

## Evaluation of the nutritive value of apple pulp mixed with different amounts of wheat straw

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### Abstract

Given the high amounts of apple rejected for commercialization its use as alternative feed for ruminants should be considered. This study was designed to investigate the nutritive value of apple pulp-wheat straw mixtures.

Chemical composition, in vitro organic matter digestibility (IVOMD) and gas production profiles of ensiled mixtures containing 85 (M85), 70 (M70), and 50% (M50) of apple pulp were studied at 0, 15, 30 and 45 days. Fermentation quality was assessed by pH, lactic acid, ethanol and acetate. The results showed that non fibre carbohydrates (NFC) decreased along the storage period while DM stabilized after 30 days. It was also shown that pH increased along the storage period and the highest values for ethanol and acetic acid concentrations were observed in the apple pulp mixtures (Apple). Within mixtures, IVOMD decreased along the storage period, being however similar across mixtures after long periods of storage. Gas production analysis indicated that easily fermentable components (VFA) and the less easily fermentable constituents (VFB) decreased along the storage period, but VFB showed no differences between mixtures for the first two storage periods.

Results suggest the possibility of reducing ethanol concentration of apple pulp silages through the incorporation of fibrous components; however silage additives to limit fermentation of sugars into ethanol should be used to ensure the course of the conservation process.

**Key words:** apple, silage, wheat straw

### Introduction

In the food chain, ruminant production from by-products offers a unique possibility of converting non edible feeds or with limited value for humans into meat and milk. Pulp from the fruit juice industry have been used for a considerable time in ruminant diets due to the high content of pectins and sugars (Alibes et al 1984; Arthington et al 2002) rapidly fermented in the rumen (Hall et al 1998). Notwithstanding low nitrogen and high free sugar content whose fermentation in the rumen may eventually lead to alcoholaemia, apples as an alternative feed for cattle have also been considered to help solving the economical disposal of industrial residues and fruits without the required characteristics to commercialization. While adverse effects of feeding apple pomace supplemented with nonprotein nitrogen to gestating beef cows and sheep have been found (Fontenot et al 1977; Rumsey et al 1979), the supplementation with cottonseed meal was comparable to corn silage diets for gestating beef cows (Oltjen et al 1977). When testing the feeding value of apple pomace silage for sheep, Alibes et al (1984) showed that this product could be included in diets containing alfalfa hay and soya bean meal as supplements. Gasa et al (1992) reported that ensiled apple pomace could be safely incorporated in roughage diets that were properly supplemented with degradable nitrogen. To prevent effluent loss in the ensiling process, Alibes et al (1984) suggested the preparation of apple pomace-cereal straw silage and,

recently, Pirmohammadi et al (2006) showed that the incorporation of wheat straw could improve apple pomace silage preservation.

In this work, the effect of different concentrations of wheat straw on the ensiling process of apple pulp residues was assessed by the *in vitro* organic matter digestibility and gas production profiles.

## Material and methods

### Silage preparation

Low calibre apples from the Douro region, North of Portugal, were ground to pass a 4mm screen (Pachancho Cutting mill, model L29025, Portugal) until a homogeneous mash was obtained. Wheat straw, chopped on a stationary chopper (Jensen & Sømmer, Agerskov, Denmark) adjusted for a theoretical cut length of 5 cm, was added and mixed by hand into mixtures containing 100 (Apple), 85 (M85), 70 (M70), and 50% (M50) of apple pulp on a fresh weight basis, respectively. Mixtures were conditioned in dark plastic bags, packed in 5 dm<sup>3</sup> plastic buckets (laboratory silos) and pressed to ensure compaction. The buckets were sealed with tight lids to prevent the entry of air and stored in the dark at ambient temperatures ranging between 20 and 25°C.

Three replicates were prepared for each treatment and each date of sampling in a total of 36 laboratory silos. The laboratory silos were opened at 15, 30 and 45 days after ensiling. Samples of mixtures, as well as wheat straw samples, were immediately dried in an air forced oven at 60°C, ground to pass 1mm screen (Retsch, Cutting mill, model SM1, Germany) and stored in airtight flasks at room temperature for subsequent chemical analysis. Fresh samples of mixtures from days 15, 30 and 45 were also collected, and immediately frozen, for chemical analysis.

### Chemical analysis

Dry samples were analysed for ash (no. 942.05, AOAC 1990) and total N as Kjeldahl N (no. 954.01, AOAC 1990). Neutral detergent fibre (NDF) acid detergent fibre (ADF) and ADL fractions were determined by the detergent procedures of Robertson and Van Soest (1981) and Van Soest et al (1991) without the use of sodium sulphite. Neutral detergent fibre and acid detergent fibre were expressed exclusive of residual ash. The acid detergent insoluble nitrogen (ADIN) was determined according to Goering and Van Soest (1970). Non fibre carbohydrates (NFC) were calculated according to NRC (2001).

Silage pH, lactic acid, ethanol and volatile fatty acids (VFA's) were determined in water extracts obtained from frozen samples according to Sheperd and Kung (1996). The pH value was measured using pH meter equipment (pH meter 632, Metrohm Ltd., Herisau, Switzerland). The concentrations of VFA's and ethanol were analysed by gas-liquid chromatography (Shimadzu GC-141 B, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (SUPELCO Nukol, 0.25 mm i.d. x 30 m x 0.25 µm) by using pivalic acid as the internal standard (Czerkawski 1976). Lactate was determined using an enzymatic assay procedure (K-late 03/06, Megazyme, Ireland). Due to problems with freezers only the samples collected at day 45 were analysed for lactic acid.

### *In vitro* incubations with rumen fluid

Rumen fluid was collected from four non-lactating rumen cannulated (Bar Diamond Inc., Parma, Idaho, USA) cows. The diet composed of meadow hay shredded to 20 cm particles through a bale gripper (JN Jensen & Sommer APS, model DK 6534 Agerskov, Denmark) and 15% soybean meal was offered in equal proportions in the morning (8.00) and afternoon (16.00). From each cow, rumen fluid was collected 2 h after the morning feeding and pooled into one in a pre-warmed insulated bottle filled with CO<sub>2</sub>. Before use in the laboratory, rumen fluid was strained and filtered through cheesecloth. All manipulations were under continuous flushing with CO<sub>2</sub>.

The *in vitro* organic matter digestibility (IVOMD) was calculated according to the procedures of Tilley and Terry (1963) as modified by Marten and Barnes (1980). The gas production incubations were conducted according to Cone et al (1996). Samples of mixtures (0.4 g dry matter) were incubated in 60 ml buffered rumen fluid in 250 ml bottles during 72 h using a fully automated system (Cone et al 1996).

For all *in vitro* incubations, each sample was incubated in duplicate in three series, performed on different days.

Gas production profiles were fitted to a two-pool logistic equation, considering that the lag time is identical for both phases (Schofield et al 1994):

$$V = V_{FA} \left\{ 1 + \exp \left( 2 + \frac{4\mu_{mA}}{V_{FA}} (\lambda - t) \right) \right\}^{-1} + V_{FB} \left\{ 1 + \exp \left( 2 + \frac{4\mu_{mB}}{V_{FB}} (\lambda - t) \right) \right\}^{-1}$$

where:

- V = the gas volume after time t (ml/g OM),
- VFA = the gas volume of the 1st phase (ml/g OM),
- $\mu_{mA}$  = maximum gas production rate of the 1st phase (ml/g OM/h),
- VFB = the gas volume of the 2nd phase (ml/g OM),
- $\mu_{mB}$  = maximum gas production rate of the 2nd phase (ml/g OM/h),
- $\lambda$  = lag time;
- t = time;
- $\mu_{mA} / VFA$  = specific digestion rate of 1st phase (h);
- $\mu_{mB} / VFB$  = specific digestion rate of 2nd phase (h).

## Statistical analysis

Data were analysed in a 4x3 factorial arrangement of treatments, with three replications, by the general linear models procedure of SAS (1990). The model included level of apple pulp incorporation (Apple, M85, M70, M50) and ensiling days (15, 30, 45), plus the interactions between the treatments. When significant differences occurred, least significant difference Student's t-test was used to compare means.

## Results

### Chemical composition

The chemical composition of feeds before ensiling is presented in Table 1. Apples showed the characteristic low content in dry matter (DM), total N and cell wall components. The incorporation of straw increased DM on 63% (M85), 127% (M70) and 211% (M50), being cell wall components also increased five, six and seven fold, respectively.

**Table 1.** Chemical composition of apple pulp, straw and mixtures before ensiling

	Apple	M85	M70	M50	Straw
Dry matter, g/kg	179	291	407	557	874
Total nitrogen, g/kg DM	3.6	3.0	2.8	3.6	3.0
Non fibre carbohydrates, g/kg DM	842	526	304	176	62
Neutral detergent fibre, g/kg DM	107	503	656	753	885
Acid detergent fibre, g/kg DM	80	317	413	470	544
Acid detergent lignin, g/kg DM	24.3	45.9	59.4	66.7	74.5
Acid detergent insoluble nitrogen, g/kg DM	13.0	3.0	2.3	2.6	21.6
pH	3.8	3.8	4.1	4.7	5.8

1 M85, M70 and M50, mixtures containing 85, 70 and 50% of apple in fresh weight basis, respectively

Changes in the chemical composition of mixtures during the course of ensilage are presented in Table 2.

**Table 2.** Chemical composition of silages after storing for 15, 30 and 45 days<sup>1</sup>

Mixture	Time	Dry matter, g/kg	Ash, g/kg DM	Total nitrogen, g/kg DM	Non fibre carbohydrates, g/kg DM	Acid detergent insoluble nitrogen, g/kg DM	Neutral detergent fibre, g/kg DM	Acid detergent fibre, g/kg DM	ADL, g/kg DM
Apple	15	78.6 <sup>b</sup>	67.4 <sup>e</sup>	7.0 <sup>d</sup>	599.3 <sup>h</sup>	17.4 <sup>d</sup>	273.2 <sup>a</sup>	226.2 <sup>a</sup>	97.4 <sup>d</sup>
	30	65.1 <sup>a</sup>	42.0 <sup>c</sup>	9.4 <sup>e</sup>	570.5 <sup>g</sup>	17.2 <sup>d</sup>	305.7 <sup>b</sup>	268.4 <sup>b</sup>	116.5 <sup>e</sup>
	45	70.7 <sup>ab</sup>	48.2 <sup>d</sup>	9.3 <sup>e</sup>	571.2 <sup>g</sup>	17.8 <sup>d</sup>	301.0 <sup>b</sup>	242.5 <sup>a</sup>	95.5 <sup>cd</sup>
M85	15	269.2 <sup>d</sup>	26.5 <sup>a</sup>	3.6 <sup>a</sup>	425.9 <sup>f</sup>	2.8 <sup>abc</sup>	515.1 <sup>c</sup>	337.1 <sup>c</sup>	58.4 <sup>a</sup>
	30	213.2 <sup>c</sup>	30.0 <sup>a</sup>	4.5 <sup>b</sup>	276.7 <sup>e</sup>	3.3 <sup>bc</sup>	653.4 <sup>d</sup>	426.6 <sup>d</sup>	76.7 <sup>b</sup>
	45	210.0 <sup>c</sup>	31.2 <sup>a</sup>	4.5 <sup>b</sup>	215.2 <sup>d</sup>	3.5 <sup>c</sup>	713.4 <sup>e</sup>	470.1 <sup>e</sup>	80.5 <sup>b</sup>
M70	15	361.5 <sup>f</sup>	28.8 <sup>a</sup>	3.4 <sup>a</sup>	210.0 <sup>d</sup>	2.1 <sup>a</sup>	730.8 <sup>e</sup>	468.4 <sup>e</sup>	80.8 <sup>b</sup>
	30	323.6 <sup>e</sup>	29.1 <sup>a</sup>	3.5 <sup>a</sup>	186.9 <sup>c</sup>	2.4 <sup>a</sup>	750.5 <sup>f</sup>	472.8 <sup>e</sup>	86.7 <sup>bcd</sup>
	45	318.8 <sup>e</sup>	30.0 <sup>a</sup>	3.3 <sup>a</sup>	152.6 <sup>b</sup>	2.2 <sup>a</sup>	785.6 <sup>g</sup>	509.4 <sup>f</sup>	78.1 <sup>b</sup>
M50	15	474.0 <sup>g</sup>	36.8 <sup>b</sup>	4.4 <sup>b</sup>	113.3 <sup>a</sup>	2.7 <sup>abc</sup>	812.3 <sup>h</sup>	530.6 <sup>g</sup>	82.0 <sup>b</sup>
	30	462.1 <sup>g</sup>	41.0 <sup>bc</sup>	5.1 <sup>c</sup>	112.6 <sup>a</sup>	2.6 <sup>ab</sup>	809.6 <sup>h</sup>	510.6 <sup>f</sup>	84.4 <sup>bcd</sup>
	45	472.6 <sup>g</sup>	41.0 <sup>bc</sup>	5.2 <sup>c</sup>	106.7 <sup>a</sup>	2.6 <sup>ab</sup>	807.6 <sup>h</sup>	527.5 <sup>fg</sup>	84.2 <sup>bc</sup>
<i>Mean values</i>									
<i>Mixture</i>									
Apple		71.5 <sup>a</sup>	52.5 <sup>c</sup>	8.6 <sup>d</sup>	580.4 <sup>d</sup>	17.5 <sup>c</sup>	293.2 <sup>a</sup>	245.8 <sup>a</sup>	103.5 <sup>c</sup>
M85		230.8 <sup>b</sup>	29.2 <sup>a</sup>	4.2 <sup>b</sup>	305.9 <sup>c</sup>	3.2 <sup>b</sup>	627.3 <sup>b</sup>	411.3 <sup>b</sup>	71.9 <sup>c</sup>
M70		334.7 <sup>c</sup>	29.3 <sup>a</sup>	3.4 <sup>a</sup>	183.2 <sup>b</sup>	2.6 <sup>a</sup>	755.6 <sup>c</sup>	483.5 <sup>c</sup>	81.9 <sup>b</sup>
M50		469.6 <sup>d</sup>	39.6 <sup>b</sup>	4.9 <sup>c</sup>	110.8 <sup>a</sup>	2.2 <sup>a</sup>	808.0 <sup>d</sup>	522.9 <sup>d</sup>	83.5 <sup>b</sup>
<i>Time</i>									
15		295.8 <sup>b</sup>	39.9 <sup>b</sup>	4.6 <sup>a</sup>	337.1 <sup>c</sup>	6.2 <sup>a</sup>	582.8 <sup>a</sup>	390.6 <sup>a</sup>	79.7 <sup>a</sup>
30		266.2 <sup>a</sup>	35.5 <sup>a</sup>	5.6 <sup>b</sup>	286.7 <sup>b</sup>	6.4 <sup>a</sup>	628.0 <sup>b</sup>	419.6 <sup>b</sup>	91.1 <sup>b</sup>
45		268.0 <sup>a</sup>	37.6 <sup>ab</sup>	5.6 <sup>b</sup>	261.5 <sup>a</sup>	6.5 <sup>a</sup>	652.2 <sup>c</sup>	437.4 <sup>c</sup>	84.6 <sup>a</sup>
<i>Standard error of means</i>									
Mixture		2.63	1.01	0.09	3.57	10.46	3.50	3.88	2.44
Time		2.28	0.87	0.08	3.10	9.06	3.03	3.36	2.17
Interaction		4.56	1.75	0.16	6.20	18.13	6.07	6.72	4.23

<sup>1</sup> M85, M70 and M50, mixtures containing 85, 70 and 50% of apple in fresh weight basis, respectively.

a,b,c,d,e,f,g,h Means in the same row with different letters differ significantly ( $P < 0.05$ ).

As expected, mixtures containing higher proportion of straw also presented higher DM and cell wall components ( $P < 0.05$ ). Inversely, the concentration of non fibre carbohydrates (NFC) decreased with straw addition ( $P < 0.001$ ), reaching the difference of 64% ( $P < 0.05$ ) between M85 and M50. The NFC content decreased along the storage period ( $P < 0.001$ ) while the DM content stabilized after 30 days ( $P < 0.05$ ). An increase in the cell components ( $P < 0.001$ ) was also observed, although this may only represent a change in proportion due to solubilisation and fermentation of soluble constituents. Excepting for ADL, significant interactions were obtained for all the chemical composition parameters. The effect observed for DM ( $P < 0.001$ ) shows that at 15 days of ensiling, mixtures M70 and M85 presented higher DM values ( $P < 0.05$ ) comparing to the other two storage periods. The mixture M50

showed no differences ( $P>0.05$ ) along the storage period. In the case of NFC, no differences ( $P>0.05$ ) were observed for M50 mixtures, and the largest variation was obtained for M85 mixtures ( $P<0.05$ ) in which the difference between the NFC in silages at 15 and 45 days was 50%.

The fermentation characteristics presented in Table 3 show increased pH values along the storage period ( $P<0.001$ ).

**Table 3.** Silage pH, ethanol, acetic and lactic acid concentrations after storing for 15, 30 and 45 days<sup>1</sup>

Mixture	Time	pH	Ethanol, g/kg DM	Acetate, g/kg DM	Lactate, g/kg DM
Apple	15	3.77 <sup>a</sup>	288.3 <sup>i</sup>	20.1 <sup>g</sup>	ND
	30	3.84 <sup>b</sup>	216.2 <sup>g</sup>	20.3 <sup>g</sup>	ND
	45	3.88 <sup>c</sup>	266.4 <sup>h</sup>	34.4 <sup>h</sup>	2.9
M85	15	3.82 <sup>b</sup>	31.3 <sup>cd</sup>	3.2 <sup>c</sup>	ND
	30	3.98 <sup>d</sup>	71.3 <sup>f</sup>	5.2 <sup>ef</sup>	ND
	45	3.99 <sup>d</sup>	75.5 <sup>f</sup>	5.6 <sup>f</sup>	9.6
M70	15	4.10 <sup>e</sup>	37.0 <sup>d</sup>	2.8 <sup>abc</sup>	ND
	30	4.18 <sup>f</sup>	33.5 <sup>cd</sup>	2.9 <sup>bc</sup>	ND
	45	4.34 <sup>g</sup>	45.8 <sup>e</sup>	5.0 <sup>e</sup>	4.5
M50	15	4.74 <sup>h</sup>	18.8 <sup>b</sup>	2.3 <sup>a</sup>	ND
	30	4.93 <sup>i</sup>	8.8 <sup>a</sup>	2.4 <sup>ab</sup>	ND
	45	4.93 <sup>i</sup>	26.8 <sup>c</sup>	4.2 <sup>d</sup>	2.8
<i>Mean values</i>					
Mixture					
Apple		3.83 <sup>a</sup>	257.0 <sup>d</sup>	24.9 <sup>d</sup>	-
M85		3.93 <sup>b</sup>	59.4 <sup>c</sup>	4.7 <sup>c</sup>	-
M70		4.21 <sup>c</sup>	38.8 <sup>b</sup>	3.6 <sup>b</sup>	-
M50		4.87 <sup>d</sup>	18.1 <sup>a</sup>	3.0 <sup>a</sup>	-
Time					
15		4.11 <sup>a</sup>	93.9 <sup>b</sup>	7.1 <sup>a</sup>	-
30		4.23 <sup>b</sup>	82.5 <sup>a</sup>	7.7 <sup>b</sup>	-
45		4.29 <sup>c</sup>	103.6 <sup>c</sup>	12.3 <sup>c</sup>	-
<i>Standard error of means</i>					
Mixture		0.006	1.47	0.11	-
Time		0.005	1.27	0.10	-
Interaction		0.010	2.54	0.19	-

<sup>1</sup> M85, M70 and M50, mixtures containing 85, 70 and 50% of apple in fresh weight basis, respectively; ND, not determined.

*a,b,c,d,e,f,g,h,i* Means in the same row with different letters differ significantly ( $P<0.05$ )

Mixtures containing only apple pulp showed the highest values for ethanol and acetic acid concentrations ( $P<0.05$ ). The presence of propionic and butyric acids was not detected. Excepting for M85, ethanol showed a tendency for a slight decrease at 30 days of ensiling, although its concentration increased at the end of the experimental period ( $P<0.05$ ).

### In vitro digestibility and gas production

The results presented in Table 4 show that IVOMD decreased along the storage period ( $P<0.001$ ). At the end of the experimental period, M70 and M50 had similar digestibility values ( $P>0.05$ ) and at 15 and 30 days of ensiling IVOMD results for M70 mixtures were not different ( $P>0.05$ ).

**Table 4.** Estimated parameters from *in vitro* gas production and organic matter digestibility of silages after storing for 15, 30 and 45 days<sup>1</sup>

Mixture	Time	VFA, ml/g OM)	mmA, ml/g OM/h	mmA / VFA, /h	VFB, ml/g OM	mmB, ml/g OM/h	mmB / VFB, /h	IVOMD, g/kg OM
Apple	15	245.4 <sup>k</sup>	27.9 <sup>i</sup>	0.114 <sup>g</sup>	169.3 <sup>c</sup>	5.2 <sup>i</sup>	0.031 <sup>g</sup>	778.4 <sup>h</sup>
	30	225.5 <sup>j</sup>	21.3 <sup>h</sup>	0.094 <sup>f</sup>	153.2 <sup>b</sup>	4.6 <sup>h</sup>	0.030 <sup>f</sup>	733.4 <sup>g</sup>
	45	216.6 <sup>i</sup>	20.1 <sup>g</sup>	0.093 <sup>ef</sup>	131.4 <sup>a</sup>	3.8 <sup>g</sup>	0.029 <sup>e</sup>	708.2 <sup>f</sup>
M85	15	177.3 <sup>h</sup>	15.6 <sup>f</sup>	0.088 <sup>e</sup>	167.5 <sup>e</sup>	3.0 <sup>f</sup>	0.018 <sup>d</sup>	578.4 <sup>e</sup>
	30	132.6 <sup>g</sup>	10.7 <sup>e</sup>	0.080 <sup>d</sup>	160.4 <sup>cd</sup>	2.8 <sup>de</sup>	0.017 <sup>d</sup>	496.9 <sup>d</sup>
	45	109.2 <sup>e</sup>	7.8 <sup>d</sup>	0.071 <sup>c</sup>	153.6 <sup>b</sup>	2.7 <sup>cd</sup>	0.017 <sup>d</sup>	438.6 <sup>c</sup>
M70	15	125.2 <sup>f</sup>	7.7 <sup>d</sup>	0.061 <sup>b</sup>	165.4 <sup>de</sup>	2.9 <sup>ef</sup>	0.018 <sup>d</sup>	439.8 <sup>c</sup>
	30	97.9 <sup>d</sup>	6.0 <sup>c</sup>	0.061 <sup>b</sup>	156.5 <sup>bc</sup>	2.6 <sup>bc</sup>	0.016 <sup>c</sup>	433.4 <sup>c</sup>
	45	78.7 <sup>c</sup>	4.7 <sup>b</sup>	0.060 <sup>b</sup>	151.6 <sup>b</sup>	2.4 <sup>b</sup>	0.016 <sup>c</sup>	386.0 <sup>a</sup>
M50	15	83.4 <sup>c</sup>	5.0 <sup>b</sup>	0.060 <sup>b</sup>	167.2 <sup>e</sup>	2.5 <sup>b</sup>	0.015 <sup>b</sup>	410.5 <sup>b</sup>
	30	68.2 <sup>b</sup>	3.6 <sup>a</sup>	0.052 <sup>a</sup>	156.9 <sup>bc</sup>	2.3 <sup>a</sup>	0.014 <sup>a</sup>	400.3 <sup>ab</sup>
	45	58.3 <sup>a</sup>	3.0 <sup>a</sup>	0.051 <sup>a</sup>	152.0 <sup>b</sup>	2.1 <sup>a</sup>	0.014 <sup>a</sup>	383.1 <sup>a</sup>

*Mean values*

Mixture							
Apple	229.2 <sup>d</sup>	23.1 <sup>d</sup>	0.100 <sup>d</sup>	151.3 <sup>a</sup>	4.5 <sup>d</sup>	0.030 <sup>d</sup>	740.0 <sup>d</sup>
M85	139.7 <sup>c</sup>	11.4 <sup>c</sup>	0.080 <sup>c</sup>	160.5 <sup>b</sup>	2.8 <sup>c</sup>	0.018 <sup>c</sup>	504.6 <sup>c</sup>
M70	100.6 <sup>b</sup>	6.1 <sup>b</sup>	0.061 <sup>b</sup>	157.8 <sup>b</sup>	2.6 <sup>b</sup>	0.017 <sup>b</sup>	419.7 <sup>b</sup>
M50	70.0 <sup>a</sup>	3.9 <sup>a</sup>	0.055 <sup>a</sup>	158.7 <sup>b</sup>	2.3 <sup>a</sup>	0.015 <sup>a</sup>	398.0 <sup>a</sup>
Time							
15	157.8 <sup>c</sup>	14.1 <sup>c</sup>	0.081 <sup>c</sup>	167.4 <sup>c</sup>	3.4 <sup>c</sup>	0.020 <sup>b</sup>	551.7 <sup>c</sup>
30	131.1 <sup>b</sup>	10.4 <sup>b</sup>	0.072 <sup>b</sup>	156.7 <sup>b</sup>	3.1 <sup>b</sup>	0.020 <sup>b</sup>	516.0 <sup>b</sup>
45	115.7 <sup>a</sup>	8.9 <sup>a</sup>	0.069 <sup>a</sup>	147.1 <sup>a</sup>	2.8 <sup>a</sup>	0.019 <sup>a</sup>	478.9 <sup>a</sup>

*Standard error of means*

Mixture	1.36	0.16	0.0010	1.10	0.03	0.0001	4.20
Time	1.18	0.14	0.0009	0.95	0.03	0.0001	3.64
Interaction	2.36	0.28	0.0018	1.91	0.05	0.0002	7.28

<sup>1</sup> OM, organic matter; M85, M70 and M50, mixtures containing 85, 70 and 50% of apple in fresh weight basis, respectively; VFA, gas volume of the 1st phase; mmA, maximum gas production rate of the 1st phase; VFB, gas volume of the 2nd phase; mmB, maximum gas production rate of the 2nd phase; mmA / VFA, specific digestion rate of 1st phase; mmB / VFB, specific digestion rate of 2nd phase; IVOMD, *in vitro* OM digestibility.

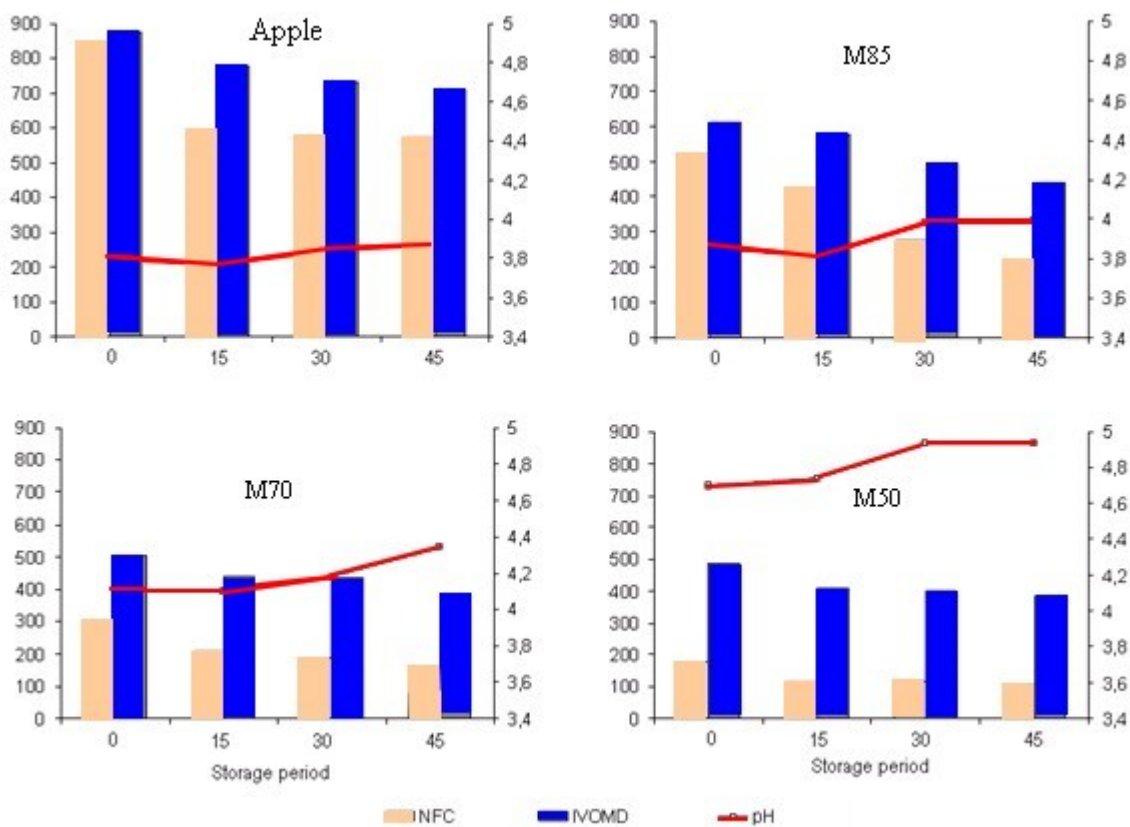
a,b,c,d,e,f,g,h Means in the same row with different letters differ significantly ( $P < 0.05$ )

The analysis of the gas production parameters (Table 4) indicated that both the easily fermentable components (VFA) and the less easily fermentable constituents (VFB) decreased along the storage period ( $P < 0.001$ ). However, for VFB there were no differences between the mixtures ( $P > 0.05$ ) for the first two storage periods, and at 45 days of ensiling only the apple pulp mixture was different ( $P < 0.05$ ) from all the other mixtures.

## Discussion

High levels of fermentable carbohydrates are considered to be an important factor in determining ensilability (Wilkinson 2005). Mixtures M85 (526 g/kg DM) and M70 (304 g/kg DM), before ensilage, showed NFC in the range values reported for apple pomace. Gasa et al (1992) indicated values varying from 220 to 352 g/kg DM, Alibes et al (1984) referred to 330 and Givens and Barber (1987) to 440 g/kg DM. More recently, values around 260g/kg DM were mentioned by Pirmohammadi et al (2006).

However, in our study, apple material did not promote a good ensiling process, notwithstanding the observed high NFC concentration. Used by the microbial population present in the mixture, as NFC clearly diminished along the ensilage process (Figure 1), fermentation of this readily available sugar fraction was not able to reduce pH to a level where silages could reach chemical and microbial stabilization.



**Figure 1.** Changes in Non fibre carbohydrates (NFC), in vitro organic matter digestibility (IVOMD) and pH of experimental silage mixtures during the storage period

The results shown in Table 3 indicate that the microbial population might have been mainly constituted by yeasts, as high levels of ethanol were determined.

Yeasts are facultative anaerobic micro-organisms and, under anaerobic conditions, they may ferment sugars to ethanol and CO<sub>2</sub> (McDonald et al 1991), decreasing the amount of fermentable carbohydrates available for lactic acid fermentation. In addition, yeasts can also use lactic acid to produce CO<sub>2</sub> and H<sub>2</sub>O, causing a rise in the pH (McDonald et al 1991). Data reported in Table 3 and Figure 1 seem to point out a close relationship between increase in ethanol production, decrease in NFC and a rise in the pH of silages, indicating high activity levels of yeasts.

At 15 days of ensiling, ethanol concentrations in M85, M70 and M50 mixtures were below the range of values reported by Alibes et al (1984) and Wyss (2003). These authors found values of ethanol ranging from 134 to 212 g/kg DM in apple pomace silages. These lower concentrations were due to the addition of straw to the mixtures and are in agreement with the study of Alibes et al (1984) who also showed that the addition of straw to apple pomace silages decreased the proportion of ethanol. The shift towards an increase in ethanol and pH values after 30 days of storage may have enhanced yeast growth. As referred by Pina and Hogg (1999), the activity of yeasts may be enhanced in silages with low initial pH values, such as in crops with high sugar content, leading to an increase in ethanol concentration. Since, in our study, pH values below 4.0 were observed in mixtures with a higher proportion of apple, low initial pH values could also have been responsible for stimulating yeast growth. We believe that this increased yeast activity explains the decrease in DM content during the first 30 days of ensilage. According to

McDonald et al (1991), it is possible to assume that approximately 49% of DM losses could be due to the fermentation of sugars into ethanol. Assuming that no ethanol was present at the time of ensiling, and using data from Tables 1 to 3, it is possible to calculate that for the first 15 days of ensilage approximately 90% of DM losses in mixtures M85 and M70 may be attributed to the metabolism of sugars by yeasts. Values obtained for M50 showed that DM losses were much higher in relation to the amount of ethanol produced, indicating that only 50% of these losses could be due to yeasts. A possible explanation, at least partially, could be the less effective compaction in the M50 mixtures due to the higher DM content. This could likely have increased the entrapment of oxygen in the silage mass thus enhancing plant cell respiration and DM loss.

Other factors that should be taken into account to explain the decrease in DM are the possibility of heterolactic fermentation of fructose and glucose as well as enterobacterial fermentation of glucose that could result up to 5, 24 and 40% DM losses, respectively (McDonald et al 1991). In addition, losses attributed to effluent production as well as loss of volatile components during oven DM determination should be referred.

As stated before, there was an increase in total nitrogen, NDF, ADF and ADL. These components became more concentrated probably due to effluent losses or soluble nutrients or DM losses. Increases in lignin could also be due to Maillard reactions, as the polymers formed have physical and chemical properties similar to lignin (Van Soest 1994).

The data obtained for IVOMD are within the values of Alibes et al (1984) who reported an organic matter digestibility of apple pomace around 75%. Givens and Barber (1987) and Gasa et al (1989) referred values of 58% and 86% for fresh apple pomace, respectively. As expected, IVOMD values decreased at higher levels of straw incorporation and although mixtures M85 and M70 were fairly digestible at the beginning of the ensilage, the IVOMD decreased along the storage period. This is in agreement with the results of chemical composition, as the decrease in NFC will limit the amount of available energy for rumen micro-organisms.

A similar trend was observed in the gas production measurements showing that prolonged storage periods lead to a decline in the volume of gas of the 1st phase (VFA). Again, these values reflected the decrease in fermentable sugars already discussed. The contribution of pectins to this soluble fraction of the mixtures could be high. In fact, in spite of the lack of data on pectin fermentation along the ensiling process, Suzzi et al (1987) and Courtin and Spoelstra (1989) already showed some pectinolytic activity in beet pulp silages. Recently, Jones et al (1992) also showed that some pectin constituents could be hydrolysed during the ensilage of lucerne.

During the second phase (VFB), the same effect was more evident in the case of Apple. This could be due to the degradation of fermentable cell wall constituents that could also have been used by the silage microbial population. Some papers have reported data suggesting that structural carbohydrates can also be used as substrate for micro-organisms during ensiling (McDonald et al 1991; Yahaya et al 2000).

The analysis of VFB along the ensiling period and across mixtures apparently shows that no differences were obtained between mixtures containing straw. However if we have in mind that there is an increase in straw proportion from M85 to M50 and that wheat straw shows a clear pattern of high VFB values, than the data are a result of different contributions of straw and apple pulp to the gas production. This is in agreement with the maximum gas production rate of the 2nd phase (mmB) that shows higher values for mixtures containing more apples, thus indicating higher concentration in more easily fermentable cell wall components.

## Conclusions

- These results demonstrate the possibility of reducing ethanol concentration of apple silages



through the incorporation of fibrous components.

- Based on the in vitro organic matter digestibility and gas production of incubated samples, mixtures containing from 15% up to 30 % of straw appear to be most appropriate for animal feeding.
- Although ensilability was improved by preparing apple-straw mixtures, silage additives to limit the fermentation of sugars into ethanol may be useful when ensiling these mixtures.

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[Go to top](#)