Effects of Chemicals on Preservation of Crab-Processing Waste and Fermentation Characteristics of the Waste−Straw Mixture

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Addition of 1.5% propionic/formic acid (1:1) prevented degradation of crab-processing waste for up to 14 days. The preserved waste was ensiled with wheat straw, sugarcane molasses, and water (32:32:16:20, wet basis) with or without 0.1% microbial inoculant. Reductions in pH and water soluble carbohydrates (WSC) and an increase in lactic acid were achieved in silages made with or without inoculant. Preserving crab waste with either 0.2% sodium hypochlorite (NaOCl) or 0.4% hydrogen peroxide (H_2O_2) prevented deterioration for up to 7 days. The concentration of trimethylamine (TMA), which indicates offensive odor in marine products, increased ($P < 0.05$) from 3.70 to 12.85 mg of N/100 g in NaOCl-treated waste, compared to no change (2.66 vs 2.71 mg of N/100 g) for H_2O_2-treated waste. The use of 1% sodium nitrite ($NaNO_2$) and a combination of 0.2% NaOCl/0.2% calcium hypochlorite [$Ca(OCl)_2$] (1:1, w/w) or 0.2% of each alone preserved the waste for a minimum of 10 days. Ammonia gas was first detected at day 12, and H_2S was not detected throughout the 15 days of preservation with 1% $NaNO_2$. Some chemicals show promise for preserving crab waste.

Keywords: *Trimethylamine; chemicals; hydrogen sulfide; ammonia; degradation*

INTRODUCTION

In Virginia and Maryland, approximately 41 million kg of crabs was harvested in 1989 (National Marine Fisheries Service, 1990). Crab-processing waste amounts to 36 million kg. The waste undergoes degradation within 5 h after harvest (Brooks, 1980). In the past, the waste was dumped into the ocean (Olsen, 1980) or was put into landfills (Brinsfield, 1980). However, these practices have been banned by the U.S. Environmental Protection Agency (EPA). The conversion of the waste into crab meal for use as livestock feed has been a marginal situation, economically, due to the cost of energy and relatively low demand for the product (Coale, 1980), perhaps due to competition from other protein supplements. Crab-processing waste is a good source of crude protein (CP) (Samuels et al., 1992) and minerals (Cantor, 1980). Fresh crab waste has been ensiled with straw with the use of large amounts of acetic acid (Samuels et al., 1992) or molasses and microbial inoculant (Abazinge et al., 1992). However, crab wastes accumulated for a period of time must be kept in a fresh condition prior to ensiling to achieve successful fermentation. The effectiveness of NaOCl in treating caged layer waste (Narasimhalu et al., 1981) and the inhibitory effect of H_2O_2 in raw milk (Lipma and Owen, 1943) and nitrite in canned cured meat (Tompkin et al., 1979) have been shown against certain bacteria.

Three experiments were conducted to evaluate the effect of chemicals on the preservation of crab-processing waste and the effects of these chemicals on fermentation characteristics of ensiled crab waste−straw mixtures.

MATERIALS AND METHODS

Experiment 1. Fresh crab waste, obtained from a crab-processing plant (Graham and Rollins Inc., Hampton, VA) was mixed thoroughly with a shovel to achieve a homogeneous mixture. The crab waste was placed in 210 L metal drums double-lined with polyethylene bags and sprayed with a mixture of propionic/formic acid (1:1, w/w) applied with a hand sprayer to achieve 1.5% of the waste. After treatment, the tops of the polyethylene bags were closed, and holes were made at the bottom of the bags and drums to allow drainage. The drums with the contents were left in an enclosed room at about 18 °C. Samples of the crab-processing waste were obtained before and after spraying and frozen for later analyses.

After 2, 7, 14, 21, and 28 days, the treated crab waste was mixed with wheat straw, sugarcane molasses, and water (32:32:16:20, w/w), with or without 0.1% microbial inoculant (*Streptococcus faecium* and *Lactobacilus plantarum*, Pioneer Hi-Bred International, Des Moines, IA). After mixing, the mixtures were packed and ensiled in 4 L cardboard containers, double-lined with polyethylene bags, which were sealed individually. Six containers were prepared for each treatment time. The ensiled mixtures were opened after a minimum of 42 days. Samples were taken before and after ensiling for analyses.

Experiment 2. Approximately 114 kg of the fresh crab waste was sprayed with NaOCl or H_2O_2 to achieve 0.2% and 0.4% (w/w), respectively, and shovel mixed. The treated wastes were placed into 210 L metal drums and prepared as described for exp 1, except the tops of the bags were not closed. After 7 days, the preserved wastes were mixed with straw,

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Table 1. Composition of Propionic/Formic Acid Treated Crab Waste (Experiment 1)

day	DM[a] (%)	ash[a,b] (%)	CP[a,b] (%)	TMA[a,c] (mg of N/100 g)
0	42.02	40.28	40.51	1.30
2	41.39	40.57	42.18	1.32
7	40.63	42.33	43.34	14.43
14	40.40	41.84	42.76	12.33
21	38.64	43.55	46.51	26.36
28	37.44	47.41	47.02	30.76
SE	0.26	0.24	0.20	0.67

[a] Linear effect of time ($P < 0.01$). [b] Dry matter basis. [c] Wet basis.

molasses, and water (32:32:16:20, w/w) and ensiled in six 4 L cardboard containers per treatment, as described in exp 1. Samples of the crab-processing waste before and after 7 days of preservation and of the mixtures before and after ensiling were obtained and frozen for later analyses.

Experiment 3. Crab-processing waste was placed in a horizontal mixer and treated with the following levels of chemicals: none, 0.2% NaOCl, 0.2% NaOCl/0.2% $Ca(OCl)_2$ (w/w), 0.4% NaOCl/0.4% $Ca(OCl)_2$ (w/w), or 1% $NaNO_2$. After thorough mixing, each mixture was placed in three 210 L metal drums per treatment and prepared as described in exp 1. The drums and the contents were stored in a semiclosed shelter. Two 1 m poly(vinyl chloride) (PVC) pipes (1.5 cm i.d.) with several holes in the lower 43 cm were placed in each drum to measure the NH_3 and H_2S. The bags were sealed tightly around the pipes, and rubber stoppers were placed at the openings. Ammonia and H_2S were obtained with a gas sampler (Gastec precision gas detector system, Japan), through the PVC pipe openings initially, at days 1, 2, and 3, and then every other day until day 10. Materials from all of the other treatments except waste treated with 1% $NaNO_2$ were discarded at day 10 due to spoilage of the mixtures. Waste treated with 1% $NaNO_2$ was sampled until day 15. Samples of the treated waste were obtained with a core sampler made of a 1.22 m PVC pipe (6.35 cm i.d.). One end of the pipe was sharpened to facilitate ease of penetrating through the mixtures. At each sampling, the PVC pipe was pushed through the mixtures from the top layer to the bottom. The samples were obtained the day following gas sampling.

Chemical Analyses. The samples of crab-processing waste obtained for expt 1−3 were analyzed for trimethylamine (TMA) according to a colorimetric procedure (Dyer, 1959) after extraction with 7.5% trichloroacetic acid. Kjeldahl N was determined on wet samples (AOAC, 1984). Dry matter (DM) was determined by drying in a forced draft oven at a maximum of 60 °C for 48 h. The dried samples were ground in a Wiley mill, and subsamples were taken for ash determination (AOAC, 1984). For the ensiled mixtures, the initial and final samples were prepared by homogenization of 25 g wet samples with 225 mL of distilled water in blender jars. These homogenates were filtered through four layers of cheesecloth. The filtered extract was used for determining pH (electrometrically), lactic acid [Barker and Summerson, 1941, as modified by Pennington and Sutherland (1956)] and water soluble carbohydrates (WSC) [Dubois et al., 1956, as adapted to corn plants by Johnson et al. (1966)].

Statistical Analysis. The data were tested by analysis of variance by the general linear model procedure of SAS (1982). For the propionic/formic acid treated crab waste−straw mixtures, effects of day, additive, and day × additive interaction were included in the model and the effect of time was tested by linear and quadratic contrasts. The treatment effect was included in the model for exp 2, and day × treatment effect was tested for exp 3. Comparisons of means were made using Tukey's HSD procedures (SAS, 1982).

RESULTS AND DISCUSSION

Experiment 1. The DM of the propionic/formic acid preserved crab waste decreased linearly ($P < 0.01$) from 42.0% at day 0 to 37.4% on day 28 (Table 1). There were concomitant increases in CP and ash. These decreases may reflect degradation of organic matter of the crab-processing waste. Abazinge et al. (1992) reported a decrease in DM and CP contents when crab waste was treated with 1.5% propionic/formic acid (1:1, w/w) and stored for 56 days. The longer storage period used by Abazinge et al. (1992) may explain the decrease in CP. The TMA levels increased linearly ($P < 0.01$) from 1.30 (day 0) to 30.8 mg of N/100 g at day 28. The large increases were on days 7 and 21. Abazinge et al. (1992) reported a TMA concentration of 55.7 mg of N/100 g for untreated crab-processing waste stored for a period of 56 days. High levels of TMA result in offensive odors, as microbes convert trimethylamine oxide to TMA.

Table 2. Fermentation Characteristics of Propionic/Formic Acid Treated Crab Waste−Straw Mixture[a] (Experiment 1)

day of storage[b]	DM[c,d]		pH[c,d]		WSC[d,f]		lactic acid[d,f]	
	none	add[e]	none	add[e]	none	add[e]	none	add[e]
pre-ensiled								
2	55.47	55.02	7.09	6.97	10.87	10.41	0.38	0.35
7	57.27	57.53	7.31	7.32	10.55	9.89	0.40	0.38
14	57.10	57.49	7.34	7.48	9.64	9.85	0.40	0.39
21	54.97	55.16	7.84	7.83	10.70	10.26	0.32	0.36
28	57.17	56.57	8.01	8.03	9.90	10.13	0.21	0.23
SE	0.29		0.02		0.41		0.03	
post-ensiled								
2	53.72	53.67	6.34	6.49	2.02	1.91	2.21	1.97
7	55.01	55.17	5.27	5.31	3.28	2.12	6.31	7.91
14	53.99	54.08	5.17	5.23	1.95	1.89	8.26	8.26
21	53.02	53.60	5.17	5.19	0.94	0.97	8.51	8.43
28	55.04	55.22	5.33	5.43	1.01	0.99	7.27	7.46
SE	0.20		0.06		0.11		0.27	

[a] Crab waste, straw, molasses, and water (32:32:16:20, wet basis). [b] Prior to ensiling. [c] Linear effect of time for pre-ensiled mixtures ($P < 0.01$). [d] Quadratic effect of time for post-ensiled mixtures ($P < 0.01$). [e] Additive (0.1% microbial inoculant, *S. faceium* and *L. plantarum*). [f] Dry matter basis.

Table 3. Composition of Crab Waste Preserved with Chemicals (Experiment 2)

	DM[a]		ash[a]		CP[b]		TMA[c−e] (mg of N/100 g)	
additive	day 1	day 7	day 1	day 7	day 1	day 7	day 1	day 7
NaOCl	39.24	39.35	36.39	39.60	44.29	44.01	3.70	l2.85
H_2O_2	40.47	41.32	36.87	36.65	43.31	43.39	2.66	2.71
SE	0.70	0.49	1.04	0.57	1.25	0.25	0.23	0.60

[a] Treatments differ for day 7 ($P < 0.05$). [b] Dry matter basis. [c] Treatments differ for day 7 ($P < 0.01$). [d] Trimethylamine. [e] Wet basis.

Dry matter and pH of the pre-ensiled mixtures increased ($P < 0.01$) gradually with time of storage of the crab waste prior to ensiling (Table 2). The elevated pH may be due to the buffer effect of the high ash and (or) TMA. After ensiling, large reductions ($P < 0.01$) in WSC and pH and an increase ($P < 0.01$) in lactic acid concentration were obtained. The reduction in pH and increase in lactic acid production were less for the waste stored for 2 days prior to ensiling (quadratic effect, $P < 0.01$). The residue of propionic/formic acid used in preservation may have restricted fermentation during storage for only 2 days. Thus, good ensiling was achieved in the waste stored for more than 2 days. There were no significant effects of microbial inoculant on any of the parameters measured. Abazinge et al. (1992) reported lower pH and higher lactic acid concentration when an inoculant was added to ensiled fresh crab waste, straw, molasses, and water. Ohyama et al.

Table 4. Fermentation Characteristics of Treated Crab Waste and Straw Silage[a] (Experiment 2)

preservative	time	pH	DM	WSC[b,c]	lactic acid[b,c]
NaOCl	pre-ensiled	7.3	56.7	11.8	0.6
	post-ensiled	5.5	54.5	1.0	8.1
H_2O_2	pre-ensiled	7.1	57.6	10.9	0.5
	post-ensiled	5.7	54.6	1.2	6.4

[a] Crab waste, wheat straw, molasses, and water (32:32:16:20, wet basis). [b] Dry matter basis. [c] Treatments differ for post-ensiled mixtures ($P < 0.05$).

(1973) showed that inoculation of ensiled ryegrass is not needed with a sufficient amount of WSC and proper sealing of the silo.

Experiment 2. Crab-processing waste treated with NaOCl was lower ($P < 0.05$) in DM and ash compared to H_2O_2 at day 7 (Table 3). The TMA concentration increased for NaOCl-treated waste, while the TMA concentration did not change for crab-processing waste preserved with H_2O_2-treated waste. Hence, at day 7 a higher ($P < 0.01$) value was observed for NaOCl-treated waste compared to H_2O_2-treated waste. However, the value of 12.85 mg of N/100 g, which is similar to the value reported by Abazinge et al. (1992), indicates minimal deterioration of the preserved waste. The low level of TMA observed with H_2O_2-treated waste reflects the strong bactericidal effect of H_2O_2 compared to NaOCl. The effectiveness of H_2O_2 in controlling the odor production in feedlots and in dairy cow and swine waste treatment operations has been reported (Ulich et al., 1975; Cole et al., 1975). The decrease in pH and WSC and the rise ($P < 0.05$) in lactic acid indicated good ensiling, regardless of the chemicals used in preserving the waste (Table 4).

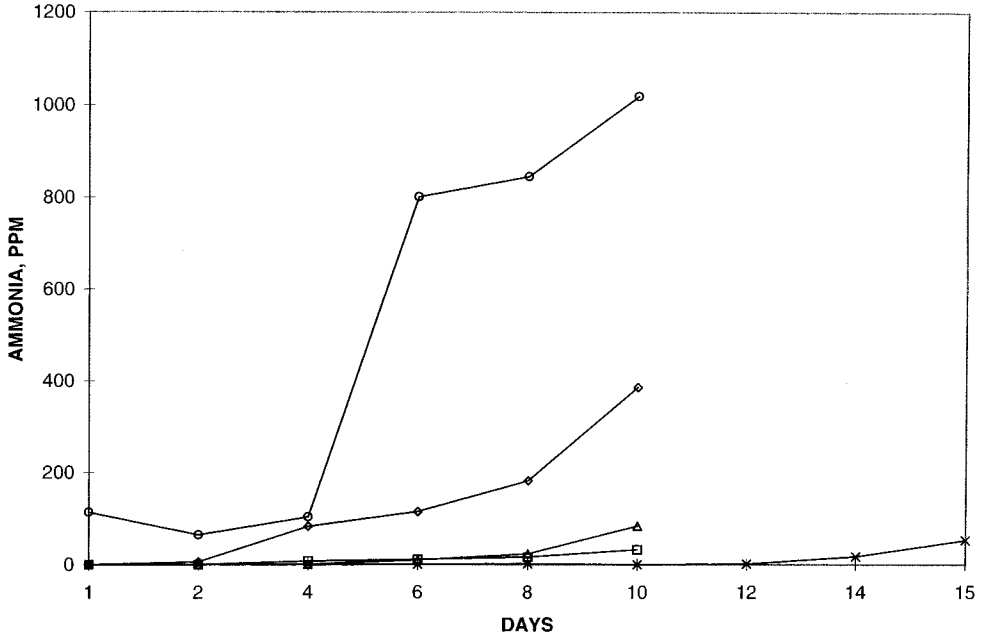

Figure 1. Effect of additives on ammonia concentration, wet basis: (◇) 0.2% NaOCl; (□) 0.4% NaOCl/$Ca(OCl)_2$; (△) 0.2% NaOCl/$Ca(OCl)_2$; (×) 1% $NaNO_2$; (○) control. Day × treatment interaction ($P < 0.01$).

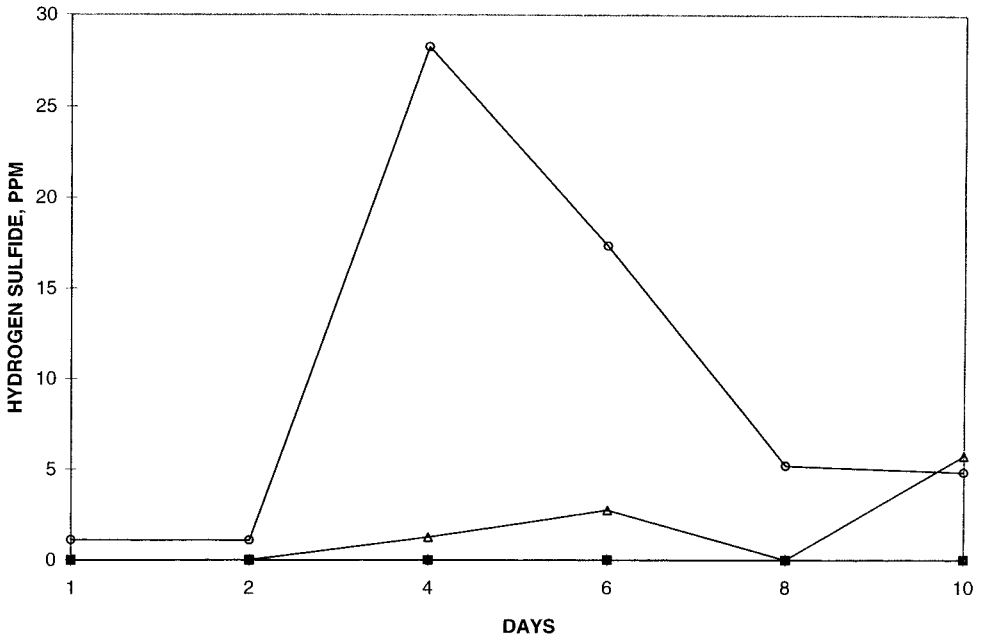

Figure 2. Effect of additives on hydrogen sulfide concentration, wet basis: (◇) 0.2% NaOCl; (□) 0.4% NaOCl/$Ca(OCl)_2$; (△) 0.2% NaOCl/$Ca(OCl)_2$; (×) 1% $NaNO_2$; (○) control. Day × treatment interaction ($P < 0.01$).

Table 5. Dry Matter, Ash, Crude Protein, and Trimethylamine Concentration in Preserved Crab Waste (Experiment 3)

day	none	0.2% NaOCl/ $Ca(OCl)_2$	0.4% NaOCl/ $Ca(OCl)_2$	0.2% NaOCl	1% $NaNO_2$	SE
			Dry Matter,[a] %			
0	39.32	38.11	31.97	36.26	37.37	0.90
5	37.26	36.13	36.88	35.35	37.52	1.35
10	38.26	35.77	36.32	36.67	39.01	1.33
15[d]					36.73	
			Ash,[a–c] %			
0	46.76	40.63	37.58	36.83	35.67	1.81
5	49.58	41.26	41.03	40.08	36.28	1.40
10	52.57	40.24	40.77	39.70	38.90	1.62
15[d]					36.42	
			Crude Protein,[a,b] %			
0	37.76	42.22	49.20	44.15	48.86	1.33
5	43.36	45.60	46.08	45.66	47.54	1.80
10	40.16	48.00	45.87	46.49	46.19	2.33
15[d]					49.71	
			Trimethylamine[a,c,e] (mg of N/100 g)			
0	1.06	0.82	0.99	0.86	0.62	0.06
5	21.00	21.49	16.95	19.71	0.87	0.51
10	31.95	26.17	19.93	26.00	2.23	0.53
15[d]					7.24	

[a] Treatments differ for day 0 ($P < 0.01$). [b] Dry basis. [c] Treatments differ for days 5 and 10, respectively ($P < 0.01$). [d] Measurement taken for $NaNO_2$ only. [e] Wet basis.

Experiment 3. The concentration of NH_3 was highest ($P < 0.01$) for the untreated crab waste, while the value for waste treated with 0.2% NaOCl was intermediate (Figure 1). Crab waste treated with 0.2 or 0.4% NaOCl/$Ca(OCl)_2$ (1:1, w/w) showed a small increase in NH_3 concentration at day 8. Treating crab waste with 1% $NaNO_2$ suppressed NH_3 production until day 12, after which time a small increase was observed. No detectable H_2S was observed with crab waste treated with 0.2% NaOCl, 0.4% NaOCl/$Ca(OCl)_2$ (1:1, w/w), or 1% $NaNO_2$ (Figure 2). The concentration was highest (28.2 ppm) for the untreated waste at day 4, after which time the level rapidly decreased to approximately 5 ppm on day 10. The use of 0.2% NaOCl/$Ca(OCl)_2$ (1:1, w/w) resulted in a small increase in H_2S concentration, which reached a peak (2.4 ppm) on day 6, decreased on day 8, and then increased again on day 10. Hammond et al. (1968) observed that treating swine waste with chlorine compounds and $NaNO_3$ lowered H_2S and NH_3 production.

At day 0, DM was highest ($P < 0.01$) for the untreated crab waste (Table 5). There were increases in ash content with time for all treatments. Values for ash content were different ($P < 0.01$) among all treatments for each sampling period. The highest value for ash was for the untreated waste.

At day 0, CP content was higher ($P < 0.01$) for chemically treated waste than for the untreated waste (Table 5). Abazinge et al. (1992) reported the lowest CP value of 35.83% in untreated crab waste kept for 56 days. In the present study, TMA values were low ($P < 0.01$) for all treatments at day 0. Increases ($P < 0.01$) were observed for all treatments at days 5 and 10; however, the increase was small for the crab waste treated with 1% $NaNO_2$. At day 10, the TMA concentration for crab-processing waste treated with 1% $NaNO_2$ was lowest (2.23 mg of N/100 g), compared to the other treatments. At day 15, there was a small increase in TMA from 2.23 to 7.24 mg of N/100 g for the $NaNO_2$-treated waste.

The results of these studies showed that NaOCl, propionic and formic acids, and H_2O_2 are effective in preserving crab-processing waste for 7–10 days prior to ensiling. The combination of NaOCl and $Ca(OCl)_2$ showed greater effectiveness against degradation of crab waste than NaOCl alone. The chemicals did not adversely affect fermentation characteristics of the crab waste–straw mixtures. The use of $NaNO_2$ for preserving crab waste for more than 10 days looks promising. Crab waste–straw silage could be fed as roughage or protein supplement to ruminants.

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