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Evaluation of sulphite in beer and spirits after the new allergen labelling rules

A new European Directive requires food manufacturers to label the potential allergenic substances sulphur dioxide and sulphites if they are used as ingredients at concentrations of more than 10 mg/l in pre-packed foods. FTIR in combination with Partial Least Squares (PLS) regression is presented as a fast (30 samples/hour), simple and inexpensive procedure for the determination of sulphite concentrations in beer (simultaneously with standard parameters like ethanol and original gravity). FTIR/PLS offers considerable advantages when measured against the conventional enzymatic reference procedure and will acquire increasing importance as an efficient high-throughput tool for screening alcoholic beverages. With information gained by FTIR screening, decisions can be made whether additional analyses (with more time-consuming and expensive but more accurate standard procedures) are required. The average concentrations expressed as SO₂ were 1.0 mg/l for spirits (n=101) and 4.2 mg/l for beer (n=195). The sulphite concentrations of spirits were found to be significantly lower than those of beer (p<0.0001). The new requirement of allergen labelling presents in few cases a problem for manufacturer of beer and spirits as only 2 spirit (1.9%) and 9 beer samples (4.6%) exceeded the limit of 10 mg/l. However, a labelling of the sulphite concentration had not been carried out in any of these cases.

Descriptors: sulphite, SO₂, allergen labelling, FTIR, enzymatic analysis

1 Introduction

From 25 November 2005, the EU Directive 2003/89/EC (amending Directive 2000/13/EC) on the indication of ingredients in food requires food manufacturers to list 12 groups of potential allergens if they are used as ingredients in pre-packed foods, including alcoholic drinks that are otherwise excluded from the declaration of an ingredients list [1]. For beer and spirits, sulphur dioxide and sulphites are the most relevant group of allergens as they are often found in fermented beverages and have been associated with the triggering of asthmatic responses in certain individuals [2]. By the new rules, sulphur dioxide and sulphites used as ingredients at concentrations of more than 10 mg/l (expressed as SO₂) must be included in the labelling [1].

In alcoholic beverages, natural sulphite concentrations may emerge during fermentation by the metabolism of yeasts. Sulphite appears as an intermediate state in the reduction of sulphate to sulphide delivering the sulphur for the sulphur-containing amino acids like methionine and cysteine in the amino acid synthesis [3].

In beer sulphite is a desired substance because of its antioxidative effect as scavenger and the binding of stale flavour causing carbonyl compounds. On the other hand, in early phases of the fermentation high sulphite concentrations may cause an undesirable flavour [3,4]. The sulphite formation is strongly influenced by the predisposition of the yeast and parameters affecting yeast growth during fermentation such as the physiological state of the yeast and the availability of nutrients and oxygen [5]. For the regulation of the sulphite concentration, measures like genetic modification of *Saccharomyces* yeasts were proposed that lead to inactivation of

enzymes responsible for the metabolism of sulphite to sulphur-containing amino acids [6-8].

Besides the natural occurrence, sulphur dioxide and sulphites are allowed to be used as food additives for beer including low-alcohol and alcohol-free beer up to a maximum level of 20 mg/l and for beer with a second fermentation in the cask up to a maximum level of 50 mg/l [9]. It must be stressed that this directive does not apply to beer produced after the German beer purity law that does not allow any food additives. The addition of sulphur dioxide or sulphites to spirits is also not authorised.

Guido concluded in his review about different methods for sulphur dioxide analysis in beer, that the brewing industry is still searching an ideal procedure [4]. A rapid method (15 min/sample) combining an enzyme reactor with HPLC and amperometric detection was presented by Weßels et al. [10]. FTIR in combination with Partial Least Squares (PLS) regression, evaluated for the first time in this study, might be another viable alternative as it is even faster (2 min/sample), simpler and inexpensive. FTIR results of a large sample collective are compared to the enzymatic reference procedure. The sulphite concentrations of alcoholic beverages are evaluated regarding the requirements of the new allergen labelling rules.

2 Materials and methods

2.1 Sample collective and reference procedure

In 2005, the CVUA Karlsruhe determined sulphite in 296 samples (101 spirits, 195 beers). The sampling was conducted by local authorities, either directly from the distilleries and breweries, or from retail trade. The reference procedure for the determination of total sulphite was an enzymatic method of the German official collection of analytical methods [11] that is based on the method of Beutler [12-14]. The test kits were purchased from R-Biopharm (Darmstadt, Germany). For validation of the reference procedure, two beers (Pilsner) and one spirit (fruit spirit) were analysed under repeatability conditions within a day (intraday) and under laboratory conditions on different days (interday).

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Tables and Figures see Appendix

2.2 Fourier Transform Infrared (FTIR) Spectroscopy

The WineScan FT 120 instrument (Foss Deutschland, Hamburg, Germany) was used to generate the FTIR spectra. The Wine Scan is a task-specific Fourier Transform Infrared Interferometer for alcoholic beverages. It scans the full infrared spectrum. The instrument has been approved for wine analysis since 1996 with ready-to-use must and wine calibrations provided by the manufacturer [15] and it was recently introduced to the analysis of beer and spirit drinks [16-18]. The conventional and part of the near-infrared range is scanned between 10.8 and 2 μm , which corresponds to the wave numbers of 926 to 5012 cm^{-1} . It acquires 1060 data points for data analysis. The spectral regions of water absorption between 1447-1887 cm^{-1} and 2971-3696 cm^{-1} were eliminated to prevent noise being included in the calculation.

No prior preparation of the samples is required for spirit drinks. The beer samples were degassed by filtration through fluted filter paper. For sampling, the injection nozzle of the spectrometer is plunged directly into the sample. The sample is then thermostated at 40°C in the analyser, so that no external thermostating is necessary. After measurement in the sample cuvette, the whole system and tubes are automatically cleaned by a built-in cleaning system.

2.3 Multivariate data analysis

As usual, the sample interferogram is Fourier transformed in the first step. Next, the water spectrum is divided from the sample spectrum to eliminate the background absorbance of water. In the third step, the sample is standardized using an equalizer sample, so that a transfer of calibrations between instruments is possible (e.g. the calibrations may be used in other laboratories that don't have the capabilities for reference analytics). The absorbance is calculated and the multivariate data analysis is performed.

For quantitative determination from the FTIR spectra (applying PLS regression), the standard software FT 120 v2.2.2 was used (Foss Deutschland, Hamburg, Germany). Prior to PLS regression, the appropriate wave length ranges for the analytes were selected using the automatic filter selection tool of the FT 120 software that applies multivariate data analysis. The ranges were selected based on the correlation between the reference results for the component in question and the sample variation in each wave number in the spectra by a non-disclosed algorithm of Foss. The optimal number of PLS factors was selected based on the lowest standard error of cross-validation (SECV). The statistical parameters were calculated using standard formulas (e.g. Ref. [19]).

3 Results and discussion

3.1 Calibration and validation of the FTIR-PLS procedure

Water, ethanol and many other compounds of alcoholic beverages contain absorptions of various functional groups in the infrared spectra. However, the constituents of spirit drinks or beer are chemically very similar and therefore display similar and overlapped absorptions, which cannot be assigned to individual compounds, i.e. the collective of beer and spirit drink spectra looks very homogenous and cannot be interpreted conventionally [18]. Therefore, chemometric techniques have to be used to calibrate the instrument against the chemical reference method, which makes FTIR a secondary analytical technique. Unfortunately, the PLS calibration for spirit drinks was unsuccessful so far ($R < 0.5$) because only low concentrations were found in spirits and many samples had not detectable sulphite concentrations. In contrast, the beer samples showed a good correlation ($R = 0.83$) according

to the criteria proposed by Shenk and Westerhaus [20]. The PLS calibration for beer is usable for a quantitative screening analysis. Because the calibration is based on a reference method for the determination of total sulphite, the FTIR results must be also interpreted as total sulphite. A differentiation between bound and free sulphite is not possible using the current calibration even if such information might be contained in the FTIR spectra.

Tables 1 and 2 illustrate information concerning the reference method and the results obtained through FTIR-PLS calibration and validation.

The fact that calibration methods will never perform better than the reference method must also be considered. FTIR should be treated as a fast, reliable screening method. Due to the calibration sets and not to the FTIR technique itself, the quantitative results have not enough confidence for official complaints against manufacturers (e.g. if the limits are exceeded). In this regard, it is mandatory that the FTIR results are confirmed using reference methods.

3.2 Evaluation of sulphite concentrations after the new allergen labelling rules

As shown in Figure 1, the sulphite concentrations of spirits were significantly lower than those of beer ($p < 0.0001$). An explanation of the low concentrations in spirits is the oxidation process during the storage of the mash (sulphurous acid reacts to non-distillable sulphuric acid), utilisation of wine yeast selected for low sulphur dioxide production, as well as the decreased importance of sulphurising in the production of fruit spirits [21,22].

The average concentrations expressed as SO_2 were 1.0 mg/l for spirits and 4.2 mg/l for beer (Table 3). Only two spirits (1.9%) exceeded the limit of 10 mg/l above which an indication on the label would be required. Such concentrations may be explained by uncontrolled fermentation without inoculation or by an accidental acidification of the mash with sulphurous acid instead of sulphuric acid [22].

Nine of the beer samples (4.6%) exceeded the limit of 10 mg/l (as verified using the reference method). This higher percentage may be explained by the fact that higher sulphite concentrations in beer are regarded positively as explained in the introduction.

Correlations between sulphite and alcohol, original gravity, methanol or propanol concentration were not verifiable. With process accompanying analytics it has to be verified if normal beer yeasts produce approximately 2-6 mg/l SO_2 . In our opinion concentrations above 10 mg/l definitely lead to suspicions of the utilisation of sulphite as an additive that has to be labelled.

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Appendix

Table 1 Validation results of the enzymatic reference method for the determination of sulphites in spirits and beer

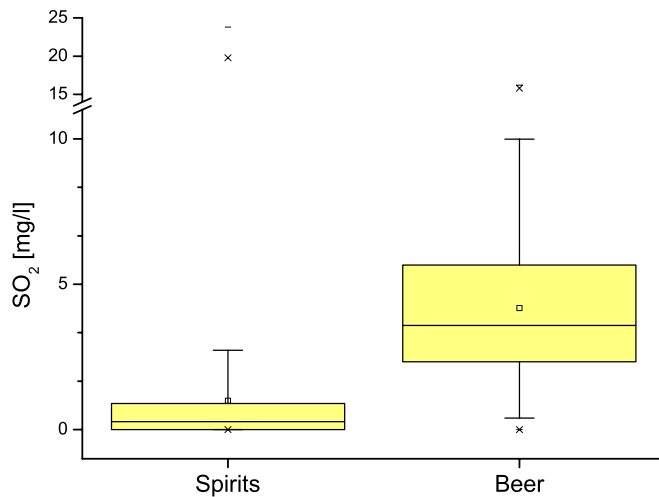
Sample	Intraday CV [%]	Interday CV [%]	Recovery [%]
Beer 1	6.3	8.3	102.5
Beer 2	1.5	2.2	96.6
Fruit spirit	2.6	3.4	96.9

Table 2 Validation results of the FTIR PLS model for the determination of sulphites in beer

Parameter	Result
PLS factors	4
SECV	2.41
Repeatability	0.04
R	0.83

Table 3 Sulphite concentrations (calculated as SO₂) in spirits and beer (results of the reference procedure)

[mg/l]	n	minimum	maximum	average	standard deviation	median	number > 10 mg/l
Spirits total	101	nn	23.8	1.0	3.9	0.3	2
Whiskey	39	nn	3.5	0.8	0.8	0.7	0
Topinambur	9	nn	1.1	0.4	0.5	0.2	0
Fruit spirits	53	nn	23.8	1.3	4.2	0.1	2
Beer total	195	nn	16.2	4.2	2.9	3.6	9
Draught beer	5	nn	2.3	1.5	1.3	2.1	0
Vollbier	146	nn	16.2	4.2	2.8	3.6	6
Brown ale	24	0.4	13.3	5.6	3.6	6.0	3
Beer mixed drinks	20	0.4	5.9	3.3	2.0	3.4	0

**Fig. 1** Boxplot of the sulphite concentrations of spirits and beer