Effect of Thermal Processing and Additives on the Kinetics of Oxytetracycline Degradation in Pork Muscle

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The degradation of oxytetracycline (OTC) was observed at 60, 70, and 80 °C in several different aqueous and tissue media. In aqueous media, the rate of OTC degradation (k_{obs}) was found to be independent of glycerol adjusted water activities in the range of 0.6 to 1.0. Orthophosphate was observed to increase k_{obs}; in contrast, polymeric phosphates (sodium pyrophosphate, sodium tripolyphosphate, and sodium hexametaphosphate) were observed to significantly decrease k_{obs}. Sodium nitrite decreased k_{obs} at 80 °C. OTC degradation was found to occur at a slower rate in porcine tissue than in aqueous media. Addition of orthophospate (0.5% as anhydrous dibasic sodium phosphate) to tissue resulted in no observable change in k_{obs}; addition of polyphosphates (equivalent to 0.5% anhydrous dibasic sodium phosphate) increased k_{obs}. Sodium nitrite (200 ppm) in tissue increased k_{obs} at 60 °C but decreased it at 80 °C. Inclusion of calcium chloride significantly decreased k_{obs} in both aqueous and tissue matrices. Thermal treatments−food additive combinations were found to have significant effects upon the rate of OTC degradation.

Keywords: *Oxytetracycline; degradation; phosphates; nitrites; kinetics*

INTRODUCTION

Agriculture and Agri-Food Canada, which is one of the Canadian government agencies responsible for the safety of the food supply, has an established drug residue monitoring program that checks for the presence of veterinary drug residues in slaughtered animals. However, this program, to date, does not check for presence of these residues in domestically processed tissues (MacNeil and Ellis, 1995). Most foods of animal origin undergo further processing prior to consumption (thermal or food additive treatments or both) for the purpose of increasing palatability and shelf-life. Commonly used food additives in meat processing include the various polymeric forms of phosphates, salts, and nitrites (Townsend and Olson, 1987). Phosphates are utilized in processing due to their ability to alter the water holding properties of meat and for their antimicrobial action (minor in this context). Nitrites inhibit the outgrowth of *Clostridium botulinum* spores and also complex with meat myoglobin to form nitrosylmyoglobin and its derivatives. These form relatively stable color compounds, thereby contributing to the aesthetic appearance of meat (Townsend and Olson, 1987).

Oxytetracycline (OTC) belongs to the tetracycline family of antibiotics. Its occurrence as a violative residue in Canadian pork is second only to the β-lactam antibiotics (MacNeil et al., 1993). Due to its inherent nature, OTC is chemically stable in tissues at refrigeration or subrefrigeration temperatures (Honikel et al., 1978). However, several research reports have indicated that it exhibits a certain degree of lability toward common cooking practices (Rutczynska-Skonieczna, 1967; Scheibner, 1969, 1972; Honikel et al., 1978; Shakaryan et al., 1976; O'Brien et al., 1980, 1981; Moats, 1988; Kitts et al., 1992; Ibrahim and Moats, 1994; Rose et al., 1996). Though these researchers have looked at the absolute levels of OTC loss as a function of these practices, there is limited information on the rate of this loss. Quantitative information on the loss of OTC as a function of different cooking variables would allow for better estimations of the potential intake of this compound and lead to a better understanding of the interactions that can occur between OTC and commonly used meat additives.

MATERIALS AND METHODS

Materials. OTC was purchased from Sigma Chemical Co. (St. Louis, MO). Tetrasodium pyrophosphate (SPP), pentasodium tripolyphosphate (STP), and sodium hexametaphosphate (SH) were obtained from Caledon Laboratories (Edmonton, AB). HPLC grade (Omnisolv) solvents, disodium hydrogen orthophosphate (SP) (AnalaR), and analytical grade (AnalaR) reagents were purchased from VWR-Canlab (Edmonton, AB). Purified water was obtained from a Milli-Q water purification system (Millipore Canada, Ltd., Missisauga, ON). Porcine tissue was obtained from the Food Product Innovation Program (University of Saskatchewan, Saskatoon, SK) and analyzed to verify the absence of OTC residues.

Methods. *(a) Aqueous Media.* The thermal stability of OTC was investigated at 60, 70, and 80 °C in the media listed in Tables 1−4. Distilled water solutions were adjusted to water activities (a_w's) of 0.9, 0.8, and 0.6 using glycerol, and the a_w's were verified by a model CX-1 Decagon water activity meter (Decagon Devices, Inc., Pullman, WA). All other media were prepared immediately before use. 3 mL of the media were dispensed into 50-mL round bottom glass centrifuge tubes (VWR-Canlab, Edmonton, AB) and 100 μL of 1 mg/mL methanolic OTC was added. The tubes were sealed with Teflon-lined caps, placed in constant temperature water baths, removed at 10, 20, 30, 40, and 60 min, and cooled immediately in ice-water. OTC was

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Table 1. Oxytetracycline Degradation Parameters k_{obs} (min^{-1}) and E_a (kcal/mol) in Aqueous Media As Affected by Water Activity

medium	temp (°C)	k_{obs} (min^{-1})[a] mean	k_{obs} (min^{-1})[a] 95% c.i.	E_a (kcal/mol) mean	E_a (kcal/mol) SE[b]
distilled water	60	−0.0043	(−0.0051, −0.0036)	30.14	2.81
	70	−0.0172	(−0.0185, −0.0159)		
	80	−0.0566	(−0.0599, −0.0532)		
glycerol/distilled water (0.9a_w at 20 °C)	60	−0.0066	(−0.0089, −0.0043)	23.99	2.93
	70	−0.0210	(−0.0240, −0.0179)		
	80	−0.0510	(−0.0556, −0.0463)		
glycerol/distilled water (0.8a_w at 20 °C)	60	−0.0061	(−0.0089, −0.0032)	26.26	3.45
	70	−0.0225	(−0.0262, −0.0188)		
	80	−0.0575	(−0.0633, −0.0517)		
glycerol/distilled water (0.6a_w at 20 °C)	60	−0.0073	(−0.0100, −0.0046)	23.59	2.81
	70	−0.0208	(−0.0231, −0.0184)		
	80	−0.0549	(−0.0595, −0.0502)		

[a] $n = 18$, linear regression of $\ln[OTC_{time=t}] = \ln[OTC_{time=0}] - k_{obs}\, t$. [b] Standard error.

Table 2. Oxytetracycline Degradation Parameters k_{obs} (min^{-1}) and E_a (kcal/mol) in Aqueous Media As Affected by Phosphate Buffer Concentration, pH, and Inclusion of Sodium Chloride

medium	temp (°C)	k_{obs} (min^{-1})[a] mean	k_{obs} (min^{-1})[a] 95% c.i.	E_a (kcal/mol) mean	E_a (kcal/mol) SE[b]
phosphate buffer (pH 5.5, 0.01 M)	60	−0.0080	(−0.0101, −0.0059)	24.23	2.39
	70	−0.0203	(−0.0215, −0.0192)		
	80	−0.0637	(−0.0669, −0.0604)		
phosphate buffer (pH 5.5, 0.05 M)	60	−0.0089	(−0.0100, −0.0082)	24.88	4.65
	70	−0.0280	(−0.0305, −0.0255)		
	80	−0.0747	(−0.0800, −0.0699)		
phosphate buffer (pH 5.5, 0.1 M)	60	−0.0198	(−0.0210, −0.0185)	18.28	1.48
	70	−0.0518	(−0.0558, −0.0479)		
	80	−0.0943	(−0.1006, −0.0880)		
phosphate buffer (pH 4.0, 0.1 M)	60	−0.0053	(−0.0058, −0.0047)	28.38	2.35
	70	−0.0195	(−0.0209, −0.0181)		
	80	−0.0600	(−0.0641, −0.0559)		
phosphate buffer (pH 7.0, 0.1 M)	60	−0.0064	(−0.0070, −0.0057)	22.36	1.80
	70	−0.0186	(−0.0201, −0.0172)		
	80	−0.0433	(−0.0453, −0.0412)		
phosphate buffer (pH 5.5, 0.05 M) + 0.1 M NaCl	60	−0.0110	(−0.0125, −0.0095)	22.12	2.12
	70	−0.0282	(−0.0318, −0.0245)		
	80	−0.0730	(−0.0819, −0.0641)		
phosphate buffer (pH 5.5, 0.05 M) + 0.4 M NaCl	60	−0.0099	(−0.0115, −0.0084)	25.52	3.04
	70	−0.0238	(−0.0266, −0.210)		
	80	−0.0705	(−0.0816, −0.0593)		

[a] $n = 18$, linear regression of $\ln [OTC_{time=t}] = \ln [OTC_{time=0}] - k_{obs}\, t$. [b] Standard error.

extracted from the aqueous matrices according to the method of Fedeniuk et al. (1996). Come-up temperature profiles were monitored using an OMEGA microprocessor thermometer with a T-type thermocouple (Omega Engineering, Inc., Laval, PQ).

(b) Porcine Tissue Media. Porcine tissue was preground in a commercial blender, frozen, and stored at −20 °C for a period no longer than one month prior to use. 5 g of ground tissue and 100 μL of 1 mg/mL methanolic OTC were consecutively added to 50-mL round bottom glass centrifuge tubes. Immediately after preparation, 500 μL of the treatment was added to the tubes (Table 5), and mixed. The tissue was packed to the bottom, sealed, and heat-treated as indicated in section a above. The come-up temperature profile of the geometric center of the tissue mass was monitored as inidicated for the aqueous media.

(c) HPLC Analysis. The HPLC system consisted of a Waters 600E pump, a Waters 715 ULTRA WISP autosampler set at an injection volume of 100 μL, and a Waters 996 photodiode array detector acquiring spectra in the wavelength range of 240−450 nm with a resolution of 1.2 nm and a data acquisition rate of 1 spectrum/4 s. Peak heterogeneity was assesed using the photodiode array software option of Millenium chromatography manager V2.10 software. Peak area counts were obtained at 350 nm. All other parameters were as indicated in Fedeniuk et al. (1996).

(d) Data Analysis. Thermal destruction constants (k_{obs}, min^{-1}) and their associated 95% confidence intervals were obtained from the linear regression equations, $n = 18$, calculated from the ln peak area counts as a function of time using Excel Version 5.0 (Microsoft Corporation, 1994). Energy of activation (E_a, kcal/mol) values and the associated standard errors were calculated according to the procedure of Hill and Grieger-Block (1980) using k_{obs} and standard errors obtained at 60 and 80 °C.

RESULTS AND DISCUSSION

Temperature come-up profiles for aqueous and meat matrices at 60, 70 and 80 °C are shown in Figure 1. In both media, the maximum temperature was attained in less than 12 min to within 1 °C of the desired temperature. The come-up times were not taken into account when calculating k_{obs} for the sake of simplifying calculations.

Thermal treatment of blank aqueous and porcine tissue media revealed no chromatographic peaks, whereas thermal treatment of the same matrices con-

Table 3. Oxytetracycline Degradation Parameters k_{obs} (min^{-1}) and E_a (kcal/mol) in Aqueous Media As Affected by Acetate Buffer Concentration and Inclusion of Monomeric and Polymeric Phosphates

medium	temp (°C)	k_{obs} (min^{-1})[a] mean	k_{obs} (min^{-1})[a] 95% c.i.	E_a (kcal/mol) mean	E_a (kcal/mol) SE[b]
acetate buffer (pH 5.5, 0.01 M)	60	−0.0063	(−0.0079, −0.0046)	22.86	2.35
	70	−0.0172	(−0.0192, −0.0152)		
	80	−0.0443	(−0.0477, −0.0409)		
acetate buffer (pH 5.5, 0.05 M)	60	−0.0064	(−0.0071, −0.0057)	26.36	2.30
	70	−0.0203	(−0.0219, −0.0189)		
	80	−0.0612	(−0.0667, −0.0556)		
acetate buffer (pH 5.5, 0.1 M)	60	−0.0071	(−0.0079, −0.0063)	23.80	2.14
	70	−0.0212	(−0.0227, −0.0198)		
	80	−0.0542	(−0.0595, −0.0489)		
acetate buffer (pH 5.5, 0.1 M) + 0.01 M SP	60	−0.0075	(−0.0089, −0.0061)	24.36	2.22
	70	−0.0233	(−0.0259, −0.0208)		
	80	−0.0602	(−0.0645, −0.0559)		
acetate buffer (pH 5.5, 0.1 M) + 0.03 M SP	60	−0.0067	(−0.0080, −0.0055)	27.50	4.20
	70	−0.0268	(−0.0310, −0.0227)		
	80	−0.0703	(−0.0748, −0.0659)		
acetate buffer (pH 5.5, 0.1 M) + 0.05 M SP	60	−0.0081	(−0.0091, −0.0070)	23.80	2.51
	70	−0.0251	(−0.0281, −0.0221)		
	80	−0.0617	(−0.0708, −0.0526)		
acetate buffer (pH 5.5, 0.1 M) + 0.01 M SAPP	60	−0.0054	(−0.0064, −0.0043)	26.88	2.61
	70	−0.0160	(−0.0172, −0.0148)		
	80	−0.0535	(−0.0592, −0.0478)		
acetate buffer (pH 5.5, 0.1 M) + 0.03 M SAPP	60	−0.0056	(−0.0064, −0.0048)	25.28	2.20
	70	−0.0182	(−0.0196, −0.0167)		
	80	−0.0486	(−0.0523, −0.0449)		
acetate buffer (pH 5.5 0.1 M) + 0.05 M SAPP	60	−0.0056	(−0.0063, −0.0048)	25.80	2.13
	70	−0.0173	(−0.0189, −0.0156)		
	80	−0.0505	(−0.0535, −0.0475)		
acetate buffer (pH 5.5, 0.1 M) + 0.01 M STP	60	−0.0060	(−0.0070, −0.0051)	24.05	2.19
	70	−0.0174	(−0.0193, −0.0156)		
	80	−0.0473	(−0.0516, −0.0430)		
acetate buffer (pH 5.5, 0.1 M) + 0.03 M STP	60	−0.0058	(−0.0063, −0.0053)	25.29	2.03
	70	−0.0188	(−0.0201, −0.0175)		
	80	−0.0448	(−0.0474, −0.0422)		
acetate buffer (pH 5.5, 0.1 M) + 0.05 M STP	60	−0.0051	(−0.0065, −0.0037)	25.37	2.95
	70	−0.0155	(−0.0180, −0.0131)		
	80	−0.0446	(−0.0512, −0.0381)		
acetate buffer (pH 5.5, 0.1 M) + 0.01 M SH	60	−0.0050	(−0.0064, −0.0036)	24.52	2.97
	70	−0.0143	(−0.0156, −0.0130)		
	80	−0.0408	(−0.0430, −0.0387)		
acetate buffer (pH 5.5, 0.1 M) + 0.03 M SH	60	−0.0052	(−0.0071, −0.0033)	23.72	2.70
	70	−0.0150	(−0.0171, −0.0130)		
	80	−0.0396	(−0.0413, −0.0379)		
acetate buffer (pH 5.5, 0.1 M) + 0.05 M SH	60	−0.0052	(−0.0060, −0.0043)	23.88	2.06
	70	−0.0146	(−0.0153, −0.0138)		
	80	−0.0400	(−0.0423, −0.0377)		

[a] $n = 18$, linear regression of $\ln [OTC_{time=t}] = \ln [OTC_{time=0}] - k_{obs}\, t$. [b] Standard error.

Table 4. Oxytetracycline Degradation Parameters k_{obs} (min^{-1}) and E_a (kcal/mol) in Aqueous Media As Affected by Calcium Chloride and Sodium Nitrite

medium	temp (°C)	k_{obs} (min^{-1})[a] mean	k_{obs} (min^{-1})[a] 95% c.i.	E_a (kcal/mol) mean	E_a (kcal/mol) SE[b]
acetate buffer (pH 5.5, 0.1 M) + 0.01 M $CaCl_2$	60	−0.0044	(−0.0072, −0.0016)	24.39	4.23
	70	−0.0112	(−0.0148, −0.0076)		
	80	−0.0357	(−0.0404, −0.0310)		
acetate buffer (pH 5.5, 0.1 M) + 0.1 M $CaCl_2$	60	−0.0028	(−0.0053, −0.0003)	16.85	6.09
	70	−0.0046	(−0.0076, −0.0015)		
	80	−0.0119	(−0.0172, −0.0066)		
acetate buffer (pH 5.5, 0.1 M) + 0.003 M $NaNO_2$	60	−0.0070	(−0.0082, −0.0058)	21.48	2.24
	70	−0.0245	(−0.0265, −0.0226)		
	80	−0.0436	(−0.0490, −0.0382)		

[a] $n = 18$, linear regression of $\ln [OTC_{time=t}] = \ln [OTC_{time=0}] - k_{obs}\, t$. [b] Standard error.

taining OTC revealed several chromatographic peaks during subsequent HPLC analysis. The chromatographic software indicated that the peak corresponding to the retention time of OTC was homogeneous in composition (i.e., one compound); additionally, the spectrum of the peak matched that for pure oxytetracycline. This was assumed to be confirmative evidence that this peak was due solely to elution of OTC, and the area counts (μV s) of the peak were used for the determination of OTC degradation parameters. The remaining peaks did not correspond to the retention times of the commercially available degradation products of OTC; specifically α and β-apooxytetracycline, and 4-epioxytetracycline (Fedeniuk et al., 1996). Studies to elucidate the identities of the peaks were not performed.

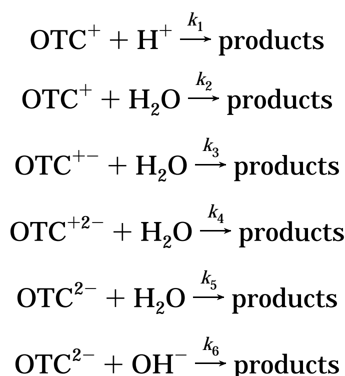
The k_{obs} and E_a values for the thermal degradation of OTC in different aqueous buffers are shown in Tables 1−4. The k_{obs} of OTC degradation is actually a com-

Table 5. Effect of Porcine Tissue Treatment upon Oxytetracycline Degradation [k_{obs} (min^{-1}) and E_a (kcal/mol)]

medium	temp (°C)	k_{obs}[a] mean	k_{obs}[a] 95% c.i.	E_a (kcal/mol) mean	E_a (kcal/mol) SE[b]
porcine tissue	60	−0.0042	(−0.0055, −0.0029)	25.52	3.04
	70	−0.0138	(−0.0157, −0.0119)		
	80	−0.0372	(−0.0415, −0.0329)		
porcine tissue + 0.4 M $CaCl_2$ (pH 5.5)	60	−0.0036	(−0.0047, −0.0024)	23.21	2.62
	70	−0.0098	(−0.0107, −0.0088)		
	80	−0.0260	(−0.0283, −0.0238)		
porcine tissue + SP (0.5% final concn)	60	−0.0065	(−0.0088, −0.0043)	19.41	2.66
	70	−0.0164	(−0.0193, −0.0136)		
	80	−0.0344	(−0.0390, −0.0297)		
porcine tissue + SAPP (equivalent to 0.5% SP)	60	−0.0060	(−0.0068, −0.0052)	23.42	1.96
	70	−0.0167	(−0.0182, −0.0152)		
	80	−0.0447	(−0.0469, −0.0424)		
porcine tissue + STP (equivalent to 0.5% SP)	60	−0.0039	(−0.0047, −0.0031)	29.43	2.99
	70	−0.0167	(−0.0186, −0.0149)		
	80	−0.0485	(−0.0546, −0.0424)		
porcine tissue + SH (equivalent to 0.5% SP)	60	−0.0081	(−0.0101, −0.0061)	21.24	2.43
	70	−0.0251	(−0.0273, −0.0229)		
	80	−0.0496	(−0.0560, −0.0433)		
porcine tissue + $NaNO_2$ (200 ppm final concn)	60	−0.0076	(−0.0091, −0.0061)	11.38	1.74
	70	−0.0121	(−0.0141, −0.0100)		
	80	−0.0200	(−0.0240, −0.0160)		

[a] $n = 18$, linear regression of $\ln [OTC_{time=t}] = \ln [OTC_{time=0}] - k_{obs}\, t$. [b] Standard error.

posite of six different degradative reactions (Connors et al., 1986):



$$OTC^{+} + H^{+} \xrightarrow{k_1} \text{products}$$

$$OTC^{+} + H_2O \xrightarrow{k_2} \text{products}$$

$$OTC^{+-} + H_2O \xrightarrow{k_3} \text{products}$$

$$OTC^{+2-} + H_2O \xrightarrow{k_4} \text{products}$$

$$OTC^{2-} + H_2O \xrightarrow{k_5} \text{products}$$

$$OTC^{2-} + OH^{-} \xrightarrow{k_6} \text{products}$$

Thus, k_{obs} is dependent upon the ionized form of OTC present, which in turn is dependent upon pH and other factors present in the medium.

The use of glycerol to adjust the a_w of the reaction media did not have a statistically significant effect upon k_{obs} at all temperatures (Table 1). The mean E_a for distilled, deionized water was higher than that obtained for the glycerol/distilled water solutions. Water is a known participant in the degradative reactions of OTC; severely limiting the presence of water in the reaction media will sharply decrease the rate of OTC degradation (Rose et al., 1996). This has been attributed to a decrease in diffusion of reactants in the media, particularly at an a_w lower than 0.6 (Bell and Labuza, 1994). In addition, a lower a_w would decrease the entropy of the reaction media and decrease the product formation constant. This would subsequently result in an increase in the E_a for degradation. At a higher a_w (greater than 0.8) dilution of the reactants would also cause a decrease in the rate of reaction.

Increasing phosphate buffer concentration from 0.01 to 0.1 M significantly increased k_{obs} at all temperatures, whereas E_a appeared to decrease (Table 2). Phosphate has been reported to be a known catalyst for the degradation of OTC, via general acid/base catalysis (i.e., Bronsted acid/base) (Connors et al., 1986). Degradation rates in 0.05M phosphate buffer, pH 5.5, were significantly higher than that observed at pH 4.0 or 7.0.

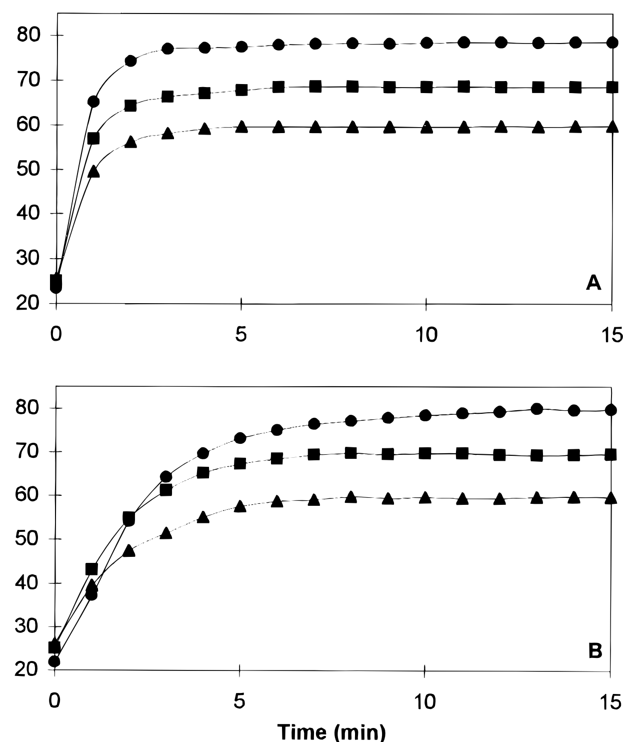


Figure 1. Come-up temperatures for aqueous media (A) and porcine tissue (B) in contant water bath temperatures at 60 (▲), 70 (■), and 80 °C (●).

Connors et al. (1986) had reviewed data illustrating that the rate of OTC degradation is relatively insensitive to pH changes in the pH range 4−7, though no statistical information was given to support this conclusion. Degradation of OTC via specific acid/base catalysis (i.e., hydronium and hydroxide ions) significantly increases beyond this pH range. In the present study, we stayed within the pH range of importance to the meat industry; therefore, the effect of pH extremes on k_{obs} was not tested.

In 0.05 M phosphate buffer, the addition of NaCl, a "non-reactive" ionic strength modifier, did not have any significant effect upon k_{obs} (Table 2). The addition of salt would effectively increase the polarity of the

medium. Increasing the polarity of the medium did not significantly affect k_{obs}, indicating that OTC degradation is proceeding predominantly via reaction mechanisms not involving transition states of an ionic nature differing from the reactant (Connors et al., 1986).

To investigate the effects of different additives upon k_{obs}, it is desirable that the reaction medium be relatively inert. Vej-Hansen et al. (1978) had reported that the rate of OTC degradation is relatively insensitive towards acetate buffer concentration in the pH range 3.5−5.6. At pH 5.5, increasing acetate buffer concentration from 0.01 to 0.1 M did not have any significant effect upon k_{obs} at 60 and 70 °C; however, at 80 °C the 0.01 M acetate buffer had a significantly lower rate of degradation than the 0.05 M and 0.1 M acetate buffers (Table 3). On the basis of these results and previous reports, subsequent testing was performed in 0.1 M acetate buffer.

Varying the concentration of SP from 0 to 0.05 M in 0.1 M acetate buffer did not have any significant effect upon the rate of degradation between 60 and 80 °C (Table 3). Increasing SAPP and STP concentrations above 0.01 M significantly lowered k_{obs} at 60 and 70 °C, but at 80 °C this effect was not as pronounced. SH significantly lowered k_{obs} at all temperature−concentration combinations. Although the concentration of the polymeric phosphates would vary as a function of time due to hydrolytic reactions, previous research indicated that the concentration of all phosphates should have remained relatively constant during the experimental time window (Van Wazer, 1958). Polymeric phosphates exhibit chelating properties which become more pronounced as the length of the polymer increases (Molins, 1991). OTC can form complexes with other molecules, such as metals, biochemicals, and other chelating agents with functional groups separated by three carbons (Gans and Higuchi, 1957; Higuchi and Bolton, 1959). It is known that small molecules binding to proteins undergo conformational changes (Nicklaus et al., 1995). Complex formation could alter the conformation of OTC, potentially altering its amenability to degradative reactions. Although previous research has shown that sodium hexametaphosphate exhibits only negligible complex formation with OTC, other unknown interactions between sodium hexametaphosphate and OTC in aqueous media may be occurring.

Addition of $CaCl_2$ to acetate buffers significantly decreased k_{obs} (Table 4). OTC is a known chelator of divalent metal ions via the oxygen atoms O_1, O_{12}, and O_{11} (Jogun and Stezowski, 1976). As these atoms are innately involved in some of the degradative reactions of OTC, their interaction with metal ions would make OTC less amenable to thermally induced decomposition.

Inclusion of 0.003M $NaNO_2$ into acetate buffer significantly increased k_{obs} at 60 °C, but at higher temperatures the increase in k_{obs} was not significant (Table 4). Sodium nitrite is a reactive molecule, promoting both oxidative and reductive reactions (Townsend and Olson, 1987). However, at pH 5.5, $NaNO_2$ degrades to several other compounds and this rate of degradation has been reported to be relatively constant between 50 and 80 °C (Okayama et al., 1991). Over the time course of the reaction, the concentration of nitrite would decrease. The exact nature of how $NaNO_2$ would influence the mechanism of OTC degradation has not been investigated.

Degradation of OTC in porcine tissue was significantly lower than in aqueous solutions (Table 5). Meat is a complex matrix of protein, minerals and fat that can all potentially influence the thermal stability of OTC via complexation, diffusional limitations, and other actions. Additionally, heat penetration of meat is influenced by its composition, and thus potential temperature profiles may be set up within the matrix, though the temperature come-up profile of meat was not qualitatively different from that for aqueous solutions (Figure 1). This phenomena would become more significant in larger cuts of meat during actual cooking procedures.

Addition of the different polymeric phosphates to meat (equivalent to 0.5% anhydrous dibasic sodium phosphate) resulted in significantly different results than those in aqueous media. The presence of SP had no effect upon k_{obs}, whereas the addition of SAPP and STP increased k_{obs} at 60 and 70 °C, and significantly increased it at 80 °C, in direct contrast to its effects in aqueous solution. Inclusion of SH significantly increased OTC degradation at all tested temperatures. These effects may be due to the chelating properties of polymeric phosphates. The phosphates can bind ions present within the meat matrix, thereby preventing complex formation with OTC. As OTC−metal complexes are thermally stable relative to free OTC, preventing this complex formation would increase k_{obs}.

Incorporation of $CaCl_2$ into meat significantly decreased k_{obs} as was also shown in aqueous media. Bones can act as storage sites for OTC after administration (Mitscher, 1978). Mechanically deboned meats do incorporate small amounts of bone into meat during manufacture (Romans et al., 1994); therefore, it is conceivable that products incorporating significant amounts of mechanically deboned meat may have sizable amounts of thermally stable OTC present.

The presence of $NaNO_2$ (200 ppm) in tissue exhibited different effects on k_{obs} than that observed for the phosphates. At 60 °C, $NaNO_2$ significantly increased k_{obs} whereas at 80 °C it significantly decreased it; in aqueous media at 80 °C $NaNO_2$ showed no observable effect on k_{obs}. The resultant E_a was substantially lower than results obtained from all other treatments. It is not known if these results are due to $NaNO_2$ or to the presence of nitrosation products in the tissue.

CONCLUSION

Initial steps have been taken to quantify OTC degradation as a function of different reaction media. Media composition has a significant effect upon OTC degradation parameters, with OTC degradation in tissue matrices being substantially slower than that occurring in aqueous media. The statistics derived from this study will supplement the few studies that have been done in this area. Several commonly used additives have been found to have significant effects upon OTC degradation parameters. This indicates that the potential dietary intake levels of OTC would vary substantially depending upon the processing OTC contaminated tissues undergo. Determination of the exact mechanism of these effects would allow for greater confidence in determining the level of intake of this residue. Application of the protocols used for this study can be modified to study the degradation of other commonly used xenobiotics.

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