



**Full Length Article**

## Nutritive, Fermentative and Environmental Characteristics of Silage of Two Industrial Broccoli (*Brassica oleracea var. Italica*) By-products for Ruminant Feed

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### Abstract

Boiled full inflorescences (BB) and Raw stems (RB) of broccoli (*Brassica oleracea var. Italica*), as industrial by-products, were ensiled in laboratory-scale silos and were sampled at different times. To evaluate its suitability as feed, the fermentative, chemical and different pesticide parameters were determined. The environmental pollution effect and the volume of released effluent were determined too and the effluent prediction equation was estimated. Both by-products presented high moisture content initial (73.2 and 103.9 g kg<sup>-1</sup> DM in RB and BB, respectively) and they produced a very high effluent volume, BB released 78.97 and RB 285.65 mL kg<sup>-1</sup>, respectively. The best equation to predict the effluent production was the reciprocal model ( $r^2 = 0.920$ ) from where can be seen that small increases on moisture content can produce very important elevations on effluent production. Both silages showed that effluent had strong potential pollution (22.97 and 39.06 gO<sub>2</sub> L<sup>-1</sup> of BOD, respectively). Levels of different pesticides were not found in BB or RB silages. The by-products showed that can be effectively ensiled under laboratory conditions. © 2014 Friends Science Publishers

**Keywords:** By-product; Silage; Broccoli; Nutritive quality; Environmental pollution

### Introduction

Different areas of the world have an adverse climate for high quality pastures production but have a high horticultural production. One of the crops that more has increased in recent years is the broccoli (19,872,263 t on the world, in 2009). The Mediterranean area and Southern Asia represent 40% of this production (Mongabay, 2013). Also the improvement of the cultivation techniques and increased consumer demand for its dietary properties has produced a greater increase in the quality and quantity of cultivated broccoli (Ouda and Mahadeen, 2008). This increase on vegetable production, through intensified agriculture, is causing a raise on the amount of by-products derived during the picking season, a very short period of time. Bearing in mind that the major vegetable production is performed in warm areas and that these by-products have high water content, it is necessary to perform their immediate disposal avoiding their fast deterioration and therefore, a powerful environmental contamination. These by-products can be seen as an alternative for small ruminants feeding, so, this use may be a solution for reducing the economic and environmental impacts (Márquez *et al.*, 2010) although a

biological transformation of them is needed. Some by-products have shown to possess features that enable their silage, when a proper management and storage targeted to prevent the production of effluents is made (Megías *et al.*, 1999).

In this sense, the high production of effluents is considered as one of the critical points in carrying out the silage of high humidity by-products because is a strong pollutant for the environment (Arnold *et al.*, 2000) and it can even act as a transmission vehicle of DNA from GMOs (Duggan *et al.*, 2000). The total volume of effluent of the ensiled crop oscillates with several important factors such as nature, maturity, pre-treatment of the crop; species, age, fertilizer regime or climate (Jones and Jones, 1995) and the effect of the use of silage additives (Haigh, 1994). Even, the respiration and fermentation process affects the effluent production (Van der Wel, 1993). Other causes that must be also taken into account are: the stored material, the nitrogen content of forage, the pre-treatment of the crop and handling and storage conditions like the degree of chopping and consolidation at ensiling, the type of the silo and the amount of rolling after ensiling. Even the pressure on the material (Waldo, 1977) and the industrial processes also increases

the effluent production when canning factories by-products are ensiled (Megías *et al.*, 2002). However, the total quantity of effluent that is produced depends principally on the dry matter content of the forage before the ensiling process (McDonald *et al.*, 1991). Guimaraes Faria *et al.* (2010) have shown decreases on volume of effluent production when dry matter content was elevated to include absorbents into the silage. The DM content must be more than 300 g kg<sup>-1</sup> for safe storage in horizontal silos and more than 350 g kg<sup>-1</sup> for safe storage in tower silos to minimize risk of seepage (Bastiman and Altman, 1985). In addition, silage effluent is the most pollutant waste produced in a farm, in terms of oxygen demand. In order to determine the prediction equation of the effluent flow released on the basis of DM content, several authors have used different models: linear (Sutter, 1957; Miller and Clifton, 1965), quadratic (Zimmer, 1967; Bastiman and Altman, 1985) or exponential (Haigh, 1999) as is described in Table 1. On the other hand, to predict the effluent production Hameleers *et al.* (1999) fitted curvilinear equations when different pressures on the material or different additives were used. The prediction equation in these cases are made for crops with low or medium content of DM but no information has been found about how is produced the flow of the effluents in high moisture content silages.

Other important problem associated with the use of by-products for animal nutrition is the variety of such by-products. Even the same plant can provide wastes with different properties depending on the part of it that is used or the treatment received in origin and the presence of different phytosanitary products (insecticides, herbicides, bactericides, fungicides, etc.) used to ensure the success of the intensive crops. The objective of this study is to know the possibility of using of two agro-industrial by-products of broccoli with different nature and processed through the use of silage. There will be an assessment of its potential as contaminant, common pesticides content and features both fermentative and nutritive for its use in feeding small ruminants.

## Materials and Methods

### By-products

Two different broccoli by-products (*Brassica oleracea var. Italica*) are the residues from the industrial process for preservation of broccoli inflorescence. The first by-product (BB) is constituted by the full inflorescences and little stems that do not pass the quality control after washing and scalding at 90°C for 20 min. The other by-product is constituted by the raw stems (RB), the major stem of the plant (20 cm length) that was chopped by hand before the plant went into the industrial process. Both materials were carried to the laboratory and drained for 12 h to allow the by-products to eliminate the liquids accumulated during its

industrial processing. RB was chopped mechanically until 4 cm long pieces to facilitate the silage compaction. After that, polyethylene containers of 12.5 L capacity and 268.8 mm bottom sections were filled with each by-product and sealed, except for a device at the base to collect the effluent.

### Methods

The silos were stored indoor and were opened and sampled after 1, 2, 3, 4, 8, 12, 24 and 50 days. The zero day corresponds to the initial material after ensiling. These days, the effluent was removed and the effluent production was measured, the analysis was carried out introducing a thermometer into the middle of the ensiled mass at 11:00 a.m. and three samples were taken from the centre of each ensiled mass and they were frozen at -20°C until analysis.

The environmental pollution capacity of the effluent and their environmental impact were studied. Parameters such as the total volume, chemical oxygen demand (COD), biochemical oxygen demand (BOD<sub>5</sub>), pH, conductivity (C) and total suspended solids (TSS) were determined using the DOE (1989) methods. Also, different pesticides (insecticides, bactericides and fungicides) were studied in the broccoli by-products, at 0, 4, 12 and 50 days. The pesticides that have been studied are: Diazinon, Chlorpyrifos-methyl, Chlorfenvinphos, Malathion, Methalaxyl, Endosulphan and Dithiocarbamates and they were determined by different methods (Meneses *et al.*, 2007).

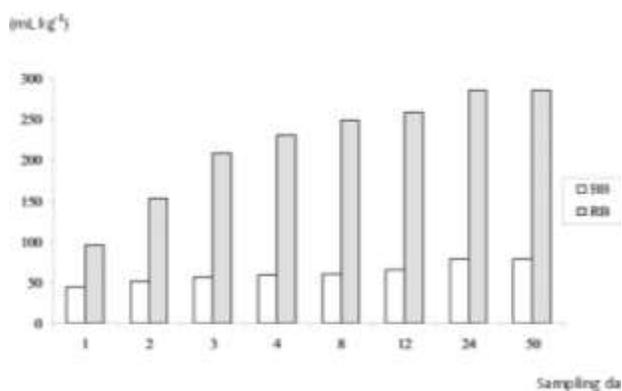
The fermentative values were determined by measurements of pH, lactic acid and ammonia-N in water extracts of fresh silage. Also, the level of volatile fatty acids (VFA) was determined in water extracts of fresh silage by capillary gas chromatography, all these methods are described in Megías *et al.* (1999). Other parameter determinations like dry matter (DM), ash and crude protein (N Kjeldahl x 6.25) (CP) contents were obtained. The water soluble carbohydrate content (WSC) by the method of anthrone, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined by the methods shown on (Megías *et al.*, 1999).

### Mathematical and Statistical Analysis

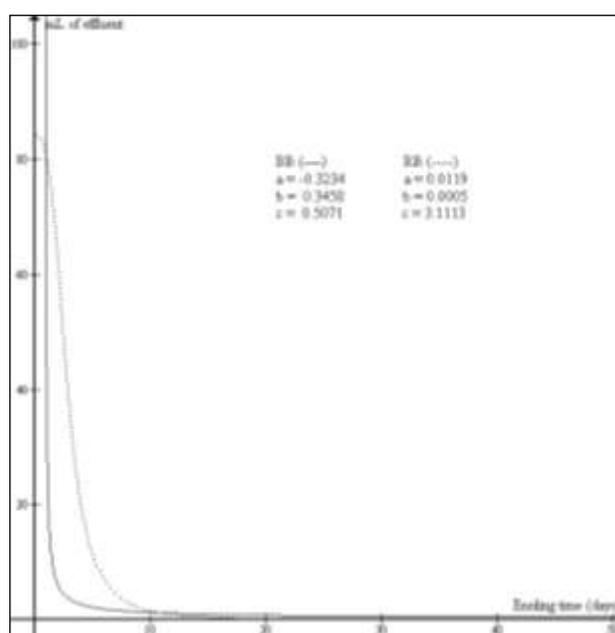
The effluent volumes released during the silage process of BB and RB were used to fit the production equation of the effluents volume. In addition, to determine the best prediction equation for effluent production based on the MS content of the forages, four different by-products were employed: BB, RB and boiled artichoke (BA) (Megías *et al.*, 1999) and raw artichoke (RA) (Meneses *et al.*, 2007) presenting the following average data: 103.8, 73.1, 162.3 and 297 g kg<sup>-1</sup> on DM content and 78.9, 285.6, 70.1 and 0 mL kg<sup>-1</sup>, on effluent volume, respectively. On both studies the Curve Expert 1 program was used (Hyans, 2005).

**Table 1:** Relationship between DM content of the forage and effluent production by different authors

Regression	Abbreviation meaning	DM for 0 effluent production	Reference
$V = 669.4 - 2.24 D$	D: % DM forage V: L of effluent 100 kg <sup>-1</sup>	299	Sutter (1957)
$V = 176.14 - 0.538 D$	D: % DM forage V: % DM lost	327	Miller and Clifton (1965)
$V = 832.6 - 5.418 D + 0.00883 D^2$	D: % DM forage V: L of effluent 100kg <sup>-1</sup>	307	Zimmer (1967)
$V = 767 - 5.34 D + 0.009360 D^2$	D: % DM forage V: L of effluent tm <sup>-1</sup>	290	Bastiman and Altman (1985)
$V = e^{(12.983-0.051D)}$	D: DM (g kg <sup>-1</sup> )V: L of effluent tm <sup>-1</sup>		Haigh (1999)



**Fig. 1:** Accumulated effluents during ensiling period of two broccoli by-products



**Fig. 2:** Evolution of effluent production of two broccoli by-products (BB and RB) during 50 days of ensiling

A bifactorial ANOVA (Steel and Torrie, 1980) have been employed to look for differences of the fermentative and chemical characteristics between the by-products or the sampling days or if there was an interaction between them. The results are displayed in several ANOVA tables that in the case of balanced designs also include SED and the degrees of freedom (DF).

## Results

### Production, Prediction and Potential Pollution of Silage Effluent

The effluent was released during the first 24 days for both by-products and no effluent was produced after this day. For BB the total volume after the experience was less than for RB with 78.97 and 285.65 Lt m<sup>-1</sup>, respectively. Moreover, the best fit found for the effluent data of these silages was the reciprocal equation Harris model (Fig. 2),  $Y = 1/(a+bX^c)$ , for both by-products, with  $r^2 = 0.768$ ; SE = 7.015 and  $r^2 = 0.900$ ; SE = 24.535, for BB and RB, respectively. Here, Y is the produced effluent (mL kg<sup>-1</sup> of fresh material) and X is the sampling day.

To predict the effluent production with respect to the initial DM content of the forages, the best equation was looked for; using the data described in section 2.3. When forages with very different content on DM were used, the Harris model was again the best model we found (Fig. 3). The fitted final prediction was  $Y = 1 / (0.4252168 + 0.08036791 X^{0.30383811})$ , with  $r^2 = 0.920$  and SE = 33.944. Here, Y is the total produced effluent (mL kg<sup>-1</sup>) and X is the DM content of the ensiled material (g kg<sup>-1</sup>). According to the bibliography (Table 1) lineal, quadratic and exponential models have been used to obtain the production prediction of the effluents and they have been compared with the Harris model. When these models were used, the best fit was found the exponential model ( $r^2 = 0.911$ ; SE= 34.499) followed by the quadratic model ( $r^2 = 0.654$ ; SE= 70.506) while the worst one was found for the linear model ( $r^2 = 0.357$ ; SE= 92.953).

In addition, the effluent is a strong pollutant for nearby land and water and creates an odour problem at the farmstead since the products are transported through the soil-plant-animal system. Effluent composition and several parameters that indicate its environmental pollution are shown in Table 2. The effluent DM content, as has already been said, was low. The CP content of the effluents was different for both by-products (P < 0.001), for BB was the same at the beginning and at the end of the process although during the process there were moderate variations but an increment from 24.7 to 62.9 g kg<sup>-1</sup> DM was found for RB. The pH of the effluent was similar (P > 0.05) for both silages and it tended to decrease (P < 0.001) with the sampling day. The TSS tendency is different for both by-products (P < 0.001). For the BB the content rose from 0.19 to 1.21 mg L<sup>-1</sup> during the experience and for the RB the content fell

**Table 2:** Environmental pollution parameters evolution of effluent of the two broccoli by-products

Sampling day	DM (g kg <sup>-1</sup> )		CP (g kg <sup>-1</sup> DM)		COD (gO <sub>2</sub> L <sup>-1</sup> )		BOD (gO <sub>2</sub> L <sup>-1</sup> )	
	BB	RB	BB	RB	BB	RB	BB	RB
1	7.0	5.1	35.1	24.7	36.9	58.5	29.80	40.89
2	2.8	4.4	33.8	33.5	25.7	45.6	19.79	46.17
3	3.2	3.4	41.9	46.8	32.2	54.3	25.63	48.83
4	3.1	3.8	61.5	41.9	42.7	65.0	32.62	37.50
8	3.5	3.7	40.8	50.1	43.0	43.2	29.06	41.80
12	3.6	3.5	40.2	64.7	43.0	32.7	21.41	43.23
24	3.8	3.5	35.1	62.9	35.0	44.0	22.97	39.06
<i>Factorial analysis</i>								
	<i>P-value</i>		<i>P-value</i>		<i>P-value</i>		<i>P-value</i>	
Sampling day	0.163		0.000		0.000		0.176	
By-product	0.000		0.000		0.000		0.001	
Day * by-product	0.000		0.000		0.012		0.008	
	pH				TSS (mg L <sup>-1</sup> )		C (μΩ cm <sup>-1</sup> )	
Sampling day	BB		RB		BB	RB	BB	RB
1	4.2		7.0		0.19	3.65	5.85	8.52
2	4.3		4.2		0.06	0.82	6.33	9.61
3	4.5		4.1		0.37	2.20	6.80	8.60
4	4.6		4.1		0.89	2.50	7.85	9.12
8	4.5		3.9		1.02	0.60	7.82	9.35
12	4.4		3.5		1.08	0.19	7.85	9.30
24	3.9		3.2		1.21	0.93	8.67	9.30
<i>Factorial analysis</i>								
	<i>P-value</i>				<i>P-value</i>		<i>P-value</i>	
Sampling day	0.000				0.000		0.008	
By-product	0.140				0.000		0.000	
Day * by-product	0.000				0.328		0.102	

**Table 3:** Multiresidues studied in two broccoli by-products before and during silage

Analytic Method	Multiresidue	Classification	BB Level detected	RB Level detected
GC-NPD	Diazinon	Insecticide-organophosphorade	ND	ND
GC-NPD	Chlorpyrifos methyl	Insecticide-organophosphorade	ND	ND
GC-NPD	Chlorfenvinphos	Insecticide-organophosphorade	ND	ND
GC-NPD	Malathion	Insecticide-organophosphorade	ND	ND
GC-NPD	Methalaxyl	Fungicide - bactericide	ND	ND
GC-ECD	Endosulphan	Insecticide-organochorade	ND	ND
Colorimetric	Dithiocarbamates	Fungicide- bactericide	ND	ND

GC-NPD: gas chromatography with nitrogen-phosphorus detector; GC-ECD: gas chromatography with electron capture detector; ND: not detected

down from 3.65 to 0.93 mg L<sup>-1</sup>. The conductivity rose from the beginning of the process until the end for both by-products (P < 0.01). The by-product with the highest (P < 0.001) conductivity was RB (9.30 μΩ cm<sup>-1</sup>). Table 3 shows that investigated pesticides were not detected (ND) in any of the by-products before or during the silage period.

### Fermentative and Nutritive Evaluation

The by-products showed their suitability for ensilage, providing a pleasant smell and good visual characteristics. During the silage process, the temperature was not affected for sampling days or by-product (P > 0.05). As regards other fermentative parameters evaluated like pH, WSC, lactic and acetic acid and N-NH<sub>4</sub> contents were affected for the sampling day while the type of by-product only affected both lactic acid and N-NH<sub>4</sub> contents. The WSC content fell drastically in both silages during the sampling period (P<0.001), from 129.4 to 41.4 g kg<sup>-1</sup> DM in BB and from 620.2 to 46.1 g kg<sup>-1</sup> DM in RB. Also, during the silage process, the BB and RB by-products showed a steady

increase in lactic acid concentration, from 0 to 65.4 g kg<sup>-1</sup> DM and from 0.3 to 102.8 g kg<sup>-1</sup> DM, respectively. In summary, as regards the fermentative parameters evaluated, the sampling day affected all of them (P < 0.008) except the temperature (P = 0.185) and the type of by-product only affected both lactic acid and N-NH<sub>4</sub> (P = 0.000). Only the pH and the lactic acid presented interaction for the two factors analysed (P = 0.000).

Sampling day and type of by-product affected (P<0.05) the DM, CP, minerals, lignin, NDF and ADF contents of silage (Table 5). The material used in the experiment had a low initial DM content of 103.8 and 73.1 g kg<sup>-1</sup> (for BB and RB, respectively) while the final DM content was of 103.0 and 69.9 g kg<sup>-1</sup> (for BB and RB, respectively). CP content of BB was higher than that of RB (P<0.001). Fibrous fractions were increased (P < 0.001) during silage process.

### Discussion

The volume produced until the 4th day was 75 and 80% for BB and RB, respectively (Fig. 1). A high effluent

**Table 5:** Nutritive content evolution of the two broccoli by-products

Sampling day	Dry matter		CP (g kg <sup>-1</sup> DM)				Minerals (g kg <sup>-1</sup> DM)		
	BB	RB	BB	RB	BB	RB			
0	103.8	73.1	314.3	145.0	60.3	107.6			
1	115.4	71.8	307.2	156.6	63.2	97.9			
2	113.8	77.8	303.4	161.3	60.6	102.6			
3	113.4	74.0	327.7	161.9	61.0	100.6			
4	111.8	72.5	324.0	163.2	65.2	103.2			
8	112.6	70.5	331.8	172.3	62.8	118.0			
12	115.2	72.3	335.5	169.0	62.4	98.7			
24	115.6	65.7	335.3	170.9	62.4	115.1			
50	103.0	66.9	336.1	177.7	64.6	111.6			
<i>Factorial analysis</i>									
	<i>P-value</i>	<i>SED</i>	<i>DF</i>	<i>P-value</i>	<i>SED</i>	<i>DF</i>	<i>P-value</i>	<i>SED</i>	<i>DF</i>
Sampling day	0.000	0.153	8	0.000	0.604	8	0.000	0.247	8
By-product	0.000	0.072	1	0.000	0.285	1	0.000	0.116	1
Day * by-product	0.000	0.217	8	0.434	0.854	8	0.000	0.349	8
Error			90			90			90
		Lignin (g kg <sup>-1</sup> DM)			NDF (g kg <sup>-1</sup> DM)			ADF (g kg <sup>-1</sup> DM)	
Sampling day	BB	RB	BB	RB	BB	RB			
0	16.0	15.3	147.9	173.5	124.0	129.4			
1	23.7	11.7	213.9	204.2	131.2	148.2			
2	20.5	22.1	217.6	325.6	136.5	167.1			
3	17.2	21.3	216.0	249.8	131.8	178.6			
4	19.9	23.0	208.9	241.3	128.9	167.9			
8	19.7	22.6	209.5	305.0	128.7	203.6			
12	30.0	19.1	233.7	287.5	143.6	212.7			
24	25.7	24.4	230.2	275.0	147.0	198.4			
50	30.2	21.5	242.5	270.9	153.4	201.1			
<i>Factorial analysis</i>									
	<i>P-value</i>	<i>SED</i>	<i>DF</i>	<i>P-value</i>	<i>SED</i>	<i>DF</i>	<i>P-value</i>	<i>SED</i>	<i>DF</i>
Sampling day	0.000	0.201	8	0.000	0.455	8	0.000	0.324	8
By-product	0.012	0.095	1	0.000	0.215	1	0.000	0.153	1
Day * by-product	0.000	0.284	8	0.000	0.644	8	0.000	0.459	8
Error			90			90			90

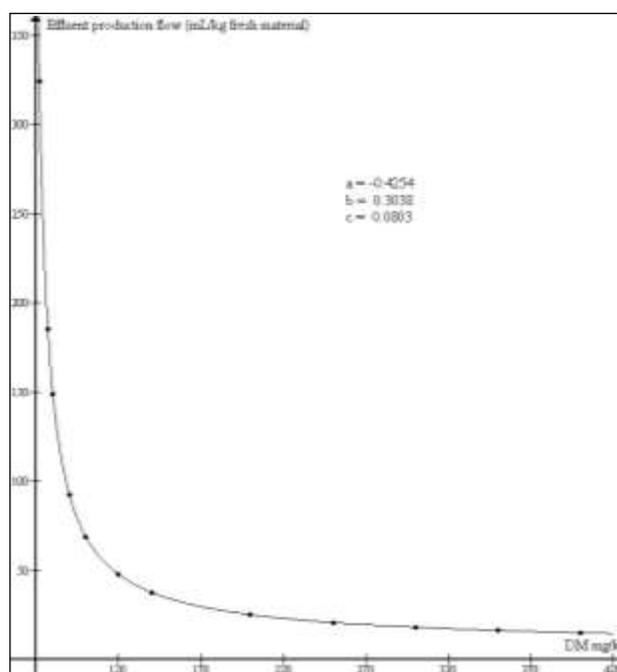
SED: Standard error of the difference of two means. DF: Degrees of freedom

production indicates increases on nutrient losses and on environmental pollution. High moisture content silages were produced with both by-products, so that there was a large amount of effluent as expected (Bastiman, 1976). There was a high intensity of fermentation during the first few days, 33% of the effluent was produced on the 3rd and 4th days in agreement with Gross (1972). In this case, the volume produced on the first 4 days are very close to those obtained by Megías *et al.* (1999) on boiled artichoke by-product silage. However, in large silos the time with a constant effluents flow was estimated for the days: 10 to 27 (Pitt and Parlange, 1987). RB produced higher volume of effluents than BB, because RB had lower DM initial content and the produced volume of effluent is inversely related to the DM content of the ensiled material; McDonald *et al.* (1991) recommended above 250 g kg<sup>-1</sup> DM content for not obtaining large amounts of effluent. Even it is known that for more than 300 g kg<sup>-1</sup> DM no effluent is released from the silos. Also, the stems of RB by-product were chopped by hand before ensiling. They were fleshy, hollow and spongy, so the mechanical treatment performed at the laboratory affected the tissue destruction and increased the effluent production into account with Savoie *et al.* (2002).

The models shown in Table 1 give good fits for the

prediction of the effluent production when used in pastures with high DM content, however when the data on DM content from the high moisture by-products were used the prediction of effluent total volume using these models was very far from the real data. All the bibliography models overestimated the effluent production, while the reciprocal Harris model fitted it better. As it can be seen in Fig. 3, little diminutions on DM content when high moisture silage are considered, can produce very important increases in effluent production. In this sense, the agro-industry by-products considered in this study presented low DM so that large amount of effluent was produced (Megías *et al.*, 1999).

The pollution capacity is in relationship with the oxygen value. The total oxygen is linked to the full organic matter oxidation by a strong chemical oxidant and is expressed in terms of the chemical oxygen demand (COD). At the end of the process the differences found were closed to those found for artichoke by-product silage effluent (Megías *et al.*, 1999). On the other hand, biochemical oxygen demand (BOD) is a potential factor for the removal of oxygen from superficial waters, because of the utilization of the organic matter for the production of energy and the multiplication of aerobic heterotrophy bacteria and mainly depends on the oxygen demand for the microbial activity as a result of the readily available carbon compounds for



**Fig. 3:** Prediction curve of production of silage effluent on the basis of forage DM content

oxidation. BOD is the most common indicator used to evaluate the contamination power of liquid wastes and its value ranges approximately from 3 g O<sub>2</sub> L<sup>-1</sup> of clean river water to 90 g O<sub>2</sub> L<sup>-1</sup> of grasses silage effluent (Woolford, 1978). For BOD, a very high pollution effect was found and RB effluents were more pollutant ( $P < 0.001$ ) than BB effluents (22.97 and 39.06 g O<sub>2</sub> L<sup>-1</sup>, for BB and RB, respectively). The values of BOD for both by-products were very close to those found in other studies; they showed BOD values of 35 and 44 g O<sub>2</sub> L<sup>-1</sup> when they included absorbents in their grass silages (Jones *et al.*, 1990; Ferris and Mayne, 1994). Hamelers *et al.* (1999) reported BOD values of around 100 g O<sub>2</sub> L<sup>-1</sup> that were found when corn silage was made. Anyway, these results were much higher than those values permitted for BOD (0.5 to 0.015 g O<sub>2</sub> L<sup>-1</sup>) of domestic sewage to be poured into a public channel. The difference between COD and BOD could suggest that the effluent did contain a certain amount of non-degradable fibre. The pH of the effluent was lower than that of the silages but very close because of the release of some anions like organic acid salts, orthophosphates, sulphates, nitrates and chlorides through the effluent (McDonald *et al.*, 1991). TSS represents the concentration of material in suspension in a liquid effluent. It is an important physical characteristic and its increment during biological treatments can also give an idea of biomass growth. The data are much lower than the highest TSS limit determined for the agricultural pollutants of watercourses and soil of 10 mg L<sup>-1</sup>. Conductivity (C) is the ability of an aqueous solution to carry an electric current. This ability depends on the

presence of ions, its total concentration, mobility, valence, relative concentrations and temperature. Conductivity rose from the beginning of the process until the end for both by-products. The results do not pass the limits of the Spanish Ministry of Agriculture which stipulates that river-water must hold less than 0.08 μΩ cm<sup>-1</sup>, although some industrial silage wastes has conductivities above 8.00 μΩ cm<sup>-1</sup> like artichoke by-product (Megías *et al.*, 1999).

The pesticide analysis of silages is necessary to guarantee the safety of the animal diets and that of the environment because of the release of their effluents. The results obtained in this study on pesticide evaluation are very similar to those found during the ensiling process for raw artichoke by-product (Meneses *et al.*, 2007).

The temperatures inside the silos remained low in all the cases, averaging around 20°C, reflecting a homo-fermentative process (McDonald *et al.*, 1991). The changes on N-NH<sub>4</sub> content indicated that our silage was of a good quality since protein degradation was low. The pH values at the end of the process showed that there were no differences and classified the silages as good quality as those forages with 300 g kg<sup>-1</sup> of DM which agrees with the results in Keady and Murphy (1998). In this sense, lactic acid is the most effective acid for reducing silage pH, thereby producing high quality forage. It is commonly accepted that ensiled forages with high lactic acid concentrations are more nutrient-dense, palatable and sweet smelling, which results in greater nutrient intake. In this case, the lactic acid content did not decrease despite the forage water content was high (Stefanie *et al.*, 1999) but the fermentative characteristics of BB showed that it was better for ensiling since it presented greater total lactic acid production, a low acetic acid content and very acceptable results for pH and N-NH<sub>4</sub>. Based on this, good silages were produced from both broccoli by-products.

As was expected, CP content of BB was higher than that of RB due to the part of the plant where each by-product comes from. The increases in ADF and NDF were connected with the water-soluble carbohydrate losses during the first phase of ensilage (Ashbell and Donahaye, 1984). The use made of the WSC by microorganisms reduced this component and this was what led to a rise in the cell walls. In addition, the RB results for mineral content were similar to those found in the bibliography without ensiling (117 g kg<sup>-1</sup>) (Megías *et al.*, 2002), while the content found in BB was far from the values reported by Kahlon *et al.* (2007), (103 g kg<sup>-1</sup>). The industrial process to which they were submitted can produce losses in the chemical-nutritive parameters of some vegetables (Megías *et al.*, 2002).

In short, when high moisture content materials are ensiled there are small decreases on DM content and a large volume of effluent is released. No pesticides traces were found at any sampling day, so the resulting of RB or BB silage does not present risk of environmental pollution, nor a health problem to be used as animal feed. Furthermore, these by-products can be effectively ensiled under laboratory conditions producing silages with good nutritious

and fermentative quality, however during the silage of RB too many effluents are released, so the silage of this by-product would not be recommended.

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## References

- Arnold, J.L., J.S. Knapp and C.L. Johnson, 2000. The use of yeast to reduce the polluting potential silage effluent. *Water Res.*, 34: 3699–3708
- Ashbell, G. and E. Donahaye, 1984. Losses in orange peel silage. *Agric. Wastes*, 14: 73–77
- Bastiman, B., 1976. Factor affecting silage effluent production. *Exp. Husb.*, 31: 40–46
- Bastiman, B. and J.F.B. Altman, 1985. Losses at various stages in silage making. *Res. Dev. Agric.*, 2: 19–25
- DOE (Standing Committee of Analysis), 1989. *Methods for Examination of Wasters and Associated Materials*, 2<sup>nd</sup> edition. HMSO Books, Publications Centre, London, Enlagland
- Duggan, P.S., P.A. Chambers, J. Heritage and J.M. Forbes, 2000. Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiol. Lett.*, 191: 71–77
- Ferris, C.P. and C.S. Mayne, 1994. Effects on milk production of feeding silage and three levels of sugar-beet pulp either as mixed ration or as an ensiled blend. *Grass Forage Sci.*, 49: 241–251
- Gross, F., 1972. The formation of fermentation liquor during ensilage and its elimination. *Bayer. Lad. Jahrb.*, 49: 964–970
- Guimaraes Faria, D.J., R.M. Garcia, R. Gonsalves Tonucci, V. Votega Tavares, O. Gomes Pereira and D. Miranda da Fonseca, 2010. Produção e composição da silagem de capim-elefante com casca de café. *R. Bras. Zootecn.*, 3: 471–478
- Haigh, P.M., 1994. A review of agronomic factors influencing grass silage effluent production in England and Wales. *J. Agric. Eng. Res.*, 57:73–87
- Haigh, P.M., 1999. Effluent production from grass silages treated with additives and made in large-scale bunker silos. *Grass Forage Sci.*, 54: 208–218
- Hameleers, A., K.A. Leach, N.W. Offer and D.J. Robert, 1999. The effects of incorporating sugar beet pulp with forage maize at ensiling on silage fermentation and effluent output using drum silos. *Grass Forage Sci.*, 54: 322–335
- Hyans, D.G., 2005. *Curve Expert version 1.4, for Windows*. Copyright 2005 all right reserved. <http://www.curveexpert.net> (Accessed: 18 February 2013)
- Jones, D.I.H. and R. Jones, 1995. The effect of crop characteristics and ensiling methodology on grass silage effluent production. *J. Agric. Eng. Res.*, 60: 73–81
- Jones, D.I.H., R. Jones and G. Mosley, 1990. Effect of incorporating rolled barley in autumn-cut ray grass silage on effluent production, silage fermentation and cattle performance. *J. Agric. Sci.*, 115: 399–408
- Kahlon, T.S., M.H. Chapman and G.E. Smith, 2007. *In vitro* binding of bile acids by spinach, kale, Brussels sprout, broccoli, mustard greens, green bell pepper, cabbage and collards. *Food Chem.*, 100: 1531–1536
- Keady, T.W.J. and J.J. Murphy, 1998. The effects of ensiling and supplementation with sucrose and fish meal on forage intake and milk production of lactating dairy cows. *Anim. Sci.*, 66: 9–20
- Márquez, M.A., F. Diáñez and F. Camacho, 2010. The use of vegetable subproducts from greenhouses (VSG) for animal feed in the Poniente region of Almería. *Renew. Agric. Food Syst.*, 26: 4–12
- McDonald, P., N. Henderson and S. Heron, 1991. *The Biochemistry of Silage*, 2<sup>nd</sup> Chalcombe Publications. Marlow Bottom, England
- Megías, M.D., F. Hernández, J. Madrid and A. Martínez, 2002. Feeding value, *in vitro* digestibility and *in vitro* gas production of different by-products for ruminant nutrition. *J. Sci. Food Agric.*, 82: 567–572
- Megías, M.D., A. Martínez-Teruel and M.R. Hernández, 1999. Potential environmental impacts of effluent from the artichoke (*Cynara scolymus L.*) by-product ensiling process with different additives. *J. Agric. Food Chem.*, 47: 2455–2458
- Meneses, M., M.D. Megías, J. Madrid, A. Martínez-Teruel, F. Hernández and J. Oliva, 2007. Evaluation of the pesticides, fermentatives and nutritives characteristics in the use of the silage of crude artichoke (*Cynara scolymus, L.*) by-product for ruminants feeding. *Small Rum. Res.*, 70: 292–296
- Miller, W.J. and C.M. Clifton, 1965. Relation of dry matter content in ensiled material and other factors to nutrient losses by seepage. *J. Dairy Sci.*, 48: 917–923
- Mongabay, 2013. <http://data.mongabay.com/commodities/> (Accessed: 18 February 2013)
- Ouda, B.A. and A.Y. Mahadeen, 2008. Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (*Brassica oleracea*). *Int. J. Agric. Biol.*, 10: 627–632
- Pitt, R.E. and J.Y. Parlange, 1987. Effluent production from silage with application to tower silos. *Amer. Soc. Agric. Eng.*, 30: 1198–1204
- Savoie, P., A. Amyot and R. Thériault, 2002. Effect of moisture content, chopping and processing on silage effluent. *Amer. Soc. Agric. Eng.*, 45: 907–914
- Steel, R.G.D. and J.H. Torrie, 1980. *Principles and Procedures of Statistics: A Biometrical Approach*, 2<sup>nd</sup> edition. McGraw–Hill Book Co, New York, USA
- Stefanie, J.W.H., O. Elferink, F. Driehuis, J.C. Gottschal and S.F. Spoelstra, 1999. Silage fermentation processes and their manipulation. In: *Silage Making in the Tropics with Particular Emphasis on Smallholders*, p: 161. ‘t Mannetje, L. (ed.). Plant Production and Protection, FAO, Rome Italy
- Sutter, A., 1957. *A Problem of Waste Effluent from Silage Making and Feeding Silage*, pp: 74–82. OEEC course (project 307. Making and feeding silage. Zurich: European Economic Commission
- Van der Wel, C., 1993. *Inkuil Verliezen by Snijmais (Ensiling Losses in Forage Maize)*. Proefstation voor de Rundvee Houderij. Schapenhouderij en Pardenhouderij, Lelystad, The Netherlands
- Waldo, D.R., 1977. Suggestion for improving silage. In *Proceeding of the 10<sup>th</sup> Research Industry Conference*, pp: 69–91. American Forage and Grassland Council Inc: Columbia MO, USA
- Woolford, M.K., 1978. The problem of silage effluent. *Herb. Abst.*, 48: 397–403
- Zimmer, E., 1967. Factors affecting silage fermentation in silo. *Das Wirtsch. Futter.*, 13: 271–286

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