RESEARCH ARTICLE

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Effect of different levels of *Saccharomyces cerevisiae* supplementation on *in vitro* gas production kinetics of some grape yield by-products

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ABSTRACT

The aim of this study was to determine the chemical composition of some grape yield by-products supplemented with *Saccharomyces cerevisiae* and gas production characteristics using *in vitro* gas production technique. The treatments contained 0, 2.5, 5 and 7.5 g yeast *Saccharomyces cerevisiae* (Sc) per kg of samples based on dry matter, respectively. The gas production profiles in triplicate fitted with the equation of $Y = A (1 - e^{-ct})$. The data was analyzed using completely randomized design. Total phenols (TP) and total tannins (TT) contents were highest for raisin waste (RW). The TP content (g/kg DM) ranged from 30.1 in grape pomace (GP) to 96.3 in RW, which also had the higher TT (72.1 g/kg DM). At the early incubation times (2 and 4 h), the treatments with Sc 7.5 g/kg DM had the highest *in vitro* gas production volume within treatments ($P<0.05$). At the all of incubation times, treatments with Sc 7.5 g/kg DM had the highest gas production compared with control treatment (treatment without Sc; $P<0.05$). It was concluded that *in vitro* gas production parameters of dried grape by-products was improved with addition of yeast *Saccharomyces cerevisiae* at all levels.

Key words: *in vitro* gas production, grape yield by-products, probiotic, *Saccharomyces cerevisiae*

Introduction

In the Middle East, animals suffer from under feeding and malnutrition in winter due to the shortage of locally produced feeds, which are not ample to cover the alimental requisites of animals. A major constraint to increasing livestock productivity in developing countries is the scarcity and fluctuating quantity and quality of the year-round supply of conventional feeds. These countries experience serious shortages in animal feeds of the conventional type. In order to meet the projected high demand for livestock products and to fulfill the future hopes of feeding the millions and safeguarding their food security, the better utilization of non-conventional feed resources which do not compete with human food is imperative. There is also a need to identify and introduce new and lesser known food and feed crops. An important class of non-conventional feeds is by-product feedstuffs which are obtained during harvesting or processing of a commodity in which human food or fiber is derived. The amount of by-product feedstuffs generally increases as the human population increases and economies grow (Besharati et al., 2008; Besharati & Taghizadeh, 2009; Besharati & Taghizadeh, 2011). Increasing agricultural industrial units for producing biscuit leads to the accumulation of biscuit waste.

Several factors have led to increased interest in by-product feedstuffs, such as pollution abatement and regulations, increasing costs of waste disposal and changes in perception of the value of by-product feedstuffs as economical feed alternatives (Besharati et al., 2008; Besharati & Taghizadeh, 2009; Besharati & Taghizadeh, 2011). The annually amount produced of agro-by-products in Iran are generous, whereas, production of grape exceeds 2.87 billion ton/yr, that proportion of grape yield is used for the production of dried grape. In this process, dried grape by-product (grape cluster stems plus rejected raisins) is produced in high level. These wastes are usually burned causing environmental pollution. The potential use of these wastes in ruminant rations will participate in reducing the shortage of feedstuffs and subsequently increase milk and meat production. However, little is known about their fermentation pattern in the rumen and a better understanding of their digestion and products of fermentation is necessary in order to properly balance their introduction into the diets (Durand et al., 1988; Sutton, 1986) and the knowledge about their potential feeding value is insufficient.

Probiotics present an attractive alternative to the use of chemical and hormonal promoters in the livestock growth production industry. The preparations contain have been used for production safe by micro-organisms, many years and thus are that in food generally accepted as both the farmer and the final consumer. *Saccharomyces cerevisiae* supplementation in ruminant diets can be increased dry matter intake (DMI), production performance, cellulose degradation, and nutrient digestibility (Callaway & Martin, 1997). The gas measuring technique has been widely used for evaluation of the nutritive value of feeds. Gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs (Getachew et al., 1998). In the gas method, kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of sample is required or a larger number of samples can be evaluated at a time. Besharati et al. (2009) showed that probiotic can improve the *in vitro* gas production.

The aim of this study was to determine the chemical composition of some grape yield by-products supplemented with *Saccharomyces cerevisiae* and gas production characteristics using *in vitro* gas production technique.

Materials and Methods

Grape yield by-products

Grape yield by-products (raisin waste (RW), grape pomace (GP) and dried grape by-product (DGB)) were obtained from raisin and grape juice production factories of Tabriz, Iran. The DGB that was collected contained grape cluster stems and rejected raisins.

Chemical composition

Feedstuffs dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30) and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (1999). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined using the methods of Van Soest et al. (1991) with sodium sulphite. NDF was analysed without amylase with ash included.

Total phenolics (TP) were measured using the Folin Ciocalteau method (Makkar, 2000). Total tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteau reagent (Makkar, 2000). Tannic acid was used as the standard to express the amount of TP and TT.

In vitro gas production trial

The DM degradability of DGB, RW and GP was determined by *in vitro* fermentation with ruminal fluid. Ruminal fluid was collected approximately 2 h after morning feeding from 2 cannulated sheep. Ruminal fluid was immediately squeezed through 4 layers of cheesecloth and was transported to the laboratory in a sealed thermos. The resulting ruminal fluid was purged with deoxygenated CO_2 before use as the inoculum. Gas production was measured by Fedorak and Hurdy (1983) method. Approximately 300 mg of dried and ground (2 mm) DGB, RW and GP samples. The treatments contained 0, 2.5, 5 and 7.5 g yeast *Saccharomyces cerevisiae* (Sc) per kg of samples based on DM, respectively. Buffered rumen fluid with McDougall's buffer (20 mL) was pipetted into each serum bottle (McDougall, 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in mL per gram of DM. The gas production profiles in triplicate fitted with the equation:

$Y = A(1 - e^{-ct})$,

where Y is the volume of gas production (mL/g DM) at time t, A is gas production from soluble and insoluble fraction, c is the gas production rate and t is the incubation time (h). The metabolizable energy (ME) contents of GP and OMD were calculated using equations of Menke et al. (1979) as:

$ME, MJ/kg\ DM = 2.20 + 0.136 \times Gv + 0.057 \times CP + 0.0029 \times CP^2$;

$OMD, g/100\ g\ DM = 14.88 + 0.889 \times Gv + 0.45 \times CP + 0.0651 \times XA$;

where OMD = organic matter digestibility (g/100 g DM), XA = ash in g/100 g DM, and Gv = the net gas production (mL) at 24 h. The VFA were calculated using below equation as:

$VFA\ (volatile\ fatty\ acid), mmol = -0.00425 + 0.0222\ Gv$.

And net energy for lactation (NE_l) was calculated using equation as:

$NE_1\ Mcal/lb = (2.20 + (0.0272 \times Gas) + (0.057 \times CP) + (0.149 \times CF))/14.64$,

where Gas is 24 h net gas production (mL/g DM), CP is crude protein (% of DM), and CF is crude fat (% of DM).

Statistical analysis

Data obtained from this study was subjected to ANOVA as a completely randomized design with 3 replicates by the GLM procedure of SAS (2002), and treatment means were compared by the Duncan test.

Results and Discussion

Table 1. *The chemical composition of feeds (g/kg DM)*[a].

Feeds	DM	CP	NDF	ADF	Crude fat	OM	Total phenols	Total tannins
Grape pomace	933	66.2	187	184	14.1	877	30.1	22.7
Raisin waste	916	62.4	280	276	12.3	927.7	96.3	72.1
Dried grape by-product	884.5	63.5	259	255	11.2	926	67	52.3

[a] DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber and OM = organic matter.

Table 2. *Total gas production volume of grape yield by-products in incubation times (ml/g DM).*

Treatments	Incubation time (h)								
	2	4	6	8	12	16	24	36	48
Grape pomace (GP)									
Control	23.13^{c}	63.84^{d}	99.76^{c}	126.79^{b}	162.60^{b}	192.43^{d}	226.42^{d}	260.47^{d}	288.59^{d}
GP + Sc 2.5 g/kg DM	24.91^{c}	71.83^{c}	103.97^{c}	134.12^{b}	174.14^{b}	202.64^{c}	238.63^{c}	275.78^{c}	239.46^{c}
GP + Sc 5 g/kg DM	36.23^{b}	86.48^{b}	124.18^{b}	155.43^{a}	194.79^{a}	217.96^{b}	253.94^{b}	291.99^{b}	325.22^{b}
GP + Sc 7.5 g/kg DM	45.55^{a}	97.14^{a}	137.50^{a}	168.53^{a}	206.11^{a}	234.61^{a}	271.48^{a}	210.42^{a}	343.87^{a}
SEM	1.48	2.43	3.57	4.08	4.20	2.93	2.89	2.40	3.81
Raisin waste (RW)									
Control	14.58^{c}	40.42^{c}	62.57^{d}	77.40^{c}	93.44^{c}	108.85^{c}	129.73^{c}	152.91^{c}	175.81^{c}
RW + Sc 2.5 g/kg DM	24.57^{b}	56.74^{b}	77.78^{c}	95.27^{b}	110.54^{b}	130.16^{b}	150.16^{b}	179.99^{b}	202.57^{b}
RW + Sc 5 g/kg DM	26.68^{b}	67.39^{a}	84.44^{b}	101.93^{a,b}	123.75^{a}	143.37^{a,b}	180.13^{a}	201.47^{a}	225.60^{a}
RW + Sc 7.5 g/kg DM	33.12^{a}	70.50^{a}	93.32^{a}	109.48^{a}	128.85^{a}	146.48^{a}	169.58^{a}	197.64^{a}	220.21^{a}
SEM	1.44	2.28	1.80	2.89	3.34	4.29	3.81	3.17	3.22
Dried grape by-product (DGB)									
Control	7.81^{d}	24.10^{d}	38.04^{d}	50.87^{d}	72.91^{d}	94.09^{d}	130.51^{d}	176.00^{d}	235.87^{d}
DGB + Sc 2.5 g/kg DM	15.36^{c}	35.42^{c}	49.81^{c}	63.97^{c}	86.67^{c}	112.07^{c}	144.83^{c}	199.97^{c}	263.01^{c}
DGB + Sc 5 g/kg DM	23.13^{b}	45.41^{b}	61.13^{b}	75.73^{b}	99.77^{b}	124.72^{b}	164.48^{b}	226.28^{b}	293.03^{b}
DGB + Sc 7.5 g/kg DM	29.79^{a}	55.18^{a}	72.89^{a}	88.83^{a}	114.42^{a}	139.82^{a}	183.13^{a}	251.92^{a}	316.67^{a}
SEM	1.68	2.10	2.46	2.60	2.90	2.96	2.13	1.65	1.46

GP = grape pomace, RW = raisin waste, DGB = dried grape pomace. [a, b, c, d] Within a column, means without a common superscript letter differ ($P < 0.05$).

The chemical composition of feeds is shown in Table 1. All of the grape yields by-products in this experiment had the same CP content, approximately. Grape pomace had the lowest ADF and NDF contents within the grape yield by-products. Total phenols and total tannins contents were highest for raisin waste. The TP content (g/kg DM) ranged from 30.1 in grape pomace to 96.3 in raisin waste (Table 1), which also had the higher TT (72.1 g/kg DM).

Total gas production volume of feedstuffs in incubation times (ml/g DM) are presented in Table 2. The cumulative volume of gas production increased with the increasing time of incubation. Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) was chosen because the relationship of its parameters with intake, digestibility and degradation characteristic of forages and concentrate feedstuffs had been documented. Sommart et al. (2000) reported that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Gas

volumes also have shown a close relationship with feed intake (Blummel & Becker, 1997) and growth rate in cattle (Blummel & Ørskov, 1993).

Table 3. *The parameters estimated from the gas production of grape yield by-products with different levels of Sc.*

Treatments	Estimated parameters					
	GP (ml/0.2 g DM)	ME (MJ/kg DM)	NE_L (MJ/kg DM)	DOMD (g/kg DOM)	SCFA (mmol/0.2 g DM)	MP (gr/kg DOM)
Grape pomace (GP)						
Control	45.28^{d}	8.39^{d}	4.91^{d}	58.24^{d}	1.00^{d}	11.24^{d}
GP + Sc 2.5 g/kg DM	47.73^{c}	8.73^{c}	5.15^{c}	60.45^{c}	1.06^{c}	11.67^{c}
GP + Sc 5 g/kg DM	50.79^{b}	9.14^{b}	5.44^{b}	63.22^{b}	1.12^{a}	12.20^{b}
GP + Sc 7.5 g/kg DM	54.30^{a}	9.62^{a}	5.78^{a}	66.39^{a}	1.20^{a}	12.81^{a}
SEM	0.58	0.08	0.06	0.52	0.01	0.10
Raisin waste (RW)						
Control	25.95^{c}	5.76^{c}	3.05^{c}	40.54^{c}	0.57^{c}	7.82^{c}
RW + Sc 2.5 g/kg DM	30.03^{b}	6.32^{b}	3.35^{b}	44.23^{b}	0.66^{b}	8.54^{b}
RW + Sc 5 g/kg DM	36.03^{a}	7.14^{a}	4.02^{a}	49.65^{a}	0.80^{a}	9.58^{a}
RW + Sc 7.5 g/kg DM	33.92^{a}	6.85^{a}	3.82^{a}	47.75^{a}	0.75^{a}	9.22^{a}
SEM	0.76	0.10	0.07	0.69	0.02	0.13
Dried grape by-product (DGB)						
Control	26.10^{d}	5.79^{d}	3.07^{d}	40.70^{d}	0.58^{d}	7.85^{d}
DGB + Sc 2.5 g/kg DM	28.97^{c}	6.18^{c}	3.45^{c}	43.28^{c}	0.64^{c}	8.35^{c}
DGB + Sc 5 g/kg DM	32.90^{a}	6.71^{b}	3.72^{b}	46.83^{b}	0.73^{b}	9.04^{b}
DGB + Sc 7.5 g/kg DM	36.62^{a}	7.22^{a}	4.08^{a}	50.21^{a}	0.81^{a}	9.69^{a}
SEM	0.43	0.06	0.04	0.36	0.01	0.07

GP = grape pomace, RW = raisin waste, DGB = dried grape pomace, ME = metabolizable energy (MJ/kg DM), OMD = organic matter digestibility (g/100 g DM), NE_1 = net energy for lactation (Mcal/lb) and VFA = volatile fatty acids (mmol), (a+b) = potential gas production (mL/g DM); c = rate constant of gas production during incubation (mL/h). $^{a, b, c, d}$ Within a column, means without a common superscript letter differ ($P < 0.05$).

treatments (P<0.05). At the all incubation times, treatments with Sc 7.5 g/kg DM had the highest gas production in compared with control treatment (treatment without Sc; P<0.05).

The addition of Sc to tannin-containing feeds increased *in vitro* gas production in all feeds. At the 2 h incubation times, the gas production volume of GP, GP + Sc 2.5, GP + Sc 5 and GP + Sc 7.5 were 23.13, 24.91, 36.23 and 45.55 ml/g DM, for RW, RW + Sc 2.5 and RW + Sc 5, RW + Sc 7.5 were 14.58, 24.57, 26.68 and 33.12 ml/g DM, for DGB, DGB + Sc 2.5, DGB + Sc 5 and DGB + Sc 7.5 were 7.81, 15.36, 23.13 and 29.79 ml/g DM, respectively. At the early incubation times (2 and 4 h), the treatments with Sc 7.5 g/kg DM had the highest *in vitro* gas production volume within

A significant increase in total gas production in the yeast treatments was observed in this study in agreement with previous studies (Lila et al., 2004, 2006). Also this result is in agreement with a previous study that *Saccharomyces cerevisiae* increase ruminal gas production (Martin & Nisbet, 1992), but others found no effect (Lila et al., 2004) or a decrease (Lynch & Martin, 2002) in batch cultures with mixed rumen microflora. The discrepancies among studies could be associated with the characteristics of the strain, diet

composition (Sullivan & Martin, 1999) and dose (Lila et al., 2006).

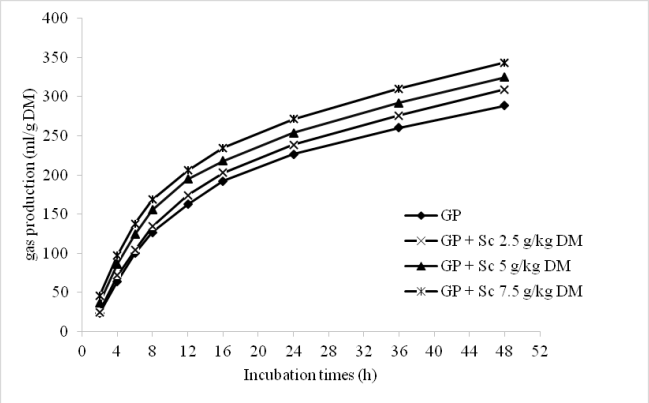

Figure 1. *The effect of different levels of Sc on gas production of grape pomace.*

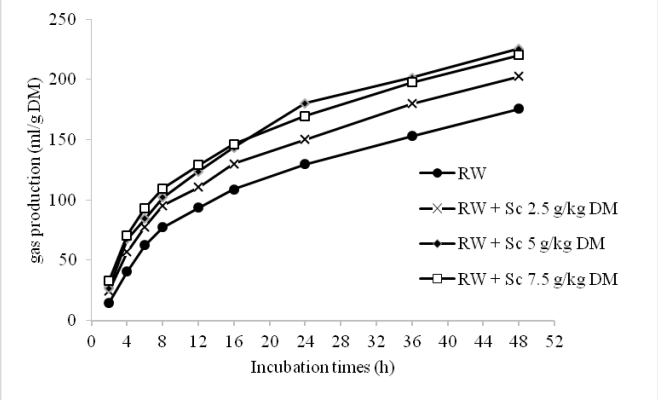

Figure 2. *The effect of different levels of Sc on gas production of raisin waste.*

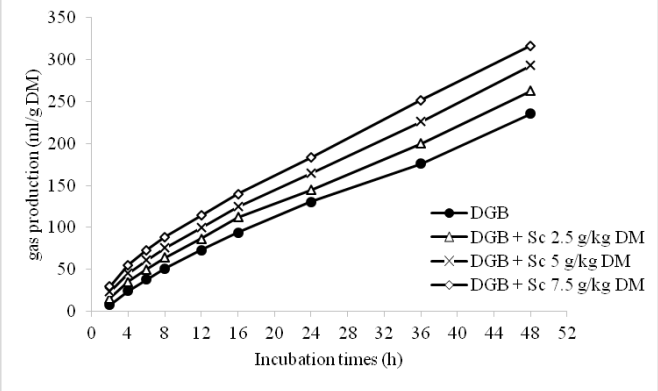

Figure 3. *The effect of different levels of Sc on gas production of dried grape by-product.*

The ability of yeast to increase *in vitro* gas production observed in the study has been reported by various authors with different roughages (Chaucheyras-Durand et al., 2008; Ando et al., 2004, 2005). Tang et al. (2008) reported an increase in the rate of gas production and *in vitro* dry matter digestibility from yeast supplementation of low quality cereal straws that was associated with an increase in protozoa and cellulolytic bacteria populations. Increase in bacterial population and activity of rumen microbes that led to higher *in vitro* dry matter digestibility as a result of yeast supplementation may be attributed to ability of yeast to remove oxygen from the rumen environment and to effects of organic acids, essential enzymes and vitamins derived from yeast activity or yeast components themselves such as peptides and amino acids (Fonty & Chaucheyras-Durand, 2006; Ding et al., 2008). Kim et al. (2005) reported a significant positive correlation between ruminal molar proportions of branched-chain fatty acids (BCFA) and the efficiency of microbial protein synthesis. The BCFA are required for resynthesis of branched-chain amino acids for microbial protein synthesis in the rumen (Allison, 1969). An *in vitro* fermentation study demonstrated that BCFA supplementation could increase microbial protein synthesis and DM digestion (Cummins & Papas, 1985). It is assumed that true protein supplementation via yeast could have been beneficial for BCFA production in the process of protein degradation in the rumen and consequently resulted in a greater increase in *in vitro* dry matter digestibility for Japanese sake yeast and Bioethanol residue yeast as compared with SP.

Wambui et al. (2010) used two strain of *Sacharomyces cereviciae* (Japanese sake yeast and Bioethanol residue yeast). Both Japanese sake yeast and Bioethanol residue yeast supplements increased the ruminal digestion of the browse foliages and the effect of JSY appeared to be significantly higher. Differences in the effect of yeast on rumen microbes and fermentation pattern are mainly associated with the strain of *Sacharomyces cereviciae* used (Ando et al., 2005). Certain strains of yeast are more effective at stimulating certain groups of bacteria and ruminal fermentation than others. The ability of yeast to influence rumen fermantation is more pronounced when live yeast cells are used as opposed to autoclaved yeast cultures or yeast derivatives (Ando et al., 2005; Wambui et al., 2010).

Ando et al. (2005) also point out that the differences in the yeasts' metabolic functions or cell wall structures can influence their degradability of roughages. Efficacy of yeast products on rumen fermentation and animal performance is also greatly influenced by the diet (Chaucheyras-Durand et al., 2008). It is postulated that factors such as the structure and biological activity of tannins and presence of other antinutritive compounds may have influenced the results observed. Further study on the effect of yeast supplementation on the nitrogen (N) degradation in the rumen and a subsequent effect on post-ruminal N digestion status are needed.

The parameters estimated from the gas production of grape yield by-products with different levels of Sc, are given in Table 3. The Sc supplementation had also a significant effect on the estimated parameters of OMD, ME, NE_l and

VFA (Table 3). In all by-products, Sc improved the amounts of OMD, ME, NE_l and VFA. For RW difference within 5 and 7.5 g/kg, DM treatments with PVP was not significant (P>0.05). In all by-products, SC increased the amounts of potential gas production (a+b) and in all by-products, Sc decreased the amount of rate constant of gas production during incubation (c). The addition of Sc significantly (P<0.05) increased the production of total VFA. Figures 1, 2 and 3 show the pattern of *in vitro* gas production of the feedstuffs.

Conclusion

It was concluded that *in vitro* gas production parameters of dried grape by-products was improved with addition of yeast *Saccharomyces cerevisiae* at all levels.

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