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# EVALUATION OF *SACCHAROMYCES CEREVISIAE* SUPPLEMENTED POMEGRANATE SEED USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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## ABSTRACT

The aim of this study was to determine the chemical composition of pomegranate seed including tannin content, and gas production characteristics using *in vitro* gas production technique. The treatment contained 0, 2.5, 5 and 7.5 g yeast *Saccharomyces cerevisiae* (Sc) per kg of pomegranate seed based on DM (dry matter), respectively. CP, ADF, NDF, EE, ASH, TP (total phenolics) and TT (total tannin) contents in pomegranate seed were 12.2 %, 44.6 %, 62.3 %, 1.6 %, 12.1 %, 1.8 % and 0.8 %, respectively. At the early incubation time (2 h), the treatments 1 and 4 (treatment with Sc, 0 and 7.5 $g.kg^{-1}$ DM, respectively) had the highest gas production volume among treatments, but after 4 h incubation the gas production volume in treatments 1 and 4 was the lowest ($p < 0.05$). The treatment 2 at the most incubation times had the highest gas production volume. It may be concluded that *in vitro* gas production parameters of pomegranate seed was improved with addition of *Saccharomyces cerevisiae* at 2.5 $g.kg^{-1}$ DM.

**Key words:** *in vitro* gas production; pomegranate seed; *Saccharomyces cerevisiae*

## INTRODUCTION

In Middle East, animals suffer from under feeding and malnutrition in winter due to the shortage of locally produced feeds which are not sufficient to cover the nutritional requirements 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 of livestock products and to fulfil 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 fibre is derived. The amount of by-product feedstuffs generally increases as the human population increases and economies grow (Besharati *et al.*, 2008; Besharati and Taghizadeh, 2009; Besharati and Taghizadeh, 2011). Increasing agricultural industrial units for producing pomegranate juice leads to the accumulation of pomegranate peel and the annual production of this by-product is approximately 120.000 metric tons in Iran (Mirzaei-Aghsaghali *et al.*, 2011). Pomegranate fruit consists of three parts: the seeds, the juice and the peels which include the husk and interior network membranes (Shabtay *et al.*, 2008).

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 and Taghizadeh, 2009; Besharati and Taghizadeh, 2011).

Probiotics present an attractive alternative to the use of chemical and hormonal promoters in the livestock growth production industry. The preparations

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have been used for production of safe by micro-organisms for many years and thus are generally accepted in food by both the farmer and the final consumer. *Saccharomyces cerevisiae* supplementation in ruminant diets can increase DMI, production performance, cellulose degradation, and nutrient digestibility (Callaway and Martin, 1997). The gas measuring technique has been widely used for evaluation of 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 probiotics can improve the *in vitro* gas production. The purpose of this study was to study the effect of adding different levels of *Saccharomyces cerevisiae* on *in vitro* gas production of biscuit by-product.

There is a little information available regarding the nutritive value of pomegranate seed (PS) produced in Iran. The aim of this study was to determine the chemical composition including tannin content of pomegranate seed supplemented with *Saccharomyces cerevisiae* and gas production characteristics using *in vitro* gas production technique.

## MATERIAL AND METHODS

### Pomegranate seed

Pomegranate seed was obtained from a fruit juice manufacturing factory in Tabriz, Iran.

### Chemical composition

Pomegranate seed 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 standard procedures (AOAC, 1999). The NDF and ADF concentrations were determined using the methods of Van Soest *et al.* (1991) without 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 dry matter degradability of each by-product was determined by *in vitro* fermentation with ruminal fluid. Ruminal fluid was collected approximately 2 h after morning feeding from two cannulated sheep consuming 400 g alfalfa hay, 300 g barley and 300 g soybean meal. Ruminal fluid was immediately squeezed through four 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 Hurdey (1983) method. The treatment contained 0, 2.5, 5 and 7.5 g *Saccharomyces cerevisiae* (Sc) per kg of pomegranate seed based on DM, respectively. Approximately 300 mg of dried and ground (2 mm) pomegranate seeds were weighed and placed into serum bottles. There were 3 replicates per treatment. Buffered rumen fluid with McDougal 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 1 g of DM. The metabolizable energy (ME) contents of treatments and OMD were calculated using equations of Menke *et al.* (1979) as:

$$ME_{(MJ\,kg^{-1}\,DM)} = 2.20 + 0.136 \times GP + 0.057 \times CP + 0.0029 \times CP^2$$

$$OMD_{(g\,100\,g^{-1}\,DM)} = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times XA$$

where XA ash is in g 100 $g^{-1}$ DM and GP is the net gas production (ml) at 24 h. The short chain fatty acids were calculated using blow equation as:

$$SCFA_{(mmol)} = -0.00425 + 0.0222GP$$

where Gas is 24 h net gas production (ml 0.2 $g^{-1}$ DM).

### Statistical analysis

Data obtained from *in vitro* gas production study was subjected to analysis of variance as a completely randomized design by the GLM procedure of SAS Institute Inc (2002) and treatment means were compared by the Duncan test.

## RESULTS AND DISCUSSION

The chemical compositions of pomegranate seeds are shown in Table 1. CP, ADF, NDF, EE, ASH, TP and TT contents in pomegranate seeds were 12.2 %, 44.6 %, 62.3 %, 1.6 %, 12.1 %, 1.8 % and 0.8 %, respectively. Chemical compositions of pomegranate seeds in the current study were inconsistent with findings of Taher-Maddah *et al*. (2012). Feizi *et al.* (2005) reported that DM, OM, CP, crude fibre, and EE values of pomegranate seeds were 94.8, 96.8, 11.4, 38.9, and 1.0 %, respectively. These differences in chemical composition of by-products may be due to a difference in cultivar, growing conditions, varieties, and different de-hulling processes (Taher-Maddah *et al*., 2012). Kamalak *et al.* (2007) reported that total and soluble condensed tannins, NDF and ADF were negatively

correlated with estimated parameters of gas production. The results of our study are consistent with those of Feizi *et al.* (2005) who suggested that pomegranate peel tannins have negative effect on *in vitro* rumen fermentation. Tannins are considered to have both adverse and beneficial effects in ruminant animals. High concentrations of tannins may reduce intake, digestibility of protein and carbohydrates, and animal performance through their negative effect on palatability and digestion. By preventing bloat and increasing the flow of non-ammonia nitrogen and essential amino acids from the rumen, low and moderate (20-45 mg/g DM) concentrations of condensed tannins in the diet improved production efficiency in ruminants, without increasing feed intake (Shabtay *et al.*, 2008). In the last few years there is an increasing interest of nutritionists in bioactive plant factors - phytofactors as natural feed additives, tannins etc. that can modify the rumen fermentation processes (e.g. defaunation), improve the protein metabolism and, at the same time, reduce ammonia production and emission, and curb methane production and emission to the atmosphere. High diversity of bioactive phytofactors contained in many plant species has been identified as a potential factor affecting the above-mentioned processes (Szumacher-Strabel and Cieślak, 2010).

**Table 1: Chemical composition of pomegranate seeds (% of DM)**

| | Components | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DM | CP | EE | NDF | ADF | ASH | TP | TT |
| Pomegranate seed | 93.8 | 12.2 | 1.6 | 62.3 | 44.6 | 12.1 | 1.8 | 0.8 |

DM = Dry matter; CP = Crude protein; EE = Ether extract; ADF = Acid detergent fibre; NDF = Neutral detergent fibre; TP = Total phenol; TT = Total tannins

Gas production volumes (ml.g$^{-1}$ DM) from *in vitro* incubation of PS supplemented with different levels of *Saccharomyces cerevisiae* at different incubation times are shown in Table 2 and Fig. 1. The cumulative volume of gas production increased with increasing time of incubation. Although there are other models available to describe the kinetics of gas production, the Ørskov and McDonald (1979) method 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 for predicting digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Gas volumes have also showed a close relationship with feed intake (Blummel and Becker, 1997) and growth rate in cattle (Blummel and Ørskov, 1993).

At the early incubation time (2 h), the treatments 1 and 4 (treatment with Sc, 0 and 7.5 ml.g$^{-1}$ DM,

**Table 2: Total gas production volume (ml/g DM) in incubation times**

| Treatments | Incubation times (h) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | 4 | 6 | 8 | 12 | 16 | 24 | 36 | 48 |
| Pomegranate seed | 31.52$^{a}$ | 33.45$^{c}$ | 38.11$^{b}$ | 57.42$^{c}$ | 82.51$^{ab}$ | 87.79$^{bc}$ | 95.11$^{b}$ | 106.50$^{c}$ | 109.47$^{c}$ |
| PS + Sc 2.5 g.kg$^{-1}$ DM | 21.31$^{b}$ | 46.22$^{a}$ | 65.86$^{a}$ | 82.06$^{a}$ | 96.42$^{a}$ | 106.14$^{a}$ | 121.68$^{a}$ | 138.73$^{a}$ | 145.25$^{a}$ |
| PS + Sc 5 g.kg$^{-1}$ DM | 21.98$^{b}$ | 43.99$^{ab}$ | 62.42$^{a}$ | 74.85$^{ab}$ | 88.76$^{ab}$ | 98.93$^{ab}$ | 112.25$^{a}$ | 127.18$^{b}$ | 135.04$^{b}$ |
| PS + Sc 7.5 g.kg$^{-1}$ DM | 33.08$^{a}$ | 36.45$^{bc}$ | 45.99$^{b}$ | 64.97$^{bc}$ | 75.05$^{b}$ | 81.86$^{b}$ | 98.28$^{b}$ | 111.33$^{c}$ | 115.86$^{c}$ |
| SEM | 2.84 | 2.85 | 3.10 | 3.57 | 4.22 | 4.27 | 3.81 | 2.52 | 2.43 |

The means within a column without common letters differ ($p < 0.05$).

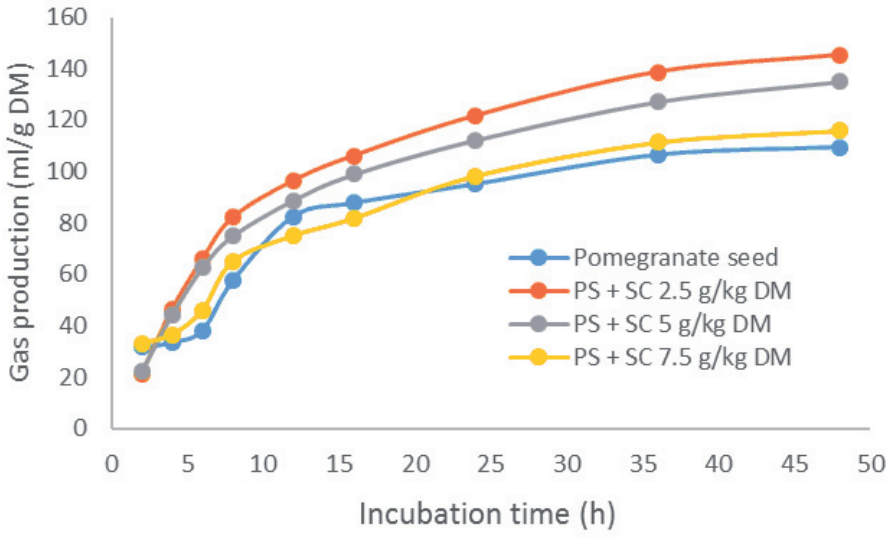


**Fig. 1: Pattern of *in vitro* gas production (fitted with exponential model) as affected by different levels of SC at different incubation times**

respectively) had the highest gas production volume among treatments, but after 4 h incubation the gas production volume in treatments 1 and 4 was the lowest ($p < 0.05$). The treatment 2 at the most incubation times had the highest gas production volume.

Mirzaei-Aghsaghali *et al*. (2011) showed that the gas production after 48 h incubation for PS was 222.3 $ml.g^{-1}$ DM, which was higher than the amount obtained in this study (109.47 $ml.g^{-1}$ DM).

These results are in agreement with a previous study that *Saccharomyces cerevisiae* increases ruminal gas production (Martin and Nisbet, 1992), but others found no effect (Lila *et al*., 2004) or a decrease (Lynch and 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 and Martin, 1999) and dose (Lila *et al*., 2006).

The ability of yeast to increase IVGP observed in the study has been reported by various authors with different roughages (Chaucheyras-Durand *et al*., 2008; Ando *et al.*, 2004; Ando *et al*., 2005). Tang *et al*. (2008) reported an increase in rate of gas production and IVRDMD (*in vitro* rumen 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 IVRDMD 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 and Chaucheyras-Durand, 2006; Ding, 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 and 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 IVRDMD for Japanese sake yeast and bio ethanol residue yeast as compared with soybean peptide (SP).

Wambui *et al*. (2010) used two strains of *Sacharomyces cereviciae* (Japanese sake yeast and bio ethanol residue yeast). Both Japanese sake yeast (JSY) and bio ethanol residue yeast supplements increased the ruminal digestion of the browse foliages and the effect of JSY appeared to be significantly higher. Differences in effect of yeast on rumen microbes and fermentation patterns 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. 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 pointed 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 studies 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.

Estimated gas production parameters of treatments are shown in Table 3. The treatment with 2.5 SC g.kg$^{-1}$ DM had the highest ME, OMD and SCFA among treatments.

**Table 3: Estimated gas production parameters of treatments**

| Treatments | Items | | | |
|---|---|---|---|---|
| | GP $_{(ml\ 0.2\ g^{-1}\ DM)}$ | ME $_{(MJ.kg^{-1}\ DM)}$ | OMD $_{(g\ 100\ g^{-1}\ DM)}$ | SCFA $_{(mmol)}$ |
| Pomegranate seed | 19.02$^{b}$ | 5.91$^{b}$ | 38.07$^{b}$ | 0.418$^{b}$ |
| PS + Sc 2.5 g.kg$^{-1}$ DM | 24.34$^{a}$ | 6.64$^{a}$ | 42.79$^{a}$ | 0.536$^{a}$ |
| PS + Sc 5 g.kg$^{-1}$ DM | 22.45$^{a}$ | 6.38$^{a}$ | 41.12$^{a}$ | 0.494$^{a}$ |
| PS + Sc 7.5 g.kg$^{-1}$ DM | 19.66$^{b}$ | 6.00$^{b}$ | 38.63$^{b}$ | 0.432$^{b}$ |
| SEM | 0.491 | 0.430 | 0.135 | 0.0101 |

ME = Metabolizable energy (MJ.kg$^{-1}$ DM); OMD = Organic matter digestibility (% DM); SCFA = Short chain fatty acid (mmoL).
The means within a column without common letters differ ($p < 0.05$).

## CONCLUSION

It was concluded that *in vitro* gas production parameters of pomegranate seed was improved with addition of *Saccharomyces cerevisiae* at 2.5 g.kg$^{-1}$ DM level.

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