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# IMPROVED MINI-SILO FOR STUDYING FERMENTATION OF SILAGE IN LABORATORY CONDITIONS

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**Abstract:** As an alternative system to glass jars, mini-silo consisting of polypropylene (PP) container for laboratory studies of fermentation dynamics in silage was proposed in our previous study. However, suitability of this mini-silo was questioned, as there was a slight probability of air penetration into container, which would lead to silage deterioration. In this study, an improvement of the original mini-silo system for ensiling was undertaken. A non-transparent PP container was replaced with a see-through container made of co-polymer poly-propylene (PP), which had a lid with layer of silicon on its edge, which made this mini-silo practically air-proof. The lid was equipped with a special water valve, for releasing of gas products of the fermentation from the silage. Moreover, the see-through container made a provision for monitoring of micro-organisms growth and thus better explanation of the obtained results on silage microbiological status. The improved silage system was tested in an experiment in which alfalfa was compacted into transparent containers with addition of two bacterial inoculants. On day 50, percentage of silage surface covered with moulds was visually estimated and samples were taken for determination of total number of moulds and volatile fatty acids. Improved mini-silo system for silage laboratory studies proposed in this paper is simple, economical, practical and capable of preventing air penetration into the silage.

**Key words:** silage, mini-silo, fermentation

## Introduction

Ruminant animals require large source of dietary fiber, which is necessary for normal rumen function and physiology. Thus, high quality forages are the inevitable ingredients of dietary rations fed to ruminants *(Driehius and Oude*

*Elferink, 2000)*. Forage crops could be preserved as fermented feedstuff during process known as ensiling process.

Presence of air in silage during fermentation enables growth of yeasts and moulds, which can cause dry matter losses and silage deterioration. Therefore, removal of air from silage during ensiling process can lead to successful inhibition of undesirable microorganisms. On the other hand, most quality changes occur during fermentation. In addition to lactic acid bacteria (LAB), which are responsible for silage preservation, other microorganisms, such as aerobic bacteria, enterobacteria, yeasts, molds, clostridia, bacilli, acetic acid bacteria and propionic acid bacteria compete for substrate in the fermentation process (*Čabarkapa, 2010*). Those microorganisms can be pathogenic, producers of toxins or can affect the quality of dairy products. Successful inhibition of those undesirable microorganisms, provide high nutritional and hygienic quality of silages *(Knicky, 2005; Čabarkapa, 2010a)*. Fermentation of silage can be supported with addition of bacterial inoculants. Microbial silage inoculants were originally used to reduce pH and to avoid, or decrease, the risk of clostridia fermentation by the native bacterial population *(Wilkinson et al., 2003)*. Nowadays, inoculants are used also to promote lactic acid production, reduce ammonia formation, reduce temperatures in the silo, reduce dry matter and energy losses while in storage, reduce protein solubilisation, prolong bunk life, i.e. increase aerobic stability and to improve animal performance *(Buckmaster and Lundmark, 2009)*.

For studying the ensiling process, small-scale mini-silos are commonly used. They make provision for evaluation of numerous experimental variables and their interactions under controlled conditions. By direct comparison of fermentation processes, it has been concluded that the small-scale silos provides a reliable prediction of field-scale fermentation process *(Cherney and Cherney, 2003; Cherney et al., 2004; McDonald et al., 1991; Meiske et al., 1975)*. On the other hand, there are numerous problems in experimental usage of mini-silos, like differences in degree of consolidation, gas exchange and heat transfer properties of silages in mini-silos and commercial silages *(Cullison, 1948; Weinberg, 1993)*.

Mini-silos have been used since beginning of the 20$^{th}$ century, comprising different types of fixed-volume vessels such as glass jars *(Autrey, 1947)*, glass cylinders *(Archibald, 1946)*, metal cans (*Nevens, 1933*), and more recently vacuum-packed polythene bags *(Johnson et al., 2005)* and poly-propylene (PP) containers *(Čolović et al., 2010)*.

The aim of this study was to investigate possibility of improving the PP mini-silo system for studying fermentation of silage in laboratory conditions.

## Materials and Methods

Container (1.8 $dm^3$) made of transparent co-polymer PP and with a silicon layer on the edge of the lid, was used as a mini-silo. Transparency of co-polymer PP allowed monitoring the interior of container, unlike the mini-silo proposed in our previous study *(Čolović et al., 2010)* (Figure 1). The role of silicone layer was to prevent air penetration into the container.

**Figure 1. Non-transparent *(Čolović et al., 2010)* and transparent mini-silo**

The lid was equipped with a special valve which was filled with water, had a rubber seal at the bottom end and was placed in the centre of the lid. Water prevented air penetration into the silage, while silage fermentation gas products could freely get out, which ensured anaerobic conditions *(Čolović et al., 2010)*.

**Figure 2. Water valve with rubber seal *(Čolović et al., 2010)***

To demonstrate possible advantages of the improved mini-silo, a simple experiment was conducted where alfalfa (0.66 ± 0.005 kg), harvest in season 2010, was compacted into co-polymer PP containers. Fresh material was divided in three parts. Two of them were sprayed with solution of commercial bacterial inoculants "Bonsilage Plus" and "Bonsilage Forte" (Schaumann, Austria) in concentration according to manufacturer's specification, and the third part was used as a control.Silages were prepared in triplicates as follows: without bacterial inoculant (K1, K2 and K3), with added "Bonsilage Plus" (BP1, BP2 and BP3) and with added "Bonsilage Forte" (BF1, BF2 and BF3). Containers were stored in dark room at the temperature of 20 ± 3□C.

At day 50, containers were opened and samples taken. Total number of moulds (TNM) was determined using Dichlor Rose Bengal Chloramphenicol agar (DRBC) after aerobic incubation at 25 °C for 5 days in the dark *(International Organization for Standardization, 2008)*. Estimation of percentage silage surface covered with moulds was done by visual inspection before opening the containers. Volatile fatty acids were also determined *(Stanaćev and Kovčin, 2003).*

## Results and Discussion

Moulds need oxygen for their development and their presence indicates inadequacy of anaerobic conditions. Thus, if the system is not sealed well, growth of moulds can be expected. Important characteristic of this improved mini-silo, its transparency, made possible the visual estimation of the of the silage surface moulds. Visual inspection of silage during experiment might be a good tool for noticing those containers in which fermentation process is not running properly. This provides for better interpretation of results.

If the containers are not sealed well, respiration process of the aerobic microorganisms causes elevation of temperature and it can reach 70°C, although mostly it is between 35 and 40°C. This can cause condensation of water at the inner surface of the lid and drops of water are visible if container is transparent. This water induces moisture increase and development of butyric acid bacteria *(Antov et al, 2004)*.

The total number of moulds, percentage of the surface of the material covered with moulds and butyric acid content (BAC) are presented in Table 1.

**Table 1. Total number of moulds (TNM), percentage of surface covered with moulds (PSCM) and concentration of butyric acid (BAC)**

| Container | TNM | PSCM, % | BAC, % |
|---|---|---|---|
| K1 | 1000 | 15 | 0.00 |
| K2 | 10000 | 30 | 0.06 |
| K3 | 7000 | 7 | 0.18 |
| BP1 | 1000 | 5 | 0.00 |
| BP2 | 5000 | 30 | 0.02 |
| BP3 | 100000 | 95 | 1.11 |
| BF1 | 20000 | 5 | 0.04 |
| BF2 | 4000 | 15 | 0 |
| BF3 | 0 | 0 | 0 |

In containers BP1, BP2 and BP3 results for TNM had good correlation with visually determined PSCM. For the control samples, the highest PSCM was determined in the container K3 which corresponded with the highest TNM, but in the container K1, TNM was lower than in K3, although it was visible that container K1 was more contaminated with moulds than K3 (Figure 3). Similar situation can be noticed for the containers BF1 and BF2. This indicates that visual inspection of the silage in the container might be very important in results interpretation. It seems that in two out of three groups of containers, sampling for TNM determination was not adequate and if containers were not transparent, incorrect conclusions about silage process could have been drawn.

**Figure 3. Mini-silos on day 50**

Interesting situation concerning moulds growth was noticed for the container BP3 (Figure 4). About 95% of surface was covered with moulds and drops of water, as a result of respiration process and increase of the temperature of silage, were visible at the lid. Also in this container the highest butyric acid content was determined (1.11%). These negative changes of the silage were visible much

before sampling date and this mini-silo could have been excluded from investigation earlier.

An example of good maintenance of anaerobic conditions was container BF3 (Figure 5), without any mould growth, which corresponds to TNM and BAC results.

**Figure 4. Silage sample BP3**

**Figure 5. Silage sample BF 3**

# Conclusion

This investigation confirms that use of small-scale mini-silos is necessary for investigation of ensiling processes. It is important to prevent air penetration into the containers in order to enable proper ensiling. In the proposed mini-silo which is made of co-polymer PP with silicon on the edge the lid, and equipped with water valve, anaerobic conditions are insured, condition on proper compaction of forage material and the careful closing of the container.

Possibility of visual inspection of silage during fermentation, due to use of transparent mini-silo, provides for exclusion of samples that are strongly contaminated with moulds or that have drops of water on the lid as a result of improper sealing or damage of the container. By use of the proposed mini-silo, those undesirable changes in silage can be noted, which prevents wrong interpretation of experimental results.

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# Poboljšan mini-silos za ispitivanje fermentacije silaže u laboratorijskim uslovima

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## Rezime

Kao alternativa staklenim teglama, za laboratorijska istraživanja procesa siliranja u našem prethodnom istraživanju predložen je novi mini-silos izrađen od polipropilena (PP). Kod ovog sistema je postojala mogućnost ulaska vazduha, što dovodi do negativnih promena u silaži. U ovom istraživanju uneta su određena unapređenja u odnosu na prethodni sistem. Neprovidni PP kontejner zamenjen je providnim, napravljenim od PP kopolimera sa silikonskim slojem na obodu poklopca. Poklopac je opremljen specijalnim vodenim ventilom čija je uloga sprečavanje ulaska vazduha u silažu, dok omogućava izlaz gasova nastalih u toku procesa siliranja. Takođe, prozirnost kontejnera omogućuje praćenje rasta mikroorganizama (pre svega plesni) i bolje tumačenje dobijenih rezultata. Poboljšani mini-silos je testiran u eksperimentu sa lucerkom koja je sabijena u providne kontejnere. Kontejneri su podeljeni u tri grupe: u dve grupe su dodati komercijalni bakterijski inokulanti, dok je treća grupa bila kontrolna. Pedesetog dana je izvršena vizuelna procena stepena pokrivenosti površine silaže plesnima i u uzorcima silaže su određeni ukupan broja plesni i isparljive masne kiseline. Predloženi mini-silos je jednostavan, ekonomičan, praktičan i sprečava prodor vazduha u silažu.

## References

ANTOV G., ČOBIĆ T., ANTOV A. (2004): Siliranje i silaže. Poljoprivredni fakultet, Novi Sad.

ARCHIBALD J.D. (1946): Studies in the chemistry of grass silage. Agr. Res., 72, 277.

AUTREY K.M., KNODT C.B., WILLIAMS P. S. (1947): Grass and legume silage studies using two-quart glass jars as miniature silos. J. Dairy Sci., 30, 775.

BUCKMASTER D., LUNDMARK B. (2009): Bacterial inoculants for silage: http://www.abe.psu.edu/extension/factsheets/i/I111.pdf .

CHERNEY J.H., CHERNEY D.J.R. (2003): Assessing silage quality. Silage Science and Technology. Buxton D.R., Muck R. and Harrison J., ed. ASA, CSSA, and SSSA, Madison, WI., 141-198.

CHERNEY J.H., CHERNEY D.J.R., COX W.J. (2004): Fermentation Characteristics of Corn Forage Ensiled in Mini-Silos. J. Dairy Sci., 87, 4238-4246.

CULLISON A.E. (1948): The effect of stage of maturity and the addition of urea and cottonseed meal on the ensiling qualities of sweet sorghum and the feeding value of the silage. PhD Thesis, University of Illinois, USA.
ČABARKAPA I., PALIĆ D., PLAVŠIĆ D., VUKMIROVIĆ Dj., ČOLOVIĆ R. (2010): The influence of a bacterial inoculant on reduction of aerobic microflora during ensiling of alfalfa. Food and Feed Research, 37, 1, 23-26.
ČABARKAPA I., PALIĆ D., MILIĆ D., PLAVŠIĆ M., PLAVŠIĆ D., VUKMIROVIĆ Dj., ČOLOVIĆ R. (2010a): The influence of Bonsilage Plus and Bonsilage Forte on microflora reduction during ensiling of alfalfa. Food and Feed Research, 37, 2, 63-68.
ČOLOVIĆ R., VUKMIROVIĆ Đ., PALIĆ D., PLAVŠIĆ M., GLAMOČIĆ D., JAJIĆ I., JEREMIĆ D. (2010): A simple system for studying the fermentation dynamics of silages. Journal on Processing and Energy in Agriculture, 14, 3, 157-159.
DRIEHUIS F., OUDE ELFERINK S.J.W.H. (2000): The impact of the quality of silage on animal health and food safety: a review. Vet. Quart., 22, 212-217.
INTERNATIONAL ORGANIZATION FOR STANDARDIZATION (2008). Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of yeasts and moulds- Part 1: Colony count technique in products with water activity greater than 0,95. ISO 21527-1
JOHANSON H.E., MERRY R.J., DAVIES D.R., KELL D.B., THEODOROU M.K., GRIFFITH G.W. (2005): Vacuum packing: a model system for laboratory-scale silage fermentations. Journal of Applied Microbiology, 98, 106-113.
KNICKY M. (2005): Possibilities to improve silage conservation: Effects of crop, ensiling technology and additives. PhD Thesis, Swedish University of Agricultural Sciences, Sweden.
MCDONALD P., HENDERSON N., HERON S. (1991): The Biochemistry of Silage. 2nd edition, Aberystwyth, UK, Chalcombe Publications.
MEISKE J.C., LINN J.G., GOODRICH R.D. (1975): Types of laboratory silos and an evaluation of their usefulness. Proc. 2nd Intl. Silage Res. Conf., National Silo Assoc., Inc. Waterloo, IA., 99-126.
NEVENS W.B. (1933): Types and varieties of corn for silage. University of Illinois Agricultural Experiment Station Bulletin, 391.
STANAĆEV V., KOVČIN S. (2003): Hraniva i tehnologija stočne hrane i osnovi ishrane domaćih životinja. Univerzitet u Novom Sadu.
WEINBERG Z.G., ASHBELL G., HEN Y., AZRIELI A. (1993): The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. J. Appl. Microbiol., 75, 6, 512-518.
WILKINSON J.M., BOLSEN K.K., LIN C.J. (2003): History of silage. Buxton D.R., Muck R. and Harrison J., ed. ASA, CSSA, and SSSA, Madison, WI., 1-30.

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