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The effects of temperature on the aerobic stability of wheat and corn silages

G Ashbell[1], ZG Weinberg[1], Y Hen[1] and I Filya[2]

[1]*Forage Preservation and By-Products Research Unit, The Volcani Center, Bet Dagan 50250, Israel;* [2]*Department of Animal Science, Faculty of Agriculture, Uludag University, Bursa 16384, Turkey*

The aim of this work was to study the effects of temperature on the aerobic stability of wheat and corn silages. Three silage samples from each crop were taken from the faces of six different commercial bunker silos immediately after unloading them. The samples were exposed to air for 3 or 6 days at 10, 20, 30 or 40ºC. The most intensive deterioration occurred at 30ºC. Samples incubated at 30ºC had the highest yeast counts, most prolific CO_2 production and greatest increases in pH. Silage samples exposed to 10 or 40ºC remained stable. The duration of exposure had a significant effect on aerobic stability, especially at 30ºC. Temperature has a significant effect on silage aerobic stability. In a warm climate, special care should be taken during unloading of silage in order to prevent intensive aerobic deterioration.
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Introduction

Ensiling is a conservation method for moist crops. It is based on natural fermentation under anaerobic conditions whereby epiphytic lactic acid bacteria (LAB) convert water-soluble carbohydrates (WSCs) into organic acids, mainly lactic acid (LA). As a result, the pH decreases and the forage is preserved. Air is detrimental to the ensiling process as it permits the activity of aerobic spoilage microorganisms [18]. Silage is often exposed to air when it is still in the silo because sealing is not hermetic, and spoilage might start as early as this stage [7,14]. During unloading, silage is normally fully exposed to air, which could result in an increase in temperature and spoilage (aerobic deterioration). The stability of silage in the presence of oxygen is a very important factor in determining its quality and value.

Many factors affect the stability (or sensitivity) of the silage while it is exposed to air. Microorganisms can be categorized, according to their ability to grow at low, moderate or high temperature, as psychrophilic, mesophilic and thermophilic. The deterioration process originates through the activities of aerobic microorganisms, mainly yeasts and moulds; therefore, factors that affect these microorganisms may affect aerobic stability of the silage. The principal factors that influence aerobic stability of silage are air, substrate availability and temperature. They are closely interdependent [13]. It is possible to improve the aerobic stability of silage by increasing compaction density, better sealing and efficient fermentation. Higher density and sealing can be achieved mainly by good technical management [12]. Improved aerobic stability of silage has been obtained by inoculation with *Lactobacillus buchneri* at ensiling. The high levels of acetic acid that it produces inhibit fungi [4,5,8,15].

Many mathematical models were developed to predict the time course of aerobic deterioration of silages (e.g., [3,17]). Such models take into account the temperature rise at the silage face due to the activity of aerobic microorganisms, but not the effect of the ambient temperature of the silage during storage and feedout. Previous studies [9,16] showed that the ensiling fermentation was affected by temperature. In the feedout phase, the silage face is exposed to ambient conditions, which may affect its stability. Temperatures above 10°C and oxygen are the main factors involved, and both have negative effects on silage stability. The objective of our present study was to evaluate the effects of temperature on wheat and corn silage deterioration intensity under aerobic conditions.

Materials and methods

Wheat and corn silage samples (of good farm quality) were taken from six different commercial bunker silos, where no additives were used at ensiling. Silages were taken from the middle of the face immediately after feedout to ensure that the best quality of material was obtained. Aerobic stability testing was carried out under laboratory conditions according to Ashbell *et al* [1]. The bottles were incubated in triplicate at 10, 20, 30, or 40°C for 3 or

Table 1 Chemical and microbiological composition of the corn and the wheat silages before exposure to air

Crop	Experiment	DM	pH	WSC	LA	AA	LAB	Yeasts
Corn	1	368	3.94	0	40	32	6.6	5.0
	2	369	4.08	0	42	20	5.2	4.5
	3	346	3.77	22	41	23	7.0	6.9
Wheat	1	376	4.03	32	52	36	7.6	5.2
	2	351	3.98	32	52	21	7.0	3.4
	3	342	3.84	21	52	25	6.3	2.2

Chemical results are in grams per kilogram DM, and microbiological results are in $\log_{10}$ of colony-forming units per gram DM.
DM, dry matter; WSC, water-soluble carbohydrate; LA, lactic acid; AA, acetic acid; LAB, lactic acid bacteria.
All silage samples contained 3.3–5.6 g kg^{-1} ethanol; the wheat silages from Experiments 1 and 3 contained also 3.3 g kg^{-1} DM butyric acid, and from Experiment 1, 4.0 g kg^{-1} DM propionic acid.
Molds were found only in the first corn silage at 6.1 $\log_{10}$ CFU g^{-1} DM.

Correspondence: Dr G Ashbell, Forage Preservation and By-Products Research Unit, The Volcani Center, Bet Dagan 50250, Israel
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Table 2 Chemical and microbiological results from corn silage exposed at different temperatures (3 experiments)

Time (days)	Temperature (°C)	pH			CO_2 (g kg^{-1} DM)			Yeasts			Molds			LA			Acetic acid		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3	10	4.0^{b}	4.1^{b}	4.0^{b}	3.1^{b}	0^{a}	2.2^{b}	5.5	NF	6.8	NF	NF	NF	35.4^{a}	–	–	12.0^{a}	–	–
	20	4.0^{b}	4.2^{b}	5.5^{a}	4.7^{b}	17.7^{a}	25.3^{b}	7.9	7.8	9.9	NF	NF	NF	29.5^{b}	46.9	50.3	9.8^{b}	12.0^{a}	1.6
	30	4.4^{a}	4.7^{a}	6.0^{a}	19.0^{a}	14.3^{a}	58.1^{a}	7.8	9.0	8.9	NF	NF	NF	17.9^{c}	35.7	45.2	4.3^{c}	4.4^{b}	1.2
	40	4.0^{b}	4.3^{ab}	4.0^{b}	3.4^{b}	0^{a}	5.0^{b}	6.0	6.6	3.5	NF	NF	NF	27.3^{b}	43.1	–	11.1^{a,b}	14.4^{a}	–
6	10	4.1^{b}	4.0^{c}	3.9^{b}	0^{b}	0^{b}	3.6^{c}	7.3	4.8	7.9	NF	2.1	6.9	38.0^{a}	33.0^{a}	34.3^{a}	16.4^{a}	20.0^{a}	21.1^{a}
	20	5.9^{a}	5.5^{b}	6.7^{a}	24.1^{a}	25.2^{a,b}	59.1^{a,b}	8.0	8.6	8.4	8.4	5.9	NF	2.0^{b}	20.0^{b}	2.0^{b}	1.0^{b}	2.5^{b}	1.5^{b}
	30	5.5^{a}	7.7^{a}	6.5^{a}	35.2^{a}	56.5^{a}	89.7^{a}	8.2	8.3	8.8	7.5	6.4	6.9	5.0^{b}	6.7^{c}	0^{a}	0^{b}	0^{b}	5.6^{b}
	40	4.0^{b}	4.2^{c}	3.9^{b}	1.0^{b}	15.3^{b}	15.4^{b,c}	6.1	5.5	3.9	8.0	3.1	3.3	39.0^{a}	39.3^{a}	27.3^{a}	15.6^{a}	17.7^{a}	16.9^{a}

Chemical results are in grams per kilogram DM and microbiological results in $\log_{10}$ of colony-forming units per gram DM. (–) not measured.
^{a,b,c}Within a column and day, means followed by different letters differ significantly ($P<0.05$).
In addition, ethanol and propionic and butyric acids were detected in some samples at levels of 0–3 , 0–1 and 0–8 g kg^{-1} in DM, respectively.

6 days. The system was constructed in two parts from recycled soft drink bottles (polyethylene terepthalate): the upper part (1 l) was filled with *ca.* 250 g (wet weight) of loosely packed silage, and the lower part with 100 ml of 20% KOH. Gas was exchanged through 1-cm holes in the upper part. Carbon dioxide produced during aerobic exposure was absorbed in the base and determined by titration with 1 N HCl. In addition, change in pH, yeast and mould counts, and visual appraisal also served as indicators of aerobic spoilage. Chemical and microbiological analyses were carried out on the silage samples, initially and after 3 or 6 days exposure to air.

Analytical procedure

Chemical analyses were performed on triplicate samples. Dry matter was determined by oven drying for 48 h at 60°C. WSCs were determined by the phenol sulphuric acid method, according to the Dubois *et al* [6]. LA was determined by a spectrophotometric method, according to Baker and Summerson [2]. Volatile fatty acids were determined with a gas chromatograph with a semi-capillary FFAP column (Hewlett Packard, Waldbronn, Germany) over a temperature range of 45–230°C. The microbiological evaluation included enumeration of lactobacilli (on pour plates of Rogosa agar; Oxoid CM627), and yeasts and moulds (on spread plates on malt extract agar acidified with LA to pH 4.0). All plates were incubated for 3 days at 30°C. The microbiological analysis was performed on a single representative sample.

Statistical analysis of the silage chemical analysis results included a one-way analysis of variance and Duncan's multiple range test, performed with the Statistical Analysis System Software (SAS, Cary, NC).

Results

The chemical and microbiological compositions of the corn and wheat silages (before exposure to air in the laboratory) are given in Table 1. All silage samples were of good quality, with low pH, and most samples had yeast and mould counts of $<10^6$ CFU (colony-forming units) g^{-1}.

Chemical and microbiological results of the corn silage experiments are given in Table 2. In the three experiments at 10 and 40°C, the pH remained stable after days 3 and 6. At 20 and 30°C, the pH was already higher after 3 days and had increased further after 6 days. Increasing temperature and time encouraged yeast development up to 30°C and inhibited it at 40°C. LA and acetic acid decreased with time of aerobic exposure, especially at 20° and 30°C; they remained highest in the 10 and 40°C treatments.

Chemical and microbiological results of the wheat silage experiments are given in Table 3. In the wheat experiments, the pH values at the various temperatures were more stable and did not change much except for an increase in pH at 30°C in the first silage (Table 3). The yeast numbers declined at 40°C between days 3 and 6. LA and acetic acid were usually lowest at 30°C.

For both corn and wheat silages, the highest CO_2 production during exposure to air was at 30°C and the lowest at 10 and 40°C. In all experiments, CO_2 production increased with time. In general, more CO_2 was produced in the corn silages than in the wheat silages, indicating that corn silages are more susceptible to aerobic exposure. The results of the current experiments indicate that temperature had a very significant effect on the aerobic stability of corn and wheat silages.

Table 3 Chemical and microbiological results from wheat silage exposed at different temperatures (3 experiments)

Time (days)	Temperature (°C)	pH			CO_2 (g kg^{-1} DM)			Yeasts			Molds			LA			Acetic acid		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3	10	4.2^{b}	4.1	3.9	2.9^{b}	0	0.9^{b}	6.6	NF	2.5	NF	NF	NF	40.8	–	–	17.7^{a}	–	–
	20	4.2^{b}	4.2	3.9	13.5^{b}	1.0	0.9^{b}	8.4	8.0	5.7	NF	NF	NF	43.3	55.5	56.7	8.0^{b}	13.7	15.6
	30	4.6^{a}	4.3	3.9	25.2^{a}	4.7	5.0^{a}	8.3	7.9	7.3	NF	NF	NF	40.7	49.1	53.0	8.0^{b}	13.6	20.7
	40	4.2^{b}	4.1	3.9	12.2^{b}	0	3.4^{a,b}	7.1	2.2	4.9	NF	NF	2.2	44.9	54.1	–	14.4^{a,b}	12.0	–
6	10	4.1^{c}	4.1	3.8^{b}	0.5^{c}	2.5	1.5^{b}	7.5	4.5	4.1	NF	NF	NF	47.0^{a}	50.0	53.0^{b}	24.5^{a}	28.0^{a}	24.5^{a}
	20	4.7^{b}	4.5	3.9^{b}	28.8^{b}	14.5	4.6^{b}	9.0	8.0	7.7	7.5	NF	NF	43.7^{a}	48.3	56.0^{b}	2.8^{b}	11.0^{b}	17.2^{b}
	30	8.3^{a}	4.2	4.1^{a}	61.8^{a}	16.1	30.4^{a}	6.7	7.6	8.8	6.1	NF	5.5	10.0^{b}	52.7	44.3^{c}	0^{b}	6.5^{b}	3.7^{c}
	40	4.1^{c}	4.1	3.8^{b}	0.5^{b}	0.7	2.5^{b}	6.1	NF	4.5	7.7	NF	2.2	51.6^{a}	39.3	65.7^{a}	21.9^{a}	23.3^{a}	17.4^{b}

Chemical results are in grams per kilogram DM and microbiological results in $\log_{10}$ of colony-forming units per gram DM. (–) not measured.
^{a,b,c}Within a column and day, means followed by different letters differ significantly ($P<0.05$).
In addition, ethanol and propionic and butyric acids were detected in some samples at levels of 0–3, 0–1 and 0–8 g kg^{-1} DM, respectively.

Discussion

It is impossible to control the ambient temperature of commercial silage under the aerobic conditions that prevail during unloading, but it is important to know the extent of damage that temperature may cause to the silage under such conditions. Such knowledge can lead to practical measures towards reduction of losses. The results of the current study indicate that at 10 or 40°C, air-exposed silage is relatively stable. Yeasts are very active in silage in the first stage of exposure to air; they use residual sugars and LA and produce CO_2 [10], and the current results indicate that their activity is strongly affected by temperature. The greatest losses and fastest yeast growth in the corn and wheat silages were found at 20 and 30°C. At 40°C, the yeast activity was reduced, and the silage was more stable. Extension of silage exposure to air from 3 to 6 days had a significant effect on stability at 30°C and less at 10, 20 or 40°C.

Mathematical models take into account the temperature rise at the silage face due to the activity of aerobic microorganisms [3,17]. However, the ambient temperature affects the rate at which the heat can be released to the surroundings, and at higher ambient temperatures, the face remains warm. Our results indicate that at 30°C, the silage is most susceptible to aerobic exposure. If the ambient temperature is very high (40°C), the face absorbs heat from the surroundings and remains stable.

In general, the silage samples did not differ markedly among themselves, in DM, pH, acetic acid content and microbial counts. The fresh wheat silages had higher WSC and LA contents and contained more VFAs (propionic and butyric acids) than the corn silages. The relatively greater aerobic stability of the former can be related to higher acid contents, which inhibit yeast and moulds [11]. From data in Table 1, it is difficult, however, to forecast the aerobic stability of the silages according to their composition. Also, it is not possible to tell whether the pH will increase during aerobic deterioration. High pH is associated with large numbers of yeasts and low concentrations of LA and acetic acid. Our hypothesis is that if residual WSCs are high, then the yeasts will use them before assimilating LA; in that case, the pH would remain low. The current data are insufficient to prove that hypothesis. It is interesting to notice, however, that the silage with the highest yeast counts (Corn 3, Table 1) had the highest pH after 3 days of aerobic exposure at 30°C, while the one with the lowest yeast counts (Wheat 3) had the lowest pH; the other silages generally followed that trend. After 6 days, that trend does not hold as well, presumably because other microbial groups, not measured, are active in aerobic deterioration of the silage. From the pH and CO_2 results, corn silage seems to be more sensitive to air exposure than wheat silage. A previous study [16] indicated that wheat silages stored at 41°C were more susceptible to aerobic spoilage than those stored at 24°C, especially when the aerobic exposure occurred at elevated temperatures (33° *vs.* 26°C). Thus, the temperature history of the silage during anaerobic storage affects its aerobic stability. Differences in aerobic stability between silages may be attributed to differences between silages and differing ensiling conditions.

The results of the present study imply that in a warm climate, special care should be taken during silage feedout in order to avoid high aerobic losses.

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