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Detection of Chloroform in the Tissues of Freshly Eviscerated Poultry Carcasses Exposed to Water Containing Added Chlorine or Chlorine Dioxide

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In the USA, chloroform was shown to occur at concentrations up to 311 µg/L in waters treated by chlorination for drinking purposes and the presence of this substance, a possible cause of human cancer, has been attributed to reactions between chlorine and naturally-occurring humic or fulvic substances (ROOK 1974; BELLAR et al. 1974; US EPA 1975).

In the food industry, super-chlorination of process water is sometimes used as an aid to controlling microbial contamination in the factory and the present study relates to its application for this purpose in the production of oven-ready poultry carcasses where concentrations of 5-20 mg/L free available chlorine may be used for in-plant chlorination with higher levels, usually up to 50 mg/L, in the water of immersion chilling systems used for cooling carcasses.

Addition of chlorine to the chilling system, frequently in the form of sodium hypochlorite, reduces significantly the microbial load of the chill-water and extends the subsequent shelf-life of chill-stored carcasses (MEAD 1980); such a treatment is said to be without any adverse effect on either the odour or flavour of the meat (RANKEN et al. 1965). However, in relation to drinking water, LARSON & ROCKWELL (1978) showed that the common metabolic intermediate, citric acid, could act as a precursor of chloroform in chlorinated water and for this reason a study has been made to determine the extent to which poultry carcasses can become contaminated with chloroform when subjected to simulated processing procedures which involve exposure to chlorinated water.

An alternative to chlorination is the use of chlorine dioxide which would be expected to reduce or eliminate the formation of trihalomethanes, including chloroform (STEVENS et al. 1976), although it may result in low levels of other by-products of possible public health significance, such as chlorite (MOORE et al. 1980). With regard to the immersion chilling of poultry carcasses, LILLARD (1979) showed that seven times more chlorine than chlorine dioxide was required to obtain the same bactericidal effect, thus allowing chlorine dioxide to be used at lower levels. Because of this possibility, treatment of carcasses with chlorine dioxide was included in the present study.

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MATERIALS AND METHODS

Preparation of carcasses

Twenty eight live broiler chickens (Starbro), including both males and females at 8-11 weeks of age, were obtained from a local processor. The birds were taken to the Institute where they were stunned electrically, killed by venesection and allowed to bleed for 3-4 min. After mechanical plucking, the birds were eviscerated manually. Eviscerated weights varied from 0.96 to 2.48 kg.

Preparation of reagents

A solution of chlorine dioxide was prepared freshly according to a standard method (Olin Corporation, Kansas City, USA) by reacting appropriate solutions of hydrochloric acid, sodium hypochlorite and sodium chlorate. The final solution was standardised by iodometric titration which was also used to determine the free available chlorine content of the sodium hypochlorite preparation.

In all cases only 'Analar' grade chemicals supplied by BDH Chemicals Ltd, Poole, Dorset were used.

Determination of residual chlorine in treatment-water

Concentrations of chlorine and chlorine dioxide in the treatment-water were determined by a standard colorimetric test (Hach Chemicals Co, Ames, Iowa, USA) and in both cases expressed in terms of free available chlorine.

Treatment of carcasses

Five freshly-eviscerated birds were taken as untreated controls, whilst batches of three from the remainder were subjected to one or more of the following treatments, using tap-water from a non-chlorinated bore-hole:

(i) Spraying with 1.5 L of water/carcass containing 20 mg/L of free chlorine (added as hypochlorite) at 13°C;

(ii) immersing for five min in 2.5 L of water/carcass containing either 5 mg/L of chlorine dioxide or 50 mg/L of free chlorine at 15-16°C;

(iii) immersing for 20 min in 2.5 L of water/carcass containing either 5 mg/L of chlorine dioxide or 5-50 mg/L of free chlorine at 1.5-2.5°C. The water temperature was maintained by the addition of a small quantity of ice.

During treatments (ii) and (iii), the water was stirred continuously. After the appropriate period, carcasses were removed from the treatment tanks and allowed to drain for 20-30 min.

One batch of birds was subjected to all three of the treatments described above, using only water with added hypochlorite (50 mg/L free chlorine for both immersion stages). After treatment, these birds were cooked in 'Roastabags' (Bacofoil Ltd, London) at 195°C for 44 min/kg + 20 min.

Sampling and determination of chloroform content

The entire skin and breast muscle were removed separately from each carcass. In addition, depot fat was collected from the abdominal cavity and neck skin of uncooked birds. With cooked carcasses, molten fat was collected in the juice drained from the 'Roastabags'.

The above samples from carcasses and 1.5-2.0 L samples from treatment-waters were stored briefly at 1°C while awaiting extraction. Each sample was extracted for one hour with petroleum ether (40-60°C) using the method of LIKENS & NICKERSON (1964). The resultant extracts were analysed by means of High Resolution Selected Ion Monitoring Mass Spectrometry, using a Pye series 104 gas chromatograph interfaced to an AEI MS 902 mass spectrometer. Results were calculated from peak heights which were compared with those obtained from a range of standard chloroform solutions.

Tests were also made to determine the proportionate recovery of chloroform from carcasses and water samples injected with standard chloroform solutions. These samples were analysed as described above.

RESULTS AND DISCUSSION

The treatment conditions used in the present study simulated those stages of commercial broiler chicken processing in which carcasses are spray-washed immediately after evisceration and then chilled by water immersion. Most commonly, the latter process is carried out in two stages. First, carcasses are passed through a tank of plain water (ca. 5 min) before being transferred for a longer period (ca. 20 min) to a tank containing iced water. The conditions used were also in accordance with requirements for water usage and temperature control specified in recent EEC legislation (Directive 78/50).

The inclusion of one batch of birds which was subjected to all the appropriate stages of simulated washing and chilling, using chlorinated water, and then cooked, enabled tests to be made for chloroform formation and persistence at the point of consumption.

In preliminary trials involving birds injected with known amounts of chlorofom, it was found that the mean percentage recovery was 89.6 for skin, 68.0 for fat and 78.4 for breast muscle; the corresponding figure for treatment-water was 93.9.

TABLE 1

Levels of chloroform in the tissues of treated carcasses

Mean and range ( ) of chloroform concentrations (μg/kg):

Treatment	Skin	Fat	Muscle
None	5 (3-7)	NT	NT
ClO_2: 5 mg/L: 5 min: 15-16°C	19 (12-25)	20 (10-30)	2 (<2-6)
ClO_2: 5 mg/L: 20 min: 1.5-2.0°C	6 (5-8)	14 (<8-20)	<2
Cl_2 : 50 mg/L: 5 min: 15-16°C	144 (102-192)	447 (53-1241)	177 (90-374)
Cl_2 : 50 mg/L: 20 min: 1.5-2.5°C	30 (17-22)	146 (56-295)	17 (13-25)
Cl_2 : 5 mg/L: 20 min: 1.5°C	3 (<3-6)	14 (<10-26)	3 (<2-4)
Cl_2 : 20 mg/L: spray at 13°C	68 (47-82)	159 (90-249)	12 (10-14)
Combined Cl_2 treatments + cooking	134 (113-164)	46 (26-82)	64 (61-71)

*Three or five samples examined in each case; data not corrected for recovery.

NT = not tested

Table 1 shows that the highest concentrations of chloroform occurred in carcasses immersed in water containing 50 mg/L of chlorine, especially those held at 15-16°C. In most cases, the chloroform concentration was higher in depot fat than in the other tissues examined.

TABLE 2

Analysis of immersion treatment water

Treatment	Initial pH	Final pH	Chloroform (µg/L)
ClO_2 : 5 mg/L: 5 min: 15-16°C	7.2	6.8	ND
ClO_2 : 5 mg/L: 20 min: 1.5-2.0°C	7.4	6.5	41
Cl_2 : 50 mg/L: 5 min: 15-16°C	7.8	7.1	575
Cl_2 : 50 mg/L: 20 min: 1.5-2.5°C	7.0	6.0	640
Cl_2 : 5 mg/L: 20 min: 1.5°C	8.0	7.8	ND

ND = not determined

Relatively low concentrations of chloroform in both carcass tissues and treatment-water (Table 2) resulted from the use of 5 mg/L of chlorine dioxide. Again, however, chloroform levels in tissues tended to be greater following immersion at 15-16°C, despite the shorter contact period than at chill temperatures. The small amounts of chloroform arising from the use of chlorine dioxide are presumed to originate mainly from the hypochlorite used in generating the reagent, whereas the even lower levels in the untreated, control carcasses, may represent merely laboratory-acquired contamination.

The apparent correlation between chlorination of water-supplies and human cancer mortality is still a matter for debate and, as MOORE et al. (1980) have emphasised, extrapolation from animal experiments to humans would require the consumption of at least 10,000 to 100,000 L of water per day, assuming chloroform concentrations of ca. 300 µg/L. Results obtained here for birds subjected to simulated washing and chilling procedures, using chlorinated water, suggest that chloroform-contamination of commercially-processed poultry carcasses may not be significantly greater than that reported for drinking water.

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