

## Nutritional Changes of Sour Cherry (*Prunus cerasus*) Kernel Subjected to *Aspergillus niger* Solid-state Fermentation

Emrah GÜNGÖR, Aydın ALTOP, Ergin ÖZTÜRK, Güray ERENER\*

Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkey

\*Corresponding author: gereener@omu.edu.tr

Geliş Tarihi (Received): 01.03.2017

Kabul Tarihi (Accepted): 15.04.2017

This study was carried out to investigate the effects of *Aspergillus niger* solid-state fermentation on main nutritional content of cherry (*Prunus cerasus*) kernel. Three *Aspergillus niger* strains (ATCC 52172, ATCC 200345, ATCC 9142) were used in this study. Cherry kernels were analyzed for crude protein (CP), total ash (TA), total fat (TF), crude fiber (CF), nitrogen free extract (NFE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) before and after fermentation to see nutritional change. CP level of the sour cherry increased by 14.1% and reached up to 41.66% from 27.56%. Fungal fermentation changed also TA, TF, CF, NFE, NDF, ADF contents of cherry kernel. These results suggest that solid-state fermentation with *Aspergillus niger* can be used for utilization nutritional properties of cherry kernels to make having potential in animal nutrition.

**Keywords:** Solid-state fermentation, cherry kernel, *Prunus cerasus*, *Aspergillus niger*, animal nutrition

### Introduction

The demand for protein foods will increase because world population is growing rapidly. It is expected that world animal product needs will increase by 58% in 2050 (Makkar et al. 2014). As it is necessary to produce more animal product (meat, egg, milk etc.) for feeding the growing population, the demand for feedstuffs used in animal nutrition will also increase. Thus, the use of feedstuffs competing with human nutrition in animal feeding rather than feeding the world population expected to rise to 9 billion in 2050 will become controversial (Cohen, 2003). For this reason, it is considered that the feed resources with low competition with human consumption should be made available in animal nutrition.

Feed costs constitute 60-70% of total costs in animal production. In feed costs, protein sources have a large share because of being very expensive and using at high levels in the ration. It is necessary that the new protein sources should be found for animal nutrition in order to reduce the pressure on the protein sources used animal nutrition made by increasing animal product demand in parallel with rising in human population.

Cherry (*Prunus cerasus* L.) is a seed fruit of Rosaceae family. The world's total production of cherry, which is being cultivated in many parts of the world, especially in Russia, Ukraine and Turkey, have reached 1.3 million tons per year (FAO, 2014). According to the National Agricultural Statistics Service, 99% of the sour cherries are consumed as processed products such as fruit juice, jam or

canned cherries (USDA, 2012). After the sour cherry has been harvested, the plant is brought to the factory and the seeds are separated by passing through the seed separating machines. 60.4-71.4% by weight of the sour cherry constitutes the fleshy portion, 13.5-18.1% the peel and 5.5-7.9% the seed (Chaovanalikit and Wrolstad, 2003). 23.5% of the sour cherry seed, which is separated as a by-product during the conversion of sour cherry to processed products, is composed of the kernel. Cherry kernel contains 30.4% crude protein, 17.6% total fat, 3.2% total ash and 9.5% crude fiber (Popa et al. 2011; Yılmaz and Gökmen, 2013). Although the sour cherry kernel is rich in lysine (5.28%), it is poor in terms of essential amino acids such as methionine, threonine and tryptophan (Yılmaz and Gökmen, 2013). It has been reported that sour cherry oil contains 46.8% oleic and 40.58% linoleic acid and thereby is also rich in unsaturated fatty acids (Yılmaz and Gökmen, 2013).

Fermentation method can be used to increase protein, amino acid, fat, mineral and vitamin contents of feed raw materials, waste and by-products (Cao, 2012; Zhang et al. 2013; Xie et al. 2016). Fermentation in which microorganisms such as bacteria, fungi or yeast are used is generally divided into liquid-state and solid-state fermentation. Solid-state fermentation is the preferred method for liquid culture fermentation because of the being economical, using abundant and cheap substrates in and having relatively less risk of contamination, which indicate the development of microorganisms on moistened

solid substrates without free water (Perez-Guerra et al. 2003; Osma et al. 2007).

The filamentous fungi, such as *A. niger*, are suitable for solid-state fermentations because they can rapidly grow in the low-water environment (Raimbault, 1998). *A. niger* is used as a probiotic in animal nutrition and is accepted as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA) (Harimurti and Hadisaputro, 2015). *A. niger* can increase the content of protein, amino acid and mineral of feedstuffs and can lose its anti-nutritional components (Iyayi and Losel, 2001; Dei et al. 2008; Okpako et al. 2008; Cao, 2012; Zhang et al. 2013). *A. niger* can also increase the digestibility of feed by producing enzymes protease, amylase, lipase, cellulase and xylanase (Milala, 2005; Betini et al. 2009; Oyeleke and Oyewole, 2011; Oliveira et al. 2016). These features of *A. niger* make it possible to enrich the cherry kernel for protein, amino acids and minerals, and to improve the digestibility of possible anti-nutritional factors. With these properties of *A. niger* it is thought that the sour cherry kernel can be enriched with respect to protein, amino acid and mineral, and also its digestibility can be increased by eliminating the possible anti-nutritional factors. In this study, the effect of solid-state fermentation with *A. niger* on crude protein (CP), total ash (TA), total fat (TF), crude fiber (CF), nitrogen free extract (NFE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of sour cherry kernel was investigated.

## Materials and Methods

### Cherry kernel supply and storage

Cherry kernels were supplied from a fruit juice factory in Turkey. The cherry kernels were stored at -20 °C until fermentation.

### Microorganisms

The microorganisms used in the study were obtained from the American Type Culture Collection (ATCC). The microorganisms were ATCC<sup>®</sup> 9142<sup>™</sup>, ATCC<sup>®</sup> 200345<sup>™</sup>, ATCC<sup>®</sup> 52172<sup>™</sup>.

### Culture media and culture conditions

*A. niger* strains obtained from ATCC were left in incubation in Potato-Dextrose-Agar (PDA, Oxoid Ltd., Basingstoke, UK) on 28 °C for 7 days according to agar plate technique. After incubation, *Aspergillus* spores were harvested by turning the

plate upside down and gently hitting the top. Spore counting was conducted according to Fuch-Rosenthal technique using a hemacytometer. After the formed spores were counted when they were inoculated into cherry kernels the same day.

### Solid-state fermentation preparation

Before the fermentation, cherry kernels were milled to a size of 2 mm (RETCHEM ZM200) and sterilized by autoclaving on 121 °C for 15 minutes. After that, the cherry kernels were divided in two groups, namely, before fermentation and after fermentation. Environment used for fermentation of cherry kernels was 1 kg cherry kernel and 1.6 l nutritional salt (glucose: urea:(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>:peptone:KH<sub>2</sub>PO<sub>4</sub>:MgSO<sub>4</sub>.7H<sub>2</sub>O 4:2:6:1:4:1). The pH of fermentation environment was calibrated into 5 using 1N NaOH and HCl. Starting humidity was 60% and, after adding nutritional salt, for each kg of the solid environment 1.4x10<sup>4</sup> *A. niger* spores were inoculated inside a sterile cabin and left in incubation on 28-30 °C. After incubation, fermented cherry kernels were placed in plastic containers, gently pressed and left for 48 hours on its heat. Since *A. niger* is a microaerobic organism, there will be enough microaerobic conditions for its growth and development even if its left in a closed environment (David et al. 2003). At the end of this period, cherry kernels were spread over a polyethylene paper in a room with 30-40 °C temperature for 6 days until reaching approximately 90% of dry matter upon which they were splintered into 0.15 mm pieces. After the fermentation period, *A. niger* strains were exposed to 60 °C temperature for 48 hours in order to be rendered inactive.

This study was designed from 3 treatment consisting of different *A. niger* strains. ATCC 52172 used group was called C1, ATCC 200345 used group was called C2, ATCC 9142 used group was called C3 and unfermented cherry kernel (control) was called CK.

### Determination of chemical composition

Ash (method, 942.05), CP (method, 976.06), ether extract (EE, method, 920.29), crude fiber (CF, method, 973.18) analyses of cherry kernels before and after solid-state fermentation were conducted (AOAC, 2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were conducted according to Van Soest (1991) using the ANKOM<sup>200/220</sup> fiber analyzer (ANKOM corporation<sup>®</sup>)

Technology Fairport, NY). Measurement for each sample was conducted 3 times and the average was taken.

### Statistical analysis

All of the experiments were carried out in triplicate, and the data were expressed as an average value and pooled SEM. Comparisons between different groups were used by one-way ANOVA and Duncan (SPSS 21.0 Statics). The level of statistical significance was preset at  $P < 0.05$ .

### Results and Discussion

Solid-state fermentation of cherry kernel with *A. niger* resulted in significant changes in its nutrient content (Table 1). CP level of the sour cherry increased by 14.1% and reached up to 41.66%. The CP level was highest in the C3 group, followed by the C2 and C1 groups. This result is in line with the results of studies on solid-state fermentation of cassava (Iyayi and Losel, 2001; Aderemi and Nworgu, 2007; Aro, 2008; Okpako et al. 2008), ginkgo leaves (Cao et al. 2012), shea nut (Dei et al.

2008), olive leaves (Xie et al. 2016), pomegranate peel and creosote bush leaves (Aguilar et al. 2008) with *A. niger*. This protein increase may be due to micelles and/or enzymes produced by *A. niger* (Onilude, 1994; Raimbault, 1998).

There was no difference in TF contents of treatments compared to the control, except a decrease in C3 group. This result is consistent with studies in which the fat content of cassava (Aro, 2008), pomegranate peel and creosote bush leaves (Aguilar et al. 2008) was not affected by fermentation while inconsistent with the studies that increased fat content of shea nut (Dei et al. 2008) and cassava (Okpako et al. 2008). Differences in the results obtained may be due to differences in the substrate used for fermentation. Besides, when the study results are interpreted, it can be seen that different *A. niger* strains can have different effects on crude oil content even in the same substrate. While the crude oil level of the sour cherry kernel was unchanged in C1 and C2, decreased in C3.

Table 1. Changes of main nutritional ingredients in sour cherry kernel with *Aspergillus niger* solid-state fermentation (% , on dry matter basis)

Component	C1	C2	C3	CK	SEM	P-value
Crude Protein	39.70 <sup>c</sup>	41.24 <sup>b</sup>	41.66 <sup>a</sup>	27.56 <sup>d</sup>	2.19	<0.001
Total Fat	26.00 <sup>a</sup>	26.93 <sup>a</sup>	22.39 <sup>b</sup>	25.78 <sup>a</sup>	0.67	0.005
Total Ash	6.90 <sup>b</sup>	7.89 <sup>a</sup>	7.79 <sup>a</sup>	2.60 <sup>c</sup>	0.71	<0.001
NFE	20.10 <sup>b</sup>	18.05 <sup>c</sup>	19.35 <sup>b</sup>	38.30 <sup>a</sup>	3.15	<0.001
Crude Fiber	7.32 <sup>ab</sup>	5.82 <sup>b</sup>	8.78 <sup>a</sup>	5.84 <sup>b</sup>	0.49	0.032
NDF	22.03 <sup>a</sup>	17.14 <sup>c</sup>	19.82 <sup>b</sup>	17.74 <sup>c</sup>	0.74	0.002
ADF	13.01 <sup>a</sup>	10.97 <sup>c</sup>	11.79 <sup>b</sup>	9.67 <sup>d</sup>	0.46	<0.001

<sup>abcd</sup>Means within a row lacking a common superscript differ ( $p < 0.05$ ); C1: cherry kernel fermented with ATCC 52172, C2: cherry kernel fermented with ATCC 200345, C3: cherry kernel fermented with ATCC 9142, CK: non-fermented cherry kernel, SEM: standard error of means

TA level was higher in all groups than control group. While there is an equal and highest TA increase in groups C2 and C3, the increase in C1 group is less than that of the others. This result is consistent with studies on cassava (Okpako et al. 2008), shea nut (Dei et al. 2008), pomegranate peel and creosote bush leaves (Aguilar et al. 2008), but inconsistent with a study on cassava (Aro, 2008) in which the amount of TA was not changed. Increase in TA level by fermentation may indicate that mineral content has been increased. It can be assumed that the enzymes produced by *A. niger* cause the sour cherry to be hydrolyzed and the mineral substances within it to become liberated

and therefore the increase in TA level is caused by this. As a matter of fact, it has been reported that the phytase secreted by *A. niger* increases the phosphorus level of the substrate by releasing the phosphorus bound in phytic acid form (Dei et al. 2008).

NFE level decreased in all groups compared to control and the highest decrease was observed in the C2 group. The decrease in the NFE may be due to the degrading of sugars by the enzymes secreted by *A. niger* for use as a carbon source (Oboh, 2006). This result is in line with the fermentation studies

on cassava (Aro, 2008, Okpako et al. 2008) and pomegranate peel (Aguilar et al. 2008).

CF was increased in group C3 but remained the same with the control group in C1, C2. CF was decreased with *A. niger* fermentation in a study on cassava (Okpako et al. 2008), while CF was increased in studies on cassava (Aderemi and Nworgu, 2007) and pomegranate peel and creosote bush leaves (Aguilar et al. 2008) in parallel with this work.

NDF and ADF were increased in all groups compared to control, except NDF in C2 group. NDF in C2 group remained the same as the control may because of having the same CF level with the control group. NDF and ADF decreased in fermentation study on cassava (Aderemi and Nworgu, 2007; Dei et al. 2008).

### Conclusions

The protein, ash and cellulose contents of Cherry kernel can be enhanced by *Aspergillus niger* solid-state fermentation. Thus, it is thought that cherry kernel, a waste product, can be fermented for increasing its protein and mineral content in making an available feedstuff for animal feeding. Besides, there has been a need to make advanced animal experiments of this fermented product in order to obtain more detailed information for suggesting animal feeding operations.

### References

- Aguilar, C.N., A. Aguilera-Carbo, A. Robledo, J. Ventura, R. Belmares, D. Martinez, R.R. Herrera and J. Contreras, 2008. Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. Food Technology and Biotechnology, 46(2): 218-222.
- AOAC, 2000. Official Methods of Analysis of AOAC International (17. Edition). ABD: AOAC International.
- Aro, S. 2008. Improvement in the nutritive quality of cassava and its by-products through microbial fermentation. African Journal of Biotechnology, 7(25): 4789-4797.
- Betini, J., M. Michelin, S. Peixoto-Nogueira, J. Jorge, H. Terenzi and M. Polizeli, 2009. Xylanases from *Aspergillus niger*, *Aspergillus niveus* and *Aspergillus ochraceus* produced under solid-state fermentation and their application in cellulose pulp bleaching. Bioprocess and Biosystems Engineering, 32(6): 819-824.
- Cao, F., X. Zhang, W. Yu, L. Zhao and T. Wang, 2012. Effect of feeding fermented *Ginkgo biloba* leaves on growth performance, meat quality, and lipid metabolism in broilers. Poultry Science, 91(5): 1210-1221.
- Chaovanalikit, A. and R. Wrolstad, 2004. Total anthocyanins and total phenolics of fresh and processed cherries and their antioxidant properties. Journal of Food Science, 69(1).
- Cohen, J.E. 2003. Human population: The next half century. Science, 302(5648): 1172-1175.
- David, H., M. Akesson and J. Nielsen, 2003. Reconstruction of the central carbon metabolism of *Aspergillus niger*. European Journal of Biochemistry, 270: 4243-4253.
- Dei, H., S. Rose, A. Mackenzie and R. Amarowicz, 2008. Growth performance of broiler chickens fed diets containing shea nut (*Vitellaria paradoxa*, Gaertn.) meal fermented with *Aspergillus niger*. Poultry Science, 87(9): 1773-1778.
- FAO, 2014. Food and Agricultural Commodities Production. <http://www.fao.org/faostat/>
- Harimurti, S. and W. Hadisaputro, 2015. Probiotics in Poultry. Beneficial Microorganisms in Agriculture, Aquaculture and Other Areas: Liong M. İsviçre: Springer.
- Iyayi, E.A. and D.M. Losel, 2001. Protein enrichment of cassava by-products through solid state fermentation by fungi. Journal of Food Technology in Africa, 6(4): 116-118.
- Makkar, H.P., G. Tran, V. Heuzé and P. Ankers, 2014. State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology, 197: 1-33.
- Milala, M., A. Shugaba, A. Gidado, A. Ene and J. Wafar, 2005. Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*. Research Journal of Biological Sciences, 1(4): 325-328.
- Oboh, G. 2006. Nutrient enrichment of cassava peels using a mixed culture of *Saccharomyces cerevisiae* and *Lactobacillus spp* solid media fermentation techniques. Electronic Journal of Biotechnology, 9(1).

- Okpako, C., V. Ntui, A. Osuagwu and F. Obasi, 2008. Proximate composition and cyanide content of cassava peels fermented with *Aspergillus niger* and *Lactobacillus rhamnosus*. *Journal of Food Agriculture and Environment*, 6(2): 251.
- Oliveira, F., C. Moreira, J.M. Salgado, L. Abrunhosa, A. Venâncio and I. Belo, 2016. Olive pomace valorization by *Aspergillus* species: Lipase production using solid-state fermentation. *Journal of the Science of Food and Agriculture*, 96(10): 3583-3589.
- Onilude, A.A. 1994. Production Characteristic and utilization of some dietary fiber degrading enzymes as additives in Broilerdiets. Ph.D. Thesis, Department of Botany and Microbiology University of Ibadan, Nigeria.
- Osma, J.F., J.L.T. Herrera and S.R. Couto, 2007. Banana skin: A novel waste for laccase production by *Trametes pubescens* under solid-state conditions. Application to synthetic dye decolouration. *Dyes and Pigments*, 75(1): 32-37.
- Oyeleke, S. and O. Oyewole, 2011. Production of protease and amylase from *Bacillus subtilis* and *Aspergillus niger* using *Parkia biglobosa* (africa locust beans) as substrate in solid state fermentation. *Advances in Life Sciences*, 1(2): 49-53.
- Pérez-Guerra, N., A. Torrado-Agrasar, C. López-Macias and L. Pastrana, 2003. Main characteristics and applications of solid substrate fermentation. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 2(3).
- Popa, V., C. Misca., D. Bordean, D.N. Raba, D. Stef and D. Dumbrava, 2011. Characterization of sour cherries (*Prunus cerasus*) kernel oil cultivars from banat. *Journal of Agroalimentary Processes and Technologies*, 17: 398-401.
- Raimbault, M. 1998. General and microbiological aspects of solid substrate fermentation. *Electronic Journal of Biotechnology*, 1(3): 26-27.
- USDA, 2012. United States Department of Agriculture, National Agricultural Statics Service, Noncitrus Fruits and Nuts.
- Xie, P., L. Huang, C. Zhang and Y.L. Zhang, 2016. Nutrient assessment of olive leaf residues processed by solid-state fermentation as an innovative feedstuff additive. *Journal of Applied Microbiology*, 121(1): 28-40.
- Yılmaz, C. and V. Gökmen, 2013. Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions. *Industrial Crops and Products*, 49: 130-135.
- Zhang, X., L. Zhao, F. Cao, H. Ahmad, G. Wang and T. Wang, 2013. "Effects of feeding fermented *Ginkgo biloba* leaves on small intestinal morphology, absorption, and immunomodulation of early lipopolysaccharide-challenged chicks", *Poultry Science*, 92(1): 119-130.