ELSEVIER

Food Chemistry 64 (1999) 377–381

Food Chemistry

Bromophenol formation as a potential cause of 'disinfectant' taint in foods

J.B. Adams*, S.J. Lock, M.R. Toward, B.M. Williams

Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK

Received 3 October 1997; received in revised form 1 May 1998; accepted 1 May 1998

Abstract

A potential cause of 'disinfectant' taints in foods is the formation of bromophenols as a result of reactions between brominating agents (residual or nascent) and phenol (food-derived or contaminating). This was studied using simple model systems containing either elemental bromine or bromochlorodimethylhydantoin (BCDMH) and phenol. Bromophenols were quantified by adding an internal standard to the model system and then analysing the compounds by solid phase micro-extraction and GC–MS. Low levels of bromophenols were generally formed in bromine mixtures, though this depended on the presence of citrate buffer and on storage of the bromine in the buffer prior to reaction with phenol. The individual bromophenol isomers were often present in considerably greater amounts than the published taste threshold levels. This was particularly evident for 2-mono- and 2,6-dibromophenol which were therefore the most likely causes of the 'disinfectant' aroma in the simple models. The BCDMH reaction with phenol led to the same bromophenol isomers as in the elemental bromine reaction. Although bromophenol levels were lower, the taste thresholds for the 2-mono-and 2,6-dibromophenol were again significantly exceeded. The relevance of these observations for taint formation in foods is discussed.

1. Introduction

'Disinfectant' taints in foods are often due to chloro- and bromophenol formation. Contact with weak halogen solutions can be made on cleansing raw materials and food processing lines and on dilution of juice concentrates. Most often, chlorine is employed but occasional use is made of solid 'bromine donors', such as BCDMH, that hydrolyse to hypobromous acid (HOBr) which can then come into direct contact with foods. HOBr can also be produced by oxidation of contaminating bromide ion either by chlorine (Sweetman and Simmons, 1980) or by ozone (Shukairy et al., 1994). Residual or nascent HOBr can then react with phenol *in situ* to yield a mixture of halophenols. Thus, if phenol is present in the water used to dissolve or dilute the disinfectant due to contamination of the supply (Onodera et al., 1977), a rapid production of halophenol is likely to occur which, if not removed completely, could be carried over and cause taints in any food product that comes into direct contact with the disinfected area. Alternatively, residual halogen, not completely removed from the processing line, could react in the food product if the latter contained phenol. This route has been considered to be the cause of chlorophenol taint in several products such as beer (Holliday, 1975), milk (Palmer, 1979) and cheese (Bosset et al., 1994).

Phenol contamination does not always appear to be necessary for a halophenol taint to occur. For example, 2,6-dichlorophenol has been identified in carrots treated with sodium hypochlorite and heated at 121°C (Fukaya et al., 1993). This suggests that the phenol was formed naturally or that the hypochlorite reacted with the carrot phenolic compounds. Tressl et al. (1977) have suggested that the phenolic acids, *p*-coumaric and ferulic acid, which occur widely in plants, are transformed into other phenolic compounds on heating. In studies with asparagus, eleven phenols were characterised and it was found that some 40 ppb of phenol itself was produced on cooking the vegetable. Although no work was carried out on cooking in the presence of halogens or hypohalous acids, it is feasible that halophenols could be formed under these conditions with consequent potential for taint production.

Bromophenols have very low sensory threshold values and have been found to cause 'disinfectant' taints at parts per 10^{12} in fish products (Whitfield et al., 1988; Boyle et al., 1992). Trace levels of bromophenols have

* Corresponding author.

0308-8146/98/$—see front matter
PII: S0308-8146(98)00131-9

also been identified in tainted fruit juices examined at CCFRA. The objective of this study was therefore to determine some of the factors involved in the formation of bromophenols in foods by using well-defined model systems.

2. Materials and methods

Elemental bromine (99% pure) was purchased from Merck and all other chemicals from Sigma/Aldrich.

Elemental bromine was mixed with de-ionised water to give 0.1, 0.01 and 0.001 mM solutions. BCDMH was dissolved in water to give 0.1 and 0.01 mM solutions. The pH of these solutions was adjusted to 3.0 using hydrochloric acid. A 1 mM solution of phenol was made up in de-ionised water and 0.1 ml was mixed with 9.8 ml of bromine or BCDMH solutions in septum-sealed glass vials. The mixtures were then held for 30 min at 30°C. Two replicate vials were analysed by solid phase micro-extraction (SPME) and gas chromatography linked to mass spectrometry (GC–MS). A third was assessed for aroma. The reactions between bromine or BCDMH and phenol were also studied using 10 mM citric acid–sodium citrate buffer, pH 3.0, in place of de-ionised water.

Loss of elemental bromine from citrate buffer solutions was determined by measuring the decrease in absorbance at 390 nm on storing solutions of bromine in 10 mM and 100 mM buffers at 30°C.

2.1. SPME/GC-MS analysis

The halophenols produced in the model system solutions were trapped using a Supelco SPME fibre assembly (85 μm polyacrylate coating) and injection device. 1 M hydrochloric acid (0.1 ml) and sodium chloride (2 g) were added to increase the hydrophobic interactions with the polyacrylate and 0.1 ml of 2.4×10^{-2} μM solution of 2-bromo-4-chlorophenol was added as an internal standard. The coated part of the fibre was then exposed to the sample at 30°C for 1 h with stirring. The trapped halophenols were immediately thermally desorbed in the injector of a Hewlett–Packard 5890 gas chromatograph (injector temperature: 300°C). A Carbowax capillary column (CP-Wax 52CB; 25 m×0.25 mm) was employed that was held for 5 min at 40°C before increasing the temperature at 15°C/minute to 220°C, and then maintaining this temperature for 10 min. The column was connected to a Hewlett–Packard 5970B benchtop mass spectrometer and eluted with helium gas at a pressure of 10 psi. The mass spectrometer was run in selected ion monitoring (SIM) mode. The following ions were employed to check the identity of the bromophenols and obtain quantitative data:- 172, 174 m/z (monobromophenols); 250, 252 m/z (dibromophenols); 330, 332 m/z (tribromophenols); 206, 208 m/z (bromochlorophenol internal standard). The MS response factors for known concentrations of each bromophenol isomer relative to the internal standard were determined. These factors were then used in calculating bromophenol levels in the model systems.

3. Results and discussion

Bromophenols generally have lower sensory threshold values than chlorophenols and therefore have much higher tainting potential (Table 1). 2-monobromophenol and 2,6-dibromophenol have particularly low thresholds and are most likely to cause taints in food products.

3.1. Bromophenol formation from phenol and bromine

Mono-, di- and tri-bromophenols were identified in simple acidic model systems containing elemental bromine and phenol (Fig. 1). Although yields were generally low (<2% conversion of phenol to bromophenols), the sensory threshold levels of individual bromophenols were frequently exceeded and strong 'disinfectant' aromas were detected (Table 2). Using freshly prepared reagents at a bromine to phenol ratio of 10:1, the major bromophenol formed was 2,4,6-tribromophenol (96% of the total bromophenols formed). Whilst a relatively low amount of 2,6-dibromophenol was formed (2.5%), the latter exceeded its taste threshold concentration by a much greater margin than the tribromophenol and would therefore be a likely

Table 1
Taste detection thresholds for bromophenols and chlorophenols in water

Compound	Abbreviation	Threshold ($\mu M\times10^3$)	Reference
Mono-halophenols			
2-Bromophenol	2-BP	0.17	Whitfield et al., 1988
4-Bromophenol	4-BP	130	Whitfield et al., 1988
Di-halophenols			
2,4-Dibromophenol	2,4-DBP	16	Whitfield et al., 1988
2,4-Dichlorophenol	2,4-DCP	1.4	Dietz and Traud, 1978
2,6-Dibromophenol	2,6-DBP	0.002	Whitfield et al., 1988
2,6-Dichlorophenol	2,6-DCP	1.0	Dietz and Traud, 1978
Tri-halophenols			
2,4,6-Tribromophenol	2,4,6-TBP	1.8	Whitfield et al., 1988
2,4,6-Trichlorophenol	2,4,6-TCP	7.0	Dietz and Traud, 1978

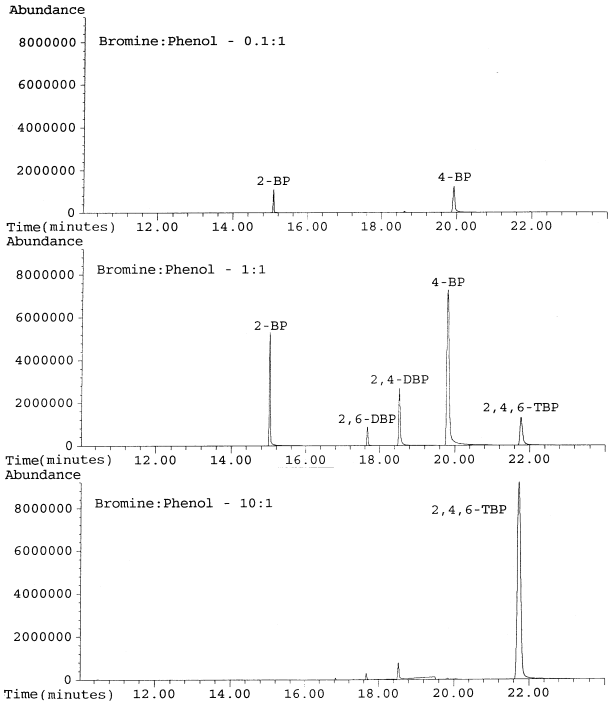

Fig. 1. GC-MS identification of bromophenols in acidified water at 30°C (pH 3.0).

cause of the 'disinfectant' aroma. At a 1:1 bromine to phenol ratio, mono-bromophenols predominated though only 2-bromophenol greatly exceeded its taste threshold. 2,6-dibromophenol represented only a small proportion of the total bromophenols (1%) but the amount formed was sufficient to exceed the taste threshold by a factor of 10,000. At a bromine to phenol ratio of 0.1:1, 2-bromophenol made the predominant taste contribution though the 2,6-dibromophenol level probably again contributed significantly to taste despite being present at only 0.1% of the total bromophenol formed.

Halogenation of aromatic systems under aqueous acidic conditions is considered to be due to electrophilic attack by halonium ion or by a positively polarised halogen-containing complex (Sykes, 1963). The inductive effect of the phenolic hydroxyl group directs attack by the positively charged species towards the 2- and 4-positions, the latter position brominating most readily due to the hydroxyl steric hindrance at position 2. In the present work, the low levels of 2,6-dibromophenol suggest that the electron-withdrawing effect of bromine at the 2- position has lowered the hydroxyl inductive influence at the 6- relative to the 4- position. Under the acidic conditions, the hydroxyl inductive effect is suppressed due to protonation of the oxygen lone-pair. This would be expected to reduce the extent of bromination and is probably the major cause of low bromophenol yields. In citrate buffer, it is proposed that *the primary salt effect* reduces the mutual repulsion of positively charged bromine and phenol species and allows a substantial increase in yield. Under these conditions, freshly prepared bromine in 10- fold excess caused 65% conversion of the phenol to bromophenols, mainly the 2,4,6-isomer (Table 3). The 2,6-isomer was formed at only 7% of the total bromophenols, but at 230,000 times the taste detection threshold, it is expected to be the main cause of the 'disinfectant' aroma observed.

Storage of bromine in citrate buffer prior to mixing with the phenol solution reduced the conversion of phenol to 1%. Moreover, the increased amounts of 2- and 4-monobromophenols and 2,4-dibromophenol relative to the 2,4,6-isomer suggested that bromine was lost rapidly from the buffer leading to low bromine to phenol ratios. This was confirmed by the higher bromine absorbance loss observed on increasing citrate buffer concentration (Fig. 2). No bromocitrate ion was detected using electrospray mass spectrometry (Adams et al., 1997), implying that conditions were not suitable for bromination of citric acid. Evaporation was therefore the most likely explanation for the bromine loss from citrate buffer solutions.

Two effects were evident in citrate buffer at pH 3.0. First, the enhanced level of phenol bromination, and second the implied greater loss of bromine compared with the loss in acidified water. The effect of citrate is

Table 2
The composition of bromophenols formed on reacting bromine with phenol in acidified water at 30°C (pH 3) over a range of bromine to phenol ratios

Bromophenol identified	Bromine:phenol = 0.1:1		Bromine:phenol = 1:1		Bromine:phenol = 10:1	
	Bromophenol concentration ($\mu M \times 10^3$)	*R*	Bromophenol concentration ($\mu M \times 10^3$)	*R*	Bromophenol concentration ($\mu M \times 10^3$)	*R*
2-BP	130 (1)	760	770 (17)	4,500	1.7 (0.6)	10
4-BP	120 (4)	0.92	880 (0)	6.8	4.3 (3.1)	0.033
2,4-DBP	1.3 (0.3)	0.081	71 (6)	4.4	1.8 (1.6)	0.11
2,6-DBP	0.34 (0.06)	170	20 (1)	10,000	6.7 (1.7)	3,300
2,4,6-TBP	0.23 (0.01)	0.13	30 (5)	17	270 (2)	150

Mean bromophenol concentration is shown with range of two replicates in parentheses.
R = Mean bromophenol concentration÷taste detection threshold concentration.

Table 3
The composition of bromophenols formed on reacting bromine with phenol in citrate buffer at 30°C (pH 3.0)

Bromophenol dentification	Bromine in citrate buffer (fresh)		Bromine in citrate buffer (stored)	
	Bromophenol concentration ($\mu M \times 10^3$)	*R*	Bromophenol concentration ($\mu M \times 10^3$)	*R*
2-BP	18 (0)	110	52 (9)	300
4-BP	140 (30)	1.1	28 (7)	0.21
2,4-DBP	480 (140)	30	24 (12)	1.5
2,6-DBP	465 (135)	230,000	1.8 (0.8)	900
2,4,6-TBP	5,400 (1,800)	3,000	14 (7)	7.8

Bromine:phenol molar ratio = 10:1; mean bromophenol concentration shown with range of two replicates in parentheses.
R = Mean bromophenol concentration÷taste detection threshold concentration.

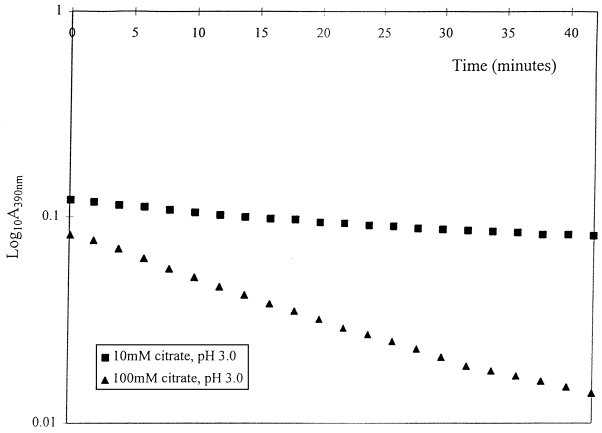

Fig. 2. The effect of citrate buffer concentration on the rate of bromine absorbance loss.

relevant to many foods containing it and other organic acids and their salts, as bromophenol formation would be expected to depend on the relative rates of evaporation and phenol bromination. It is proposed that taints are only produced in cases where 2-mono- or 2,6-dibromophenol formation exceeds the threshold level for the foodstuff concerned.

3.2. Bromophenol formation from phenol and BCDMH

The hypobromous acid released on hydrolysis of BCDMH (Table 4) gave rise to lower yields of bromophenols in water than found when using elemental bromine. This could be due to either a low rate of protonation of the hypobromous acid to bromonium ion, or to hypobromous acid itself having only a low brominating effect on phenol. The latter explanation is supported by the low yield in citrate buffer, which is in contrast to the high yield in the elemental bromine system where *the primary salt effect* of citrate ion was considered to enhance the bromination rate.

The relative bromophenol isomer levels depended on the ratio of BCDMH to phenol in a similar way to the relative levels in the presence of bromine. At a 10:1 ratio of BCDMH to phenol, 2,4,6-tribromophenol was produced in the highest yield though 2,6-dibromophenol probably contributed most to the 'disinfectant' aroma detected. At an equimolar ratio, the monobromophenol levels greatly exceeded the tribromophenol levels formed at the 10:1 ratio with the 2-isomer probably making the major contribution to the 'disinfectant' aroma.

Whilst there is significant potential for residual or nascent brominating agents to be present at low levels in foods, the potential for phenol to be present or to be formed is less evident. Further work is required on the formation of phenol from naturally occurring phenolic compounds in foods with a view to defining which products give rise to the highest phenol levels and are therefore susceptible to bromophenol taint formation.

4. Conclusions

Bromine caused bromination of phenol in acidic model systems at 30°C. The levels of bromophenol isomers

Table 4
The composition of bromophenols formed on reacting BCDMH with phenol in water and in citrate buffer at 30°C (pH 3.0)

Bromophenol identification	BCDMH:phenol = 10:1 (in water)		BCDMH:phenol = 10:1 (in citrate)		BCDMH:phenol = 1:1 (in citrate)	
	Bromophenol concentration ($\mu M \times 10^3$)	*R*	Bromophenol concentration ($\mu M \times 10^3$)	*R*	Bromophenol concentration ($\mu M \times 10^3$)	*R*
2-BP	1.5 (0.8)	8.8	0.048 (0.010)	0.28	410 (84)	2,400
4-BP	5.1 (2.0)	0.039	0.19 (0.11)	0.0015	300 (85)	2.3
2,4-DBP	9.8 (3.2)	0.61	2.4 (0.8)	0.15	2.0 (0.70)	0.12
2,6-DBP	1.2 (0.2)	600	0.42 (0.30)	210	0.30 (0.050)	150
2,4,6-TBP	24 (0.0)	13	12 (1.0)	6.7	0.016 (0.0060)	0.089

Mean bromophenol concentration shown with range of two replicates in parentheses.
R = Mean bromophenol concentration÷taste detection threshold concentration.

formed were generally low though this depended on the presence of citrate buffer and the length of bromine storage in the buffer. The bromophenol levels were frequently in excess of the published taste threshold levels, particularly for 2-mono- and 2,6-dibromophenol which were therefore expected to have made the greatest contributions to the 'disinfectant' aroma detected.

Mono- and dibromophenols were present in the highest proportions when the bromine to phenol ratio was $\leq$1:1 ratio. Evaporation of bromine from citrate buffer solutions was suggested as leading to low bromine to phenol ratios, thereby causing high relative amounts of the tainting mono- and dibromophenols.

Bromochlorodimethylhydantoin reaction with phenol led to lower levels of bromophenol being formed in comparison with bromine but these again greatly exceeded the taste thresholds for 2-monobromophenol and 2,6-dibromophenol. The relative amounts of bromophenol isomer formed depended on the BCDMH to phenol ratio. A 10:1 ratio favoured the tribromophenol isomer though 2,6-dibromophenol probably contributed most to the 'disinfectant' aroma detected. Equimolarity led to a greater amount of monobromophenols being formed, of which the 2-isomer was most likely to have made the major contribution to the 'disinfectant' aroma.

References

Adams, J. B., Lock, S. J., Toward, M. R. and Williams, B. M. (1997) Potential microbial and chemical causes of 'disinfectant' taint formation in foods. CCFRA R&D Report No. 44.

Bosset, J. O., Sieber, R. and Schmutz, F. (1994) Occurrence and origin of chlorophenols in cheese rind: a review. *Schweizerische-Milchwirtschaftliche-Forschung* **23**, 47–52.

Boyle, J. L., Lindsay, R. C. and Stuiber, D. A. (1992) Bromophenol distribution in salmon and selected seafoods of fresh and saltwater origin. *Journal of Food Science* **57**, 918–922.

Dietz, F. and Traud, J. (1978) Geruchs- und geschmacks -schwellenkonzentrationen von phenolkörpern. *Gas-Wasserfach, Wasser Abwasser* **119**, 318–325.

Fukaya, T., Ishiguro, Y., Yokoyama, M., Hiroi, S. and Muraoka, A. (1993) Presence of 2,6-dichlorophenol in carrot treated with sodium hypochlorite. *Journal of the Japanese Society of Food Science and Technology* **40**, 244–249.

Holliday, A. G. (1975) Phenols and beer flavour. Abstracts of papers, American Chemical Society, **170**, AGFD 4.

Onodera, S., Kato, J., Kamonzeki, Y. and Ishikura, S. (1977) Chemical changes of some organic compounds in chlorinated water II. Reaction of residual free chlorine with phenol and chlorophenols. *Journal of Hygienic Chemistry* **23**, 331–338.

Palmer, J. (1979) Care is needed to prevent chemical off-flavours in liquid milk. *Farm and Food Research* **10**, 120–121.

Shukairy, H. M., Miltner, R. J. and Summers, R. S. (1994) Bromide's effect on disinfection by-products formation, speciation, and controls. I. Ozonation.. *Journal American Water Works Association* **86**, 72–87.

Sweetman, J. A. and Simmons, M. S. (1980) The production of bromophenols resulting from the chlorination of water containing bromide ion and phenol. *Water Research* **14**, 287–290.

Sykes, P. (1963) *A Guidebook to Mechanism in Organic Chemistry*, Longmans, Green & Co., London.

Tressl, R., Bahri, D., Holzer, M. and Kossa, T. (1977) Formation of flavour components in asparagus 2. Formation of flavour components in cooked asparagus. *Journal of Agricultural and Food Chemistry* **25**, 459–463.

Whitfield, F. B., Last, J. H., Shaw, K. J. and Tindale, C. R. (1988) 2,6-Dibromophenol: the cause of an iodoform-like off-flavour in some Australian crustacea. *Journal of the Science of Food and Agriculture* **46**, 29–42.