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Mouldy and musty off-flavour in garlic is caused by the presence of 2,4,6-trichloroanisole

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ABSTRACT

The occurrence of garlic with expressed mouldy, musty and cork taint-like off-flavour is of serious concern for the food industry as it may cause significant economic damage. In this study, we investigated a total of 42 garlic samples for their concentrations of 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA). The TCA odour thresholds in two garlic matrices were determined to evaluate the impact of the TCA presence on garlic's flavour properties. While TCA was identified in more than 45% of the samples in concentrations of up to 78 μg kg^{-1}, TBA was not detected in any of the samples. In approx. 35% of the garlic samples, the concentrations were higher than the odour threshold. It is noteworthy that garlic preparations that are to be further processed by the food industry, on average, show higher contamination rates than samples drawn from retail. The results of this survey demonstrate that it is of the utmost importance for food processing companies to perform a sensory evaluation of the garlic or garlic formulations prior to their use in order to avoid economic damage and to minimize food waste.

1. Introduction

Garlic (*Allium sativum* L.) is a vegetable and spice that has been used in kitchens worldwide since ancient times due to its attractive and intense flavour. The chemistry of garlic flavour is well under-stood. Alliin (S-allyl-L-cysteine sulfoxide), a non-proteinogenic amino acid, is the predominant odourless precursor in garlic which is located in the garlic cytoplasm. Cell disruption caused by garlic processing leads to the release of the enzyme alliinase from the vacuoles and catalyses the alliin degradation under the formation of 2-propene sulfenic acid which dimerises and forms diallyl thiosulphinate ('allicin'). Allicin itself is not stable and immediately reacts further – depending on the specific conditions – resulting in a large number of different sulphur containing compounds which are made responsible for the characteristic garlic aroma (Lanzotti, 2006; Christensen et al., 2007; Abe et al., 2020). Organosulfur compounds reported from allium species exhibit health promoting and health beneficial effects with respect to metabolic and cardiovascular diseases and also cancer prevention (Pérez-Rubio et al., 2022; Hossain et al., 2022; Sánchez-Gloria et al., 2022). The antibacterial and fungicidal effects of fresh garlic and garlic juice are also described (Lanzotti, 2006; Li et al., 2023). The combination of the intense flavour and the health beneficial effects make the consumption of garlic popular in almost every region of the world.

In 2021, worldwide garlic production amounted to approx. 30 million metric tons per year, with China being the largest garlic producer with an annual production of approx. 23 million tons (www.atlasbig.com), followed by India with an approximate production of 2.9 million tons per year (www.worldatlas.com). Even though garlic flavour is very intense, the flavour associated with the consumption of garlic is often offset by the perception of a distinct mouldy and musty off-flavour that is most likely caused by the presence of the haloanisole 2,4,6-trichloroanisole (TCA) (e.g., www.metrovino.com). TCA is well known as an off-flavour causing compound in various food commodities. The occurrence of TCA in white and red wine and its relevance for the perception of the so-called cork taint has been reported extensively in the literature. Recent reviews give surveys about the occurrence and detection of cork taint in wines (Callejón et al., 2016; Cravero, 2020; Tarasov et al., 2022). TCA occurrence is also described in many other food commodities, such as drinking water, fresh and dried fruits and vegetables, sake and rice koji, chicken and eggs (Whitfield, et al., 1987; Mottram, 1998; Miki et al., 2005; Dietrich and Burlingame, 2020; Zahraei et al., 2021). The occurrence of medicinal and phenolic

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off-flavour caused by the presence of TCA in coffee even carries a specific name and is generally known as the 'Rio defect' (Spadone et al., 1990; Romano et al., 2022). In addition to the consumers' disappointment or disgust due to the sensory perception of the tainted food, the economic damage is incredibly high. The Rio defect affects approx. 20% of the Brazilian coffee harvest (Romano et al., 2022); with reference to wine, cork taint affects approx. 5% of the wines bottled in Europe, resulting in annual losses of 700 million euros (https://cordis.europa.eu/project/id/262319).

Interestingly, TCA does not only evoke cork-like taint in various food matrices. The presence of TCA quenches the perception of other odour-active compounds by suppressing cyclic nucleotide-gated channels in the olfactory receptor cells (Takeuchi et al., 2013). This behaviour was reported in cases of extremely low TCA concentrations and implies that, in addition to the formation of a pronounced off-odour in foods, the presence of TCA in sub-threshold concentrations may lead to a significant reduction of the typical sensory properties of the respective food.

The source of the mouldy, cork-like taint in garlic is not yet completely understood. Several ways of contamination or the formation, respectively, of TCA are conceivable, including the bioformation of haloanisoles by microorganisms from suitable precursor compounds, but also contamination from any exterior sources. 'Fusarium dry rot' of garlic is a postharvest disease of great concern for garlic producers. In recent studies, the occurrence of dry rot on stored garlic caused by contamination with Fusarium proliferatum was reported as a problem of serious concern. An up to 30% loss of garlic bulbs during storage was reported in France and Italy (Tonti et al., 2012; Leyronas et al., 2018). In addition to the economic damage, contamination with Fusarium proliferatum is of toxicological concern due to its production of fumonisins, which are rated as potentially carcinogenic to humans (Mondani et al., 2021). The fungicide prochloraz [N-propyl-N-[2-(2,4,6-trichlorophenoxy)-ethyl]imidazole-1-carboxamide] is a broad-spectrum fungicide that has frequently been applied to prevent fruit from deterioration. Residues were described in a broad range of produce, such as apples, oranges, mushrooms, green tea drinks, bananas and Chinese garlic sprouts. High efficiency against garlic dry rot was reported when garlic was treated with prochloraz (Horáková et al., 2021; Mondani et al., 2021). 2,4,6-Trichlorophenol as the final metabolite of prochloraz may then serve as a precursor for TCA (Fang et al., 2017). The role of filamentous fungi, which had been isolated from cork, on the formation of TCA from 2,4,6-trichlorophenol by *O*-methylation was already described in the literature from two decades ago (Álvarez-Rodríguez et al., 2002). Similar reactions are most likely to occur in garlic cloves when a Fusarium infection is observed which requires the treatment with prochloraz.

Paper and cardboard used to package garlic and to produce storage boxes represent further potential sources of haloanisoles. Halophenols are described in the literature as a base for wood preservatives. In the presence of fungi or other microorganisms, these halophenols may be dehalogenated followed by O-methylation to finally form TCA (Martendal et al., 2007). Migration of the haloanisoles into the garlic cloves during storage or transportation in cardboard and paper packages is a serious problem in food logistics. Furthermore, the contamination of tap water in Chinese cities has become an emerging problem over the last decades (Zhang et al., 2016). A potential contamination of garlic with haloanisoles from water at any time of the production (e.g. through irrigation of the field or washing of the garlic after harvest) is likely to occur and might be considered an alternative source of 2,4,6-trichloroanisole contamination.

The occurrence of garlic samples with a corky off-flavour is a serious problem for food processing companies as it may cause significant economic damage. It is also an annoying fact for consumers in private households and gastronomy. As a consequence, we performed a survey on the presence of 2,4,6-trichloroanisole in garlic. Due to similarities in their odour profiles, we included the analysis of 2,4,6-tribromoanisole in this study. Samples were purchased at random from local supermarkets and farmers' markets and were also provided by a local food processing company that purchases dried and deep-frozen garlic in large amounts from different international sources. To evaluate the sensory impact of the haloanisoles in the garlic samples, we determined the TCA odour threshold in garlic in addition to the quantitative analysis. With this investigation, we aim to build a basis for the evaluation of garlic quality and to demonstrate the importance of this topic to the food industry.

2. Material & methods

2.1. Sample material

In order to perform a survey on the potential occurrence of haloanisoles, the concentrations and the sensory impact on commercially available garlic, we collected a total of 42 garlic samples for this study. Sampling was performed at random. Different types of garlic material were analysed: (i) 11 samples of dried garlic granule – the samples were provided by a local food processing company or purchased at random from different sources (supermarkets and farmers' markets); (ii) 13 samples of fresh garlic purchased in local supermarkets or farmers' markets; (iii) 9 samples of deep-frozen garlic provided by a local food processing company or purchased in local supermarkets and supermarkets for catering supply; (iv) 9 samples of garlic paste provided by a local food processing company. A detailed description of the sample material, along with the respective TCA concentrations as well as odour activity values, is given in Table 1 The fresh garlic samples were analysed as soon as possible after purchase or delivery, respectively; the garlic granules were stored in closed glass containers at room temperature until the analysis, while the deep-frozen products were kept at − 26 °C until further use.

3. Sensory evaluation – determination of the odour thresholds of TCA in garlic matrices

The odour threshold of TCA was determined for the garlic granules and deep-frozen garlic. Due to similarities of the matrices fresh garlic, deep-frozen garlic and garlic paste, deep-frozen garlic was considered as suitable representative for these three sample types. For the garlic granules, a TCA-free sample (sample DG 9) was used as a matrix. The matrices fresh garlic, deep-frozen garlic and garlic paste short large similarities. Thus, deep-frozen garlic was considered as suitable representative for these three sample types. In sample DF 9, the garlic was cut into equally sized garlic cubes with an edge-length of approx. 2 mm. DG 9 and DF 9 were available in quantities that were large enough to produce homogenous samples that were necessary for duplicate threshold determinations. In DF 9, TCA was present in low concentrations (0.11 μg kg^{-1}, Table 2); however, in a sensory pre-test, TCA was not perceivable to the panellists from this sample. For the calculation of the sensory threshold in this matrix, the background concentration was included in the calculation of the final threshold value.Table 2.

Sensory experiments were performed under standardized conditions in the sensory laboratory. The study was conducted in accordance with the Declaration of Helsinki. 13 well-trained panellists participated in the experiments. All participants provided their informed consent at the beginning of the study. All subjects fulfilled the requirements given in DIN EN ISO 8586 for sensory experts. In addition, each panellist had

Table 1
Odour threshold of TCA in garlic granule and in garlic paste prepared from deep frozen garlic; 13 trained panellists; each threshold determination was carried out in duplicate (in total n = 26 per matrix).

Sample matrix	Odour threshold [μg kg^{-1}]
Garlic granule	0.24
Fresh garlic/garlic paste	0.42

Table 2
Sample list and concentrations and OAVs of TCA in garlic samples; n = 3; 'n.d.' not detected; 's′ standard deviation; concentrations that are higher than the odour thresholds are printed in bold; 'DG' dried garlic, 'FG' fresh garlic, 'DF' deep-frozen (cut garlic cubes, deep-frozen garlic cloves), 'GP' deep-frozen garlic paste), 'OQ' organic quality.

Sample Code	*Sample type*	*Source*	*TCA concentration ± s [μg kg⁻¹]*	*OAV*[a]
DG 1	Granule	Local supermarket	n.d.	
DG 2	Granule	Local supermarket	0.03 ± 0.002	< 1
DG 3 (OQ)	Granule	Local supermarket	n.d.	
DG 4	Granule	Local supermarket	n.d.	
DG 5 (OQ)	Granule	Local supermarket	n.d.	
DG 6	Granule	Local supermarket	n.d.	
DG 7	Granule	Local supermarket	n.d.	
DG 8	Granule	Supermarket for catering supplies	n.d.	
DG 9	Granule	Provided by a local food producer	n.d.	
DG 10	Granule	Provided by a local food producer	0.23 ± 0.09	~ 1
DG 11	Granule	Provided by a local food producer	0.06 ± 0.02	< 1
FG 1 (OQ)	Fresh garlic bulb	Local supermarket	n.d.	
FG2	Fresh garlic bulb	Local supermarket	n.d.	
FG 3	Fresh garlic bulb	Local supermarket	n.d.	
FG 4	Fresh garlic bulb	Local supermarket	n.d.	
FG 5 (OQ)	Fresh garlic bulb	Local supermarket	n.d.	
FG 6 (OQ)	Fresh garlic bulb	Local supermarket	n.d.	
FG 7	Fresh garlic bulb	Supermarket for catering supplies	n.d.	
FG 8	Fresh garlic bulb	Provided by a local food producer	n.d.	
FG 9	Fresh garlic bulb	Local supermarket (provenance China)	**20.4 ± 1.6**	**48**
FG 10	Fresh garlic bulb	Local supermarket (provenance Spain)	**8.9 ± 0.3**	**21**
FG 11	Fresh garlic bulb	Local farmers' market	**2.4 ± 0.1**	**6**
FG 12	Fresh garlic bulb	Local farmers' market	**1.8 ± 01**	**4**
FG13	Fresh garlic bulb	Local farmers' market	**0.9 ± 0.05**	**2**
DF 1	Deep-frozen chopped garlic	Provided by a local food producer	n.d.	
DF 2	Deep-frozen chopped garlic	Provided by a local food producer	n.d.	
DF 3	Deep-frozen garlic cloves	Provided by a local food producer	n.d.	
DF 4	Deep-frozen garlic pellets	Provided by a local food producer	0.40 ± 0.02	~ 1
DF 5	Depp-frozen chopped garlic	Provided by a local food producer	0.12 ± 0.03	< 1
DF 6	Deep-frozen garlic puree	Provided by a local food producer	n.d.	
DF 7	Deep-frozen garlic	Provided by a local food producer	**25.2 ± 1.7**	**60**
DF 8	Deep-frozen garlic	Provided by a local food producer	**9.7 ± 0.2**	**23**
DF 9	Deep-frozen chopped garlic	Supermarket for catering supplies	0.11 ± 0.01	< 1
GP 1	Deep-frozen garlic paste	Provided by a local food producer	**28.9 ± 0.8**	**69**
GP 2	Deep-frozen garlic paste	Provided by a local food producer	n.d.	
GP 3	Deep-frozen garlic paste	Provided by a local food producer	0.42 ± 0.2	~ 1
GP 4	Deep-frozen garlic paste	Provided by a local food producer	**1.24 ± 1.1**	**3**

Table 2 (*continued*)

Sample Code	*Sample type*	*Source*	*TCA concentration ± s [μg kg⁻¹]*	*OAV*[a]
GP 5	Deep-frozen garlic paste	Provided by a local food producer	n.d.	
GP 6	Deep-frozen garlic paste	Provided by a local food producer	**34.5 ± 1.6**	**82**
GP 7	Deep-frozen garlic paste	Provided by a local food producer	**78.0 ± 7.5**	**185**
GP 8	Deep-frozen garlic paste	Provided by a local food producer	n.d.	
GP 9	Deep frozen garlic paste	Provided by a local food producer	8. **± 2.3**	**61**

[a] The OAV was calculated by relating the concentration to the odour threshold in the respective matrix (i.e. odour threshold of 0.24 μ/kg for the samples DG1-DG11; odour threshold of 0.42 μg kg^{-1} for all other samples)

long standing experience in analytical sensory evaluation. Based on their sensory knowledge, they were familiar with the odour of TCA and the odour of other compounds expressing mouldy, musty or corky odour. In the specific training phase for this task, they were familiarised with the different qualities of garlic granules and fresh/deep-frozen garlic.

For the odour-threshold determination, TCA-containing samples were produced in the laboratory by spiking the garlic samples DG 9 and DF 9 with adequate amounts of TCA (>99%, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). To perform this, a TCA stock solution (1.25 g L^{-1}) was prepared in non-denatured ethanol (95%, Merck KGaA, Darmstadt, Germany) and, stepwise, further diluted with non-denatured ethanol to obtain the necessary concentrations. To estimate the appropriate TCA concentrations for the threshold determination test, off-odourous samples with known TCA concentrations were evaluated prior to the threshold determination experiments by a limited number of panellists to narrow the number of samples that were required for the threshold determination.

The garlic granule matrix DG 9 was a very homogenous sample with very small and equally-sized garlic granules which could be used for the preparation of the spiked samples as such. For the preparation of the spiked garlic granule samples, 2 g of garlic granule was weighed into each sample container. Dilutions of the TCA stock solution were prepared in adequate concentrations and added to reach the following concentrations in the garlic samples by adding 10 μL of the respective TCA-dilution: 0.125 μg kg^{-1}, 1.25 μg kg^{-1} and 12.5 μg kg^{-1}. For the duplicate determination, the concentration range was narrowed down to concentrations between 0.125 μg kg^{-1} to 1.25 μg kg^{-1} based on the results from the first experiment. For the garlic reference samples, 10 μL of ethanol was added without TCA to account for any impact from the used solvent. After spiking, each sample was thoroughly shaken for a homogenous distribution of TCA in the garlic granules. Samples were left to equilibrate for 24 h before the sensory evaluation.

For the preparation of the fresh garlic samples, the deep-frozen garlic cubes were thawed and homogenized in a laboratory scale blender (BÜCHI mixer B400, BÜCHI Labortechnik AG 9230 Flawil, Switzerland) to obtain a homogenous matrix. For each TCA concentration, sample homogenization and spiking were performed in one operation step. 100 g of garlic sample was homogenized with the addition 100 μL of the adequate TCA solution dissolved in ethanol to reach the final concentrations of 0.125 μg kg^{-1} up to 12.5 μg kg^{-1}. To compensate for any potential solvent effect, 100 μL of ethanol was added to 100 g of the reference garlic sample. For sensory evaluation, 5 g of (spiked) garlic samples was put into each sample container.

The sensory threshold was determined by offering the TCA spiked garlic samples in order of increasing concentrations together with the garlic references. As the overall intensity of the samples was very high, we sought to keep the number of samples as low as necessary (i.e., the reference sample plus three samples per test in increasing TCA concentrations). The garlic samples were offered in small glass containers with screw caps. Each panellist obtained his/her own sample sets. All samples were coded with a 3-digit randomized number. In order to keep the odour background in the sensory laboratory at a low level, the panellists were asked to only open the lids immediately before sniffing the samples and to close them again immediately after the evaluation. Panellists were asked to note down if an off-odour was perceived and to describe the perceived off-odour as precisely as possible.

The odour threshold concentrations were determined as best estimate thresholds (BET) (Meilgaard et al., 2016). Individual odour thresholds were calculated as geometric means for each single panellist from the concentration where the off-flavour was perceived and the concentration of the preceding sample. The final odour threshold was determined as the group threshold (BET) by calculating the geometric means of all individual thresholds.

4. Quantitative determination of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole

With respect to the quantitative determination of halogenated anisoles, the dried garlic granules had to be treated slightly differently than the fresh and deep-frozen garlic as well as garlic paste, respectively. For all sample types, isotopically labelled compounds were used as internal standards for the quantification (2,4,6-trichloroanisole-d5 (TCA-d5), purity > 99.5%, Dr. Ehrenstorfer GmbH, Augsburg. Germany; 2,4,6-tribromoanisole-d5 (TBA-d5), purity > 99.5%, EQ Laboratories GmbH, Augsburg, Germany). A dilution of both deuterated compounds was prepared in methanol for further use (1 μg L^{-1} per compound; methanol, 99.8%, Sigma Aldrich, Austria). For the dried garlic granules, two different approaches were chosen for reasons of quality assurance; for all samples, both ways of sample preparation were carried out in parallel. In both cases, sample preparation was carried out directly in 20 mL headspace vials to avoid excess sample manipulation: (i) 100 μL of water was added to 10 mg of garlic granules; (ii) direct extraction of the analysis from the dried granules (sample weight 10 mg). In both cases, the internal standards were added (10 pg absolute per compound equivalent to 1 μg kg^{-1} sample). For the determination of the analytes from fresh garlic, the garlic cloves were peeled; the deep-frozen garlic was thawed, and the garlic paste was used as is. 10 g of garlic product was mixed thoroughly with 100 mL of water using a lab-scale blender (BÜCHI mixer B400, BÜCHI Labortechnik AG 9230 Flawil, Switzerland). An aliquot of 100 μL was transferred into a 20 mL glass vial with the addition of internal standards (10 pg absolute per compound equivalent to 1 μg kg^{-1} sample). All samples were analysed in triplicate.

For the quantitative determination of TCA and TBA in the garlic samples, gas chromatography triple quadrupole mass spectrometry (GC-MS/MS with multiple reaction monitoring MRM) with stable isotope dilution was used. Prior to the gas chromatographic separation, the volatiles were enriched from the headspace above the samples using headspace solid phase microextraction (HS-SPME). The sampling was performed with a CTC Combi PAL sampler (CTC Analytics, Switzerland). An SPME 'arrow' CWR/PDMS (1.1 mm outer diameter, 120 μm phase thickness, sorption phase: 44.0 mm^2 (surface)/ 3.8 μL (volume); CTC Analytics, Switzerland) was used. For the enrichment of the analytes, the samples were put into the sample oven of the autosampler (Single Magnetic Mixer, Chromtech, Germany) and equilibrated at 80 °C while stirring the samples thoroughly with a glass coated magnet stirrer. The SPME arrow was exposed into the headspace for 20 min. After enrichment, the arrow was transferred directly into the GC injection port for thermodesorption (splitless injection, 270 °C injector temperature). The GC separation and detection were carried out on a Shimadzu System (GC-2010 coupled to triple quadrupole TQ 8050; both components Shimadzu, Japan). The GC separation was performed on a semi-polar column (Rxi®−5 ms, 30 m × 0.25 mm × 0.25 μm, Restek Corporation, USA) using the following conditions: a temperature program starting at 70 °C (1 min) with a temperature ramp of 10 °C min^{-1} up to 300 °C (holding time 3 min). Helium was used as a carrier gas with a constant flow rate of 35 cm sec^{-1}. The interface temperature was set to 280 °C, quadrupole resolution for Q1, and Q3 was set to 'high resolution'. Electron impact ionisation (EI 70 eV) with an ion source temperature of 200 °C was used. For mass spectrometric identification and quantification, triple quadruple MS/MS-MRM was used with a detector voltage of 1.8 kV. The following transitions (collision energy [eV]) were used for the detection of the analytes and internal standards, respectively: TCA-d5: 217.00 > 198.90 (18); 215.00 > 197 (12); TCA: 211.90 > 197.00 (15); 209.90 > 194.90 (15); TBA-d5: 350.80 > 332.70 (17); 348.70 > 330.70 (17); TBA: 345.80 > 330.80 (17); 343.70 > 328.70 (17). Detailed information on the development of the whole method was described earlier (Leis, 2021).

The linearity of the system was validated over two concentration ranges. All calibration points were analysed in triplicate. For lower concentrations, five-point calibrations were used between 0.01 μg kg-1 and 5 μg kg^{-1} TCA and TBA in the garlic matrix (r^2 =0.9998 for TCA, r^2 =0.9989 for TBA), and the higher concentration ranges were also calibrated in five-point calibrations between 5 μg kg^{-1} and 100 μg kg^{-1} (r^2 =0.9988 for TCA and r^2 =0.9978 for TBA). The limits of quantitation were calculated based on the calibration curves of the lower concentration ranges. A limit of quantitation of 0.02 μg kg^{-1} was obtained for TCA and 0.03 μg kg^{-1} for TBA in the garlic matrix.

5. Results and discussion

5.1. TCA odour thresholds in garlic matrices

It is well known that odour thresholds are highly dependent on the sample matrix. For most compounds, odour threshold values have been published on the matrix of air or on the liquid matrices of water and oil (Van Gemert, 2011). The sensory threshold is not only dependent on the matrix, but also on the familiarity of the panellists with the respective type of off-flavour and on the sensory techniques that are applied for the threshold determination. As a consequence, the odour threshold values given in the literature for a single compound usually vary over several orders of magnitude (Bi and Ennis, 1998; Schranz et al., 2017). Despite all these limitations, the determination of the sensory thresholds is indispensable when judging the impact of single compounds on the perceived off-odour of a specific food. The determination of the odour-active compound's concentration alone is not sufficient to evaluate the impact of the compounds on the flavour or off-flavour of the product under investigation. The commonly used odour activity value (OAV) concept that was published several decades ago (Grosch, 1994) and relates the compound's concentration to its sensory threshold also accounts for this correlation (i.e., OAV=concentration of the compound / odour threshold of the compound in the respective matrix; only compounds with OAV>1 contribute to the odour of the product).

In addition to TCA odour thresholds in water, TCA odour thresholds have been reported in wine as the occurrence of cork taint in wine is a serious problem for the wine industry. On average, the TCA odour threshold values that are given in the literature range between 8 and 30 pg L^{-1} in water and between 30 pg L^{-1} and 22 ng L^{-1} in different wine samples (Van Gemert, 2011; Prescott et al., 2005; Mazzoleni & Maggi, 2007; Teixeira et al., 2016; Callejòn et al., 2016). In comparison to water and wine, garlic is a food matrix with a very high genuine flavour intensity. Due to the high odour potency, particularly of the organosulfur compounds (which are responsible for garlic flavour), the masking of any off-flavour caused by haloanisoles is likely to be expected. Furthermore, garlic as a food matrix is completely different to aqueous samples or wine in its composition — with respect to macromolecules that may lead to a modification of the flavour release. Thus, we assumed that the use of published threshold values of 2,4,6-trichloroanisole in the matrices of water or wine are not applicable for the assessment of TCA concentrations in garlic samples. As a consequence, we decided to determine the odour threshold for TCA in garlic samples. As TBA was not detected in any of the investigated samples, we refrained from determining the sensory threshold for the brominated analogue.

The determination of the sensory threshold of TCA in garlic was performed by a well-trained panel. Specific training on mouldy, musty and earthy odours as well as on different types of garlic samples with different TCA levels was performed prior to the threshold tests. The sensory thresholds were determined (i) in garlic granules and (ii) in a garlic paste that was produced from deep-frozen garlic. Off-odourous samples were prepared as described above. The selection of the TCA spiking levels was based on a sensory evaluation of garlic samples as listed in Table 2 and on threshold values described in the literature in other matrices. Due to the high intensity of the garlic flavour and the fact that the combination of garlic flavour with cork taint was perceived as highly unpleasant by the panellists, the number of samples that had to be evaluated per session was kept at a very low level. Just one sample set was evaluated per session. Samples were evaluated only by smelling the samples. gives the odour threshold values that were determined in this study for garlic granules and fresh garlic, respectively.

In comparison to the sensory thresholds for TCA in water and wine, with values of 0.24 μg kg^{-1} for garlic granules and 0.42 μg kg^{-1} for fresh/deep-frozen garlic, respectively, the obtained sensory thresh-olds for TCA are significantly higher in garlic matrices than in other matrices. This corresponds to our expectations and is most probably caused by the significantly stronger odour of the matrix itself. It is also interesting to note that for the sensory detection of cork taint in fresh/deep-frozen garlic, a significantly higher concentration (i.e., 2-fold) is necessary as compared to dried garlic granules. We assume that the garlic flavour in dried garlic granules is less intense than in fresh/deep-frozen garlic due to the loss of garlic volatiles during the drying process; furthermore, the overall odour of dried garlic granules is perceived as less pungent, which might also be caused by chemical alterations of the garlic sulphur compounds during thermal processing. It is noteworthy that the corky and typical TCA taint was addressed as such in most tainted samples; however, in samples that showed concentrations of higher than approx. 5 μg kg^{-1}, the odour of the samples was described as rotten. The typical corky TCA taint could not be addressed when high TCA concentrations were present.

5.2. Quantitative determination of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole in garlic samples

The suitability of solid phase microextraction (SPME) for the enrichment of the haloanisoles from the headspace (HS) of aqueous food samples had been demonstrated some years ago (Martendal et al., 2007; Callejón et al., 2016). In this investigation, we did not use conventional SPME fibres, but we applied SPME Arrows that provide a significantly increased capacity in comparison to conventional SPME fibres. Zhang et al., 2020 reported a 24-fold increased sorption volume of SPME Arrows in comparison to conventional SPME fibres (Zhang et al., 2020; Lisanti et al., 2021). In addition, due to the different construction of SPME fibres and SPME Arrows, a longer lifespan of the devices has to be expected due to their more rigid design. For the enrichment of TCA and TBA, a sample temperature of 80 °C was applied. This comparably high temperature for a period of 20 min during the enrichment of the volatiles turned to out to be the optimum design for the enrichment of TCA and TBA from food samples (Leis, 2021). The two analytes and also the corresponding deuterated analogues show high thermal stability; no degradation reactions had to be taken into account during the enrichment phase. Results from the investigations on various off-odourous compounds in foods performed in our laboratory (results not shown) also confirmed the suitability of HS-SPME Arrows for this purpose. Even though the concentrations of haloanisoles as contaminants in food samples are usually very low, the use of a complex food matrix such as garlic does not impact the sensitivity of the method as a potential background caused by garlic volatiles is minimised by the tandem GC-MS/MS technique. The limit of quantification of TCA in the garlic matrix was one order of magnitude lower than the odour threshold (i.e. LOQ_{TCA} 0.02 μg kg^{-1} compared to the odour $threshold_{TCA}$ 0.24 μg kg^{-1} in the garlic granules and 0.42 μg kg^{-1} in fresh garlic).

5.3. TCA and TBA concentrations in garlic samples and the impact on the sensory properties

The quantitative determination of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole was performed in a total of 42 garlic samples (i.e. garlic granules, fresh garlic, deep-frozen garlic and garlic paste/pu-ree). Fig. 1 gives a survey (box plot) over the TCA concentrations in the different garlic sample types. The detailed values are listed in Table 2. We detected TCA in concentrations higher than the LOQ (i.e. 0.02 μg kg^{-1}) in 45% of the samples. In approx. 35% of the samples, the OAV was 1 or higher than 1, indicating a sensory impact of the TCA presence on the flavour of the garlic samples.

It is important to note that there are significant differences in the level of TCA contamination depending on the sample type. TCA was detected in only 3 of the 11 analysed dried garlic samples. In all dried garlic samples, the OAV was lower than 1, indicating that the presence of TCA does not significantly impact the overall flavour of these samples. Fresh garlic was mainly purchased in local supermarkets or at local farmers' markets. 35% of the samples were contaminated with TCA in concentrations higher than the odour threshold. Interestingly, only samples (FG 9 and FG 10), which were obviously not derived from local production sites, showed high concentrations and OAVs, respectively. Deep-frozen garlic (chopped, sliced or processed into a garlic puree or paste) is a product that is usually not sold in conventional Austrian supermarkets. All of these samples, with the exception of DF 9, were provided by a local food producer who purchases their raw materials from various sources. A total of 18 different deep-frozen garlic products were investigated; the results show that the probability of purchasing TCA-contaminated deep-frozen garlic is very high. 61% of these samples were 'TCA-positive', 39% of which showed OAVs significantly higher than 1.

The data shown in Fig. 1 and Table 2 demonstrate that the probability of purchasing bad garlic quality is highest in deep-frozen garlic products. Very low TCA concentrations were detected in dried garlic powder or granule. We do not assume that the quality of the raw material is different from that of other products. Rather, it can be assumed that the thermal load during the drying process, with the possible application of reduced pressure, leads to evaporation and thus a reduction in quantities of TCA. Fresh garlic bulbs that were purchased in local supermarkets also showed a low TCA load. Usually, the requirements of supermarkets for fresh fruit and vegetables are very high, which might explain the low TCA concentrations in these types of samples. Conversely, the contamination of deep-frozen products – particularly those of garlic paste prepared for food processing companies – is of great concern. Even though garlic is used in rather small portions in most food formulations, the addition of tainted garlic will most

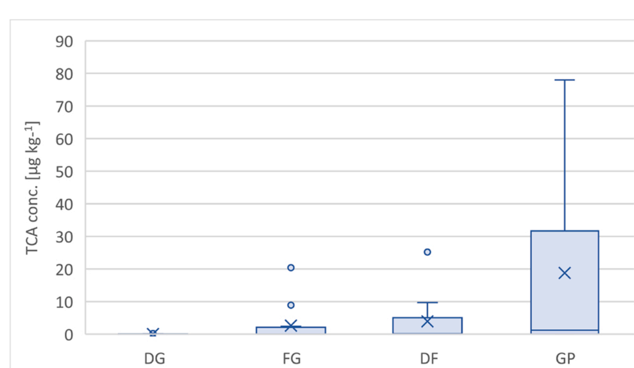

Fig. 1. Box plot of the TCA concentrations [μg kg^{-1}] in the investigated garlic samples; DG dried garlic, FG fresh garlic, DF deep frozen garlic, GP garlic paste; x average concentrations; ◦ outliers.

probably negatively influence the overall flavour of the final product. Our cooperation with the local food processing company, which provided a large portion of the deep-frozen samples, already showed that the use of TCA contaminated garlic may lead to major product recalls. In the case of low TCA concentrations, the characteristic TCA odour in food commodities might not be addressed by its consumers. However, keeping in mind that TCA suppresses the perception of other odour active compounds (Takeuchi et al., 2013), the flavour of the food commodity will be negatively influenced by the reduction of the perceived product flavour.

In contrast to TCA, TBA was not identified in any of the investigated samples. Under the assumption that either the fungicide prochloraz (with its final degradation product 2,4,6-trichlorophenol) or irriga-tion with TCA contaminated water are both potential sources or precursors to the TCA in garlic, the absence of 2,4,6-tribromoanisole in the investigated samples is not surprising.

6. Conclusion

The results of this study show that the contamination of garlic or garlic formulations by TCA represents a problem of serious concern. To the best of our knowledge, this is the first study addressing the presence of TCA in garlic and evaluating the impact on the flavour of the product. In this investigation, TCA was identified in approx. 45% of the investigated samples; in 35% the TCA concentrations exceeded the odour threshold leading to a mouldy, musty off-flavour in garlic. Fortunately, according to current scientific knowledge, there is no evidence that haloanisoles cause detrimental health effects to consumers. However, the use of garlic with a distinct musty and mouldy off-flavour may lead to significant economic damage. Furthermore, we live in a time when, in addition to considerations regarding economic damage, considerations regarding how to avoid food waste are becoming increasingly important.

Our results suggest a cautious conclusion that lower quality garlic is processed into deep-frozen chopped garlic or garlic paste designated for the food industry. If highly contaminated garlic is processed into any type of product for convenience, the probability of the perception of an off-flavour in the end product is very high, resulting in consumer complaints and product recalls. The reasons for the contamination of garlic with haloanisoles are not clear. Depending on the origin, along with storage and processing conditions of the garlic tubers, various contamination routes are also conceivable. However, for those who further process or consume the tainted garlic, the contamination pathway plays a subordinate role. Nevertheless, future investigations with well-described samples are required to understand sources and contamination routes in develop specific avoidance strategies. It was demonstrated from wine matrices that TCA – once present – can rarely be removed from food matrices (Cravero, 2020). Thus, it is of the utmost importance for food processing companies to perform sensory evaluation of garlic raw material prior to processing. Sensory evaluation to detect the potential presence of off-flavour needs to be implemented in quality assurance concepts. For other off-flavour causing compounds, such as geosmin and 2-isobutyl-3-methoxypyrazine, it was reported that 3–5% of the population is anosmic to these compounds (Cravero, 2020). This data demonstrates that is has to be guaranteed that personnel responsible for the sensory evaluation of garlic are sensitive to TCA in order to avoid false results.

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CRediT authorship contribution statement

Sabrina Stranig.: Investigation, Data curation, Writing. **Erich Leitner:** Conceptualization. Validation, Writing – review & editing. **Dorothea Leis:** Methodology. **Barbara Siegmund:** Conceptualization, Supervision, Writing – original draft as well as review & editing.

Declaration of Competing Interest

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. Furthermore, we declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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