Validation of a HPLC-DAD method for quantification of ascorbic acid in mixed juice powder of acerola and seriguela

Validação de um método HPLC-DAD para quantificação de ácido ascórbico de suco misto em pó de acerola e seriguela

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ABSTRACT
The objective of this work was to optimize extraction process and to validate the HPLC-DAD method for quantification of ascorbic acid content in mixed juice powder of acerola and seriguela with different dextrose equivalent maltodextrin (5, 10, 15) as an encapsulating agent. The influence of the extraction variables was studied through factorial design. Control variables were mass, percent of orthophosphoric acid and stirring time. Chromatographic method was validated in terms of precision, selectivity, linearity and accuracy. Chromatographic and titrimetry results were compared for all dextrose equivalent. Mass and percentage of orthophosphoric acid presented $p < 0.05$. There were no significant differences among the dextrose equivalents ranging from 2,830.1 mg/100 g to 2,931.61 mg/100 g of ascorbic acid. Both values, intra and intermediary precision test coefficients of variation were less than 5%. The correlation coefficient was 0.99. Respective value of limit of detection was 0.001 mg/mL and for quantification was 0.003 mg/mL. The application of HPLC-DAD method can be considered acceptable for quantification ascorbic acid in fruit powder.

Keywords: factorial design, validation, hplc-dad, vitamin c, spray drying.

RESUMO
O objetivo deste trabalho foi otimizar o processo de extração e validar o método HPLC-DAD para quantificação do teor de ácido ascórbico em suco em pó misto de acerola e seriguela com diferentes dextrose equivalente maltodextrina (5, 10, 15) como agente encapsulante. A influência das variáveis de extração foi estudada por meio de planejamento fatorial. As variáveis de controle foram massa, porcentagem de ácido ortofosfórico e tempo de agitação. O método cromatográfico foi validado em termos de precisão, seletividade, linearidade e exatidão. Os resultados cromatográficos e de titulação foram comparados para todos os equivalentes de dextrose. A massa e a porcentagem de ácido ortofosfórico apresentaram $p < 0,05$. Não houve diferenças significativas entre os equivalentes de dextrose variando de 2.830,1 mg / 100 g a 2.931,61 mg / 100 g de ácido ascórbico. Ambos os valores, coeficientes de variação do teste de precisão intra e intermediário foram menores que 5%. O coeficiente de correlação foi de 0,99. O respectivo valor do limite de detecção foi 0,001 mg / mL e para quantificação foi 0,003 mg / mL. A aplicação do método HPLC-DAD pode ser considerada aceitável para quantificação do ácido ascórbico em pó de frutas.

Palavras-chave: planejamento fatorial, validação, hplc-dad, vitamina c, spray drying

1 INTRODUCTION

Brazil has diverse native fruits with great nutritional, sensory and economic potential that are not exploited as food. By 2050, the availability and production of food

should double. The interest in native fruits and their products provides greater diversification in food systems, increases production and consequently reduces waste. Some native Brazilian fruit species provide relevant sensory characteristics and high concentration of nutrients and bioactive compounds, attracting the interest of the international market and the scientific community (CURI et al., 2019; INFANTE et al., 2016).

Acerola is one of the most important natural sources of vitamin C, with amounts ranging from 342.47 and 2,649.72 mg/100g (MARANHÃO RIBEIRO et al., 2018; SOARES; OLIVEIRA, 2021). Seriguela (*Spondias purpúrea* L.) is considered a source of phenolic compounds (SILVA, R. V. et al., 2016). The mixing of fruit juices may add nutritional value. Several studies have used acerola as a vitamin C enrichment agent in various fruit products (PRAKASH, A. et al., 2016; SILVA, et al., 2017). However, these fruits are highly perishable and require processing to increase their shelf life.

The microencapsulation of fruit juice and spray drying can bring numerous advantages, such as extending the shelf life, preserving bioactive compounds, reducing packaging costs and the possibility of using them as ingredients in other products. Vitamin C is a necessary nutrient for humans, its deficiency can cause scurvy and consumption in appropriate amounts can reduce the risk of cancer. However, a high consumption of vitamin C leads to diarrhea, urinary stones, and stomach convulsion. However, its activity can be impaired when exposed to high temperatures, light and oxygen, during processing and storage (TARRAGO-TRANI; PHILLIPS; COTTY, 2012; TASHKHOURIAN; VALIZADEH; ABBASPOUR, 2019). Encapsulating agents, such as maltodextrin, have been widely used to preserve vitamin C during drying. Maltodextrin is the most commonly used encapsulating agent because it is inexpensive and suitable for ingredients that have the potential for oxidation.

There are several methods for quantification of ascorbic acid which is divided into conventional (official) methods and instrumental methods. Choosing a method is an important step in food analysis. Official methods that have been most recommended for fruit juices is that from the Association of Official Analytical Chemists - AOAC. This is based on the reduction of a 2.6 dye solution dichloro indofenol-DCFI by ascorbic acid. Chromatographic methods are able to selectively detect various compounds and have been widely used to quantify ascorbic acid in various foods, however it is not used as a standard method (BRAININA et al., 2020; TARRAGO-TRANI; PHILLIPS; COTTY, 2012).

The daily dose to prevent scurvy is 46mg, in Brazil the recommended daily intake is 60mg. However, this indicator depends on the accuracy of the food composition data, which are still imprecise and contradictory for micronutrients. The main challenge in the determination of vitamin C is to quantify it with analytical safety due to its oxidation potential. Considering that mixed juice powder (MJP) of acerola and seriguela has a high content of ascorbic acid and that this compound is susceptible to oxidation, this study aimed to evaluate the extraction process of ascorbic acid using factorial design (mass, percent of orthophosphoric acid and stirring time); to apply to the HPLC-DAD method for quantification of this compound in MJP of acerola and seriguela with different dextrose equivalent maltodextrin (5, 10, 15) as an encapsulating agent; besides, to compare with official method (OFFICIAL METHODS OF ANALYSIS, 2005).

2 MATERIALS AND METHOD

2.1 MATERIALS

Seriguela (*Spondia purpurea* L.) and acerola (*Malphighia emarginata* DC) from the Sertaneja variety of mature maturation stage were purchased from the Food Supply Center of Pernambuco. The carrier agents used were as follows: maltodextrin with 4-7% dextrose equivalent (5DE) GLOBE from Ingredion (São Paulo, Brazil); maltodextrin with 9-12% dextrose equivalent (10DE) MOR-REX® 1910 from Corn Products (Mogi-Guaçu, Brazil); and maltodextrin 15-17% dextrose equivalent (15DE) MOR-REX® 1914 from Corn Products (Mogi-Guaçu, Brazil). Two commercial brands of orange juice powder (OJP 1 and OJP 2) were purchased from supermarkets to be used in the evaluation of the analytical protocol used.

Reagents and solvents

HPLC-grade orthophosphoric acid (H_3PO_4), potassium phosphate monobasic (KH_2PO_4), acetonitrile (CH_3CN) and methanol (CH3OH) were obtained from Merck (Switzerland). L- ascorbic acid was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was obtained from a 5 MilliQ integral system (Millipore). An Innoval C_{18} (4.6 mm x150 mm, 3 µm, 100 Å) column was used.

2.2 METHODS

Preparation of feed mixture and spray drying conditions

For the preparation of the feed mixture, the pulps were mixed in the proportion of 60% of acerola and 40% of seriguela, maltodextrin (5 DE, 10 DE, 15 DE) was added at a concentration of 20% and water in a 1:1 pulp-to-water ratio. This mixture was homogenized in a kitchen blender (Wallita) to obtain the mixed Juice (MJ). Drying was performed in a mini spray dryer, model LM MSD 1.0 (Labmaq do Brasil, Riberão Preto, SP, Brazil), with a nozzle atomization system under the following conditions selected from previous studies: temperature of 140 ºC, flow rate of 0.60 L/h, nozzle of 1.2 mm in diameter, air flow of 30 M3/h and air pressure of 0.6 bar. After drying, the MJP of acerola and seriguela with different dextrose equivalents was weighed and stored in hermetically sealed glass pots (250 mL) protected from light in a dry environment containing silica gel as a desiccant agent at a temperature of 22± 2 °C until analyses were performed.

Extraction procedure (pilot study)

The procedure of ascorbic acid extraction was based on the methodology by Islam et al. (ISLAM et al., 2016) with some modifications. The following conditions were evaluated: sample mass; use of centrifugation; and number of filtrations. Initially, two sample mass quantities (5 g and 1.5 g) of MJP of acerola and seriguela were tested, and approximately 80 mL of 3% orthophosphoric acid was added. This material was vortexed for 1 minute and then vacuum filtered using fast-speed quantitative filter paper (Fmaia brand retention of 20-25 microns). However, due to the formation of a suspension on the surface of the filter, good filtration was not achieved. To overcome this difficulty, the following steps were added: shaking for 3 minutes; centrifugation at 4000 rpm for 15 minutes; and vacuum filtration. However, in the chromatographic step, the obtained peaks presented purity of less than 90%, and a second filtration step was necessary before injection into the apparatus using a 0.45 µm cellulose acetate membrane. For a better adjustment of the absorptivity, masses of 0.5, 0.05 and 0.005 g were tested.

Factorial design

The factorial design was conducted to verify the effect of the extraction variables on the ascorbic acid quantification. In this study, a 23 complete factorial design with 8 factor points and 3 central points was used. A total of 11 assays was proposed to evaluate the influence of the following independent variables: sample mass (0.06, 0.1 and 0.14 g); percent of orthophosphoric acid (1, 2 and 3%); and stirring time (1, 2 and 3 minutes). All possible combinations were investigated considering the effects of interactions. For this

case, the factorial scheme was complete. The interactions were evaluated at two levels, namely low (-1) and high (+1), providing a greater possible number of degrees of freedom for the residue. A first order model was used to adjust the experimental data (Eq. 1) as follows:

$$Y = \beta 0 + \beta 1M + \beta 2A + \beta 3\,T + \beta 4\,MA + \beta 5\,MT + \beta 6\,AT \quad \text{(Eq 1)}$$

where Y is the ascorbic acid content; β1, β2 and β3 are the linear regression coefficients; and β4, β5 and β6 are the coefficients of interactions.

The analysis of variance (ANOVA) with the lack of fit test (F- test) as well as the regression coefficient determination and the response surface were processed using the *Statistica* program (*Statistica* 10.0, Statsoft, Tulsa, USA).

Preparation of samples for analysis

For the analysis, the best assay condition was used. Approximately 0.14 g of sample was weighed, and 80 ml of 3% orthophosphoric acid was added. The sample was homogenized using a Vortex for 1 minute followed by centrifugation at 4000 rpm for 15 minutes at 4 °C. The supernatant was vacuum filtered (Fmaia brand, 20-25 µm, fast-speed quantitative filter paper). The filtrate was measured in a 100 ml volumetric flask. An aliquot was removed and placed in a vial. The injection volume was 20 µl. Throughout the analysis, the temperature was controlled between 23 and 25 °C, and the sample was protected from light. To verify the quality of the processing, quantification of ascorbic acid was also performed in the MJ, which was subjected to spray drying for calculating the retention. Retention of the ascorbic acid content (%) was calculated using the following formula:

$$AAR = [(\text{ascorbic acid content in the MJP of acerola and seriguela x } 100/ \text{ ascorbic acid content in the MJ of acerola and seriguela}] \quad \text{(Eq 2)}$$

Chromatographic analysis

The HPLC system was a Shimadzu (Shimadzu, Japan) composed of a SPD-20AV diode array detector (DAD), C-20 AT model quaternary pump, CTO-20AT column oven and SIL 20AT autoinjector. The entire system was monitored by a CBM-20A model controller and operated with LC Solution software (version 1.25, from Shimadzu, Japan). Samples, standards and tests were measured at a wavelength of 254 nm. The mobile phases used were phosphate buffer (pH 3.0) and methanol at a ratio of 95:5 (v/v). All solutions were prepared at 25 °C and were filtered before use with a Millipore filter (pore

size of 0.22 µm). The elution was performed in the isocratic mode with the total operating time not exceeding 10 min.

Validation of the method

To evaluate the method developed, the following parameters were assessed: selectivity, linearity, limit of detection (LD), limit of quantification (LQ), precision and recovery rate. The selectivity was evaluated for separating the compound of interest from other compounds. To ensure that the method generated reliable and interpretable information about the sample, a spectral scan was performed in three regions of the peak (beginning, apex and end) to confirm the maximum detection wavelength (DAD), purity index and similarity of the samples with the standard.

The stock solution for the calibration curve was prepared at the concentration of 2mg mL^{-1} ascorbic acid. The standard was diluted with 2 ml of 3% orthophosphoric acid, and its volume filled with mili-Q water. The linearity of the method was estimated using a 5-point calibration curve (0.05, 0.01, 0.008, 0.005 and 0.001mg/mL) within the expected concentration range. The linearity of the curve was evaluated by linear regression analysis, plotting peak areas versus standard concentrations. The standard deviation of the angular coefficient b, the intercept of the linear coefficient a, and the value of r were measured to verify the quality of the calibration curve.

The LD represents the lowest concentration of analyte that can be detected in the calibration curve, obtaining a signal-to-noise ratio of 3:1, while the LQ represents the lowest concentration that can be quantified of the analyte, producing a signal-to-noise ratio of 10:1. The LD was expressed as follows: LD = 3.3 * s/S; where s is the estimate of the standard deviation of the response, which can be the estimate of the blank standard deviation, the regression line or the linear coefficient of the equation; and S is the slope or angular coefficient of the analytical curve. The LQ was expressed as follows: LQ = 10 * s/S, which is determined by the relationship between the standard deviation of the calibration curve and its angular coefficient, using the multiplier factor 10 as suggested by ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION- ICH, 1997).

Reproducibility was determined by calculating the standard deviation of five injections of the sample (MJP, MJ and OPJ) on the same day and on different days. Relative standard deviation (RSD) and standard deviation (SD) of the replicates were calculated.

The recovery value was estimated using the following equation: $R = (C1 - C2) / C3 * 100$; where C1 is the concentration measured in a sample with ascorbic acid addition; C2 is the concentration measured in a sample without addition of ascorbic acid; and C3 is the concentration added. The concentration added was 2 mg/mL ascorbic acid.

Ascorbic acid analysis by titration

The results were validated by comparing the ascorbic acid concentrations obtained using the chromatographic method with those obtained using the official AOAC titrimetric method (OFFICIAL METHODS OF ANALYSIS, 2005). The ascorbic acid content was quantified using 2,6-dichlorophenol indophenol (DCFI) with modification using oxalic acid solution as solvent, replacing metaphosphoric acid. The samples were extracted with 5 mL of 80% oxalic acid and 20% acetic acid and titrated with 0.05% 2,6-dichlorophenol-indophenol solution in sodium bicarbonate, until the presence of a rosy color equal to the standard, for more than 5s. The indophenol solution was standardized at each analyze with the L-AA solution. All the determinations were repeated three times. The results were expressed as g ascorbic acid per 100 g of dry base (db).

Statistical analysis

The results obtained for the vitamin C concentrations for the different concentrations of maltodextrin were evaluated using ANOVA, and the difference between the means was analyzed using Tukey's test at a confidence interval of 0.95. Statistica software (version 10.0) was used for all statistical analyses. The data were transformed on a logarithm basis after homoscedasticity assessment and are presented as the mean ± standard deviation (SD) and relative standard deviation (RSD) of the samples.

3 RESULTS AND DISCUSSION

Factorial design

The significant variables in this study were the sample mass, extracting solution concentration, mass interaction with extracting solution concentration and the interaction of the extracting concentration with stirring time because they presented p-values <0.05. The pure error was 0.00001, revealing that the values of the central points were close. The percentage of explained variation was 99.6%, and F calculated was greater than F tabulated. Thus, it can be concluded that the model fit well the experimental data.

In the present study, the ascorbic acid content increased with the concentration of the extracting solution (Figure1), and similar behavior was observed by (GERGORIC; BARRIER; RETEGAN, 2019). These authors explain that the influence of the concentration of orthophosphoric acid can be explained by the drop in pH, which allows better extraction conditions. This increase in the ascorbic acid content is due to the presence of H + in the extraction solution, which hinders its ionization and prevents irreversible hydrolysis to 2,3-diketogonic acid.

Fig 1. Effect of orthophosphoric acid concentration and mass under ascorbic acid content. Aa ascorbic acid

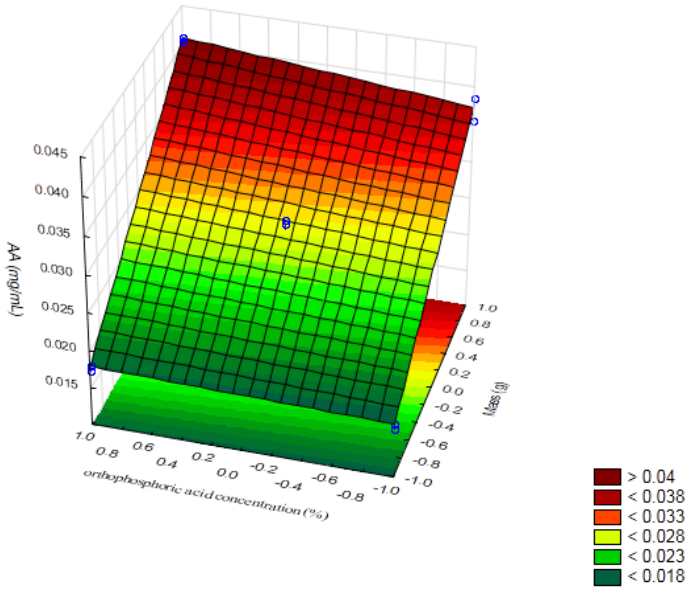
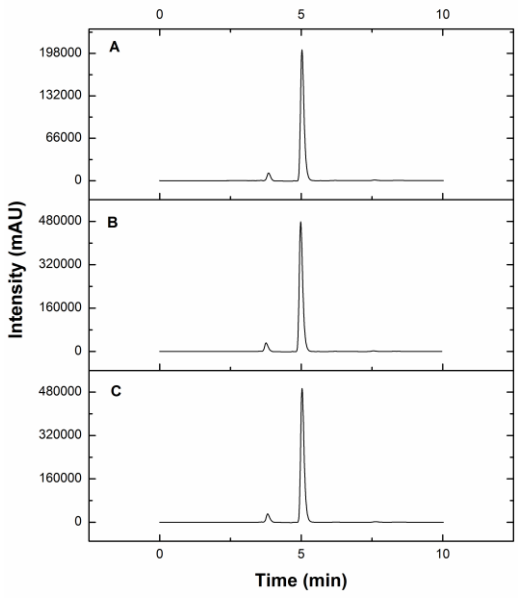


Figure 2 shows the chromatograms obtained from the three main conditions evaluated in factorial design. Figures 2B and 2C corroborate the statistical results. With a mass of 0.14 g and 3% orthophosphoric acid, the best extraction results were obtained as confirmed by the intensity of the ascorbic acid peaks. The stirring time did not influence the protocol optimization as the same concentration of ascorbic acid was obtained with 1 to 3 minutes of stirring. Grosso et al. (GROSSO et al., 2014) reported that various factors can influence the extraction, such as stirring time, type of extractor solution, type of extractor solvent and solid rate. The highest ascorbic acid levels were obtained with the following combinations in relation to the complete factorial design: 0.14 g of sample mass, 3% extracting solution concentration, and stirring times of 1 and 3 minutes

Fig.2. Chromatograms obtained from the three main conditions evaluated in the factorial design. (A) Concentration of ascorbic acid with 0.06 g of mixed juice powder of acerola and seriguela, 1% orthophosphoric acid and stirring time of 1 minute. (B) Concentration of ascorbic acid with 0.14 g of mixed juice powder of acerola and seriguela, 3% orthophosphoric acid and stirring time of 1 minute. (C) Concentration of ascorbic acid with 0.14 g of mixed juice powder of acerola and seriguela, 3% orthophosphoric acid and stirring time of 3 minutes.



Influence of the addition of maltodextrin as an encapsulating agent

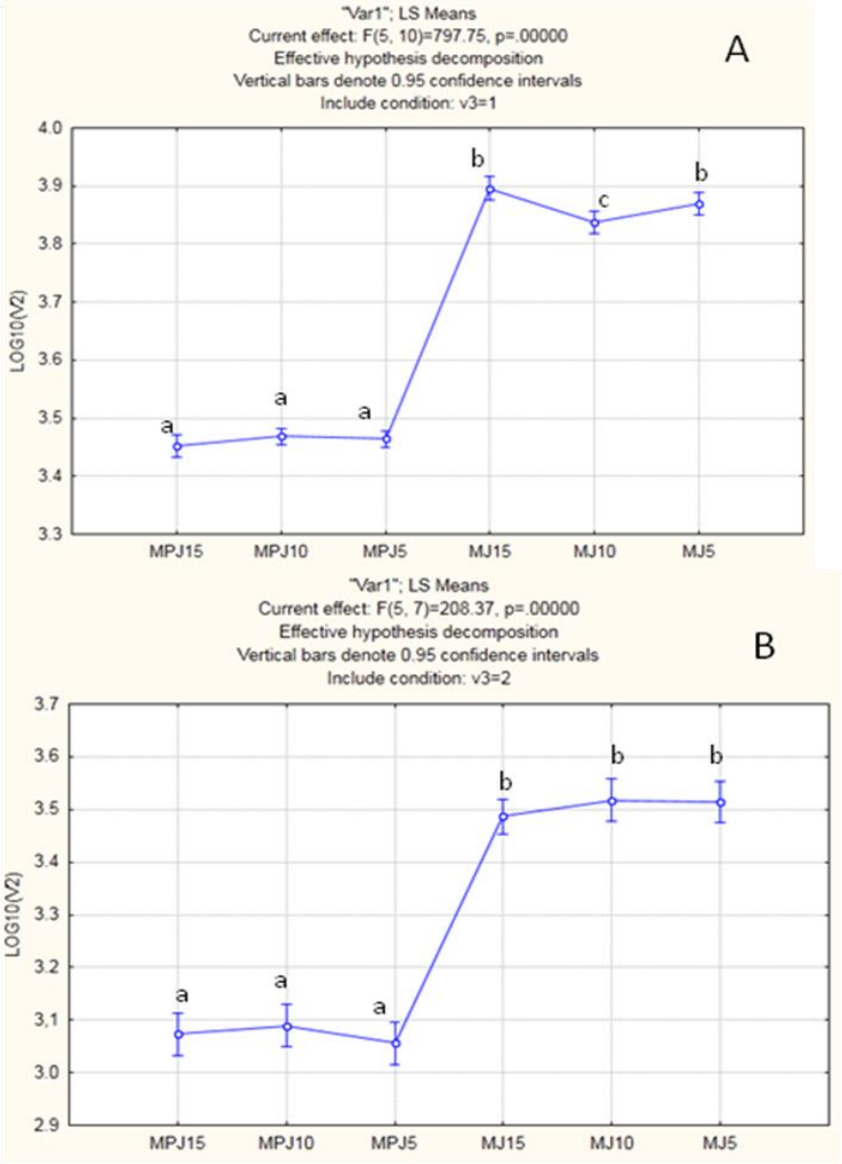
Figure 3 shows that there was no significant difference between MJP produced with different DEs (5, 10, 15). These results also corroborated the results reported by.(IGUAL et al., 2014; PATIL; CHAUHAN; SINGH, 2014) The protective potential of maltodextrin is associated with its ability to form films and act as a reducing agent, thereby protecting ascorbic acid from oxidation (VANIN; CARVALHO, 2020). Ascorbic acid levels in the MJP of acerola and seriguela with 5 DE, 10 DE and 15 DE maltodextrin ranged from 3.451 to 3.468 on a log base corresponding to 2,830.1 mg/100 g to 2,931.61 mg/100 g MJP on a dry basis for samples analyzed on the same day (Fig 3A and Table 1). These results were similar to those found by Moreira et al.(MOREIRA et al., 2010), who reported a value of 3,645.53 mg/100 g of ascorbic acid in acerola extract using cashew gum and maltodextrin as an encapsulating agent.

The ascorbic acid levels in MJ with 15 DE and 10 DE maltodextrin analyzed on the same day differed statistically from 3.89 to 3.83 on the log base corresponding to 7,872.07 to 6,882.41 mg/100 g of ascorbic acid (Figure 3). This difference may have been due to the handpicked selection of fruits. Figure 3B shows that there was no significant difference in the MJ analyzed on different days. These values were higher than those

found by Moreira et al. (MOREIRA et al., 2010) in acerola pulp. There was a reduction in ascorbic acid content in MJ with 5 DE, 10 DE and 15 DE maltodextrin, which ranged from 55 to 56.2% for samples analyzed on the same day compared to samples analyzed on different days (Fig 3A and 3B). This reduction occurred after six months of freezing the pulps in a conventional freezer. The similar result was found by Phillips et al. (PHILLIPS et al., 2016) when studying the effect of freezing on various fruits. These authors reported that this result occurred because the chemical structure of ascorbic acid can be easily oxidized in aqueous solutions and that this phenomenon can occur through an enzymatic and/or non-enzymatic process.

The retention of ascorbic acid in the first day ranged from 36.22 to 44.81%, and the retention of ascorbic acid after 6 months of storage ranged from 34.13 to 35.33%. The results indicated that there was approximately 60% loss of ascorbic acid during the drying process. Ribeiro et al. (RIBEIRO, C. M. C. M. et al., 2019) explained that this loss is due to the use of temperature and exposure to oxygen during drying, thereby causing oxidation of ascorbic acid. Despite this loss, these mixed juices powder were still considered as good sources of ascorbic acid because they had an ascorbic acid content well above the recommended daily intake of 75 mg/day (IOM, 2010).

Fig. . 3. Chromatograms obtained from the three main conditions evaluated in the factorial design. (A) Concentration of ascorbic acid with 0.06 g of mixed juice powder of acerola and seriguela, 1% orthophosphoric acid and stirring time of 1 minute. (B) Concentration of ascorbic acid with 0.14 g of mixed juice powder of acerola and seriguela, 3% orthophosphoric acid and stirring time of 1 minute. (C) Concentration of ascorbic acid with 0.14 g of mixed juice powder of acerola and seriguela, 3% orthophosphoric acid and stirring time of 3 minutes.



Validation of the chromatographic method

The precision results for the MJP on the same day showed that the relative standard deviation (RSD) ranged from 0.17 to 2.57%, and the precision results for the MJP on different days showed that the RSD ranged from 0.83 to 6.0%. In mixed juice (MJ), these variations ranged from 1.38 to 2.38% for samples analyzed on different days. These results were similar to those reported by Boonpangrak et al. (2016) and within the international criteria (SHAH et al., 1992). Thus, it can be concluded that the method was precise.

The selectivity of the chromatographic method was verified by similarity and purity. These tools allow one to verify if the identification is reliable. The similarity ranged from 80 to 99% with the ascorbic acid standard. The purity of all treatments for all MJPs produced with 5 DE, 10 DE and 15 DE maltodextrin was low (below 61%), indicating that the ascorbic acid peak obtained had matrix interferers. However, the lack of purity did not occur in the MJ of acerola and seriguela with 5 DE, 10 DE and 15 DE maltodextrin. Crystallization and gelatinization of maltodextrin occurred during drying, which was evidenced by differential scanning calorimetry (DSC) analysis (RIBEIRO et al., 2020). When subjected to heat, maltodextrin may undergo crystallization and/or retrogradation, which interferes with the purity of the chromatographic peak (KRISHNAIAH; NITHYANANDAM; SARBATLY, 2014; PYCIA et al., 2016).

The linearity of the calibration curve was evaluated by four injections of the standard concentration of ascorbic acid for a period of four months. The mean of the regression equation was as follows: y = 10.2 * 107 x 14.0 * 103. The coefficient of determination (R2) was 0.9831, which indicated a good suitability for quantification of ascorbic acid. Boonpangrak et al. (BOONPANGRAK et al., 2016) found a LD value of 1.8 µg mL^{-1}, which was higher than that found in the present study (0.001 mg/mL), which was sufficient to be applied in fruit juice powder. The LQ obtained in the present study (0.003 mg/mL) was mg/mLlower than that (1.2 mg/g) described by ABE-MATSUMOTO et al. (2020).

The accuracy of the method was achieved by the addition of a known concentration of ascorbic acid mentioned above in item 2.2.5. In the MJP of acerola and seriguela with 5 DE, 10 DE and 15 DE maltodextrin, the recovery rate ranged from 107.62 to 115.96% for the samples analyzed on the same day and from 104.42 to 116.96% for the samples analyzed on different days. These results were consistent with those predicted for methodological validation tests, which should be between 80 and 120%, indicating that the accuracy of the method was acceptable (DEPARTAMENTO DE ALIMENTAÇÃO E NUTRIÇÃO, 2012).

Comparison of the determination method for ascorbic acid by HPLC and titration

A similar result was found by (CUNHA-SANTOS et al., 2019) in four samples of Camu-camu pulp. These authors explain that the titration method, despite being routine, has a number of disadvantages, including interference at the inflection point of the indicator agent, which is caused by color samples and low specificity, the presence of

other reducing substances that can reduce indophenol, like sugars and organic acids, and cause an overestimation of the ascorbic acid content.

4 CONCLUSION

The factorial design allowed the elaboration of an extraction protocol capable of quantifying ascorbic acid content in MJP of acerola and seriguela. The validation parameters were within the acceptable range, which attested to the reliability required for the use of this protocol in quality control laboratories. The method of quantification by HPLC was precise, accurate and reproducible as well as accessible and easily executed. This ease of execution and accessibility are associated with the speed of analysis and the low cost of reagents. Therefore, it can be concluded that the chromatographic method can be used with analytical safety, constituting an important tool in the evaluation of ascorbic acid in fruit juice powder and fresh juices. Although the titration method is recommended for the determination of ascorbic acid in fruit juice, it overestimated the ascorbic acid content for all analyzed samples in the present study. Maltodextrin dextrose equivalent (DE) did not influence the ascorbic acid content. The addition of maltodextrin was effective in preserving ascorbic acid during the drying process. Despite the losses of ascorbic acid during the process, the MJP of acerola and seriguela presented a high ascorbic acid content well above the recommended daily intake (RDI).

Declaration of Competing Interest

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

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