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A fast and sensitive HPLC method for sulfite analysis in food based on a plant sulfite oxidase biosensor

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ABSTRACT

A reliable and sensitive analysis of sulfites in food is essential in food monitoring. However, the established methods exhibit deficiencies in the very low concentration ranges (below 10 mg/L SO_2), especially with more complex food matrices. With a focus on these challenges, an HPLC method with immobilized enzyme reactor (HPLC–IMER) for the analysis of sulfites in food was optimized and compared to a standard method. A modulated sample preparation procedure and the use of a novel sulfite oxidase from *Arabidopsis thaliana* were explored to make the method applicable for most food samples. The plant sulfite oxidase turned out to be superior to the commercially available animal sulfite oxidase in terms of detection limit (0.01 mg/L SO_2), linear range (0.04–20 mg/L SO_2) and stability. In a small scale comparison within our laboratory, as well as in a standardized proficiency testing, the HPLC–IMER was compared to an established distillative method. The enzyme-based method is not only more sensitive and specific, it also yields higher sulfite recoveries in almost all samples while exhibiting better statistic method parameters.

1. Introduction

Sulfur dioxide is one of the oldest food additives, and probably the most versatile one. However, due to known adverse health effects and its pseudoallergic potential, the application of sulfur dioxide and sulfites as additives in foodstuffs is legally restricted in Europe, as well as in other countries. The determination of sulfites is not yet completely satisfying regarding sensitivity, specificity and trueness. A correct analytical determination of sulfites in foodstuffs, especially in the concentration range below 10 mg/L SO_2 (amount of all sulfite forms, calculated as SO_2) is important for several reasons: (1) the European food law requires a declaration of sulfur dioxides above the legal limit of 10 mg/L or 10 mg/kg SO_2, respectively; (2) some people suffer from severe pseudoallergic reactions after the ingestion of sulfite containing foods. For them, a correct declaration of even trace amounts of sulfites is essential; (3) claims like "no sulfites added" or "free from food additives" have to be verifiable. Despite the need of a sensitive sulfite analysis and despite a large number of different methods, a reliable, sufficiently sensitive and easy-to-perform method covering the concentration range below 10 ppm is still owing.

1.1. Existing methods

Sulfur dioxide and sulfites are easily detected by various analytical methods. However, sulfites may undergo reactions with certain food components, e.g. aldehydes, ketones or polyphenols, and become reversibly or irreversibly bound (Wedzicha, 1984; Berké et al., 1998). Whereas the reversibly bound adducts are decomposed only slowly upon acidification, the decomposition takes place more rapidly when heated to boiling temperature or in alkaline media. In the analytical quantification of sulfites, this behavior is used to determine the free, as well as the bound sulfites. Especially the bound sulfites may account for too low analytical findings, if they are not – or not completely – released.

Probably the most common and widespread method for sulfite analysis in food until now is the one that was originally developed by Monier-Williams (1927). This method is composed of a distillation with a titrimetric quantification of the distilled sulfur dioxide. It can be applied to almost all kinds of foods for the determination of free, as well as of total SO_2. For many years, this method was considered to be the most reliable method for the determination of SO_2 in food (Joslyn and Braverman, 1954). For certain matrices, though, the method yielded too high or too low findings, and the laborious procedure was considered a drawback of the method.

There have been innumerable attempts to find a better suited method for the determination of sulfites in food matrices. Some

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of these newer methods include ion-chromatography (Anderson et al., 1986; Kim and Kim, 1986; Lindgren and Cedergren, 1982; Ruiz et al., 1994), flow-injection analysis (Carballo et al., 2003; Cardwell and Christophersen, 2000; Matsumoto et al., 1989; Groom et al., 1993; Mana and Spohn, 2001; Möller and Winter, 1985; Ruiz-Capillas and Jiménez-Colmenero, 2009; Tsukatani and Matsumoto, 2000; Su et al., 1998), and numerous electroanalytical methods, like the cyclic voltammography (Raoof et al., 2007; Safavi et al., 2008), only to mention a few.

Most of the methods mentioned above show great advantages in some aspects, but require either some very expensive laboratory equipment or a laborious calibration or sample preparation procedure. Many of the methods are found to be inaccurate or have limited uses, or their detection limits are above the legal limit of 10 mg SO_2 per liter or kilogram, especially with more complex matrices like foodstuffs.

In most aspects, the HPLC–IMER presented in this paper is able to overcome these drawbacks, as it is applicable for almost all kinds of food samples with little sample preparation, short times for analyses and a very low detection limit of 0.01 mg/L SO_2.

1.2. HPLC with immobilized enzyme reactor (IMER)

The HPLC–IMER coupling combines the benefits of chromatographic separation with the specificity of the enzyme sulfite oxidase and the sensitivity of an electrochemical detector to easily yield accurate results with an extremely low detection limit.

For the sample preparation, adjustment of the sample to an alkaline pH value is important for several reasons: (1) the release of reversibly bound sulfites is most effective in alkaline media; (2) the sulfite oxidase is solely able to process the sulfite ion (SO_3^{2-}), which dominates at high pH values; (3) the maximum activity of the sulfite oxidase is at pH 8.6. Therefore, prior to injection into the HPLC, the sample is treated with alkaline solution in order to release bound sulfites and to turn all forms of sulfur(IV)-oxo species into the desired ionic sulfite (SO_3^{2-}) form.

Separated by an anion exchange column, the sulfite ions pass the enzyme reactor. The immobilized enzyme sulfite oxidase specifically oxidizes sulfite ions to sulfate, producing equivalent amounts of hydrogen peroxide:

$$SO_3^{2-} + H_2O + O_2 \xrightarrow{\text{sulfite oxidase}} H_2O_2 + SO_4^{2-}$$

The hydrogen peroxide is electrochemically detected by an amperometric detector at 0.2 V (for setup of the HPLC–IMER, see Fig. 1).

An electrochemical detection of sulfite instead of hydrogen peroxide is possible as well. However, sulfite oxidation requires higher potentials than the oxidation of hydrogen peroxide, and may thus lead to interferences in the chromatogram by other oxidized substances. The use of the enzyme reactor leads to more specificity and 40-fold better sensitivity.

The general principle of postcolumn derivatization and its advantages in food analysis was thoroughly described by Galensa in the book "Food Biosensor Analysis" (Wagner and Guilbault, 1994). In his working group, the specific coupling of an HPLC system with an integrated sulfite oxidase reactor between the column and the detector was first described in the dissertation thesis of Pabel (1993). Several modifications and advancements of the method were later presented by Weßels (Weßels et al., 1995; Weßels, 1997) again in the working group of Galensa. Further optimization of the method was presented by Patz et al. (1997).

However, an interlaboratory comparison based on the method presented in the work of Weßels (1997) had shown difficulties in finding the true total amount of sulfites, especially in samples that were rich in polyphenols as, i.e. wines and grape juices. This led to the presumption, that the release of the polyphenol-bound sulfites still needed improvement.

In the presented work, the optimization was done with focus on sample preparation and the comparison of two different types of sulfite oxidase, respectively. The sample preparation procedure was modified in order to determine the best conditions for a maximum release of bound sulfites and a minimum loss of free sulfites. The commercially available sulfite oxidase (cSO; derived from chicken liver) was compared with a new sulfite oxidase from *Arabidopsis thaliana*. The plant sulfite oxidase (pSO) was employed in an enzyme-based sulfite analysis method for the first time.

After optimization, the HPLC–IMER was compared to a well established distillative method (IFU 7a (International Federation of Fruit Juice Producers (IFU), 2000), which is based on the Monier-Williams Method, but optimized for fruit juices) regarding sulfite recovery rates and standard deviation. The comparison was performed by our laboratory, as well as by an official reference laboratory in a DRRR[1] proficiency testing.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of reagent grade and obtained from Merck (Darmstadt, Germany). All solutions were prepared with purified water. Purified water was produced with Millipore Direct-Q® 3UV (Molsheim, Germany).

Plant sulfite oxidase (pSO) gene was cloned from *A. thaliana* (Eilers et al., 2001) and the recombinant enzyme was expressed and purified according to Hänsch et al. (2006).

Animal sulfite oxidase (cSO) from chicken liver (EC 1.8.3.1; S9526; CAS Number: 9029-38-3; Lot-Nr. 2005-30282) was obtained from Sigma–Aldrich (Steinheim, Germany) as a suspension in 3.2 mol/L ammonium sulfate containing 1.6 mM molybdic acid, pH 7.5, containing 20–70 units/mg protein. Polycarbonate cartridges were from Trace, Braunschweig, Germany and the immobilizing agent, Eupergit® C 250 L, oxirane acrylic beads, was obtained by Röhm (Darmstadt, Germany).

Carbonate buffer (A) for the eluent was prepared of 0.43 g/L sodium carbonate and 3.04 g/L sodium bicarbonate ($c = 0.04$ mol/L; pH 9.1). Carbonate buffer (B) for the sulfite standard solution was prepared of 4.7 g/L sodium carbonate and 2.4 g/L sodium bicarbonate ($c = 0.06$ mol/L; pH 10.6). Sulfite standard solution contained 0.4 mg/L of SO_2 in carbonate buffer (pH 10.6) and was stabilized with 0.18 g/L EDTA and 0.23 g/L fructose.

2.2. Enzyme immobilization

For one enzyme reactor, 50 units of active protein were immobilized on 40 mg of the carrier material Eupergit® C 250 L. The carrier material with the immobilized enzyme was then filled into a polycarbonate cartridge and sealed at both sides with a porous HDPE plate. When not in use, the enzyme reactor was stored at 4 °C in carbonate buffer (B).

2.3. HPLC–IMER

2.3.1. General procedure

The enzyme reactor was employed into an isocratic HPLC system, consisting of a pump with degasser (ICS-3000), an autosampler (AS 50), an anion exchange column (CarboPac PA-100 Guard, 4 mm × 50 mm, room temperature. All devices by Dionex,

[1] German Reference office for food proficiency testing and reference materials (Deutsches Referenzbüro für Lebensmittel-Ringversuche und Referenzmaterialien).

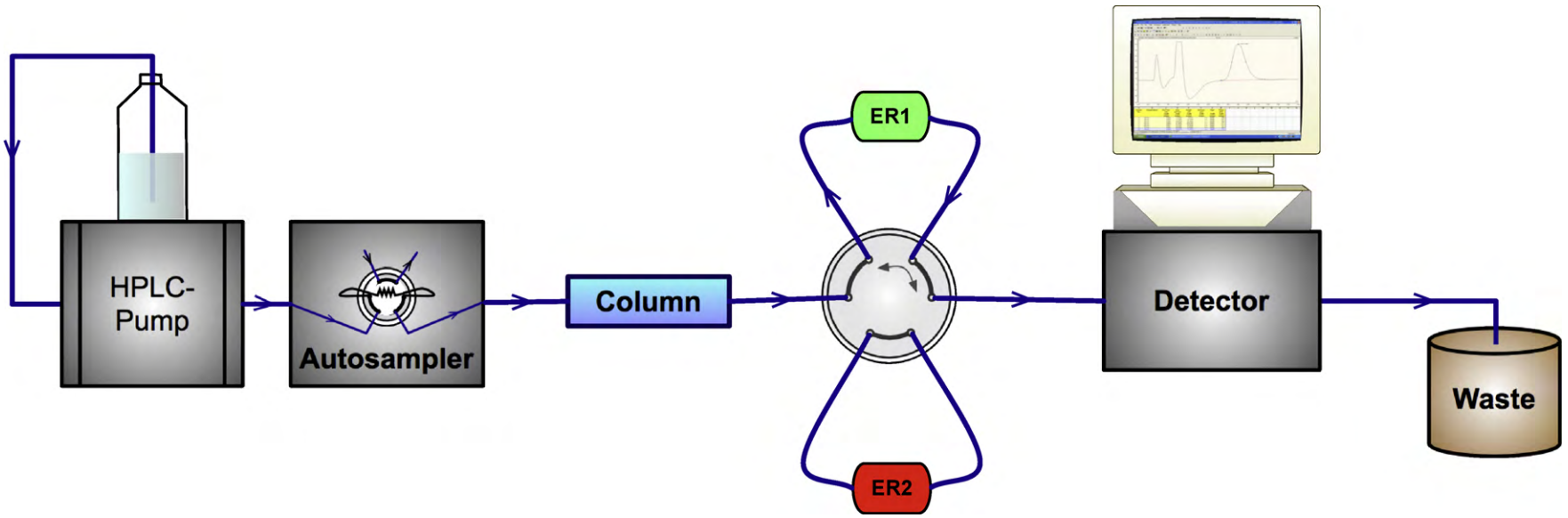

Fig. 1. Setup of the HPLC–IMER. The switch valve is integrated between the column and the electrochemical detector. ER1 and ER2 represent two different enzyme reactors.

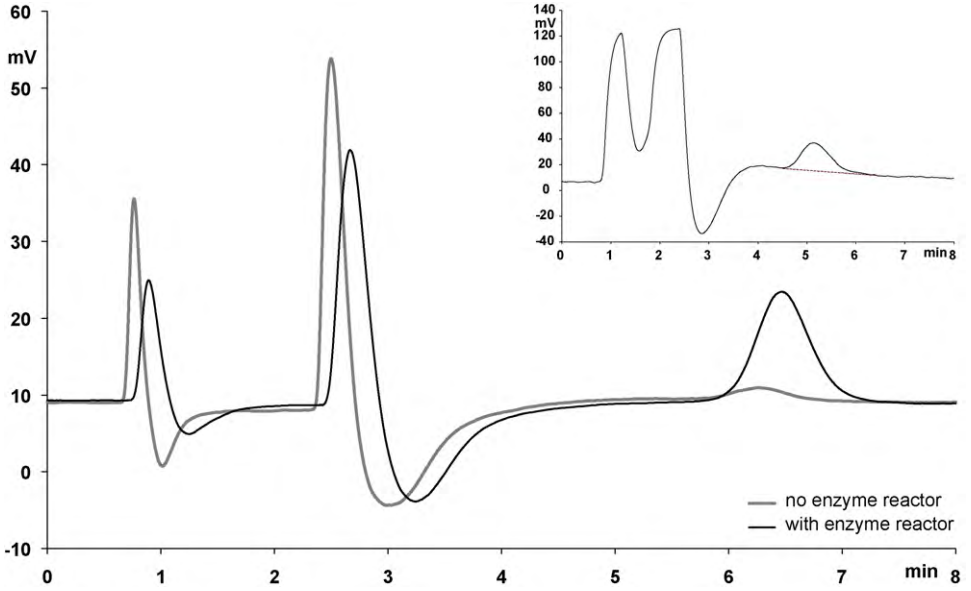

Fig. 2. HPLC–IMER chromatograms of sulfite standard solution ($c = 0.4$ mol/L), analyzed with and without the enzyme reactor. Upper right: chromatogram of a red grape juice (13.6 mg/L SO_2; diluted 1:25).

Idstein, Germany) and an electrochemical detector (TED 4020, Trace, Braunschweig, Germany) with a platinum flow-through electrode. A switch valve was used for consecutive analyses of samples in two different ways, allowing either a comparison of two different enzyme reactors or a comparison of measurements with and without enzyme reactor. A schematic view of the setup with two integrated enzyme reactors is presented in Fig. 1.

After sample preparation, 25 μL of the sample solution were injected into the HPLC. The flow rate was set at 0.6 mL/min. Detection at the platinum cell was at 200 mV at a range of 20 nA. One run was finished within 8 min. Two chromatograms of a sulfite standard solution ($c = 0.4$ mol/L SO_2) analyzed with and without enzyme reactor, respectively, are shown in Fig. 2. A chromatogram of a grape juice sample is displayed in the top right of Fig. 2.

Several fruit juices and alcoholic beverages (red wine, white wine, apple cider) were treated with different alkaline solutions to examine the best conditions for sulfite liberation. The liquids were diluted 1:100 (v/v) with either carbonate buffer (pH 9.1 and pH 10.6), sodium hydroxide solution (pH 10.3) or with a mixture of 90% carbonate buffer (pH 10.6) and 10% NaOH (1 mol/L). After dilution, the mixture was injected into HPLC–IMER seven times within 11 h to monitor the changes in recovery over time. Each sample was analyzed in quintuplicates.

In a second experiment, two parts of sodium hydroxide solution ($c = 1$ mol/L) were added to one part of the sample, and 22 parts of carbonate buffer (B) were added after different waiting periods, immediately prior to injection. Sulfite peak areas of the chromatograms were compared to determine the minimum reaction time for maximum sulfite recovery.

All sample preparation was carried out by the autosampler. Thus, it was possible to execute experiments over long periods of time without interruptions and with very high reproducibility.

2.4. Enzyme comparison

The properties of two different sulfite oxidase enzymes were compared.

For these experiments, the HPLC–IMER was equipped with a switch valve and two enzyme reactors, one filled with immobilized plant sulfite oxidase and one with animal sulfite oxidase (see Fig. 1).

Both reactors were prepared the same day with 50 units of sulfite oxidase enzyme each. By injecting each sample twice, and switching from one reactor to the other between two runs, both enzyme reactors were equally stressed. Thus, a direct comparison of both enzymes under identical conditions regarding standing times, temperatures, flow rates and number of injected samples, was possible.

For the determination of the linear range, sulfite standards with the following concentrations were prepared: 0.04 mg/L, 0.08 mg/L, 0.2 mg/L, 0.4 mg/L, 0.8 mg/L, 2 mg/L, 4 mg/L, 8 mg/L, and 10 mg/L. Each sulfite standard was analyzed twice with both enzyme reactors in the same sequence.

For comparison of the long-term stability, two reactors were stressed with 250 injections of grape juice samples and standard solutions within two months. Every 50 injections, the sulfite peak areas of a standard solution ($c = 0.4$ mol/L SO_2) were determined for both enzymes and calculated relative to each other.

2.5. IFU 7a

The IFU 7a method is a modified version of the Monier-Williams distillation, designed for the analysis of fruit juices. In a Lieb–Zacherl apparatus, 15 mL of phosphoric acid was added to 50 mL of the sample. The flask was heated to boiling temperature, so bound sulfites were released and gaseous sulfur dioxide was distilled into the receiver flask, which was equipped with 3 mL of hydrogen peroxide solution ($c = 0.3\%$), where SO_2 was oxidized to sulfate. The resulting sulfuric acid was quantified by titration with sodium hydroxide solution ($c = 0.01$ mol/L).

2.6. Method comparison and validation

In a pre-testing phase, 35 commercially purchased fruit juices were tested for sulfite residues. Of them, 15 were found to contain sulfites. These were chosen for the comparison experiments. All juices were analyzed in duplicates with both methods, the optimized HPLC–IMER and the IFU 7a, respectively.

One red grape juice and one white grape juice were each analyzed five times with both methods, and variance, standard deviation and relative standard deviation were calculated.

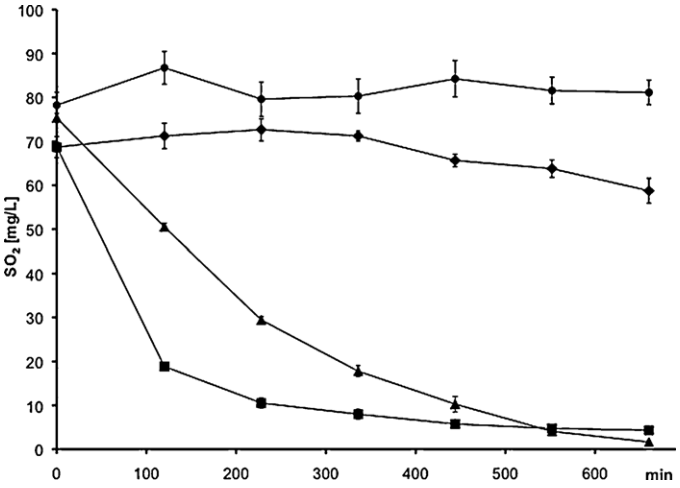

Fig. 3. Sulfite amounts of a red grape juice (Blauer Portugieser), diluted (1:100, v/v) with four different solutions: (•) carbonate buffer (pH 10.6) with 10% NaOH (1 mol/L), (♦) NaOH (pH 10.3), (▲) carbonate buffer (pH 9.1), (■) carbonate buffer (pH 10.6).

In a DRRR Proficiency Testing (PT RVEP 0970) both methods, the IFU 7a as well as the HPLC–IMER (and three other methods for sulfite analysis[2]), were subjected to an ability test. 24 laboratories located within Europe were involved in total. The IFU 7a was performed by 16 laboratories, the HPLC–IMER by 5 laboratories. The reference material was an industrially produced red grape juice.

3. Results and discussion

3.1. Method optimization

As presented in Fig. 3, addition of carbonate buffer solution to the sample leads to a decrease in sulfite findings over time. Whereas at the beginning, about 70 mg/L SO_2 was detected, the findings decreased to less than 10 mg/L SO_2 within 10 h. This effect was stronger with the more concentrated and more alkaline carbonate buffer (pH 10.6).

The use of sodium hydroxide solution with a pH value of 10.3 instead of carbonate buffer, showed comparable sulfite concentrations at the beginning. However, the decrease in the findings was a lot less intense.

Best results were obtained with a mixture of sodium hydroxide solution with a carbonate buffer. After a rise within the first 100 min, the level of detected sulfites did not change within the next 10 h.

Similar results were also found with a number of other food samples, e.g. other grape juices, wines, or apple ciders. In all cases, a mixture of sodium hydroxide solution with carbonate buffer lead to the highest results.

The decrease in sulfite recoveries seems to be caused by a slow reaction of the sample matrix with the carbonate buffer. Apparently, this effect does not occur when sodium hydroxide solution is present. So we concluded, that sodium hydroxide solution is best suited for sample treatment, leading to higher sulfite recoveries than carbonate buffer. However, sodium hydroxide solution was used in combination with carbonate buffer, in order to ensure the same pH value, and therefore matching retention times for different samples.

In another experiment, the sulfite-liberating effect of adding pure NaOH ($c = 1$ mol/L) to the sample prior to dilution with carbonate buffer, was shown to be even stronger than the effect of adding NaOH and buffer at the same time. Generally, the maximum sulfite concentration was detected after about 60–100 min. The increase in sulfite findings over time was not the same for all sample materials. Whereas for a grape juice, the effect was very small, the bound sulfites of the rosé wine were liberated only upon treatment with sodium hydroxide solution prior to addition of buffer.

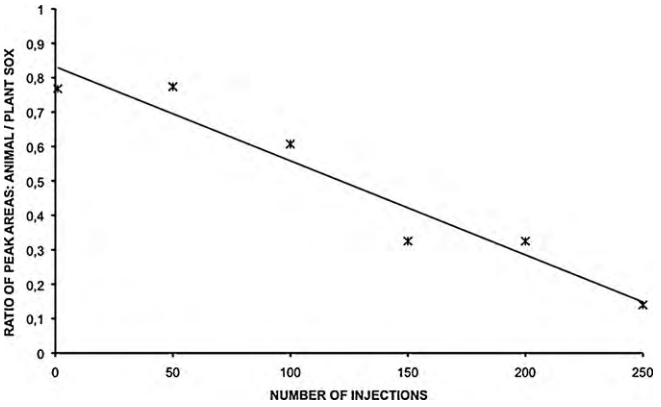

Fig. 4. Comparison of sulfite oxidase performances. Ratio of the peak areas is displayed (area cSO/area pSO) over 250 injections.

3.2. Enzyme comparison

3.2.1. Linear range

The plant sulfite oxidase (pSO) shows a linear range from 0.04 mg/L to 2 mg/L SO_2 with $R^2 = 0.99996$. Higher amounts than 2 mg/L were above the maximum detection capacity of the detector and were thus not quantifiable. In contrast, the results with animal sulfite oxidase (cSO) in the same concentration range do not show a linear correlation ($R^2 = 0.88809$), but rather form a curve. Thus, the pSO seems to have either greater capacity for the reaction with sulfites or its reaction time (in the immobilized state) is much faster than that of the cSO.

3.2.2. Stability

The two enzyme reactors were equally stressed under identical conditions with 250 grape juice samples as well as sulfite standard solutions. Peak areas of sulfite standard solution ($c = 0.4$ mg/L SO_2) obtained with animal and plant sulfite oxidase were compared. At the beginning of the experiment, the animal sulfite oxidase reactor showed an activity level of 77% compared to the plant sulfite oxidase reactor (data displayed in Fig. 4). After 50 injections, the ratio was still about the same. From thereon, the activity of the cSO decreased compared to that of the plant sulfite oxidase. After 200 injections, the peak areas of a standard solution with the animal sulfite oxidase reactor were only 34% of that of the pSO.

After two months and 250 injections of standard sulfite solutions and food samples, the performance of the animal sulfite oxidase reactor became too weak for sensitive sulfite analysis with HPLC–IMER. The peak area of a standard solution with 0.4 mg/L SO_2 was then only 13% of its initial value.

In this experiment, it was necessary to compare both enzyme reactors relative to each other, rather than to compare the absolute values, as the absolute peak area of a reactor may vary greatly due to different factors. For example, the performance of the enzyme reactor weakens due to its exposure to samples, but it can be recovered to a large extend by placing the reactor in a carbonate buffer solution in the fridge for the night. Also, the sensitivity of the platinum cell decreases over time, leading to smaller peaks in the chromatogram. By cleaning the cell, its initial sensitivity can be

[2] §64 LFGB L00.00-46.1; Rebelein; STN EN 13196.

Table 1
Sulfite contents of 15 fruit juices, each analyzed with HPLC–IMER and IFU 7a.

Sample juice	IFU 7a [mg/L]	HPLC–IMER [mg/L]	%
Grape (1)	6.6	4.6	−30
Grape (2)	6.1	4.9	−20
Grape, white (1)	6.5	7.2	+11
Grape (3)	14.1	18.9	+34
Grape (4)	7.9	11.0	+39
Grape (5)	8.8	13.2	+50
Grape (6)	13.5	20.2	+50
Grape, white (2)	5.1	8.5	+67
Grape (7)	5.9	11.5	+95
Grape (8)	9.5	20.1	+112
Grape (9)	2.4	6.4	+167
Lemon	0.5	1.5	+200
Peach	1.2	4.1	+242
Tomato (1)	0.3	2.3	+667
Tomato (2)	0	2.6	–

The last column displays the difference of the results with IFU 7a and HPLC–IMER in percent.

restored. Thus, it is not possible to determine the exact decrease of one sulfite oxidase reactor's reactivity.

However, the plant sulfite oxidase reactor still showed approximately 93% of its initial performance at the end of this experiment.

3.3. Comparison with the IFU 7a

3.3.1. Laboratory scale

In this experiment, 15 commercially purchased juices with sulfites were analyzed with both methods, HPLC–IMER and IFU 7a, respectively. The results of this comparison are displayed in Table 1. The juices contained 0–20 mg/L sulfite, calculated as SO_2. In the last column of the table, the % value of the difference in the results is displayed.

The sulfite results of both methods are not alike, neither in respect to absolute, nor to percentage amounts. In all but two samples (both products of the same producer), the HPLC–IMER method yielded higher results than the IFU 7a method. In 10 out of the 15 juices, the result of the HPLC–IMER was more than 50% higher than that of the IFU 7a. Analyzed with HPLC–IMER, the two tomato juices and the lemon concentrate contained low, but well quantifiable amounts of sulfites, whereas the distillation method yielded only trace amounts or even no sulfites at all. Four of the grape juices (4, 5, 7, 8) would not have been legally objectionable according to the results of the IFU 7a. However, regarding the results of the HPLC–IMER, these juices actually contained more sulfites than officially allowed (i.e. more than 10 mg/L).

In another experiment, the data and statistics obtained from the analyses of two grape juices were determined. Not only does the enzyme-based method yield higher results, the HPLC–IMER also shows better reproducibility, when comparing the method parameters variance, standard deviation and relative standard deviation of both methods. For example, the relative standard deviation of the HPLC–IMER was 1.68% for a red grape juice and 2.36% for a white grape juice. In contrast, the relative standard deviation of the IFU 7a was 8.79% for a red grape juice and 7.04% for a white grape juice. Especially in the low concentration range of about 10 mg/L SO_2, a good reproducibility of the method is essential for a reliable judgment of the marketability of the products.

3.3.2. Interlaboratory comparison

The results of the interlaboratory comparison PT RVEP 0970 are displayed in Fig. 5.

In accordance with our own lab tests, the mean value with the HPLC–IMER (10.12 mg/L SO_2) was again higher than the best estimate for the true value with the IFU 7a (8.95 mg/L SO_2). Additionally, the standard deviation of the IFU 7a method was more than two times higher (2.42 mg/L) than that of the HPLC–IMER (0.95 mg/L). These results support the findings of our laboratory's tests. In the interlaboratory comparison, the discrepancy between both methods was smaller, probably due to deviant experimental setups of some laboratories or due to this specific sample material.

However, the concentration range around the legislation limit of 10 mg/L is very important for the food industry, e.g. fruit juice producers, as fruit juice products with SO_2-contents above 10 mg/L have to be rejected by the consumer protection authorities. The IFU 7a is the accepted method for testing SO_2. To judge products, the reproducibility $R = 3.82$ mg/L of that method has to be added to the limit of 10 mg/L.

The proficiency testing (PT) RVEP 0970 showed that the IFU7a can be operated under routine conditions within the limits of R. Nevertheless, it may be questioned whether the reproducibility R is still up to date. Within the PT RVEP 0970, the HPLC–IMER showed promising results. But in order to compare the proficiency of both methods, the HPLC–IMER needs to be validated and precision data

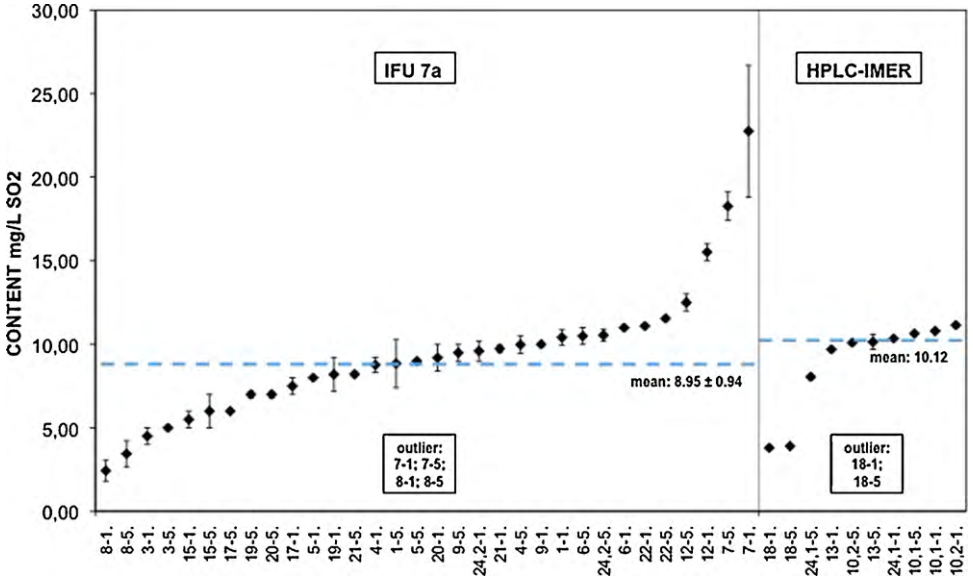

Fig. 5. DRRR proficiency testing results of report RVEP 0970 fruit juice (grape juice).

has to be determined. The HPLC–IMER showed high potential to surpass the IFU 7a as a future reference method for the determination of SO_2.

After all, the HPLC–IMER is almost immune against false positive results due to the specificity of the enzyme sulfite oxidase and the chromatographic separation. As the chance of false positives with the HPLC–IMER is negligible, one must conclude, that the higher results of the HPLC–IMER are likely to be much closer to the true amounts of sulfites than the results of the IFU 7a.

4. Conclusions

The optimized HPLC–IMER method was successfully employed in the analysis of sulfur dioxide in various liquid food samples as, i.e. fruit juices, beer and wine as well as in solid foods like dried fruits, jams, jellys and sirups. The method is not only simple to perform and fast but it also shows a high reliability, as falsely positive results are highly unlikely due to the specificity of the enzyme. It shows a promising potential to become a high precision method.

This work shows several advantages of the HPLC–IMER over the Monier-Williams type distillation:

- Time efficiency:
  One HPLC run takes only 8 min. The sample preparation can easily be executed manually or by an autosampler, and the standing time of 60 min prior to injection into the HPLC requires no manual action. Analyzing one sample with IFU 7a takes about 30 min of manual action.
- Automatic sample preparation:
  The entire sample preparation can be automated by an appropriate autosampler, making this method simple to perform even for unexperienced staff. In contrary, the distillation methods require thorough experience in handling, in order to obtain reliable results.
- Less dangerous and toxic chemicals:
  The only critical chemical in the HPLC–IMER is the sodium hydroxide solution. If the sample preparation is done by the autosampler, no contact with any chemicals is necessary at all (except for preparing the solutions and refilling of the reservoirs), whereas the distillation requires a manual refill of caustic acid for every run.
- No false positive results:
  The specificity of the enzyme makes false positive results almost impossible. Analyzing one sample without the enzyme reactor leads to a chromatogram without the characteristic sulfite peak. If the peak does not disappear, it is not a sulfite peak. In contrast, the distillation holds the risk of overestimation of the sulfite content.
- Better reproducibility:
  Lower possibility of variances and errors leading to an increased reproducibility of the HPLC–IMER compared to the distillation methods.
- Much lower detection limit:
  Probably the major advantage of the HPLC–IMER is the quantification limit of 0.01 mg/L. The distillation methods are a lot less sensitive, as they do not lead to reliable results below 10 mg/L.

Acknowledgements

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