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Fermentative profile of corn silages with the inclusion of chemical additives or bacterial inoculant

[Perfil fermentativo de silagens de milho com a inclusão de aditivos químicos ou inoculante bacteriano]

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ABSTRACT

The objective was to evaluate the inclusion of chemical additives or bacterial inoculant in corn silage. The experimental design was completely randomized with five treatments: silage without additive; silage added with urea (3.0%); silage with limestone (3.0%); silage added with crystal sugar (3.0%); and silage with a bacterial inoculant. Five of them were opened during the fermentation process (15 days) to measure pH, and three were opened 60 days after ensiling to evaluate the ammonia nitrogen and organic acids content. For the pH at the end of the fermentation process, higher values were found for the limestone additive, followed by the silage with urea, which had higher participation of lactic acid, 19.06 ppm for limestone and 18.95 ppm for urea. Higher concentrations of acetic acid were observed in inoculant silages (18.49ppm) or silage without additive (18.46ppm). The ammonia nitrogen content was higher in the silage with urea (23.74mg dL-1), followed by the silage without additive (7.54mg dL-1), which also had the highest concentration of butyric acid (4.19ppm). The use of additives reduced the concentration of butyric acid in the silage. The bacterial inoculant was the most efficient in decreasing the pH of the ensiled material.

Keywords: lactic acid, acetic acid, butyric acid, ammoniacal nitrogen, dry matter recovery

RESUMO

Objetivou-se avaliar a inclusão de aditivos químicos ou inoculante bacteriano na silagem de milho. O delineamento experimental foi inteiramente ao acaso, com cinco tratamentos: silagem sem aditivo; silagem aditivada com ureia (3,0%); silagem aditivada com calcário (3,0%); silagem aditivada com açúcar cristal (3,0%); e silagem com inoculante bacteriano. Cinco sacos de silagem foram abertos durante o processo fermentativo (15 dias) para mensuração de pH, e três 60 dias após a ensilagem, para avaliação do teor de nitrogênio amoniacal e de ácidos orgânicos. Para pH no final do processo fermentativo, maiores valores foram verificados para o aditivo calcário, seguido da silagem com ureia, os quais apresentaram maior participação de ácido lático, 19,06 ppm para calcário e 18,95 ppm para ureia. Maiores concentrações de ácido acético foram observadas nas silagems inoculante (18,49 ppm) ou sem aditivo (18,46 ppm). O teor de nitrogênio amoniacal foi superior na silagem com ureia (23,74mg dL⁻¹), seguida pela silagem sem aditivo (7,54mg dL⁻¹), que também apresentou maior concentração de ácido butírico na silagem. O inoculante bacteriano foi mais eficiente que aditivos químicos no declínio do pH do material ensilado.

Palavras-chave: ácido lático, ácido acético, ácido butírico, nitrogênio amoniacal, recuperação de matéria seca

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INTRODUCTION

The use of preserved forages, especially corn silage, is a practice present in many properties to supply the lack of food in critical periods for ruminants. However, several factors can influence the fermentation process and impact the quality of this food. According to Muck et al. (2018), the main challenge of ensilage is to conserve forage through a fermentation process that results in high nutritional and microbiological quality, minimizing fermentative losses.

Failures in the fermentation process can promote losses of dry matter and nutritional principles and low aerobic stability. In addition, low-quality silage will be less acceptable to the animal, with reduced consumption and, consequently, lower animal performance and net farm profit (Kung Jr., 2018). In this way, various additives can be applied at the beginning of the ensiling process to ensure that fermentation occurs appropriately, reducing losses and providing adequate conservation conditions (Muck *et al.* 2018). A range of products or substances can be used as additives; however, there is a lack of studies on their efficiency.

In this context, the search for alternatives that can improve the fermentation process and the conservation of silage, such as bacterial inoculants or chemical additives, must be constant. Therefore, studies are needed to verify and quantify the efficiency of these products in the conservation of silage characteristics. To contribute to reducing the lack of research results related to the use of additives in corn silage, the objective of this study was to evaluate the fermentation process and dry matter losses of corn silage made with different additives.

MATERIAL AND METHODS

The present study was carried out by the Study and Research Group in Animal Health, Production and Reproduction (GPqPRA) in the experimental area of the Federal University of Fronteira Sul (UFFS), Realeza campus. The municipality of Realeza is in the Southwest region of Paraná, Brazil, at an altitude of 520 m, 25° 46' South latitude, and 53° 31' West longitude. The prevailing climate in the region, according to the Köppen classification, is humid subtropical (Cfa). The summers are hot, with temperatures above 22°C, and during winters, the temperatures vary from -3 to 18°C (Alvares et al., 2013). The soil is characterized as a typical Dystroferric Red Latosol with a clayey texture (Bognola, et al., 2011).

The area used for planting corn was 50.0 meters long by 6.0 meters wide (50.0m x 6.0m), totaling $300.0m^2$. Prior to implementing the experiment, physical and chemical analysis of the soil was carried out. According to the granulometric analysis performed, the soil of the experimental area was classified as type 3, very clayey, with a percentage of 17.50% sand, 17.50% silt, and 65% clay. For chemical analysis, soil collection was from 0-20 cm and the results obtained are described in Table 1.

Table 1. Soil chemical characteristics of the UFFS experimental area, Realeza

Soil Analysis										
Phosphorus (mg dm ⁻³)	Organic matter (g dm ⁻³)		Sortive Complex							
		pH (CaCl ₂)	H+A1	K	Ca	Mg	SB	CEC	Al	V
			cmol _c dm ⁻³ %						%	
11.25	26.23	4.70	7.20	0.49	3.66	1.58	5.73	12.93	2.86	44.32

Source: The author, 2022

The experiment was carried out from September 2019 to March 2021, with two years of data collection. During the first year of evaluation, the corn crop planting was carried out on September 30th, and before its establishment, the area was plowed and 3.0t. ha⁻¹ of limestone was applied.

Later, it was harrowed for breaking up, leveling the soil, and eliminating weeds. In the second year of evaluation, the implantation of the crop took place on November 6th. The planting was carried out with a seeding density of 56,800 plants/hectare, with a spacing between rows of 45cm, carried out with a tractor-coupled hydraulic seeder. After planting, the area was fertilized with 10t ha⁻¹ of chicken litter. A fertilizer sample was collected and sent to the laboratory to determine NPK (nitrogen, phosphorus, and potassium). The poultry litter was characterized by 17.77 (g kg⁻¹) of nitrogen, 25.13 (g kg⁻¹) of phosphorus, and 9.85% (g kg⁻¹) of potassium.

Genetically modified corn hybrid seeds were not used for planting. Weed control was carried out manually using brush cutters and manual weeding. For the control of fall armyworm and insects, *Bacillus thuringiensis* (concentration of $32g \text{ kg}^{-1}$) in the proportion of 500g ha⁻¹ and Azadirachtin (concentration of 2.4g L⁻¹) in the proportion of 500mL ha⁻¹ were used, both applied in the quantity of 150L of spray per hectare. Two applications were carried out in each year of cultivation, approximately 30 and 60 days after planting. Chemical herbicides, insecticides, and fungicides were not applied. Therefore, the cultivation was carried out organically.

Harvest was carried out when the plants reached the point for silage, with the grains in the farinaceous stage (100 and 98 days after planting in the first and second year, respectively). All plants were harvested manually, at the height of 25cm from the ground level, and fragmented in a forage harvester coupled to the tractor. Samples of the crushed material were collected, stored in paper bags, weighed, and placed in a forced-air oven at 55°C until constant weight in view to estimate the dry matter content of the material at the time of ensiling. At the time of ensilage closing, the crushed material presented an average value of 34.65% of dry matter (DM).

Subsequently, the crushed material was stored in silage bags using a silage packaging and compactor machine. The silage bags were 200 microns thick and were compacted with a density equivalent to 550kg m⁻³, similar to the compaction density in trench or surface silos, with about 12kg of material per bag. Also, they were hermetically sealed utilizing plastic seals.

Different additives were added to the chopped material during the packaging process, namely: urea, crystal sugar, limestone, microbial additive, or without additive. The additives urea, commercial crystal sugar, and limestone were incorporated in the proportion of 3.0% of the green material weight to be ensiled. The bacterial inoculant used was the commercial inoculant Total Silo[®], which had the following composition, according to the manufacturer's instructions: lactic acid bacteria Lactobacillus plantarum (homofermentative). Lactobacillus buchneri (heterofermentative). Pediococcus acidipropionici (homofermentative), Pediococcus acidilactici (homofermentative) at a concentration of 1.0x10⁹ CFU mL⁻¹. The inoculant was incorporated in the proportion of 1.0 liter of the additive diluted in 100 liters of water per ton of green material for silage. For each additive evaluated, eight silage bags were produced, of which three were weighed to obtain the weight of the silage bags at closing.

At the time of making the silage, the pH of the was evaluated. crushed material The methodology described by Silva and Queiroz (2006) was used to measure the pH, consisting of the dilution of 9.0 grams of the fresh sample in 60mL of distilled water. The pH was read after the sample rested for 30 minutes using a digital potentiometer (Peagameter). The pH readings were retaken at 24 hours (day 1), 96 hours (day 4), 168 hours (day 7), 240 hours (day 10), and 336 hours (day 14) after silage preparation. For each day of pH evaluation, a new silage bag not yet opened for each evaluated additive was used.

After storing the silages for about 60 days, the three bags were weighed again to calculate the dry matter recovery index (DMR), obtained by the method proposed by Jobim *et al.* (2007), using the following equation:

DMR (%) = $(fFM \times fDM)/(iFM \times iDM) \times 100$

Where: DMR – dry matter recovery rate (%); fFM – forage mass at opening (kg); fDM – dry matter content of forage at opening (% DM); iFM – forage mass at closing (kg); iDM – dry matter content of forage at closing (% DM).

After weighing, three silage bags were opened per treatment, and the contents of the end and sides of the bags were discarded. Part of the collected silage was subjected to pressing utilizing a mechanical press to extract the silage juice, which was previously filtered with the aid of gauze. 9.0 mL of the total collected juice was taken, and 1.0 mL of 20% sulfuric acid was added to it. Then, the sample was frozen at -18° C. Later, according to the methodology of Silva and Queiroz (2006), the ammonia nitrogen content was determined (N-NH₃) by distillation with magnesium oxide and calcium chloride, using boric acid receptor solution and titration with 0.1 N hydrochloric acid.

Another part of the juice collected, about 8.0 mL, was placed in recipients containing 2.0mL of the 20% metaphosphoric acid solution and frozen at -18°C to evaluate the levels of organic acids (lactic, acetic, propionic, and butyric acids) by gas chromatography. The silage juice samples with the metaphosphoric acid solution were centrifuged for 10 minutes at 5,000 rpm to decanting possible sediments present in the sample. After that, the samples were filtered through a Pes 0.45 µm membrane syringe filter, placed in 2.0mL Vials flasks, and later taken to the analysis center of the Foundation to Support Education, Research, and Scientific and Technological Development at UTFPR, Pato Branco campus.

The experimental design used was completely randomized. Data were subjected to analysis of variance by the following mathematical model:

 $Yij = \mu + A_i + T_j + \epsilon ij$

Where: Yij represents the dependent variables; μ is the overall mean of the observations; There is the effect of the year of evaluation (used as a covariate); Tj is the effect of the additive used; and ϵ ij is the random residual error.

Data were submitted to analysis of variance using the "F" test (ANOVA). When significance was observed in the evaluated parameter, the means were compared using the Student "t" test, with $\alpha = 0.05$. For the orthogonal contrasts analysis, the F test was Applied considering $\alpha =$ 0.05, in which the comparison of the silage without additive was carried out in relation to the silages with the inclusion of additives (bacterial inoculant, sugar, limestone, or urea). The orthogonal contrast of bacterial inoculant use versus the other chemical additives evaluated was also carried out (sugar, limestone, or urea). Analyzes were performed using the SAS statistical program version 9.2 (SAS, 2009).

RESULTS AND DISCUSSION

At the time of ensiling, the ground material had a pH of 5.93, and during the fermentation process, it decreased, as expected. There was an effect (P<0.05) of the additives on the pH values in the corn silage during the 14 days of the fermentation process after closing the silage bags (Table 2).

Table 2. Hydrogen potential	(pH) of corn silages p	oroduced with the addition of different additives
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	Additive					_	P-value		
Variables	Sugar	Limestone	Bacterial inoculant	No additive	Urea	Standard error	Additives	Contrast	
								No additive	Bacterial inoculant
								х	х
								additives	Additives
pH, day 0	5.93	5.93	5.93	5.93	5.93	0.01	1.0000	1.0000	1.0000
pH, day 1	4.15 d	5.00 a	4.20 d	4.42 c	4.67 b	0.05	< 0.0001	0.1944	< 0.0001
pH, day 4	3.79 c	4.11 a	3.78 c	3.82 c	4.27 b	0.02	< 0.0001	< 0.0001	< 0.0001
pH, day 7	3.78 c	5.07 a	3.85 c	3.79 c	4.06 b	0.11	< 0.0001	0.0028	0.0012
pH, day 10	3.77 c	4.23 a	3.85 c	3.81 c	4.10 b	0.04	< 0.0001	0.0009	0.0008
pH, day 14	3.82 b	4.59 a	3.85 b	3.81 b	4.42 a	0.06	< 0.0001	< 0.0001	< 0.0001

^{a, b, c} Means followed by different letters on the line differ with P<0.05 by Student's "t" test

On the first day after ensiling, the lowest pH values were obtained in silages with sugar (4.15) or bacterial inoculant (4.20). Therefore, we can affirm that both additives provided conditions for the rapid establishment of homolactic bacteria, intensifying lactic acid production. It is assumed that commercial crystal sugar stimulated the growth of lactic acid bacteria by increasing sucrose availability. Meanwhile, the inoculant

provided an increase in the population of desirable bacteria in the fermentation process, triggering a rapid pH decline in the initial phase of the fermentation process.

During the period evaluated, the highest pH values were verified for the limestone additive followed by the silage additive with urea, which is related to the characteristics of these products

since they are alkaline and have a buffering action. According to Nascimento et al. (2016), both are classified as basic chemical additives and tend to influence the decrease in pH. According to Santos et al. (2008), when alkaline additives dissociate into atoms, they produce ionic charges that neutralize hydrogen ions derived from organic acid produced during silage fermentation. It confers resistance to pH reduction, thus requiring more time for the pH to drop and the silage to stabilize. In the case of silages with urea additives, higher pH values may be related to the fact that urea is transformed into ammonia, which in the ensiling process prevents the decline in pH of the ensiled mass and alters the course of fermentation (Kung Jr. et al., 2003).

After 14 days of fermentation, the silages treated with urea and limestone differed statistically (P<0.05) from the others, with pH values higher than those recommended by Kung Jr. et al. (2018) for corn silage, varying from 3.7 to 4.0. Despite the pH values above the standard range recommended by the literature, its quality was not affected, as there were high concentrations of lactic acid and low concentrations of butyric acid for the silages with these additives (Table 3). Considering that the butyric acid-producing microorganisms develop in environments with higher pH and that the butyric content was low, we can deduce that there was no significant population increase of these bacteria in this pH range. According to Henderson (1993), depending on the type of additive and/or chemical treatment added to the silage material, its pH can be higher than 4.0 without altering the quality of the resulting material.

By contrast analysis, the pH averages of the silage without additive did not differ from the silages with additive only on the first day of the fermentation process. On the other evaluation days, it was verified that the silage without additive had a lower pH value, which can be explained by the fact that the inclusion of additives results in modifying the fermentation process. In this way, buffering products such as urea and limestone cause a higher final silage pH, while the bacterial inoculant maintains higher pH values since it provides greater acetic acid production, which is considered a weak acid. Also, although sugar provides a rapid decline in pH in the initial phase, there is less production of organic acids in the fermentation process (Table 3). It may justify the increase in pH averages during the final fermentation phase once the quantity of organic acids produced was insufficient to maintain acidity stability. In the contrast analysis of bacterial inoculant versus other additives (sugar, limestone, or urea), there was a significant effect in all evaluations during the fermentation process (P < 0.05), in which it is verified that the inoculant was more efficient in declining the pH than the chemical additives used. This result is related to the microbiological composition of the inoculant, which presents homofermentative bacteria and acts quickly in the initial phase of the fermentation process as lactic acid is produced. Furthermore, according to the contrasts analysis, the use of bacterial inoculant provided a higher concentration of acetic acid (Table 3) than chemical additives, which promoted a greater decline in silage pH.

Concerning organic acids, the highest participation of lactic acid was observed in silages with the addition of limestone (19.06ppm) or urea (18.95 ppm), while the lowest concentration (9.48 ppm) was for the silage produced with sugar (Table 3). In contrast, silages without additive or with bacterial inoculant showed intermediate values. A high concentration of lactic acid reduces pH and positively affects silage by inhibiting the growth and activity of undesirable bacteria. In Other words, lactic acid is the main responsible for the pH decline (Muck et al., 2018). However, in this research, the high buffering capacity of urea and limestone additives prevented the reduction of the pH of these silages, although they present high concentrations of lactic acid.

Regarding acetic acid production, its highest concentration was observed in silages with the addition of bacterial inoculant (18.49ppm) or without the use of additive (18.46ppm). The high concentration of acetic acid demonstrates the activity of heterofermentative lactic acid bacteria. It is related to increased aerobic stability, as this acid acts as an inhibitor of spoilage organisms (yeasts and filamentous fungi) (Kung Jr. *et al.*, 2018).

			Additive			P-value			
		Limestone	Bacterial inoculant	No additive	Urea	Standard error		Contrast	
Variables	Sugar						Additives	No	Bacterial
								Additive	inoculant
								X	X
								Additives	Additives
Lactic acid (ppm)	9.48 b	19.06 a	14.71 ab	14.82 ab	18.95 a	2.11	0.0139	0.7913	0.6472
Acetic acid (ppm)	7.29 b	8.34 b	18.49 a	18.46 a	7.30 b	2.91	0.0053	0.0161	0.0022
Propionic acid (ppm)	11.74	13.55	15.37	20.73	14.93	2.86	0.2508	0.0371	0.5545
Butyric acid (ppm)	2.17 b	1.38 b	2.04 b	4.19 a	0.91 b	0.57	0.0025	0.0002	0.4073
Ammonia									
Nitrogen	2.83 c	4.83 c	4.46 c	7.54 b	23.74 a	0.72	< 0.0001	0.0813	< 0.0001
(mg/ dL)									
Dry matter	86.50	80.47	84.29	83.86	80.29	176	0.0987	0.6284	0.3689
recovery (%)						1.70			
recovery (%)	86.50	80.47	84.29	83.86	80.29	1.76	0.0987	0.6284	0.3689

Table 3. Concentrations of organic acids (ppm), ammoniacal nitrogen (mg dL⁻¹), and dry matter recovery of corn silages with the addition of different additives

^{a, b, c} Means followed by different letters on the line differ with P<0.05 by Student's "t" test

 $ppm = parts per million; mg dL^{-1} = milligrams per deciliter.$

It is noteworthy that the inoculant used in this study contained Lactobacillus buchneri, a heterofermentative bacteria, which, according to Muck et al. (2018), can ferment lactic acid to acetic acid, which helps to explain high concentrations of acetate in these silages. An increase in acetic acid concentration was also observed by Ranjit et al. (2002). The authors affirm that adding L. buchneri in corn silage decreased its lactic acid concentration while it increased the acetic acid concentration, as well as significantly reduced the number of yeasts present in it. However, in heterofermentative fermentation, there is a tendency for greater loss of DM since for each molecule of acetic acid formed, an equivalent molecule of carbon dioxide is generated (Silva et al., 2017). Thus, there could be considerable dry matter loss with heterofermentative fermentation for treatments without additive and bacterial inoculant. Nevertheless, observing the DM recovery rates (Table 3), this effect was not verified.

The lowest concentration of acetic acid was observed in silages with sugar (7.29ppm), urea (7.30ppm), and limestone (8.34ppm). Baytok *et al.* (2005) observed that the addition of molasses, a source of sugar, in corn silage decreased acetic acid levels. Santos *et al.* (2018) found that 0.5% or 1.0% urea levels exert an inhibitory effect on acetic acid-producing microorganisms, as there was low production of it in sorghum silages, indicating that urea favors the reduction of acetic and heterofermentative bacteria action in the

fermentation process. We can infer from data obtained in this research that the additives urea, limestone, and sugar impacted the growth of these organisms. After opening the silages with these additives, they will be more prone to deterioration due to the low concentration of acetic acid.

As for the butyric acid content, there was a statistical difference (P<0.05) between the treatments. In silages that received additives, butyric acid concentrations were significantly lower than those without additives. In other words, the higher concentration of butyric acid obtained in the silage without additive may signal greater action of unwanted microorganisms in these silages, highlighting the development of species of the genus Clostridium spp. The activity of these microorganisms is undesirable for several reasons, such as the loss of acceptability and reduced forage consumption. According to Ávila and Carvalho (2020), the Clostridium tyrobutiricum group is the main species responsible for butyric fermentation in silage, and they can grow in environments with lower pH values (< 4.30).

Considering the contrast between silages with additives and silages without additive, a lower concentration of butyric acid (P<0.05) was observed when adding additives, which indicates that they decreased its concentration. Reductions ranged from 51% for silage with added sugar and up to 78% with the addition of urea. Among the

additives, urea promoted lower levels of butyric acid (0.91ppm). This result agrees with the studies by Santos *et al.* (2021), who found a lower concentration in corn silage with a higher share of urea (0.29g kg⁻¹ DM) and a higher concentration of butyric acid in silage without urea (0.39g kg⁻¹ DM).

According to the results obtained, the concentration of propionic acid did not differ (P>0.05) between the evaluated additives, with values between 20.77 ppm (silage without additive) and 11.74 ppm (silage with sugar), indicating that the amount of propionic acid produced by silages was higher than butyric acid concentrations. The recommendation is that silages have reduced concentrations of this acid, and according to Kung Jr. *et al.* (2018), it is generally undetectable (especially in drier silages) or at very low concentrations (<0.1%) in good silages.

Ammoniacal nitrogen (N-NH₃) levels were respectively 23.74mg dL⁻¹ and 7.54mg dL⁻¹ for silages produced with urea or without additive. The addition of limestone, bacterial inoculant, and sugar resulted in silages with lower N-NH₃ values, with means of 4.83; 4.46, and 2.83mg dL , respectively. The addition of urea in the silage promoted a significant increase in N-NH₃ levels, which can be explained by the inclusion of an ammonia source. The results found by Santos et al. (2018) validate this justification, as these authors observed a linear increase in ammoniacal nitrogen values as they raised the levels of urea addition in sorghum silages. However, the ammonia nitrogen content alone is not enough to assess the quality of urea-treated silages since they tend to have higher concentrations due to the availability of non-protein nitrogen. It means that the values found in silages with urea, 23.74 mg dL⁻¹, cannot be used as the only indication to classify the silage as unsatisfactory.

The dry matter recovery rates (DMR) found in this study, with average values from 80.29 to 86.50%, were not influenced (P>0.05) by the addition of additives (Table 3). Santos *et al.* (2020) found a dry matter recovery of 96.14%, 95.67%, and 95.15% for corn silages treated with urea, activated inoculant, and activated inoculant with urea, respectively. The authors associated the high DMR values with the effect of the antimicrobial action of urea, which reduces the development of yeasts in the ensiled mass, and with the greater number of lactic acid bacteria populations present in the activated inoculant. However, this action by urea was not observed in this study. On the contrary, the silage with urea had a DM recovery value of 80.29%.

According to Kung Jr. (2018), in the fermentation process, the energy losses occur depending on the type of fermentation that predominates. For example, heterolactic glucose fermentation has a theoretical DM recovery of 76%, while homolactic fermentation results in a theoretical DM recovery of 100%. Thus, it can be mentioned that the lower averages of DMR may be related to the occurrence of heterolactic fermentation, with higher productions of acetic, propionic, and other acids. However, lower levels of RMS impact the chemical composition of the silage. According to Carvalho et al. (2014), dry matter losses directly influence the nutritional quality of the silage, as they proportionally increase the fibrous constituents and, consequently, reduce the dry matter digestibility.

CONCLUSION

The use of chemical or microbiological additives in corn silage alters silage fermentation process and reduces butyric acid production. The use of limestone or urea in corn silage demonstrates a buffering effect, delays pH decline, and increases lactic acid concentrations, while adding crystal sugar reduces the content of organic acids in silage.

The addition of bacterial inoculant is more efficient in the pH decline during the silage fermentation process and increases the concentration of acetic acid in relation to chemical additives.

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