Microbial status, aerobic stability and fermentation of maize silage sealed with an oxygen barrier film or standard polyethylene film

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An experiment was conducted to compare a bunker silo sealing system comprising an oxygen barrier film (OB: 45µm thickness) with protective woven polypropylene with one comprising standard black polyethylene film (S, 125µm thickness) with protective tyres. Analysis of samples taken to 30 cm depth after 365 days of storage showed no differences in pH or lactic acid between the two sealing systems. There were no differences in aerobic bacterial count between silages. Whilst 2.56 $\log_{10}$ CFU moulds g^{-1} fresh weight were found in samples of silage sealed with S, no moulds were found in samples of silage sealed with OB. Aerobic stability, averaged 249 hours and 184 hours for OB and S, respectively. The OB system probably inhibited the development of the micro-organisms responsible for the initiation of aerobic deterioration to a greater extent than the standard silo sealing system.

Key words: maize silage, oxygen barrier film, fermentation, moulds, aerobic stability

Introduction

Many factors can affect the deterioration and loss of nutrients during the conservation and feed-out of silage, including crop maturity, the use of additive, particle size, rate of silo filling, packing density, type of plastic sealing and the fermentation profile of the ensiled material (Johnson et al. 2002, Holmes and Bolsen 2009). Maize silage is particularly susceptible to aerobic deterioration when it is exposed to oxygen in the silo or in the feed bunk (Ashbell and Weinberg 1992, Kung et al. 1998). According to Pahlow et al. (2003) and Uriarte-Archundia et al. (2002) yeasts that metabolize lactic acid are the primary spoilage microorganisms in maize silage, although the acetic acid bacteria and moulds can also cause aerobic spoilage (Spoelstra et al. 1988).

Borreani et al. (2007) found that loss of dry matter from the upper 40 cm layer was 10% for maize ensiled with no additive treatment and covered with a coextruded oxygen barrier (OB) film (125 mm thickness, 100 cm^{-3} m^{-2} per 24 h oxygen permeability at 1 bar, 23 °C, 85% relative humidity) under farm-scale conditions in Italy. Comparable loss of dry matter was higher, averaging 38%, for the same crop ensiled under standard polyethylene film (180 mm thickness, 990 cm^3 m^{-2} per 24 h oxygen permeability at 1 bar, 23 °C, 85% relative humidity). Coextruded oxygen barrier (OB) film with thickness of 45mm is 100 times more of a barrier to oxygen than a standard 125 mm polyethylene film (oxygen transmission rate: 3 *vs*. 400 cm^{-3} m^{-2} per 24 h 21% O_2) due to its special chemical composition and physical structure. Studies on thinner, 45mm thickness, OB film have shown positive effects on both grass (Wilkinson and Rimini 2002) and maize silages (Berger and Bolsen 2006).

Wilkinson and Rimini (2002) reported virtually no visible surface mould or spoilage and a lower percentage of inedible silage for triple co-extruded OB film sealed small-scale silos compared to single and double standard 125 mm thickness polyethylene film-sealed silos. Kuber et al. (2008) found that OB film was more effective than standard polyethylene film in preventing the entry of oxygen into large silos of ensiled maize. Bolsen and Bolsen (2006) found that maize silage and high mositure maize grain in the top 0 to 45 cm under the OB covering had better fermentation profiles and lower estimated additional spoilage losses of OM compared to the crops stored under standard plastic film.

The aim of this large-scale experiment was to compare the effect of a standard sealing system comprising a single layer of standard plastic film with a system comprising an oxygen barrier film and protective woven polypropylene tarpaulin, on fermentation characteristics, microbial status and aerobic stability of the top layer (30 cm), under conditions found in commercial practice in Hungary.

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Materials and methods

Ensiling

The trial was carried out on a commercial large-scale farm in Hungary (47°10'29'' N, 19°18'25''E, altitude: 99 m). The size of the bunker silo used in the trial was 25 wide and 70 m in length, with walls along both sides (3 m height) made from pre-cast concrete blocks. Forage maize was harvested without additive on 16 September 2009 with a precision chop harvester fitted with a kernel processor (Claas Jaguar 840). Stubble height was in the range of 30 to 40 cm above the soil level. Consolidation was carried out using two tractors with a weight of 10 tonnes per tractor (Raba Steiger). Sealing was completed within 240 minutes after terminaton of consolidation. The average height of the silage after filling was 3.5 m.

The oxygen barrier (OB) silo sealing system consisted of a transparent thin co-extruded plastic film of 45 µm thickness ("Silostop", 2Gamma, Mondovi, Italy), a close weaved dark green anti-UV polypropylene net (190 g m^{-2}) to protect the film (UV light protection), and gravel bags. The net designed with a woven structure which allows wind to pass through rather than lifting the sheet. The special woven structure also ensures that the nets do not accumulate heat and do not cause the silage the heat. Moreover the net protects the OB film from many types of physical damage (birds, dogs and hail). The gravel bags not only sealed the edges but they also prevented the film and net blowing off the silage. The standard (S) sealing system comprised a single black coloured standard plastic sheet (thickness of 125 µm, 1020 $cm^3 m^{-2}$ per 24 h oxygen permeability) covered with an average of 1.6 used car tyres m^{-2} (edge-to-edge). A central area of 10 m length was sealed with overlapping OB and S films (Fig. 1) and gravel bags were placed around its periphery. The peripheral edges of the entire silo were sealed with gravel bags. Both film sheets were laid transversally across the silo. The layout of the two silo sealing treatments and the sampling protocol are shown in Figure 1.

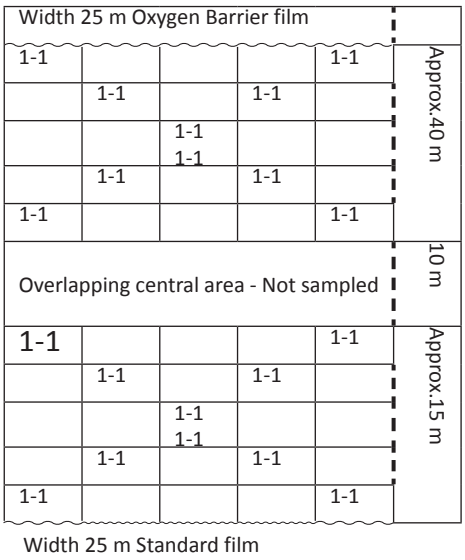

Fig. 1. Sampling design in split bunker silo. One sample derived from fresh, packed material and one sample from silage after 12 months.

Sampling

Ten initial samples, each of 2.5 kg fresh matter (FM) were taken by corer in both the OB and S areas of the silo before sealing, as shown in Figure 1, after the completion of consolidation but before sealing, to a depth of 30 cm from the top surface, for laboratory analyses and density estimation. A further set of ten samples were taken on the 16 September 2010 (12 months after filling and first sampling) from both the OB and S areas, according to the Figure 1, within 50 cm of the place where the initial samples were taken. These samples, also 2.5 kg FM, were also taken to a depth of 30 cm from the top surface for laboratory analyses, density estimation and assessment of aerobic stability.

Physical measurements

Temperature measurements were undertaken on the whole sample to depth of 30 cm with a digital infra-red thermometer immediately after the samples were taken. Fresh matter density was determined by the following method: Each sample was unloaded from the sample corer (approximately 30 cm depth ×15 cm × 15 cm). The volume of each core was then determined by filling the corer with water to the same depth as that of the core and the weight of the core determined using a digital mobile scale. The samples were then divided, with 1 kg retained for laboratory analyses and 1.5 kg for aerobic stability.

Aerobic stability was measured in a special model silo system in a constant temperature room at Szent István University, in Gödöllő. Each sample of silage was mixed and put into a model silo (density 120 g DM m^{-3}) leaving a 5 cm air layer above the top of the silo into which air was passed continually through a 10 mm diameter hole. Temperature sensors were built into the model silos, which were indvidually insulated with a polyethylene coat and stored in a controlled temperature room at 20 ± 1 °C. Aerobic stability was defined as the number of hours the silage remained stable before rising by 2 °C above the ambient temperature (Ranjit and Kung 2000).

Laboratory analyses

Laboratory analyses were executed according to the Hungarian National Standards: dry matter HNS ISO 6496:1993, crude protein HNS 6830-4:1981, crude ash HNS ISO 5984, lactic and volatile fatty acids: HNS 6830-39: 1986 (Hungarian Feed Codex 2004). Fiber fractions were determined according to Van Soest (1963). Aerobic bacteria were analysed by the method of STRF-MIKR-LB-1: 1999 (Hungarian Feed Codex, 2004), moulds were detected by LACTOAC-MIKR-LB-1:1999 (Hungarian Feed Codex, 2004). *Clostridium perfringens* were identified by HNS EN 13401:2000. (Hungarian Feed Codex, 2004).

Statistical analyses

The chemical compositional data of the fresh chopped whole crop maize and the silage samples (n=10, S vs. OB samples), fermentation profile and microbial counts (except mould counts) of the silage samples (n=10, S vs. OB samples) were analyzed for their statistical significance with IBM SPSS (version PASW Statistics 18). All microbial counts were log10 transformed to obtain log-normal distributed data. Chemical composition, fermentation profile, microbial counts (except mould counts) and aerobic stability (number of hours at 2 °C above the ambient temperature, n=10, S *vs*. OB samples) were analyzed for their statistical significance by ANOVA. Significant differences between variances were identified by the P-values of ANOVA (Levene's test for equality of variances), and the effects were considered significant at $p \leq 0.05$. When calculated values of F were non-significant (equal variances), Student t-test for equality of means was used ($p \leq 0.05$), when calculated values of F were significant (non-equal variances), Welch's t test ($p \leq 0.05$) was used to interpret any significant differences among the mean values. Mould counts were analyzed for their statistical significance by the Wilcoxon test (n=8, 36 T+ and 0 T-).

Results

The dry matter and nutrient content of samples of fresh maize in the upper 30 cm layer under either OB or S films are shown in Table 1.

Table 1. Composition of the consolidated chopped fresh crop (top 30 cm) under either standard (S) or oxygen barrier (OB) film (n=10)

		Consolidated fresh crop (top 30 cm)				Silage			
Sealing system		S	OB	SED	Sig.	S	OB	SED	Sig.
DM	g kg^{-1}	367	376	7.36	NS	359	362	5.82	NS
Crude protein	g kg^{-1} DM	77	76	1.48	NS	74	75	0.49	NS
Crude ash	g kg^{-1} DM	43	42	1.23	NS	42	44	0.91	p =0.042
NDF	g kg^{-1} DM	443	442	10.71	NS	431	417	7.28	NS
ADF	g kg^{-1} DM	214	208	6.30	NS	238	227	5.50	NS
ADL	g kg^{-1} DM	25	24	1.51	NS	25	25	2.16	NS

DM = dry matter, SED = standard error of difference. NS = not significant p >0.05, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin

The physical parameters of the fresh crop and silages sealed with either the OB or S sealing systems are shown in Table 2.

Table 2. Physical parameters of the consolidated chopped fresh crop (top 30 cm) and of silage under either standard (S) or oxygen barrier (OB) film (n=10)

		Consolidated fresh crop (top 30 cm)				Silage			
Sealing system		S	OB	SED	Sig.	S	OB	SED	Sig.
Fresh matter density	kg m^{-3}	528	517	35.2	NS	546	540	30.8	NS
Dry matter density	kg m^{-3}	194	195	15.3	NS	196	196	12.2	NS
Temperature	°C	23.3	23.0	0.6	NS	25.5	25.0	0.3	NS

SED = standard error of difference, NS -not significant $p>0.05$

Fermentation characteristics, microbiological composition and aerobic stability of the silages in the top 30 cm stored under the two sealing systems are shown in Table 3.

Table 3. Fermentation characteristics, microbiological composition and aerobic stability of silage under standard (S) or oxygen barrier (OB) film (n=10)

Sealing system		S	OB	SED	Sig.
pH		3.73	3.80	0.038	NS
Lactic acid	g kg^{-1} DM	45.0	47.1	3.28	NS
Acetic acid	g kg^{-1} DM	32.3	24.7	2.11	p =0.002
Propionic acid	g kg^{-1} DM	0.9	0.4	0.19	p = 0.017
Butyric acid	g kg^{-1} DM	0.0	0.0	-	-
Ethanol	g kg^{-1} DM	11.3	6.5	1.01	p=0.005
Volatile fatty acids	g kg^{-1} DM	33.2	25.1	2.06	p =0.001
Total organic acids	g kg^{-1} DM	78.2	72.2	3.62	NS
Total fermentation products	g kg^{-1} DM	88.7	78.7	4.69	p =0.042
Lactic acid/acetic acid		1.4	2.0	0.23	p =0.024
Lactic acid/ total fermentation products		0.5	0.6	0.02	p =0.001
AEMB	log_{10} CFU g^{-1} FM	4.71	4.12	0.54	NS
Moulds	log_{10} CFU g^{-1} FM	2.56	0.0	0.43	p =0.008
Yeasts	Number of positive samples	1	3	-	-
		-	-	-	-
Clostridium perfringens	log_{10} CFU g^{-1} FM	1.93	0.56	0.46	p =0.008
Aerobic stability	No. hours to +2 °C above ambient	184	249	16.63	p =0.002

DM = dry matter,
SED = standard error of difference, NS = not significant $p>0.05$, FM - fresh material, AEMB = aerobic mesophilic bacteria

Temperature profiles of the aerobic stability phase are shown in Figure 2.

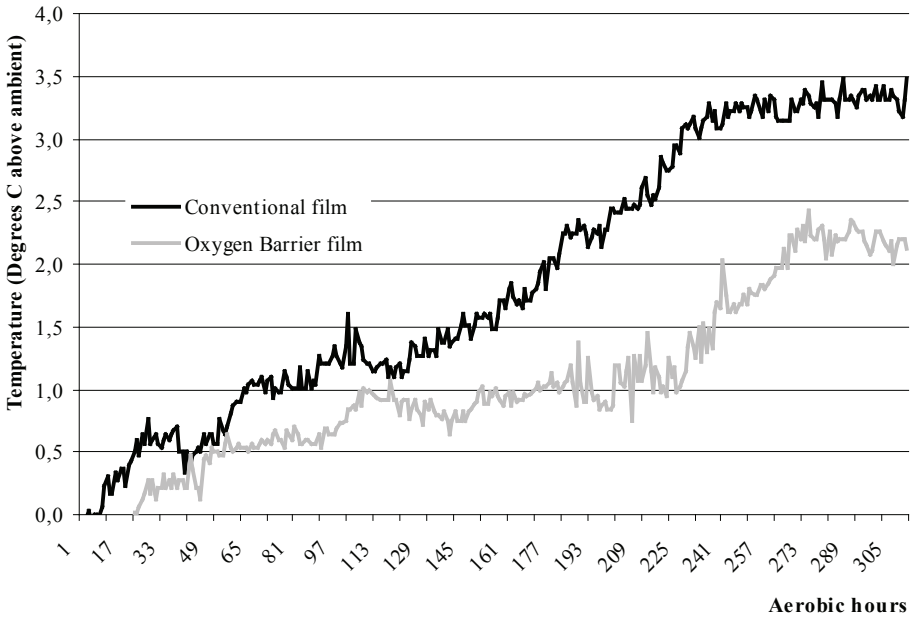

Fig. 2. Temperature changes of maize silages during exposure to air (n=3)

Discussion

There were no significant differences in the concentrations of dry matter, crude protein, crude ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) or acid detergent lignin (ADL) in the initial fresh maize crop at the point of ensilement under either the OB or the S sealing systems. This indicated that the trial design had been successful at ensuring that the starting point for maize quality was the same across the two treatments.

After 12 months storage in the silo there were no significant differences in the concentrations of dry matter, crude protein, NDF, ADF or ADL in samples of silage from in the upper 30 cm layer under either the OB or S sealing systems. However, the crude ash content was higher, by 2.1 g kg^{-1} dry matter, in the maize silage covered by the OB sealing system than in maize silage stored under the S sealing system. Whilst this was statistically significant the difference was numerically small and probably of little importance biologically or chemically.

There were no significant differences between the two sealing treatments in either the density of the top 30 cm or the temperature at 30 cm depth. Both fresh matter and dry matter densities were similar between the two treatments which indicated consistency across the two treatments with respect to compaction during filling.

Silage density is important for the exclusion of air from the silo to ensure an anaerobic environment where nutrients are preserved. In the present experiment, the average fresh matter density of the silage in the top 30 cm layer was 196 kg DM m^{-3}, reflecting the relatively high tractor packing capacity of 20 tonnes, but was lower than the recommendation of Muck and Holmes (1999) of maize silage of 225 kg DM m^{-3}, This indicates the difficulty in achieving high silage density in the uppermost layer of the silo even when the tractor packing capacity is considered to be adequate for consolidation.

There were no significant differences in pH or lactic acid between the two sealing treatments. Further, the low pH values indicate that the overall silage quality irrespective of treatment was generally good indicating acceptable silo management and forage preservation. In contrast, Borreani et al. (2007) and Kuber et al. (2008) found more lactic acid and lower pH values in silage stored under OB film than under a standard sealing system.

In the present study, the concentration of acetic acid was lower in silage sealed with the OB system than in silage covered with the S system. The concentration of propionic acid was also lower in the silage stored under the OB than in that stored under the S sealing system. Butyric acid was not found in any of the samples. In contrast, Borreanni et al. (2007), found a higher concentration of propionic acid in untreated maize silage in the peripheral area of one farm bunker silo sealed with OB than with S, but not in another silo where the maize had been treated with an additive containing *Lactobacillus buchneri*.

Total concentrations of volatile fatty acids and fermentation products (FP) and the ratios of lactic to acetic acid and lactic acid to FP were higher for silage stored under OB than S, indicating that the fermentation had been enhanced by the OB method of sealing. Berger and Bolsen (2006) also reported a better fermentation profile in maize silage in the top 0 to 46 cm under a 45 µm OB film compared with maize silage stored under a 150 µm polyethylene.

The concentration of ethanol was lower in the silage stored under the OB than under the S sealing system, indicating lower yeast activity in the material stored under OB than S film (Pahlow et al. 2003, Rooke and Hatfield 2003). Borreani et al. (2007) and Borreani and Tabacco (2008) found no differences in concentrations of ethanol and yeast counts of less than 1 $\log_{10}$ cfu g^{-1} in the peripheral areas of untreated maize ensiled under either OB or S sealing systems in a similar split-bunker silo design to that of the experiment reported here. In the present study, counts of yeasts were positive in three of the ten samples of silage covered by the S system (mean 4.91 $\log_{10}$ cfu g^{-1} fresh matter, FM), whereas only one sample of silage covered by the OB system contained a measurable count of yeasts (4.31 $\log_{10}$ cfu g^{-1}). Yeast counts lower than 3.1 $\log_{10}$ cfu g^{-1} indicate a likelihood of aerobic stability in excess of 50 hours of in maize silage (Muck 2004). Considering that the majority of the samples were negative for yeasts, good aerobic stability would be expected.

There was no significant difference in aerobic bacteria count between silages. However, a mean mould count of 2.56 $\log_{10}$ cfu g^{-1} FM were found in silage stored under the S system while there were no moulds found in any of the ten samples of silage stored under the OB system. Borreani and Tabacco (2008) also found that the lower oxygen permeability of the OB film was reflected in a lower mould count in the peripheral areas of untreated maize silage stored in a farm bunker silo under OB than under S.

Lower populations of *Clostridium perfringens* were found in the silage stored under OB than under S. *C. perfringens* is both saccharolytic and proteolytic (Woolford 1984), and is one of a group of undesirable species of silage bacteria, the *Clostridia*, which can degrade amino acids to ammonia, ferment lactic acid to butyric acid and cause the "late blowing" of cheese (Pahlow et al. 2003). Borreani and Tabacco (2008) found much higher counts of butyric acid bacteria (BAB) spores in untreated maize silage in the peripheral areas of a farm bunker silo. The authors found that the higher counts of BAB spores were associated with higher mould counts, lower concentrations of nitrate in silage and higher temperature differences between the silage and ambient, indicating that clostridial sporulation can be encouraged in silage which is exposed to oxygen, as Kwella and Weissbach (1991) observed.

The time taken for the temperature of the silage samples to rise by 2 °C above ambient averaged 249 hours for material stored under the OB system compared to 184 hours in the case of silage stored under the S system - a difference of 65 hours. Aerobic stability data should be considered as relative values obtained under laboratory conditions. Borreani et al. (2007) found an average aerobic stability of untreated maize silage of 72 and 69 h (+2 °C above ambient) for OB and S systems, respectively. The values observed here, although greater than those reported by Kleinschmit and Kung (2006) for uninoculated corn silage (25 h), were similar to those recorded by Borreani et al. (2007) with maize silage treated with an additive containing *L. buchneri* (355 and 178 h for OB and S, respectively). The higher aerobic stability of silage stored under OB than under S can be explained by the significantly lower mould count in the OB silage.

Johnson et al. (2002) stated that the aerobic stability of maize silage can be increased by kernel processing which in turn is reflected in increased FM packing density compared with unprocessed corn silage. The greater FM packing density limits the exposure of processed silage to oxygen during the initial period of the storage phase compared with unprocessed silage. Therefore, the growth of aerobic microorganisms is minimal in processed corn silage before the opening of the silo. In the present study, the crop was processed and there was no significant difference in the density of the silages between the two sealing treatments, so the differences in aerobic stability must be attributed to differences in oxygen exposure of the upper layers of the silages due to the different oxygen permeability of the two plastic films. The significantly higher aerobic stability in the silage sealed by the OB system cannot be due to differences in concentrations of either acetic acid or propionic acid (significantly higher in S than in OB) which are both known to inhibit yeasts and moulds (Woolford 1975, Pahlow and Muck 2009). Therefore, the improved aerobic stability must be a consequence of less aerobic microbial activity (yeasts and moulds) during the storage phase before silo opening.

It can be summarized that the OB silo sealing system had a beneficial effect on the hygienic status of the top 30 cm of silage with lower counts of moulds, yeasts and *Clostridium perfringens*. Possibly most importantly, the OB system improved the aerobic stability of the maize silage. These results are particularly interesting as it would generally be expected that the silage with the higher acetic acid concentration would provide a greater degree of stability. However, in this experiment the silage under the OB system contained less acetic acid than that under the S sealing system. Whilst oxygen concentrations in the silo were not measured, and no differences were found in aerobic bacterial numbers, differences in the number of samples containing moulds and yeasts were found. Therefore, it is likely that the increased aerobic stability in the upper layer of silage stored under the OB system was due to reduced oxygen permeation through the silo seal during the storage period.

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