# Potential use of high-temperature and low-temperature steam treatment, sodium hydroxide and an enzyme mixture for improving the nutritional value of sugarcane pith

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## Abstract

The effectiveness of different treatment methods to improve the nutritional value of the sugarcane by-products (pith or bagasse) has been evaluated. The treatment methods included a high-pressure steam treatment (HPST; 19 bar, 3 min), treating the products with sodium hydroxide, sulphuric acid plus an enzyme mixture, or low-temperature steam treatment (LTST) under different conditions. Gas production (GP), two-step *in vitro* digestibility (IVD) and *in situ* degradability (ISD) techniques were used to monitor the effectiveness of the treatments. HPST resulted in a significant increased in the total soluble sugar (TSS) content of unsteamed pith (USP), 20 *vs.* 123.75 mg/100 mL. Except for the enzyme treatment, the other treatments led to a significant improvement in the nutritional value of sugarcane by-products, as measured by the IVD method. LTST resulted in an increase in potential GP (*B*) at higher temperature, reaction time and amount of acid. The highest potential GP (110.92 mL/300 mg DM) was achieved under the conditions, 134 °C, 18 g acid/kg DM, 120 min, and the lowest (72.4 mL/300 mg DM) under the conditions, 121 °C, no acid, 40 min. *In situ* dry matter degradability (ISDMD) was unaffected by LTST. Dry matter digestibility results indicated that the optimal treatments for treating pith were HPST and NaOH, but that enzymes were ineffective. Furthermore, considering treatment cost (creating high-pressure are more expensive than low temperature treatments), potential environmental health problems and the relative improvement in the nutritional value of pith achieved by the LTST + acid method, compared to the HPST method (as measured using GP), these results suggested that the methods based on the use of LTST and acid (especially under harsher conditions), have the best potential to improve the nutritive value of sugarcane by-products.

**Keywords:** *Saccharum officinarum*, steam, bagasse, raw or unsteam-treated pith
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## Introduction

Sugarcane bagasse and sugarcane pith, the residues after rind removal, are lignified by-products of the sugar and paper industries (Muller, 1978). Annually large quantities of these products are produced worldwide (Horton *et al.*, 1991), and also in Iran. One potential use of bagasse and pith, and to avoid their accumulation in nature, is as feedstuff for ruminants (Pate, 1982). However, their low digestibility, high lignin and very low nitrogen content are considered the main reasons for the unsatisfactory performance of animals fed these roughages (Chapman & Palmer, 1972; Osorio & Cruz, 1990).

The key to maximising the nutritional value of lignocellulosic materials lies in disrupting the plant cell walls so as to allow complete access of microorganisms to their nutrients, but not creating extra anti-nutritional factors. The disruption conditions of choice will always be a compromise between the use of harsh processes that afford high levels of access, but simultaneously the formation of anti-nutritional factors, or milder but less disruptive processes (Castro *et al.*, 1994).

Many methods have proved successful in disrupting cell wall material, e.g. use of sodium hydroxide (Jackson, 1977), lithium chloride in hydrochloric acid (Shambe & Kennedy, 1984), hydrogen peroxide (Gould, 1984; Adebowale *et al*., 1989; Amjed *et al.*, 1992) and high-pressure steam alone (Dekker & Wallis, 1983; Castro & Machado, 1990) or combined with enzyme treatment (Liu & Ørskov, 2000). In particular, steam and pressure treatments alone or in conjunction with chemical treatments are known to disrupt lignocellulosic material in a way that allows for the improved utilisation of cell wall polysaccharides by cell-free enzymes (Fan *et al*., 1981; Grohmann *et al.*, 1985; Brownell & Saddler, 1987) and rumen microbes

(Castro & Machado, 1990). Treatments using steam and pressure alone require harsh conditions (180 – 220 °C) (Knappert *et al*., 1981; Ullal *et al*., 1984). Under these conditions acetyl groups are released from the hemicellulose (HCL) matrix and suitable levels of cell wall disruption are achieved (Muzzy *et al*., 1983). This also results in the formation of furfural by secondary dehydration reactions of hemicellulosic pentoses (Brownell *et al*., 1986; Morjanoff & Gray, 1987) and soluble phenolic compounds (Wayman & Chua, 1979; Toussaint *et al*., 1991). Both furfural (Kyuma *et al*., 1991), and phenolics (Britton, 1978) inhibit the activity of rumen microbes and cell-free enzymes (Brownell *et al*., 1986; Puls *et al*., 1986; Sutcliffe & Saddler, 1986). The use of lower temperatures in conjunction with an acid can lead to cell wall disruption comparable to that achieved through using steam treatment at high temperatures (Grohmann *et al*., 1985; Castro *et al*., 1994), and afford lower quantities of toxic compounds (Clausen & Gaddy, 1983).

The objective of this study was to determine the effects of the use of different levels of low-temperature steam treatments, sulphuric acid, and reaction duration on the utilisation of sugarcane pith by rumen microbes.

## Materials and Methods

The sugarcane bagasse and pith samples were subjected to the following treatments:

Low-temperature steam treatment: During low-temperature steam treatment (LTST) sulphuric acid solutions (0, 6, 12 and 18 g $H_2SO_4$/kg DM of USP) were added to sugarcane pith that had not been steam treated ('unsteamed pith', USP) (100 g, about 92% DM), to obtain samples of *ca*. 30% dry matter (DM). The samples were then autoclaved at 121 °C and 134 °C for 40, 80 and 120 min. The pressures used at 121 °C and 134 °C were 1.1 and 2.2 bar, respectively. The samples were oven-dried at 55 °C for 48 h.

High-pressure steam treatment: The high-pressure steam-treated (HPST) sugarcane pith was prepared at 19 bar for 3 min (70% moisture) in a Monel pressure vessel (Emamkhomeini Co., Khuzestan-Iran). Before each treatment, air was purged from the pressure vessel with steam at 100 °C. Then the vessel was quickly heated by pressurising with saturated steam from a steam generator. Cooling was achieved either by venting steam from the top of the vessel or by explosive discharge of the entire contents through a nozzle and into a stainless steel collection vessel (Morjanoff & Gray, 1987).

Enzyme and chemical treatment: Four replicates from substrates (USP, HPST pith, and bagasse) were treated with NaOH (SH; 4 g/kg DM). The method involved suspending 100 g substrate and 4 g NaOH in 100 mL distilled water, and then leaving the mixture at room temperature for 48 h. The selected enzyme mixture (Bioproton Pty. Ltd. Co.), which contained cellulase, xylanase, β-glucanase, α-amylase, pectinase, phytase, protease, amyloglycosidase, hemicellulase and pentosanase, was added in dry form to bagasse, and to unsteamed, steam-treated and NaOH-treated sugarcane pith (1 g/kg DM). Enzyme activities were the following (MIU/kg): cellulase 4.2, xylanase 2.5, β-glucanase 0.5, α-amylase 0.95, pectinase 0.07, phytase 0.3, protease 0.003, amyloglycosidase 0.015, hemicellulase 0.015 and entosanase 0.015.

To monitor the effectiveness of the different treatments, the following techniques were used: Gas production (GP) was analysed in triplicate by the Menke & Steingass (1988) technique, using 100 mL glass syringes (model Fortuna; Haberle Labortechnik, Germany) filled with 200 – 500 mg of the USP, HPST and LTST samples, 30 mL of artificial saliva and 15 mL of rumen fluid. Rumen content was removed through a permanent fistula from sheep maintained on a diet of hay. The fluid was strained through four layers of muslin cloth into a flask and $CO_2$ was passed into the flask to displace air above the rumen fluid, which was then kept at 39 °C. Syringes were incubated at 39 °C and GP was measured after 3, 6, 12, 24, 48, 72 and 96 h. The cumulative *in vitro* gas production data were fitted to the exponential equation $GP = B (1 - e^{-Ct})$, where *B* and *C* are the GP values from the fermentable fraction (mL for 96 h) and the GP rate constant (mL/h), respectively.

*In vitro* dry matter digestibility (IVDMD) of bagasse, untreated and steam-treated pith, with and without enzyme and NaOH treatment was determined by the two-stage method of Tilley & Terry (1963). Samples were incubated in rumen fluid for 48 h and then for 48 h in a pepsin-HCl solution. IVDMD was calculated from difference between the amount of the initial and final residue of the samples. *In situ* degradability of the DM of the substrates was measured by an *in situ* technique, using two fistulated Holstein steers (400 ± 25 kg). The animals were fed a 40 concentrate : 60 forage diet. The experimental samples were milled (2 mm screen) and weighed (5 g DM) into four bags (12×19 cm, 52 µm pore size) made of polyester cloth. Samples were prepared for each incubation time. The bags were incubated in the rumen for 2, 4, 8, 16,

24, 48, 72 and 96 h. Four bags were washed with cold tap water to determine the wash-out at zero time. After each incubation time the removed bags were hand-washed with cold tap water, and then dried in a forced-air oven (55 °C, 48 h). The degradable coefficients of DM (*a*, *b* and *c*) were determined using the exponential equation of Ørskov & McDonald (1979).

The following procedure was followed for the extraction of the soluble sugar content of the USP and HPST pith. Samples of 10 – 40 mg DM were soaked in 15 mL distilled water at 40 °C for 60 min, with intermittent stirring, and then filtered through a 300-mesh nylon fabric (pore size 48 μm). The soluble sugar content was measured by the phenol–sulphuric acid method (Dubois *et al*., 1956). To 0.5 mL of sample (five replicates for each treatment) containing 0 - 200 mg of total sugar/L, 0.5 mL of a 0.5% phenol solution was added and vortex mixed. Concentrated $H_2SO_4$ (2.5 mL) was quickly added and vortex mixed. The $A^{490}$ of the final solution was recorded. Total sugar content was calculated from the standard curve obtained from a series of glucose solutions (0, 25, 50, 100 and 200 mg/L).

Data were analysed statistically using the GLM procedure of SAS (1999). Digestibility data were analysed according to a 2×2×2 factorial experimental design using the effect of steam, NaOH and enzyme as treatments:

$$Y_{ijkl} = \mu + S_i + N_j + E_k + SN_{ij} + SE_{ik} + NE_{jk} + SNE_{ijk} + \varepsilon_{ijkl}$$

where: $Y_{ijkl}$ = observation, $\mu$ = overall mean, $S_i$ = steam effect, $N_j$ = NaOH effect, $E_k$ = enzyme effect and $\varepsilon_{ijkl}$ = error.

Results obtained in LTST trial were analysed according to a 4×2×3 factorial experimental design, using the effect of acid, temperature and reaction time as treatments. After a significant F test ($P < 0.05$), the difference in means was tested using Duncan's multiple range test.

## Results

Total soluble sugar (TSS) and *in vitro* GP of USP and HPST sugarcane pith are shown in Table 1. Total soluble sugar of USP increased (20 *vs*. 123.75 mg/100 mL) significantly ($P < 0.05$) when using the HPST treatment. Furthermore, steam treatment resulted in an increasing trend ($P < 0.1$) in 24 h GP (GP24), potential GP (*B*), and a decreasing rate of GP (*C*) after 96 h incubation.

**Table 1** *In vitro* gas production and total soluble carbohydrates in untreated and HPST pith

| Item | Treatments | | s.e.m. | P-value |
|---|---|---|---|---|
| | USP | HPST pith | | |
| TSS (mg/100 mL) | 20[b] | 123.75[a] | 15.2 | * |
| GP 24 h (mL/500 mg) | 37.30 | 40.33 | 1.62 | NS |
| B (mL/500 mg) | 110.56 ± 2.66 | 122.50 ± 5.81 | 6.9 | NS |
| C (mL/h) | 0.018 ± 0.001 | 0.015 ± 0.001 | 0.0017 | NS |

GP - gas production; *B* - potential gas production (mL for 96 h); USP - unsteamed pith; TSS - total soluble sugar; *C* - *g*as production rate constant (mL/h); HPST - high-pressure steam-treated; s.e.m. - Standard error of mean.
* $P < 0.05$; NS - non-significant.

*In vitro* digestibility of bagasse, USP and HPST pith, and the effects of different treatment methods, as well as the main effects of diverse treatment procedures, including the use of sodium hydroxide, enzyme and steam on the IVDMD of bagasse USP and HPST pith are presented in Tables 2 and 3, respectively. In the non-treatment situation (Table 2), the highest and lowest IVDMD values, 340 and 237 g/kg DM, were for HPST pith and bagasse ($P < 0.05$), respectively, achieved by adding the mixed enzyme and NaOH. The range of IVDMD was between 240 and 350 g/kg DM and 370 and 490 g/kg DM for bagasse and HPST pith,

respectively. Therefore, there was a significant difference (P <0.05) for IVDMD, between sugarcane by-products before and after treatment with NaOH and enzyme.

**Table 2** The effect of treatment method on IVDMD (g/kg DM) of bagasse, USP and HPST pith

| Feed | Treatment method | | | | s.e.m. | | | P | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Non | E | SH | SH+E | E | SH | SH+E | E | SH | SH+E |
| USP | $^{b}260^{B}$ | $^{b}300^{A}$ | $^{a}380^{A}$ | $^{a}400^{B}$ | 21.6 | 30.6 | 30.4 | * | * | * |
| HPSTP | $^{b}340^{A}$ | $^{b}350^{A}$ | $^{a}490^{A}$ | $^{a}470^{A}$ | 21.6 | 30.6 | 30.4 | NS | * | NS |
| Bagasse | $^{b}237^{B}$ | $^{b}240^{B}$ | $^{a}370^{B}$ | $^{a}400^{A}$ | 21.6 | 30.6 | 30.4 | NS | * | NS |
| s.e.m. | 16.3 | 18.0 | 9.2 | 28 | - | - | - | - | - | - |
| Effect | * | * | * | * | - | - | - | - | - | - |

NS - non-significant; s.e.m. - standard error of mean; E - enzyme; SH - sodium hydroxide; *P <0.05.
USP - unsteamed pith; HPSTP - high-pressure steam-treated pith; IVDMD - *In vitro* dry matter digestibility.
Superscripts a – b indicate significant differences (P <0.05) between means within columns, and superscripts A – B indicate significant differences (P <0.05) between means within rows.

**Table 3** The main effects of sodium hydroxide, enzyme and steam on IVDMD of bagasse USP and HPST pith

| Treatment | Condition | | s.e.m. | P-value | Effect |
|---|---|---|---|---|---|
| | Unused | Used | | | |
| Sodium hydroxide | $273^{b}$ | $423.3^{a}$ | 12.0 | 0.0001 | * |
| Enzyme | 306 | 327.5 | 10.8 | 0.864 | NS |
| Steam | $305^{b}$ | $344.4^{a}$ | 12.5 | 0.040 | * |

Superscripts, a – b, indicate significant differences (P <0.05) between means within rows.
USP - unsteamed pith; HPSTP - high-pressure steam-treated pith.
IVDMD - *in vitro* dry matter digestibility.
* P <0.05; s.e.m. - standard error of mean; NS - non-significant.

The best result was related to HPST pith, which means that steam treatment was an efficient method for the processing of pith. Regardless of the NaOH and enzyme effects, the use of steam pressure resulted in a 14% increase in the digestibility of USP (Table 2, see difference between rows). Furthermore, the highest IVDMD, achieved after treatment with NaOH, indicated the effectiveness of this method (Table 2, see deference between columns). Overall, the treatment methods used in present experiments, except for the use of the enzyme mixture, led to significant improvements in the nutritional value of sugarcane by-products, (Table 3). Hence, irrespective of the type of treatment used here, there was an improvement in IVDMD (P <0.05) (Table 4).

Table 5 presents the gas production coefficients of sugarcane pith treated at a low temperature and with commercial $H_2SO_4$ at different times. Generally, there was an increase in potential GP (*B*) with an increased in temperature, reaction time and amount of acid. The highest potential GP (110.92 mL/300 mg DM) was obtained under the conditions, 134 °C, 18 g/kg acid and 120 min, and the lowest (72.4 mL/300 mg DM) under the conditions, 121 °C, no acid, 40 min.

**Table 4** The sum effect of treatment (irrespective of the type) on IVDMD of bagasse USP and HPST pith

| Feedstuff | Treatment | | s.e.m. | Effect |
|---|---|---|---|---|
| | Non-treated | Treated | | |
| USP | $^{b}266^{B}$ | $^{a}386.6^{B}$ | 10.9 | * |
| HPST pith | $^{b}324^{A}$ | $^{a}450^{A}$ | 11.2 | * |
| Bagasse | $^{b}237^{B}$ | $^{a}336.6^{C}$ | 8.6 | * |
| s.e.m. | 14.9 | 18.9 | - | - |
| P-value | 0.018 | 0.048 | - | - |
| Effect | * | * | | |

Superscripts a – b indicate significant differences (P <0.05) between means within columns;
Superscripts A – B indicate significant differences (P <0.05) between means within rows.
USP - unsteamed pith; HPST - high-pressure steam-treated; IVDMD - *in vitro* dry matter digestibility.
NS - non-significant; s.e.m. - standard error of mean; * P <0.05.

**Table 5** Gas production coefficients (mean ± s.e. per 300 mg DM) of low-temperature treated pith and the effect of acids

| Temp (°C) | Time (min) | | Acid (g/kg DM) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 6 | 12 | 18 |
| 134 | 120 | *B* | 78.4 ± 2.3 | 80.4 ± 1.6 | 107.3 ± 2.6 | 110.9 ± 3.5 |
| | | *C* | 0.06 ± 0.001 | 0.03 ± 0.001 | 0.04 ± 0.002 | 0.04 ± 0.008 |
| 134 | 80 | *B* | 74 ± 0.99 | 84.6 ± 2.8 | 92.3 ± 1.4 | 107.1 ± 5 |
| | | *C* | 0.06 ± 0.002 | 0.03 ± 0.002 | 0.05 ± 0.002 | 0.03 ± 0.004 |
| 134 | 40 | *B* | 72.8 ± 1.2 | 72.6 ± 0.7 | 79.2 ± 0.7 | 95.3 ± 0.9 |
| | | *C* | 0.07 ± 0.002 | 0.02 ± 0.004 | 0.05 ± 0.003 | 0.06 ± 0.002 |
| 121 | 120 | *B* | 77.5 ± 3 | 77.9 ± 3 | 82 ± 1.5 | 99.2 ± 4 |
| | | *C* | 0.03 ± 0.003 | 0.03 ± 0.005 | 0.04 ± 0.002 | 0.02 ± 0.002 |
| 121 | 80 | *B* | 73 ± 0.55 | 73.4 ± 0.58 | 77.9 ± 1.5 | 85.4 ± 1.1 |
| | | *C* | 0.05 ± 0.002 | 0.04 ± 0.002 | 0.04 ± 0.004 | 0.04 ± 0.003 |
| 121 | 40 | *B* | 72.4 ± 0.91 | 74.8 ± 2 | 80.4 ± 0.54 | 86.5 ± 1.0 |
| | | *C* | 0.02 ± 0.003 | 0.06 ± 0.006 | 0.03 ± 0.002 | 0.1 ± 0.005 |

*B* - potential of gas production (mL for 96 h); *C* - gas production rate constant (mL/h); s.e. - standard error.

**Table 6** The main effects of acid, temperature and reaction time on cumulative gas production (mL per 300 mg) of low-temperature treated pith

| | Acid (g/kg DM) | | | | | Temp (°C) | | | Time (min) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 6 | 12 | 18 | s.e.m. | 121 | 134 | s.e.m. | 40 | 80 | 120 | s.e.m. |
| *B** | $74.5^{c}$ | $77.3^{c}$ | $86.5^{b}$ | $97.4^{a}$ | 0.23 | $80.0^{b}$ | $87.9^{a}$ | 0.16 | $79.3^{b}$ | $83.3^{ab}$ | $89.2^{a}$ | 0.20 |

*B* - gas potential of gas production (mL for 96 h); s.e.m. - standard error of mean.
Means with different superscripts, a, b and c, in each section differ significantly (P < 0.05).

The main effects of acid, temperature and reaction time on the cumulative gas production of LTST pith are presented in Table 6. Increasing the amount of $H_2SO_4$ significantly ($P < 0.05$) increased the GP of LTST pith (74.52 and 97.41 mL, respectively, for 0 and 18 g acid/kg DM). Temperature (79.97 *vs*. 87.91 mL at 121 and 134°C, respectively), and the treatment time, especially at 120 min (79.27 *vs.* 89.22 mL at 40 and 120 min, respectively) had a noticeable effect on GP ($P < 0.05$). There was a significant interaction between experimental conditions, whereas the effect of LST was clearer in the presence of acid ($P < 0.05$).

**Table 7** *In situ* DM (mean ± s.e.) rumen degradation coefficients (a, b, c) of low-temperature treated pith under various conditions

| Temp (°C) | Time (min) | | Acid (g/kg DM) | | | |
|---|---|---|---|---|---|---|
| | | | 0.0 | 6 | 12 | 18 |
| 134 | 120 | *a* | 0.16 ± 0.02 | 0.17 ± 0.02 | 0.19 ± 0.02 | 0.5 ± 0.02 |
| | | *b* | 0.47 ± 0.03 | 0.47 ± 0.04 | 0.46 ± 0.03 | 0.55 ± 0.07 |
| | | *c* | 0.03 ± 0.007 | 0.04 ± 0.008 | 0.03 ± 0.007 | 0.02 ± 0.008 |
| | | *PD* | 0.63 | 0.64 | 0.65 | 0.7 |
| | | *ED* | 0.41 | 0.42 | 0.43 | 0.4 |
| 134 | 80 | *a* | 0.12 ± 0.02 | 0.13 ± 0.02 | 0.13 ± 0.02 | 0.17 ± 0.02 |
| | | *b* | 0.48 ± 0.03 | 0.46 ± 0.04 | 0.47 ± 0.04 | 0.45 ± 0.04 |
| | | *c* | 0.04 ± 0.008 | 0.03 ± 0.008 | 0.03 ± 0.008 | 0.03 ± 0.008 |
| | | *PD* | 0.6 | 0.59 | 0.6 | 0.62 |
| | | *ED* | 0.39 | 0.38 | 0.38 | 0.39 |
| 134 | 40 | *a* | 0.1 ± 0.15 | 0.13 ± 0.02 | 0.15 ± 0.04 | 0.14 ± 0.02 |
| | | *b* | 0.53 ± 0.17 | 0.47 ± 0.03 | 0.46 ± 0.08 | 0.46 ± 0.03 |
| | | *c* | 0.08 ± 0.07 | 0.04 ± 0.008 | 0.03 ± 0.01 | 0.04 ± 0.008 |
| | | *PD* | 0.61 | 0.6 | 0.61 | 0.6 |
| | | *ED* | 0.47 | 0.4 | 0.37 | 0.4 |
| 121 | 120 | *a* | 0.14 ± 0.05 | 0.14 ± 0.02 | 0.14 ± 0.02 | 0.14 ± 0.02 |
| | | *b* | 0.45 ± 0.06 | 0.49 ± 0.03 | 0.47 ± 0.04 | 0.48 ± 0.04 |
| | | *c* | 0.05 ± 0.02 | 0.03 ± 0.007 | 0.03 ± 0.009 | 0.03 ± 0.007 |
| | | *PD* | 0.6 | 0.63 | 0.61 | 0.63 |
| | | *ED* | 0.42 | 0.4 | 0.38 | 0.39 |

*PD* - potential degradability; *ED* - effective degradability; *a* - rapidly degradable fraction; *b* - slowly degradable fraction; *c* - fractional degradation rate constant (1/h) of *b* fraction; s.e. - standard error.

**Table 8** The main effects of acid, temperature and reaction time on the potential of degradation of DM in low-temperature treated pith, measured by an *in situ* method

| | Acid (g/kg DM) | | | | | Temperature (°C) | | | Time (min) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 6 | 12 | 18 | s.e.m. | 134 | 121 | s.e.m. | 40 | 80 | 120 | s.e.m. |
| *PD* | 0.58 | 0.59 | 0.59 | 0.60 | 0.07 | 0.58 | 0.60 | 0.05 | 0.57 | 0.58 | 0.61 | 0.06 |

*PD* - potential degradability; s.e.m. - standard error of mean.

Coefficients of *in situ* dry matter degradability (ISDMD) (Table 7) and the main effects of acid, temperature and reaction time on ISDMD (Table 8) show that, when supplemented with acid, an increased reaction time and temperature had no significant effect ($P > 0.05$), but there was a trend towards improvement under harsher conditions ($P < 0.1$).

## Discussion

An increase in TSS content as a result of steam treatment may be due to partial (Schultz *et al.*, 1984) or complete hydrolysis of the HCL fraction of raw pith (USP), changing it into more soluble components (Pate, 1982; Toussaint *et al.*, 1991) and partial depolymerisation of lignin (Morjanoff & Gray, 1987; Toussaint *et al.*, 1991). Similar changes in total soluble carbohydrates of rice straw (Liu *et al*., 1999; Liu & Ørskov, 2000) were observed following treatment by steam pressure. Although the TSS of HPST pith increased significantly above that of unsteamed pith, GP between USP and HPST was not significantly different, even though the GP parameter reflects the sugar contents of samples. Significant positive linear relationships have been observed between the GP parameter and soluble carbohydrate content (Liu *et al.*, 1999). Little effect of steam treatment on the GP parameters of steam-treated rice straw was observed by Liu *et al.* (1999). They demonstrated that the inhibitory effect on the GP at the higher steam pressures might be attributed to some anti-nutritional compounds produced during steam treatment. Castro *et al.* (1994) observed that low (134 °C) and high (210 °C) temperature steam-treatment produced some compounds that could inhibit rumen microbial activity and, under increasingly harsher treatment conditions, greater quantities of these anti-nutritional factors were produced.

The use of steam pressure resulted in about a 14% increase in the IVDMD of USP (Table 2). This was confirmed by Pate (1982), when steers were fed (560 g/kg DM diet) with steam pressure-treated (SPT) bagasse (19 bar, 4.3 min), where the digestibility of DM and OM in the SPT bagasse diet were, respectively, 7% and 8% higher than in the raw bagasse. In studies with sheep, Campbell *et al*. (1973) fed a diet containing 400 g SPT bagasse/kg DM and reported 15 and 25% increases in the digestibility of OM and total digestible nutrient, respectively, above similar diet containing raw bagasse. Pate (1982) found that crude protein (CP) digestibility was 55% lower in the HPST pith bagasse diet compared to that in the raw bagasse diet. He suggested that metabolic agents were formed in SPT bagasse that inhibited the digestibility of CP, and hence DM digestibility increased little. The probability that anti-nutrients were produced is in agreement with reports of Castro *et al*. (1994). However, the CP response differed from results of studies with sheep (Campbell *et al.*, 1973) where CP digestibility in a diet containing SPT bagasse was 25% higher than in a raw bagasse diet. Wong You Cheong *et al*. (1974) concluded from *in vitro* digestibility studies that any improvement in digestibility of bagasse resulting from SPT was due to the formation of water soluble substances and that the lignocellulose in SPT bagasse was almost totally indigestible. This is in agreement with results reported for *in vitro* studies by Liu *et al.* (1999; 2000) and Chaji & Naserian (2006).

Although the HCL fraction of roughage was mostly hydrolysed after steam treatment (Liu *et al*., 1999; Chaji & Naserian, 2006), the improvement in nutritional value, which was measured by *in vitro* GP in the present study, was less than what was expected. Furan derivatives (furfural and hydroxyl methyl furfural) and phenolic compounds can be formed during steam treatments, and these may be toxic to rumen microbes (Forsberg *et al*., 1986; Kyuma *et al.*, 1991) or have an inhibitory activity on cell-free enzymes (Mes-Hartree & Saddler, 1983, Sharma *et al*., 1985). This would provide an explanation for our reasoning. On the other hand, Zahedifar (1996) indirectly demonstrated that the phenolic compounds and furan derivatives were not toxic to rumen microbes. Castro (1994) also observed in wheat straw (treated at low temperatures), that rumen microbes were tolerant, and could quickly metabolise both furfural and hydroxyl methyl furfural. He found that inhibitory compounds present in steam-treated samples are phenolic-type compounds, though in his study these negative effects were observed after exposure to harsh treatment conditions. These compounds were not measured in the present study, but the effects of enzymic hydrolysis should be inhibited by these possible harmful products due to the relatively high content of lignin in pith and the harsh conditions under which the HPST pith was prepared (19 bar, 3 min). Furthermore, the improvement of IVDMD in USP when using enzyme was more than the enzyme treatment on HPST pith (Table 2). On the other hand, in the present study, the limited effect of enzyme treatment on the IVDMD of HPST pith may be due to the short incubation time (about 2 h) with the enzymes, the amount of enzyme used (1 g/kg DM), the type of enzyme (mixed) used, and other factors. Yang *et al.* (1999) demonstrated that, apparently, the

inconsistent results obtained from enzyme use can be attributed to a number of factors such as diet composition, type of enzyme preparation, complement of enzyme activities, quantity of enzyme, enzyme stability and method of enzyme application. Mohammadabadi *et al*. (2008) for instance used 5 g/kg DM of a mixed enzyme, similar to the enzyme we used, but it contained 20% and 50% more cellulase and xylanase, respectively, and used a high fat (165 g/kg DM) sunflower meal. They observed a significant improvement in GP (167 *vs*. 197 mL/500 mg DM). Hence, different results for effect of enzyme are obtained when using different amounts and types of enzymes.

Sodium hydroxide resulted in a significant increase in the digestibility of bagasse, USP and HPST pith (mean 20%) (Table 2), and had the highest main effect for improving digestion (Table 3). Processing and treatment of fibrous material by alkali treatment such as with NaOH and/or hydrogen peroxide causes silica and hemicellulose to dissolve, but has no effect on cellulose, and the amount of solubilisation depends on the type of fibrous material and its characteristics (Jackson, 1977). All of these changes resulted in an increase in the digestibility of the DM and NDF of the by-products (Amjed *et al.*, 1992).

Currently, the use of ammonia and NaOH in the treatment of straws and crop residues are the most widely used chemical techniques for enhancing their quality (Liu *et al*., 1999). Treating roughages with NaOH generally increases the daily feed intake and digestibility in ruminants, though sodium contamination of soil and water from the urine of animals fed NaOH-treated material is a potential problem. Due to its high price, the widespread use of NaOH to process crop residues is unlikely to be economically feasible in most regions of the world (Liu *et al*., 1999). Yet another important prerequisite for a pretreatment method which is largely ignored, is that it has to be effective for a large quantity of biomass materials (Schultz *et al*., 1984). Therefore, the result of the present study may indicate that, although NaOH resulted in a higher IVDMD than steam (20% *vs*. 14%), when taking associated problems into account, the use of steam treatment would be preferable as it is more environmentally friendly, usable on a large scale for treatment, and is at least cheaper than chemical treatments in Iran.

The use of acid increased the reaction time, and temperature had no significant effect on ISDMD ($P > 0.05$). However, the higher potential of DM degradability ($PD = a+b$) at 134 °C for 120 min, and the increasing trend with an increase in acid (63 *vs*. 70% for 0 and 18 g acid/kg DM, respectively) (Table 7) indicated that the best conditions during the low-temperature or pressure steam treatment in the present study were 134 °C, 18 g acid/kg DM and 120 min. On the other hand, according to the GP data it was possible to improve the nutritional value of pith by using temperatures higher than 134 °C (Tables 5 and 6). Chaji & Naserian (2006) confirmed this. In addition, the PD of DM in LTST pith (Table 7; 0.70 for 134 °C, 18 g acid/kg and 120 min) was significantly higher than the PD of DM (0.59) of raw pith (Chaji & Naserian, 2006). Nevertheless, the improvement in nutritional value of raw pith, which was measured by ISDMD in the present study, was much less than what was expected. This is in accordance with the findings of Castro (1994), who used the same conditions for treatment of wheat straw as was used in the present study. The effect of LST and $H_2SO_4$ used in our experiments was significantly lower than reported in other studies conducted at similar temperatures (Cunningham & Carr, 1984; Grohmann *et al*., 1985). However, the results of Cunningham & Carr (1984) and Grohmann *et al*. (1985) can be explained by the higher levels of $H_2SO_4$ used (>45 g $H_2SO_4$/kg on DM basis). Two further aspects that might have influenced the response of the steam treatment in our study and that of Castro are the following: (1) Type of substrate: the pith used in the present study and the wheat straw in that of Castro (1994) showed a low bio-utilization by cell-free enzymes (1.5% after 2 h and 9% after 48 h of incubation), (2) Amount of water: the higher DM content after acid addition (30% in this study and 20% in Castro's study) compared to the 10% described in the studies of Cunningham & Carr (1984) and Grohmann *et al*. (1985) might have influenced the efficiency of heat transfer and acid impregnation. This is in agreement with a report by Morjanoff & Gray (1987). In their experiment a tests were carried out with dried bagasse, undried whole bagasse and different water : solid ratios to determine the effect of drying on the steam treatment process. Their results indicated that the optimum treatment time for fresh wet bagasse was significantly shorter than for dried bagasse, and that reducing the water : solids ratio from 4 to 1 increased the optimum treatment time and decreased the sugar yield. In particular, the rate of HCL hydrolysis was higher for the undried sample. Wilke (1979) stated that the drying of HCL can change its conformation, and cause denaturation and the irreversible adsorption of xylan onto cellulose. This would explain the longer treatment times required for dried bagasse.

## Conclusion

Use of high-pressure steam treatment and sodium hydroxide appears to improve the nutritional values of raw pith, as determined by *in vitro* GP and digestibility, respectively. Enzyme treatment had no significant effect on IVDMD of HPST pith; its effect was higher in USP. Overall, the use of low temperature and acid was more suitable for improving the nutritional value of USP, especially when taking the cost of treatment and environmental health into consideration. Further studies on the suitability of using low temperatures, which include consideration of the use of different conditions and methods, are required.

## Acknowledgements

We are grateful to the Emamkhomeini Co. for the high-pressure, steam-treatment pith.

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