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Chemical Composition, Physical Characteristics, Digestibility and Degradability of Grape Pomace Processed with *Neurospora sitophila*

Research Article

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ABSTRACT

The nutritive values of non- processed and processed grape pomace with *Neurospora sitophila* were evaluated. The chemical compositions and physical characteristics of samples were evaluated by laboratory analysis. An *in vitro* digestibility experiment was done to determine digestibility coefficients of dry matter, organic matter and also digestible organic matter in dry matter (DOMD) to estimate the metabolizable energy (ME) content of grape pomace samples. In addition, dry matter (DM), organic matter (OM) and crude protein (CP) disappearance of samples were determined by an *in situ* method by using 9 bags for each sample at 0, 3, 6, 12, 24, 48 and 72 h after incubation and their kinetics were described using the equation $P= a + b(1-e^{-ct})$. The nutritive value index (NVI) of samples was calculated using the equation; NVI= a + 0.4b + 200c. The collected data were analyzed in a completely randomized design. The average total phenolic compounds and extractable tannins, NDF, water holding capacity, soluble dry matter and digestion coefficients of DM, OM and DOMD were decreased ($P<0.05$) by processing of grape pomace, but percentage of CP, ash, acid detergent lignin, functional specific gravity, bulk density; effective degradability and NVI of DM, OM, CP were increased ($P<0.05$). After processing, water-soluble portion (a) for DM, OM and CP were increased, but the slow degradation rate and degradation rate of b were decreased ($P<0.05$). It can be concluded that, processing of this by product with *Neurospora sitophila*, increased protein content and decreased its tannins and phenols and therefore will improve the efficiency of using grape pomace in animals nutrition.

KEY WORDS degradability, grape pomace, *Neurospora sitophla*.

INTRODUCTION

In recent decades, population growth, economic and social development caused higher demand for livestock products in many developing countries. A large portion of agricultural by-products has no direct human consumption but it can be used indirectly to produce human food. Effective usage of agricultural by-products as animal feed depends on some factors such as nutrients composition compared to animal needs (McDonald *et al.* 1995) and cost-effective processing of byproducts (Ammerman and Henry, 1991). Grape pomace is a lignocellulolytic product with low level of protein and high tannin content which is being produced after extraction of grape juice in some regions of Iran. Grape pomace has low economic value and its processing wastes will cause environmental pollution. However, processing of this by product in order to increase protein content and decrease its tannins and phenols will increase its efficiency in ruminant nutrition. There are various methods such as using microorganisms, like fungi and yeasts, to

increase the protein content of grape pomace (Forage and Richelato, 1979). Some researchers used fungi to increase protein content in citrus pulp (Barreto de Menezes *et al*. 1989; Madadi-nuei, 1997; Grewal *et al*. 1990; Labaneiah *et al*. 1979; Nazem *et al*. 2008) and beet pulp (Dashti-Saridregh *et al*. 2010). Therefore, the aim of this study was to evaluate the effect of processing with *Neurospora sitophila* on chemical composition, physical characteristics, digestion coefficients and protein degradation of grape pomace.

MATERIALS AND METHODS

Chemical composition and physical characteristics

Non processed and processed grape pomace were dried in a forced-air oven (60 ˚C) and ground to pass a 1 mm screen in a Willy Mill (Arthuer H. Co. and Thomas, Philadelphia, PA). Nitrogen (N) content was measured by the Kjeldahl method (Kjeltec 2300 Auto-analyzer, Foss Tecator AB, Hogans, Sweden) and crude protein (CP) was calculated as N × 6.25. Acid detergent lignin (ADL) was determined based on AOAC (2000). Neutral detergent fiber (NDF) was determined with methods described by Van Soest *et al*. (1991). Ash was determined by the method of AOAC (2000). Phenolic compounds and total tannin compounds of grape pomace were determined (Makkar *et al*. 1993).

Physical characteristics of samples such as bulk specific gravity (Giger-Reverdin, 2000), water holding capacity (WHC) (Robertson and Eastwood, 1981), dry matter and soluble ash (AOAC, 2000) and functional specific gravity (Wattiaux *et al*. 1992) were determined.

Inoculant preparation

A loop of mycelium of fungal was inoculated under completely sterilized condition to each medium of PDA and were kept in 30 ˚C for 48h and then refrigerated in 4 ˚C. The composition of preserving medium and inoculant in 1 liter were as below (Griffiths and Done, 1991): glucose, 10 g; yeast extract (medium) 2 g; potassium hydrogen phosphate (KH_2PO_4) 0.714 g; urea 0.86 g; ammonium sulphate ($(NH_4)_2SO_4$) 0.47 g; magnesium sulphate ($MgSO_4.7H_2O$) 0.2 g; calcium chloride 0.2 g; zinc sulphate ($ZnSO_4.7H_2O$) 4.4 g; boric acid (H_3BO_3) 0.144 mg; ammonium molybdate ($(NH_4)_6\ Mo_7O_{24}.4H_2O$) 0.48 mg; copper sulphate ($ZnSO_4.7H_2O$) 4.4 mg; magnesium chloride ($MnCl_2.4H_2O$) 0.144 mg; ferric chloride ($FeCl_3$) 3.2 mg. The preservative culture was made by taking 100 mL of medium, with above mentioned conditions, and transferred to a 250 mL Erlenmeyer flask. For maximum growth of fungi the pH was adjusted to be 5.5 and sterilized under 121 ˚C and 15 psi pressure for 15 min. Then few pieces of purified mycelium were transferred into flask of containing preservative culture and were shaked for 24h in 35 ˚C. Finally, inoculated culture kept under 4 ˚C.

Processing of grape pomace

Grape pomace was dried in the sun and sieved through sieves with 10 and 35 meshes. The DM content and pH of grape pomace were 90% and 3.4, respectively. To bring the pH to 5.5, which is essential for protein production in a single cell, 0.6 mL per 10 g grape pomace sieved through a sieve with 10 mesh and 0.7 mL per 10 g of sample sieved through a sieve with 35 mesh, was added to the samples. One milliliter of inoculated liquid was added per 10 g of dried pomace. Twenty gram of grape pomace sieved by 35 mesh sieve was added to each of the two 250 mL erlenmeyer flask and the other two 250 mL flasks, each 20 grams of pomace sieved through a sieve with 10 mesh and then 53.2 mL of mixture of ammonia and water was added to each of the flasks. In order to investigate the effect of sample volume on increasing the protein percentage in each flask, 40 g of sample sieved with 40 mesh sieve was added to a separate erlenmeyer flask, and 10 g of sample sieved with 10 and 35 were added to two other flasks. Appropriate amount of mixture of water and ammonia was added to achieve 75% moisture content and pH 5.5. After sterilization of the flasks and their contents, 1 ml of fungi culture medium per 10 g of grape pomace was inoculated under hood in sterile conditions. Then, the flasks were incubated at 35 ˚C for 120 h. After incubation, samples within the flasks were transferred to Petri dishes. These samples were dried at a temperature of 45-50 ˚C to prevent decreasing the quality of protein due to high temperature (Shojaosadati *et al*. 1999). After complete drying, samples were grounded and mixed and their protein content was determined.

Determination of digestion coefficients by using *in vitro* method

Three ruminally fistulated Kermani male sheep (47±3 kg) were fed twice daily with a total mixed ration containing alfalfa hay (60%) and concentrate (40%). The concentrate consisted of barley (73%), soybean meal (25%), calcium carbonate (0.6%), salt (0.4%) and vitamin and mineral mixture (1%) (vitamin-mixture contains: 5000000 IU of vitamin A; 5000000 IU of vitamin D and 500000 IU of vitamin E per kg and mineral mixture composition: 82.500% dynamad, 12.260% Mn, 4.12% Cu-sulfate, 1.0160% Zn-sulfate, 0.060%, ethylene diamine dihydriodide EDDI-80% and 0.044% Na-selenide).

The ground non-processed and processed grape pomace samples were incubated with rumen fluid following the procedures of Tilly and Terry (1963). Whole ruminal contents were collected from different parts of rumen before the morning feeding (08:00 h) by vacuum pump and filtered

through 4 layers of cheesecloth into a warmed thermos bottle that had been flushed with CO_2. The incubation inoculum was prepared by diluting the digesta inoculum with the artificial saliva (Tilly and Terry, 1963) in a 1:4 (vol/vol) ratio and stirring water bath at 39 ˚C with purging CO_2 until its use (10-15 min later). From each sample, 0.5 g dry weight was weighed and added to sterile plastic tubes (six replicates for each) and then 20 mL of the incubation inoculum was added. Tubes were sealed with rubber stoppers and incubated for 48 h at 39 ˚C. Tubes were gently swirled by hand four times every 12 h. At the end of the 48 h incubation period, tube contents were acidified by adding 6 *M* HCl to reach a final pH of 1.3-1.5. After the foam subsided, pepsin powder was added to a final concentration 0.2% (wt/vol). The tubes were reincubated for an additional 48 h. The tubes were centrifuged at 2500 × g for 15 min, and the supernatant was discarded. Fifty mL of H_2O was added to the pellets and were recentrifuged to wash out the residual acid.

The tubes containing the pellets were dried in a forced-air oven at 60 ˚C for 48 h to determine the residual DM weights. *In vitro* digestibility of DM and OM were calculated as the DM and OM which disappeared from the initial weight inserted into the tubes. The ME values of samples were calculated using the following equation (AFRC, 1993):

ME (MJ/kg DM)= 0.016 × DOMD (g/kg DM).

In situ ruminal degradability of DM, OM and CP

Three ruminally fistulated Kermani male sheep (47±3 kg) consuming 1.2 ± 0.2 kg DM/d were used. The sheep were fed a total mixed ration containing alfalfa hay (60%) and concentrate (40%) twice daily at 08:00 and 17:00 h. The *in situ* technique (Orskov and McDonald, 1979) was used to measure the kinetics of DM, OM and CP degradation of non- processed and processed grape pomace samples. Dried samples (2 g) were weighted into 5 cm × 13 cm nylon bags (50 µ pore size) and 9 bags were prepared for each sample and each incubation time. Ruminal incubation times were 0, 3, 6, 12, 24, 48 and 72 h.

The bags were removed after incubation in the rumen and were washed in cold running water until the washing ran clear and colorless. Zero time disappearance was obtained by washing unincubated bags in a similar way. All washed bags were dried in a forced-air oven at 60 ˚C for 48 h. The DM, OM and CP disappearance were calculated using the equation:

$$P= a + b(1-e^{-ct})$$

Where:
P: disappearance rate at time t.
a: rapidly degradable DM, OM or CP fraction.
b: slowly degradable DM, OM or CP fraction in the rumen.
c: rate constant of degradation of b and t is the time of incubation.

The effective degradability values of DM, OM and CP were calculated using the equation:

$$P= a + [(b\times c) / (c+r)]$$

Where:
P: effective degradability of nutrients.
a: water-soluble fraction.
b: potentially degradable fraction.
c: degradation rate of parameter.
r: passage rate of the digest out of the rumen at 0.02 h-1, which is an average value for animals fed at approximately maintenance level (AFRC, 1993).

The nutritive value index (NVI) of each nutrient for samples was calculated using the equation of Orskov and McDonald (1979) as:

$$NVI= a + 0.4b + 200c$$

Where:
a: water-soluble fraction.
b: potentially degradable fraction.
c: degradation rate of parameter.

Statistical analysis

For experiments data were analyzed by SAS (2002) using the general linear models procedure as a completely randomized design as:

$$Y_{ij}= \mu + T_i + e_{ij}$$

Where:
Y_{ij}: each observed value.
µ: mean of measured trait.
T_i: effect of treatment.
e_{ij}: random error.

Statistical differences between the non- processed and processed grape pomace were determined using Tukey's multiple range test (Pearse and Hartly, 1966). Mean differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical composition and physical characteristics

The chemical composition of grape pomace before and after processing is presented in Table 1. The average DM and

OM of grape pomace were not affected by processing with fungi.

However, Dashti-Saridregh *et al*. (2010) and Nazem *et al*. (2008) reported a reduction in DM of beet pulp and citrus pulp processed with *Neurospora sitophila* fungi. They mentioned that this reduction might be due to usage of pulp as a feed source by fungi. Some of the carbon in the pulp, following inhalation of fungi, is released as carbon dioxide into the environment and thus the DM content may decrease after processing (Shojaosadati *et al*. 1999).

Table 1 Chemical analysis (DM basis) of non processed and processed grape pomace

Constituents	Grape pomace		SEM	P-value
	Non-processed	Processed		
DM (%)	93.41	90.25	0.525	NS
OM (%)	91.70	89.60	0.275	NS
Ash (%)	8.30	10.40	0.242	*
CP (%)	10.50	18.35	0.950	*
NDF (%)	51.30	35.33	0.255	*
ADL (%)	41.63	49.62	1.030	*
Total phenolic compounds (%)	2.50	1.02	0.013	*
Total tannic compounds (%)	1.50	0.61	0.042	*

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber and ADL: acid detergent lignin.
SEM: standard error mean.
* (P<0.05).
NS: non significant.

The ash content of grape pomace was increased after processing with fungi (P<0.05) which is likely due to usage of organic matter such as cell wall and soluble carbohydrates by fungi (Shojaosadati *et al*. 1999). Some studies reported that ash in beet root pulp and citrus pulp processed with *Neurospora sitophila* increased (Dashti-Saridregh *et al*. 2010; Nazem *et al*. 2008; Madadi-nuei, 1997).

In present experiment, crude protein percentage content of grape pomace was (P<0.05) increased by processing. Dashti-Saridregh *et al*. (2010), Nazem *et al*. (2008) and Madadi-nuei (1997) also reported that CP in the processed beet pulp and citrus pulp increased with *Neurospora sitophila*, which might be due to the growth of fungi on them. Increase in fungal biomass during fermentation process increases crude protein content of grape pomace, because fungi use easily fermentable and lignocellulosic materials in the grape pomace by its extracellular enzymes and produce energy, protein and carbon dioxide (Shojaosadati *et al*. 1999).

During processing of grape pomace fungi uses structural carbohydrates, as a source of carbon, and nitrogen sources will be used for protein synthesis, which means that grape pomace through bio-conversion method is being enriched (Gibriel *et al*. 1981; Nigam, 1994). On the other hand, the fungal biomass *Neurospora sitophila* has about 45 percent crude protein (Moo-Young *et al*. 1993). The protein content of other agricultural by-products processed by different fungi cultures were also increased, similar to the results reported in the present work (Dashti-Saridregh *et al*. 2010; Nazem *et al*. 2008; Madadi-nuei, 1997; Xue *et al*. 1992; Illanes *et al*. 1992; Lena and Quaglia, 1992). The percentage of ADL in grape pomace increased after processing (P<0.05), but NDF content decreased (P<0.05). Cell wall reduction in processed beet pulp and citrus pulp with *Neurospora sitophila* fungi has been reported by Dashti-Saridregh *et al*. (2010) and Nazem *et al*. (2008), which is likely due to the high content of digestible carbohydrates. Fungi can use cellulose and hemicellulose in cell wall efficiently, but they are not able to degrade lignin. After processing, both total average of phenolic and tannic compounds were decreased down to 59% (P<0.05), which might be related to the use of phenolic compounds and tannins, or breaking down of tannin-protein complex or polysaccharides present in grape pomace by fungi (Alipour and Rouzbehan, 2006). White fungi such as mushrooms were used for biological degradation of tannins, and with *Sporotrichum pulverulentum* culture, the total tannin and condensed tannin were decreased (Makkar *et al*. 1993). The physical characteristics and mass densities of grape pomaces are shown in Table 2.

Table 2 Physical characteristics and bulk density of non-processed and processed grape pomace

	Grape pomace		SEM	P-value
	Non-processed	Processed		
Water holding capacity (g water/g DM)	3.60	3.20	0.030	*
Functional specific gravity (g/mL)	0.98	1.26	0.014	*
Soluble DM (% of DM)	19.30	14.28	0.238	*
Soluble DM (g/g DM)	0.16	0.09	0.008	*
Soluble ash (% of ash)	23.69	28.40	2.069	NS
Soluble ash (g/g DM)	0.25	0.30	0.028	NS
BD 50	0.36	0.42	0.003	*
BD 100	0.37	0.41	0.002	*

BD 50 and BD 100: determination of bulk density by using 50 and 100 mL cylinder, receptively.
SEM: standard error mean.
* (P<0.05).
NS: non significant.

The average water holding capacity of grape pomace decreased after processing with fungi (P<0.05). This is probably due to the degradation of cellulose and hemicellulose by fungi and the destruction of intracellular spaces. It has been reported by Giger-Reverdin (2000), that feed with a low mass density are being hydrated in water and the extracellular matrix is replaced with water, and therefore, water hold-

ing capacity will decrease. Processing forages has a reduction effect on water holding capacity (Wattiaux *et al*. 1992).

The average specific gravity of grape pomace after processing by fungi increased (P<0.05). It is likely because of partially degradation of cell wall which reduces free spaces by fungi. Therefore, the spaces are being filled by gas, water or even finer material and all this will increase gravity. Chaji *et al*. (2008) reported that processing pit cane with water vapor increased its gravity. After processing, the average of DM solution of grape pomace decreased (P<0.05), which might be due to consumption of cell wall and dissolved compounds inside the cell by fungi, as a food source.

The average bulk density of grape pomace after processing with fungi increased (P<0.05). It is because of low levels of soluble intracellular compounds, the high amount of lignin in the cell wall which is not being consumed by the fungi. Giger-Riverdin (2000) reported that particles with low mass density usually have high cell wall content. According to hotel theory (Van Soest, 1975), bulk density will increase by breaking down of the samples' cell wall because the empty spaces in cellulose decreases as they are being filled with air, water and finer particles. Chaji *et al*. (2008) reported that processing sugarcane pit with water vapor increased bulk density.

Coefficients of digestibility

Digestibility coefficients and metabolizable energy of grape pomace before and after processing are shown in Table 3.

Table 3 Digestibility coefficients (DM basis) and metabolisable energy of non-processed and processed grape pomace

	Grape pomace		SEM	P-value
	Non-processed	Processed		
DM (%)	54.25	50.10	0.265	*
OM (%)	44.81	40.30	0.525	*
DOMD (%)	37.90	30.50	0.370	*
ME (Mcal/kg DM)	1.43	1.28	0.297	NS

DM: dry matter; OM: organic matter; DOMD: digestible organic matter in dry matter and ME: metabolizable energy.
SEM: standard error mean.
* (P<0.05).
NS: non significant.

The mean digestibility coefficients of DM, OM and OM in DM of grape pomace decreased after processing (P<0.05). However, different results were found by Dashti-Saridregh *et al*. (2010) and Nazem *et al*. (2008). They found higher digestibility coefficients of DM, OM and OM in DM of beet pulp and citrus pulp processed with *Neurospora sitophila* fungi. In this research, lower digestibility coefficients might be related to lignin content increase. Nazem *et al*. (2008) and Durand and Chereau (1988) reported that there is a negative correlation between the amount of lignin and feed digestibility.

Processing has reduced the percentage of cell wall but lignin was increased. Decreased digestion coefficients might be related to *Neurospora sitophila* activity, which was not able to break down the lignin in processed grape pomace.

Degradability

The data related to the degradation parameters, effective degradability and NVI of DM of samples are given in Table 4.

Table 4 Dry matter disappearance (%) of non -processed and processed in the rumen by *in situ* method

	Grape pomace		SEM	P-value
	Non-processed	Processed		
Estimated parameters				
a (%)	30.20	41.00	0.550	*
b (%)	22.63	12.30	1.163	*
c (h-1)	0.05	0.03	0.001	*
Effective degradability				
k= 2% (% h-1)	27.03	41.00	0.400	*
k= 5% (% h-1)	23.40	35.01	0.310	*
k= 8% (% h-1)	18.02	29.50	0.570	*
NVIDM (%)	46.28	51.22	0.465	*

a: rapidly degradable fraction; b: slowly degradable fraction; c: rate constant of degradation of the b fraction and NVIDM: nutritive value index of dry matter.
k: passage rate (% h-1).
SEM: standard error mean.
* (P<0.05).

DM degradability coefficients from grape pomace (P<0.05) were affected by processing. Water soluble fraction (a) increased but the fraction with slow degradation rate (b) and degradation rate of b (h^{-1}) decreased. The reason of increasing soluble fraction (a) might be due to the high quantity of crude fiber and soluble compounds which are being used by fungal enzyme systems during processing and converted into soluble material. By increasing feed water-soluble contents, more energy can be available for growth to rumen microorganisms, and therefore degradability of feed will increase (McDonald *et al*. 1995); however, slowly degradable fraction (b) decreased after processing. Most common structural compounds in grape pomace are cellulose and hemicellulose, which are insoluble.

Reduction of DM degradation rate (c) of grape pomace might be related to increase of lignin and reduction of cellulose and hemicellulose in cell wall. NVI of DM of processed grape pomace increased (P<0.05) (Table 4), which might be due to increase of DM soluble fraction (a) of grape pomace.

The OM degradation parameters was similar to DM degradation (Table 5) as processing of grape pomace increased water soluble fraction (a), but fraction with slow degradation rate (b) and degradation rate of fraction b were reduced (P<0.05). Because most of the DM (90%) in grape pomace is composed of OM, therefore, changes in OM degradation

parameters of grape pomace were similar to DM degradation. After processing with fungi, NVI of OM of grape pomace (P<0.05) increased due to increase of water soluble fraction (a). Passage rate from the rumen (k) is affected by the amount of feed, and by increasing the level of feed intake, this amount will increase. Also, increasing the k value decreases the access time of rumen microorganisms to feed as a result of decreased effective degradability of DM and OM.

Table 5 Organic matter disappearance (%) of non- processed and processed in the rumen by *in situ* method

	Grape pomace		SEM	P-value
	Non-processed	Processed		
Estimated parameters				
a (%)	19.93	25.41	0.071	*
b (%)	30.25	25.54	0.624	*
c (h-1)	0.05	0.03	0.001	*
Effective degradability				
k= 2% (% h-1)	26.30	32.72	0.220	*
k= 5% (% h-1)	18.00	25.33	0.101	*
k= 8% (% h-1)	15.01	21.08	0.320	*
NVIOM (%)	38.74	40.53	0.077	*

a: rapidly degradable fraction; b: slowly degradable fraction; c: rate constant of degradation of the b fraction and NVIOM: nutritive value index of organic matter.
k: passage rate (% h-1).
SEM: standard error mean.
* (P<0.05).

As noted in Tables 4 and 5, increasing k value from 2% to 8% caused a reduction in DM and OM degradability percentages. Lower cell wall content and higher crude protein content increased effective degradability of DM and OM in processed grape pomace. The structural and non-soluble carbohydrate content in processed grape pomace decreased as those compounds were used during fermentation by fungi, however the effective degradability of DM and OM increased. Results obtained in this study, were compatible with the results reported by Dashti-Saridregh *et al*. (2010) and Nazem *et al*. (2008) for sugar beet pulp and citrus pulp processed by *Neurospora sitophila* fungi, respectively.

The degradability and effective degradability parameters and NVI of crude protein of grape pomace are shown in Table 6. Protein degradability coefficients of grape pomace after processing (P<0.05) increased for water soluble fraction (a) but fraction with slow degradation rate (b) and degradation rate of part b decreased. Increasing water-soluble protein is probably due to a lower percentage of cell wall in processed grape pomace, as there is a positive correlation between cell wall digestibility and nitrogen digestion in many feed sources in which nitrogen is surrounded by the cell wall. In other words, higher cell wall in unprocessed grape pomace prevents protein degradation. Therefore, increasing level of feed intake will increase k value, and, therefore, feed materials in the rumen have less time to be degraded.

Protein degradability coefficient in the rumen depends on CP content and protein degradability percentage.

Table 6 Crude protein disappearance (%) of non -processed and processed in the rumen by *in situ* method

	Grape pomace		SEM	P-value
	Non-processed	Processed		
Estimated parameters				
a (%)	38.30	47.05	0.090	*
b (%)	26.42	21.45	0.135	*
c (h-1)	0.04	0.03	0.001	*
Effective degradability				
k= 2% (% h-1)	34.32	47.50	0.053	*
k= 5% (% h-1)	30.07	42.40	0.230	*
k= 8% (% h-1)	25.50	36.20	0.150	*
NVICP (%)	52.07	60.25	0.194	*

a: rapidly degradable fraction; b: slowly degradable fraction; c: rate constant of degradation of the b fraction and NVICP: nutritive value index of crude protein.
k: passage rate (% h-1).
SEM: standard error mean.
* (P<0.05).

Since CP and protein degradability percentages were higher in processed grape pomace the coefficient of protein effective degradability in the rumen increased (P<0.05).

Also, by increasing the rate of passage (k) there will not be enough time for feed to be degraded in the rumen. In general, reducing the percentage of protein degradation and increasing feeding level decreases the effective degradability coefficient of protein in the rumen. The results of water soluble protein in processed grape pomace were in agreement with Dashti-Saridregh *et al*. (2010) and Nazem *et al*. (2008) for sugar beet pulp and citrus pulp, respectively.

CONCLUSION

Processing grape pomace with *Neurospora sitophila* increased its CP content and degradability of DM. But, in contrast, phenolic and tannic compounds in the grape pomace were decreased. In conclusion, processed grape pomace can be used as valuable animal feed source.

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