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Evaluation of storage of sunflower pellets in silo bags

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Abstract

Silo bags are a temporary and potentially hermetic storage system widely used for grains, oil seeds, legumes and other products. Oil extraction of sunflower seeds through the extrusion-expelling process generates a high protein sub product, which is pelletized for animal consumption. Storing sunflower pellets in silo bags could bring logistical advantages to animal production farms and the sunflower processing industry. Thus, the objectives of this work were to: 1) study the effect of silo bag storage on chemical composition and fungal biota evolution on sunflower pellets; and 2) quantify the mechanical damage due to the loading and unloading operation on pellet stability. The study was carried out in a facility near Crespo (Entre Rios Province, Argentina), between February and October of 2018. Samples were collected during the loading (initial) and unloading (final) of the pellets to/from the silo bag. Samples were analyzed for fungal biota, mycotoxins, and composition (moisture content, protein, fat, and fiber). Temperature, relative humidity and CO_2 concentration were measured during storage. Mechanical damage was evaluated by measuring pellet length and dust percentage. Storage moisture content was around 9% (dry basis) and did not change during storage, while relative humidity remained below 60%. The evolution of CO_2 concentration was related to the pellet temperature inside the bag (summer time 14.3% CO_2 and 30°C, winter time 6.0% CO_2 and 15.3°C). Fungal colony counts in the initial samples were low ($1.2x10^1$ CFU/g DM) and slightly increased during storage ($3.4x10^2$ CFU/g DM). Low concentrations of DON (12.5 µg/kg) and Zearalenone (5.5 µg/kg) were found in initial sampling, and no increase was detected during storage. Pellet length before bagging was 27.1 mm and decreased to 24.0 mm after storage, while dust percentage did not change (14.9%). These results indicated the feasibility of storing sunflower pellets in silo bags without quality deterioration.

Keywords: Sub product storage, Chemical composition, Carbone dioxide, Fungal colonies, Moisture content, Mechanical damage, Hermetic storage

Introduction

In the last decade (2009-2019), global sunflower oil production has increased 72% (13.4 to 23.1 million tonnes) and sunflower meal (main sub-product of oil extraction) also increased from 14.8 to 24.7 million tonnes. In this context, Argentina is the third world producer of sunflower seeds and sunflower by-products, and an important trader in the global market (USDA, 2020).

Protein and crude fiber are the main components in sunflower meal, ranging around 20-60% and 5-34%, respectively. The residual oil varies depending on the extraction process, starting from less than 3% when solvent extraction is applied (Pedroche, 2002). The sunflower meal is usually pelletized through a high pressure and temperature process (Arelovich, 2003). Pelletizing meal works to maximize bulk density and minimize dusting, which thereby reduces the storage and transportation cost of the sunflower meal. Pelletizing also increases the physical stability of the meal and increases the allowable storage time before consumption (Heuzé et al., 2020). Consequently, minimization of pellet damage during transportation, handling and conveying is highly desirable (Aarseth and Prestl, 2003). Additionally, it is important to preserve the chemical stability and prevent mycotoxins production during feed storage (Pereyra et al., 2019).

The silo bag is a temporary storage system widely employed for grain storage in Argentina and several countries (Bartosik, 2012). With the increase of sub products availability (WDGS, meals and pellets), silo bags began to be considered as an alternative storage system for animal feed (Alvarez, 2016). Thus, the objectives of this work were to: 1) study the effect of silo bag storage on chemical composition and fungal biota evolution on sunflower pellets; and 2) quantify the mechanical damage due to the loading and unloading operation on pellet stability.

Materials and methods

The study was carried out in a facility near Crespo city (Entre Rios Province, Argentina) between February and October of 2018. A standard silo bag of 2.7 m diameter and 60 m long (with recommended stretching) was filled with approximately 160 t of sunflower pellets. During the loading operation, 5 kg of pellet were collected directly in the flow from the wagon to the bagging machine (Mainero 2230, Bell Ville, Argentina) in four sites (every 10 linear meters approximately). Temperature and relative humidity (RH) were registered (every 3 h) with integrated data loggers (Ibutton DS 1923, Maxim, San Jose, USA) placed in two sites at 20 m from the ends of the bag, at one meter deep from the bag surface. Carbon dioxide concentration was measured every month along every 6 m of the bag, starting the first measuring point at 3 m from the end employing a portable gas analyzer (Silcheck, Junín, Argentina). After eight storage months the silo bag was unloaded and pellet samples were collected directly in the flow from the unloading machine (Richiger Ea 180, Sunchales, Argentina) to a truck. The degree of wear of the augers of the bagging and unloading machines was documented.

Fungal biota (filamentous fungi and yeasts) was evaluated using the method of counting in Petri dishes in agar, potato dextrose (Britania®), with the addition of chloramphenicol (0.1% Anedra®). Plates were incubated in an oven at 28°C for 5 d (Pitt and Hocking, 2009). Counts were expressed as colony forming units per gram of pellets dry matter (CFU/g DM). Mycotoxin concentrations (Aflatoxins, Fumonisins, Zearalenone, Ochratoxins, Deoxynivalenol (DON)) were analyzed using LC MS/MS method (UPLC Acquity H Class / detector MSMS: Xevo TQ-S micro, Waters, USA).

Crude fats, protein and fiber were analyzed by NIRS (FOSS DS 2500, USA). Moisture content (m.c.) was determined with the oven method: three sub-samples of 100 g were dried at 103°C during 24 h (ASAE, 2003). Chemical composition results were expressed in dry basis percentage (% d.b.). Mechanical damage was determined by measuring the length of 70 pellets (randomly selected) with a caliper (Hamilton C 10, China), and dust fraction was separated by employing a grain sieve (1,8 mm opening diameter). Then, dust fraction was collected and weighted (OHAUS, Pioneer PA 214, USA).

Comparisons of treatments were performed with ANOVA (R software version 3.6.3). Tukey's HSD ($\alpha = 0.05$) post hoc test was also used for mean comparison. Figures were created with Excel (Microsoft Office Professional Plus 2016).

Results and discussion

Figure 1 shows that the temperature at the beginning of storage (middle of the summer) was above 30°C while the mean ambient temperature was 25°C. During the two following months, the pellet temperature inside of the silo bags decreased until reaching 25°C, while the mean ambient temperature remained almost stable. Then, pellets and ambient temperature decreased towards the wintertime, reaching a minimum value of 11 and 14°C for ambient and pellets, respectively. In September (beginning of spring), both temperatures started to gradually increase, equilibrating to the same temperature (18°C) at the end of September. The RH at the beginning of storage was of 59%, trended to decrease towards winter (52%) and increased in early spring (54%). The maximum CO_2 concentration (15%) was registered after one month of storage (end of March), decreased in winter reaching a minimum of 6% by the end of July and then increased to 12% by September.

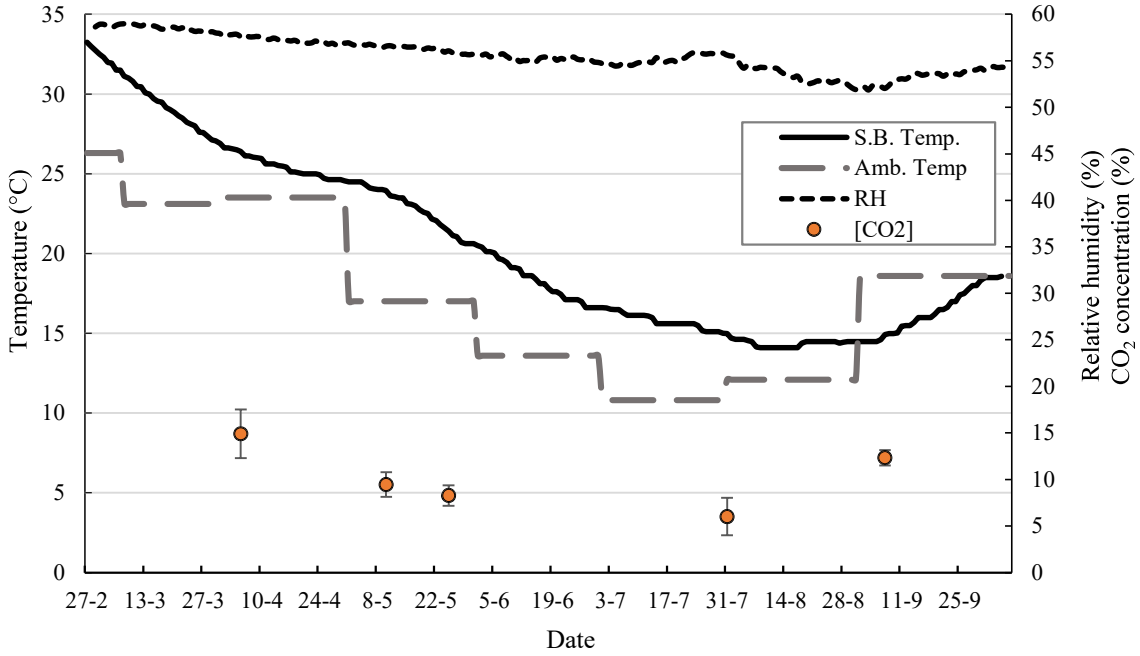

Fig. 1. Evolution of CO_2 concentration ([CO_2], %), relative humidity (RH, %), pellet temperature in silo bags (S.B. Temp., °C) and mean ambient temperature (Amb. Temp., °C), during silo bag storage of sunflower pellets. Vertical bars indicate SD.

Table 1 shows that pellets m.c. was near 9% (d.b.), the variability between sampling sites was low (SD less 0.5%) and did not change with storage time ($p>0.05$). Crude protein (mean= 36.9% d.b.), crude fiber (mean= 27.2% d.b.) and fat content (mean= 1.62% d.b.) also showed no variations with storage time ($p>0.05$).

Mean initial pellet length was approximately 27 mm (Table 1), and after pellet extraction the length decreased to 24.4 mm ($p= 0.0013$). Statistical analysis of dust fraction showed no variation between sampling time (mean= 14.8%; $p>0.05$). Variation between sampling sites at the beginning of storage (samples collected during the loading operation) was larger than at the end of storage (samples collected during the unloading operation) (SD= 7.7 vs 4.4 %).

Table 1. Chemical composition, pellet length and dust content (mean ± SD) during bagging (Initial sampling) and unloading operation (Final sampling)

Parameter	Initial sampling*	Final sampling
Moisture Content (% d.b.)	9.04 ± 0.44 **a**	8.60 ± 0.13 **a**
Fats (% d.b.)	1.81 ± 0.16 **a**	1.43 ± 0.52 **a**
Crude Fiber (% d.b.)	27.43 ± 1.26 **a**	27.04 ± 0.50 **a**
Crude Protein (% d.b.)	36.33 ± 2.87 **a**	37.37 ± 1.92 **a**
Pellet length (mm)	26.90 ± 0.61 **a**	24.40 ± 0.47 **b**
Dust (%)	13.55 ± 7.67 **a**	16.29 ± 4.39 **a**

*Different letters in same row indicate statistically significant differences between sampling times (p-value <0.05).

The initial count of fungal biota was 1.2 CFU/g DM (Table 2), and was mainly composed of *Alternaria sp.* and *A. flavus*. After storage, CFU count increased to 350 CFU/g DM ($p<0.0001$), while its fungal biota was composed of *A. flavus, A. niger,* and *Eurotium sp*. Table 2 also shows that the initial samples were contaminated with DON (12.5 µg/kg) and Zearalenone (5.5 µg/kg).

In both cases, the mean level of mycotoxin did not show variation with storage time ($p>0.05$). Moreover, the variation of contamination among sampling sites (SD) was larger than their mean values in both sampling times.

Table 2. Mold colony forming unit counts and mycotoxin contamination (mean ± SD) during bagging (initial sampling) and unloading operation (final sampling).

Parameter		Initial sampling*	Final sampling
Molds (CFU/g DM)		1.20 ± 1.40 **a**	347 ± 1.50 **b**
Mycotoxins (µg/kg)	DON	12.50 ± 16.20 **a**	10.25 ± 20.00 **a**
	Zearalenone	5.50 ± 6.47 **a**	3.80 ± 7.60 **a**
	Aflatoxin	**nd**	**nd**
	Fumonisin	**nd**	**nd**
	Ochratoxin	**nd**	**nd**

*Different letters in same row indicate statistically significant differences between sampling times (p-value <0.05); nd = mycotoxin non detected (detection limit: Ochratoxin: 10 µg/kg, others = 1 µg/kg).

The initial difference between ambient and pellet temperature could be explained by the high initial pellet temperature (due to the extrusion process). The temperature at which a product is bagged affects its temperature in the first weeks of storage (Cardoso et al., 2008). During the remaining storage time, the average pellet temperature followed the pattern of the average ambient air temperature. This influence of ambient temperature over the temperature of the product stored in silo bags had been previously documented (Bartosik et al., 2008; Bartosik 2012).

Temperature has a direct effect on microbial growth and also an indirect effect through the equilibrium RH (Magan et al., 2003). As temperature decreased toward winter, so did the equilibrium RH, even when the pellet m.c. did not change during storage.

The biological activity of the pellet, measured as CO_2 concentration, also followed the temperature pattern throughout the season (Fig. 1), this being a consequence of the effect of the temperature on the respiration rate of grains and byproducts (Ochandio et al., 2017). Rodríguez et al. (2008) found a similar pattern in the evolution of the CO_2 concentration through the seasons in wheat silo bags.

The CO_2 concentration in the first few storage months was similar to that reported by Bartosik et al. (2008) for sunflower seed stored in silo bags at a similar m.c. (16.5% CO_2 for 9.2% m.c. seed (d.b.)). However, since the oil content of the sunflower seed is substantially higher (around 50%) than that of the pellet (<2%), the equilibrium RH for 9% sunflower seed is also higher (close to 70% according to Maciel et al. (2018) than that measured for pellet in this study (52-58%). Therefore, it would be expected that for the same m.c., both the microbiological activity and the respiration of the sunflower pellets would be lower than that reported for sunflower seeds. The low initial CFU count could be due to the previous extrusion-expelling process and the subsequent pelletizing. The high temperature and pressure of those processes affect vegetative microorganisms to a great extent, and mold spores to a lesser extent (ICMSF, 2006). Therefore, when pelletizing is carried out correctly, the product results in low mold CFU counts ($<10^2$ CFU/g; IAG, 1993).

The initial biota was mainly composed of *Alternaria* and *Aspergillus* species, typical oilseed fungi (Stroka and Gonc, 2019). While *Alternaria* and *Aspergillus* species can colonize the grain in the field and produce mycotoxins, *Alternaria flavus*, in particular, is regarded as a storage mold (Magan and Lacey, 1988). However, the presence of this mold was not related to aflatoxin contamination of the initial samples. Zearalenona and DON are produced by the *Fusarium graminearum* and *F. culmorum* species (Miller, 1995) that typically colonize grain in the field (Fleurat-Lessard, 2017). The *Fusarium* species were not identified in initial samples; thus, it could be speculated that the contamination was produced before harvest.

Even though the equilibrium RH of the sunflower pellet was below the limit reported for filamentous fungi growth (70%) (Magan and Lacey, 1988), in the final samples some *Eurotium* sp. and *Aspergillus* sp. (xerotolerant storage mold (Fleurat-Lessard, 2017)) were found. During long term storage, the daily and seasonal changes in temperature often result in moisture migration in the product mass, creating favorable conditions for fungal growth in the silo bag periphery (Cardoso et al., 2007). In fact, during pellet extraction, a slight moisture condensation was observed in some areas where an air chamber between the plastic cover and the pellets was created. The equilibrium RH limit for fungal growth (70%) is lower than the limit for mycotoxins production (83-85%) (Fleurat-Lessard, 2017). Thus, it can be hypothesized that the xerophilic conditions during storage allowed the survival of *A. flavus* but prevented the development of Aflatoxin. Zearalenone and DON levels found in this study were lower than those reported by Boevre et al. (2012) in sunflower meal (81 µg/kg and 23-24 µg/kg, respectively). In general, the mycotoxin levels detected in the current study were below the maximum level recommended for feed by the European Commission (DON: 900 µg/kg; Zearalenone: 100 µg/kg, Aflatoxin: 5 µg/kg and Ochratoxin: 10 µg/kg), according to Stroka and Gonc (2019). On the other hand, the high variability in mycotoxin concentration among replicates could be due to a sampling method error or the heterogeneity of the matrix (FAO, 2003).

The moisture and fat contents of this study were lower than those reported by other authors for sunflower pellets (Arelovich, 2003) and sunflower meal (Heuzé et al., 2020), and this could be the reason for the high stability of the pellets during storage (Le Cleft and Kemper, 2015).

The reduction in the length of the pellets during the loading and unloading operation, although statistically significant, was only 2.5 mm (from 26.9 to 24.4 mm), remaining within what is considered to be a standard size (between 10 and 30 mm) according to Clef and Kemper (2013). This was probably due to the good condition of the bagger and extractor machine augers. Hidalgo et al. (2009) reported that auger characteristics, such as wear condition, length, and angle of operation, were the most important factors in grain breakage during rice loading and unloading operations. The amount of dust in the initial samples was high because the pellets were previously stored in a metal bin, so there was a previous handling of the product. It is also possible that the previous handling of the pellets caused a localized dust concentration in some sectors of the metal bin, which subsequently translated into dust concentration variability in the silo bag.

These results showed the feasibility of storing sunflower pellets in silo bags without quality deterioration.

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