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Aspergillus niger may improve nutritional quality of grape seed and its usability in animal nutrition through solid-state fermentation

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ABSTRACT

Effects of different *Aspergillus niger* strains on main nutritional components of grape seed in solid-state fermentation were investigated in this study. Grape seeds were fermented with three different *A. niger* strains which are ATCC 9142, ATCC 200345 and ATCC 52172. Fermented and unfermented grape seeds were analyzed for crude protein, ether extract, ash, crude fiber, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Unfermented grape seeds (control group) and fermented groups: FG1 (ATCC 9142), FG2 (ATCC 200345) and FG3 (ATCC 52172) were compared each other depending on the results of chemical analyses. Crude protein increased ($p < 0.001$) with fermentation in all groups and the highest increases were observed in FG2 and FG3 groups. Ether extract was similar with control in FG1 group but decreased ($p < 0.001$) in FG2 and FG3 groups. Ash content increased ($p < 0.001$) through fermentation in all groups, the highest increases were noted in FG2 and FG3 groups. Crude fiber, ADF, NDF and nitrogen-free extracts (NFE) were decreased with fermentation in all groups ($p < 0.001$). Whereas the highest decreases of NFE were observed in FG1 and FG2 groups, the highest reduction in crude fiber, ADF and NDF were ocurred in FG2 group. These results showed that nutritional quality of grape seeds can be improved by *A. niger* solid-state fermentation and the best results were taken from ATCC 52172.

1. Introduction

Grape is one of the most produced fruits in the world with 77 million tons of annual production [1]. Grape seed is a by-product emerges by processing of grapes in fruit juices and wine factories [2]. It is rich in polyphenols such as proanthocyanidins (catechin, epicatechin, epicatechin-3-O-gallate; [3]). These polyphenols have antimicrobial, antioxidant and anticarcinogenic properties [4].

Positive results have been obtained from animal experiments on grape seed. Grape seed has antioxidant effects on lamb meat [5] sheep milk [6], poultry meat [2, 7] and egg [8]. Besides, it enhanced growth performance in *Eimeria tenella* infected broilers [9], increased antibody titer against Newcastle Disease Virus [2] and inhibit the deterioration of jejunal epithelial cells in the bulls exposed to heat stress [10].

Grape seed can increase milk production [11, 12], milk calcium, iron [12] and fatty acid content [6] in sheep. It has been also reported to increase the fatty acid levels in egg yolk [13] and chicken meat [14].

Grape seed can reduce methane emission from ruminants. Studies showed that grape seed can diminish methane production by 20% without affecting microbial fermentation in rumen [15, 16]. Condensed tannins in grape seed can increase the efficiency of feed protein by preventing proteins from ruminal digestion [17] and reduce blood urea level hereby [18].

Grape seed having positive effects on animals with its active components has been reported to be used at limited level in animal feed because of having less digestibility due to high crude fiber content [19, 20]. Fermentation is a method that can be used to improve digestibility of agricultural by-products [21]. Enzymes produced by microorganisms during fermentation can break down cellular structure of the substrate and thus increase its digestibility [22]. Besides microorganisms can increase protein, amino acid, ether extract, mineral and vitamin

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content of substrate with fermentation [23]. Fermentation is divided into liquid-state and solid-state fermentation. Solid-state fermentation is preferred method due to low cost, suitability for working with agricultural by-products and less risk of bacterial contamination [24]. Solid-state fermentation refers to microbial reproduction in moist substrates without free water [25]. *Aspergillus niger* is a filamentous fungus that can grow rapidly in low-water environments [26] and, therefore, used widely in solid-state fermentation of agricultural by-products [27]. In this study, it was aimed to enhance the nutritional composition of grape seed by *A. niger* solid-state fermentation for making available to be used in animal nutrition.

2. Material and Methods

2.1 Microorganisms and substrate

A. niger strains were obtained from the American Type Culture Collection (ATCC). The strains were ATCC 9142 (FG1), ATCC 200345 (FG2) and ATCC 52172 (FG3).

Grape seeds were provided from a grape juice factory in Turkey and stored at -20 °C till fermentation.

2.2 Solid-state fermentation

Grape seeds were milled to a size of 2 mm before being sterilized by autoclaving at 121 °C for 15 min. The nutritional salt (glucose:urea:(NH4)2SO4:peptone: KH2PO4:MgSO4.7H2O=4:2:6:1:4:1) were mixed to the substrate to encourage microorganism to grow after sterilizing phase. Each *A. niger* strain cultured in Potato-Dextrose-Agar (PDA) was added to grape seed substrate at 10^4 spores and uninoculated grape seeds were assigned as control. Samples were incubated at 60 °C for 48 hours. Afterwards, samples were dried at room temperature for 6 days in which samples reached approximately 90% dry matter. Three replicates were prepared for each treatment.

2.3 Main nutritional components analysis

Ash (method, 942.05), crude protein (method, 976.06), ether extract (method, 920.29), crude fiber (method, 973.18) analyses of grape seeds before and after solid-state fermentation were conducted [28]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted according to Van Soest et al. [29] using the ANKOM^{2000} fiber analyzer (ANKOM corporation® Technology Fairport, NY). Measurement for each sample was conducted three times.

2.4 Statistical analysis

All of the experiments were carried out in triplicate, and results were expressed as means with pooled standard error of means (SEM). Differences between treatments were tested using ANOVA and Duncan's multiple range test (SPSS 21.0 Statistics). p values ≤ 0.05 were considered statistically significant.

3. Results and Discussion

Nutritional changes in grape seed by *A. niger* solid-state fermentation are given in Table 1 and photos before, during and after fermentation are shown in Figure 1. Nutritional composition of grape seeds changed with *A. niger* solid-state fermentation. Crude protein content increased (p < 0.001) in all groups whereas the higher increases were obtained in FG2 and FG3. Crude fiber content was decreased (p < 0.001) in all groups and the highest reduction was observed in FG2. Ether extract content was decreased in FG2 and FG3 but remained the same in FG1. Ash content was increased in all groups with the higher increases in FG2 and FG3. Nitrogen-free extract (NFE) was decreased in all groups with the higher decreases in FG1 and FG3. NDF and ADF were decreased in all groups, and the higher decreases occurred in FG2 and FG3.

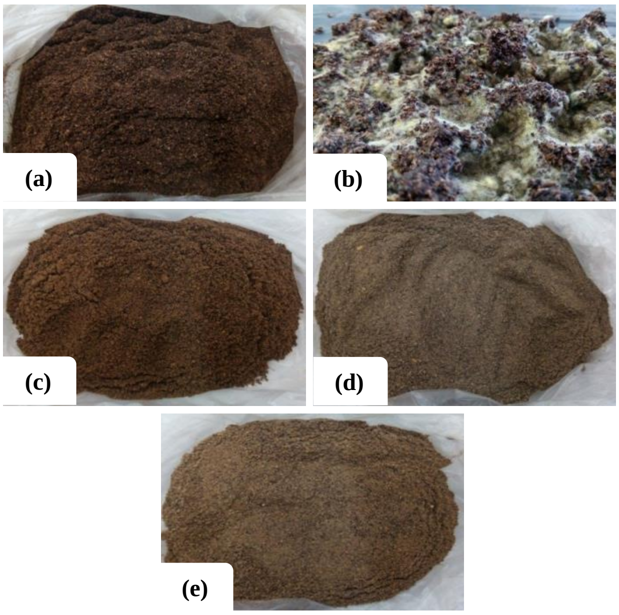

Figure 1. Photos before, during and after fermentation, a) unfermented grape seed, b) fermentation stage, c) grape seed fermented by ATCC 9142 (FG1), d) grape seed fermented by ATCC 200345 (FG2), e) grape seed by fermented ATCC 52172 (FG3)

Table 1. Nutritional changes in grape seed by *Aspergillus niger* solid-state fermentation

Composition (%)	G	FG1	FG2	FG3	SEM	P
Crude Protein	10.13^c	25.94^b	29.50^a	29.49^a	2.415	***
Ether Extract	12.50^a	13.21^a	9.82^c	11.68^b	0.394	***
Ash	3.88^c	7.99^b	8.72^a	8.84^a	0.614	***
NFE	26.06^a	13.17^c	15.72^b	12.71^c	1.632	***
Crude Fiber	47.43^a	39.69^b	36.25^d	37.30^c	1.325	***
NDF	64.01^a	52.30^b	48.98^c	49.13^c	1.857	***
ADF	57.14^a	47.85^b	43.21^c	43.34^c	1.708	***

***: <0.001; a,b,c: Means having different superscripts differ (p < 0.05)
G: unfermented grape seed, FG1: grape seed fermented by ATCC 9142, FG2: grape seed fermented by ATCC 200345, FG3: grape seed fermented by ATCC 52172

Nutritional composition of agricultural by-products can be enhanced with solid-state fermentation [30]. Aderemi and Nworgu [31] reported that cassava root and peels' crude protein content increased while crude fiber, NDF, and hemicellulose content decreased with fermentation. Similarly, cassava starch residues' crude protein, ether extract, ash content increased and crude fiber, NDF, ADF content decreased with fermentation [21]. Similar findings were reported in the studies on olive leaves [30] and *Gingko biloba* leaves [23, 27]. In this study, nutritional composition of grape seed was improved by *A. niger* solid-state fermentation with increases in crude protein, ether extract and ash content, decreases in crude fiber, NDF, ADF and NFE content.

Protein content of feed is an important factor affecting growth and yield of animals. Productivity losses are seen in animals when their protein requirement is not supplied by diet [32]. However, protein sources used in animal feeding are costly, therefore, increasing feed costs. In this study, crude protein content of grape seed was increased by fermentation. This increase may be due to mycelia and/or enzymes produced by *A. niger* [26]. Similar findings were reported in the studies on cassava [21, 31, 33-35], shea nut [36], *Ginkgo biloba* leaves [23, 27], pine needle [37], olive leaf [30] and sour cherry kernel [38].

Structural carbohydrates such as cellulose, hemicellulose, and lignin reduce digestibility of feeds because they are difficult to digest by animals [39]. For this reason, structural carbohydrate content is an important sign for estimating the feed's digestibility [30]. In this study, structural carbohydrate content of grape seeds such as crude fiber, NDF, and ADF decreased by fermentation. *A. niger* has been reported to produce cellulase in solid-state fermentation [30] It is thought that cellulase degraded the structural carbohydrates and it caused reduction in crude fiber, NDF and ADF content. Similar results were obtained from the studies on cassava pulp and peels [31], cassava starch residues [21] and shea nut [36]. However, these results are different from the results of the studies on cassava peels [35] and sour cherry kernel [38]. Differences in the results may be due to the fact that different strains were used in the studies or fermentation conditions were different. Indeed, Güngör et al. [38] reported that different strains had different effects on the same substrate.

Increase in ash content is line with the studies on cassava peels [35], cassava starch residues [21], shea nut [36] and sour cherry kernel [38] but not in line with the study that cassava pells' ash content was not changed with fermentation [21].

It has been reported that different *A. niger* strains affect substrate differently with regard to ether extract content [38]. Similarly, grape seed was affected differently by different *A. niger* strains in this study. Ether extract was decreased in FG2 and FG3 while remained at the same level in FG1. These results are consistent with the studies on cassava [35], pomegranate peel and creosote bush leaves [40] and sour cherry kernel [38]. However it was reported that the ether extract content was increased with fermentation of shea nut [36] and cassava [21], unlike our study.

Soluble carbohydrates are firstly preferred to other nutrients for carbon sources by fungi [41]. In fact, NFE level of grape seed decreased with fermentation in this study. This result is in an agreement with the studies on cassava peels [21, 35] and cassava bagasse [33].

4. Conclusion

Nutrient enrichment in the grape seed was achieved by fermentation. Crude protein and ash contents were increased with fermentation, and higher increases were obtained from ATCC 200345 and ATCC 52172. Crude fiber, ADF, and NDF contents were reduced in all groups and the highest reduction was obtained by ATCC 200345. Ether extract level remained at the same level in ATCC 9142 used group but decreased by ATCC 200345 and ATCC 52172. NFE level decreased in all groups; the higher decreases occurred by ATCC 9142 and ATCC 200345. These results showed that *A. niger* can improve nutritional composition of grape seed. Also, different *A. niger* strains (ATCC 9142, ATCC 200345, ATCC 52172) had different effects on grape seed, suggesting that ATCC 200345 may be used for nutritional enrichment of grape seed.

References

1. FAO. Food and Agriculture Organization, 2016. Access date: 25.04.2018. Available from: http://www.fao.org/faostat.
2. Farahat, M., F. Abdallah, H. Ali, and A. Hernandez-Santana, *Effect of dietary supplementation of grape seed extract on the growth performance, lipid profile, antioxidant status and immune response of broiler chickens.* Animal, 2017. **11**(5): p. 771-777.
3. Perumalla, A. and N.S. Hettiarachchy, *Green tea and grape seed extracts—Potential applications in food safety and quality.* Food Research International, 2011. **44**(4): p. 827-839.
4. Garavaglia, J., M.M. Markoski, A. Oliveira, and A. Marcadenti, *Grape seed oil compounds: Biological and chemical actions for health.* Nutrition and Metabolic Insights, 2016. **9**: p. 59-64.
5. Jerónimo, E., C.M. Alfaia, S.P. Alves, M.T. Dentinho, J.A. Prates, V. Vasta, J. Santos-Silva, and R.J. Bessa, *Effect of dietary grape seed extract and Cistus ladanifer L. in combination with vegetable oil supplementation on lamb meat quality.* Meat Science, 2012. **92**(4): p. 841-847.
6. Correddu, F., A. Nudda, G. Battacone, R. Boe, A.H.D. Francesconi, and G. Pulina, *Effects of grape seed supplementation, alone or associated with linseed, on ruminal metabolism in Sarda dairy sheep.* Animal Feed Science and Technology, 2015. **199**: p. 61-72.

7. Brenes, A., A. Viveros, I. Goni, C. Centeno, S. Sáyago-Ayerdy, I. Arija, and F. Saura-Calixto, *Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens.* Poultry Science, 2008. **87**(2): p. 307-316.

8. Hou, F., M. Xiao, J. Li, D.W. Cook, W. Zeng, C. Zhang, and Y. Mi, *Ameliorative effect of grape seed proanthocyanidin extract on cadmium-induced meiosis inhibition during oogenesis in chicken embryos.* The Anatomical Record, 2016. **299**(4): p. 450-460.

9. Wang, M., X. Suo, J. Gu, W. Zhang, Q. Fang, and X. Wang, *Influence of grape seed proanthocyanidin extract in broiler chickens: effect on chicken coccidiosis and antioxidant status.* Poultry Science, 2008. **87**(11): p. 2273-2280.

10. Li, X., Y. Yang, S. Liu, J. Yang, C. Chen, and Z. Sun, *Grape seed extract supplementation attenuates the heat stress-induced responses of jejunum epithelial cells in simmental×qinchuan steers.* British Journal of Nutrition, 2014. **112**(3): p. 347-357.

11. Gessner, D., C. Koch, F.-J. Romberg, A. Winkler, G. Dusel, E. Herzog, E. Most, and K. Eder, *The effect of grape seed and grape marc meal extract on milk performance and the expression of genes of endoplasmic reticulum stress and inflammation in the liver of dairy cows in early lactation.* Journal of Dairy Science, 2015. **98**(12): p. 8856-8868.

12. Mokni, M., M. Amri, F. Limam, and E. Aouani, *Effect of grape seed and skin supplement on milk yield and composition of dairy ewes.* Tropical Animal Health and Production, 2017. **49**(1): p. 131-137.

13. Omidi, M., S. Rahimi, and M.A.K. Torshizi. *Modification of egg yolk fatty acids profile by using different oil sources*. in *Veterinary Research Forum*. 2015. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

14. Francesch, A. and M. Cartañà, *The effects of grape seed in the diet of the Penedes chicken, on growth and on the chemical composition and sensory profile of meat.* British Poultry Science, 2015. **56**(4): p. 477-485.

15. Wischer, G., J. Boguhn, H. Steingaß, M. Schollenberger, and M. Rodehutscord, *Effects of different tannin-rich extracts and rapeseed tannin monomers on methane formation and microbial protein synthesis in vitro.* Animal, 2013. **7**(11): p. 1796-1805.

16. Moate, P., S. Williams, V. Torok, M. Hannah, B. Ribaux, M. Tavendale, R. Eckard, J. Jacobs, M. Auldist, and W. Wales, *Grape marc reduces methane emissions when fed to dairy cows.* Journal of Dairy Science, 2014. **97**(8): p. 5073-5087.

17. Bruno-Soares, A., A. Soares-Pereira, T. Matos, and J. Ricardo-da-Silva, *Preliminary results on the effects of grape (Vitis vinifera) seed condensed tannins on in vitro intestinal digestibility of the lupin (Lupinus angustifolius) seed protein fraction in small ruminants.* Journal of Animal Physiology and Animal Nutrition, 2011. **95**(4): p. 456-460.

18. Kronberg, S. and C. Schauer, *Cattle and sheep develop preference for drinking water containing grape seed tannin.* Animal, 2013. **7**(10): p. 1714-1720.

19. Spanghero, M., A. Salem, and P. Robinson, *Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces.* Animal Feed Science and Technology, 2009. **152**(3-4): p. 243-255.

20. Baumgärtel, T., H. Kluth, K. Epperlein, and M. Rodehutscord, *A note on digestibility and energy value for sheep of different grape pomace.* Small Ruminant Research, 2007. **67**(2): p. 302-306.

21. Aro, S., *Improvement in the nutritive quality of cassava and its by-products through microbial fermentation.* African Journal of Biotechnology, 2008. **7**(25): p. 4789-4797.

22. Couri, S., S. da Costa Terzi, G.A.S. Pinto, S.P. Freitas, and A.C.A. da Costa, *Hydrolytic enzyme production in solid-state fermentation by Aspergillus niger 3T5B8.* Process Biochemistry, 2000. **36**(3): p. 255-261.

23. Zhang, X., L. Zhao, F. Cao, H. Ahmad, G. Wang, and T. Wang, *Effects of feeding fermented Ginkgo biloba leaves on small intestinal morphology, absorption, and immunomodulation of early lipopolysaccharide-challenged chicks.* Poultry Science, 2013. **92**(1): p. 119-130.

24. Pérez-Guerra, N., A. Torrado-Agrasar, C. López-Macias, and L. Pastrana, *Main characteristics and applications of solid substrate fermentation.* Electronic Journal of Environmental, Agricultural and Food Chemistry, 2003. **2**(3): p. 343-350.

25. Osma, J.F., J.L.T. Herrera, and S.R. Couto, *Banana skin: A novel waste for laccase production by Trametes pubescens under solid-state conditions. Application to synthetic dye decolouration.* Dyes and Pigments, 2007. **75**(1): p. 32-37.

26. Raimbault, M., *General and microbiological aspects of solid substrate fermentation.* Electronic Journal of Biotechnology, 1998. **1**(3): p. 26-27.

27. Cao, F., X. Zhang, W. Yu, L. Zhao, and T. Wang, *Effect of feeding fermented Ginkgo biloba leaves on growth performance, meat quality, and lipid metabolism in broilers.* Poultry Science, 2012. **91**(5): p. 1210-1221.

28. AOAC, *Official Methods of Analysis of AOAC International (17. Edition)*. 2000, AOAC International: ABD.

29. Van Soest, P.v., J. Robertson, and B. Lewis, *Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition.* Journal of Dairy Science, 1991. **74**(10): p. 3583-3597.

30. Xie, P., L. Huang, C. Zhang, and Y.-l. Zhang, *Nutrient assessment of olive leaf residues processed by solid-state fermentation as an innovative feedstuff additive.* Journal of Applied Microbiology, 2016. **121**(1): p. 28-40.

31. Aderemi, F. and F. Nworgu, *Nutritional status of cassava peels and root sieviate biodegraded with Aspergillus niger.* American-Eurasian Journal of Agricultural and Environmental Science, 2007. **2**(3): p. 308-311.

32. Bregendahl, K., J. Sell, and D. Zimmerman, *Effect of low-protein diets on growth performance and body composition of broiler chicks.* Poultry Science, 2002. **81**(8): p. 1156-1167.

33. Vandenberghe, L.P., C.R. Soccol, A. Pandey, and J.-M. Lebeault, *Solid-state fermentation for the synthesis of citric acid by Aspergillus niger.* Bioresource Technology, 2000. **74**(2): p. 175-178.

34. Iyayi, E.A. and D.M. Losel, *Protein enrichment of cassava by-products through solid state fermentation by fungi.* The Journal of Food Technology in Africa, 2001. **6**(4): p. 116-118.

35. Okpako, C., V. Ntui, A. Osuagwu, and F. Obasi, *Proximate composition and cyanide content of cassava peels fermented with Aspergillus niger and Lactobacillus*

rhamnosus. Journal of Food Agriculture & Environment, 2008. **6**(2): p. 251-255.

36. Dei, H., S. Rose, A. Mackenzie, and R. Amarowicz, *Growth performance of broiler chickens fed diets containing shea nut (Vitellaria paradoxa, Gaertn.) meal fermented with Aspergillus niger.* Poultry Science, 2008. **87**(9): p. 1773-1778.
37. Wu, Q., Z. Wang, G. Wang, Y. Li, and Y. Qi, *Effects of feed supplemented with fermented pine needles (Pinus ponderosa) on growth performance and antioxidant status in broilers.* Poultry Science, 2015. **94**(6): p. 1138-1144.
38. Güngör, E., A. Altop, E. Öztürk, and G. Erener, *Nutritional changes of sour cherry (Prunus cerasus) kernel subjected to Aspergillus niger solid-state fermentation.* Journal of Tekirdag Agricultural Faculty, 2017: p. 99-103.
39. Graminha, E., A. Gonçalves, R. Pirota, M. Balsalobre, R. Da Silva, and E. Gomes, *Enzyme production by solid-state fermentation: Application to animal nutrition.* Animal Feed Science and Technology, 2008. **144**(1): p. 1-22.
40. Aguilar, C.N., A. Aguilera-Carbo, A. Robledo, J. Ventura, R. Belmares, D. Martinez, R. Rodríguez-Herrera, and J. Contreras, *Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (Punica granatum) peel and creosote bush (Larrea tridentata) leaves.* Food Technology and Biotechnology, 2008. **46**(2): p. 218-222.
41. Papagianni, M., *Advances in citric acid fermentation by Aspergillus niger: biochemical aspects, membrane transport and modeling.* Biotechnology Advances, 2007. **25**(3): p. 244-263.