SPECTROPHOTOMETRIC ANALYSIS OF VITAMIN C IN DIFFERENT MATRICES UTILIZING POTASSIUM PERMANGANATE

Diego José Zanini[1], Matheus Henrique Silva[1], Elizama Aguiar-Oliveira[2], Mônica Roberta Mazalli[3], Eliana Setsusko Kamimura[3], Rafael Resende Maldonado [1,4,*]

[1]Integrated College Maria Imaculada, R. Paula Bueno, Centro, Postal Code: 13080-040, Mogi Guaçu, São Paulo, Brazil.

[2]Exact Sciences and Technology Department, Santa Cruz State University, R. Jorge Amado, km 16, Salobrinho, Postal Code: 45.662-900, Ilhéus, Bahia, Brazil

[3] Food Engineering Department, Faculty of Zootecnic and Food Engineering, University of São Paulo, *campus* Pirassununga, Av. Duque de Caxias Norte, 225, Campus da USP, Postal Code: 13635-900, Pirassununga, São Paulo, Brazil.

[4]Food Department, Technical College of Campinas, University of Campinas, R. Jorge Figueiredo Corrêa, Parque Taquaral, Postal Code: 13087-261, Campinas, São Paulo, Brazil.

*** Corresponding Author:**
Email: ratafta@yahoo.com.br

ABSTRACT

Vitamin C has a great importance for metabolism and can be found in fruit and vegetables; due to its oxidation, it is necessary to efficiently monitor its concentration. The aim of this study was to evaluate a spectrophotometric method utilizing potassium permanganate to quantify vitamin C in different matrices. The method was efficient when applied to vitamin C pills (error range for -4 to +2%).Nevertheless, it was not efficient when applied to industrialized fruit juice, for the results were much higher than values mentioned in the labels and with regard to the other methods utilized for comparison. It was possible to conclude that the method is applicable to less complex matrices (vitamin C solution and pills), with linear range between 5 and 100 mg/L, coefficient of variation minor than 5% until 50 mg/L, limits of detection (0.25 and 125 mg/L) and limits of quantification (5.0 and 100 mg/L).

Key-words: Vitamin C, Juice, Oxidation, Reduction, Spectrophotometric.

1. INTRODUCTION

Vitamin is a class of complex compounds that can be found in the majority of edibles in form of micronutrients and, although in small amounts, they are essential to the function of several physiological processes of the human body. The concentration of vitamins in foods varies according to different conditions, such as weather its origin is animal or vegetal, and in the latest case, the species or variety of the plant, the harvest season, conditions of cultivation, ripeness, processing, storage e preparation each type of food (Moreira &Sant'anna,2010).

Among the different types of vitamins, the vitamin C has important properties for the human health. It is a water-soluble vitamin and is not synthesised by the human organism. Its molecular formula is $C_6H_8O_6$ and its molar mass is 176.13 g/mol. The vitamin C is also known as ascorbic acid and is mainly found in nature as isomer L. The isomer D may also be encountered in nature, although with low biological activity (Tomita, 2013; Whitney&Rolfes, 2013). The chemical structure of vitamin C may be seen in Figure 1.

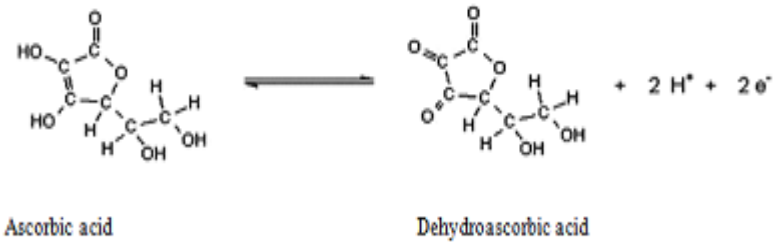

Figure 1 – Chemical structure of vitamin C (adapted from Villâsboas et al., 2016)

The vitamin C possesses a great antioxidant power. Due to this property, it is easily converted from its reduced form (ascorbic acid) to its oxidised form (dehydroascorbic acid). It acts as an anti-scurvy disease; antioxidant: intercepting e eliminating free radicals, which minimises damage to lipids, proteins and nucleic acids. It also acts on the synthesis of collagen and conjunctive tissues, increases the bioavailability of iron, among several other biological activities of the human body. The recommended daily doses are 25 to 30 mg/1000 kcal (Tomita, 2013; Moreira &Sant'anna, 2010; Bianchi &Antunes, 1999).

The main sources of vitamin (circa 90%) are citric fruits and vegetables, especially acid fruits such as the tomatoes and its derivatives (juice and sauce), oranges, lemons, tangerines, amid others. However, to a better utilization of vitamin C, it is recommended that these edibles are fresh when consumed (Tomita, 2013).

Despite the ascorbic acid being easily encountered in fruit and vegetables, there may be a significant loss caused by ripeness, cut, cooking, washing, thermic processes and exposure to atmospheric oxygen (Bender, 2010). The addition of vitamin C to food is allowed in Brazil, since it does not surpass the limits of recommended daily ingestion (Brazil, 1998).

Due to its importance and also to its facility of degradation, it is of great importance to quantify correctly the levels of vitamin C in nourishments. For that purpose, there are innumerable reports in the literature regarding various methods for the determination of this substance, being them titrimetric (da Silva et al., 2017;Villâsboas et al., 2016;Zenebon et al., 2005), spectrophotometric (da Silva et al., 2017;Shyla&Nagendrappa, 2013;Gamboa-Santos et al., 2014;Kukoc-Modun et al., 2012;Lenghor et al., 2012;Gúzman&Seron, 1993), chromatographic (Maia et al., 2007; Valente et al., 2011), amidst a great number of techniques.

The titrimetric method of Tillmans based on the reduction of DCPI (2,6-dichlorophenolindophenol) and methods utilizing ultra-performance liquid chromatography (UPLC) have been reported as standard methods for quantification of vitamin C in edibles (Cardoso, 2013; Tomita, 2013). Nonetheless, spectrophotometric methods have also been applied successfully for the quantification of vitamin C in different matrices and with the utilisation of different reagents (Ameta and Singh, 2014;Lenghor et al., 2011; Li et al., 2008;Arya et al., 2001;Grudpan et al, 1999).

Therefore, the aim of this study was to apply a spectrophotometric method based on the reduction of the potassium permanganate by vitamin C in acid medium for quantification of vitamin C in industrialized fruit juice and drugs in order to assess the applicability of the method for different matrices and compare its efficiency regarding other analytical methods described in the literature.

2. MATERIAL E METHODS

2.1. Spectrophotometric method for quantification of vitamin C

The quantification of vitamin C was realized utilizing a spectrophotometric method, by which the reduction of the absorbance of a potassium permanganate was measured when reacted with a vitamin C solution in acid medium, as shown in Equation 1. In this reaction, the vitamin C consumes the potassium permanganate (violet coloration) causing a decrease in the absorbance at 525 nm. This method was based in a previous study (Lenghor et al., 2012).

$2KMnO_4 + 5C_6H_8O_6 + 3H_2SO_4 = 2MnSO_4 + 5C_6H_6O_6 + K_2SO_4 + 8H_2O$ **Equation 1**

The described method was applied to: (i) standard ascorbic acid solution P.A. (Merck), (ii) vitamin C pills, (iii) industrialized fruit juice [flavours: pineapple (410 mg/L), cashew (225 mg/L), orange (190 mg/L), passion fruit (160 mg/L) and strawberry (140 mg/L)]. The levels of vitamin C stated by each juice label are in parentheses. The samples of vitamin C were acquired in chemist shops and the industrialized juice samples were acquired in supermarkets in the Mogi-Guaçu area (São Paulo, Brazil).

The analyses were realized with samples containing from 1 to 100 mg/L of vitamin C. The standard solutions of ascorbic acid were also prepared within the same concentration range. With regard to the remaining samples (vitamin C pill and industrialized juice), the dilution was realized based on the information obtained from the labels or the literature, in order to comprise the concentration of the diluted solutions within the established range (1 to 100 mg/L).

After the preparation of the samples, aliquots of 10.0 mL of each solution were pipetted volumetrically, mixed and homogenised with 10.0 mL of potassium permanganate ($KMnO_4$) previously dissolved in sulphuric acid (H_2SO_4) 0.1 mol/L. This mixture caused the reaction of reduction of the potassium permanganate. Immediately after the mixing, the absorbance of the samples was read in the spectrophotometer (Quimis Q108U2M) in a glass cuvette at 525 nm. In each analysis, the same reaction analysis system was utilized to calculate the blank of the apparatus by the substitution of the sample containing vitamin C by one containing the same amount of distilled water.

The vitamin C reacts with the solution of potassium permanganate, which causes a diminution on the absorbance of the system, ergo, the relation between the reduction of the absorbance of the $KMnO_4$ solution *versus* the concentration of vitamin C was evaluated. The reduction of the absorbance (ΔA) is defined according to Equation 2, in which A_{blank} is the absorbance of the KMnO4 solution (100 mg/L at 525 nm) and A_{sample} is the absorbance of the system after the reaction with the vitamin C.

$\Delta A = A_{blank} - A_{sample}$ **Equation 2**

2.2. Validation

The selected method was initially validated regarding to the following aspects: linearity, precision, limit of detection and limit of quantification utilizing vitamin C standard solution. The results of the reduction of absorbance *versus* concentration of vitamin C were plotted in order to determine in which interval the response (reduction of absorbance) is linear with the concentration based on a value of R^2 greater than 0.90, as recommended by INMETRO (Aragão et al., 2009;Ribani et al., 2004). To determine the precision of the method, samples with a similar concentration of vitamin C were analyzed in triplicate and the results were utilized to calculate the average, standard deviation and coefficient of variation to assess the reproducibility of the analytical response. The limits of detection and quantification were determined from the concentrations of vitamin C utilized in the study of linearity. The limit of detection was defined as the minor concentration that the method was able to measure (analytical response different from zero) and the limit of quantification was defined as the minor concentration that the method was able to measure with reliability (Aragão et al.,2009;Ribani et al., 2004).

2.3. Influence of color and reducing sugar

The color of the juice can influence the values of absorbance. Hence, for each analysis involving the industrialized juice, two systems were evaluated: (i) potassium permanganate reaction system + diluted juice and (ii) blank of reaction, in which the solution of potassium permanganate was substituted by distilled water in equal volume. The absorbance values of the blank of reaction were utilized to correct the absorbance values of the reaction systems.

The influence of reducing sugar on the quantification of vitamin C was also evaluated. For such, two systems were analyzed: (i) reaction system containing potassium permanganate 100 mg/L + vitamin C, utilizing concentrations between 1 and 20 mg/L and (ii) reaction system containing potassium permanganate 100 mg/L+ vitamin C (1 to 20 mg/L) and reducing sugar (0.8 to 16 g/L).

2.4. Standard addition method

The standard addition method was applied in order to determine the capacity of the analytical method of quantifying correctly the concentration of vitamin C contained in the samples. For such, vitamin C was added to the industrialized juice samples in the same quantity indicated on the label. Therefore, the final concentration of the samples was the double of that declared on the label. From the results obtained, the percentage of recuperation of vitamin C in each analyzed matrix could be calculated.

2.5. Comparison with other analytical methods

The results obtained from the analyzed samples were compared with two other analytical methods: one titrimetric and one chromatographic. The titrimetric method utilized in the comparison was the method of Tillmans (Zenebon et al., 2005). For this quantification, 10 mLof the solution of DCPI (2,6-dicholophenolindophenol) were pipetted volumetrically and transferred to an Erlenmeyer. Then, 10 mL of a solution of oxalic acid 2 % (g/100 mL) were added and the mixture was titred with the fruit juice samples until the pink coloration of the DCPI solution disappeared. For the second verification, the samples were centrifuged at 30,000 rpm for 30 minutes at 15ºC in Micro Ultracentrifuge (Hitachi Himac model CS 120 GXII) and then filtered in a membrane of 0.45 µm. The

quantification of the samples was then realized by ultra-performance liquid chromatography (UPLC) in chromatograph Shimadzu-Prominence (UFLC, LC 20 AD) with diode array detection and column C18, 100 x 4.6 mm x 4 µm (Chormolith, Merck) utilizing a mobile phase of monobasic sodium phosphate 0.2 mol/L pH = 2.5 with flow of 0.5 mL/min (Rosa et al. 2007). The results were also compared to the values declared on the labels of the products and to the literature.

3. RESULTS AND DISCUSSION

3.1. Validation

3.1.1. Linearity

The linearity of the method was evaluated in the range of 0.25-125 mg/L of vitamin C reacting with a solution of potassium permanganate of 100 mg/L. The data obtained, as well as the adjusted curves, are presented in Figure 2, which displays three adjustments realized for the set of data obtained: adjustment 1 (all analyzed points between 0.25 and 125 mg/L of vitamin C); adjustment 2 (analyzed points between 5.0 and 125 mg/L of vitamin C) and adjustment 3 (analyzed points between 5.0 and 100 mg/L of vitamin C). It is noticeable that all three adjusted equations were linear and with a high coefficient of correlation ($R^2 > 0.90$). Among the three adjustments, the third one had the best coefficient of correlation ($R^2 = 0.995$) and, consequently, its range (5 to 100 mg/L) was established as the linear range of the method.

Based on the experimental data, it was possible to observe that from 5 mg/L, the reduction of the absorbance is near 0.100, which may be considered more reliable value. For smaller concentrations, the difference between the absorbance of the samples and the absorbance of the blank of reaction is rather small and the results may be affected by the sensitivity of the equipment as it distinguishes the absorbance of the blank of the reaction from that of the evaluated samples.

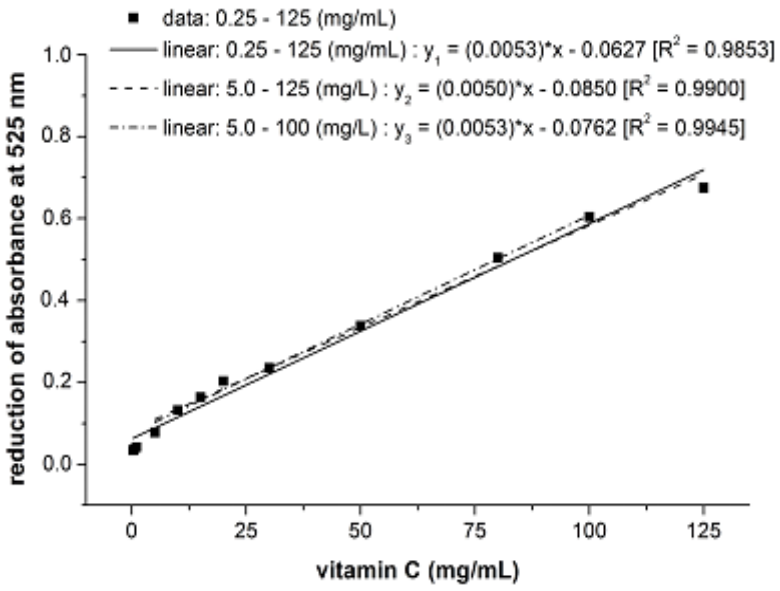

Figure 2 – Reduction of the absorbance at 525 nm on the solution of $KMnO_4$ (100 mg/L) when reacted with standard solution of vitamin C at different concentrations (from 0.25 to 125 mg/mL). Symbols represent the obtained data and lines represent the linear fitting for different groups of points.

Nevertheless, at the concentration of 125 mg/L of vitamin C, practically all the potassium permanganate was consumed and the absorbance value of the sample is very low, which also affects the reliability of the result due to the sensitivity of the equipment. The established vitamin C linear range (5 to 100 mg/L) is inferior to that encountered for the titrimetric method utilizing DCPI (10 to 200 mg/L) and superior to that for the spectrophotometric method utilizing cupric ions with cuproine (2 to 12 mg/L)(da Silva et al., 2017). Spectrophotometric methods are usually more sensitive than titrimetric methods, which results in lower linear ranges

In the comparison with other studies, it is possible to state that the linear range encountered in this method is within the values cited in the literature for different methods of quantification of vitamin C, which vary from $1.4.10^{-2}$ to $8.0.10^{4}$ mg/L (Stojanović et al., 2013;Kukoc-Modun et al., 2012;Karayannis, 1975)

3.1.2.Precision

Besides the evaluation of the linearity of the method, the utilized data also allowed an estimation of precision, for the measurements were realized in triplicate. The experimental data, average values, standard deviation and coefficient of variation may be viewed on Table 1. The results present coefficients of variation between 0.30 and 22.35%. For analytical methods in general, the results of coefficients of variation up to 5% may be considered acceptable. The ideal condition is that they are inferior to 1% (Aragão et al., 2009;Ribani et al., 2004).

Table 1 – Experimental results for the measurement of the absorbance or the reaction systems containing $KMnO_4$ (100 mg/L) + vitamin C (in different concentrations).

Concentration of vitamin C (mg/L)	Absorbance at 525 nm			Average	Standard deviation	Coefficient of variation (%)
Blank	0.704	0.700	0.701	0.702	0.002	0.30
0.25	0.679	0.649	0.677	0.668	0.017	2.51
0.50	0.670	0.656	0.670	0.665	0.008	1.21
1.00	0.663	0.660	0.658	0.660	0.003	0.38
5.00	0.628	0.619	0.624	0.624	0.005	0.72
10.0	0.570	0.564	0.577	0.570	0.007	1.14
15.0	0.531	0.543	0.540	0.538	0.006	1.16
20.0	0.501	0.497	0.500	0.499	0.002	0.42
30.0	0.479	0.465	0.456	0.467	0.012	2.48
50.0	0.362	0.359	0.372	0.364	0.007	1.87
80.0	0.219	0.191	0.185	0.198	0.018	9.15
100	0.080	0.103	0.110	0.098	0.016	16.07
125	0.022	0.034	0.026	0.027	0.006	22.35

For concentrations of vitamin C higher than80 mg/L, the coefficient of variation were much more elevated, which may be attributed to the small absorbance values measured in such conditions, indicating that practically all the $KMnO_4$ of the system was consumed by the addition of the vitamin C. Based on the data contained in Table 1, it is recommended to deal with a concentration of standard solution of vitamin C up to 50 mg/L in order to ensure the analytical results are precise. The results for concentrations until 50 mg/L (CV between 0.30 and 2.50%) are compatible to those obtained by

Lenghor et al. (2012) that achieved a coefficient of variation of 2.9% (n= 5 replicates) for spectrophotometric method utilizing potassium permanganate in the concentration of 400 mg/L.

3.1.3. Limit of detection and limit of quantification

The systems were evaluated as function of the reduction of the absorbance caused by the addition of vitamin C. Ergo, very elevated concentrations of vitamin C result in small values of absorbance, hampering its detection by the method. The data on Table 1 and the adjustments displayed on Figure 1 indicate a limit of detection (LOD) for the method at 125 mg/L. Despite the absorbance being very small and the coefficient of variation being very large, it was possible to achieve an analytical response in this concentration. The limit of quantification (LOQ) may be established in 100 mg/L. Notwithstanding the high coefficient of variation, the absolute value of the standard deviation is small and the method was quite linear in the concentration range between 5 and 100 mg/L of vitamin C.

Nonetheless, when very low concentrations of vitamin C were applied, the samples are practically equal to the blank of reaction. So, the limits of detection and quantification can also be defined in function of the reduction of absorbance as 0.25 mg/L (LOD) and 5.0 mg/L (LOQ). Therefore, inferior and superior limits of detection and quantification can be defined for the method: inferior LOD = 0.25 mg/L and superior LOD = 125 mg/L; inferior LOQ = 5.0 mg/L and superior LOQ = 100 mg/L.

The values obtained are superior to those obtained by chromatographic method utilizing acetonitrile, methanol and metaphosphoric acid as mobile phase with LOD = 0.17 and LOQ = 0.55 mg/L of vitamin C (Maia et al., 2007). However, the LOD and LOQ values are similar to those obtained by Stojanović et al. (2013) utilizing chromopotentiometric method (LOD = 21 and LOQ = 69 mg/L).

3.2.Quantification of vitamin C in drugs

The assessed method was applied for the quantification of vitamin C in drugs (pills of vitamin C). The pills of vitamin C were dissolved and diluted in different concentrations within the range from 5 to 50 mg/L of vitamin C (values comprised in the linear range of the method). The dilutions were realized regarding the declared value of vitamin C on the label of the product. The results, which can be visualised on Table 2, indicated that the dilutions that were situated in the central area of the linear range of the method (20 to 40 mg/L) were the more adequate for the quantification of vitamin C in drugs, for presented coefficients of variation and error in relation to the declared value inferior to 5%.

Table 2 – Quantification of vitamin C in pill of vitamin C by spectrophotometric method utilizing potassium permanganate as oxidant.

Presumed value (mg/L)	Experimental value (mg/L)	CV (%)	Error (%)*
5.0	(1.5 ± 0.3)	20.0	-70
10.0	(8.5 ± 0.2)	2.4	-15
20.0	(20.4 ± 0.7)	3.4	+2.0
30.0	(29.2 ± 0.5)	1.7	-2.7
40.0	(38.4 ± 0.7)	1.8	-4.0
50.0	(44.5 ± 0.6)	1.3	-11.0

*The error was calculated in relation to the presumed value based on the label of the product.

Lenghor et al. (2011) also evaluated the concentration of vitamin C in drugs (vitamin C tablets) of ten different brands and errors obtained related to the declared value between - 5.0 e + 15 %, utilizing potassium permanganate as reagent in spectrophotometric method. Therefore, it is possible to quantify vitamin C in drugs accurately with the evaluated method when utilizing an adequate dilution.

3.3.Quantification of vitamin C in fruit juice

3.3.1.Preliminary evaluation

The method was primarily applied for the analysis of industrialized strawberry juice. The results may be seen on Table 3. The sample of strawberry juice was diluted based on the concentration indicated on the label aiming to reach concentrations between 5 and 100 mg/L (within the linear range). Nonetheless, when realising the dilutions, the absorbance values obtained were quite small. This fact occurred probably because the actual concentration of vitamin C in the product is greater than that indicated on the label. It is not rare that the actual concentration is greater than the declared concentration, for the vitamin C degrades with time and the producer must ensure the quantity declared on the label until the end of the storage period. Therefore, in order to apply the method for the analysis of the strawberry juice, greater dilutions had to be realized. The presumed concentration range of the strawberry juice, which permitted the measurement of absorbance, was between 1 e 20 mg/L.

The experimental values obtained with the dilutions described presented satisfactory reproducibility of the analysis with coefficients of variation lower 5 %, except for the most diluted sample. However, the errors calculated in relation to the values contained on the label were elevated, which led to some hypotheses regarding possible interfering factors on the application of the method utilized for the analysis of the fruit juice:

(i) The presence of reducing substances in the juice, such as reducing sugar.

(ii) The coloration of the sample, which can absorb the luminous radiation on the wave length utilized in the analysis.

(iii) The disparity between the actual concentration of vitamin C in the sample and the concentration indicated on the label of the product. The Brazilian legislation permits variations in relation to the label of ± 20 %. Besides, the producers, aware of the loss of vitamin C during the storage period, add this substance in excess to the product.

(iv) The method evaluated may not be efficient to quantify vitamin C in this sort of matrix (fruit juice).

Facing these hypotheses, a series of evaluations was realized in order to discover the causes of so great discrepancies between the concentration obtained on the analysis and that provided by the label. This issue was also verified in a previous study in which the errors in relation to the declared values were superior to 200 % in the quantification of vitamin C utilizing an iodometric method applied to the same brand of strawberry juice (Villâsboas et al., 2016).

Table 3 – Quantification of vitamin C in industrialized strawberry juice by spectrophotometric method utilizing potassium permanganate as oxidant.

Presumed value (mg/L)	Experimental value (mg/L)	CV (%)	Error (%)
1.0	(3.0 ± 1.0)	32.7	212.5
5.0	(22.4 ± 0.5)	2.25	348.3
10.0	(42.8 ± 0.1)	0.29	327.5
15.0	(58.0 ± 2.0)	3.75	284.2
20.0	(67.9 ± 0.3)	0.38	239.6

3.3.2.Interference of reducing sugar

The first hypothesis investigated was the interference of reducing sugar. This substance may occasionally react with potassium permanganate, which may cause error on the analysis, increasing the (quantity) of vitamin C obtained.

To evaluate this possibility, two standard curves were constructed: (i) standard curve of vitamin C (1 to 20 mg/L) and (ii) standard curve of vitamin C (1 to 20 mg/L) with the addition of glucose (from 0.8 to 16 g/L). The addition of glucose was intended to simulate the concentration of reducing sugar on the juice samples evaluated. The results are displayed on Figure 3.

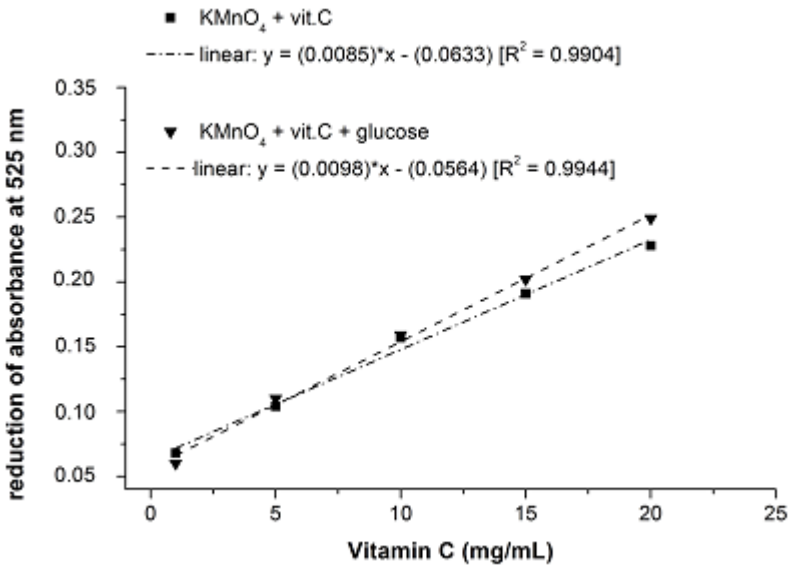

Figure 3 – Comparison for the reduction of absorbance at 525 nm of a $KMnO_4$ solution (10 mg/mL) reacted with vitamin C (square) and vitamin C + glucose (inverted triangle). Lines represent the linear fitting of data.

For all the concentrations of vitamin C evaluated (except the lowest of them, of 1 mg/L), the reduction of absorbance value was slightly higher on the systems which contained glucose than those which contained only vitamin C. This confirms that the reducing sugar interferes on the quantification of vitamin C in fruit juice, which requires the correction of the results obtained.

By comparing the standard curves (Figure 3) and applying them to the experimental data obtained for the strawberry juice, an increase of approximately 10% on the quantity of vitamin C was verified when glucose was added to the system.

3.3.3.Interference of juice coloration on the quantification of vitamin C

The interference of the coloration was evaluated for the five types of juice assessed: pineapple, cashew, orange, passion fruit and strawberry. The samples were diluted in accordance with the label for presumed values of vitamin C between 5 and 20 mg/L. Measurements of two systems were made: (i) reaction system composed by potassium permanganate + diluted samples of juice and (ii) control system composed by distilled water (which substituted the solution of potassium permanganate) + diluted samples of juice. The values of absorbance for both systems can be viewed on Table 4.

Table 4 – Evaluation of the impact of the coloration of the fruit juice on the application of the spectrophotometric method utilizing potassium permanganate.

Presumed value of vit. C (mg/L)	Pineapple		Cashew		Orange		Passion fruit		Strawberry	
	A R	A C	A R	A C	A R	A C	A R	A C	A R	A C
5	0.597	0.014	0.546	0.072	0.533	0.029	0.583	0.044	0.524	0.027
10	0.507	0.024	0.408	0.142	0.386	0.058	0.458	0.077	0.363	0.044
15	0.422	0.033	0.320	0.193	0.271	0.087	0.357	0.122	0.229	0.066
20	0.336	0.045	0.313	0.277	0.202	0.117	0.293	0.165	0.131	0.090
25	0.280	0.059	0.335	0.334	0.167	0.149	0.263	0.211	0.086	0.109
30	0.227	0.067	0.424	0.424	0.180	0.178	0.266	0.250	0.095	0.128

A R = absorbance (525nm) of the reaction system; A C = absorbance (525 nm) of the control system

As shown on this table, it is perceivable that the influence of the coloration is quite significant for cashew, orange, passion fruit and strawberry juice. As the sample is less diluted, the concentration of vitamin C increases, which diminishes the absorbance of the potassium permanganate due to its consumption. However, the color of the juice is intensified, which increases the quantity of radiation absorbed by the juice. Only the sample of pineapple juice presented a minor influence on the result, as in the lowest dilution, the absorbance of the control system (0.067) corresponds to circa 30% of the absorbance of the reaction system (0.227). For the further samples, the lowest dilution has a practically identical absorbance in both systems.

From these results it is noticed that the application of the method evaluated for the fruit juice assessed necessitates the correction of the value of the absorbance measured to calculate the content of vitamin C and that higher dilutions minimise the effect of the coloration of the juice on the analysis. Previous centrifugation and/or filtration may also minimise the problem, removing the material in suspension from the samples.

A further issue to be considered is that, for presenting a higher concentration of vitamin C (410 mg/L), the pineapple juice was which suffered more dilutions to reach the linear range of the method on the analyses realized, and this may be due to the minor interference of the coloration on the execution of the analytical method. The dilutions of the pineapple juice varied from 1:13.7 to 1:82, whilst the strawberry juice (the one with the lowest concentration of vitamin C) the dilutions varied from 1:4.7 to 1:28. In a previous study, da Silva et al. (2017) verified that the spectrophotometric method utilizing cuproine and cupric ions was more accurate for the quantification of vitamin C in orange and pineapple juice than the titrimetric method utilizing DCPI, due to the high factor of dilution utilized in the spectrophotometric method (1:100).

3.3.4.Evaluation of the efficiency of the method in the detection of vitamin C – standard addition method

The capability of the method to detect vitamin C in the industrialized juice samples was evaluated with the standard addition method, from which the recuperation of a known quantity of vitamin C added to the samples was determined. The results may be seen on Table 5.

Table 5 – Recovery factors of vitamin C in fruit juice by the standard addition method

Sample	Recovery factor (%)
Pineapple	88.5
Cashew	92.3
Orange	129.0
Passion fruit	103.1
Strawberry	98.6

The analyses were realized by diluting the juice samples to the concentration of 5.0mg/L in order to minimise the interference of the coloration of the juice and the concentration of reducing sugar on the quantification of vitamin C. In general, the results demonstrate that the method is efficient to quantify the vitamin C accurately in the majority of the samples evaluated with variation of detection of vitamin C between -11.5 and +3.1%, in relation to the quantity added, except for the orange juice in which a quantity of vitamin C circa 30% higher than the added was detected.

In a previous study, Villasbôas et al. (2016) detected lower detection factors for cashew (59%), passion fruit (89%) and strawberry juice (76%) when evaluated juice samples from the same brand by the iodometric method. The results obtained in this analysis indicate that the method evaluated was capable of detecting, with relative precision, a known quantity of vitamin C added to the majority of the matrices analyzed, in other words, elevated errors in the quantification of vitamin C are not directly related to the capability of the method of quantifying the analyte in the sample accurately.

3.3.5.Comparison among methods for quantification of vitamin C in fruit juice

Samples of the five juice flavours studied were analyzed by three distinct analytical methods – titrimetric, spectrophotometric and chromatographic, and the values were compared among each other and with the values specified on the labels of the products. The results may be seen on Table 6.

Table 6 – Quantification of vitamin C in fruit juice by three distinct analytical methods – spectrophotometric (with potassium permanganate), titrimetric (with DCPI) and chromatographic (with column C-18).

	Vitamin C (mg/L)			
Juice	**Spectrophometric method**	**Titrimetric method**	**Chromatography method**	**Declared value**
Pineapple	(1098 ± 36)	(437 ± 4)	(726 ± 2)	410
Cashew	(896 ± 7)	(470 ± 20)	(719 ± 7)	225
Orange	(1020 ± 70)	(379 ± 6)	(495 ± 3)	190
Passion fruit	(667 ± 52)	(226 ± 2)	(287,0 ± 0,7)	160
Strawberry	(770 ± 57)	(216 ± 3)	(250 ± 0,7)	140

The comparison among the three methods reveals that the concentration of vitamin C in the samples evaluated is greater than the value declared on the label, regardless the method utilized. Industrialized juice receives an addition of vitamin C before being thermically processed to ensure a minimum amount of vitamin C in the final product. In general, this excess added is quite high to guarantee that by the end of the expiration date, the content of vitamin C is greater than the indicated on the label.

The results that more closely match the values indicated on the label, although much higher, are those obtained by titrimetric method utilizing as reagent the compound DCPI, which is of easy execution and is largely promulgated for application on edibles.

The samples utilized also influence the results. For the orange, passion fruit and strawberry juice, the results between the titrimetric and chromatographic methods are relatively close among each other, which is not true for the cashew and pineapple juice.

Regarding the spectrophotometric method evaluated, it is noticeable that the results obtained are quite superior to those indicated on the labels, and they are considerably higher than those obtained by the titrimetric and chromatographic methods. The fact of being the potassium permanganate a strong antioxidant may contribute to explain this difference. It is highly probable that the potassium permanganate reacts with other reducing substances present in the juice and not only with the vitamin C.

The results encountered in the literature for the quantification of vitamin C are rather variable in function of the matrix analyzed and the method utilized. Some examples may be cited, such as: concentration of 2190 mg/L of vitamin C in passion fruit of organic and conventional cultivations measured by ultra-performance liquid chromatography (Pertuzatti et al. 2015); 60 to 280 mg/L of vitamin C in currant juice (Matilla et al. 2011); over 212 mg/L of vitamin C in orange juice (Zuo et al., 2015); circa 13000 mg/L of vitamin C in acerola pulp (Yamashita et al., 2003); 220 mg/L in whole pasteurised pineapple juice (Matsuura &Rolim, 2002); 356 and 420 mg/L in orange and pineapple juice, respectively (da Silva et al., 2017); 380, 535 and 502 mg/L in cashew, passion fruit and strawberry juice, respectively (Villâsboas et al., 2016).

4. CONCLUSION

Through this study, the linear range of detection of vitamin C was established as from 5 to 100 mg/L, however, the lowest range of coefficient of variation (< 5%) was obtained in the range from 0.25 to 50 mg/L. Two limits of detection and two limits of quantification were obtained: inferior and superior (inferior LOD and LOQ were 0.25 and 5.0 mg/L; superior LOD and LOQ were 125 e 100 mg/L, respectively). The spectrophotometric method was effective for the quantification of vitamin C in drugs (vitamin C pills) with an error range between -4.0 and +2.0% and CV up to 3.7%. However, it was inadequate for the quantification of vitamin C in industrialized fruit juice, with values quite superior to those declared on the label and in comparison to other analytical methods (titrimetric and chromatographic).

All the three methods employed indicated that the concentration of vitamin C in industrialized juice is rather superior to that indicated on the labels of the products. The presence of reducing sugar (up to 16 g/L) had an average influence of 10% on the content of vitamin C by the spectrophotometric method utilizing potassium permanganate and the coloration of the juice samples evaluated interfered on the spectrophotometric analysis, whereas the minor the dilution, the major the impact caused by the color. The coloration interference was minimized only for the pineapple juice, due to more dilutions realized. The recuperation factors for the spectrophotometric method varied between 88 and 130% depending on the type of juice analyzed, which indicate that the method is relatively satisfactory to quantify vitamin C in the juice samples analyzed and that the overestimated values obtained must be related to the presence of further reducing substances than vitamin C in the juice samples.

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