

## Influence of distillation time and sample mass on sulfur dioxide analysis in passion fruit juice through Monier-Williams method

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### Abstract

This study aimed to evaluate the effect of the distillation time and the sample mass on the total SO<sub>2</sub> content in integral passion fruit juice (*Passiflora* sp). For the SO<sub>2</sub> analysis, a modified version of the Monier-Williams method was used. In this experiment, the distillation time and the sample mass were reduced to half of the values proposed in the original method. The analyses were performed in triplicate for each distilling time x sample mass binomial, making a total of 12 tests, which were performed on the same day. The significance of the effects of the different distillation times and sample mass were evaluated by applying one-factor analysis of variance (ANOVA). For a 95% confidence limit, it was found that the proposed amendments to the distillation time, sample mass, and the interaction between distilling time x sample mass were not significant ( $p > 0.05$ ) in determining the SO<sub>2</sub> content in passion fruit juice. In view of the results that were obtained it was concluded that for integral passion fruit juice it was possible to reduce the distillation time and the sample mass in determining the SO<sub>2</sub> content by the Monier-Williams method without affecting the result.

**Keywords:** sulfur dioxide; analytical methods; passion fruit juice; sulfites; design of experiments.

**Practical Application:** This study can be applied in analyses of sulfur dioxide in juices with reduced amounts of time and quantities of samples.

### 1 Introduction

Sulfites, or sulfite agents, are additives that release sulfur dioxide (SO<sub>2</sub>) in conditions of use. They have various functions, such as antimicrobial agents, enzyme inhibitors, antioxidants, controlling enzymatic and non-enzymatic browning reactions, and in the modification of the structure and functional properties of proteins; their main function is as conservatives or antioxidants in foods and beverages (Walker, 1985; Fazio & Warner, 1990; Wedzicha, 1992; Machado et al., 2006).

Permitted sulfite agents include sulfur dioxide and sulfite salts such as sodium/potassium metabisulfite and sodium/potassium/calcium bisulfite. These are extensively used in the processing of foods and beverages because, apart from the aforementioned reasons, they are cheap, effective and versatile (Walker, 1985).

Sulfites may be present in foods in three different forms: free sulfite, which is not bound to the food molecules; reversibly-bound, which is released under certain pH and temperature conditions; and sulfite that is irreversibly-bound to foods (Walker, 1985; World Health Organization, 2009).

Reversibly-bound sulfites are formed when foods contain high amounts of glucose, xylose and L-xylose, arabinose, galacturonic acid, acetaldehyde, pyruvic acid and 2-ketoglutaric acid. Irreversibly-bound sulfites are formed when the matrix contains greater numbers of alkenes and aromatic compounds

(Nisida, 1991; Swales & Wedzicha, 1992; World Health Organization, 2009).

Despite its many positive functions, SO<sub>2</sub> intake can cause some adverse effects in sensitive individuals, such as breathing difficulties, gastric irritation and the induction of asthmatic reactions (Fazio & Warner, 1990; Taylor & Hefle, 2001; Vally et al., 2009). In 1974, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) for sulfites of 0-0.7 mg/kg body weight, expressed as SO<sub>2</sub> (World Health Organization, 1974). Consequently, the quantification of SO<sub>2</sub> in different commercial products is extremely important.

Resolution n° 04/88 of the National Health Council of the Brazilian Ministry of Health established the maximum limit of sulfites in fruit juices and nectars as 0.02 g/100 g or g/100 mL, expressed as residual SO<sub>2</sub> for conservation purposes (Brasil, 1988). For cashew juice, Resolution RDC n° 12/02 of the ANVISA/MS established a limit of 0.30 g/100 mL for juice with high pulp content (dilution of 1:9) and a limit of 0.033 g/100 mL for juice that is ready for consumption (Brasil, 2002). These limits were amended by Resolution RDC n° 08/13 to 0.005 g/100 g or g/100 mL for juices, nectars, fruit pulps and tropical juices except for cashew pulp and cashew integral juice, where the maximum limit is 0.02 g/100 mL for antioxidative properties. According to this technical regulation, in the case

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of concentrated or dehydrated juices, the dilution's factor must be considered for result comparison with the maximum limit established. Companies have a period of two years to adapt to this technical regulation (Brasil, 2013).

Thus, it can be seen that it is necessary to control this additive in foods, and particularly in beverages, because of its greater intake compared to other foods. One of the ways of monitoring this additive is the Monier-Williams method, which was developed in 1927, and has been widely used both in industry and in research centers because it is simple to implement, low-cost, stable and applicable to many matrices (Leclercq et al., 2000; Suh et al., 2007; Machado et al., 2008; Cressey & Jones, 2009). In addition, the Monier-Williams method can be used as a reference to compare the accuracy and precision of other methods (Holak & Specchio, 1989; Perfetti et al., 1989; Kim et al., 1990; Lawrence et al., 1990; Sullivan et al., 1990; Pizzoferrato et al., 1998; Lowinson & Bertotti, 2001). These factors enable this method to be routinely used in quality control laboratories (Fazio & Warner, 1990; Machado et al., 2008). Moreover, it is a classic method that is used as a reference by the Association of Official Analytical Chemists (2012), US Food and Drug Administration - FDA (CFR Title 21, Appendix A to Part 101; Food and Drug Administration, 1985), European Union (EN 1988-1;1998, European Food Safety Authority, 2013), and the Brazilian Ministry of Agriculture, Livestock and Supply (Brasil, 2005), as well as being widely used by public health laboratories and food industries. The Monier-Williams method determines the levels of free sulfite and reversibly-bound sulfite efficiently and with high levels of repeatability. However, one of the drawbacks of this method is its practical application because its distillation time can take up to 2 hours.

In order to improve the Monier-Williams method, the present study evaluated the effect of distillation time and sample mass reduced to half of the values proposed in the original method, on the level of sulfur dioxide in integral passion fruit juice (*Passiflora* sp).

## 2 Materials and methods

### 2.1 Fruit sample

A commercial nationwide-marketed integral passion fruit juice (*Passiflora* sp) was used in the experiments. The samples (two PET bottles of 500 mL) were purchased in a local market in the city of São Paulo, Brazil, and they contained sodium metabisulfite (INS 223) in the ingredients list. As stated on the label, the dilution's factor 9 should be used in juice reconstitution with water. The bottles of juice were well mixed and homogenized and the mixture was kept in a closed 1 L plastic bottle until chemical analysis.

### 2.2 Design of experiment and physicochemical analysis

A 2<sup>2</sup> full factorial design with three replications in the center point was used to assess the impact of distillation time and sample mass (independent variables) on the content of sulfur dioxide in integral passion fruit juice (dependent variable). The experiment was conducted in a completely randomized fashion (Table 1) in order to avoid carry-over effects (Rodrigues

& Iemma, 2009). The analyses were performed in triplicate for each distilling time x sample mass binomial (105 min and 25 g; 105 min and 50 g; 50 min and 25 g; 50 min and 50 g), which resulted in a total of 12 trials that were performed on the same day under repeatability conditions.

This experiment evaluated the distillation time and sample mass, which were reduced to half of the values proposed by the M-W method. For the sulfur dioxide analysis, a modified version of the Monier-Williams method was used, as referenced by Nagato et al. (2013) and the Association of Official Analytical Chemists (2012), which established a sample mass of 50 g and a total distillation time of 120 min (105 min of boiling) as reference experimental conditions to quantify the sulfur dioxide in beverages. The integral juice was also characterized with regard to pH value, soluble solids content (°Brix) and total acidity expressed as citric acid (g/100 g) using the methods described by Instituto Adolfo Lutz (2005a, b, c).

### 2.3 Statistical analysis

Experimental results were initially evaluated for normality and homogeneity of variances using the Shapiro-Wilk's test and Levene's test, respectively. Finally, the significance of the effects of the different distillation times and sample mass were evaluated by applying one-factor analysis of variance (ANOVA) (Granato et al., 2014). A Pareto chart was generated to show the statistical significance of the factors on the response (Statsoft, 2013). All statistical analysis was performed using the Statistica v.11 software (Statsoft, Tulsa, USA).

## 3 Results and discussion

The physicochemical characterization of the commercial integral passion fruit juice showed 11.3 °Brix, pH 3.0 and 2.54 g/100 g total acidity expressed as citric acid. The results of the SO<sub>2</sub> concentration in juice in relation to the independent variables (factors) ranged from 0.0212-0.0224 g/100g, as shown in Table 2.

**Table 1.** Design of experiment proposed to assess the distillation time and sample mass in the determination of sulfur dioxide in integral yellow passion fruit juice.

Order of analysis	Coded values		Real values	
	Distilling time (x <sub>1</sub> )	Sample mass (x <sub>2</sub> )	Distilling time (min)	Sample mass (g)
6	1	-1	105	25
10	1	-1	105	25
4	1	1	105	50
11	-1	1	50	50
8	1	1	105	50
5	-1	-1	50	25
12	1	1	105	50
3	-1	1	50	50
9	-1	-1	50	25
2	1	-1	105	25
7	-1	1	50	50
1	-1	-1	50	25

According to the statistical results (one-way ANOVA), for a 95% confidence limit, it was found that the proposed amendments to distillation time ( $p = 0.056$ ), sample mass ( $p = 0.757$ ) and interaction between distilling time x sample mass ( $p = 0.477$ ) were not significant in the determination of the  $\text{SO}_2$  content in passion fruit juice. The Pareto chart (Figure 1) that was generated from the experimental results clearly shows the isolated effect of the distillation time and the sample mass, as well as the interaction of distilling time x sample mass on the level of  $\text{SO}_2$  content in integral passion fruit juice.

These results show that reducing the distillation time and using smaller samples may be adopted to quantify the level of  $\text{SO}_2$  in integral passion fruit juice. This can be explained by the type of connection that the  $\text{SO}_2$  makes with the matrix components (Walker, 1985).

Several studies evaluated some slight experimental modifications in the Monier-Williams method such as: the use of other acids and indicators, apparatus used in the distillation procedure,

titrant concentration (NaOH) and treatment of the sample prior to distillation (Yabiku et al., 1987; Lafeuille et al., 2007; Cressey & Jones, 2009; Nagato et al., 2013). However, our work contributed to the literature by showing the effects of sample mass and distilling time on the sulfur dioxide analysis. Thus, for the official Monier-Williams method to be modified in terms of reduced sample mass and distillation time, a study of the impact of all the effects, both alone and combined (distillation time x sample mass) must be performed. If there is statistical significance ( $p < 0.05$ ), *i.e.* in the sample mass, the distillation time, or the interaction between the distillation time and the sample mass, it is recommended that the conventional Monier-Williams method is used without alterations.

The residual  $\text{SO}_2$  level (0.0212-0.0224 g/100 g) obtained for integral passion fruit juice indicated that the current use of sulphites by industry was well below the value legally permitted by the Brazilian legislation (0.005 g/100 g) considering the dilution factor (9) for juice reconstitution.

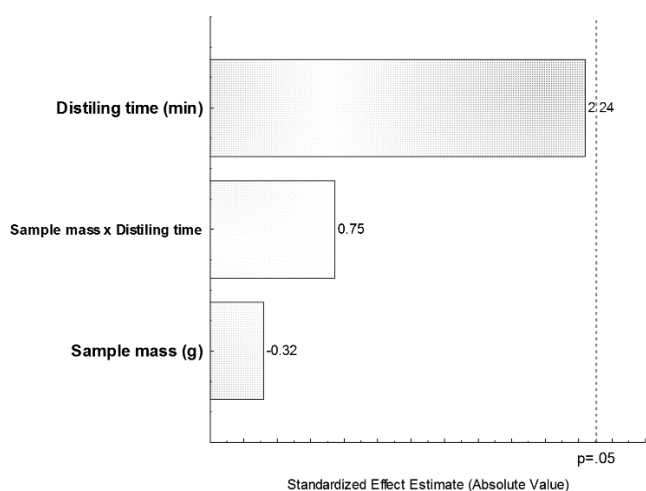
#### 4 Conclusions

This study showed that it was possible to alter the distillation time and sample mass in terms of  $\text{SO}_2$  analysis using the Monier-Williams method for the analysis of integral passion fruit juice. These modifications are of great importance, especially for public health laboratories, which use this method as a reference, and where time and the amount of sample available for analysis are critical factors.

It is important to stress that this modification was possible for this particular matrix, but depending on the composition of other types of food/drinks there may be a greater or lesser interaction between sulfite salts with the intrinsic matrix components. For that reason, studies of the variables (distilling time x sample mass) in each case are necessary. The current work showed, for the first time in the literature, an experimental design combined with statistical analysis for the assessment of the effects of each factor to be modified in the official Monier-Williams method.

**Table 2.** Sulfur dioxide content in relation to distilling time and sample mass.

Order of analysis	Distilling time (min)	Sample mass (g)	$\text{SO}_2$ (g/100 g)
6	105	25	0.0217
10	105	25	0.0220
4	105	50	0.0224
11	50	50	0.0216
8	105	50	0.0218
5	50	25	0.0214
12	105	50	0.0216
3	50	50	0.0216
9	50	25	0.0218
2	105	25	0.0219
7	50	50	0.0212
1	50	25	0.0217



**Figure 1.** Pareto chart showing the non-significance of the distillation time and sample mass on the sulfur dioxide content of integral passion fruit juice.

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