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Development of microorganisms during storage of wet brewery waste under aerobic and anaerobic conditions

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ABSTRACT - This research study was conducted to evaluate, by means of microbiological analyses, the preservation of wet brewery waste stored under aerobic and anaerobic conditions, regarding the development of filamentous fungi, yeasts and lactic-acid bacteria. The following treatments were used: untreated brewery waste, pre-dried brewery waste silage, brewery waste silage with effluent drainage and BW silage without effluent drainage. Silos made of PVC and equipped with Bunsen valves to allow gases to escape were used. Dry matter (DM), crude protein (CP), temperature (°C) and pH in the untreated BW, in the stored brewery waste and in the brewery waste silage upon silo opening, after 60 days of ensilage were analyzed. A completely randomized design was used. The data were subjected to analysis of variance, and the means were compared by the Tukey test at the 5% probability level. The preservation of brewery waste packaged under aerobic conditions was not appropriate due to the development of filamentous fungi and yeasts; however, storage under anaerobic conditions proved to be an effective conservation process.

Key Words: filamentous fungi, lactic acid bacteria, silage, yeasts

Introduction

The need to produce feed for ruminants, whilst at the same time to reduce costs and maximizing profitability, propels the search for new food sources and highlights the possibility of using agro-industrial by-products for this purpose without compromising animal production.

Wet brewery waste is an alternative food source widely used for ruminants, which is generated in large amounts during the production of beer (Cabral Filho et al., 2007). It can be obtained throughout the year at a low cost, minimizing the costs of the animal feeding.

The main limiting factor for effective use of wet brewery waste is its low dry matter content, which hinders the transport, storage and preservation of this residue. Data published in the literature showed dry matter levels ranging from 9% to 30% (López & Pascual, 1981; Cardoso et al., 1982; Geron et al., 2007). The high moisture content and storage of wet brewery waste under aerobic conditions, as commonly used on farms, can provide ideal conditions for the development of microorganisms such as filamentous fungi and yeasts, promoting the degradation of the waste stored under these conditions (Allen et al., 1975).

An efficient alternative for the storage of wet brewery waste would be the ensilage process, which ensures anaerobic fermentation, the production of organic acids, reduction in pH and, consequently, preservation of the quality of ensiled material.

According to Arcuri et al. (2003), the presence of filamentous fungi has pronounced effect on the nutritional value of silage because they degrade it and can produce toxins that affect the animal metabolism. They can utilize lactic acid and sugars, competing with lactic acid bacteria at the start of the fermentation process, forming ethanol, which causes a loss in the dry matter content and has no useful properties for the preservation of the silage (Rotz & Muck, 1994).

Few assessments of microorganism populations present in preserved foods and their impact on the nutritional quality of food have been undertaken in Brazil, perhaps because of the shortage of specialized laboratories in this area (Jobim et al., 2005). The identification of such microorganisms could assist in assessing the quality of feed for ruminants. Thus, this research was performed in order to assess the microbiological characteristics of wet brewery waste under aerobic and anaerobic storage conditions.

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Material and Methods

This experiment was performed in the Estação Experimental Prof. Dr. Antônio Carlos dos Santos Pessoa, in the Laboratórios de Análises de Alimentos, Nutrição Animal, Microbiologia e Bioquímica, Universidade Estadual do Oeste do Paraná *campus* of Marechal Cândido Rondon - Paraná.

The wet brewery waste used was obtained from a brewing industry in the city of Marechal Cândido Rondon - Paraná. Microbiological analyses were performed to evaluate the preservation of wet brewery waste under aerobic and anaerobic conditions.

In order to simulate aerobic conditions, the wet brewery waste was placed into plastic buckets with a capacity of approximately 10 kg, and the microbiological analysis was performed by periodically counting the filamentous fungi and yeasts.

There were six treatments with three replicates, distributed in a completely randomized design. The treatments were: untreated wet brewery waste with a microbial count before storage; wet brewery waste placed into buckets without a lid, where the microbes were counted after 7 days; and treatments with wet brewery waste packed into buckets with a lid, where the microbes were counted after 7 days , 14 days, 21 days and 28 days .

The microorganisms in the treatment without a lid were only counted on the 7 days because extensive deterioration of the wet brewery waste prevented their evaluation after this period.

In order to simulate anaerobic conditions, the wet brewery waste was ensiled and the development of microorganisms was evaluated by counting the filamentous fungi, yeasts and lactic acid bacteria. The treatments were: untreated wet brewery waste, with a microbial count before ensilage wet brewery waste; wet brewery waste silage that was pre-dried in the sun; wet brewery waste silage with effluent drainage and wet brewery waste silage without effluent drainage.

This part of the experimental design was completely randomized with four treatments and four replicates.

Ensilage of the wet brewery waste was performed in experimental silos consisting of PVC, 50 cm high and 10 cm in diameter, with a capacity of about 3 kg. The wet brewery waste was compacted and the silos were sealed with caps and fitted with Bunsen valves for the free escape of gases (Schefer de Rojas, 1976).

Before ensilage, to obtain the silage of wet brewery waste pre-dried in the sun, the wet brewery waste was pre-dried in the sun and the dry matter content was determined in a microwave oven (Pastorini et al., 2002). For the drainage of effluent, in the silage of wet brewery waste with effluent drainage, adapted to an 8-mm tube at the bottom of the silo. For the silage of wet brewery waste without effluent drainage, the wet brewery waste was ensiled under natural conditions.

After 60 days of storage, silos were opened and contents were removed and placed into trays for homogenization of the silage. Samples were only collected from the central portion of the silage mass in order to avoid the influence of silage extremities affecting the results of the analysis.

The pH was measured using a digital pH meter, where 100 mL of distilled water were added to 10 g of the sample, according to the method of Cherney and Cherney (2003), and was left for 1 hour before reading. Temperature was measured using a portable thermometer, and the microbiological analysis of the wet brewery waste untreated was performed before storage, in the wet brewery waste placed in buckets and in the ensiled wet brewery waste.

For the microbiological analysis, 450 mL of sterile distilled water were added to 50 g of the sample under agitation. From the solution obtained, 1 mL or 0.1 mL was removed through a pipette to provide successive dilutions of 10^1 to 10^7 in test tubes containing 9 mL of distilled water. After this, 0.1 mL of the diluted extracts was placed onto each Petri dish. Three Petri dishes were used for each culture and dilution medium and the microbial count and results were analyzed as described by González & Rodríguez (2003).

Microbial populations in the wet brewery waste untreated and the wet brewery waste stored under aerobic and anaerobic conditions were determined using culture techniques according to Silva et al. (1997). PDA agar (potato dextrose agar) was used, acidified with 10% tartaric acid solution, and yeasts and filamentous fungi were distinguished by the physical characteristics of the colonies; Petri dishes were kept at room temperature for 5 to 7 days. In order to quantify lactic acid bacteria, MRS agar (De Man, Rogosa and Sharpe) was used after incubation in an oven for 48 hours at 37 °C.

After the incubation period, colonies were counted using a "Quebec" colony counter, counting Petri dishes alone with 25 a 250 CFU (Colony Forming Unit); results were obtained through average from the Petri plates, for the dilutions selected, and expressed in log.

Data were transformed into logarithms and subjected to analysis of variance, and the Tukey test was used to compare the means at the 5% probability level.

Results and Discussion

The average dry matter (DM) content of the wet brewery waste was higher (P<0.05) than in the other treatments (Table 1). This result was due to the large population of yeasts, which had developed in the residue during the fermentation period (Figure 1). According to McDonald et al. (1991), alcoholic fermentation is intense in silage with a high content of soluble carbohydrates and a large population of epiphytic yeasts, which convert the carbohydrates into ethanol, CO_2 and water. This process causes an excessive loss of DM, which can cause a loss of up to 48.9% DM (McDonald et al., 1991), and reduces the nutritional value of the silage (Alli et al., 1982).

The highest value of CP, observed at 28 days, was due to the prevalence of homofermentative microorganisms, which are characterized by a faster rate of fermentation with less proteolysis (Zopolatto et al., 2009). Antunes et al. (2006), working with maize hybrids at different times of silo opening, also observed a reduction in the CP content during the first few days of fermentation for one of the hybrids studied. Generally, the CP content remains stable during ensilage (McDonald et al., 1991). However, the biochemical profile of the CP radically changes, due to intense proteolysis promoted by proteases of the ensiled material itself and the microorganisms in the silo, on the first day (Ohshima & McDonald, 1978).

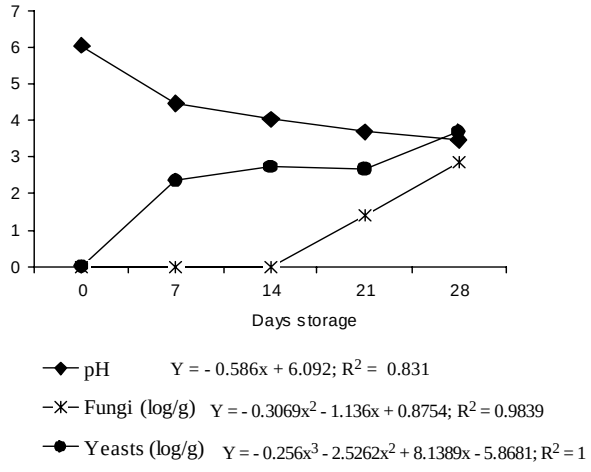

Figure 1 - Average pH values and the development of filamentous fungi and yeasts (log/g residue), as periodically evaluated in the wet brewery waste.

The temperature of the treatment with microbes counted after 7 days was higher (P<0.05) than wet brewery waste packed into bucks with lid, with microbes counted after 7, 14, 21 days, and equivalent to that of 28 days, which did not differ (P>0.05) from the others. The highest (P<0.05) pH value was observed for wet brewery waste, and the smallest, for the wet brewery waste in buckets with lids and microbes counted after 28 days. This did not differ from that with microbes counted after 21 days, and the latter was equivalent to that of 14 days. The pH decreased in the treatment with microbes counted after 7 days; however, it did not prevent the development of filamentous fungi and yeasts (Figure 1), because yeasts can even develop equally in 2.0-pH environments (McDonald et al. 1991).

According to McDonald (1991), high temperature and non-reduction of the pH stimulates the growth of undesirable microorganisms. However, the optimal pH for wet brewery waste preservation is dependent on the humidity and temperature of the material. In silages with a dry matter content greater than 20%, pH 4 is acceptable for satisfactory preservation, because the pH range that characterizes good quality silage is from 3.8 to 4.2 (McDonald, 1991).

No difference (P>0.05) was found between wet brewery waste, the treatment with microbes counted after 7 days and 14 days in the development of filamentous fungi (log/g of residue), possibly due to the short storage period and the low level of air exposure. However, there was accentuated growth of filamentous fungi (P<0.05) as a consequence of the greater exposure to the air in the treatment with microbes

Table 1 - Average contents of dry matter (DM%), crude protein (CP% DM), temperature (T) and pH and the development of filamentous fungi and yeasts (log/g residue) in wet brewery waste (BW)

Parameters	BW	BWiL7	BWL7	BWL14	BWL21	BWL28	CV%
DM	24.15a*	22.46b	23.15b	20.99c	19.64d	20.71c	1.43
CP	27.35a	26.91a	25.01b	24.06b	26.51a	26.57a	2.35
T	27.00b	32.00a	29.00b	28.00b	28.00b	29.50ab	3.46
pH	6.03a	5.42b	4.45c	4.02d	3.69de	3.48e	3.46
Fungi	0.00d	6.92a	0.00d	0.00d	1.38c	2.84b	3.77
Yeasts	0.00e	7.79a	2.36d	2.72c	2.66c	3.67b	2.32

BWiL7 - BW placed in buckets without a lid for 7 days.
BWL - BW placed in buckets with lids for 7, 14, 21 and 28 days.
CV - coefficient of variation.
*Means followed by the same letter in the row do not significantly differ (P>0.05) by Tukey test.

counted after 7 days (6.92 log/g residue) and the longest storage periods in the treatment with microbe count after 21 (1.38 log/g residue) and 28 days (2.84 log/g of residue).

According to Pereira & Reis (2001), the observation of fungi in silage is mainly associated with inadequate compaction, which allows air to enter the silo. Jobim and Gonçalves (2003) reported that the presence of oxygen in the silage mass promotes the action of spoilage microorganisms and the reduction of soluble sugars and organic acids, resulting in an increased pH level.

In the wet brewery waste, no yeasts were found, but a higher number was counted ($P<0.05$) in the treatment with microbes counted after 7 days (7.79 log/g of residue), possibly due to the higher level of air exposure and the higher temperature and pH. These characteristics reflect the deterioration of the material, which is usually manifested by an elevation in temperature, a change in the odor and appearance of mould (McDonald et al., 1991). The optimum development of yeast occurs at temperatures from 20 to 30 °C (Ashbell et al., 2002). In the treatment with microbes counted after 28 days, a higher number of yeasts ($P<0.05$) were found compared with that of 21 days, which showed an equivalent number to the treatment with counting after 14 days, probably due to the longer storage period.

The high population of yeasts in the treatment with counting after 7 days could cause deterioration of the wet brewery waste and promote loss of dry matter.

The wet brewery waste stored under aerobic conditions for 7 days presented visible changes, including unpleasant odor and high temperature and pH, which could contribute to a marked proliferation of filamentous fungi and yeasts, causing large nutrient losses in the wet brewery waste and feeding risks to the animals due to the likely production of toxins.

T values of wet brewery waste and wet brewery waste ensiled did not differ ($P>0.05$) (Table 2).

Significant differences were found in the pH values ($P<0.05$). wet brewery waste presented a higher pH, whereas the silages (wet brewery waste pre-dried in the sun, with and without effluent drainage) showed lower values, evidencing a proper fermentation pattern. The pH values of the silages were lower than pH 4.2, which is considered ideal because it is indicative of the possible inhibition of undesirable microorganisms responsible for secondary fermentation (McDonald, 1991).

The final pH in the silage cannot be considered an isolated criterion for evaluating the silage because the inhibition of secondary fermentation also depends on the velocity of reductions in the pH, ionic concentration and humidity of the ensiled wet brewery waste (Woolford, 1984). Thus, one must assess the speed of pH reduction by assessing the silage fermentation profile. However, according to Cherney & Cherney (2003), the pH can still be considered a good indicator of the quality of fermentation in silage with a low DM content.

An increase ($P<0.05$) was found in the development of filamentous fungi in the silages (wet brewery waste pre-dried in the sun, with and without effluent drainage) compared with wet brewery waste. The results of the development of filamentous fungi are similar to those obtained by Jobim et al. (1999) for corn cob silage (2.6 log/g silage) and surpass those obtained for grain silage (1.9 log/g silage).

According to Guerra et al. (2005), the increase in silage pH can promote the proliferation of undesirable microorganisms (fungi). In silages with a pH>4.0, the presence of large quantities of *Listeria* (10^4 CFU/g) can be observed (Fenlon, 1996; Skovaard & Morgen, 1988; Ryser et al., 1997).

Yeast was not observed in wet brewery waste or silage with effluent drainage or silage without effluent drainage. It is possible that the size of the particles in the wet brewery waste provided better compaction, inhibiting the growth of yeast as a consequence of lower oxygenation, as reported by McDonald (1991). On the other hand, materials with a high DM content can compromise compaction and promote oxygenation, which explains the growth of yeasts ($P>0.05$) in silage of wet brewery waste pre-dried in the sun.

Yeast development results found in this study were lower than those obtained by Jobim et al. (1999), who observed the development of yeast in the order of 6.4 log/g for high-moisture corn silage.

Table 2 - Averages of dry matter (DM%), crude protein (CP% DM), temperature (T) and pH and development of filamentous fungi, yeasts and lactic acid bacteria (LAB) (log/g residue)

Parameters	BW	SilPDS	SilED	SilWED	CV%
DM	23.85	49.62a*	24.13b	20.41c	5.94
CP	24.79	23.73ab	23.24b	25.40a	3.82
T	27.00a	30.00a	30.00a	30.00a	6.24
pH	6.03a	4.05b	2.86c	3.57b	6.69
Fungi	0.00b	2.89a	2.33a	2.45a	22.29
Yeasts	0.00b	1.71a	0.00b	0.00b	
LAB	6.21ab	6.62ab	5.78b	7.26a	8.64

BW - untreated wet brewery waste.
SilPDS - silage of wet brewery waste pre-dried in the sun.
SilED - silage with effluent drainage.
SilWED - silage without effluent drainage.
CV - coefficient of variation.
* Means followed by the same letter in the row do not differ ($P>0.05$) by Tukey test.

Woolford (1990) noted that silages with more than 5.0 log of yeast/g of silage are the most susceptible to deterioration. The presence of yeasts in the order of 10^6 CFU/g in the ensilage process is considered undesirable because it does not contribute towards acidification and is associated with the aerobic deterioration of silage and high dry matter losses due to consumption of soluble sugars (Alli et al., 1983).

After opening the silos, if the silage contains 10^6 yeast CFU/g can, in two or three days time, reach 10^9 CFU yeast/g, and the silage be considered low stability (Muck et al., 1992).

The lactic acid bacteria population was higher ($P<0.05$) in the silage without effluent drainage and lower in the silage with effluent drainage, and did not differ from wet brewery waste and silage of wet brewery waste pre-dried in the sun (Table 2). The results found in this survey were lower than those recommended by McDonald et al. (1991), about 8.0 log/g in the ensiling, a population considered appropriate for sharp fall in pH.

However, Driehuis et al. 2001 and Whiter & Kung Junior (2001) observed initial populations of LAB between 3.7 and 6.3 log/g in various materials, without compromising the conservation of the material. Thus, the population observed in this study (Tabela 2) is located within a good range for wet brewery waste silage preservation.

In forages, the population of lactic acid bacteria is low, and the preservation of forage in the silo depends on the activity of these microorganisms, which ferments sugars to produce lactic acid and other products (Reis et al., 2004). Bernardes (2003) observed that the population of lactic acid bacteria present in the material, during ensilage, was not appropriate for an adequate production of lactic acid; however, from the first day of fermentation, the number of lactic acid bacteria increased, providing rapid acidification.

Data on the microbial development in the sorghum, wheat, corn, alfalfa and grass silages in general have been widely reported by several researchers (Lindgren et al., 1985; Umana et al., 1991; Taylor & Kung Junior, 2002; Kung Junior et al., 2003; Filya et al., 2004; Nishino et al., 2004). The results of these studies were similar regarding both yeast and lactic acid bacteria populations; however, each material ensiled had a different microbial profile, depending on the epiphytic flora of the substrate available for fermentation and the DM content.

Conclusions

The storage of wet brewery waste under aerobic conditions is not appropriate due to the pronounced development of filamentous fungi and yeasts, as characterized by their deterioration. However, ensiling is an efficient process for the preservation of wet brewery waste.

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