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Changing Nutritional Composition of Pomegranate (*Punica granatum* L.) Seed by *Aspergillus niger* Solid State Fermentation to Making Suitable for Poultry Nutrition

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Abstract

Nutritional changes in pomegranate (*Punica granatum* L.) seed by *Aspergillus niger* solid state fermentation were investigated in this study. Pomegranate seed was fermented by two different *A. niger* strains (ATCC 20345 and ATCC 9142). Crude protein, ether extract and ash were increased ($P<0.001$), nitrogen-free extract, crude fiber, neutral detergent fiber and acid detergent fiber were decreased ($P<0.001$) by fermentation in both strains. Hemicellulose was not changed ($P>0.05$) by ATCC 20345 but decreased ($P<0.01$) by ATCC 9142. This results showed that *A. niger* solid state fermentation can be used to improve nutritional composition of pomegranate seed in order to make a suitable feedstuff for poultry nutrition.

Key words: *Aspergillus niger, pomegranate seed, poultry nutrition, Punica granatum L., solid state fermentation*

Introduction

Utilization of agricultural residues has become having particular attention in recent years in order to reduce the dependence of poultry nutrition on the cereals that are used in human nutrition (Saki et al., 2014). Agricultural residues, which can be supplied at cheap prices, can reduce feed costs with using in poultry diets. In addition, environmental pollution caused by agricultural residues can also be lowered.

Pomegranate is one of the most ancient edible fruits (Manterys et al., 2016). Annual pomegranate production of Turkey reached 656.200 tons in 2016 (TUIK, 2016). Pomegranate seed has antioxidant effect on broiler chicks and laying hens (Saki et al., 2014, Ahmed et al., 2015). It can increase egg production (Saki et al., 2014) and improve egg yolk color (Kostogrys et al., 2017). Morever, harmful effects of mycotoxins on broiler chicks can be eliminated with pomegranate feeding (Hussein, 2015).

Cellulose is one of the major factors affecting digestibility of feedstuffs (Graminha et al., 2008). Poultry is more sensitive to cellulose level of the diet than ruminants. Pomegranate seed contains 13.2% crude protein (CP), 35.3% crude fiber (CF) and 27.2% ether extract (EE) on dry weight basis

(El-Nemr et al., 1990). High CF content of pomegranate seed is thought to limit its use in poultry nutrition. Solid state fermentation can be used for nutritional enrichment of agricultural residues (Aro, 2008). *Aspergillus niger* is recommended for solid state fermentation because of growing ability in the low-water environment (Raimbault, 1998). It was aimed to improve nutritional composition of pomegranate seed and reduce its CF content by two different *A. niger* strains (ATCC 200345 and ATCC 9142) for making a feedstuff that can be used in poultry diets.

Materials and Methods

Pomegranate seeds were obtained from a juice factory in Turkey. *A. niger* strains were ATCC 200345 and ATCC 9142. Pomegranate seeds were milled and sterilized with autoclave at 121°C for 15 min. The nutritional salt (glucose: urea:$(NH4)_2SO_4$:peptone:KH_2PO_4:$MgSO4.7H_2O$=4:2:6:1:4:1) was added to support microbial development. *A. niger* was cultured in Potato-Dextrose-Agar (PDA) and inoculated at 10^5 spores/kg pomegranate seed. Afterwards, samples were incubated at 60 °C for 48 hours and dried at room temperature for 6 days till reaching %90 dry matter.

CP, EE, ash and CF were analyzed according to AOAC (2000) before and after fermentation. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted as reported by Van Soest et al. (1991). Hemicellulose was calculated as NDF minus ADF. All of the experiments were carried out in triplicate. Differences between treatments were tested using ANOVA and Duncan's multiple range test (SPSS 21.0 Statistics). The level of statistical significance was declared at $P \leq 0.05$.

Results and Discussion

Nutritional composition of pomegranate seed was changed by *A. niger* solid-state fermentation. CP, EE and ash were increased ($P<0.001$) by fermentation whereas nitrogen-free extract (NFE), CF, NDF and ADF decreased ($P<0.001$) in both groups (P1: fermented pomegranate seeds with ATCC 200345, P2: fermented pomegranate seeds with ATCC 9142). However, hemicellulose did not change ($P>0.05$) in P1 but decreased ($P<0.01$) in P2.

A. niger increased CP contents of pomegranate peel (Aguilar et al., 2008), shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008), palm kernel cake (Iluyemi et al., 2006, Lawal et al., 2010), sour cherry kernel (Güngör et al., 2017) and grape seed (Altop et al., 2017). In this study, crude protein of pomegranate seed was increased ($P<0.001$) by fermentation from 16.12% to 31.82% (P1) and 32.63% (P2). This increase may be due to enzymes and mycelia produced by *A. niger* (Raimbault, 1998).

EE increased (P<0001) from 1.55% to 5.72% (P1) and 6.17% (P2). Similar findings have been obtained from the studies on shea nut (Dei et al., 2008), whereas there was no difference in ether extract in the studies on palm kernel (Iluyemi et al., 2006, Lawal et al., 2010), pomegranate peel (Aguilar et al., 2008) sour cherry kernel (Güngör et al., 2017), grape seed (Altop et al., 2017) and decrease in the studies on mango kernel (Kayode and Sani, 2008), sour cherry kernel (Güngör et al., 2017), grape seed (Altop et al., 2017).

Ash content was increased (P<0.001) by solid state fermentation (P<0.001) from 3.05% to 8.19 (P1) and %8.33 (P2). This result consistent with the studies on pomegranate peel (Aguilar et al., 2008), shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008), sour cherry kernel (Güngör et al., 2017) and grape seed (Altop et al., 2017).

A. niger prefers soluble carbohydrates to other nutrients for using as a carbon source (Papagianni, 2007). NFE content of pomegranate seed was decreased (P<0.001) by fermentation from 41.12% to 28.65 (P1) and 26.49 (P2) in this study. This result is in line with the studies on pomegranate peel (Aguilar et al., 2008), shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008), sour cherry kernel (Güngör et al., 2017) and grape seed (Altop et al., 2017).

CF decreased (P<0.001) from 38.16% to 25.62% (P1) and 26.39% (P2). NDF decreased (P<0.001) from 51.38% to 41.32% (P1) and 37.77 (P2). ADF decreased (P<0.001) from 37.66% to 28.87% (P1) and 27.04% (P2). Hemicellulose did not change (P>0.05) in P1 (control:13.72%, P1:12.44%) while decreased (P<0.01) in P2 from 13.72% to 10.73%. *A. niger* has been reported to produce cellulase in solid state fermentation (Xie et al., 2016). These decreases can be attributed to the production of cellulase that breaks down the structural carbohydrates.

Conclusion and References

Nutritional composition of pomegranate seed was improved by *A. niger* solid state fermentation. CP, EE and ash were increased while NFE, CF, NDF and ADF were decreased (P<0.001) by ATCC 20345 and ATCC9142 in solid state fermentation. Hemicellulose was not changed (P>0.05) by ATCC 20345 but decreased (P<0.01) by ATCC 9142. This results showed that *A. niger* solid state fermentation can be used to improve nutritional composition of pomegranate seed in order to make a suitable feedstuff for poultry nutrition. However, detailed fermentation studies and animal experiments are needed to recommend to use of fermented pomegranate seeds in poultry diets.

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