The nutritive values of non-processed and processed pistachio peel with *Neurospora sitophila* were evaluated. The chemical composition of samples was evaluated by laboratory analysis. An *in vitro* digestibility study was done to determine digestibility coefficients of dry matter (DM), organic matter (OM). Digestible organic matter in the dry matter (DOMD) to estimate the metabolizable energy (ME) content of pistachio peel samples. In addition, the disappearance of DM, OM and crude protein (CP) of the samples was determined by an *in situ* method. For each sample 12 bags were incubated for 0, 3, 6, 12, 24, 48 and 72 h and their kinetics were described using the equation $p = a + b(1-e^{-ct})$. The nutritive value index (NIV) of samples was calculated using the equation: $NIV = a + 0.4b + 200c$. The collected data were analyzed in a completely randomized design. The average total phenolic and total tannin compounds, DM percentage and digestion coefficient of DM in pistachio peel decreased ($P<0.05$) by processing. However, the percentage of CP, acid detergent fiber (ADF), acid detergent lignin (ADL), effective degradability of DM, OM and CP, and NIV of DM, OM and CP increased ($P<0.05$). The results showed that processing of pistachio peel with *Neurospora sitophila*, decreased compounds of phenol and tannin and increased its CP and effective degradability.

**KEY WORDS** degradability, *Neurospora sitophila*, pistachio peel.

**INTRODUCTION**

In recent decades, population growth, economic and social development has caused a higher demand for livestock products in many developing countries, which is outrunning the resources available to meet it. A large portion of agricultural by-products unsuitable for human consumption can contribute to the food chain via livestock. Effective usage of agricultural by-products as animal feed depends on matching their nutrient composition to the needs of the animal (McDonald *et al.* 1995). Cost-effective processing of by-products may be an option for their improvement (Ammerman and Henry, 1991). Annual production of pistachio in Iran is approximately 478000 tons (FAO, 2014). Pistachio peel (the soft external hull) remains after dehulling process of harvested pistachio (0.8-1.29 kg pistachio peel/kg dry pistachio; (Shakeri and ForoughAmeri, 2008)). Using pistachio peel as an alternative animal feed will not only meet the feed shortage but also reduce the risk of environmental pollution (Gholizadeh *et al.* 2010). Pistachio peel is a by-product with a low level of crude protein (CP) and a high content of phenolic and tannin compounds. Processing of this by product is justified to increase protein content and decrease its tannins and phenols, which will increase the value of pistachio peel in animal nutrition. Methods include the use of microorganisms, such as fungi...
and yeasts (Forage and Richelato, 1979), to increase the protein content of pistachio peel. Fungi have also been used to increase the protein content of citrus pulp (Barreto de Menezes et al. 1989; Grewal et al. 1990; Labaneiah et al. 1979; Madadi-nuei, 1997; Nazem et al. 2008), beet pulp (Dashti-Saridreg et al. 2010), date tops fronds (Dayani et al. 2013) and grape pomace (Dayani et al. 2014). Their results showed that processing of citrus pulp, beet pulp and grape pomace with fungi increased their protein content.

The aim of this study was to evaluate the effect of processing pistachio peel with microscopic fungi Neurospora sitophila and its effect on chemical composition, digestion coefficients and DM, OM and CP degradation of this by-product.

**MATERIALS AND METHODS**

**Inoculant preparation for processing**

A loop of fungal mycelium was inoculated under completely sterilized condition in each medium of potato dextrose agar (PDA), which were kept at 30 °C for 48 h and then refrigerated in 4 °C.

The contents of a liter of preserving medium and inoculant were (Griffiths and Done, 1991): glucose, 10 g; yeast extract (medium) 2 g; potassium hydrogen phosphate (KH₂PO₄) 0.714 g; Urea 0.86 g; ammonium sulphate ((NH₄)₂SO₄); magnesium sulphate (MgSO₄.7H₂O) 0.2 g; calcium chloride 0.2 g; zinc sulphate (ZnSO₄.7H₂O) 4.4 mg; boric acid (H₃BO₃) 0.144 mg; ammonium molybdate ((NH₄)₆ Mo₇O₂₄.4H₂O) 0.48 mg; copper sulphate (CuSO₄.5H₂O) 4.4 mg; magnesium chloride (MnCl₂.2H₂O) 0.144 mg and ferric chloride (FeCl₃) 3.2 mg.

In order to prepare the preservative culture 100 mL of medium, as described above, and transferred to a 250 mL erlenmeyer flask. For maximum growth of fungi the pH was adjusted to be 5.5 and sterilized at 121 °C until used.

**Processing of pistachio peel**

During the harvest season, pistachio peel samples were supplied by a pistachio de-hulling factory in Kerman, Iran. Pistachio peel was dried in the sun and sieved through 2 mm and 0.5 mm. The DM of the pistachio peel was 90 per cent and the pH 3.4. To bring the pH to 5.5 (suitable pH for production of proteins within a single cell), 0.6 mL per 10 g pistachio peel sieved through a sieve with 2 mm and 0.7 mL per 10 g of sample sieved through a sieve with 0.5 mm was added to the sample. One mL of inoculated liquid was added per 10 g of dried pistachio peel. Twenty g of pistachio peel sieved through a 0.5 mm was added to each of two of the 250 mL erlenmeyer flask. Each of the other two 250 mL flasks contained, 20 g of pistachio peel sieved through a 2 mm sieve, to which 53.2 mL of mixture of ammonia and water was added. In order to investigate the effect of sample volume on increasing the protein percentage in each flask, 40 g of sample sieved through a 0.47 mm sieve was added to a separate erlenmeyer flask and 10 g of sample sieved through 2 mm and 0.5 mm were added to two other flasks. Appropriate amount of the water and ammonia mixture was added to achieve 75% moisture content and 5.5 pH. After sterilization of the flasks and their contents, 1 mL of fungi culture medium per 10 g of pistachio peel was inoculated under a fumehood in sterile conditions. The flasks were transferred to the incubator for 120 h at 35 °C. After incubation samples within the flasks, they transferred to petri dishes and dried at a 45-50 °C to prevent decreasing the quality of protein due to over heating. After complete drying, samples were ground and mixed and their protein content determined.

**Determination of digestion coefficients by using an in vitro method**

Three Kermani male sheep, with rumen fistulas and weighing 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%) and vitamin and mineral mixture (1.4%) (each kg of vitamin and mineral mixture contained 0.30 g CoSO₄, 20.1 g CuSO₄, 10 g FeSO₄, 50 g ZnO₂, 40.2 g MnSO₄, 0.75 g KI, 878 g NaCl, 50 0000 IU vitamin A, 50 0000 IU vitamin D and 10 000 IU vitamin E.

Ground non-processed and processed pistachio peel samples were incubated with rumen fluid following the procedures of Tilley and Terry (1963).

Rumen contents were collected from different sites within the rumen before the morning feeding (08:00 h) by vacuum pump and filtered through four layers of cheesecloth into a warmed thermos bottle that had been flushed with CO₂. The incubation inoculum was prepared by diluting the digesta inoculum with the artificial saliva (Tilley and Terry, 1963) in a 1:4 (vol:vol) ratio and stirring, using a water bath to maintain a temperature of 39 °C and flushing with CO₂ until its use (10-15 min later). A sample with a dry weight of 0.5 g was weighed into sterile plastic tubes (six replicates for each) and 20 mL of the incubation inoculum 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 to 1.3-1.5.
After the foam subsided, pepsin powder was added at a concentration of 0.2% (wt/vol). The tubes were then reincubated for an additional 48 h after which they were centrifuged at 2500 × g for 15 min and the supernatant was discarded. To the pellet 50 mL of H₂O was added and then centrifuged again 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):

\[
\text{ME (MJ/kg DM)} = 0.0157 \times \text{DOMD (g/kg DM)}
\]

In situ ruminal degradability of DM, OM and CP

Three Kermani male sheep, with rumen fistulas and weighing 47 ± 3 kg and consumed 1.2 ± 0.2 kg DM 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 pistachio peel samples. Dried samples (2 g) were weighted into 5 cm × 13 cm nylon bags (50 μm pore size) and nine bags were prepared for each sample and each incubation time. Rumen incubation times were 0, 3, 6, 12, 24, 48 and 72 h. The bags were removed after incubation and washed in cold running water until the washing ran clear and colourless. 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 was 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.
- \(t_l\): lag time (h).

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 (NIV) of each nutrient for samples was calculated using the equation of Orskov and McDonald (1979) as:

\[
NIV = a + 0.4b + 200c
\]

Where:

- \(a\): water-soluble fraction.
- \(b\): potentially degradable fraction.
- \(c\): degradation rate of parameter.

Chemical composition

Non-processed and processed pistachio peel was dried in a forced-air oven (60 °C) and ground to pass a 1-mm screen in a Willy Mill (Arthur H. Co. and Thomas, Philadelphia, PA).

Nitrogen (N) content was measured by the Kjeldahl method (Kjeltec 2300 Auto-analyzer, Foss Tecator AB, Hogsan, Sweden) according AOAC (2000), 935.11. Crude protein (CP) was calculated as N × 6.25. Acid detergent lignin was determined by the method described in AOAC (2000), 973.18. Neutral detergent and acid detergent fiber (NDF and ADF) were determined by methods described by Van Soest et al. (1991). Sodium sulfite and an alpha amylase were not used in the NDF and ADF assays (Uden et al., 2005).

Ash was determined by the method of AOAC (2000), 942.05. Phenolic compounds and total tannin compounds of pistachio peel were determined according to Makkar et al. (1993).

Statistical analysis

Experimental data was analyzed by SAS (2002) using the general linear models procedure as a completely randomized design:

\[
Y_{ij} = \mu + T_i + e_{ij}
\]

\(Y_{ij}\): each observed value.
\(\mu\): mean of measured trait.
\(T_i\): effect of treatment.
\(e_{ij}\): random error.

Statistical differences between the non-processed and processed pistachio peel were determined using Tukey’s multiple range test (Pearse and Hartly, 1966). Mean differences were considered significant at (P<0.05).
Fungi can use cellulose and hemicellulose in cell walls efficiently, but cannot degrade lignin (Shojaosadati et al. 1998). After processing, the total phenolic compounds and extractable tannin in pistachio peel were reduced by 24 and 50 per cent, respectively (P<0.05). Processing of walnut hulls by Neurospora sitophila caused a reduction in the total amount of tannin and phenolic compounds (Takalloozadeh et al. 2015). Moreover, Dayani et al. (2014) reported a reduction in the total phenolic compounds and extractable tannin of treated grape pomace, which might be related to using phenolic and tannic compounds or breaking down of the tannin-protein complex or polysaccharides by fungi (Shojaosadati et al. 1999). For biological degradation of tannins white fungi such as mushrooms was used also, after culturing with Sporotrichum pulverulentum the total tannin and condensed tannin were decreased (Makkar et al. 1993).

**Coefficients of DM and OM digestibility**

Digestibility coefficients of DM and OM and metabolizable energy of pistachio peel before and after processing are shown in Table 2. The mean digestibility coefficient of DM of pistachio peel reduced after processing (P<0.05). Lower digestibility coefficient of DM of pistachio peel processed with fungi might be related to increase of ADL content as processing increased the percentage of lignin (Table 1). Nazem et al. (2008) and Durand et al. (1988) reported that there is a negative correlation between the amount of lignin and feed digestibility. Decreased digestion coefficients might be related to Neurospora sitophila activity, which was not able to break down the lignin in processed pistachio peel. This finding is supported by data of Dayani et al. (2014) and Takalloozadeh et al. (2015), which they processing walnut hulls and grape pomace with Neurospora sitophila, respectively. In contrast, Dashiti-Saridregh et al. (2010) and Nazem et al. (2008) reported that digestibility of DM, OM and DOMD for processed beet pulp and citrus pulp with Neurospora sitophila fungi were higher than unprocessed pulp. Metabolizable energy of pistachio peel was not affected by processing. An inverse relationship between ADF content and digestibility was reported by Durand et al. (1988). Treating with Neurospora sitophila increased ME of citrus pulp (Nazem et al. 2008). An ME content of 2.5 and 3.1 (MJ/kg DM) for untreated and treated beet pulp, respectively, was reported by Dashiti-Saridregh et al. (2010). Takalloozadeh et al. (2015) indicated that treating with Neurospora sitophila decreased the ME of walnut hull.

**Degradability**

The results for degradation parameters, effective degradability and NIV of DM of samples are given in Table 3.
The water soluble fraction (a) increased, but with slow degradation rate fraction (b) and degradation rate of c (h⁻¹) decreased. The cause of the increase in the soluble fraction (a) might be because of the high quantity of crude fiber and soluble compounds, which are used by fungal enzyme systems during processing and are converted into soluble materials.

By increasing water-soluble materials, more energy can be available for growth of rumen microorganisms and therefore degradability of feed will increase (Orskov, 1992; McDonald et al. 1995); however, the slowly degradable fraction (b) decreased after processing. Most common structural compounds in pistachio peel are cellulose and hemicellulose, which are insoluble. Reduction of DM degradation rate (c) of pistachio peel might be related to the increase of lignin and reduction of cellulose and hemicellulose in cell wall. Pistachio peel DM degradability coefficients were significantly (P<0.05) affected by processing.

The NIV of DM of processed pistachio peel increased significantly (P<0.05) (Table 3), which could be due to the increase of the DM soluble fraction (a) of pistachio peel.

The OM degradation parameters are shown in Table 4. Processing of pistachio peel increased the water soluble fraction (a), but the degradation rate fraction (b) and degradation rate of fraction b were both reduced (P<0.05). Because most of the DM in pistachio peel is composed of OM, changes in OM degradation parameters of pistachio peel were very similar to DM degradation. After processing with fungi, NIV of OM of pistachio peel significantly (P<0.05) increased due to an increase of the 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 (Orskov, 1992). Increasing the k value also decreases the access time of rumen microorganisms to feed as a result of decreased effective degradability of DM and OM (Orskov, 1992).

### Table 1 Chemical analysis (DM basis) of non-processed and processed pistachio peel (n=5)

<table>
<thead>
<tr>
<th>Constituents (g/kg)</th>
<th>Non-processed</th>
<th>Processed</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>940.26</td>
<td>920.85</td>
<td>3.1</td>
<td>0.037</td>
</tr>
<tr>
<td>OM</td>
<td>880.46</td>
<td>870.56</td>
<td>3.3</td>
<td>0.275</td>
</tr>
<tr>
<td>Ash</td>
<td>110.54</td>
<td>120.44</td>
<td>3.3</td>
<td>0.232</td>
</tr>
<tr>
<td>CP</td>
<td>80.45</td>
<td>100.41</td>
<td>2.3</td>
<td>0.0045</td>
</tr>
<tr>
<td>NDF</td>
<td>250.73</td>
<td>250.79</td>
<td>3.2</td>
<td>0.341</td>
</tr>
<tr>
<td>ADF</td>
<td>170.09</td>
<td>200.05</td>
<td>4.0</td>
<td>0.0036</td>
</tr>
<tr>
<td>ADL</td>
<td>80.04</td>
<td>110.85</td>
<td>9.7</td>
<td>0.0017</td>
</tr>
<tr>
<td>Total phenolic compounds</td>
<td>140.05</td>
<td>100.7</td>
<td>13.5</td>
<td>0.0366</td>
</tr>
<tr>
<td>Total tannic compounds</td>
<td>100.7</td>
<td>50.3</td>
<td>12.0</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber exclusive of residual ash; ADF: acid detergent fiber exclusive of residual ash and ADL: acid detergent lignin.

SEM: standard error of the means.

### Table 2 Digestibility coefficients and metabolizable energy of non-processed and processed pistachio peel (n=5)

<table>
<thead>
<tr>
<th>Digestibility</th>
<th>Non-processed</th>
<th>Processed</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>85.70</td>
<td>81.88</td>
<td>1.03</td>
<td>0.044</td>
</tr>
<tr>
<td>OM (%)</td>
<td>76.38</td>
<td>75.46</td>
<td>0.55</td>
<td>0.167</td>
</tr>
<tr>
<td>DOMD (%)</td>
<td>66.67</td>
<td>66.75</td>
<td>0.53</td>
<td>0.263</td>
</tr>
<tr>
<td>ME (Mcal/kg DM)</td>
<td>10.46</td>
<td>10.47</td>
<td>0.32</td>
<td>0.341</td>
</tr>
</tbody>
</table>


SEM: standard error of the means.

### Table 3 Dry matter disappearance (%) of non-processed and processed pistachio peel in the rumen by in situ method

<table>
<thead>
<tr>
<th>Items</th>
<th>Non-processed</th>
<th>Processed</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (%)</td>
<td>53</td>
<td>65.50</td>
<td>2.02</td>
<td>0.04</td>
</tr>
<tr>
<td>b (%)</td>
<td>34.06</td>
<td>25.06</td>
<td>2.66</td>
<td>0.0019</td>
</tr>
<tr>
<td>c (h⁻¹)</td>
<td>0.12</td>
<td>0.095</td>
<td>0.004</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

a: rapidly degradable fraction; b: slowly degradable fraction; c: rate constant of degradation of the b fraction and NIVDM: nutritive value index of dry matter.

k: passage rate (% h⁻¹).

SEM: standard error of the means.
As noted in Tables 4 and 5, passage rate from the rumen (k) is affected by the amount of feed, and by increasing the level of feed intake. With an increase in k value from 2 to 8 percent of the time, the percentage of effective degradability of DM and OM decreased.

Higher CP content increased effective degradability of DM and OM in processed pistachio peel. The structural and non-soluble carbohydrate content in processed pistachio peel decreased as those compounds were used during fermentation by fungi, however the effective degradability of DM and OM increased (Dashti-Saridregh et al. 2010). Results obtained in this study, were compatible with the results reported by Takalloozadeh et al. (2015), Dayani et al. (2014), Dashti-Saridregh et al. (2010) and Nazem et al. (2008) for walnut hull, grape pomace, beet pulp and citrus pulp, respectively, all processed by Neurospora sitophila fungi.

The degradability and effective degradability parameters and NVI of CP of pistachio peel are shown in Table 5. Protein degradability coefficients of pistachio peel after processing significantly (P<0.05) increased for water soluble fraction (a), but fraction with slow degradation rate (b) and degradation rate of part b decreased. Increasing the water-soluble protein is probably due to increase in fungal biomass protein during fermentation process.

In other words, a part of CP of processed pistachio peel was fungal protein, which has different degradation characteristics. 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 (Orskov, 1992). Since CP and protein degradability percentages were higher in processed pistachio peel, the coefficient of protein effective degradability in the rumen increased significantly (P<0.05).

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 pistachio peel were in agreement with Dayani et al. (2014), Dashti-Saridregh et al. (2010) and Nazem et al. (2008) for grape pomace, beet pulp and citrus pulp, respectively.
CONCLUSION

Processing pistachio peel with *Neurospora sitophila* increased its CP content and degradability of DM and CP were improved. But, in contrast, phenolic and tannin compounds in the pistachio peel, decreased. In conclusion, processed pistachio peel is more proper feed source for animals. Additional *in vivo* experiments can be conducted to further evaluate this fungus for processing of pistachio peel as a feedstuff for ruminants.

ACKNOWLEDGEMENT

The authors thank Mrs. Teimori for her technical assistance during the experiment.

REFERENCES


