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Influence of Heat and Cure Preservatives on Residues of Sulfamethazine, Chloramphenicol, and Cyromazine in Muscle Tissue

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Animals were given medications to produce incurred residues of sulfamethazine or chloroamphenicol in swine and cyromazine and melamine metabolite residues in cattle. This muscle tissue was subjected to combinations of curing or noncuring and temperatures of 2–3, 68 (casing), and 122 °C (canning) with the analytes of interest quantitated before and after processing. Sulfamethazine survived in pork cured and thermally processed, although losses of up to 50% were observed. Half the chloramphenicol concentration in pork was lost in uncured processed tissue, with greater losses observed after curing and complete loss for canned product. Cyromazine losses of approximately 35% occurred in beef after processing and curing. Cyromazine levels increased when the beef was processed at 68 °C in casing, due to water loss. In canned product, melamine was present at 1 ppm for the dosed and nondosed tissue. The melamine contamination may be due to the melamine–formaldehyde resin in the can lining.

Various drugs or chemical agents have been used for controlling diseases and as growth promotants in livestock, but unacceptable concentrations of residues may remain if animals are not properly withdrawn prior to slaughter. Information is available from the manufacturers of various veterinary agents indicating appropriate withdrawal times of the compound in uncooked animal tissue. However, before meat products are consumed, they are cooked and/or cured. No information is currently available concerning the fate of residues in such products. The study objective was to evaluate the influence of cure, preservatives, and heating on chemical residues in processed meats. Chemical information for the three compounds chosen for this study, sulfamethazine, chloramphenicol, and cyromazine, is listed in Table I.

Sulfamethazine (110 mg/kg) in feed is widely used in the swine industry in combination with other antibiotics with growth-promotant properties. The U.S. Food and Drug Administration (FDA) established a tolerance of 0.1 ppm (Code of Federal Regulations, 1987) for sulfamethazine in edible swine tissue. However, sulfamethazine contamination of nonmedicated feed occurs when medicated feed is prepared at the feed mill, resulting in animals with sulfamethazine residues. In addition, a recycling of the sulfamethazine residues can occur through residues in the feces and urine.

Chloramphenicol, a potent antibiotic, is not approved for use in food-producing animals in the United States. Residues of chloramphenicol, in edible tissue, is a public health concern because it can cause aplastic anemia in man (Meyer et al., 1974; Settepani, 1984).

Cyromazine is an insecticide approved for use by the U.S. Environmental Protection Agency (EPA) in chickens. Cyromazine is administered as a feed-through larvicide incorporated into feed at 0.50 mg/kg for laying hens to prevent flies from hatching in the manure (Federal Register, 1984). The tolerance of cyromazine and the melamine metabolite is 0.05 ppm each (Code of Federal Regulations, 1985) in edible poultry tissue. Cyromazine is used in other animals species by other meat-producing countries.

EXPERIMENTAL SECTION

Materials. In this study four market-weight hogs averaging 81.4 kg live weight were selected for dosing, one with chloramphenicol, two with sulfamethazine, and one animal served as a control. Similarly, three yearling cattle weighing 200–250 kg were used; two were fed cyromazine as a Larvadex premix to produce cyromazine-incurred tissues, and the third served as a control.

Two market-weight hogs were fed ASP-250 (American Cyanamid, Inc., Princeton, NJ). This antibiotic blend contains chlortetracycline, penicillin, and sulfamethazine. Sulfamethazine was fed to swine at 110 mg/kg in feed for a period of 7 days. One hog was slaughtered 48 h and the second hog 72 h after placement on nonmedicated feed to produce target sulfamethazine residue concentrations in muscle tissue of 0.5 and 0.2 ppm, respectively (Randecker et al., 1987).

Chloramphenicol was purchased from Sigma Chemical Co., St. Louis, MO, and prepared at 150 mg/mL in propylene glycol. One hog was dosed at 0.2 mg/kg intrave-

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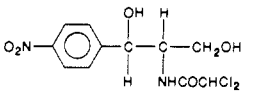
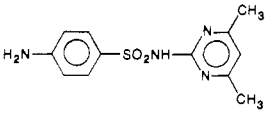
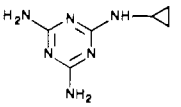
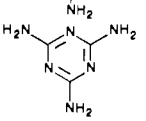
Table I. Compounds Evaluated in Tissue-Processing Study

name	M_r	empirical formula	structure	chemical name
chloramphenicol	323.1	$C_{11}H_{12}C_{12}N_2O_5$		D-*threo*-(−)-2,2-dichloro-*N*-[β-hydroxy-α-(hydroxymethyl)-*p*-nitrophenethyl]acetamide
sulfamethazine	278.3	$C_{11}H_{13}N_3O_3S$		*N*′-(4,6-dimethyl-2-pyramidinyl)sulfanilamide
cyromazine	166.2	$C_6H_{10}N_6$		*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine
melamine	126.1	$C_3H_6N_6$		1,3,5-triazine-2,4,6-triamine

nously (iv) via ear vein and slaughtered after 4 h to produce a target residue of 50 ppb in muscle tissue (Epstein et al., 1986).

Larvadex (cyromazine) premix (0.30%) from CIBA-Geigy Corp., Greensboro, NC, was fed in a growth ration at the rate of 0.50 and 5.0 mg/kg of body weight per day to two yearling cattle that were slaughtered without withdrawal-producing residues in muscle tissue of approximately 0.2 and 2.0 ppm, respectively (Code of Federal Regulations, 1985).

Processing. All animals were slaughtered according to standard practices and the carcasses chilled for 48 h at 0 °C. The hams, loins, and shoulders from the swine were deboned and trimmed to about 0.64 cm of surface fat. Beef rounds were also trimmed of surface fat and used for product processing. A 1-kg muscle tissue sample from each animal hindquarter, after chilling, was immediately frozen at −40 °C without any additional processing.

Approximately 10 kg of muscle tissue from each animal was ground separately on a commercial grinder fitted with a 1.9-cm grinder plate. A standard curing mixture was then added to 5 kg of tissue and the mixture finely minced in a silent cutter until it had the consistency of an "emulsion" type product such as frankfurters or bologna. The second 5-kg portion of tissue was treated equally but without the addition of cure ingredients. Homogenization of the sample ensured complete diffusion of cure ingredients, a uniform meat mass for uniform thermal penetration, and a minimum preparation of samples for subsequent chemical analysis. Each processing began with the control first followed by low and high dose incurred tissue concentrations, respectively. All equipment was cleaned thoroughly between each treatment combination to reduce the risk of residue cross-contamination.

The experimental design included matched pairs of sample sets for all control and dosed animals, one cured and one uncured. The curing mixture was as follows: 2.25% sodium chloride, 156 ppm sodium nitrite, 550 ppm sodium erythorbate, spices, and 25% added water (based on batch weight). Samples from each cured or noncured muscle tissue treatment were then subjected to the following processing: chilling at 2–3 °C; stuffing into a fibrous casing and cooking to an internal temperature of 68 °C in a temperature-programmable smokehouse; or canning and processing at 122 °C for 95 min with a 453.6-g (300 × 407) can size. There were six sample types per dose treatment as a result of these processes.

Analytical Methods. Sulfamethazine-dosed pork samples and control tissues were analyzed by thin-layer fluorescence chromatography (Thomas et al., 1981; AOAC, 1984). Briefly, 2.5 g of tissue was homogenized in ethyl acetate, sulfapyridine added at 0.1 ppm as an internal standard, and the mixture centrifuged. The ethyl acetate layer was partitioned with 1 N hydrochloric acid and the aqueous layer adjusted to pH 6.5. The sulfamethazine was then extracted with methylene chloride, evaporated to dryness, and reconstituted in methanol. An aliquot was spotted on a LK6D thin-layer plate and chromatographed with chloroform–butanol (80:20). The plate was then dried and reacted with fluorescamine. The fluorescence bands were quantitated on a scanning densitometer with a practical detection limit of 0.02 ppm and a method repeatability for sulfonamides of less than 12% (USDA, 1985).

Chloramphenicol-dosed swine and control samples were extracted from tissue and quantitated by gas chromatography (GC) (Simpson et al., 1985). A 5-g sample spiked with an internal standard of monochloramphenicol at 50 ppb was extracted with ethyl acetate and evaporated to dryness. The residue was redissolved in 4% aqueous sodium chloride, the aqueous phase partitioned three times with hexane, and the hexane layer discarded. Chloramphenicol was extracted from the aqueous phase with ethyl acetate and evaporated to dryness. After formation of the trimethylsilyl derivative, chloramphenicol concentrations were determined by use of GC electron-capture detection with a sensitivity of 5 ppb and method repeatability of less than 12% (USDA, 1985).

Cyromazine and melamine concentrations in dosed and control beef muscle samples were determined by high-performance liquid chromatography (Smith et al., 1984). Briefly, 25 g of muscle tissue was homogenized in 90% acetonitrile in water for 1 min. An aliquot of the extract was filtered through a C18 Sep Pak (Waters Association, Waltham, MA), followed by additional filtration and ion-exchange chromatography using Dowex 1X-8 and 50WX-4 (Aldrich Chemical Co., Milwaukee, WI) resins. The analytes were eluted with methanol and evaporated to dryness. Cyromazine and melamine were quantitated by HPLC on a Zorbax-NH_2 column (Dupont Instruments, Wilmington, DE) with 95% acetonitrile–water as the mobile phase and an ultraviolet (UV) detection unit set at 214 nm. The limit of detection was 0.01 ppm, and method repeatability was less than 20% (USDA, 1985). The HPLC melamine eluants for designated samples were confirmed as the trimethylsilyl derivative by both positive-ion chemical ionization (PICI) and full-scan electron-impact (EI) mass spectrometry.

Table II. Effect of Processing, Curing, and Thermal Preparation on Sulfamethazine Residues in Pork Muscle Tissue

	sulfamethazine,[a] ppm					
		0.2 ppm target dose animal			0.5 ppm target dose animal	
cured	nondosed animal prep[b]	2–3 °C chilled	68 °C casing	122 °C canning	2–3 °C chilled	68 °C casing	122 °C canning
no	nd[c]	0.09	0.07	0.11	0.50	0.45	0.68
yes	nd	0.07	0.07	0.09	0.26	0.36	0.41
nonprocessed tissue	nd	0.10	na[d]	na	0.62	na	na

[a] Mean of two determinations on separate samples within 95% confidence limits. [b] Residues in nondosed animal preparation were not detected in 2–3 °C chilled, 68 °C casing, or 122 °C canning treatments. [c] nd = not detected below 0.02 ppm. [d] na = not applicable.

Table III. Effect of Processing, Curing, and Thermal Preparation on Chloramphenicol Residues in Pork Muscle Tissue

	chloramphenicol,[a] ppb			
		50 ppb target dose animal		
cured	nondosed animal prep[b]	2–3 °C chilled	68 °C casing	122 °C canning
no	nd[c]	21.9	14.9	nd
yes	nd	10.6	nd	nd
nonprocessed tissue	nd	48.8	na[d]	na

[a] Mean of two determinations on separate samples within 95% confidence limits. [b] Residues in nondosed preparation were not detected in 2–3 °C chilled, 68 °C casing, or 122 °C canning preparations. [c] nd = not detected below 5 ppb. [d] na = not applicable.

RESULTS AND DISCUSSION

Mincing the uncured pork or beef muscle tissue in the silent cutter to form a 2–3 °C chilled emulsion product resulted in a loss of analyte of approximately 10–15% for sulfamethazine, 50% for chloramphenicol, and 20% for cyromazine (Tables II–IV). Melamine concentrations were near the 0.01 ppm detection limit for the 0.2 ppm Larvadex beef tissues (Table IV); therefore, no conclusion could be made concerning analyte loss during processing. Percentages for analyte loss were calculated as the mean of two values: computing the analyte difference subsequent to treatment, dividing by the initial tissue concentration, and multiplying by 100.

The lower dose of sulfamethazine (0.2 ppm) in pork muscle tissues was not affected by either emulsifying or curing nor by the combination of curing and heating at 68 or 122 °C. Tissues containing sulfamethazine that were minced and chilled or canned at 122 °C without cures showed no apparent loss of sulfamethazine, i.e. within analytical method experimental error of 6.6% for sulfamethazine in swine muscle (Thomas et al., 1983). Small losses of sulfamethazine prior to curing or heat treatment may be due to the formation of the N^4-glucopyranosyl derivative, which has been noted in swine liver (Parks, 1984) and shown to be reversible under slightly acidic conditions. Addition of cure resulted in a loss of sulfamethazine for the 0.5 ppm dosed pork muscle of approximately 48, 20, and 40% for the chilled, 68 °C, and 122 °C treatments, respectively (Table II).

Chloramphenicol residue concentrations in product from pork product, summarized in Table III, were markedly affected by emulsifying and heating as well as by the curing process. Successive losses of 50% for each processing step were noted. This reduced the chloramphenicol concentration in the chilled treatment from an initial 48.4 to 10.6 ppb. Product processed in casings at 68 °C without added cure also resulted in a 25% reduction of chloramphenicol, while the combined effect of curing and heating accelerated the loss of chloramphenicol. Chloramphenicol did not survive the 122 °C canning process, for both cured and uncured product.

The data from the Larvadex (cyromazine) dosing and processed product studies are summarized in Table IV. Successive processes of forming an emulsified beef product and curing causes approximately a 20% loss of cyromazine and melamine at each step, resulting in a total loss of approximately 35%. The same losses for cyromazine were noted for both the 0.2 or 2.0 ppm target dosed animals in chilled processed tissue. The melamine values in beef decreased from 0.13 to 0.09 ppm (2.0 ppm target tissue) in the chilled samples.

For cyromazine-dosed tissue in casings subjected to a 68 °C processing temperature, there was an apparent elevation of the cyromazine concentration. This was due to a decrease in the moisture level of the beef product from a mean of 78 to 64%, a change of at least 14%, in part explaining the higher cyromazine values. Mass spectrometry studies on the nondosed, cured, and noncured canned beef tissues (Table IV) confirmed the presence of melamine. These data include both PICI and EI scans. In the dosed beef tissue (2.0 ppm cyromazine target), the melamine metabolite quantitation was 0.09–0.17 ppm in noncanned tissues. This is consistent with the difference

Table IV. Effect of Processing, Curing, and Thermal Preparation on Cyromazine and Melamine Metabolite Residues in Beef Muscle Tissue

	nondosed animal preparation			0.2 ppm target dose animal			2.0 ppm target dose animal		
cured	2–3 °C chilled	68 °C casing	122 °C canning	2–3 °C chilling	68 °C casing	122 °C canning	2–3 °C chilled	68 °C casing	122 °C canning
				Cyromazine,[a] ppm					
no	nd[e]	nd	nd	0.12	0.17[b]	0.08	1.41	2.44[b]	1.49
yes	nd	nd	nd	0.10	0.13[b]	0.10	1.26	1.63[b]	1.19
nonprocessed tissue	nd	na[f]	na	0.15	na	na	1.90	na	na
				Melamine Metabolite,[a] ppm					
no	nd	nd	1.02[c,d]	0.01	0.02	1.03[c]	0.12	0.17[b]	1.27[c,d]
yes	nd	nd	1.11[c,d]	0.02	0.02	1.05[c]	0.09	0.12	1.17[c,d]
nonprocessed tissue	nd	na	na	0.03	na	na	0.13	na	na

[a] Mean of two determinations on separate samples within 95% confidence limits. [b] Elevated values due to 20% decrease in the moisture after processing resulting in a higher analytical weight basis. [c] Melamine formed during canning from the melamine–formaldehyde resin in the can lining. [d] Confirmed by positive-ion chemical ionization (isobutane gas) and electron-impact mass spectrometry. [e] nd = not detected below 0.01 ppm. [f] na = not applicable.

in melamine concentrations in the dosed canned beef of 1.17–1.27 ppm versus melamine in nondosed canned tissue of 1.02–1.11 ppm. The melamine in the canned beef tissues may be due to the migration of the melamine from the melamine–formaldehyde resin in the can lining.

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Registry No. Sulfamethazine, 57-68-1; chloramphenicol, 56-75-7; cyromazine, 66215-27-8; melamine, 108-78-1.

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Thermal Inactivation Kinetics of Potato Tuber Lipoxygenase

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The thermal inactivation kinetics of two isozymes, crude and reconstituted enzyme preparations of potato tuber lipoxygenase, was investigated in solution. Kinetic parameters of thermal inactivation were determined graphically and by a least-squares method using nonlinear regression analysis. Each of the two isozymes followed first-order kinetics with different inactivation rate constants. However, the apparent inactivation kinetics of crude and reconstituted lipoxygenases exhibited second-order kinetics, and this could be explained by the presence of two isozymes having different heat resistances. Heat inactivation data (percent residual activity) of crude and reconstituted lipoxygenase could also be adequately calculated from the two isozymes' first-order inactivation rate constants and their enzyme reaction rate constants. The results suggested the possible utilization of parameters derived from the apparent second-order kinetics for predicting the extent of thermal inactivation of isozymic mixture of potato tuber lipoxygenase.

Lipoxygenase is an ubiquitous enzyme in plant kingdom (Pinsky et al., 1971). It acts upon free fatty acids in foodstuffs, destroying essential fatty acids such as linoleic, linolenic, and arachidonic acids, and produces mainly hexanal and pentanal. These volatile compounds are known to be a major cause of rancidity and off-flavor occurring in inadequately processed, stored foodstuffs, especially of plant origin (Grosch, 1972). The enzyme appears in most plants as multiple isozymes, some of which are more heat-resistant than the others of the same plant. Thus, incomplete inactivation of the enzyme during heat treatment of foodstuffs has been explained by the presence of heat-resistant isozymes (Chenchin and Yamamoto, 1973; Delincee et al., 1975; Ling and Lund, 1978; Naveh et al., 1982).

Kinetic studies of thermal inactivation have been carried out for lipoxygenases of various origin to rationalize the thermal-processing conditions of foodstuffs to inactivate the enzyme, thus preventing the eventual occurrence of rancidity and off-flavors during storage and distribution

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