QUANTITATION OF SULPHUR AROMA COMPOUNDS IN MAILLARD MODEL REACTION SYSTEMS OF DIFFERENT COMPOSITION


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Abstract

The development of a SPME-GCMS method aimed to quantify 2-methyl-3-furanthiol (MFT), furfurylthiol (FFT) and bis(2-methyl-3-furanyl) disulphide (MFT-MFT) in model reaction systems of various amino acid and sugar composition is presented. However, the concentration of cysteine was demonstrated to have a major impact on the thiol stability and the partition of sulphur species between thiols and their disulfides forms. The impact of cysteine was only partially compensated by stable isotopic labelled standards. We concluded that thiol and disulfide compounds can not be simultaneously measured reliably in model reaction systems containing an undefined cysteine concentration via analytical methods based on partition.

Introduction

Thiol compounds such as 2-methyl-3-furanthiol (MFT) and 2-furfurylthiol (FFT) exhibit very potent meat aroma characters. The analysis of these two thiols is complicated due to their instability, in particular their ability to oxidise into disulfides like MFT-MFT or FFT-FFT (1), also displaying meaty characters. To understand the impact of composition of the starting material and thermal condition on the formation of such meaty aroma compounds, an analytical method, allowing the quantification of targeted sulphur compounds in food matrices of different composition was desired. A method based on SPME-GCxGC-TOFMS was selected as a good compromise between sensitivity, automation, minimal sample preparation. Also, SPME-GCMS has already been reported for thiols and disulfides quantification (2). The experiments to validate the method were performed on GC-MS and are reported in this paper.

Experimental

MFT, FFT, and MFT-MFT were purchased from Aldrich (Zwijndrecht, The Netherlands) and the respective stable-isotope labelled internal standard (labelling degree >98%) MFT* ([2H3] 2-methyl-3-furanthiol) and MFT*-MFT* ([2H6] bis(2-methyl-3-furanyl)disulphide) from AromaLAB AG (Munich, Germany). These chemicals were spiked either in a pyrophosphate buffer (pH 6) or in a sulphur free model reaction system composed of glutamate (0.3%), glycine (2.5%), xylose (0.5%), glucose (0.5%) and fructose (0.5%) reacted at 100ºC under reflux for 2 hours in the same buffer.

Analysis were performed via SPME-GC-MS, using 30 min absorption time at 60ºC on a PDMS fibre (100µm coating) desorbed at 230ºC in a Trace GC (Thermo).
Separation was performed with a Restek DB5 (30m x 0.25mm x 0.5μm film thickness) coupled to a 70 eV Ei-trace DSQ MS (Thermo) operated in SIM mode.

**Results and discussion**

The chemical pathway of MFT and FFT formation is known to originate from the reaction of the sulphur amino acid cysteine with pentose (3). The sulfhydryl group of cysteine is a very strong reducing agent used frequently to prevent enzyme inactivation, by breaking disulfide bridges formed via oxidation. Since cysteine is a precursor of MFT and FFT, it is likely to be not fully degraded at the end of our thermal conditions (100ºC for maximum 3 hours) and could exhibit an antioxidant activity influencing the equilibrium of sulphur species between their thiol and disulfide form.

To assess this hypothesis, various cysteine concentrations up to 1% were added into a sulphur-free reacted model system, in which standards of MFT and FFT (100 ppb) were spiked. A similar experiment was also performed with the addition of MFT-MFT (100 ppb), and the combined results are presented in Figure 1. For the thiol species, the signal intensity greatly increased with cysteine concentration, stabilising above cysteine concentration of 0.5%. The disulfide behaved in an opposite manner, with the signal intensity dropping dramatically between 0 and 0.25% cysteine and remained low and stable with increasing cysteine concentrations.

![Figure 1. Influence of addition of Cysteine to the signal intensity of standard MFT, FFT and MFT-MFT spiked into a sulphur-free reacted model system. (MFT and FFT were measured in a separate experiment than MFT-MFT).](image)

The result in Figure 1 shows that the reducing activity of cysteine influences the equilibrium between thiols and their disulfide forms. Moreover cysteine has also been shown to impact the stability of thiols over time (1), and its stabilising effect was studied (4). Using similar experimental conditions as in Figure 1, the effect of cysteine (0.1 and 0.4%) on MFT and FFT stability was monitored over 28 hours at room temperature and at 4ºC with 0.4% cysteine. The results are presented in Figure 2. The samples kept at room temperature showed that a higher cysteine concentration improved the stability of MFT and FFT over time. A further improvement was obtained by keeping the sample at 4ºC, although none of the conditions used here were sufficient to stop totally the degradation of MFT and FFT. To ensure reliable analytical results, it is important to select conditions where as little thiol degradation as possible occurs.
Beside cysteine; the composition of the model reaction system also influences the headspace concentration of MFT and FFT. This was demonstrated by spiking MFT and FFT into aliquots of a sulphur free model reaction systems, collected at regular interval during heating at 100°C, to simulate compositional changes. In such experiment (data presented in the poster), the signal intensity of MFT and FFT declined in a significant manner as the reaction time increased. For instance, the signal intensity of MFT after 60 minutes was only 15% of the intensity measured at the start of the reaction.

A consequence from Figure 1, Figure 2 and this last experiment is that quantification of MFT and FFT is not possible with external calibration in model reaction systems using a partition method like SPME. The use of stable-isotope labelled internal standard MFT* and MFT*-MFT* was attempted to compensate for the matrix effect and the effect of cysteine. Calibration lines for MFT and MFT-MFT in buffer were determined between 0 and 10 ng/g relative to their labelled internal MFT* and MFT*-MFT* (7 ng/g), using 0 and 0.2% cysteine. In the case of MFT-MFT, the calibration lines obtained are presented in Figure 3.
MFT-MFT behaved linearly in the absence of cysteine, but drifted severely from linearity at 0.2% cysteine. An examination to understand this loss of linearity showed that the peak area of MFT*-MFT* decreased with an increased concentration of MFT-MFT. This was explained by the formation of an increasing amount of a single substituted labelled species MFT-MFT*, as a result of an exchange of deuterated MFT* between the labelled and non labelled standard of MFT-MFT. Such result is in accordance with the cysteine effect observed in (Figure 1) and shows the dynamic nature of the equilibrium between MFT and its dimmer. Similar limitations to the application of labelled internal standards were reported to quantify mercaptans in wine (6). The consequence is that MFT* and MFT*-MFT* can not be used together for quantification in a single analysis via a partition technique if the cysteine concentration is not known.

Conclusion

The aim of this work was to develop an analytical method based on SPME-GC-MS capable of quantifying thiols and disulfides in Maillard systems of different composition. However, the method development has revealed that without prior knowledge of the cysteine concentration such quantification was not possible. The reducing activity of cysteine had a strong influence on the stability over time of thiol and governed the equilibrium of sulphur species between their thiol and disulfide forms. The use of stable-isotope labelled internal standards proved of limited contribution as they also reacted in an uncontrolled manner.

In the light on these results, quantification of thiols and disulfides generated from thermal reaction in a model reaction system was questioned as the concentrations of thiol and disulfide measured at the end of a reaction are greatly influenced by the redox state in each sample. A solution to suppress such effect would be to control the redox state of the sample and totally reduce disulfides into their thiols form, using a reducing agent such as Dithiothreitol (DTT). Doing so, the total potential thiol concentration formed via thermal generation would be measured rather than the actual thiol concentration in a sample. Such analysis would simplify thiol analysis resulting in more stable and reliable thiols measurements. The development of such analytical method to quantify total potential thiol concentration formed via thermal generation would be reported in a later stage.

References