Ensiling Characteristics of Crab Waste and Wheat Straw Treated with Different Additives†

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Three experiments were conducted in which crab waste and wheat straw were ground and ensiled in 1:1 proportions. The crude proteins of the crab waste and wheat straw were 44.1 and 4.0%, dry matter basis, respectively. In the first experiment 0, 10, or 20% dry molasses, 0 or 0.1% microbial silage inoculant; and 0 or 5.4% phosphoric acid were added. In the second experiment, silages were prepared with 10, 15, or 20% molasses, 0 or 0.1% microbial silage inoculant, and 0 or 20% water. For each experiment a 3 × 2 × 2 factorial arrangement was used. After ensiling, the pH of the mixtures without added molasses or acid was higher than it was initially. Lactic acid levels increased ($p < 0.05$) and pH decreased ($p < 0.05$) linearly with increasing levels of molasses. Trimethylamine (TMA), which indicates offensive odor in marine products, was lower ($p < 0.01$) for molasses- and inoculant-treated silages. Total volatile fatty acid, acetic, propionic, and isobutyric acid, concentrations were higher ($p < 0.01$) in the mixtures with added water and molasses. Addition of 10–20% dry molasses to crab waste–straw mixtures prior to ensiling resulted in silages with substantial amounts of lactic acid. In a third study (large silos), pH was lower and lactic acid was higher ($p < 0.05$) for ensiled mixtures with added molasses and inoculant, compared to molasses alone.

INTRODUCTION

Crabs represent the fourth most important seafood product in the United States, with a total annual catch of about 149 000 metric tons (NMFS, 1975). After processing, 80–90% of the crab remains as waste material (Brinsfield, 1980). Disposal of the waste presents problems due to the high moisture content, which averages 60% (Miles, 1981). In the past, the major outlet was the production of crab meal (Olsen, 1980); however, the economic feasibility of this method is questionable under current energy prices. An alternative method of disposal that requires minimal energy input is ensiling.

Previous efforts in this laboratory to ensile crab waste were unsuccessful due to offensive odor and increased pH (Samuels et al., 1992). However, crab waste and wheat straw mixtures were stabilized by adding 16% glacial acetic acid, but this practice is not economical.

Crab waste is high in minerals and protein (Cantor, 1980), which contribute buffering activity to the mixtures. Trimethylamine, a volatile product, buffers and imparts offensive odor to the waste (Beatty and Gibbons, 1937). To successfully ensile crab waste, substantial amounts of fermentable sugars may be required to produce large quantities of lactic acid to lower the pH and stabilize the product (Raa and Gilberg, 1982). There is evidence that ammonia is produced early in the fermentation process before the pH has dropped (Raa and Gilberg, 1982).

This study was undertaken to investigate the additives necessary to produce acceptable silages of crab waste and straw.

MATERIALS AND METHODS

Small Silo Studies. Crab waste was obtained from a processing plant (Graham and Rollins, Inc., Hampton, VA) after the meat was hand-picked from steamed blue crabs. The wet crab waste was ground in a high-speed hammermill without screen. Wheat straw was ground in a tub grinder (Sperry-New Holland, New Holland, PA) through a 2-cm screen. The nutritional characteristics of the crab waste and wheat straw are shown in Table I.

Two experiments were conducted with 3 × 2 × 2 factorial arrangement of treatments. In the first experiment, ground crab waste and wheat straw were mixed in 1:1 proportions, wet basis, with the addition of 0, 10, or 20% dry sugarcane molasses, 0 or 5.4% H_3PO_4, and 0 or 0.1% microbial silage inoculant (*Streptococcus faecium* and *Lactobaccilus plantarum*) obtained from Pioneer Hi-Bred International, Inc., Des Moines, IA. The amount of acid added was estimated to lower the pH of the mixtures to 7.0 to enhance streptococci activity. Water was added at 20% to all mixtures. The water was added to lower the dry matter (DM) level to 50–60% and provide an aqueous medium for microbial activity. In the second experiment, 10, 15, or 20% molasses, 0 or 20% water, and 0 or 0.1% microbial silage inoculant were added.

For both experiments, the mixtures were prepared by slowly adding the wheat straw to the crab waste in a horizontal mixer. The inoculant was stirred into the molasses, and the molasses was slowly added to the crab waste–straw mixtures and allowed to mix for 30 min. In the first experiment, three laboratory silos, consisting of 1-L cardboard containers double-lined with polyethylene bags, each containing approximately 0.7 kg of the initial mixture, were prepared for each treatment. Six silos, consisting of 3.8-L cardboard containers double-lined with polyethylene bags and each containing approximately 2 kg of the initial mixtures, were prepared for each treatment in the second experiment. Initial samples were taken for dry matter (DM) determination. Mixtures were firmly packed, and each bag was individually sealed after elimination of air. The silos were weighed before and after ensiling.

After a minimum of 42 days in an enclosed building with a temperature of 10–15 °C during September and November, the silos were weighed, opened, and observed for appearance and odor. The top 5 cm of the ensiled material was removed prior to sampling. Water extracts of the initial and fermented mixtures were prepared by homogenizing duplicate 25-g samples with 225 mL of deionized water in 0.5-L jars in a Waring blender at full speed for 2 min. The homogenate was filtered through four layers of cheesecloth. The extracts of the initial and fermented mixtures were used for measurement of pH (electrometrically), lactic acid

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Table I. Chemical Composition of the Crab Waste and Wheat Straw Used in Experiments

	crab waste			wheat straw		
	small silos		large	small silos		large
component	expt 1	expt 2	silos	expt 1	expt 2	silos
dry matter, %	45.22	44.80	44.92	89.20	89.50	90.12
crude protein[a]	44.13	44.52	44.36	4.02	4.04	3.94
neutral detergent fiber[a]	22.53	21.82	22.40	86.72	85.90	86.62
acid detergent fiber[a]	17.05	16.94	16.85	58.15	58.10	58.30
cellulose[a]	0.14	0.16	0.18	46.52	46.48	46.60
hemicellulose[a]	5.48	5.24	5.36	28.57	28.62	28.70
lignin[a]	0.01	0.03	0.03	10.38	10.28	10.34
ash[a]	39.25	39.15	39.18	5.57	5.62	5.54
water-soluble carbohydrates[a]	3.42	3.38	3.46	4.08	4.13	4.14

[a] Percent of dry matter.

[Barker and Summerson, 1941, as modified by Pennington and Sutherland (1956)], water-soluble carbohydrates (WSC) [Dubois et al., 1956, as adapted for corn plants by Johnson et al. (1966)], and volatile fatty acids (VFA) (Varian 6000 gas chromatograph, column packed with 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW, Varian Associates, Sugarland, TX). Trimethylamine was determined on initial and fermented mixtures according to a colorimetric procedure (Dryer, 1959) after extraction in 7.5% trichloroacetic acid. Dry matter of the mixtures was determined by drying duplicate 200-g samples in a forced-draft oven at a maximum of 60 °C for 48 h. The samples were allowed to air equilibrate and were composited and ground in a Wiley mill through a 1-mm screen and analyzed for DM and ash (AOAC, 1980). Kjeldahl nitrogen (N) was determined on wet silage samples (AOAC, 1980).

Large Silo Study. On the basis of data from the small silo studies and previous work in this laboratory, a study was conducted to further determine fermentation characteristics. Crab waste and wheat straw (1:1 proportions) were ensiled with the following additives: (1) 20% dry molasses, (2) 20% dry molasses and silage inoculant (*S. faecium* and *L. plantarum*), and (3) 16% glacial acetic acid. In previous work (Samuels et al., 1991) palatability and digestibility of acetic acid treated crab waste–straw mixtures were satisfactory. Water was added at 20% to the mixtures prior to ensiling to lower the DM level to about 50%. Thus, the final mixtures contained 16% dry molasses and 13% acetic acid, representing 20 and 16%, respectively, before water was added. Wheat straw was ensiled alone with 40% added water also.

Crab waste and wheat straw were ground and mixed in a horizontal mixer, and the various additives were added slowly. After mixing for about 30 min, the mixtures were augered into 210-L metal drums double-lined with 0.08-mm polyethylene bags. All mixtures were firmly packed by trampling, and the bags were individually sealed after exclusion of air. Initial samples of the ingredients and mixtures were collected, composited, subsampled, and frozen.

The mixtures were allowed to ensile in an open shed in January and February at an average temperature of 10 °C for a minimum of 42 days. The top 5 cm was removed when the silos were opened, and samples were taken from several areas of the silo, subsampled, and frozen for subsequent analyses. Procedures used for the determination of fermentation characteristics, DM, and N were the same as described for the small silo studies. Samples were analyzed also for neutral detergent fiber (NDF) (Van Soest and Wine, 1967), acid detergent fiber (ADF) (Van Soest, 1963), lignin, cellulose, (Van Soest and Wine, 1968), and ash (AOAC, 1980). Hemicellulose was determined by difference (NDF − ADF).

Statistical Analyses. Analyses of variance were performed using the general linear model (GLM) procedure of the Statistical Analyses System (SAS, 1982). For the small silo experiments, the results were analyzed as 3 × 2 × 2 factorial arrangements of treatments. The effect of level of molasses was tested by linear and quadratic contrasts. For the large silo study, the following comparisons were made: (1) straw vs crab waste–straw silages, (2) acetic acid treatment vs molasses and molasses plus inoculant, and (3) molasses vs molasses plus inoculant.

RESULTS AND DISCUSSION

Small Silo Studies. The DM of the crab waste was 45.0% (Table I), which is slightly higher than the value of 40.2% reported by Samuels et al. (1992). The crude proteins (CP) of the crab waste and straw were 44.3 and 4.0%, DM basis, respectively. The CP content of the crab waste was higher than the value of 28% reported by Cantor (1980) but similar to those reported by Watkins (1982) and Samuels et al. (1992). The crab waste used in the present study was from the same plant as the waste used by Samuels et al. (1992). Neutral detergent fiber and ADF were 22.2 and 17.0%, respectively, for the crab waste, and the ash content was 39.2%. Water-soluble carbohydrates (WSC) for the crab waste and wheat straw were low, averaging 3.4 and 4.0%, respectively.

The silages with pH in the range 5.0–6.0 had a pleasant aroma. The control mixtures had a pungent and offensive odor after ensiling. The initial pH in the first experiment were near neutral for all mixtures, ranging from 6.8 to 7.4 (Table II). After ensiling, all mixtures showed a decrease in pH except for those with no additives and the inoculated treatment alone. The pH for these increased to 8.26 after ensiling. Adding molasses decreased ($p < 0.05$) post-ensiled pH, suggesting enhanced fermentation.

Initial WSC concentrations were low except in the mixtures with added molasses. There was a decrease in WSC after ensiling, especially for mixtures containing added molasses. Post-ensiled WSC was higher ($p < 0.05$) for mixtures with added phosphoric acid (H_3PO_4). Appreciable amounts of lactic acid (>1.5%) were observed only for the mixtures ensiled with molasses. Addition of acid resulted in lower ($p < 0.05$) lactic acid levels in the post-ensiled mixtures, indicating that fermentation was decreased. A large decrease occurred when it was added to mixtures with added molasses (acid × molasses interaction, $p < 0.05$). Adding molasses and microbial silage inoculant increased ($p < 0.05$) lactic acid levels of the ensiled mixtures, compared to molasses alone, but the effect was noted only when the H_3PO_4 was not added.

The post-ensiled pH of the mixtures decreased linearly ($p < 0.05$) with increasing levels of molasses. Water-soluble carbohydrate disappearance was highest for the mixtures with added 20% molasses, indicating a greater extent of fermentation. Lactic acid levels increased linearly ($p < 0.05$) with level of molasses.

Initial mixtures had concentrations of TMA of about 7.0–8.0 mg/100 g of silage (Table II). After ensiling, essentially no change occurred in TMA for the mixtures with 20% molasses without H_3PO_4. Addition of microbial inoculant further decreased ($p < 0.05$) TMA concentration. High levels of TMA result in very offensive odor. The high concentrations of TMA in the silages without molasses are congruent with the results of Tatterson (1980), who suggested that in the absence of free sugar putrefying microbes actively degrade proteins, resulting in the production of amines. Watson (1939) reported that microbes in marine animals, including crabs, used trimethylamine oxide as a terminal electron acceptor and reduced it to TMA in the absence of appropriate substrates such as pyruvate and glucose.

Addition of molasses, water, and microbial inoculant (experiment 2) decreased ($p < 0.05$) the pH of the mixtures after ensiling (Table III), but the effect of inoculant alone was small. Water decreased pH only in mixtures with 15 and 20% molasses (molasses × water

Table II. Fermentation Characteristics of Crab Waste–Wheat Straw Silages, Small Silos, Experiment 1

	treatment[a]												
item	none	acid[b]	inoculant[c]	10% molasses	20% molasses	acid[b] + 10% molasses	acid[b] + 20% molasses	acid[b] + inoculant	inoculant + 10% molasses	inoculant + 20% molasses	acid[b] + inoculant + 10% molasses	acid[b] + inoculant + 20% molasses	SE[d]
pH													
pre-ensiled	7.34	7.14	7.36	6.87	7.13	7.06	6.96	6.86	7.18	6.95	6.81	6.99	0.06
post-ensiled[e–g]	8.30	6.81	8.23	6.83	5.31	6.49	6.47	6.55	6.07	5.34	6.64	6.42	0.08
water-soluble carbohydrates[h]													
pre-ensiled[f,g,i]	2.25	2.53	2.16	10.73	22.70	10.40	20.76	2.84	12.11	19.65	10.87	20.71	0.33
post-ensiled[e,f]	2.10	2.42	1.99	3.62	4.81	5.82	4.39	2.68	3.84	3.79	3.99	6.71	0.11
lactic acid, post-ensiled[e–i]	0.47	0.41	0.59	3.03	6.85	1.55	2.80	0.69	4.88	11.07	1.19	2.67	0.07
trimethylamine, mg/100 g													
pre-ensiled	8.57	7.86	7.65	7.32	7.18	7.29	7.11	7.48	7.19	7.16	7.23	7.11	0.18
post-ensiled[f,g,j]	20.55	14.97	18.84	16.84	8.37	14.44	15.15	15.16	12.08	8.11	15.52	14.85	0.56

[a] Crab waste and wheat straw (1:1, wet basis). [b] Phosphoric acid (5.4%). [c] Silage inoculant, *S. faecium* and *L. plantarum* (0.1%). [d] Standard error of means. [e] Acid effect ($p < 0.05$). [f] Molasses effect ($p < 0.05$). [g] Acid × molasses interaction ($p < 0.05$). [h] Percent of dry matter. [i] Inoculant × molasses interaction ($p < 0.05$). [j] Inoculant effect ($p < 0.05$).

Table III. Fermentation Characteristics of Crab Waste–Wheat Straw Mixtures, Small Silos, Experiment 2

	treatment[a]												
	10% molasses				15% molasses				20% molasses				
item	none	H_2O[b]	inoculant[c]	H_2O + inoculant	none	H_2O	inoculant	H_2O + inoculant	none	H_2O	inoculant	H_2O + inoculant	SE[d]
dry matter[j]	71.82	49.04	70.30	50.12	72.15	59.61	71.80	60.14	73.52	58.40	73.15	59.54	
pH													
pre-ensiled	7.55	7.25	7.32	7.29	7.24	7.19	7.22	7.23	7.13	7.21	7.12	7.22	0.17
post-ensiled[e–i]	6.97	6.75	6.81	6.65	6.77	5.74	6.78	5.49	6.17	5.35	6.41	5.17	0.06
water-soluble carbohydrates[j]													
pre-ensiled[e,h]	12.02	12.32	12.46	11.98	18.39	18.76	18.18	18.27	22.31	22.28	23.44	23.57	0.26
post-ensiled[e–i]	10.22	6.82	9.59	8.39	12.49	10.37	12.14	7.84	14.62	6.94	13.75	5.50	0.12
lactic acid post-ensiled[e–j]	0.94	3.04	1.17	4.46	0.88	4.46	1.27	5.13	0.80	6.97	1.05	11.06	0.11
volatile fatty acids													
acetic[e–l]	1.55	2.75	1.57	2.87	1.39	2.72	2.14	3.70	1.18	2.58	1.10	2.39	0.14
propionic[f,h–k]	0.06	0.11	0.06	0.13	0.05	0.07	0.12	0.08	0.05	0.11	0.05	0.11	0.01
isobutyric[e,f,h–k]	0.08	0.04	0.06	0.13	0.06	0.14	0.06	0.09	0.06	0.07	0.07	0.07	0.01
total[e–l]	1.69	2.90	1.69	3.13	1.50	2.93	2.32	3.87	1.29	2.76	1.22	2.57	0.14

[a] Crab waste and wheat straw (1:1, wet basis). [b] Water (20%). [c] Silage inoculant, *S. faecium* and *L. plantarum* (0.1%). [d] Standard error of means. [e] Molasses effect ($p < 0.05$). [f] Water effect ($p < 0.05$). [g] Inoculant effect ($p < 0.05$). [h] Molasses × water interaction ($p < 0.05$). [i] Molasses × inoculant interaction ($p < 0.05$). [j] Percent of dry matter. [k] Water × inoculant interaction ($p < 0.01$). [l] Quadratic effect of molasses ($p < 0.05$).

interaction, $p < 0.05$). Water addition increased lactic acid at all levels of molasses, but the absolute effect was greater at the higher levels of molasses (molasses × water interaction, $p < 0.05$). Molasses had little effect if water was not added. The effect of water may have been to permit more effective packing or to make the soluble carbohydrates more readily available to the bacteria. Adding inoculant usually decreased pH and increased lactic acid at all levels of molasses with or without added water; however, a molasses × inoculant interaction ($p < 0.05$) was obtained.

The predominant VFA in all mixtures was acetic acid (Table III). Addition of water to the mixtures increased ($p < 0.05$) acetic, propionic, and isobutyric acid concentrations and subsequently resulted in higher ($p < 0.05$) total VFA concentration. Adding microbial silage inoculant increased ($p < 0.05$) the levels of acetic acid and total VFA in the mixtures. Addition of molasses increased ($p < 0.01$) acetic acid, isobutyric acid, and total VFA. The absence of butyric acid in these silages is in contrast to the findings of Samuels et al. (1992), in which appreciable quantities were reported for the crab waste silages. Butyric acid is associated with "clostridial" type of fermentation and usually indicates the breakdown of amino acids. The absence of butyric acid in the present study agrees with the findings of Roa (1965) that free glucose represses the production of deaminating enzymes by putrefying microbes. Quadratic ($p < 0.05$) effects of level of molasses were observed for acetic acid and total VFA.

Table IV. Chemical Composition of Initial Mixtures of Crab Waste–Wheat Straw, Large Silos

	additives to mixtures[a]			
item	16% acetic acid	20% molasses	20% molasses + 0.1% inoculant[b]	wheat straw
dry matter, %	49.30	60.82	60.58	53.90
crude protein[c]	12.04	12.68	12.74	3.92
neutral detergent fiber[c]	60.04	55.74	54.84	86.72
acid detergent fiber[c]	43.08	40.54	41.98	58.12
cellulose[c]	33.02	31.39	33.21	46.50
hemicellulose[c]	16.96	15.20	12.86	28.60
lignin[c]	8.53	8.32	8.15	10.40
ash[c]	20.28	15.89	15.82	5.57

[a] Crab waste and wheat straw (1:1, wet basis). [b] *S. faecium* and *L. plantarum*. [c] Percent of dry matter.

Large Silo Study. The crude protein content of the crab waste–straw silage mixtures averaged between 12 and 13%, DM basis, while that of the wheat straw averaged 3.9% (Table IV). Fiber components were higher for the wheat straw silage, compared to the crab waste–straw silages, a reflection of the low fiber of the crab waste and additives. Ash content was higher in the crab waste mixtures.

Table V. Fermentation Characteristics of Crab Waste–Wheat Straw Silages, Large Silos

item	additives to mixtures[a]				
	16% acetic acid	20% molasses	20% molasses + 0.1% inoculant[b]	wheat straw	SE[c]
pH					
pre-ensiled[d]	4.35	6.97	7.02	7.05	0.03
post-ensiled[d,e]	4.36	5.28	4.70	4.75	0.04
water-soluble carbohydrates[f]					
pre-ensiled[d,g]	6.42	26.82	27.12	4.04	0.22
post-ensiled[e,g]	5.47	6.42	5.12	1.28	0.10
lactic acid,[f] post-ensiled[d,e,g]	0.55	10.84	12.76	2.46	0.16
volatile fatty acids[f]	34.68	3.65	1.78	1.49	0.47
acetic[d,e,g]	34.68	3.65	1.78	1.49	0.47
propionic[d,g]	0.07	0.18	0.16	0.25	0.02
isobutyric[d,g]	0.02	0.03	0.03	0.00	0.02
butyric[g]	0.00	0.00	0.00	0.04	0.00
total[d,e,g]	34.77	3.86	1.97	1.78	0.47
trimethylamine, mg/100 g					
pre-ensiled[g]	7.82	7.78	7.80	0.00	0.08
post-ensiled[g]	8.12	8.14	7.96	0.00	0.07

[a] Crab waste and wheat straw (1:1, wet basis). [b] *S. faecium* and *L. plantarum*. [c] Standard error of means. [d] Acetic acid and molasses treatments differ ($p < 0.05$). [e] Molasses and inoculant treatments differ ($p < 0.05$). [f] Percent of dry matter. [g] Wheat straw and crab waste silages differ ($p < 0.05$).

The initial pH values were lower ($p < 0.05$) for the acetic acid treated silage (Table V), a reflection of added acetic acid. After ensiling, there was no apparent change in pH for the acetic acid treated silage. Other silages showed marked decreases in pH and were characterized by a pleasant aroma. The molasses/inoculant-treated silage had a lower ($p < 0.05$) post-ensiled pH, compared to molasses alone (4.70 vs 5.28). There was a high disappearance of WSC in the crab waste–straw silages (from about 27 to 6%), suggesting a high rate of fermentation in these mixtures. Addition of microbial silage inoculant resulted in the production of higher ($p < 0.05$) amounts of lactic acid, compared to molasses alone. Lactic acid concentration was higher ($p < 0.05$) for the crab waste–straw silages, compared to ensiled wheat straw silage. However, the higher concentration of lactic acid was observed only for mixtures with added molasses.

The predominant VFA, as in the small silos, was acetic acid, which was higher ($p < 0.05$) in the acetic acid treated silages, a result of the acid addition. The concentration of acetic acid was higher ($p < 0.05$) in the 20% molasses mixture, compared to the molasses/inoculant mixture (3.65 vs 1.78%). This observation suggests that inoculation may be beneficial in directing fermentation to lactic acid formation. Propionic acid was higher ($p < 0.05$) in the wheat straw, compared to the crab waste–straw silages, although all values were low. Isobutyric acid was detected only in the crab waste–straw silages and butyric acid only in the wheat straw silage.

There were no significant differences ($p > 0.05$) in TMA among the crab waste–straw silages. The concentrations remained about the same after ensiling (averaging 8 mg/100 g) and are in the range for acceptable smell.

Conclusion. The results of this study show that crab waste and wheat straw mixtures can be ensiled with the addition of molasses. Addition of 20% molasses appears to be the optimum level that is required to promote fermentation as well as for the development of low pH, the inhibition TMA formation, and, consequently, the elimination of offensive odor. Moisture is critical in the ensiling process, probably due to the fact that it allows for better packing of the mixtures and solubilization of the fermentable sugars. Although acetic acid was used successfully to stabilize crab waste–straw mixtures, the economic feasibility of using acetic acid is questionable. However, crab waste is produced at the coastal areas where the price of molasses is generally low. It appears that the low price of molasses and its enhancement of palatability of the mixtures should be an advantage over the use of acetic acid. The ensiled crab waste mixtures with added molasses have potential for use as feed for ruminants. Consequently, feeding trials should be conducted to establish the level at which these mixtures can be fed without adversely affecting animal performance.

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