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# *Aspergillus niger* IMPROVES THE NUTRITIONAL COMPOSITION OF APPLE POMACE BY SOLID-STATE FERMENTATION

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**Abstract:** The effect of solid-state fermentation using *Aspergillus niger* on the nutritional composition of apple pomace was investigated in this study. Apple pomace was milled to 2 mm and sterilized at 121 °C for 15 min before fermentation. *Aspergillus niger* strain (ATCC 200345) was cultured and inoculated to apple pomace $10^4$ spores/ml. Raw and fermented apple pomace were analyzed for determination of the crude protein (CP), ash, ether extract (EE), crude fiber (CF), neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and acid-detergent lignin (ADL) content. The CP ($P<0.001$) and ash ($P<0.05$) contents of apple pomace were increased after solid-state fermentation. However, *A. niger* decreased ($P<0.001$) the CF, NDF, ADF, hemicellulose, NFE and EE ($P<0.05$) contents of apple pomace. The ADL content of apple pomace was not affected by solid-state fermentation. The results demonstrated that solid-state fermentation using *A. niger* can improve the nutritional composition of apple pomace.

**Keywords:** Agricultural by-product, Nutritional enrichment, *Malus domestica*, *Aspergillus niger*

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## 1. Introduction

The increasing world population increases the demand for protein foods. Animal production is important in meeting the need for protein. Feed costs in animal production constitute 60-70% of operating costs (Güngör et al., 2017). Therefore, alternative feed sources should be made available for animal nutrition.

Apple pomace is an agricultural waste product from the production process of feed mills. Apple pomace is rich in minerals and carbohydrates, but it has low crude protein (CP) and high crude fiber (CF) content (Beigh et al., 2015). These characteristics limit the use of apple pomace in animal nutrition. Apple pulp can be used in 5% of diets in broiler chicks without adverse effects on growth performance, but higher levels impair the feed conversion ratio (Ayhan et al., 2009). Similarly, Beigh et al. (2015) noted that apple pomace can be used in animal nutrition at limited levels because of its low CP content.

Solid-state fermentation means microbial growth in the insoluble solid substrates immersed in free water (Yasar and Tosun, 2020). Solid-state fermentation can improve the nutritional quality of agricultural residues (Altop et al., 2018). *Aspergillus niger* was used in solid-state fermentation of apple pomace to produce organic acids (Shojaosadati and Babaeipour, 2002) and enzymes (Dhillon et al., 2012). However, recent studies showed that *A. niger* can improve the nutritional composition of apple pomace (Yang et al., 2022). Tosun and Yaşar (2021) demonstrated that *A. niger* increased ash and EE content and decreased the crude fiber content of apple pomace by solid-state fermentation for 3 days. However, there is a lack of study on the effect of solid-state fermentation using *A. niger* for a longer period on the nutritional composition of apple pomace. This study aimed to investigate the effect of *A. niger* solid-state fermentation for 7 days on the main nutritional composition of apple pomace.

## 2. Materials and Methods

Apple pomace was supplied from a local juice factory in Samsun, Turkiye. *Aspergillus niger* (ATCC 200345) was obtained from the American Type Culture Collection (ATCC). The apple pomace was dried at 75 °C for 48 hours and ground to a size of 2mm. Dried apple pomace was enriched by the nutritional salt (glucose: urea: $(NH_4)_2SO_4$: peptone: $KH_2PO_4$: $MgSO_4$. $7H_2O$= 4: 2: 6: 1: 4: 1) and sterilized by autoclave at 121 °C for 15 min. *Aspergillus niger* was cultured in Potato-Dextrose Agar and spore suspension was prepared at $10^4$ spores/ml. Sterilized apple pomace samples were inoculated by *A. niger* spores and incubated at 30 °C for 7 days. After the incubation period, apple pomace was dried at room temperature 30-35 °C for 6 days to reach 90% dry matter (DM). Unfermented and fermented apple pomace were analyzed to determine the DM, ash, CP, EE and CF contents according to AOAC (2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid

detergent lignin (ADL) contents were determined according to the methods of Van Soest et al. (1991).

### 2.1. Statistical Analysis

The experiment was conducted in triplicate, and the results were presented as means and pooled standard error of mean (SEM). Obtained data were analyzed with the Student t test (SPSS 21.0 Statistics). Results were considered significantly different at P≤0.05.

## 3. Results

Solid-state fermentation increased the CP and ash contents of apple pomace (P<0.001 and P=0.044, respectively). However, fermented apple pomace had a lower CF, NDF, ADF and hemicellulose contents than those of unfermented apple pomace (P=0.004, P<0.001 and P<0.001, respectively). Similarly, *A. niger* decreased the EE and NFE content (P=0.019 and P<0.001, respectively). However, the ADL content was not changed (P>0.05) by solid-state fermentation (Table 1).

## 4. Discussion

Feed costs in animal production constitute an important part of operating costs (Güngör et al., 2017). Finding alternative feedstuffs to the current feedstuffs will reduce feed costs. Solid-state fermentation can be used to improve the nutritional composition of agricultural wastes obtained at affordable prices (Tosun and Yaşar, 2021). The nutritional composition of apple pomace was improved by solid-state fermentation using *A. niger* for 7 days in the present study. Similarly, Tosun and Yaşar (2021) showed that *A. niger* improved the nutritional quality of apple pomace by increasing ash and EE content and decreasing CF, ADF and NDF content with solid-state fermentation for 3 days. Similar results were taken from the studies on solid-state fermentation of apple pomace using *A. niger* (Zhong-Tao et al., 2009; Yang et al., 2022).

The prices of protein-rich-feed stuffs, which are essential for meeting the protein needs of animals, are higher than other feed raw materials. Therefore, the use of economical feed raw materials in order to meet the protein deficit in the diet of animals makes a significant contribution to reducing feed costs. Apple pomace is used at a limited level in animal feeds due to its low CP content (Beigh et al., 2015). Solid-state fermentation using *A. niger* increased the CP content of apple pomace in this study. Similarly, Yang et al. (2022) noted that *A. niger* elevated the CP, pure protein and amino acid contents of apple pomace in solid-state fermentation. Zhong-Tao et al. (2009) also reported that the crude protein content of apple pomace was increased by solid-state fermentation using *A. niger*. Various microbial enzymes such as proteinase, cellulase and pectinase can be produced by *A. niger* during solid-state fermentation (Zhong-Tao et al., 2009). Similarly, Yang et al. (2022) showed that *A. niger* enriched produced cellulase in apple pomace solid-state fermentation. The increase in CP content of apple pomace can be related to the enzymes produced by *A. niger* during fermentation. Indeed, the results of the study conducted by Yang et al. (2022) demonstrated that the increase in the CP level of apple pomace is related to the cellulase production capacity.

Crude fiber content of a feedstuff is an important factor affecting its digestibility. In general, feeds with low CF content have high digestibility, while feeds with high CF content have low digestibility. The reduction of CF, ADF, NDF and hemicellulose levels in apple pomace by solid-state fermentation in the present study is in accordance with the results of the various studies (Tosun and Yaşar, 2021). Tosun and Yaşar (2021) reported that *A. niger* decreased the CF content of apple pomace in solid-state fermentation. Similarly, solid-state fermentation using *A. niger* reduced the CF level in pomegranate pomace (Gungor et al., 2021a). Yang et al. (2022) reported that *A. niger* can produce cellulase in apple pomace during solid-state fermentation. Decreases in the CF, ADF, NDF and hemicellulose levels can be because of the cellulase activity in apple pomace through fermentation.

Microorganisms can accumulate some minerals in the fermentation medium during solid-state fermentation and increase the ash content of substrate (Xiao et al., 2021).

**Table 1.** Nutritional composition of unfermented and fermented apple pomace by *Aspergillus niger* in solid-state fermentation

| Nutrients (%, dry matter basis) | UAP | FAP | SEM | P |
|---|---|---|---|---|
| Crude Protein | 5.39 | 17.55 | 2.721 | <0.001 |
| Ether Extract | 2.78 | 2.39 | 0.100 | 0.019 |
| Ash | 3.34 | 7.62 | 1.163 | 0.044 |
| NFE | 62.31 | 48.33 | 3.142 | <0.001 |
| Crude Fiber | 26.14 | 22.77 | 0.792 | 0.004 |
| Hemicellulose | 12.78 | 7.00 | 1.295 | <0.001 |
| NDF | 51.78 | 43.99 | 1.746 | <0.001 |
| ADF | 39.00 | 36.99 | 0.454 | <0.001 |
| ADL | 21.52 | 19.94 | 0.685 | 0.294 |

UAP= unfermented apple pomace, FAP= fermented apple pomace, SEM= standard error of mean, NFE= nitrogen-free extract, NDF= neutral-detergent fiber, ADF= acid-detergent fiber, ADL= acid-detergent lignin.

Tosun and Yaşar (2021) reported that solid-state fermentation increased the ash content of apple pomace starting on the first day. Similarly, the ash level of apple pomace was increased by solid-state fermentation using *A. niger* for 7 days in this study. Increase in the ash content of apple pomace after fermentation can be due to the accumulation of minerals by *A. niger* during fermentation. Gungor et al. (2021b) also reported that *A. niger* increased the ash content of grape pomace in solid-state fermentation.

*Aspergillus* spp. can enrich the substrates with microbial lipids by producing them during solid-state fermentation and increasing the EE content of the fermentation medium (Hui et al., 2010). Solid-state fermentation using *A. niger* increased the EE content of apple pomace (Tosun and Yaşar, 2021). However, *A. niger* reduced the EE content of apple pomace by fermentation in this study. Similarly, Gungor et al. (2021b) showed that solid-state fermentation using *A. niger* decreased the EE content of grape pomace.

Microorganisms try to meet the carbon needed to sustain their growth mainly from the easily degradable carbohydrates in the fermentation medium (Papagianni, 2007). The sharp decrease in the NFE level of apple pulp may be due to the fact that *Aspergillus niger* may consume the easily digestible carbohydrates in apple pomace and use them for mineral accumulation and production of microbial proteins such as enzymes and mycelium. Similarly, Gungor et al. (2021b) reported a reduced NFE content in *A. niger*-fermented grape pomace compared to unfermented grape pomace. However, *A. niger* increased the total reduced sugar content of apple pomace after solid-state fermentation (Tosun and Yaşar, 2021). The different results may be due to the differences in *A. niger* strains and fermentation conditions.

## 5. Conclusion

The results of the present study demonstrated that solid-state fermentation using *A. niger* improved the nutritional quality of apple pomace by increasing CP and ash contents with reducing CF, ADF and NDF levels. The optimum fermentation conditions need to be determined to obtain higher quality fermented apple pomace in further studies. In addition, the effect of fermented apple pomace on ruminant and non-ruminant animals can be investigated in future studies.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | A.A. | E.G. | G.E. |
|---|---|---|---|
| C | 40 | 30 | 30 |
| D | 40 | 40 | 20 |
| S | 50 | | 50 |
| DCP | 30 | 60 | 10 |
| DAI | 30 | 70 | |
| L | 50 | 50 | |
| W | 40 | 50 | 10 |
| CR | 40 | | 60 |
| SR | 20 | 60 | 20 |
| PM | 100 | | |
| FA | 100 | | |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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