Summary

Since sulphites have been implicated as the cause of adverse reactions such as bronchospasms in some subjects with asthma, their use as food additives may represent a risk for sensitive individuals. Moreover, it was reported by the Joint Expert Committee on Food Additives in its fifty-first meeting that the consumption of certain foods and beverages such as fruit juices may result in sulphite intakes above the Acceptable Daily Intake (ADI) of 0.7 mg.kg⁻¹ body weight, leading the Codex Committee on Food Additives to recommend Member States to monitor the sulphite intake in their countries based on the current use of these additives and on individual food consumption. Therefore, the present work aimed at determining the residual sulphite contents in a variety of fruit juices available on the Brazilian market using the optimized Monier-Williams distillation method, in order to check whether current uses of these preservatives are in accordance with the Brazilian legislation. Thirty-nine samples of fruit juices, either fruit juices to be consumed after dilution (37) or ready-to-drink juices (2), were collected on the market and analysed for sulphites using the optimized Monier-Williams method. The residual sulphite levels, expressed as sulphur dioxide, in ready to drink juices ranged from <10.0 mg.L⁻¹ in grape and guava juices to 98.5 mg.L⁻¹ in mango juice. The mean recovery was 89.1% with a CV of 6.8%. All samples presented residual sulphite levels below 50% of the national maximum permitted level of 200 mg.L⁻¹ (333 mg.L⁻¹ for cashew apple juice). These findings indicate that the current use of sulphites by the fruit juice industry is well below the values legally permitted by the Brazilian legislation.

Key words: Sulphiting agents; Food additives; Preservatives; Monier-Williams distillation method.
Resumo

Embora os sulfitos sejam utilizados amplamente na indústria de alimentos, a ingestão desses aditivos têm sido associada a reações adversas em humanos, tais como broncoespasmos em indivíduos asmáticos sensíveis. Além disso, em uma avaliação da exposição aos sulfitos conduzida pelo JECFA (Joint FAO/WHO Expert Committee on Food Additives) durante sua 51ª reunião em 1998, concluiu-se que o consumo de certos alimentos e bebidas, incluindo os sucos de frutas, pode resultar em valores de ingestão de sulfitos acima de sua Ingestão Diária Aceitável (IDA) de 0,7 mg.kg⁻¹ peso corpóreo. Com base nessas informações, o Comitê do Codex sobre Aditivos de Alimentos recomendou que a exposição diária a sulfitos a partir de todos os alimentos e bebidas seja novamente avaliada pelos Estados Membros com base em dados reais de uso e em dados individuais de consumo. Dessa forma, o presente trabalho teve como objetivo a determinação analítica dos níveis de sulfito residual em sucos de frutas disponíveis comercialmente no Brasil, utilizando para isso o método oficial de Monier-Williams otimizado. Foram adquiridas 39 amostras de sucos de frutas nacionais entre sucos de frutas a serem consumidos após diluição com água (37) e sucos prontos para o consumo (2). Os níveis residuais de sulfitos, expressos como dióxido de enxofre em sucos prontos para o consumo, variaram de <10,0 mg.L⁻¹ em suco de uva e goiaba a 98,5 mg.L⁻¹ em suco de manga. A recuperação média obtida foi de 89,1% com coeficiente de variação de 6,8%. As amostras de sucos de frutas apresentaram concentrações de sulfitos abaixo de 50% do limite máximo permitido de 200 mg.L⁻¹ (333 mg.L⁻¹ para suco de caju). Os resultados obtidos nesse estudo indicam que as concentrações de uso dos sulfitos pela indústria de sucos de frutas estão abaixo dos limites máximos estabelecidos pela legislação brasileira.

Palavras-chave: Agentes sulfitantes; Aditivos alimentares; Conservadores de alimentos; Destilação Monier-Williams.
1 Introduction

Sulphites or sulphiting agents have been added to foods for centuries and include sulphur dioxide (SO₂) and the many forms of inorganic sulphites that release SO₂ under the conditions of use. These substances are used worldwide as food additives due to their function as antimicrobial agents, enzyme inhibitors, antioxidants and inhibitors of enzymatic and non-enzymatic browning (TAYLOR et al., 1986; LUCK and JAGER, 1997).

Among the browning mechanisms, the one that involves the action of polyphenol oxidase (PPO) is of the greatest importance since this enzyme is widely found in fruits and vegetables. PPO activity is high in foods that are particularly sensitive to oxidative browning, such as potatoes, apples, mushrooms, bananas, peaches, fruit juices and wines. Although alternatives are being searched for, sulphites are still the additives most widely used for controlling browning in the food industry. Due to their multiple functions, sulphiting agents are used as preservative in fruit juices the world over (TAYLOR et al., 1986; IYENGAR and MCEVILY, 1992; WARNER et al., 2000).

From a toxicological point of view, residual levels of sulphites in food and beverages have become implicated as causing adverse reactions such as local gastric irritation, nausea, headache, urticaria and asthmatic reactions (bronchospasms) in some subjects with asthma (YANG and PURCHASE, 1985; TAYLOR et al., 1986; ANIBARRO et al., 1992; WÜTHRICH et al., 1992; PERONI and BONER, 1995; WARNER et al., 2000). Although the physiological basis for asthmatic responses is still not fully understood, clinical observations have established that certain medical conditions, such as severe asthma and steroid-dependence, are associated with a predisposition to sulphite-hypersensitivity (ANIBARRO et al., 1992; PERONI and BONER, 1995; WARNER et al., 2000). It was also observed that sulphite in the form of sulphur dioxide is the agent that causes the physiological response (FAZIO and WARNER, 1990; WARNER et al., 2000). In addition, several neurological abnormalities, seizures and lens subluxation were diagnosed in individuals presenting a deficiency of sulphite oxidase. This enzyme is responsible for the final oxidation of sulphite to inactive sulphate that can rapidly be excreted in the urine (KISKER et al., 1997; EDWARDS et al., 1999).

The use of food additives in different countries is limited by specific regulations established on the basis of their safety for use and technological needs. Brazil, like many other countries, has followed the recommendations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) on the safe use of food additives. According to the exposure assessment of sulphites conducted by JECFA, based on the proposed maximum limits for sulphites in the draft “General Standard for Food Additives”, in conjunction with the data on food intake provided by national governments, certain foods and beverages such as fruit juices, dried fruits and wines were identified as major contributors to the overall intake of sulphites. In addition, it was considered that the consumption of these foods and beverages by certain individuals may result in intakes of sulphites above the Acceptable Daily Intake (ADI) of 0.7 mg.kg⁻¹ body weight, expressed as sulphur dioxide, leading the Codex Committee on Food Additives and Contaminants (CCFAC) to recommend Member States to monitor the sulphite intake in their countries (WHO, 2000; CAC, 2006, 2007).

National data concerning sulphite intake based on the measurement of residual sulphite levels in foods and beverages, are scarce in Brazil. Most of the information available on sulphite contents in foods and beverages has been generated in order to control the use of these preservatives by the food industry. In this sense, Yabiku et al. (1987) determined the content of sulphites in 473 samples of fruit juices available on the Brazilian market using the Monier-Williams method as modified by Shipton. Fifty-one percent of the samples analysed presented residual sulphite levels above the national permitted level of 200 mg.L⁻¹. The highest level of residual sulphite was found in cashew apple juice (1439 mg.L⁻¹). Recently, Popolim and Penteado (2005) estimated the intake of sulphite by 14 to 19 year-old Brazilian students based on the national maximum levels for use and on individual consumption data. The authors reported that 4.5% of the individuals studied presented sulphite intakes above 50% of the ADI, and that the main contributors for these intakes were manufactured fruit juices.

The fruit juice sector in Brazil is characterized by a continuous growth in sales (around 15% per year). The fruit juice segment intended for direct sale to the consumer for the preparation of ready-to-drink fruit juice after dilution with water accounts for 22% of this sector (630 million L in the year 2002) (ARAÚJO, 2003). Moreover, data from a household budget survey conducted by the Brazilian Institute of Geography and Statistics from July 2002 to June 2003 showed a notable increase in the consumption of manufactured fruit juices in recent years (IBGE, 2004), which makes it increasingly important to know the levels of residual sulphites in these beverages. Since the sulphite levels typically used do not reflect the concentration remaining in a food at the time of ingestion, owing to losses during the processing and storage of the treated foods (TAYLOR et al., 1986), the monitoring of sulphite usage and intake based on the analytical data of residual sulphite levels would certainly result in a more accurate assessment of exposure to these preservatives. As a consequence, the determination of residual sulphite, particularly in foods and beverages that are consumed without pre-treatment, such as fruit juices, wine and dried foods, is of the greatest importance since this enzyme is widely found in foods that are particularly sensitive to oxidative browning.
fruits, has become an issue of concern in recent years (WHO, 2000).

The Monier-Williams method, which is a distillation-titration procedure, is the procedure most widely used for the analytical determination of sulphite in foods and beverages (WARNER et al., 1986; FAZIO and WARNER, 1990; AOAC, 1995). Although time consuming, this method is considered as a reference method for other procedures, due to its accuracy and precision (HILLERY et al., 1989; FAZIO and WARNER, 1990). In Brazil, the Ministry of Agriculture, Livestock and Food Supply has adopted the Monier-Williams procedure as modified by Shipton (PEARSON, 1962), as the official method to analyze sulphites in non-fermented beverages (BRASIL, 2005).

Since 1986, the United States Food and Drug Administration (FDA) has required warning labels on any food containing more than 10 mg.kg⁻¹ or beverage containing more than 10 mg.L⁻¹ of sulphites. For this purpose, Hillery et al. (1989) published a collaborative study using the Monier-Williams method, optimized by the FDA, for determining sulphites at the level of 10 mg.kg⁻¹ or mg.L⁻¹ in foods and beverages. The minor modifications that were introduced by the FDA did not change the chemistry of the Monier-Williams procedure but established additional specifications to achieve a lower level of quantification as required by the current labelling regulation in the USA (CFR, 2006a,b).

The present work aimed to determine the residual sulphite content in a variety of fruit juices available on the Brazilian market using the optimized Monier-Williams distillation method, in order to check whether the current use of these preservatives is in accordance with Brazilian legislation. These data will be further used to estimate the contribution of fruit juices to the intake of sulphites in Brazil.

2 Material and methods

2.1 Reagents

All chemicals were of analytical grade and the nitrogen gas used was over 99.9% pure. The water was obtained from a Millipore Milli-Q water purification system. The solutions used for the residual sulphite determination were the following:

a) 4 mol.L⁻¹ aqueous hydrochloric acid (HCl), prepared by diluting 90 mL of concentrated hydrochloric acid (37%) to 270 mL;

b) 0.25% solution of methyl red in ethanol;

c) Standardized titrant of 0.01 mol.L⁻¹ sodium hydroxide (NaOH), prepared by diluting 100 mL of 0.1 mol.L⁻¹ NaOH (Titrisol® Merck) to 1000 mL and standardized with 0.01 mol.L⁻¹ potassium hydrogen phthalate;

d) 3% hydrogen peroxide (H₂O₂) solution, prepared daily by diluting 10 mL of 30% H₂O₂ to 100 mL in water; and

e) 5% aqueous ethanol.

2.2 Sampling

For the purpose of this study, national brands of fruit juices whose label declared the addition of sulphites were purchased from supermarkets and specialized stores in the city of Campinas, State of São Paulo, between August 2004 and April 2005. Most of the samples collected were available as fruit juices to be consumed after dilution with water, with only one brand of cashew apple juice and one brand of tomato juice being commercialized as ready-to-drink juices.

The types and brands of juices collected were the following: Antilles cherry (2 brands), cashew apple (7 brands), grape (3 brands), graviola (sour sop) (1 brand), guava (5 brands), mango (6 brands), orange (1 brand), passion fruit (5 brands), peach (1 brand), pineapple (5 brands), tamarind (1 brand), tomato (1 brand), and a mixed blend of orange, papaya and banana juices to be added to milk (1 brand).

2.3 Sample preparation

Each sample comprised three different batches of the same type and brand of juice, which were carefully mixed before analysis. The samples were analysed in duplicate.

The analyses were carried out using the undiluted juice. The volume used for analysis ranged from 10 to 50 mL, depending on the sulphite level in the sample.

Sample preparation and analysis were carried out as quickly as possible to avoid the loss of labile forms of sulphites.

2.4 Determination

The analyses were performed according to the AOAC Official Method 990.28 (AOAC, 1995), which measures free sulphite plus a reproducible portion of bound sulphite.

The sample, in 5% aqueous ethanol, was quantitatively transferred to a 1000 mL round-bottomed distillation flask followed by the addition of 90 mL of 4 mol.L⁻¹ hydrochloric acid. The acidified sample was refluxed for 1 h and 45 min. Any liberated SO₂ was carried through the apparatus by a stream of nitrogen gas into a solution of 3% H₂O₂, where the SO₂ was oxidized to sulphuric acid (H₂SO₄). The nitrogen flow rate was adequate to keep a
positive pressure in the round-bottomed flask, avoiding the reflux of matrix into the trap.

The $\text{H}_2\text{SO}_4$ generated was quantified by titration with standardized NaOH solution using 0.25% solution of methyl red in ethanol as the indicator. Blank determinations on the reagents were performed using distilled water and sulphite-free fruit juices. The residual sulphite levels, expressed as sulphur dioxide, were computed as follows (Equation 1):

$$\text{SO}_2 (\text{mg.L}^{-1}) = \frac{(32.03 \times (V_t - V_b) \times M \times 1000)}{V_a}$$ \quad (1)

where:
- $V_t$ = volume (mL) of standardized NaOH solution required to reach the sample end point
- $V_b$ = volume (mL) of standardized NaOH solution required to reach the blank end point
- $M = \text{molar concentration of standardized NaOH solution}$
- $1000 = \text{converts grams to milligrams}$
- $V_a = \text{volume (mL) of sample used for the distillation}$

As the Brazilian legislation considers the use of sulphites in fruit juices on a ready to drink basis, the residual sulphite concentrations in the juices as served to the consumer were further calculated according to the dilution instructions recommended by the manufacturers.

2.5 Gravimetric confirmation procedure

In order to verify if the acid content determined from titration was provided only by distilled $\text{SO}_2$, the barium sulphate ($\text{BaSO}_4$) gravimetric confirmation procedure was performed with the contents of the 3% hydrogen peroxide trap (AOAC, 1995). The trapping solution was transferred to a 400 mL beaker and 4 drops of 1 mol.L$^{-1}$ HCl and an excess of 10% barium chloride ($\text{BaCl}_2$) solution added. The mixture was allowed to stand overnight at room temperature. After decantation the precipitate was washed with hot water through a weighed Gooch crucible and then dried to constant weight at 103-105 °C. Blank determinations on the reagents were also performed.

SO$_2$ recovery was computed as follows (Equation 2):

$$\text{SO}_2 (\mu\text{g} \cdot \text{mL}^{-1} \text{ or mg L}^{-1}) = \frac{\text{BaSO}_4 \times 0.274476 \times 1000}{V_a}$$ \quad (2)

where:
- $\text{BaSO}_4 = \text{barium sulphate precipitate formed (mg)}$
- $0.274476 = \text{obtained by division of the } \text{SO}_2 \text{ molar mass by the } \text{BaSO}_4 \text{ molar mass (SO}_2/\text{BaSO}_4)$
- $1000 = \text{converts milligram to microgram}$
- $V_a = \text{volume (mL) of sample used for the distillation procedure}$

2.6 Recovery

The recovery assays were carried out by fortifying samples of sulphite-free fruit juices with standard solutions that were prepared fresh daily and appropriately diluted just before use. The standard solutions were made by diluting sodium metabisulphite Granular AR (ACS) grade (Mallinckrodt Baker, Inc., Phillipsburg, USA) in purified water. The amount of sulphite added, expressed as sulphur dioxide, ranged from 10.0 to 1192.0 mg.L$^{-1}$.

The repeatability of the method was evaluated from the coefficients of variation (CV) associated with the measurements of SO$_2$ obtained during the recovery tests for two fortification levels of 25.6 mg.L$^{-1}$ ($n = 6$) and 68.0 mg.L$^{-1}$ ($n = 4$).

3 Results and discussion

Samples fortified at levels ranging from 10.0 to 1192.0 mg.L$^{-1}$ of sulphur dioxide presented average recoveries of 89.1% with a coefficient of variation of 6.8%. Table 1 shows the detailed results obtained from the recovery studies, which were performed with cashew apple, passion fruit and grape juices, without added sulphites. These results are comparable with the findings obtained by Warner et al. (1986), who reported recoveries higher than 90% for sulphite added to foods such as table grapes, hominy, dried mangos, and lemon juice, using the optimized Monier-Williams method. The CVs obtained from recovery tests performed on different days for the two fortification levels, 25.6 mg.L$^{-1}$ ($n = 6$) and 68.0 mg.L$^{-1}$ ($n = 4$), were 6.0 and 4.5%, respectively. These results are below the maximum limit of 15% for determinations at mg.L$^{-1}$ levels, recommended by Horwitz et al. (1980).

Table 1. Recovery and coefficient of variation (CV) for sulphur dioxide (SO$_2$) in fruit juice samples fortified with sodium metabisulphite and analysed by the AOAC official method (AOAC, 1995).

<table>
<thead>
<tr>
<th>Added SO$_2$ levels (mg.L$^{-1}$)</th>
<th>n$^a$</th>
<th>R$^b$ (%)</th>
<th>CV$^c$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-50</td>
<td>7</td>
<td>80.0-89.0</td>
<td>4.4</td>
</tr>
<tr>
<td>51-100</td>
<td>7</td>
<td>80.8-94.2</td>
<td>5.4</td>
</tr>
<tr>
<td>&gt;100</td>
<td>6</td>
<td>91.4-99.0</td>
<td>3.2</td>
</tr>
</tbody>
</table>

$^a$Number of fortified samples analysed in this range of SO$_2$ concentration.

$^b$Range of recovery.

$^c$Coefficient of variation amongst the recovery values obtained for each range of SO$_2$ fortification.

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All the samples presented residual sulphites below the national maximum permitted level of 200 mg.L\(^{-1}\) for ready to drink juices (333 mg.L\(^{-1}\) for cashew apple juice). Comparing these results with those reported by Yabiku et al. (1987) a great decrease in the use of sulphites in fruit juices by the industry was noted.

Sulphite intake from foods and beverages has been implicated as one of the main causes of inducing are in accordance with the AOAC recommendation that establishes recoveries of ≥80% for food matrices fortified at the 10 mg.L\(^{-1}\) level, in order to ensure accurate sulphite analytical data using the optimized Monier–Williams method (HILLERY et al., 1989; AOAC, 1995). Based on these results, a quantification limit of 10 mg.L\(^{-1}\) was established for the method employed. For the samples that presented sulphite levels below the quantification limit, it was assumed that such samples contained 50% of this limit (5.0 mg.L\(^{-1}\)) in order to calculate the average concentrations for each type of fruit juice.

Table 2 shows the gravimetric confirmations that were carried out with the contents of the 3% hydrogen peroxide trap after the titration of eight analyses (4 samples in duplicate). The average relative recovery was 96.6% with a CV of 2.3%. These findings confirm that the titrated acid content was provided only from distilled SO\(_2\).

The sulphite levels, expressed as sulphur dioxide, found in the fruit juices without the addition of water ranged from <10.0 mg.L\(^{-1}\) in the grape and guava juices to 589.3 mg.L\(^{-1}\) in the cashew apple juice (Table 3). In Brazil, a maximum permitted level of residual sulphites of 200 mg.L\(^{-1}\) in ready-to-drink juices was established for most fruit juices, with the exception of cashew apple juice for which two levels were established: 333 mg.L\(^{-1}\) for ready to drink juice and 3000 mg.L\(^{-1}\) for high pulp juice (BRASIL, 1988, 2002). According to the results, the six samples of high pulp cashew apple juice presented concentrations below the permitted level. Considerable variability in sulphite levels was found within different brands of the same fruit juice, suggesting a lack of control in the addition of this preservative to these products, differences in the quality of the processed fruits and or the use of different processing conditions.

The residual sulphite concentration in the juices as served to the consumer was calculated by applying the factor of dilution recommended on the product labels. The levels found ranged from <10.0 mg.L\(^{-1}\) in grape and guava juices to 98.5 mg.L\(^{-1}\) in mango juice (Table 4).
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bronchospasms in some asthmatic subjects (ANIBARRO et al., 1992; PERONI and BONER, 1995). Data available in the literature indicate that about 5-10% of all asthmatics are affected, and that sulphite sensitive asthmatics are adversely affected mainly when these substances are inhaled (SIMON et al., 1982; ANIBARRO et al., 1992; NANNINI and HOFER, 1997; WARNER et al., 2000). Taking these considerations into account, it is important to point out that due to the release of gaseous SO_2, from a non-diluted fruit juice such as high pulp cashew apple juice, which may contain up to 600 mg.L\(^{-1}\) of sulphites, the mere handling of these products may represent a risk for sulphite-sensitive subjects.

Since its thirtieth meeting, the Joint FAO/WHO Expert Committee on Food Additives has recommended that alternative methods of preservation should be encouraged, particularly in those applications in which the use of sulphites may lead to high levels of acute intake, and which have most commonly been associated with life-threatening adverse reactions (WHO, 1987).

In Brazil, research has been carried out with the purpose of developing new technologies to produce fruit juices with minimized amounts of sulphite (MAIA et al., 2001; CIANCI et al., 2005). Thus the use of combining processes such as heat treatment, chemical preservatives and nitrogen stripping have been studied, in order to produce high pulp cashew apple juice with reduced amounts of sulphite (MAIA et al., 2001).

4 Conclusions

The optimized Monier-Williams method was suitable for determining sulphites in fruit juices. Although time consuming, this procedure is of low cost and could be used as a reference method in Brazil to obtain the real sulphite contents of other commercially available sulphited products.

All the fruit juices analysed presented residual sulphite levels within the maximum values established by the Brazilian legislation in force, indicating the adequate use of these preservatives by the industry.

The data generated in this work will contribute to a databank on the levels of sulphites in foods and beverages in Brazil, which has been developed in order to allow the assessment of sulphite intake by Brazilians.

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