Optimization of Steam Treatment Conditions for Improving the Nutritive Value of Date Leaves

M. Zahedifar[1*], N. Karimi[2], H. Fazaeli[1], and S. A. Mirhadi[1]

ABSTRACT

The aim of this study was to investigate the effect of steam treatment on nutritive value of date (*Phoenix dactylifera*) leaves. Date leaves were chopped and mixed with water or sulfuric acid solution to contain 50% moisture with or without 1% sulfuric acid. Steam treatment of the date leaves was carried out using three levels of steam pressure (14, 17 and 20 bar), three reaction times (120, 180, and 240 seconds) and two levels of acid (0 and 1 percent). The treated samples were analyzed for chemical composition including: cell wall components, ash, total extractable phenolics, water soluble sugars, and reducing sugars. Dry matter loss (DML), enzymic hydrolysis, and *in vitro* gas production of the samples were also measured. Results showed that steam treatment significantly affected (P< 0.05) cell wall components. An increasing trend was observed in DML by increasing harshness of treatment conditions. The lowest DML (12.7 g kg^{-1}) was observed in the auto-hydrolyzed (steam treatment without addition of exogenous acid) sample treated at 14 bar pressure and 120 seconds reaction time and the highest DML (78.8 g kg^{-1}) was observed in the acid-hydrolyzed (addition of 10 g kg^{-1}acid prior to treatment) samples treated at 20 bar pressure and 180 and 240 seconds reaction times. Steam treatment significantly (P< 0.05) decreased neutral detergent fiber (NDF) content but increased acid detergent lignin (ADL). Maximum changes in hemicellulose and water soluble sugars were observed in acid-hydrolyzed samples, in which hemicellulose decreased from 264.6 g kg^{-1} in control to 72.2 g kg^{-1} in the sample treated at 20 bar and 240 seconds and water soluble sugars increased from 14.0 g kg^{-1} in the control to 101.8 g kg^{-1} in the sample treated at 17 bar and 240 seconds. Enzymic hydrolysis of date leaves was improved after steam treatment and higher improvement was observed in acid-hydrolyzed samples. Gas production was significantly increased (P< 0.05) in all incubation times after steam treatment. The maximum increase in metabolizable energy (ME) estimated by gas production was from acid-hydrolyzed sample treated at 20 bar and 240 seconds. In auto-hydrolyzed samples, the biggest increase in ME was observed in the sample treated at 20 bar and 180 seconds. The results suggest that steam treatment could be used for upgrading the nutritive value of date leaves in the regions where date is grown and animals are encountered with severe feed shortage.

Keywords: Chemical composition, Hemicellulose solubilization, Lignin depolymerization, Enzymic hydrolysis, *in vitro* gas production.

INTRODUCTION

Date is the main agricultural crop production in some parts of south-east Iran, where livestock production is also an important component of the farming system. However, in that area, feed limitation is the main constraint for livestock production. Date palm frond could be a significant source of roughage that may be treated and used as ruminant feed (Belal *et al*., 1999). The yearly maintenance of date palm trees causes leaving out large quantities of leaves, about 20 kg per tree (Pascual et al., 2000; Arhab et

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[1] Animal Science Research Institute of Iran, Department of Animal Nutrition, Karaj, Islamic Republic of Iran.
[*] Corresponding author; email: zahedifar44@yahoo.com
[2] Islamic Azad University, Pishva Branch, Varamin, Islamic Republic of Iran.

al., 2006). It is estimated that the annual date leaves production in Iran is over 500000 tones (Kardooni, 2012). Nowadays, very little of the leaves is being used as fuel or for making arts and the rest is being disposed of as a waste material, which cause environmental pollution. The information available on chemical composition of date leaves are inconsistent. The values for crude protein (CP), NDF, acid detergent fiber (ADF), and ash reported by Medjekal *et al.* (2011) were 52.0, 852.0, 609.0, and 87.0 g kg⁻¹, respectively, while the values reported by Arhab *et al.* (2006) for the respective parameters were 64.8, 586.1, 422.8 and 109.5 g kg⁻¹. Such variations in chemical composition may be related to the differences between cultivars of date plant. Bukhaev *et al.* (1985) reported that the *in vitro* digestibility of the three fractions of date leaves including foliole, petiole, and base was 67, 129 and 84 g kg⁻¹, respectively. Little is known about improving the nutritive value of date leaves as ruminant feed. Kawamoto *et al.* (2001) studied the digestibility of dry, pelleted, ensiled and NaOH treated date leaves and found that voluntary intake in pelleted form was higher,, but with lower digestibility, than that of the other treatments. No difference was found between digestibility of dried and ensiled leaves, but digestibility of NaOH treated leaves was higher compared to the both dried and ensiled leaves.

The conventional methods used for upgrading the nutritive value of roughages such as urea treatment has not shown acceptable results in date leaves (Sadeghi *et al.*, 2001), while results of steam treatment has been promising for improving the nutritive value of some low quality roughages (Pate, 1982; Castro *et al.*, 1993a; Chaji, *et al.*, 2010a). When lignocellulosic materials are subjected to high steam pressure, at initial stage of treatment, hemicellulose and pectin are partially hydrolyzed and acetyle and formyl groups are released. The release of endogenous organic acids, e.g. acetic and formic acids will help to catalyze further reaction which depolymerizes part of

hemicellulose and lignin. Solubilization of hemicellulose and depolymerization of lignin are the main chemical changes that occur during steam treatment. Depolymerization of cellulose can also occur during the treatment, but more severe conditions are needed as compared to hemicellulose (Baugh and McCarty, 1988). Disrupting of cell wall structure by steam treatment makes it more susceptible to the action of rumen microorganisms (Chaji, *et al.*, 2011). There are detailed studies on steam treatment of wheat straw (Castro, 1994) and sugar cane by products (Chaji *et al.*, 2013) but very little information is available on steam treatment of date leaves.

The aim of this study was to assess the effect of different conditions of steam treatment including pressure, reaction time, and acid on nutritive value of date leaves.

MATERIALS AND METHODS

Preparation of Sample

Date (*Phoenix dactylifera)* leaves were chopped into 2-5 cm length and mixed with water or sulfuric acid solution to get homogenous samples containing 50% moisture with or without 1% acid (dry matter basis). The prepared samples were stored in plastic bags for 24 hours at room temperature prior steam treatment.

Steam Treatment

Steam treatment was accomplished using steam-treatment equipment designed for research purposes at Animal Science Research Institute of Iran. Steam was generated in a boiler and released into the hydrolyzer chamber (110 liter capacity) containing substrate. Samples (1,000 g wet) were put into stainless steel net containers. For each treatment, the chamber was preheated and filled with maximum 6 containers. The chamber was then closed and charged with steam to reach the

appropriate pressure by adjusting the steam releasing valve. In this study, effect of two levels of acid (0 and 1%), three levels of pressure (14, 17 and 20 bar), and three levels of reaction time (120, 180 and 240 seconds) were studied. Each treatment was carried out in three replications. After treatment, samples were removed and dried in an air-forced oven at 60°C for 48 hours. The dried samples were kept at room temperature for further analysis. .

Dry Matter Loss and Chemical Compositions

Dry matter loss (DML) of the samples during steaming process was measured by subtracting dry weight of the samples before and after the treatment. Samples were analyzed for N content by Kjeldahl method (ID 7.015), ether extract (EE) by Soxtech, and Ash (ID 7.009) by burning at 550 ℃ (AOAC, 2000). Neutral detergent fiber (NDF) assayed without amylase and expressed inclusive of residual ash as described by Van Soest *et al.* (1991). Acid detergent fiber (ADF) expressed inclusive of residual ash and acid detergent lignin (sa) (ADL) determined by solubilization of cellulose with sulfuric acid as described by Robertson and Van Soest (1981).

Total Extractable Phenolics

Five ml 70% acetone solution (v/v) was added to a test tube containing 100 mg sample (DM basis) to extract phenolic compounds. Nine hundred µl water, 0.5 ml 1M Folin and Ciocauteu phenol reagent and 2.5 ml 20% Na_2CO_3 were added to 0.1 ml extract . The mixture was vortex mixed after the addition of each reagent. It was kept for 35 minutes at room temperature, then; the absorbance was recorded at 725 nm spectrophotometer. A standard curve was constructed using 0, 10, 20, 40, 80 and 100 µl of 0.5 mg ml^{-1} tannic acid in 70% acetone solution (Makkar *et al.*, 1992).

Enzymic Hydrolysis

Enzymic hydrolysis was performed according to the method described by Castro *et al.* (1993b). Volume of 50 ml acetate buffer (pH 5.0, 0.1M) with 0.1% sodium azide as a preservative was added to 250 ml conical flask containing 1 g of washed sample (DM basis). One g of Cellulase enzyme (Onozuka, EC: 3.2.1.4, xylanase activity 10^5 UI/mg, cellulase activity 1 IU mg^{-1} as carboxy methyl cellulose-CMC, Merck product) was dissolved in 50 ml acetate buffer and 3.2 ml of which (containing 64 IU cellulase as CMCas activity) was added to the conical flask. Enzymic hydrolysis for each sample was performed in 3 replications. The flasks were stoppered and incubated in water bath at 120 rev min^{-1} at 37°C for 48 hours. Aliquots (2 ml) were withdrawn from each flask after 48 hours and quickly filtered through Whatman No. 1 filter paper. The filtrates were heated at 100°C for 10 minutes to stop enzymic activity. The liquid hydrolysates were analyzed for reducing sugars (Nelson, 1944).

Reducing Sugars Analysis

Reducing sugars were measured by the Somogyi method as adapted by Nelson (1944). A volume of 0.5 ml of water extract from the sample containing 0-100 mg l^{-1} reducing sugar was mixed with 0.5 ml of Nelson reagent in a test tube, then, it was vortex mixed and 0.5 ml of Hardings reagent was added, vortex mixed again and kept for 10 minutes at 100°C. The mixture was transferred to an ice bath and kept for about 5 minutes, then, 0.5 ml of Somogyi reagent was added and the reaction mixture was vortex mixed. Finally, 3 ml of distilled water was added and the solution vortex mixed. The A_{600} of the final solution was recorded. Reducing sugar content (mg l^{-1}) was calculated from the standard curve obtained from a series of glucose solutions (25, 50, 75, and 100 mg l^{-1}).

Bioavailability to Rumen Microbes

Samples (200 mg) were incubated in triplicate in 100 ml calibrated glass syringes according to the method described by Menke and Steingass (1987). Rumen liquor was collected from the three fistulated local breeds of cattle (Taleshi breed) fed at maintenance (2.07 Mcal kg^{-1} ME and 10.20% CP. Diet contained 30% alfalfa, 35% wheat straw, 20% wheat bran, 14.5% barley grain, 0.2% vitamin and mineral supplement, and 0.3% salt). Gas production was recored at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after incubation. Net gas production for each treatment was calculated by subtracting the gas produced in blank. Cumulative gas production data were fitted to the exponential equation: $Y= b(1-e^{-ct})$ of Ørskov and McDonald (1979) modified by Blummel and Becker(1977) in which *a* parameter was ommited from the equation. Where *Y* is the gas produced at *t* time, *b* is the potential of gas production (after 96 hours) from the insoluble but fermentable fraction (ml g^{-1} OM), *c* the gas production rate constant for *b,* and *t* the incubation time. The ME and OMD contents were calculated using equations of Menke and Steingass (1987) as:

$$ME \text{ (MJ kg}^{-1} \text{ DM)}= 2.20+(0.136\times Gp)+(0.0057\times CP)+(0.00029\times EE^2) \quad (1)$$

$$OMD \text{ (g 100 g}^{-1} \text{ DM)}= 14.88+(0.889\times Gp)+(0.45\times CP)+(0.0651\times Ash) \quad (2)$$

Where, *CP* is crude protein in g 100 g^{-1} DM, *EE* is ether extract in g 100 g^{-1} DM, ash in g 100 g^{-1} DM, and *Gp* is the net gas production (ml) from 200 mg after 24 hours of incubation.

Statistical Analysis

A completely randomised design with 2×3×3 factorial arrangement was used to assess the effect of two levels of sulfuruic acid (0 and 1%), three levels of pressure (14, 17, and 20 bar) and three levels of reaction time (120, 180, and 240 seconds) with 3 replicates per treatment on date leaves. Statistical analysis was performed using GLM procedure of SAS system (SAS Institute, 2000) and tested for significance using Duncan multiple range test at $P< 0.05$. The adopted statistical model is shown below:

$$Y_{ijkj}= \mu+S_i+P_j+T_k+(SP)_{ij}+(ST)_{ik}+(PT)_{jk}+(SPT)_{ijk}+e_{ijk} \quad (3)$$

Where, Y_{ijkj}= Dependent variable, μ= Overall mean, S_i= Fixed effect of the ith acid level (i= 0 or 1), P_j= Fixed effect of the jth steam pressure level (j= 1-3), T_k= fixed effect of the kth reaction time (k= 1-3), $(SP)_{ij}$= Fixed effect of the interaction between i^{th} acid level and j^{th} steam pressure Level, $(ST)_{ik}$= Fixed effect of the interaction between i^{th} acid level and k^{th} reaction time, $(PT)_{jk}$ = fixed effect of the interaction between j^{th} pressure level and k^{th} reaction time, $(SPT)_{ijk}$= Fixed effect of the interaction between the ith acid level, the jth steam pressure level and the kth reaction time, e_{ijk}= Random error.

A separate statistical analysis was performed for auto-hydrolyzed samples (steam treatment of samples without addition of exogenous acid) and acid-hydrolyzed samples (steam treatment of samples contaning 1% sulfuric acid). For this purpose, a completely randomised design with 3×3 factorial arrangement was used to assess the effect of three levels of pressure and three levels of reaction time on chemical composition of the date leaves.

RESULTS

Chemical Composition

The treatment variables tested in this study, namely, pressure, acid, and reaction time affected all the chemical parameters (Table 1). Pressure and acid showed more effective than reaction time on chemical composition. The interaction between acid and time had the least effect on chemical composition.

All levels of pressure and acid significantly increased DML, which was significantly increased (P< 0.05) by increasing time from 120 to 180 seconds, but it was not affected when reaction time changed from 180s to 240s. Addition of acid also increased (P< 0.05) DML. The separate statistical analysis for both auto-hydrolyzed and acid-hydrolyzed samples showed (Table 2) that, in both cases, increasing pressure from 14 to 20 bar increased DML.

The NDF content of samples was significantly (P< 0.05) decreased by the three factors and their interactions (Table 1). In auto-hydrolyzed samples (Table 2), changes in pressure and reaction time significantly affected NDF, but, in the case of acid-hydrolyzed samples, no effect was observed when reaction time changed from 180 to 240 seconds. Maximum reduction in NDF content for both auto-hydrolyzed and acid-hydrolyzed samples was observed at 20 bar pressure.

As is seen in Table 1, all treatments and interactions, except pressure×acid, affected

ADF content.

Effect of different treatments on DML and chemical composition of the date leaves are shown in Table 3. The minimum ADF content was in the acid-hydrolyzed sample treated at 20 bars for 120 seconds.

None of the interactions, except pressure×time, affected (P< 0.05) hemicellulose content (Table 1). The least hemicellulose content was observed in acid-hydrolyzed sample treated at 20 bars pressure for 240 seconds reaction time (Table 3).

In both auto-hydrolysis and acidhydrolysis, all treatments significantly reduced hemicellulose content (P< 0.05), except for $P \times T$ in acid hydrolysis.

The content of cellulose in treated samples was affected (P< 0.05) by levels of pressure and acid (Table 1). Reaction time also affected (P< 0.05) cellulose, but its effect was not consistent.

All the treatments significantly (P< 0.05) increased the content of water soluble sugars (WSS). The highest level of WSS was

Table 1. Effect of pressure, time, and acid on dry matter loss and chemical compositions of date leaves (g kg^{-1} DM).

Treatments	Levels	Parameters								
		DML [a]	NDF [b]	ADF [c]	HEM [d]	CELL [e]	WSS [f]	R. Sug. [g]	TEP [h]	ADL [i]
Ctrl	-	-	717.0	519.3	264.6	406.5	14.0	60.3	12.6	81.3
Pressure (P)	14	22.2[c]	650.1[ak]	526.7[a]	194.2[a]	364.7[a]	72.7[c]	77.7[b]	53.0[c]	148.3[b]
	17	40.8[b]	612.4[b]	521.9[a]	166.9[b]	352.5[b]	88.6[b]	93.9[a]	65.6[b]	147.1[b]
	20	58.3[a]	562.0[c]	506.9[b]	120.2[c]	344.8[c]	94.8[a]	92.4[a]	76.9[a]	159.6[a]
SE		1.57	3.31	4.01	4.08	4.20	1.02	2.16	0.83	2.34
Probability> *F*		**	**	*	**	**	**	**	**	**
Time (T)	120	32.5[b]	624.0[a]	509.9[b]	182.3[a]	345.4[b]	78.8[b]	78.8[b]	60.5[c]	142.2[c]
	180	41.5[a]	605.9[b]	517.7[ab]	158.2[b]	357.0[a]	87.0[b]	90.9[a]	64.6[b]	150.4[b]
	240	44.6[a]	594.7[c]	527.9[a]	141.0[c]	349.6[b]	90.4[a]	94.3[a]	70.5[a]	162.3[a]
SE		1.57	3.31	4.01	4.08	4.20	1.02	2.16	0.83	2.34
Probability> *F*		**	**	**	*	**	**	**	**	*
Acid (A)	0	25.9[a]	632.4[a]	532.7[a]	172.7[a]	367.8[b]	79.7[b]	76.5[b]	63.0[b]	156.0[a]
	1	51.3[b]	584.0[b]	504.3[b]	148.2[b]	333.5[a]	91.0[a]	99.5[a]	67.4[a]	147.3[b]
SE		1.28	2.70	3.27	3.33	3.43	0.83	1.77	0.67	1.99
Probability> *F*		**	**	**	**	**	**	**	**	*
P×*T*		NS	**	**	**	NS	**	NS	**	*
P×*A*		**	*	NS	NS	NS	**	*	**	NS
T×*A*		NS	**	*	NS	NS	*	NS	NS	NS
P×*T*×*A*		NS	**	**	NS	NS	**	NS	*	*

[a] Dry Matter Loss; [b] Neutral Detergent Fiber; [c] Acid Detergent Fiber; [d] Hemicellulose; [e]: Cellulose, [f] Water Soluble Sugars; [g] Reducing Sugars; [h] Total Extractable Phenolics, [i] Acid Detergent Lignin, [k] Means for each parameter with different superscript are significantly different (P< 0.05), **P<0.01 , *P<0.05, NS: Not Significant (P> 0.05).

Table 2. Effect of pressure and reaction time on dry matter loss and chemical composition (g kg^{-1} DM) of date leaves during auto-hydrolysis and acid hydrolysis.

Type of hydrolysis	Treatments	Levels	Parameters								
			DML^a	NDF^b	ADF^c	HEM^d	CELL$_e$	WSS$_f$	R. Sug.$_g$	TEP^h	ADL^i
Auto-hydrolysis	Ctrl		-	717.0	519.3	264.6	406.5	14.0	60.3	12.6	81.3
	Pressure (P)	14	16.9^{ck}	675.8^a	541.8^a	200.3^a	386.7^a	65.7^c	71.5^b	43.3^c	154.0^b
		17	28.9^b	641.8^b	533.3^{ab}	183.7^b	367.3^b	81.5^b	78.2^a	66.2^b	150.6^{ab}
		20	42.8^a	579.6^c	522.9^b	134.3^c	349.4^c	92.0^a	80.0^a	79.4^a	163.6^a
	SE		1.62	5.87	6.68	5.12	4.57	1.18	2.34	1.33	4.21
	Probability> *F*		**	**	NS	**	**	**	**	**	*
	Time (T)	120	26.2^b	654.4^a	526.4	192.4^a	360.5	70.9^c	70.1^b	58.0^b	151.6^b
		180	30.9^{ab}	639.6^b	539.1	172.0^b	374.2	81.9^b	79.2^a	61.2^b	151.9^b
		240	31.5^a	605.8^c	532.4	153.8^c	368.7	86.4^a	80.2^a	69.7^a	164.7^a
	SE		1.62	5.87	6.68	5.12	4.57	1.18	2.34	1.33	4.21
	Probability> *F*		*	**	NS	**	NS	**	*	**	NS
	P×T		NS	NS	NS	**	NS	**	NS	**	NS
Acid-hydrolysis	Pressure (P)	14	27.6^c	624.4^a	511.6^a	188.2^a	342.7^a	79.8^b	83.9^b	43.3^c	154.0^b
		17	52.7^b	583.1^b	510.4^a	150.2^b	337.7^a	95.7^a	109.6^a	66.2^b	151.9^b
		20	73.8^a	544.4^c	490.9^b	106.2^c	320.2^b	97.6^a	105.0^a	79.4^a	163.6^a
	SE		2.68	3.07	0.443	6.37	7.06	1.66	3.64	0.97	2.44
	Probability> *F*		**	**	**	**	NS	**	**	**	**
	Time (T)	120	44.3^c	593.6^a	493.3^b	172.1^a	330.2	86.6^b	87.6^c	59.6^c	151.6^b
		180	52.1^b	574.9^b	496.3^b	144.3^b	339.8	92.1^a	102.5^b	61.2^b	151.9^b
		240	57.7^a	583.6^b	523.3^a	128.2^c	330.5	94.4^a	108.4^a	69.8^a	164.7^a
	SE		2.68	3.07	4.43	6.37	7.06	1.66	3.64	0.97	2.44
	Probability> *F*		**	**	**	**	NS	**	**	**	**
	P×T		NS	**	**	NS	NS	**	NS	**	**

^{a} Dry Matter Loss; ^{b} Neutral Detergent Fiber; ^{c} Acid Detergent Fiber; ^{d} Hemicellulose; ^{e}: Cellulose, ^{f} Water Soluble Sugars; ^{g} Reducing Sugars; ^{h} Total Extractable Phenolics, ^{i} Acid Detergent Lignin, ^{k} Means for each parameter with different superscript are significantly different (P< 0.05), **P<0.01 , *P<0.05, NS: Not Significant

Table 3. Effect of treatments on dry matter loss and chemical composition (g kg^{-1} DM) of date leaves.

Treatments			Parameters ^{a}								
Acid	Pressure	Time	DML	NDF	ADF	HEM	CELL	WSS	R. Sug.	TEP	ADL
	Untreated		---	710.7	519.3	264.6	406.5	14.0	60.3	12.6	81.3
0	14	120	12.7	683.3	530.0	200.0	381.4	61.3	67.1	37.8	150.7
		180	19.3	684.0	552.7	205.2	392.7	64.0	72.3	39.0	149.3
		240	18.7	660.0	542.7	195.6	386.1	71.7	75.0	53.2	162.0
	17	120	28.7	661.3	527.3	201.0	364.4	69.6	70.6	64.7	145.3
		180	27.7	655.3	536.0	186.8	375.3	81.8	81.3	65.2	140.3
		240	30.2	608.7	536.7	163.2	362.2	93.1	82.8	68.6	166.0
	20	120	37.2	618.7	522.0	176.3	335.8	81.9	72.6	71.3	158.7
		180	45.6	571.3	528.7	124.0	354.5	99.9	84.1	79.5	166.0
		240	45.6	548.7	518.0	102.5	357.8	94.3	82.9	87.4	166.0
1	14	120	23.6	656.0	546.0	195.0	344.3	68.9	75.1	62.1	143.3
		180	25.4	608.0	494.8	190.8	341.3	79.8	83.4	63.5	140.7
		240	33.7	609.3	494.0	178.9	342.2	90.7	93.1	62.5	144.0
	17	120	27.6	578.0	472.0	179.6	344.7	89.3	96.6	61.7	128.7
		180	52.0	592.7	520.0	137.7	335.2	96.0	110.7	65.5	146.7
		240	57.2	578.7	539.3	133.4	333.2	101.8	121.4	67.7	155.3
	20	120	60.4	546.7	462.0	141.7	301.5	101.6	91.0	65.4	126.7
		180	78.8	524.0	474.0	104.5	343.0	100.6	113.4	74.6	159.3
		240	78.8	562.7	536.7	72.2	316.0	90.5	110.7	83.2	180.7
	S.E.		2.77	6.77	4.12	5.70	3.75	1.87	2.42	1.75	2.19

^{a} As defined under Table 2.

observed in the samples treated at 20 bar pressure. The increasing trend in WSS in auto-hydrolyzed and acid-hydrolyzed samples was not similar. In auto-hydrolysis all levels of pressure and reaction time significantly ($P< 0.05$) increased WSS, but in acid hydrolysis no further increase in WSS was observed by applying 20 bar pressure for 240 seconds reaction time (Table 2).

A big difference (more than 6 times) was found between the control and the treated samples in content of total extractable phenolics (TEP), but such difference was not observed among the treatments. Quantity of TEP was affected by all the treatments, except for acid×time (Table 1). As is shown, TEP content significantly ($P< 0.05$) increased by increasing the levels of the three factors. In auto-hydrolyzed samples, TEP significantly ($P< 0.05$) increased by levels of pressure, but, in acid hydrolyzed samples, this change was not consistent. In acid-hydrolyzed samples, increasing reaction time significantly ($P< 0.05$) increased TEP, but in auto-hydrolyzed samples no change was observed up to 180 seconds. The highest level of TEP for both auto-hydrolyzed and acid-hydrolyzed samples was observed at 20 bar pressure.

A big difference was observed in ADL content between control and all the treated samples, but such difference was not found among the treatments. The content of ADL was not affected up to 17 bar pressure, but was significantly increased at 20 bars.

Enzyme Hydrolysis

As is shown in Table 1, the three factors significantly affected quantity of reducing sugars. Among the interactions of the three factors, only effect of pressure ×acid was significant ($P< 0.05$). Although quantity of reducing sugars was affected ($P< 0.05$) by both pressure and reaction time, but increasing harshness of treatment conditions from 17 bars for 180 seconds up to 20 bars for 240 seconds did not improve hydrolysis

of cell wall carbohydrates. Similar pattern in reducing sugars changes was observed for both auto-hydrolysis and acid hydrolysis, but with higher values for acid hydrolyzed samples (Table 2). For both auto-hydrolyzed and acid-hydrolyzed samples, the difference in reducing sugar content between the treatments was smaller than that between the control and the treatments; such difference was greater in acid-hydrolyzed samples.

Bioavailability to Rumen Microbes

Results of gas test of steam-treated date leaves are shown in Table 4. A significant increase was observed in gas production between the control and steam treated samples. Similar to the results of chemical analysis, in all cases, the difference between the control and treatments were greater than the difference between the levels of the studied factors. All of the parameters measured were significantly affected by acid and levels of pressure. In case of reaction time, none of the parameters were affected when time increased from 180 to 240 seconds.

DISCUSSION

Changes in Chemical Composition

Steam treatment of lignocellulosic materials is associated with dry matter loss of the treated materials. The acetic and formic acids which are formed during steam treatment are volatile compounds and contribute in dry matter loss (Baugh and McCarty, 1988). Another reason for reduction in dry matter content is formation of browning compounds. During steaming, some part of the monosaccharaides, which are formed from hydrolysis of hemicellulose and cellulose, are converted to furans (furfural and 5-hydroxymethl furfural) through dehydration process (Tipson and Horton, 1988). Since the steam treatment temperature is higher than the evaporation

Table 4. Effects of the main factors on *in vitro* gas production, fermentation characteristics, organic matter digestibility, and metabolizable energy of date leaves.

Treatments	Levels	Incubation times					OMD[a]	ME[b]	Fermentation characteristics	
		6	12	24	48	96			b	c
Ctrl		11.14^c	15.82	21.36	28.56	35.51	34.74	5.13	31.43	3.15
	14	15.05^c	20.42^c	29.65^c	42.27^c	50.43^c	41.89^c	6.01^c	46.46^b	3.05^c
	17	16.72^b	22.41^b	32.47^b	45.34^b	52.83^b	44.41^b	6.53^b	47.87^a	3.42^b
Pressure	20	17.45^a	23.30^a	33.53^a	46.33^a	53.69^a	45.37^a	6.79^a	48.10^a	3.56^a
S.E.		0.122	0.160	0.206	0.218	0.258	0.183	0.029	0.274	0.034
Probability>F		**	**	**	**	**	**	**	**	**
	120	15.92^b	21.38^b	30.90^b	43.74^b	51.35^b	43.00^b	6.34^c	46.86^b	3.25^b
Time	180	16.51^a	22.45^a	32.20^a	44.90^a	52.79^a	44.17^a	6.43^b	47.87^a	3.34^{ab}
	240	16.78^a	22.50^a	32.55^a	45.29^a	52.81^a	44.49^a	6.61^a	47.71^a	3.44^a
S.E.		0.122	0.160	0.206	0.218	0.258	0.183	0.029	0.274	0.034
Probability>F		**	**	**	**	**	**	**	*	*
	0	15.19^b	20.73^b	30.47^b	43.37^b	50.73^b	42.63^b	6.62^b	46.96^b	3.26^b
Acid	1	17.63^a	23.36^a	33.30^a	45.93^a	53.47^a	45.15^a	7.00^a	48.00^a	3.43^a
S.E.		0.100	0.131	0.168	0.218	0.210	0.149	0.024	0.223	0.027
Probability>F		**	**	**	**	**	**	**	**	**
P × T		*	*	**	**	**	**	**	**	**
P × A		NS[d]	NS	NS	NS	NS	NS	**	NS	NS
T × A		NS	NS	NS	NS	NS	NS	**	NS	NS
P × T × A		NS	NS	NS	*	NS	NS	**	*	*

[a] Organic matter digestibility, [b] Metabolizable Energy (MJ kg^{-1} DM), [c] Means with different superscripts in each column are significantly different (P< 0.05), **P<0.01 , *P<0.05, [d] Not Significant (P> 0.05).

temperature of the furans, most of the furans leave the hydrolyzer after opening its lid. Hemicellulose is mainly made up of pentose (Zahedifar, 1996). Depending on treatment conditions during steam treatment, part of the pentose is hydrolyzed and, consequently, converted to furans. Therefore, the role of hemicellulose in formation of furans is more remarkable than cellulose. (Figure 1-a) shows correlation between DML and percentage of losses from hemicellulose and

cellulose during auto-hydrolysis. As is seen, for a given dry matter loss, the values for hemicellulose are greater than that for cellulose, which means that hemicellulose is more affected by dry matter loss compared to cellulose.

The same trend was observed in acid hydrolysis but with greater values for both hemicellulose and cellulose (Figure 1-b). Dry matter loss is a disadvantage and inevitable process during steam treatment
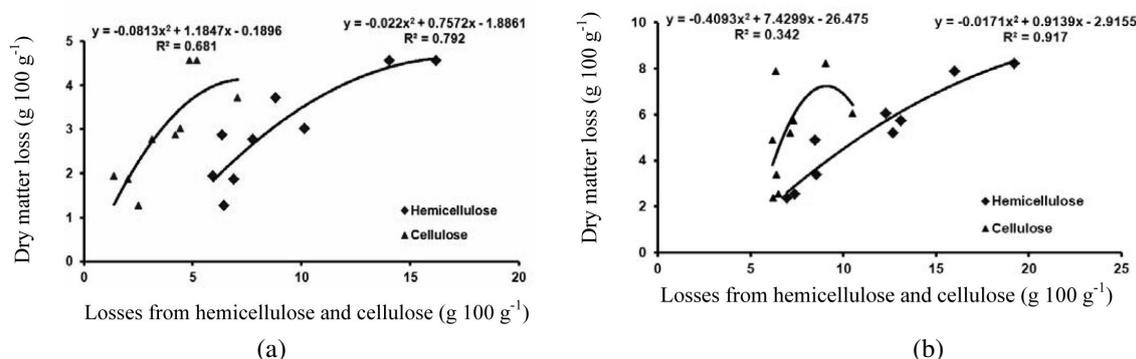

Figure 1. Correlation between dry matter loss (%) and losses of hemicellulose and cellulose (%) during (a) auto-hydrolysis, (b) acid hydrolysis

(Zahedifar, 1996; Liu *et al.*, 1999). The important point in optimization of treatment conditions is a compromise between DML and nutritive value. No report is available on DML from date leaves during steam treatment.

Steam treatment significantly reduced NDF and, consequently, increased soluble fraction. Apart from NDF solubilization, other factors should be taken into consideration when the purpose of steam treatment is feed production. Declining trend in NDF content was lower in auto-hydrolysis than for acid hydrolysis (Table 2). Effect of steam treatment conditions on some roughages such as sugar cane bagasse (Pate, 1982; Zahedifar *et al.*, 2004; Chaji *et al.*, 2010a, 2010b, 2013), wheat straw (Castro *et al.*, 1993b), and corn stover (Oji and Mowatt, 1978) has been reported, but no report is available on steam treatment of date leaves to compare the results of this study with.

Another important change in chemical composition was the increase in ADF content (Table 4). This is probably explained by occurrence of browning reactions and formation of insoluble polymers (Hodge, 1953). Occurrence of such reactions is a disadvantage process because it increases the loss of sugars previously released from hemicellulose, and also decreases the bioavailability of nitrogen to rumen microorganisms. Oji and Mowatt (1979) observed an increase in ADF content (from 44.5 to 50.3%) of steam treated corn stover as compared to the control. This increase was attributed to production of lignin-like compounds. In another report by Castro, (1994), the ADF content of wheat straw increased from 46.5% up to 53.8% after steam treatment.

Soluble Sugars

The correlation between water soluble sugars and losses from hemicellulose and cellulose in auto-hydrolyzed and acid-hydrolyzed samples are shown in (Figure 2 a-b), respectively. In both cases, the figures of lost hemicellulose for a given WSS are greater than that of cellulose. About 61 and 73% of the original hemicellulose was hydrolyzed in auto-hydrolyzed and acids-hydrolyzed samples, respectively. These values for cellulose were 12 and 22%, respectively. These figures show that hydrolysis of cellulose is limited as compared to hemicellulose and also confirm that the main source of soluble sugars in steam treated sample is hemicellulose. Grohmann *et al*. (1985) and Cunningham and Carr (1984) have shown extensive hydrolysis of cell wall carbohydrate (> 95%) by acid hydrolysis of straw when high level of sulfuric acid was used (> 4.5% H_2SO_4 on DM basis).

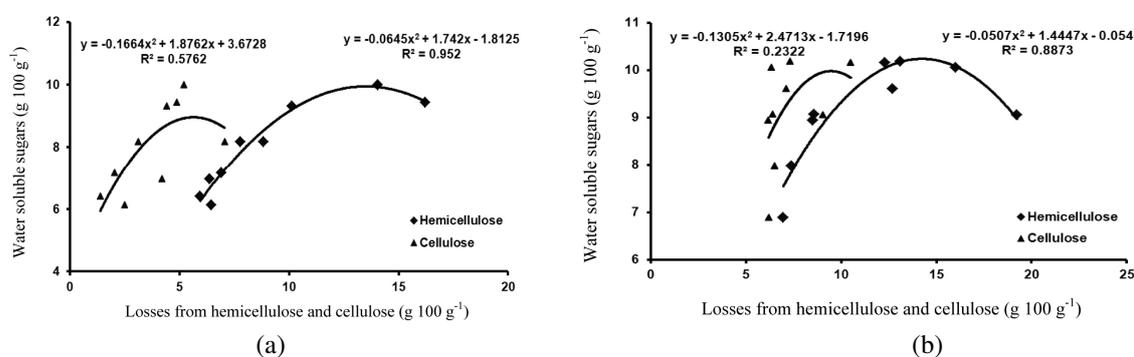Hemicellulose solubilization is clearly shown in Tables 2 and 3. About 60 percent

Figure 2. Correlation between water soluble sugars (%) and losses of hemicellulose and cellulose (%) during (a) auto-hydrolysis, (b) acid hydrolysis

of hemicellulose was depolymerized at the harshest condition used in this study. However, WSS at severe conditions do not correspond with loss of hemicellulose. Although harsher treatment conditions cause more hydrolysis of cell wall carbohydrates, but at the same time, such conditions may accelerate conversion of soluble carbohydrates to browning compounds (Zahedifar, 1996). Increasing harshness of treatment conditions up to a specific point increases concentration of soluble sugars but, afterward, applying more severe conditions will negatively affect concentration of soluble sugars (Castro, 1994). The decline in concentration of sugars by increasing harshness of treatment conditions is clearly shown in (Figure 2 a-b).

Cellulose

As mentioned earlier, cellulose is hydrolyzed to a lower extent than hemicellulose. It is shown in Table 3 that difference in cellulose content between the treated samples was not as big as the difference between the control and the treated samples. Hydrolysis of cellulose during steam treatment is not uniform. This is attributed to internal and external factors present in cellulose (Mosier, *et al.*, 2002). Crystallinity of cellulose is the most important factor affecting its hydrolysis kinetic during steam treatment because glycosidic linkages present in amorphous region are more susceptible to hydrolysis compared to the organized region and require less energy for breakdown (Carrasco, *et al.*, 1994; Puri, 1984). Therefore, by hydrolyzing the amorphous regions, percentage of crystalline form is increased. This implies that harsher treatment conditions are needed for achieving complete hydrolysis of cellulose and the treatment conditions used in this study was not harsh enough for such process. External factor is related to the degree of cell wall lignification.

According to Dekker and Wallis (1983), following separation of cellulose from lignin, the adjacent chains of cellulose is ordered into an organized form. These changes reduce rate of cellulose hydrolysis (Van Soest, 1994).

Phenolic Compounds

Quantity of TEP and ADL were significantly (P< 0.05) increased in all of the treatments compared to the control. It is reported that the increase in TEP is due to deploymerization of lignin to lower molecular weight phenolic compounds (Wayman and Chua, 1979). Zahedifar *et al.* (2008) reported that quantity of TEP in sugarcane bagasse increased from 0.06 percent in the control up to 5.58 percent after treatment at 14 bar for 360 seconds. Although deploymerization of lignin to low molecular weight of phenolics is an inevitable process during steaming (Wayman and Chua, 1979), but increasing both TEP and ADL content suggest that production of new phenolic compounds may be another process happening during steam treatment. As mentioned earlier, furan derivatives are the most abundant soluble sugar decomposition products formed during treatment. A wide variety of compounds can be formed by aromatization reactions of furan derivatives (Hodge, 1953; Popoff and Theander, 1972; Theander and Nelson, 1978). At later stages of browning reactions, these aromatic compounds can polymerize, eventually becoming an insoluble matrix. This fraction exhibits chemical similarities to lignin, i.e. alkali solubility, acid insolubility, and optical activity typical of phenolics. This fraction has been classified as lignin-like compounds (Van Soest, 1982). Chua and Wayman (1979) reported that high molecular weight phenolics can contribute up to 60% of the extracted phenolics. A dramatic increase in ADL content is very

likely due to contribution of these compounds to lignin.

Bio-availability of Steam-Treated Date Leaves to Cell Free Enzyme and Rumen Microbes

An increasing trend was observed in enzymic hydrolysis of steam-treated date leaves by increasing the levels of the three factors. The difference in quantity of reducing sugars between untreated and the treated samples were greater than the difference among the treatments. A similar trend has been reported by Castro (1994) and Liu and Ørskov (2000) in enzymic hydrolysis of steam treated wheat straw and rice straw, respectively, by increasing harshness of treatment conditions. The highest quantity of reducing sugars was from the acid-hydrolyzed sample treated at 20 bars for 180 seconds and no further increase in reducing sugars was observed by increasing reaction time.

Depolymerization of lignin reduces the barrier effect of lignin in cell wall, therefore, improves carbohydrate utilization by rumen microbes. In untreated forages, the soluble fraction is assumed readily available to rumen microbes (Ørskov and McDonald, 1979), but in steam-treated roughages some parts of this fraction, such as browning reaction products and low molecular weight phenolics, are not fermented (Zahedifar, 1996). Bio-availability of steam-treated samples to rumen microbes is shown in Table 4. Increasing levels of pressure significantly increased gas production and rate of ruminal fermentation, which was in agreement with the results reported by Castro, *et al*. (1993a). Similar to the data reported by Chaji *et al*. (2010b), addition of exogenous acid significantly ($P< 0.05$) increased the potential of gas production and rate of fermentation. However, the difference between the control and all treatments was much bigger than the difference between the treatments. It seems that those parts of the cell wall in date leaves

that were sensitive to steam treatment responded to 14 bars pressure, while for hydrolyzing the remaining parts more severe treatment conditions would be needed. The rate of gas production was significantly affected by reaction time, but increasing the time from 180 to 240 seconds did not affect gas production. This could imply that more improvement in nutritive value may be obtained by increasing the levels of pressure and acid than reaction time. OMD and ME followed a similar pattern for all treatments, except for $P\times T$, $T\times A$ and $P\times T\times A$ treatments.

CONCLUSION

Steam treatment effectively disrupted cell wall components of date leaves and significantly changed the chemical compositions. Steam treatment significantly improved bio-utilization of date leaves by cell free enzymes and rumen microbes. Improvements in bio-utilization is mainly explained by depolymerization of cell wall components, mainly hemicellulose and lignin.

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تعیین شرایط بهینه عمل آوری برگ درخت خرما با بخار آب برای افزایش ارزش غذایی آن

م. زاهدی فر، ن. کریمی، ح. ،فضائلی، و س. ا. میرهادی

چکیده

هدف از انجام این تحقیق مطالعه اثر بخار آب تحت فشار بر ارزش غذایی برگ درخت خرما بود. برگ درخت خرما پس از خرد کردن با محلول اسید سولفوریک و یا آب مخلوط گردید به نحوی که دارای ۵۰ درصد رطوبت و حاوی یا بدون ۱ درصد اسید سولفوریک باشد. اثر سه سطح فشار بخار آب (۱۴، ۱۷ و ۲۰ بار فشار)، سه سطح زمان عمل آوری (۱۲۰، ۱۸۰ و ۲۴۰ ثانیه) و ۲ سطح اسید سولفوریک (۰ و ۱ درصد) در یک طرح کاملاً تصادفی با آرایش فاکتوریل بر روی افت ماده خشک، ترکیبات شیمیایی (دیواره سلولی، دیواره سلولی منهای همی سلولز، لیگنین نا محلول در شوینده اسیدی، همی سلولز، سلولز، خاکستر، کل ترکیبات فنلی قابل استخراج، قندهای محلول در آب، قندهای احیا کننده) هیدرولیز آنزیمی و آزمون گاز مورد بررسی قرار گرفت. نتایج نشان داد که عمل آوری با بخار آب به طور معنی داری (P<0.01) اجزاء دیواره سلولی را تحت تاثیر قرار داد. یک روند افزایشی در افت ماده خشک با افزایش شرایط عمل آوری مشاهده گردید. کمترین مقدار افت شستشو (۱۲/۷ گرم بر کیلوگرم) در نمونه عمل آوری شده در شرایط ۱۴ بار فشار و ۱۲۰ ثانیه و بیشترین مقدار افت شستشو

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(۷۸/۸ گرم بر کیلو گرم) در نمونه عمل آوری شده در شرایط عمل آوری ۲۰ بار فشار و ۲۴۰ ثانیه مشاهده شد. عمل آوری با بخار آب به طور معنی داری (P<0.01) موجب کاهش دیواره سلولی (از ۷۱۰/۷ گرم در کیلوگرم در نمونه شاهد تا ۵۲۴/۰ گرم در کیلوگرم در نمونه عمل آوری شده) و افزایش مقدار لیگنین نامحلول در شوینده اسیدی (از ۸۱/۳ گرم در کیلوگرم نمونه شاهد تا ۱۸۰/۷ گرم در کیلوگرم نمونه عمل آوری شده) گردید. تغییرات در مقدار دیواره سلولی منهای همی سلولز روند مشخصی نداشت. مقادیر همی سلولز و قندهای محلول در آب به طور مؤثری تحت تاثیر شرایط عمل آوری قرار گرفتند. مقدار همی سلولز از ۲۶۴/۶ گرم در کیلوگرم در نمونه شاهد به ۷۲/۲ گرم در کیلوگرم بعد از عمل آوری کاهش، و مقدار قندهای محلول از ۱۴/۰ به ۱۰۱/۸ گرم در کیلوگرم بعد از عمل آوری افزایش یافت. هیدرولیز آنزیمی برگ خرما بعد از عمل آوری با بخار آب افزایش یافت و مقادیر بیشتر هیدرولیز آنزیمی (از ۶۰/۳ گرم در کیلوگرم در نمونه شاهد تا ۱۲۱/۴ گرم در کیلوگرم در نمونه عمل آوری شده) در نمونه های هیدرولیز اسیدی (نمونه های عمل آوری شده حاوی ۱ درصد اسید سولفوریک) مشاهده شد. نتایج آزمون گاز نشان داد که عمل آوری برگ خرما با بخار آب موجب افزایش معنی دار (P<0.01) تولید گاز در کلیه ساعات تخمیر گردید. بیشترین افزایش در مقدار انرژی متابولیسمی قابل تخمیر برآورد شده با آزمون گاز مربوط به نمونه هیدرولیز اسیدی عمل آوری شده در شرایط ۲۰ بار فشار و ۲۴۰ ثانیه بود. بیشترین افزایش در مقدار انرژی قابل متابولیسم در نمونه های خود هیدرولیز (نمونه های عمل آوری شده بدون اضافه نمودن اسید) در شرایط ۲۰ بار فشار و ۱۸۰ ثانیه مشاهده شد. نتایج این تحقیق نشان داد که عمل آوری با بخار آب می تواند برای بهبود ارزش غذایی برگ خرما در مناطق خرما خیز که دامهای آن با شرایط دشوار کمبود مواد خوراکی مواجه هستند مورد استفاده قرار گیرد .