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Solid-state Fermentation of Wheat Bran by *Aspergillus niger* Strains: Effect on the Nutritional Composition and *In vitro* Digestibility

Buğday Kepeğinin *Aspergillus niger* Suşları ile Katı Faz Fermantasyonu: Besin Madde Kompozisyonu ve *In vitro* Sindirilebilirliği Üzerine Etkisi

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SOLID-STATE FERMENTATION OF WHEAT BRAN BY *ASPERGILLUS NIGER* STRAINS: EFFECT ON THE NUTRITIONAL COMPOSITION AND *IN VITRO* DIGESTIBILITY

ABSTRACT

This study aimed to investigate the effect of *Aspergillus niger* on the nutritional composition and *in vitro* dry matter digestibility of wheat bran (WB) in solid-state fermentation. Two *A. niger* strains (ATCC 200345 or ATCC 52172) were used as inoculants in solid-state fermentation of WB. Wheat bran was sterilized at 121°C for 15 min and inoculated with *A. niger* strains at 10^5 spores kg^{-1} WB. Samples were incubated at 60°C for two days and dried at room temperature until reaching 90% dry matter. Crude protein (CP), ether extract (EE), ash, crude fiber (CF), hemicellulose, neutral detergent fiber (NDF) and acid detergent fiber (ADF) content and *in vitro* dry matter digestibility of WB and fermented WB were determined. The CP and ash levels were increased (P<0.001) by solid-state fermentation using *A. niger* strains. However, both *A. niger* strains decreased (P<0.001) the CF, NDF, ADF, and hemicellulose content of WB. The EE content of WB was reduced (P=0.027) by ATCC 200345 but was not affected (P=0.145) by ATCC 52172. The *in vitro* dry matter digestibility of WB was increased (P=0.013) by both *A. niger* strains. The greater increases in CP and ash content and the greater decrease in hemicellulose were obtained from ATCC 52172 (P<0.001). However, greater decreases in CF and ADF content were observed in fermented WB with ATCC 200345 compared to fermented WB with ATCC 52172 (P=0.037 and P=0.013, respectively). The results of the present study showed that *A. niger* improved the nutritional composition and *in vitro* dry matter digestibility of WB with solid-state fermentation. ATCC 52172 can be recommended for higher CP and ATCC 200345 for lower CF and ADF and in solid-state fermentation of WB.

Keywords: Fermentation, Fungus, Wheat Bran, Nutrient Composition, Digestibility.

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BUĞDAY KEPEĞİNİN *ASPERGİLLUS NİGER* SUŞLARI İLE KATI FAZ FERMANTASYONU: BESİN MADDE KOMPOZİSYONU VE *IN VİTRO* SİNDİRİLEBİLİRLİĞİ ÜZERİNE ETKİSİ

ÖZ

Bu çalışmada, buğday kepeğinin (BK) besin madde kompozisyonu ve *in vitro* kuru madde sindirilebilirliği üzerine *Aspergillus niger* kullanılan katı faz fermantasyonun etkileri araştırılmıştır. BK'nin katı faz fermantasyonunda iki farklı *A.*

niger suşu (ATCC 200345 ve ATCC 52172) inokulant olarak kullanılmıştır. BK, 121°C'de 15 dakika steril edilmiş ve *A. niger* suşları 10^5 spor kg^{-1} BK düzeyinde inokule edilmiştir. Örnekler 60°C'de 2 gün inkübe edilmiş ve %90 kuru maddeye ulaşana kadar oda sıcaklığında kurutulmuştur. BK ve fermente BK'nin, ham protein (HP), ham yağ (HY), ham kül (HK), ham selüloz (HS), hemiselüloz, nötr deterjanda çözünmeyen lif (NDF), asit deterjanda çözünmeyen lif (ADF) düzeyleri ile *in vitro* kuru madde sindirilebilirliği belirlenmiştir. BK'nin HP ve HK düzeyleri, *A. niger* suşlarının kullanıldığı katı faz fermantasyonu ile artmıştır (P<0.001). Buna karşın, her iki *A. niger* suşu da BK'nin HS, NDF, ADF ve hemiselüloz düzeylerini azaltmıştır (P<0.001). BK'nin HY içeriği, ATCC 200345 ile azaltılırken (P=0.027) ATCC 52172'den etkilenmemiştir (P=0.145). BK'nin *in vitro* kuru madde sindirilebilirliği her iki *A. niger* suşu ile artırılmıştır (P=0.013). BK'nin HP ve HK düzeyindeki en büyük artış ile hemiselüloz düzeyindeki en büyük düşüş ATCC 52172'den elde edilmiştir (P<0.001). Bununla birlikte, ATCC 200345 kullanılarak fermente edilen BK'nin HS ve ADF düzeyleri ATCC 52172 ile fermente edilen BK'den daha düşük olmuştur (sırasıyla P=0.037 ve P<0.013). Çalışmanın sonuçları, *A. niger* kullanılan katı faz fermantasyonu ile BK'nin besin madde kompozisyonunun ve *in vitro* kuru madde sindirilebilirliğinin iyileştirilebileceğini göstermiştir. BK'nin katı kültür fermantasyonunda daha yüksek HP içeriği için ATCC 52172, daha düşük HS ve ADF içeriği için ATCC 200345 önerilebilir.

Anahtar Kelimeler: Fermantasyon, Mantar, Buğday Kepeği, Besin Madde Kompoziyonu, Sindirilebilirlik.

1. INTRODUCTION

Feed costs account for 70% of the total costs in poultry operations. Protein sources have a significant share in feed costs because they are more expensive than other feeds and are used in high amounts in poultry diets. Therefore, it is necessary to improve the nutritional composition of low-protein feeds and convert them to high-protein feeds to reduce feed costs (Güngör et al., 2017).

Wheat bran (WB) is a by-product of the milling process and contains 17.1% crude protein (CP), 44.6% crude fiber (CF), and 5.8% ash (Shang et al., 2020). The CP content of WB is lower than that of oilseeds. The high CF in WB results in lower digestibility and limits the use of WB in poultry diets (Gallardo et al., 2018).

Solid-state fermentation can improve the nutritional composition and increase the *in vitro* digestibility of feedstuff (Zhang et al., 2006). *Aspergillus niger* is a probiotic microorganism used in poultry diets (Harimurti and Hadisaputro, 2015). Imelda et al. (2008) reported that *A. niger* increased the CP and ash content of WB in solid-state fermentation. However, there is a lack of information on the effect of *A. niger* on the *in vitro* digestibility of WB. Furthermore, Güngör et al. (2017)

reported that different strains of *A. niger* have different effects on the nutritional composition of feedstuffs in solid-state fermentation. The hypothesis of this study was that different *A. niger* strains in solid-state fermentation have different influence on the nutritional composition of WB. The effect of two *A. niger* strains (ATCC 200345 or ATCC 52172) on the nutritional composition and *in vitro* dry matter digestibility of WB was evaluated in this study.

2. MATERIALS AND METHODS

2.1. Treatments, Microorganisms and Substrate

The study was conducted with three treatment groups with three replicates in each group. The treatments were unfermented WB (control), fermented WB (FWB) using ATCC 200345 (FWB1) and FWB using ATCC 52172 (FWB2).

The *A. niger* strains used in the study (ATCC 200345 and ATCC 52172) were obtained from the American Type Culture Collection (ATCC). Wheat bran was provided by a local feed mill factory.

2.2. Solid-state Fermentation

Wheat bran was milled to 2 mm and autoclaved at 121°C for 15 min for sterilization prior to fermentation. The nutrient salt ($(NH_4)_2SO_4$:glucose:KH_2PO_4:urea:-peptone:$MgSO_4.7H_2O$=6:4:4:1:1) was added to the WB to support fungal growth. *A. niger* was inoculated at 10^5 spores kg^{-1} WB. Samples were then incubated for 48 hours at 60°C and dried for six days at room temperature until having 90% dry matter, according to Güngör et al. (2020).

2.3. Nutritional Composition

WB and FWB were analyzed to determine the dry matter, ether extract (EE), ash, CP, and CF content according to AOAC (2000). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) analyses were performed according to the methods of Van Soest et al. (1991). The hemicellulose content of WB and FWB was obtained by subtracting ADF from NDF.

2.4. In Vitro Digestibility

A pepsin-pancreatin enzymatic assay was performed to determine the *in vitro* dry matter digestibility of WB and FWB according to Yegani et al. (2013). Samples were milled to pass through a 1 mm sieve, and 500 mg were placed in *in vitro* flasks. Then, 25 mL of 0.1 M phosphate buffer and 10 mL of 0.2 M HCl were added to

each flask. The flasks were shaken to mix the samples with the solution. Then 1 mL of porcine pepsin (25 mg mL^{-1}, P-7000, Sigma-Aldrich) was added to the flasks. Chloramphenicol (0.5 mL) was added to each flask to minimize the effects of any bacterial fermentation during the incubation period. The flasks were shaken again and then placed in a shaking incubator (100 rpm, 41°C) for 2 hours.

At the end of the incubation, 5 mL of 0.6 M NaOH and 10 mL of 0.2 M phosphate buffer were added to each flask. After shaking the flasks, 1 mL of porcine pancreatin (100 mg mL^{-1}, P1750; Sigma-Aldrich) containing protease, lipase, and amylase was added to each flask. The flasks were shaken and incubated for 4 hours (100 rpm, 41°C). After removing the flasks from the incubator, 5 mL of 20% sulfosalicylic acid was added to each flask. The flasks were kept at room temperature for 30 min, and then the contents of the flasks were filtered through Whatman No. 54 filter papers (Whatman Inc., Florham Park, NJ). The filter papers before the process and residues collected on the filter papers were dried at 80°C overnight. The *in vitro* dry matter digestibility was calculated using the following formula:

$$\textit{In vitro}\ \text{dry matter digestibility}\ (\%) = 100 - \left(\frac{\textit{in vitro}\ \text{residue weight}\ (g)}{\text{sample weight}\ (g)}\right) \times 100$$

2.5. Statistical Analysis

All experiments were performed with three replicates. Differences between treatments were tested by ANOVA using SPSS software (SPSS 21.0 Statistics). The means of the treatments were separated by the TUKEY test. The level of statistical significance was set at $P \leq 0.05$.

3. RESULTS

The nutritional composition of unfermented and fermented WB using *A. niger* is shown in Table 1. The CP and ash contents of WB were increased ($P<0.001$) by solid-state fermentation. However, solid-state fermentation decreased ($P<0.001$) the CF, NDF, ADF, hemicellulose, and nitrogen-free extract (NFE) contents of WB. The EE content of the FWB1 group was higher ($P=0.027$) than that of the WB group, but it is similar ($P=0.145$) to that of the FWB2 group. The FWB2 had higher ($P<0.001$) CP and ash content than FWB1. In addition, CF and ADF levels were lower in FWB1 compared to FWB2 ($P=0.037$ and $P=0.013$, respectively). However, FWB1 had higher ($P=0.027$ and $P=0.003$, respectively) hemicellulose and NFE content than FWB2. Fermented wheat brans had similar ($P=0.856$) NDF contents.

Table 1. Nutritional composition of WB and FWB using two *A. niger* strains

Nutrients (%, dry matter)	WB	FWB1	FWB2	SEM	P
Crude Protein	16.87^{c}	31.53^{b}	33.51^{a}	2.626	<0.001
Ether Extract	3.15^{a}	2.04^{b}	2.46^{ab}	0.194	0.031
Ash	5.51^{c}	10.57^{b}	11.22^{a}	0.904	<0.001
NFE	63.53^{a}	46.87^{b}	43.01^{c}	3.157	<0.001
Crude Fiber	10.95^{a}	8.97^{c}	9.79^{b}	0.300	<0.001
NDF	43.52^{a}	29.87^{b}	30.01^{b}	2.265	<0.001
ADF	13.46^{a}	10.46^{c}	11.87^{b}	0.449	<0.001
Hemicellulose	30.06^{a}	19.41^{b}	18.14^{c}	1.893	<0.001

WB: wheat bran, FWB1: fermented wheat bran by *Aspergillus niger* ATCC 200345, FWB2: fermented wheat bran by *Aspergillus niger* ATCC 52172

The *in vitro* dry matter digestibility of WB and FWB using *A. niger* is shown in Table 2. Both *A. niger* strains increased (P<0.001) the *in vitro* dry matter digestibility of WB. In addition, the *in vitro* dry matter digestibility of FWB1 tended (P=0.060) to be higher than that of the FWB2.

Table 2. *In vitro* dry matter digestibility of WB and FWB using two *A. niger* strains

Digestibility (%)	WB	FWB1	FWB2	SEM	P
Dry Matter	39.73^{b}	56.30^{a}	48.10^{a}	2.661	0.001

WB: wheat bran, FWB1: fermented wheat bran by *Aspergillus niger* ATCC 200345, FWB2: fermented wheat bran by *Aspergillus niger* ATCC 52172

4. DISCUSSION

Solid-state fermentation is a useful method for improving the nutritional composition of feedstuffs and enhancing nutrient digestibility (Gungor and Erener, 2020). In the present study, the nutritional composition of WB was improved through solid-state fermentation using *A. niger*. Similarly, *A. niger* improved the nutritional quality of WB by increasing CP, ash, and amino acid content through solid-state fermentation (Imelda et al., 2008). *Aspergillus niger* also increased the *in vitro* dry matter digestibility of WB in this study. Similar to the results of the present study, *in vitro* dry matter digestibility of pomegranate seed was improved by solid-state fermentation using *A. niger* (Güngör et al., 2020).

Protein is an important nutrient that animals need to maintain their development. The protein content of feed is an important factor in determining its price. Solid-state fermentation using *A. niger* increased the CP level of WB in this study.

Similarly, Imelda et al. (2008) reported increased CP and amino acid levels in WB through solid-state fermentation. *Aspergillus niger* can produce various digestive enzymes such as protease, cellulase, and hemicellulase (Kang et al., 2004; de Castro et al., 2015). Several studies have shown that WB is a suitable substrate for *A. niger* to produce protease and xylanase enzymes (Couri et al., 2000; de Castro et al., 2015). The increase in the CP content of WB may be due to microbial proteins such as filamentous fungi (*A. niger*) and enzymes produced during solid-state fermentation.

Aspergillus niger can produce cellulolytic enzymes such as cellulase and hemicellulase in WB during solid-state fermentation (Kang et al., 2004). Both *A. niger* strains reduced the CF, hemicellulose, NDF, and ADF content of WB in this study. Similarly, *A. niger* reduced the CF, NDF, and ADF content of hazelnut kernel meal (Altop et al., 2019). The hemicellulose content of cottonseed, sunflower, and hazelnut kernel meals was reduced by solid-state fermentation using *A. niger*. Cellulolytic enzymes can degrade the structural carbohydrates such as cellulose and hemicellulose in WB and lignin and cause a decrease in the CF, hemicellulose, NDF and ADF content of WB.

Aspergillus niger can enrich the substrates with various digestive enzymes such as protease, cellulase, and hemicellulase (Kang et al., 2004; de Castro et al., 2015). Solid-state fermentation can increase the nutrient digestibility of feeds by reducing the level of structural carbohydrates in feeds that are difficult to digest (Güngör et al., 2020). Similarly, chickens received diets supplemented with fermented sour cherry kernel had higher dry matter digestibility than the control chickens due to reduced CF content and enzymes produced during fermentation (Gungor and Erener, 2020). Both *A. niger* strains increased WB's *in vitro* dry matter digestibility in the present study. Similarly, Güngör et al. (2020) showed an increased *in vitro* dry matter digestibility of pomegranate seeds by *A. niger* solid-state fermentation. The increased *in vitro* dry matter digestibility may be due to the reduced CF content and digestive enzymes produced in WB during solid-state fermentation.

Aspergillus spp. can produce microbial lipids during solid-state fermentation and increase the EE content of the substrate after fermentation (Hui et al., 2010). Solid-state fermentation using *A. niger* increased the EE content of WB up to the first four days, but the longer fermentation times caused a decrease in the EE content of WB, similar to the results of the present study (Imelda et al., 2008). Similarly, reduced EE content was reported by *A. niger* after solid-state fermentation in cottonseed meal and sunflower meal (Altop et al., 2019). In addition, *A. niger* ATCC 200345 reduced the EE content of WB; however, *A. niger* ATCC 52172 did not affect the EE content of WB in this study. This result confirms the hypothesis of the present study that different strains have different effects on the nutrient content of the substrate. Similarly, two studied *A. niger* strains did not change the EE content of sour cherry kernels, but one *A. niger* strain decreased the EE content in solid-state fermentation (Güngör et al., 2017).

Some minerals can be accumulated by the microorganism during solid-state fermentation, leading to an increase in the ash content of the substrates (Xiao et al., 2021). Both *A. niger* increased the ash content of WB in this study. Imelda et al. (2008) also showed that the ash content of the WB increased from the first day of the solid-state fermentation with *A. niger*. Similar results were also reported in the studies on cottonseed meal, sunflower meal, and hazelnut kernel meal (Altop et al., 2019).

Microorganisms prefer to use carbohydrates as a carbon source rather than other nutrients. In this study, solid-state fermentation caused a decrease in the NFE content of WB. Similarly, Imelda et al. (2008) found that the total carbohydrate content of WB was reduced by solid-state fermentation using *A. niger*. The decrease in the NFE content of WB can be attributed to the consumption of the carbohydrate content of WB by *A. niger*. Similarly, the NFE content of sunflower meal and hazelnut kernel meal was reduced after solid-state fermentation (Altop et al., 2019).

5. CONCLUSION

In conclusion, solid-state fermentation using *A. niger* can increase the *in vitro* dry matter digestibility and improve the nutritional composition of WB by increasing the CP and ash content and decreasing the structural carbohydrate content. ATCC 52172 can be recommended for the higher CP content and ATCC 200345 for the lower CF and ADF content of WB in solid-state fermentation. Further detailed studies need to be conducted to verify the results of the present study.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethics

This study does not require ethics committee approval.

Author Contribution Rates

Design of Study: AA(%30), EG(%40), GE(%30)

Data Acquisition: AA(%20), EG(%40), ŞÖ(%30), GE(%10)

Data Analysis: AA(%15), EG(%30), ŞÖ(%40), GE(%15)

Writing Up: AA(%20), EG(%40), ŞÖ(%20), GE(%20)

Submission and Revision: AA(%10), EG(%60), ŞÖ(%20), GE(%10)

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