized water and 50 µL of 2 M potassium carbonate were added following the centrifugation at 10,000 g for 1 min. After centrifugation, 50 µL of supernatant was collected and 50 µL of deionized water and 40 µL of 2,5-dimethyl-1H-pyrrole-3,4-dicarbaldehyde were added and 10 min were waited. Finally, 360 µL of 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, Japan) was fit with C18 column (250 x 4.6 mm ACE), guard column (5- 131 mm, 50 x 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.

2.5 STATISTICAL ANALYSIS

To identify differences in the L-citrulline and L-arginine content between cucumber (rind and pulp) and watermelon (rind and pulp) a two-way ANOVA was performed. In addition, to identify differences in the levels of amino acids (L-citrulline and L-arginine) during storage a two-way repeated measures 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.

3 RESULTS AND DISCUSSION

At initial day, a lower L-citrulline concentration in CP compared to WP (CP: 0.83 ± 0.34 vs. WP: 9.59 ± 2.15, p<0.000) and in CR compared to WR (CR: 0.60 ± 0.34 vs. WR: 7.52 ± 0.98, p<0.000) was observed. In addition, a lower L-arginine concentration in CP compared to WP (CP: 1.17 ± 0.19 vs. WP: 4.33 ± 0.54, p<0.000) and in CR compared to WR (CR: 0.68 ± 0.25 vs. WR: 2.15 ± 0.11, p<0.000) was observed. (Table 1). The findings are in agreement with Fish et al. (2012) that observed higher content of L-citrulline in watermelon than cucumber. L-citrulline is an important amino acid involved in nitric oxide synthesis and studies have shown positive effects of watermelon juice (a food source of L-citrulline) on cardiovascular health (Figueroa et al., 2013). In addition to its properties in human health, L-citrulline has also important functions for plants. Akashi et al. (2001) showed that L-citrulline contributes to oxidative stress tolerance under dry climates as a hydroxyl radical scavenger and Wang et al. (2014) showed that L-citrulline metabolic genes were found to be upregulated during dry climate.

Table 1. Values of L-citrulline and L-arginine in cucumber and watermelon at initial day.

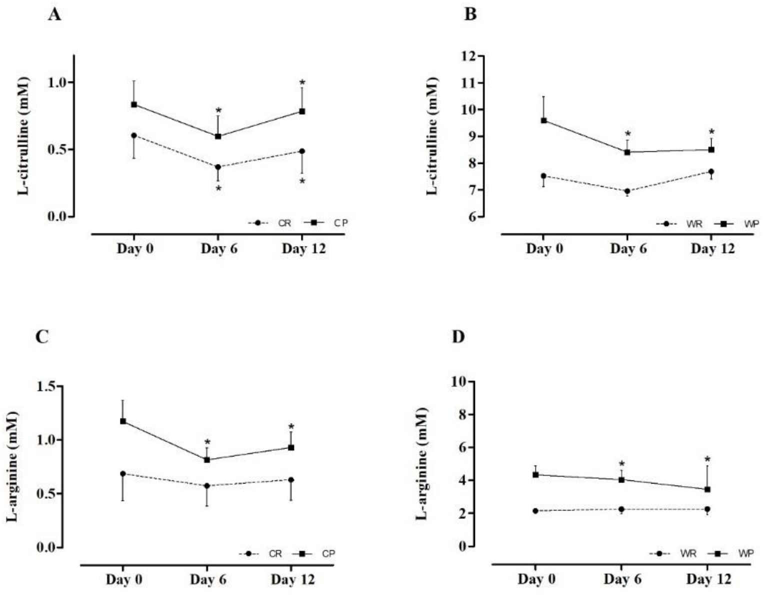
	L-citrulline (mM)	**L-arginine (mM)**
Cucumber pulp	0.83 ± 0.34	1.17 ± 0.19
Cucumber rind	0.60 ± 0.34	0.68 ± 0.25
Watermelon pulp	9.59 ± 2.15$^{\#}$	4.33 ± 0.54$^{\#}$
Watermelon rind	7.52 ± 0.98$^{*\#}$	2.15 ± 0.11$^{*\#}$

The values are expressed as mean ± SD. The symbol * denotes significant difference from pulp and the symbol # denotes significant difference from cucumber.

Concentration of bioactive compounds has been evaluated in pulp and rind of fruits, since rind could also be an important source of nutrients. In the current study, differences in L-citrulline (CP: 0.83 ± 0.34 vs. CR: 0.60 ± 0.34, p=0.811) and L-arginine (CP: 1.17 ± 0.19 vs. CR: 0.68 ± 0.25, p=0.060) between CP and CR was not observed. However, WP presented a higher L-citrulline (WP: 9.59 ± 2.15 vs. WR: 7.52 ± 0.98, p=0.016) and L-arginine (WP: 4.33 ± 0.54 vs. WR: 2.15 ± 0.11, p<0.000) when compared to WR. Previous studies have evaluated L-citrulline content in different parts (rind vs pulp) of watermelon (Akashi et al., 2017; Tarazona-Díaz et al. 2011; Rimando and Perkins-Veazie 2005). Akashi et al. (2017) and Rimando and Perkins-Veazie (2005) showed that watermelon rind presented higher L-citrulline content when compared to pulp. However, Joshi et al. (2019) showed that there was no difference in L-citrulline content between watermelon rind and pulp. The difference in the L-citrulline distribution between rind and pulp is still not well understood. However, it has been suggested that growing conditions (i.e. temperature, water supply) may strongly influence L-citrulline content between pulp and rind (Fish 2012).

Cucumber and watermelon ingestion in juice form have been stimulated due to possible effects on human health (Figueroa et al., 2013; Mukherjee et al., 2013). For this reason, it is important to evaluate L-citrulline stability in cucumber juice and watermelon juice during storage. During storage, it was observed that CP showed a reduction in L-citrulline content at day 6 (0.59 ± 0.30 vs. 0.83 ± 0.34 mM, p=0.011) and 12 (0.78 ± 0.35 mM, p=0.013) compared to day 0. The same characteristic was observed in CR at day 6 (0.37 ± 0.20 vs. 0.60 ± 0.34 mM, p=0.012) and at day 12 (0.48 ± 0.33 mM, p<0.000). WP during storage showed reduction in L-citrulline content at day 6 (7.52 ± 1.54 vs. 9.59 ± 2.15 mM, p<0.000) and at day 12 (7.37 ± 1.37 mM, p<0.000). However, changes in L-citrulline in WR at day 6 (6.96 ± 0.49 vs. 7.52 ± 0.98 mM, p=0.182 and at day 12 (7.68 ± 0.67 mM, p=1.000) were not observed (Figure 1). Although a higher L-citrulline content was observed in WP on the initial day, L-citrulline was not stable over the 12-day period; whereas, WR presented lower L-citrulline content, in spite of being stable over 12 days.

Figure 1. L- Mean of L-citrulline in (A) cucumber pulp and rind, (B) watermelon pulp and rind, and L-arginine (C) cucumber pulp and rind, (D) watermelon pulp and rind during storage for 12 days. * denotes significative difference from Day 0.



Previous studies have demonstrated the potential effect of L-citrulline supplementation (> 3 g of citrulline/day) on cardiovascular health (Bailey et al., 2016; Figueroa et al., 2013). In the present study, it was observed that approximately 23% loss of L-citrulline content in watermelon pulp after 12 days of storage (Day 0: 9.59 ± 2.15 vs. Day 12: 7.37 ± 1.37 mM, p<0.000). This loss may be relevant in clinical studies investigating the health benefits of watermelon as a dietary source of L-citrulline. For example, Bailey et al. (2016) observed increased plasma nitrite (a biomarker of NO synthesis) after consuming watermelon juice (containing 3.4 g of L-citrulline) for 16 days. Assuming 23% losses in L-citrulline, a decline might be expected in the content of L-citrulline from 3.4 g to 2.6g using the Bailey et al.'s study as an example, which is indeed a lower concentration than that reported to promote changes in vascular health (> 3 g of citrulline/day).

To promotes greater L-citrulline stability in CR, CP and WP, food technologies might be applied, such as microencapsulation, which is an important method to preserve bioactive compounds in several juices (Paulo and Santos, 2017). For example, Gurak et al. (2013) showed that the phenolic compound content in grape juice powder produced by freeze-drying (a microencapsulation method) remained stable during storage at room temperature for 120 days. Regarding L-citrulline stability in WR and WP, the differences observed may be explained by differences in the action of enzymes involved in its catabolism. Gou et al. (2013) suggested that L-citrulline in WP can be regulated by arginosuccinate synthase and arginosuccinate lyase-like genes, but it is unlikely to occur in WR.

L-arginine is an amino acid produced during L-citrulline catabolism (Joshi et al., 2019) and, for this reason, it is important evaluate L-arginine content during storage. It was observed that L-arginine course during storage was similar for cucumber and watermelon. L-arginine content in CP reduced at day 6 (0.81 ± 0.19 vs. 1.17 ± 0.19 mM, p<0.000) and at day 12 (0. 93 ± 0.14 mM, p=0.001) compared to day 0. However, L-arginine content in CR was stable at day 6 (0.57 ± 0.18 vs. 0.68 ± 0.25 mM, p=0.088) and at day 12 (0.63 ± 0.19 mM, p=0.373) compared to day 0. L-arginine content in WP reduced at day 6 (4.05 ± 0.53 vs. 4.33 ± 0.54 mM, p=0.008) and at day 12 (3.46 ± 1.39 mM, p=0.028) compared to day 0. However, L-arginine in WR was stable at day 6 (2.25 ± 0.26 vs. 2.15 ± 0.11 mM, p=0.590) and at day 12 (2.26 ± 0.35 mM, p=1.000) (Figure 1). The reduction in L-arginine content in CP and WP may be explained by enzyme activity. Arginase is an enzyme that acts on L-arginine, which leads to production of other amino acids, such as ornithine (Joshi et al., 2019). In addition to arginase, nitric oxide synthase (NOS) can also act upon the arginine content in plants. In this reaction, nitric oxide and L-citrulline are produced. In plants, nitric oxide performs important roles for the establishment of symbiosis, the induction of phytoalexin production, and the regulation of plant growth and development (Yamasaki et al., 2016).

4 CONCLUSION

In conclusion, the results demonstrated that L-citrulline present in cucumber (rind and pulp) and watermelon pulp were not stable during storage at refrigerated conditions for 12 days. However, L-citrulline in watermelon rind remained stable for 12 days. Considering that fruits and vegetable ingestion has been encouraged due to their possible effects on health, the results of the current study suggest that refrigerated conditions were not effective in promoting L-citrulline stability in cucumber juice and watermelon pulp during storage for 12 days.

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