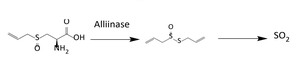
Investigation of *Allium* Containing Food Products for a False Positive Sulfite Response by LC-MS/MS

Katherine S. Carlos, Stephen M. Conrad, Sara M. Handy and Lowri S. de Jager

Center for Food Safety and Applied Nutrition, Food and Drug Administration, 5001 Campus Drive, College Park, MD 20740 USA

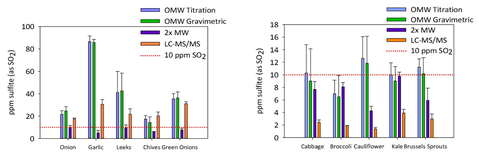
Introduction

Sulfites are a common food preservative used to reduce microbial growth and prevent both enzymatic and non-enzymatic browning. However, a small subset of the human population is sensitive to this class of compounds. As a result, the US Food and Drug Administration (FDA) mandated that all sulfites be declared on the label if they are present at levels greater than 10 mg/kg as sulfur dioxide (SO_2). Furthermore, the FDA stated that the AOAC Official Method #990.28, the optimized Monier-Williams (MW) method, be used for the quantitation of sulfites in foods. For most sulfite-containing matrices, the MW method gives accurate results.



Alliin → Allicin

One of many possible reactions involving garlic's endogenous sulfur compounds breaking down to form sulfur dioxide which would be quantified as sulfite in sulfite methods once released.



False positive sulfite response of Allium and Brassica vegetables when analyzed using the OMW method, a modified MW method (2x MW) and the LC-MS/MS method.

Since most products analyzed as regulatory samples for sulfite are not the vegetable itself but instead a food product containing the vegetable, a follow up study was conducted to determine the false positive response at lower percentages in potential matrices.

Methodology

Sample Preparation

Hummus

A basic recipe was used for hummus. Olive oil, chickpeas, tahini, fresh lemon juice, and salt were blended until smooth.

Phyllo shells, quinoa, chips

Frozen shells were baked according to manufacturers directions. Shells and potato chips were ground to a powder. Quinoa was used as is.

Extraction

Sulfites are reacted with 0.2% formaldehyde solution to form stable hydroxymethylsulfonate (HMS).
A heating step converts all sulfite-carbonyl adducts to HMS.
A C18 SPE cleanup removes any lipids or other matrix components that might interfere with the analysis.

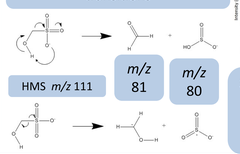
LC-MS/MS

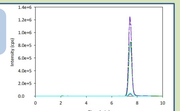
Waters Acquity LC System

Sciex 4000 QTRAP

Column: 150 x 2.1 mm SeQuant ZIC HILIC, 5 µm thermostatted at 30 °C
Gradient Elution:
(a) 90% ACN/10% H_2O in 10 mM ammonium acetate
(b) 50% ACN/50% H_2O in 10 mM ammonium acetate
Monitored transitions:
(a) 111 → 81 (b) 111 → 80

Explanation of monitored transitions





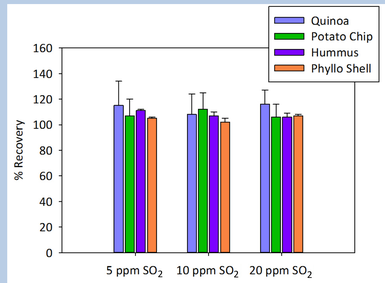
Sample chromatogram of a 0.4 mg/kg Na_2SO_3 standard

Species Identification

Ten samples of garlic were analyzed via polymerase chain reactions (PCR) using 4 traditional DNA barcoding markers including 3 from the chloroplast and ITS2 from the nuclear genome. All amplified products were bi-directionally sequenced on an 3500xl DNA Analyzer. Sequences were compared to Genbank using BLASTn to make a species determination.

Results

Recovery of sulfite from 4 different matrices



Four different matrices were spiked at 5 ppm SO_2, 10 ppm SO_2, and 20 ppm SO_2.

All recoveries were within the acceptable range of 80-115%. Repeatability was also within the acceptable range.

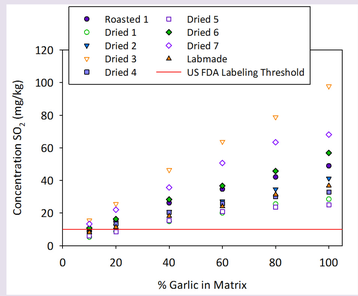
These results demonstrate that added sulfite can be accurately quantified with this method.

False positive response of 9 garlic powders were quantified by increasing the powder concentration in a quinoa-garlic mixture.

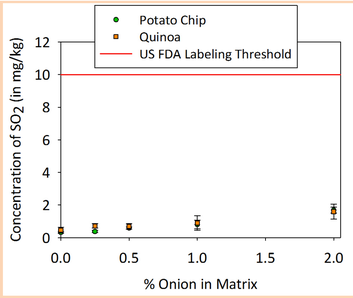
▲Labmade garlic powder was also analyzed to compare this garlic powder used in spiking experiments with commercial garlic powders.

Variability between powders did exist. At 100% garlic, concentrations ranged from 25-98 ppm SO_2.

Determination of sulfite from 9 garlic samples in quinoa at increasing concentrations



False positive sulfite response from onion powder in potato chips and quinoa



Lab made garlic powder was added at increasing concentrations to four different matrices up to 8% garlic.

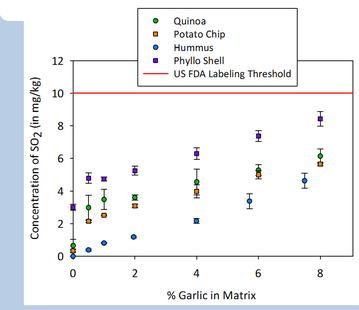
There was more variability by matrix than was observed with the onion powder addition. Hummus was slightly lower than the quinoa and potato chip samples and phyllo shell was slightly higher. Phyllo shell also had a higher background sulfite concentration.

Labmade onion powder was added at increasing concentrations to potato chip and quinoa matrices.

Even at 2% onion powder, there was a very small release of SO_2. The concentration of SO_2 released was never greater than 2.5 ppm SO_2.

Responses were very similar in potato chip and quinoa.

False positive sulfite response from garlic powder



Species identification was conducted on all 9 garlic powders plus a lab dried elephant garlic. All powders were *Allium sativum* with the exception of the Elephant garlic which was *Allium scorodoprasum var. viviparum*. Due to the difference in species, Elephant garlic was not used in the addition experiments.

Conclusions

Onion powders do not appear to pose a risk for producing false positive concentrations of SO_2 when added as an ingredient into a product.

There was some variability of SO_2 produced in the commercial garlic powders that were tested. The labmade garlic used for the spiking experiments was in the middle of all those tested.

Based on the sample set that was investigated, it would appear that the concentration of SO_2 released by the garlic powders would have to be 5-25% by weight before a false positive result was achieved. The researchers do not believe there are many products on the market that would have such high garlic concentrations.

The released SO_2 concentrations will be lower the higher the moisture concentration of the product. It is believed that the SO_2 will have already offgassed to some extent in these products as was observed in the hummus samples.

Future Work

Since these are agricultural products, additional sampling may improve confidence in our results. The analysis could be repeated in a few years time in order to gauge whether these differences reflect growing year and production time.

Four matrices were chosen as representative samples of products that might have garlic or onion added to them and could be tested as regulatory samples for sulfites. There are additional products that might be encountered as regulatory samples but before proper conclusions can be drawn about the feasibility of their analysis by LC-MS/MS more experimentation should be conducted.

Elephant garlic and other *Allium* species were not tested as part of this study since they hadn't shown high concentrations in the initial study. It might be instructive to collect some data on vegetables such as chives or leeks to verify that the trends observed in garlic and onion could be applied to the whole species.

References

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Robbins, KS; Shah, R; MacMahon, S; de Jager, LS. (2015) Development of a Liquid Chromatography-Tandem Mass Spectrometry Method for the Determination of Sulfite in Food. *J Ag Food Chem.*

Lafeuille, J.-F.; Lefevre, S.; Achouri, D. (2007) Determination of Added Sulfites in Dried Garlic with a Modified Version of the Optimized Monier-Williams Method. *J AOAC Int.* 90 (4).

Additional Studies:

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