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ASPERGILLUS NIGER AND *BACILLUS SUBTILIS* CHANGE THE NUTRITIONAL COMPOSITION OF SWEET CHERRY KERNEL (*Prunus avium* L.)

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Abstract

The effects of *Aspergillus niger* and *Bacillus subtilis* on the nutritional composition of sweet cherry kernel (CK) were investigated in this study. Cherry kernels were milled to the size of 2 mm and fermented with *A. niger* and *B. subtilis* for 7 days. The crude protein (CP), ether extract (EE), ash, crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was determined before and after fermentation. Hemicellulose (HC) was calculated as NDF minus ADF. Both *A. niger* and *B. subtilis* increased ($p<0.001$) CP and ash content, decreased CF ($p<0.001$), HC ($p<0.001$) and NFE ($p<0.01$) content and did not change EE and ADF content of CK. The NDF content was decreased ($p<0.01$) by *A. niger* but was not changed by *B. subtilis*. *Aspergillus niger* cause more increase ($p<0.001$) of CP and ash and a higher decrease in CF ($p<0.001$) and HC ($p<0.05$) than *B. subtilis*. These results showed that the nutritional composition of CK can be improved by *A. niger* and *B. subtilis* through solid state fermentation. *Aspergillus niger* shows better performance compared with *B. subtilis*.

Keywords: Sweet cherry kernel, *Prunus avium* L., Solid state fermentation, Nutritional enrichment, *Aspergillus niger*, *Bacillus subtilis*

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1. Introduction

Development in animal husbandry with the rapid increase in the world population leads to an increase in feed requirement. This demand on animal feed cannot be met with cereal grains, legumes, etc. This reason forces the researchers to find new alternative feed sources. The utilization of agricultural residues has been taking interest recently due to billions of tons of waste produced every year (Xie et al., 2016).

Sweet cherry (*Prunus avium* L.) is produced at 2.4 million tonnes of annual production worldwide (FAOSTAT, 2017). Cherry is generally consumed as fresh fruit and also as jam, jelly, stewed fruit and marmalade (Vursavuş et al., 2006). Sweet cherry kernel (CK) contains 13.81% crude protein (CP), 26.28% ether extract (EE), 3.34% ash, 21.65% crude fibre (CF), 27.21% neutral detergent fiber (NDF), 16.24% acid detergent fiber (ADF; Altop, 2019). Although CK having the potential of using in animal nutrition, Altop (2019) reported that dietary 1% addition of CK affect feed conversion ratio negatively in broiler chickens, which may be due to high cellulose content or antinutritional components.

Solid state fermentation can improve the nutritional composition of agricultural residues by increasing crude protein and decreasing the structural carbohydrate contents (Dei et al., 2008; Gungor and Erener, 2019a). *Aspergillus niger* and *Bacillus subtilis* are used as probiotics in animal nutrition and accepted as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA). Both are preferred microorganisms in solid state fermentation. *Aspergillus niger* improved the nutritional composition of sweet (Altop, 2019) and sour cherry kernel (Güngör et al., 2017). However, there is not any study comparing the effects of *A. niger* and *B. subtilis* on the nutritional composition of sweet cherry kernel. This study aimed to investigate the effects of *A. niger* and *B. subtilis* on the nutritional composition of sweet cherry kernel.

2. Materials and Methods

2.1. Cherry Kernel and Microorganisms

Cherry kernels were obtained from a juice factory in Tokat, Turkey. *Aspergillus niger* (ATCC 9142) and *B. subtilis* (ATCC 21556) supplied from American Type Culture Collection (ATCC).

2.2. Solid State Fermentation

Cherry kernels were ground to the size of 2 mm. After autoclave sterilization at 121 °C for 15 minutes, kernels were enriched with the 1.6 l nutrient salt (glucose:urea:$(NH_4)2SO_4$:peptone:KH_2PO_4:$MgSO_4.7H_2O_4$:2:6:1:4:1) for each 1 kg sample. *A. niger* and *B. subtilis* were inoculated to CK at 10^5 spores/ml and 10^{10} cfu/ml, respectively. Samples were gently mixed to disperse spores and incubated at 28-30 °C for 7 days in plastic containers. Kernels were dried on a bench at room temperature until they reached approximately 90% dry matter after the fermentation process.

2.3. Chemical Composition

The CP, EE, ash, and CF contents of CK were determined according to AOAC (2000). The NDF and ADF were analyzed as reported by Van Soest et al. (1991). Hemicellulose (HC) was calculated as NDF minus ADF. Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of CP, EE, CF and ash from 100%.

2.4. Statistical Analysis

All data were analyzed by one-way ANOVA. The statistical differences between treatments were determined by a Tukey test. Values are presented as the mean and pooled standard error of means (SEM). The level of significance was preset at p<0.05.

3. Results and Discussion

The nutritional composition of unfermented and fermented CK is given in Table 1. Fermentation increased CP (p<0.001) content of CK. *Aspergillus niger* caused more (p<0.001) CP increase than *B. subtilis*. Similarly, increased crude protein by *A. niger* has been reported in sweet (Altop, 2019) and sour cherry kernel (Güngör et al., 2017). *Aspergillus niger* increased CP of mango kernel (Kayode and Sani, 2008), grape seed (Altop et al., 2018), shea nut (Dei et al., 2008) and palm kernel cake (Iluyemi et al., 2006; Lawal et al., 2010). *Bacillus subtilis* also increased CP content of rapeseed meal (Fazhi et al., 2011), cottonseed meal (Sun et al., 2012), soybean meal (Teng et al., 2012) and mixed feed (Shi et al., 2017). The increase in CP content may be due to the microbial protein produced by *A. niger* and *B. subtilis* (Raimbault, 1998; Shi et al., 2017).

Table 1. Nutritional composition of unfermented and fermented CK

Composition	CK	CK-B	CK-A	SEM	p
Crude protein	13.2^c	28.8^b	30.7^a	2.77	***
Ether extract	27.5	27.4	32.7	1.35	ns
Ash	3.1^c	7.4^b	8.1^a	0.78	***
NFE	35.4^a	20.8^b	16.8^b	3.00	**
Crude fiber	20.8^a	15.6^b	11.8^c	1.32	***
NDF	25.6^a	23.7^a	19.7^b	0.95	**
ADF	13.7	14.4	12.9	0.33	ns
Hemicellulose	11.9^a	9.3^b	6.8^c	0.76	***

^{abc}Means that have no superscript in common are significantly different from each other (p<0.05), **= p<0.01, ***= p<0.001, ns= not significant

NFE= nitrogen-free extract, SEM= standart error of means

CK= cherry kernel; CK-B= fermented cherry kernel by *B. subtilis*; CK-A= fermented cherry kernel by *A. niger*

Fermentation did not alter the EE content of CK in this study. Similar results were reported in the studies on sour cherry kernel (Güngör et al., 2017), palm kernel (Iluyemi et al., 2006; Lawal et al., 2010) and grape seed (Altop et al., 2018). However, EE content was increased in the studies of shea nut (Dei et al., 2008) and decreased in mango kernel (Kayode and Sani, 2008), sour cherry kernel (Güngör et al., 2017; Gungor and Erener, 2019b) and grape seed (Altop et al., 2018).

Ash content was increased (p<0.001) by solid state fermentation. *Aspergillus niger* resulted in a higher (p<0.001) ash content compared with *B. subtilis*.

Similarly, *A. niger* increased ash content of sour cherry kernel (Güngör et al., 2017), shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008) and grape seed (Altop et al., 2018). *Bacillus subtilis* also increased ash content of cottonseed meal (Sun et al., 2012) and mixed feed (Shi et al., 2017). The increase in ash content possibly was relative due to the decrease in other nutrients rather than an actual increase.

Soluble carbohydrates are preferred by the microorganism to other nutrients for carbon sources (Papagianni, 2007). Solid state fermentation decreased ($p<0.01$) the NFE content of CK in this study. Similarly, Güngör et al. (2017) reported a decreased NFE in sour CK by *A. niger*. This result is in line with the studies on shea nut (Dei et al., 2008), mango kernel (Kayode and Sani, 2008) and grape seed (Altop et al., 2018).

Solid state fermentation decreased the CF ($p<0.001$), and HC ($p<0.001$) but did not change ADF content. *A. niger* decreased ($p<0.01$) NDF content although *B. subtilis* did not change NDF content of CK. *Aspergillus niger* (Xie et al., 2016) and *B. subtilis* (Ritter et al., 2018) can produce cellulase in the conditions of solid state fermentation, which can be the reason of the degradation and decrease of the structural carbohydrates in this study. Similarly, *A. niger* decreased CF (Gungor and Erener, 2019b), NDF and ADF (Güngör et al., 2017) content of sour cherry kernel. *A. niger* also decreased CF, NDF and ADF content of grape seed (Altop et al., 2018), shea nut (Dei et al., 2008) and palm kernel cake (Iluyemi et al., 2006; Lawal et al., 2010). Similarly, *B. subtilis* diminished CF in cottonseed meal (Sun et al., 2012) and decreased NDF in the mixed feed (Shi et al., 2017).

4. Conclusion

In conclusion, these results showed that solid state fermentation can be used to improve the nutritional composition of cherry kernel and *A. niger* shows better performance compared with *B. subtilis*.

Conflict of interest

The authors declare that there is no conflict of interest.

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