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A preliminary comparative study of conventional and hermetic storage of wet distillers grains with solubles

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ABSTRACT

The bioethanol industry has valuable by-products: for each litre of ethanol, 2 kg wet distillers grains with solubles (WDGS) and 0.75 kg of CO_2 are produced. WDGS is a product rich in proteins, which makes it valuable as feed for beef, milk, poultry and hog production, although its high moisture content (35% dry matter) reduces its storability. The objective of this study was to evaluate hermetic storage for extending storage time of WDGS. Two storage systems (hermetic and non-hermetic) and two storage times (10 and 20 days) were proposed and three replicates were considered for each combination of treatments. The WDGS samples were collected at the beginning of the experiment, 10 and 20 days of storage, and analysed for moisture content, appearance and odour, colony forming units (moulds and yeasts), *p*H, crude protein, ammonia nitrogen, ruminal degradability and intestinal digestibility of protein. It was concluded that WDGS could be hermetically stored without quality losses for at least 20 days, while in non-hermetic conditions, spoilage became noticeable after 10 days of storage.

Key words: Ensiling, Intestinal digestibility of protein, Moisture content, Moulds, Ruminal degradability of protein, Yeasts

In the past decade, production and consumption of biofuels had increased considerably; in 2009, global ethanol production reached nearly 75.7 billion litres in more than 40 countries (Rodriguez, 2013). Argentina is the seventh largest producer of bioethanol in the world, with an estimated production of 900 million litres in 2016, of which half are corn (*Zea mays* L.)-based ethanol. The bioethanol industry has valuable by-products: for each litre of ethanol, 2 kg wet distillers grains with solubles (WDGS) and 0.75 kg CO_2 are produced (Calzada and Frattini, 2015). WDGS is a product rich in proteins, which makes it valuable as feed for beef, milk, poultry and hogs production, although its high moisture content (m.c.) (35% dry matter) reduces its storability. The WDGS can be dried to obtain dried distillers grains with solubles (DDGS; 88% dry matter) increasing its storability; however, because of the drying cost, most of the time ethanol by-product is quickly sold as WDGS in a radius of approximately 300 km around the processing plants. The WDGS is stored at environmental conditions in piles on the bare ground or on concrete floor for 7 to 14 days (depending on ambient temperature) before intake (Di Lorenzo, 2013). This short storage time requires an almost continuous supply of WDGS from the ethanol plants to animal production farms. The logistics involved become very complex and expensive and prevents the extensive use of this material. In Argentina, the silobag technology is widely used for grains and silage storage (Bartosik, 2012), and it could also be adapted for storing WDGS. If WDGS storage time can be substantially extended using hermetic storage, logistic distribution could be improved and the cost reduced, expanding the use of this product. The objective of this study was to evaluate hermetic storage for extending storage time of WDGS.

MATERIALS AND METHODS

Samples of WDGS were collected in June of 2015 from three-bioethanol plants production, two of them located in Cordoba province and the last located in San Luis province, Argentina.

Samples were stored in sterilized plastics bins of 30 l capacity, and 12 to 24 h later (depending of sampling order) they arrived to INTA Balcarce Postharvest

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Laboratory. The samples were placed inside plastic bags, made with the constituent liners of a standard silobag (mix of linear and non-linear polyethylene, 235 microns thickness); each bag can hold 6 kg of WDGS. The bags for the hermetic treatments were thermo-sealed, and a pressure decay test (PDT) was done for checking its air tightness (Navarro and Zettler, 2000). The bags for the ambient condition treatments were left open, so normal gas (O_2 and CO_2) exchange was allowed. The bags were randomly placed in a storage chamber at 16°C.

A randomized complete block design was followed with two factors: storage type (hermetic and non-hermetic) and storage time (10 and 20 days). Each treatment was a combination of the two factors, and the three different WDGS origins (processing plants) were considered replicates (block).

During the sampling procedure (at 0, 10 and 20 days of storage), WDGS was transferred from the bag to sterilized plastic recipients, mixed and immediately analyzed:

– Moisture content of WDGS was determined with the Oven Method (ASABE, 2003): Three subsamples of 100 g weight in small metallic trays at 103°C during 24 h (constant weight).
– Sensory inspection (colour, odour, texture, foreign materials presence) was carried out as per Gallardo (2014).
– Fungal biota (moulds and yeasts) was evaluated using the method of counting in Petri dishes in potato dextrose agar (Britania®), with the addition of chloramphenicol (0.1% Anedra®). Plates were incubated in an oven at 28°C for 5 days (Pitt and Hocking, 2009). Counts were taken as colony forming units/gram of WDGS dry matter (CFU/g DM).
– Total nitrogen was determined by Dumas´ direct combustion method, according to Horneck and Miller (1998). Crude protein (% DM) was calculated multiplying total nitrogen by 6.25.
– Ruminal degradability and intestinal protein digestibility were carried out Gargallo et al. (2006).
– Ammonia nitrogen, as a percentage of total nitrogen content was determined using the colorimetric method, according to Weatherburn (1967).
– Relative humidity (r.h.) and temperature (outside and inside of bags) were hourly measured (Ibutton, Hygrochrom, EEUU).
– Gas composition (CO_2 and O_2) was measured every 48-72 h, employing a portable gas analyzer (Dan Sensor, Denmark).

The *p*H of samples was determined using a digital pH meter (Oakton, Singapore) as per Kaiser and Piltz (2003).

The data were analyzed with STATISTICA software, version 7 (Statsoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

The moisture content of WDGS was 71.4%, 73.0%

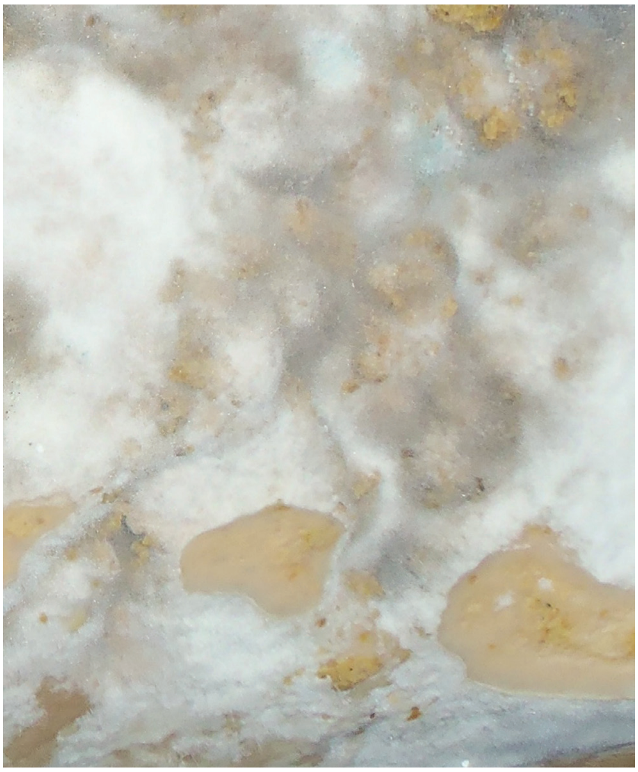

Fig. 1. Sensory evaluations of wet distillers grains with solubles after 10 days of storage for the hermetic (*left*) and non-hermetic (*right*) conditions

and 67.8% for the production facilities A, B and C, respectively. This m.c. range was found between the values reported by Rosentrater and Lehman (2007) (52–53%) and by Lehman and Rosentrater (2013) (75%). During the experiment no variation in moisture content was observed for hermetic and non-hermetic treatments.

During the experiment, the mean ambient r.h. was 55%, while inside the hermetic bag it was100% and 97–98% in the non-hermetic bag. Almost saturated r.h. conditions were also reported by Rosentrater and Lehman (2008) for storage of WDGS at 53% m.c. Average ambient temperature was 17°C, and mean temperature inside the bags was around 16.1°C.

At the beginning of the experiment WDGS, had an orange-yellow colour, texture compact and homogeneous, absence of moulds or yeasts, and soft and nice fermentation aroma (Fig. 1). After 10 days of storage, a discoloration of WDGS was seen in both storage conditions. Rosentrater Lehman (2008) also reported a colour change of WDGS after few days of storage. Hermetic bags showed presence of some visible yeast on product surface and no change in texture, while in non-hermetic bags unpleasant odours and fungi growth on product surface were evident and texture changed to a compacted mass with some lumps. After 20 days of storage, no change was observed in the WDGS hermetically stored, while total colonization of fungi, easily disaggregated texture and aggressive and putrid odors were observed for non-hermetic storage.

Oxygen (O_2) in all hermetic bags was depleted in a few hours. Fig. 2 shows the CO_2 concentration in hermetic bags of WDGS from the three bioethanol plants. The CO_2 concentration after 10 days of hermetic storage ranged from 30 to 37%, the highest value was from production facility C. Considering that in all hermetic treatments r.h.was 100%, regardless of the moisture content, differences in CO_2 production can be due to sample composition, *p*H, or initial inoculum concentration. For instance, mean *p*H value for facility C samples was about 4.3, while for the other samples the value was lower, around 4.0 (higher acidity). Probably this resulted in WDGS samples from facilities A and B to have a more restrictive pH condition for microbial development than WDGS from facility C.

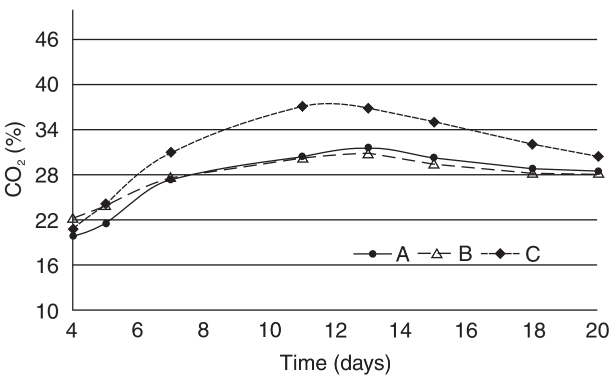

Fig. 2. Concentration of CO_2 (%) in hermetic bags for wet distillers grains with solubles from three bioethanol plants (A, B and C) from 4 to 20 storage days.

Table 1 Average across all storage times for moulds and yeasts counts (CFU/g DM) for hermetic and non-hermetic WDGS storage

	Hermetic storage	Non-hermetic storage
Moulds (CFU/g DM)	13.1^{a}	2×105^{b}
Yeasts (CFU/g DM)	7×105^{a}	9×106^{b}

Values in the same line with different letters denote significant differences (α=0.05).

Average initial microbiological contamination was 2.9 ×10^3 CFU /g DM for mould sand 1×10^6 CFU/g DM for yeasts. After 10 days of storage, mould and yeast counts decreased in hermetic storage and increased in the non-hermetic storage. The statistical analysis between 10 and 20 days showed that there were no significant differences in mould and yeast counts in any storage system. Table 1 reveals the average across storage time (10 and 20 days) for mould and yeast counts, showing that in hermetic bags there were smaller counts than in non-hermetic. In a similar study, Rosentrater and Lehman (2007) reported a lower initial count of total CFU (3 and 8 ×10^2 CFU/g DM of moulds and yeasts) at the beginning of storage, and after nine days of non-hermetic storage, counts increased to 1×10^8 CFU/g DM, but still below the counts reported in this study.

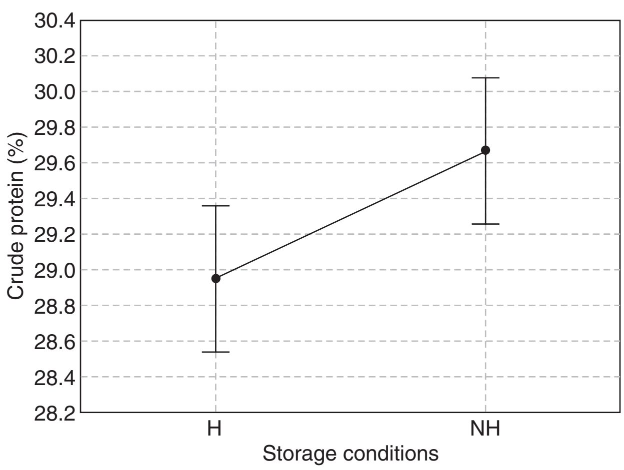

Fig. 3. Average across storage time for crude protein of wet distillers grains with solubes for hermetic (H) and non-hermetic (NH) storage conditions. [Bars denote confidence interval (0.95)]

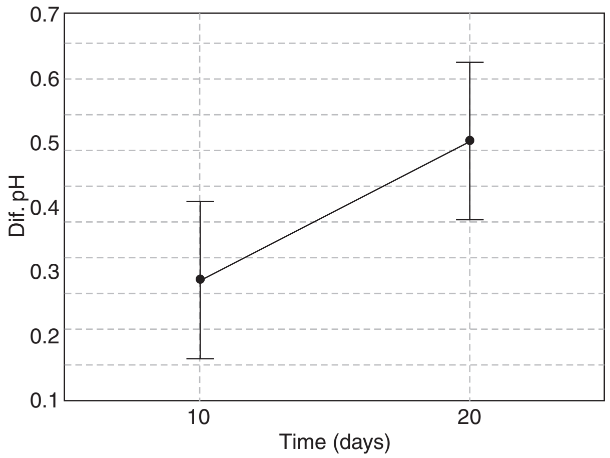

Fig. 4. Mean pH difference (Dif. pH) of 10 and 20 days of storage with the initial condition (time 0). [Bars denote confidence interval (0.95)].

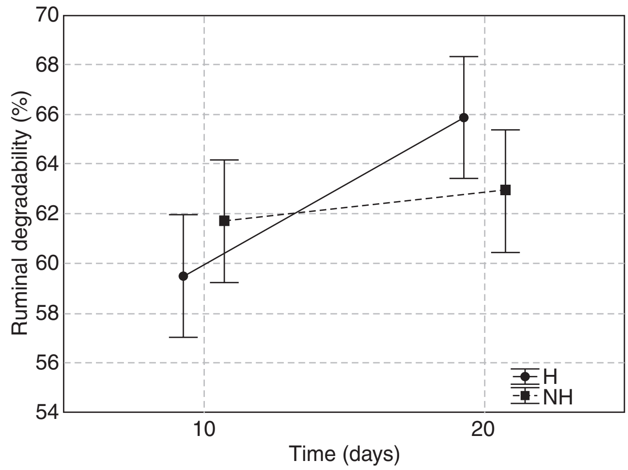

Fig. 5. Ruminal degradability of wet distillers grains with solubles in hermetic (H) and non-hermetic (NH) storage conditions, for 10 and 20 days of storage. [Bars denote confidence interval (0.95)].

The difference among storage systems for crude protein, is depicted in Fig. 3, indicating that under non-hermetic conditions it was about 0.7 percentage points higher. It could be speculated that under non-hermetic storage conditions, a larger DM loss proportionally increased the crude protein value.

Initial pH was 4.57. Typically, pH in WDGS is low due to the addition of sulfuric acid to stop fermentation during ethanol production (Mjoun et al., 2011). However, pH further decreased to almost 4 after 20 days of storage (Fig. 4). No difference was found in *p*H between hermetic and non-hermetic storage conditions.

Ruminal protein degradability increased from 10 to 20 days in hermetic storage condition from 60 to 66%, while in non-hermetic, it remained between 62 and 63% (Fig. 5). Intestinal protein digestibility was not affected by the storage system and storage time (mean value = 63.8%). If the trend of increasing ruminal degradability and intestinal digestibility remains stable over time, total digestible protein could increase in hermetic storage conditions. This is an important benefit from the nutritional point of view. Ammonia nitrogen was not affected by the storage system and storage time (mean value= 1.10%).

The rapid O_2 depletion in hermetic bags decreased strict aerobic microorganism, but not facultative aerobic microorganism, like yeasts. Oxygen depleting conditions and low *p*H allowed the ensiling process, which was also reported by other authors (Muck, 1988; Arias et al., 2012). Garcia and Kalscheur (2001) indicated that hermetic storage of WDGS succeeded most likely owing to the initial low *p*H rather to the subsequent fermentation process (little changes in volatile fatty acids). Along the same line, Mjoun et al. (2011) indicated that ensiled WDGS resulted in good conservation parameters during129 days of storage.

Superficial mouldy aspect and putrid odour increased during non-hermetic storage. However, inside the product, less deleterious changes were observed. Superficial spoilage can be explained by the presence of O_2, which most likely decreased towards the centre of the product mass due to the low gas diffusion of the compacted WDGS, which resulted in certain stability of the evaluated parameters. The high m.c., high product compaction and low pH allow speculation that some degree of ensiling process occurred inside the WDGS mass. This would indicate that the surface area exposed to the ambient air in non-hermetic storage of WDGS is critical, since spoilage reduces the palatability and nutritional value of WDGS and increases the potential for toxic effects (Rosentrater and Lehman, 2007).

CONCLUSION

Based on the results of this study, it could be concluded that WDGS could be stored at least for 20 days in hermetic conditions without significant quality losses and that an increase in ruminal protein degradability was found over time, while in non-hermetic conditions, spoilage and quality losses become evident at the surface after 10 day of storage.

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