

## Aspects of sugarcane laboratory ensiling and analysis techniques

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**Introduction** Fermentation in sugarcane silages is dominated by yeasts, resulting in high ethanol content in the forage and high dry matter (DM) losses. Usually, aspects of silage fermentation are evaluated in laboratory silos but studies on the effects of differences in laboratory silos on silage fermentation are scarce. Analysis of fermentation products is normally carried out in water extracts produced in a blender or stomacher and, less frequently, in silage juices produce by means of a hydraulic press. A direct comparison of these methods could not be found on the literature. The aim of this trial was to test the hypothesis that fermentation in sugarcane silages is not affected by the type of laboratory silo but, the recovery of ethanol and other fermentation products may be affected by the presence of valves in the silos and the method used to produce silage extracts.

**Materials and Methods** Sugarcane was ensiled in three different types of laboratory silos: 9.7 x 30 cm PVC tubes with tight lids fitted with rubber O-rings (seal rings), equipped or not with Bunsen valves, and 20 L plastic buckets with tight lids and Bunsen valves. The minisilos (five replicates) were weighed and the forage sampled on day 0 and 139 days after ensiling. Gas losses (GL) were estimated by difference between the weight of the minisilos immediately after sealing and at the end of the ensiling period, after removal of the adhesive tapes. Total DM loss (TDML) was calculated by DM weight loss in the silages. Three methods were used to produce silage extracts: a hydraulic press, a stomacher or a blender. Dry matter, CP, NDF, ash, GL and TDML data were analyzed as a complete randomized design and ethanol, acetic acid, lactic acid and pH as a complete randomized design on a 3 x 3 factorial design, with six treatments (three types of minisilos and three methods of silage extract preparation) and five replicates. All data were subjected to ANOVA by the GLM procedure of SAS (SAS, 2003). Differences among means were tested using LSMEANS and the *t* test ( $P < 0.05$ ).

**Results and Discussion** The type of laboratory silo did not affect DM, CP, NDF, ash and TDML in the silages but affected GL (Table 1). Gas losses corresponded to about 50% of TDML in the three types of silos and did not differ between PVC tubes with valves and plastic buckets with valves. Gas losses in the PVC tubes without valves were equal to buckets with valves and smaller than in PVC tubes with valves, indicating that the rubber O-rings did not prevent gases from escaping after the adhesive tapes were removed, at the time the silos were weighed with lids on. No interaction between silo type and method of silage extract production was observed for ethanol and organic acids contents in the silages but it was detected for pH. The type of silo affected ethanol content but did not affect the concentration of lactic and acetic acids in the silages (Table 1). Considering the similar composition and TDML of silages produced in the three types of silos, it may be inferred that fermentation was not affected by silo type and that ethanol production was the same in the different silos. Therefore, the variation in ethanol but not in lactic and acetic acids contents among silages might be explained by differences in the volatility of these components and in the ratio between the silage top surface area and the silage

mass in the different silos (TSA:SM). The PVC silos with valves had a higher TSA:SM what may have allowed more ethanol to escape through the valves relatively to the amount of DM in the silos, reducing its concentration compared to the silages in the buckets.

**Table 1** Chemical composition (g/kg DM; except when otherwise stated) and losses of sugarcane silages produced in different types of laboratory silos.

	PVC with valve	PVC without valve	Bucket with valve	SE
DM (g/kg FF)	262	262	255	7.2
CP	33.4	32.4	31.2	2.44
NDF	622	598	604	34.8
Ash	29.6	29.2	28.2	1.80
Gas losses	133a	118b	124ab	9.6
TDML	225	223	244	22.8

a, b, c, d - Means within a row with different letters differ by *t* test ( $P < 0.05$ ); FF: fresh forage

The method used to produce silage extracts affected the recovery of all fermentation products in the silages (Table 2). Recovery of ethanol and acetic acid was higher in silage extracts produced using a blender, compared to recovery in extracts produced using a stomacher or a hydraulic press. For lactic acid recovery, the hydraulic press method was superior to the other two methods. In an overall view, the recovery of fermentation products was more effective with the blender, intermediate with the hydraulic press and lower with the stomacher.

**Table 2** Fermentation parameters (g/kg DM) in sugarcane silages as affected by different types of laboratory silos and methods of silage extract production.

	Ethanol	Acetic acid (g/kg DM)	Lactic acid
PVC with valve	81.3B	38.2A	30.8A
PVC without valve	106.8A	34.2A	31.9A
Bucket with valve	116.3A	35.8A	33.2A
Blender	128.7a	43.3a	33.0b
Stomacher	75.1c	30.3b	18.9c
Hydraulic press	105.3b	34.5b	44.1a
SE	16.5	6.5	5.5

A, B, C ; a, b, c - Means within a column with different letters differ by the *t* test ( $P < 0.05$ ): capital letters within silo type and lowercase letters within type of silage extract.

All silages presented adequate pH but values were lower ( $P < 0.05$ ) in juices produced with the hydraulic press (3.36), compared to blender (3.57) and stomacher (3.61) for the three types of silos. The more efficient extraction of lactic acid may have led to a lower pH in these extracts compared to the other two methods. Among silos, pH was lower for silage produced in buckets, in extracts produced with a blender or a stomacher but not with a hydraulic press. This result cannot be explained since there was no difference in the concentration of acids among silos.

**Conclusion** It may be concluded that fermentation was not affected by type of silo but both type of silo and method of silage extract production influenced the recovery of fermentation products in the silages. Therefore, caution must be taken when comparing inter-experimental results and efforts should be made aiming the standardization of experimental procedures.