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Encapsulated propionic acid as a silage additive

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ABSTRACT

Various propionic acid (PA) based additives are used to successfully inhibit fungi in silages. These additives are all introduced directly, and an encapsulated formulation of PA has not yet been examined for its antifungal abilities. The current study's objective was to test the possibility of using encapsulated PA as a silage additive. Carboxymethylcellulose (CMC)-based films (film A) and CMC/β-cyclodextrin-based films (film B) were used as biodegradable matrix platforms for encapsulated PA delivery and tested on whole-crop wheat and corn silages. Films were added as a mixture combined with the silage or divided at the top and bottom of the bulk silage system. A *Lactobacillus plantarum* inoculation procedure was also examined for its effects. In the wheat ensiling experiment, film B resulted in the highest PA concentrations after 2 weeks (1.4% and 1.1% in dry matter for the mixed and divided films, respectively). Mixed film A also produced high levels of PA after 2 weeks. Lactic acid (LA) concentrations peaked after 2 weeks and the highest final concentrations were obtained in the *L. plantarum* treatment. The highest PA concentrations in the corn silages were measured at the end of the experiment. Film B tended to result in slightly higher PA concentrations than film A. LA concentrations peaked after 2 weeks and the highest final content was obtained with film B. Overall, this study demonstrates that addition of encapsulated PA to biodegradable CMC films may provide an advanced safe approach for retaining silage quality and wastage reduction.

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Carboxymethylcellulose; β-cyclodextrin; encapsulation; propionic acid; silage

Introduction

Ensiling is a preservation method for moist crops. It is based on anaerobic fermentation, whereby lactic acid (LA) bacteria, found in decomposing plants, metabolize soluble sugars into organic acids. LA is the main metabolic product of this process; as a result the silage's pH decreases (the biomass becomes acidic) and the crops are preserved as long as no air penetrates. Air is detrimental to silages because it enables aerobic microorganisms to spoil it, especially yeasts and molds (Woolford 1990). The latter might also produce mycotoxins, which are detrimental to the health of animals and humans. In a bunker silo the areas adjacent to the walls, the top and the corners between the walls and the top were found to be most susceptible to air penetration. If silage preparation procedures are not met precisely, aerobic intervention leads to common spoilage. Our previous studies have revealed that air penetrates the silage as far as 1–2 m past the silage face (Weinberg & Ashbell 1994). A rapid consumption of the silage helps to minimize face spoilage and losses. Top dry matter (DM) losses might reach over 75% of the top layers as compared to only 12% in the center which is furthest from the walls and top (Ashbell & Weinberg 1992). In whole-crop wheat, corn, and sorghum silages, the major spoilage microorganisms are yeasts followed by fungal molds.

Silage additives have been developed and used for many years. The functions of such additives vary according to the nature and composition of the silages. Overall, they are applied in order to enhance fermentation, reduce losses, improve the silages' nutritional values, and stabilize it upon aerobic exposure at feed out (Kung et al. 2003). Volatile fatty acids such as acetic acid (AA), propionic acid (PA), and butyric acid (BA) inhibit aerobic yeasts and molds, whereas LA enhances their development during aerobic exposure of silages (Moon 1983; Weinberg et al. 1993). *Lactobacillus buchneri*, which converts LA to AA and 1,2 propanediol in silages, is therefore sometimes

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included in inoculants for susceptible silages (Driehuis et al. 1999) as well as various PA-based additives (Kung et al. 2003). PA has been used in order to inhibit yeasts and molds and thus improve aerobic stability. Because of its pungent offensive odor and corrosive nature, commercial products usually contain the acid salts (mainly calcium). PA is not usually produced in the ensiling fermentation (Weinberg et al. 1995) and has to be added manually. Due to its volatile nature, PA is not recommended to be added by spraying it onto the silage's face.

Encapsulation of volatile molecules is known to help reduce their volatility, as well as protect active agents from external factors such as light, oxidation, and heat, and mask undesired accompanying odors and aftertaste (Lakkis 1998; Lopez-Rubio et al. 2006). One form of encapsulation may be accomplished by trapping volatile agents in a polymer matrix, thus inhibiting their natural evaporation rate. The "molecules" are then forced to diffuse their way through the polymer network. Additional materials in the selected formulation may contribute to PA's encapsulation by further retarding its release rate. In our previous study it was discovered that β-cyclodextrin (β-CD) facilitates in encapsulating PA in cellulose-based films. β-CD containing formulations were successful in increasing PA's uploading capacity as well as slowing down its natural release rate (Rutenberg et al. 2016).

The objective of the current study was to test the effect of encapsulated PA in biodegradable carboxymethylcellulose (CMC)-based films on quality and aerobic stability of wheat and corn silages. The effect of adding β-CD as an auxiliary encapsulation molecule to the CMC matrix on the films' performance was also studied.

Materials and methods

Ensiling experiments

Two ensiling experiments, one with whole-crop wheat (at the milk stage of maturity) and the other with whole-crop corn (at half milk line), were performed in order to test the films into which PA was incorporated. The crops were chopped into 2−4 cm pieces and 20 g were placed in 96 50 mL plastic tubes with screw caps (Cellstar®, Greiner Bio-One, Kremsmünster, Austria). Seventy-two tubes were used for a time-course study in which three tubes per treatment were sampled 1, 2, 4, and 13 weeks after ensiling. The remaining 24 tubes were used to study the effect of rate of application: 0, 2.5, 5.0, and 10.0% w/w (on wet weight basis, in triplicate). The latter tubes were sampled after 3 months. The tubes were incubated at 30 °C throughout the storage period of each treatment.

In the time-course study the following treatments were included: control (no additives); *Lactobacillus plantrarum* MTD1 (Ecosyl, Stokesly, Yorkshire, UK); film A and film B mixed in the silage; and film A and film B divided between the bottom and top of each tube. In the experiment with corn silages the films were placed at the bottom of the tubes only. All of the film treatments were applied at 10% w/w and were combined with the *Lactobacillus*.

In the application rate study the films were divided between the bottom and top of each tube (in the corn silages the films were placed at the bottom of the tubes only). All the film treatments were combined with the *Lactobacillus*. The bacterial inoculant was applied by suspending 100 mg of powder (which contained 2×10^{10} colony forming units [CFU] per g) in 20 mL water and mixing thoroughly with 2 kg of chopped forage before placing into the tubes. Thus, 10^6 CFU per g forage were applied.

At the end of each study the silages were separated from the films, and 4 g from each tube were used for chemical analyses; the rest of the silage was loosely re-placed in the tubes which were left open at room temperature (25 ± 2 °C) for 3 days to assess aerobic stability.

Film preparation

All reagents were of analytical grade and used without further purification. CMC (sodium salt) and PA were purchased form Alfa Aesar (Heysham, UK). β-CD was purchased from Chem-Impex Int'l Inc. (Wood Dale, IL).

For both film formulations (A and B), PA was dissolved in double distilled water (DDW) at a 15% w/w concentration. The solutions were then heated to 50 °C with a stopper over the flask's top. At this stage β-CD was added to film B formulation at a 5% w/w concentration and stirred for 1 h. CMC was then added (2% w/w) and the reaction was stirred for 2 h at 50 °C. All films were obtained by pouring 9 mL portions of the film-forming solutions into Teflon Petri dishes (9 cm in diameter). All films spontaneously dried at 23 °C for 2 days in a chemical hood at relative humidity (RH) of 65 ± 2%. The prepared films were stored at −20 °C until their application in the experiments.

Analytical procedures

DM was determined by oven drying for 48 h at 60 °C. LA was determined by a spectrophotometric method according to Barker & Summerson (1941). Ethanol and volatile fatty acids were determined in aqueous extracts by means of gas chromatograph equipped with a semi-capillary FFAP (nitroterephthalic acid modified polyethylene glycol) column (Hewlett Packard, Waldborn, Germany) over a temperature range of 40−230 °C.

Statistical analyses

Statistical analyses of the chemical parameters included ANOVA and TUKEY student range test which were performed with the JMP 7.0 software (Statistical Discovery™ from SAS, Cary, NC) with silage treatment and time as the main effects.

Results and discussion

In the current study two CMC-based film formulations were tested, which encapsulated PA in wheat and corn silages in order to protect them against aerobic yeasts and molds. Our hypothesis was that the films would release PA gradually, thus preventing fungal development in the silages. Treatments also included the incorporation of homolactic *L. plantarum* bacterium that produces only LA in the silage. LA can be utilized by lactate assimilating aerobic yeasts and in fact enhances aerobic spoilage of whole crop cereal silages (Weinberg et al 1993). The reason for including this bacterium along with all film treatments was to challenge the films in the silage. Films were applied in two different ways: mixed with the silage; or divided between the top and bottom of the tubes. In the latter, it was possible to separate the films from the silage particles, whereas when mixed, this was not possible and the measured PA might still have been in part absorbed by the films.

In the wheat experiments, silages with both films placed on top of the silages (under the tube caps) had molded. This result was not expected, and our explanation is that when PA evaporated from the films at the top, the net CMC served as a good medium for molds, which developed in the upper part of the silage because the caps were probably not air tight. However, the corn silages in which the films were placed only at the bottom did not mold. The different kinetics of PA build-up between the wheat and corn silages could also be attributed to the manner in which the films were placed in each trial.

Film B, which had β-CD, resulted in accumulation of higher PA concentrations in the silage than film A. This was also apparent from the dose-response trials (Tables 1 and 2). This is because β-CD promotes PA encapsulation (Poverenov et al. 2013). This was also reflected by the initial PA concentrations in films A and B (2.6% and 6.4% in DM, respectively). Cyclodextrins are key components in a large number of encapsulation systems (Hedges 1998; Del Valle 2004). β-CD has a torus like shape with an inner hydrophobic cavity and external OH groups that allow hydrogen bond interactions.

Both CMC (cellulose derivative) and β-CD are used in the food and pharmaceutical industries (Tehatiparti et al. 2010) and are considered as GRAS (Generally Recognized as Safe). No harmful effects on ruminants should be expected by using these additives in silages.

Table 1. Effect of film type and propionic acid dose on the chemical composition of wheat silages (% in DM).

Treatment	pH	Ethanol	AA	PA	LA	BA
Control	4.3	2.2	1.0^{b}	0.1^{b}	2.7	2.3^{a}
L. plantarum	4.2	2.3	1.9^{ab}	0.1^{b}	3.8	0.8^{b}
Film A − 2.5%	4.5	1.4	1.8^{ab}	0.4^{ab}	3.4	0.7^{b}
Film A − 5.0%	4.1	1.9	1.7^{ab}	0.4^{ab}	3.9	0.1^{b}
Film A − 10.0%	4.0	3.0	1.1^{ab}	0.4^{ab}	3.8	0.2^{b}
Film B − 2.5%	3.9	2.4	1.9^{ab}	0.3^{ab}	4.0	0.4^{b}
Film B − 5.0%	4.5	1.3	2.0^{a}	0.4^{ab}	2.1	0.7^{b}
Film B − 10.0%	3.9	1.6	1.8^{ab}	0.8^{a}	4.4	0.0^{b}

Notes: Within columns, means followed by different superscript letters differ significantly ($P < 0.05$). AA, acetic acid; BA, butyric acid; LA, lactic acid; PA, propionic acid.

Table 2. Effect of film type and propionic acid dose on the chemical composition of corn silages (% in DM).

Treatment	pH	LA	Ethanol	AA	PA
Control	4.2^{c}	0.3	0.8	4.7^{a}	0.7^{bc}
L. plantarum	4.25^{bc}	0.2	0.5	3.8^{ab}	0.5^{c}
Film A − 2.5%	4.3^{ab}	0.3	0.5	3.7^{b}	0.6^{c}
Film A − 5.0%	4.3^{ab}	0.4	0.6	4.2^{ab}	0.8^{bc}
Film A − 10.0%	4.4^{a}	0.3	0.5	3.5^{b}	0.9^{bc}
Film B − 2.5%	4.25^{bc}	0.5	0.5	4.6^{a}	1.3^{b}
Film B − 5.0%	4.25^{bc}	0.3	0.7	3.5^{b}	1.1^{bc}
Film B − 10.0%	4.3^{ab}	0.5	0.7	3.9^{ab}	2.2^{a}

Notes: Within columns, means followed by different superscript letters differ significantly ($P < 0.05$). Butyric acid concentrations were negligible. AA, acetic acid; LA, lactic acid; PA, propionic acid.

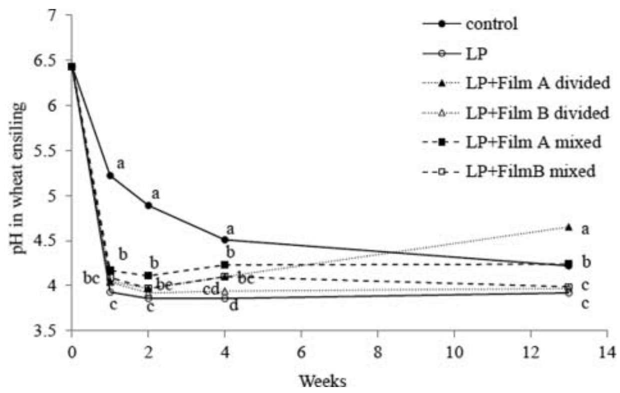

Figure 1. pH decrease during wheat ensiling. LP stands for *Lactobacillus plantarum*. For each sampling date, data points accompanied by different letters are significantly different ($P < 0.05$).

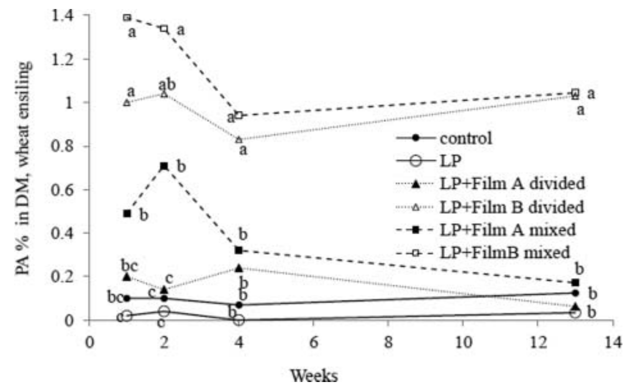

Figure 3. Propionic acid (PA) changes during wheat ensiling. LP stands for *Lactobacillus plantarum*. For each sampling date, data points accompanied by different letters are significantly different ($P < 0.05$).

PA content in the films was tested immediately after their preparation using a 2 h extraction in 30 mL DDW and an acid-base titration with sodium hydroxide (0.1 M) as the titrant and phenolphthalein as the pH indicator. Films A and B were found to hold 2.6% and 6.4% w/w PA, respectively.

The DM content of the fresh wheat and corn plants was 34.8% and 31.8%, respectively. Tables 1 and 2 provide results for the wheat and corn silages treated with different levels of film doses (2.5, 5, 10% w/w). In the wheat silages the concentrations of AA, PA, and LA were not affected by film A at any application dose; film B at 10% application resulted in higher PA concentration than the control silages. This treatment also resulted in the numerically lowest pH value and highest LA concentration. The control wheat silage contained a high level of BA, which is unusual in such high DM silages. The fermentation profile of the corn silages has generally revealed unusually low concentrations of LA and high concentrations of AA with no relation to the treatment.

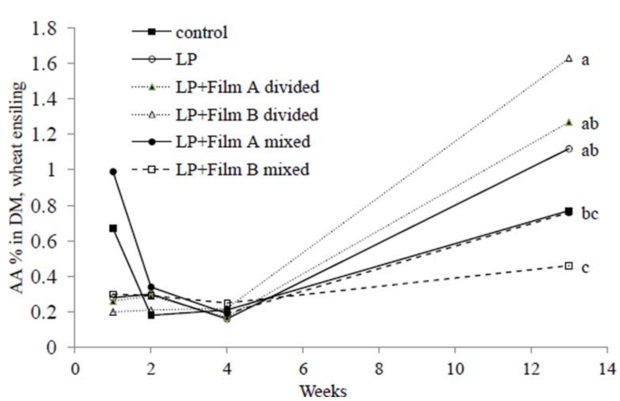

Figure 2. Acetic acid (AA) build-up during wheat ensiling. LP stands for *Lactobacillus plantarum*. For each sampling date, data points accompanied by different letters are significantly different ($P < 0.05$).

After 3 months of incubation, wheat silages with films placed at the top and bottom of the silages were moldy only at the top. Corn silages (with films either mixed or at the bottom only) did not mold at all.

Figure 1 presents the pH decrease during the wheat ensiling experiment. The control silages' pH decreased the slowest and the silages with film A's pH values remained higher than those with film B. Figures 2 and 3 present changes in AA and PA during the wheat silages' storage periods. AA developed between 4 and 13 weeks of storage, and the mixed films resulted in its lowest concentrations. Film B resulted in the highest PA concentrations, which were observed after 2 weeks (1.4% and 1.1% in DM, for the mixed and divided film, respectively), after which they decreased. Mixed film A also produced high levels of PA after 2 weeks with LA concentrations also peaking after 2 weeks. LA concentrations were not affected by the type of treatment selected. The highest final LA concentrations were measured in the *L. plantarum* treatment (3.6% in DM after 13 weeks).

Figure 4 presents the change in pH during the corn ensiling experiment. It can be seen that pH values of most of the silages were above 4.0, which indicates accumulation of low LA concentrations. Figures 5 and 6 present the change in AA and PA during the corn silages' storage period. AA concentrations built up with time in most treatments and were relatively high (3.0%−4.5% in DM), except for when film B was mixed with the corn silages. PA concentrations in the corn silages were significantly higher in the film treatments

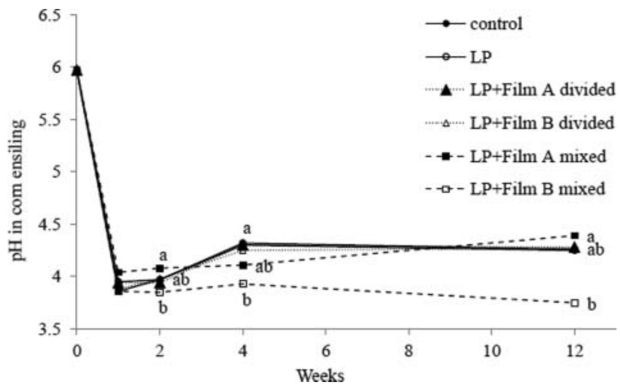

Figure 4. pH change during corn ensiling. LP stands for *Lactobacillus plantarum*. For each sampling date, data points accompanied by different letters are significantly different ($P < 0.05$).

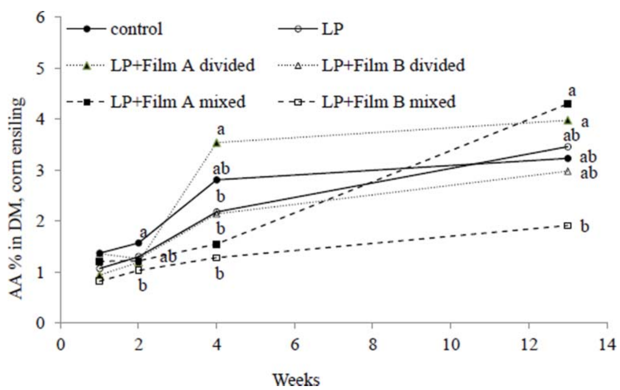

Figure 5. Acetic acid (AA) build-up during corn ensiling. LP stands for *Lactobacillus plantarum*. For each sampling date, data points accompanied by different letters are significantly different ($P < 0.05$).

than those of the control and *L. plantarum* treatments. In most film treatments PA increased after 2 weeks and the highest concentrations were measured at the end of the experiment. Film B tended to result in

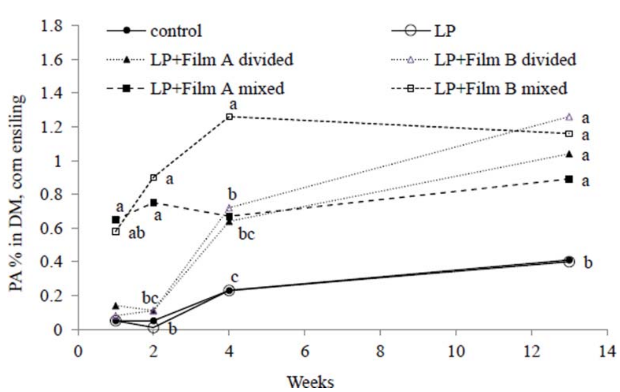

Figure 6. Propionic acid (PA) build-up during corn ensiling. LP stands for *Lactobacillus plantarum*. For each sampling date, data points accompanied by different letters are significantly different ($P < 0.05$).

slightly higher PA concentrations than film A. LA concentrations peaked after 2 weeks and then decreased to unusual levels for corn silages (0.5% in DM after 13 weeks); the highest final LA content was obtained with film B when mixed (1.8% in DM), in accordance with this treatment's lowest pH value (3.75).

The concept of using films of encapsulated PA as a silage additive seems feasible. The matrix that comprised CMC and was augmented by β-CD (film B) seems more efficient than film A (CMC only). However, more research is required in order to develop commercial scale films. The application mode should also be engineered. This study's findings may contribute and encourage the research and utilization of encapsulated PA films as an advanced green and safe approach for silage.

Disclosure statement

No potential conflict of interest was reported by the authors.

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