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Solid-state Fermentation with *Aspergillus niger* Strains Improves the Nutritional Composition of Grape Seed, Cherry Kernel, and Maize Seed Mixture

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ABSTRACT

The nutritional composition of fermented grape seed, cherry kernel, and maize seed mixture (GCM) with *Aspergillus niger* strains was investigated in this study. The fermentation substrate was prepared by mixing 20 g of maize seed, 40 g of grape seed, and 40 g of cherry kernel. A mixture of the different *A. niger* strains (ATCC 200344, 200345, 201572, 52172) was used in this study. The nutrient salt was added to the substrate and mixed. The mixture was sterilized at 121 °C for 15 min using an autoclave. Each *A. niger* suspension containing 106 spores per ml was prepared and inoculated into the sterilized substrate. The substrates were incubated at 30 °C for seven days. Crude protein, crude ash, ether extract, and crude fiber were analyzed in the unfermented and fermented substrates. Nitrogen-free extract was estimated by subtracting the percentages of crude protein, ether extract, crude ash, and crude fiber from 100%. The crude protein content of GCM was increased ($P<0.001$) after solid-state fermentation with *A. niger*. Solid-state fermentation also increased ($P<0.001$) the ether extract content of GCM. In addition, fermented GCM had higher ($P=0.001$) crude ash content than unfermented GCM. However, crude fiber content of GCM was reduced ($P<0.001$) by solid-state fermentation. Similarly, *A. niger* decreased ($P<0.001$) the nitrogen-free extract in GCM. The results showed that solid-state fermentation with *A. niger* improved the nutritional quality of GCM.

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Introduction

Grape (*Vitis vinifera* L.) is one of the most produced fruit crops in the world, with an annual production of 72 million tonnes (FAOSTAT, 2023). Grape seed is a by-product emerging from the juice extraction by pressing grapes in the wine and fruit juice production (Viveros et al., 2011). It contains 10.1% crude protein, 12.5% ether extract, 3.88% crude ash, 47.4% crude fiber (Altop et al., 2018). It is rich in amino acids, minerals, and polyphenols (Costa et al., 2022). It also has *in vitro* antioxidant and antimicrobial properties (Sarıca & Urkmez, 2018).

Cherry (*Prunus avium* L.) is a seed fruit of the Rosaceae family. Cherry is produced at 2.9 million tonnes each year worldwide (FAOSTAT, 2023). Cherry can be consumed as fresh fruit, jam, marmalade, and jelly (Vursavuş et al., 2006). Cherry kernel is a by-product of the processing of the cherries into commercial products in the factories. It has 14% crude protein, 26% ether extract, 3% crude ash, and 22% crude fiber (Altop, 2019). It has an antimicrobial effect on broiler chickens (Altop, 2019).

Maize (*Zea mays* L.) is an important cereal crop for human and livestock diets. It is produced into various commercial products such as corn flour, semolina, breakfast cereals, snacks, and starch (Rouf Shah et al., 2016). It is produced in quantities exceeding 1.24 billion tonnes worldwide (FAOSTAT, 2023). It is also one of the main ingredients of poultry diets. It has 9.8% crude protein, 1.6% crude ash, 4.5% ether extract, and 2.6% crude fiber (Abiose Sumbo & Victor, 2014). Maize is the most preferred energy source due to its high energy content, low crude fiber content, and also having coloring pigments and essential fatty acids (Panda et al., 2011).

Fermentation applications utilizing microorganisms, including bacteria, fungi, and yeasts, can generally be divided into submerged and solid-state fermentation (Bhargav et al., 2008). In recent years, solid-state fermentation has been used to utilize the agricultural residues in animal nutrition by improving nutritional quality, which is produced at billions of tonnes every year (Güngör et al., 2017). The nutritional quality of agricultural

residues can be improved by solid-state fermentation through increasing the protein level and decreasing the crude fiber content (Dei et al., 2008). Solid-state fermentation refers to the growth of microorganisms on moistened solid substrates without free water (Wang et al., 2025). This process is the preferred fermentation method due to its economic, abundant, and cheap substrates and its relatively lower risk of contamination (Karimi et al., 2021).

Cherry kernel, grape seed, and maize seed have been used in solid-state fermentation separately (Altop, 2019; Altop et al., 2018; Cui et al., 2021). However, Mala et al. (2007) reported that using a mixture of different substrates as a fermentation medium enhances microorganism growth and increases fermentation efficiency compared to using a single substrate. Although agricultural by-products are produced in large quantities, their potential use as feedstuff is limited due to production quantities (Malenica et al., 2022). Considering the limited production of agricultural wastes, it may be more feasible to use these residues in mixtures in animal diets than to use them alone. Therefore, a mixture of cherry kernel, grape seed, and maize seed was used as a substrate in the present study.

Aspergillus niger is a commonly used probiotic in broiler diets and is favored for solid-state fermentation thanks to its ability to grow successfully in low-moisture environments (Gungor & Erener, 2020a). The previous studies showed that different *A. niger* strains (ATCC 200344, 200345, 201572, and 52172) had different effects on the nutritional composition of agricultural residues (Altop et al., 2018, 2021, 2023a). A synergistic effect can be achieved by using a mixture of the *A. niger* strains. This study hypothesized that solid-state fermentation with *A. niger* strains would improve the nutritional composition of grape seed, cherry kernel, and maize seed by increasing crude protein and decreasing crude fiber content. This study investigated the effect of solid-state fermentation with *A. niger* on the nutritional composition of grape seed, cherry kernel, and maize seed.

Materials and Methods

Substrate and Microorganism

Grape seed and cherry kernels were obtained from a local fruit juice factory. Maize seed was supplied from a local feed mill. *A. niger* strains (ATCC 200344, 200345, 201572, 52172) were obtained from American Type Culture Collection (ATCC).

Fermentation

Solid-state fermentation was conducted according to Gungor and Erener (2020b). Grape seed and cherry kernel were sun-dried and kept at 4 °C until fermentation. The samples were ground to a particle size of 1 mm before fermentation. To prepare the substrate, 40 g of grape seed and 40 g of cherry kernel were mixed, and then 20 g of maize seed was added to the substrate. The amount of the maize seed (20 g) was determined according to Cui et al. (2021). A total of 80 g of agricultural waste, consisting of grape seeds and sour cherries, has been added in equal amounts, each weighing 40 g (Cui et al., 2021). The substrate (100 g) was enriched with 100 ml of nutrient salt consisting of 20 g glucose, 10 g urea, 30 g $(NH_4)_2SO_4$, 5 g peptone, 20 g KH_2PO_4, and 5 g $MgSO_4.7H_2O$, and 1 liter of distilled water. The samples were sterilized at 121 °C for 15 min using an autoclave.

A. niger was cultivated at 30 °C for 7 days using potato dextrose agar. Suspensions containing 10^6 spores per ml for each *A. niger* strain were prepared and mixed prior to use in the fermentation. The suspensions were prepared by washing sterile distilled water containing 0.1% Tween 80. The *A. niger* spores were counted using a Thoma counting chamber. The substrates were inoculated with 1 ml of suspension of mixed *A. niger* strains for each 100 g sample.

Chemical Analysis

Grape seed, cherry kernel, and maize seed mixture (GCM) was analyzed to determine dry matter (930.04), crude ash (942.05), crude protein (976.06), ether extract (920.29), and crude fiber (973.18) content according to AOAC (2000). Nitrogen-free extract was calculated on a dry matter basis by subtracting the percentages of crude protein, ether extract, crude ash, and crude fiber from 100%.

Statistical Analysis

The data were analyzed with a student's t test using the statistical software SPSS, version 21. All experiment was conducted in triplicate. The differences were considered significant at a probability level of $P<0.05$.

Results

Nutritional composition of fermented GCM with *A. niger* strains is presented in Figure 1. Solid-state fermentation with *A. niger* strains increased the crude protein, ether extract, and crude ash content of GCM ($P<0.001$, $P<0.001$, $P=0.001$, respectively). The crude fiber content of GCM was decreased ($P<0.001$) by solid-state fermentation. Solid-state fermentation with *A. niger* decreased ($P<0.001$) the nitrogen-free extract content of the substrate.

Discussion

Solid-state fermentation with *A. niger* improves the nutritional quality of agricultural residues. Indeed, solid-state fermentation with *A. niger* improved the nutritional composition of cherry kernel (Altop, 2019) and sour cherry kernel (Gungor & Erener, 2020a). Altop et al. (2018) also reported that *A. niger* improved the nutritional composition of grape seed.

Crude Protein

Crude protein is an essential nutritional component that indicates the total nitrogen content of a feed, including true protein and non-protein nitrogen. Protein is an important nutrient for the growth and muscle development of animals. Solid-state fermentation with *A. niger* can increase the crude protein of cherry kernel (Altop, 2019) and sour cherry kernel (Gungor & Erener, 2020a). Similarly, crude protein content of grape seed increased by treatment with *A. niger* strains (Altop et al., 2018). In this study, *A. niger* strains raised the crude protein content of GCM after solid-state fermentation. *A. niger* can produce various enzymes such as protease, cellulase, lipase, phytase, tannase, and pectinase during solid-state fermentation (Meini et al., 2021; Sousa et al., 2024; Zheng-Yu et al., 2007).

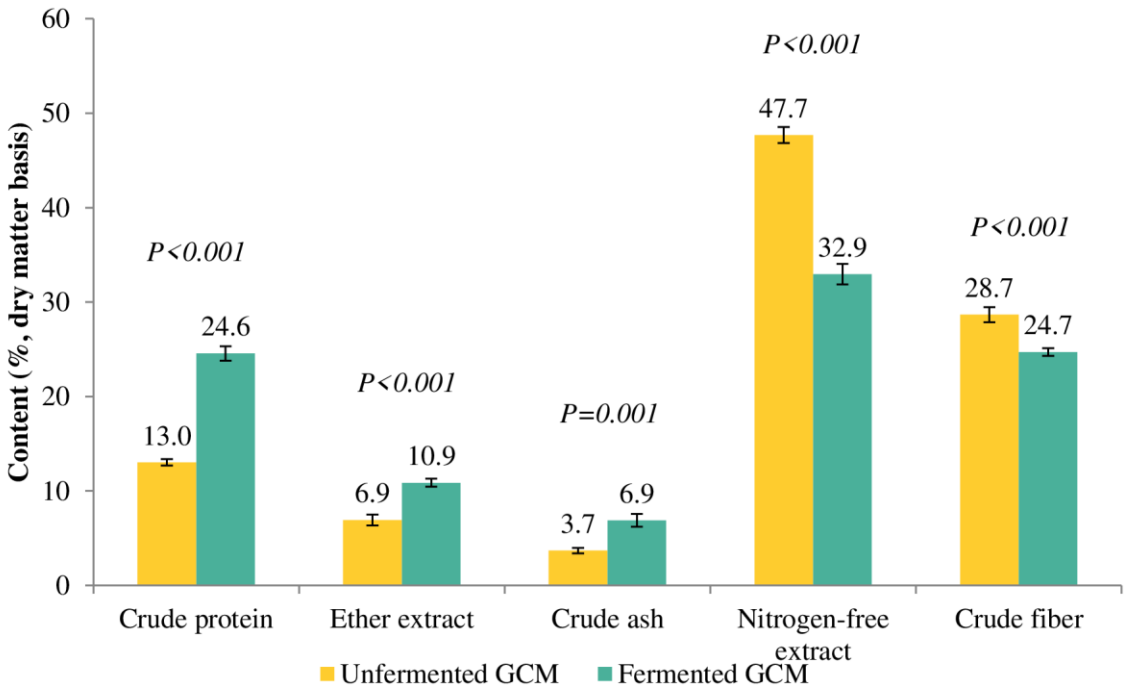

Figure 1. Effect of solid-state fermentation on the nutritional composition of grape seed, cherry kernel, and maize seed mixture (GCM)

The increase in the crude protein content can be due to the enzymes produced by *A. niger* during solid-state fermentation or other secondary metabolites in the protein structure, owing to their protein composition (Altop et al., 2023a). Indeed, solid-state fermentation with *A. niger* increased the crude protein content of various substrates, including rapeseed cake (Shi et al., 2015), pineapple waste (Omwango et al., 2013), soybean husk (Ihtifazhuddin et al., 2016), apple pomace (Altop et al., 2023b), and sesame seed (Altop et al., 2023a).

Ether Extract

Ether extract indicates the total fat content of a sample, including oils and other fat-soluble substances. These compounds are essential for meeting energy requirements and providing the essential fatty acids for animals. Solid-state fermentation can increase the ether extract content of agricultural residues. It established that solid-state fermentation with *A. niger* increased the ether extract content of cherry kernel (Altop, 2019) and sour cherry kernel (Gungor & Erener, 2020a). Similarly, *A. niger* strains increased the ether extract content of grape seed in solid-state fermentation (Altop et al., 2018). Shi et al. (2015) also reported that solid-state fermentation with *A. niger* raised the ether extract content of rapeseed cake. *A. niger* can produce microbial lipid during solid-state fermentation, which increases the ether extract content of the substrates (Hui et al., 2010). The increase in the ether extract content of GCM in the present study can be due to the microbial lipid that was possibly produced by *A. niger* during solid-state fermentation. The reduction in the nitrogen-free extract and crude fiber content of GCM may also have caused the increase in the ether extract content. However, solid-state fermentation with *A. niger* decreased the ether extract content of apple pomace (Altop et al., 2023b). Different results can be attributed to the difference in *A. niger* strains and the substrates between the studies.

Crude Ash Content

Crude ash content provides an indication of the total mineral content of a feed sample. Solid-state fermentation can cause an increase in the crude ash content of the substrates. It was reported that *A. niger* strains increased the crude ash content of grape seed by solid-state fermentation (Altop et al., 2018). Similarly, crude ash content of cherry kernel and sour cherry kernel was increased by *A. niger* in solid-state fermentation conditions (Altop, 2019; Gungor & Erener, 2020a). *A. niger* can accumulate some minerals during solid-state fermentation and cause an increase in the crude ash content of the substrates (Xiao et al., 2021). Accumulation of the minerals by *A. niger* can be a reason for the increased crude ash content of GCM in this study. The increase in the crude ash content of GCM can also be due to the reduction in the nitrogen-free extract and crude fiber level. Similarly, solid-state fermentation with *A. niger* increased the crude ash content of pineapple waste (Omwango et al., 2013). Altop et al. (2023b) also reported an increased crude ash content in apple pomace after solid-state fermentation with *A. niger*.

Crude Fiber

Crude fiber refers to the indigestible part of plants, which consists mainly of cellulose, hemicellulose, and lignin, which are structural carbohydrates. *A. niger* can produce cellulase enzyme during solid-state fermentation (Meini et al., 2021). Producing cellulase by *A. niger* in solid-state fermentation can cause a reduction in crude

fiber content of the substrates by breaking down cellulose, hemicellulose, and lignin compounds. Similar to the result of the present study, the crude fiber content of grape seed was decreased by solid-state fermentation with *A. niger* (Altop et al., 2018). It was also reported that *A. niger* decreased the crude fiber content of cherry kernel and sour cherry kernel in solid-state fermentation (Altop, 2019; Gungor & Erener, 2020a). Shi et al. (2015) also reported that *A. niger* decreased the neutral-detergent fiber content of rapeseed cake in solid-state fermentation conditions. Similarly, solid-state fermentation with *A. niger* decreased the crude fiber content of various substrates, including pineapple waste (Omwango et al., 2013), soybean husk (Ihtifazhuddin et al., 2016), apple pomace (Altop et al., 2023b), and sesame seed (Altop et al., 2023a).

Nitrogen-Free Extract

Nitrogen-free extract contains easily digestible carbohydrates such as sugars and starches. Microorganisms preferred the easily digestible carbohydrates to the other nutrients in the substrates to use as a carbon source (Papagianni, 2007). Therefore, solid-state fermentation causes a reduction in the NFE content of the substrates (Altop et al., 2018). It was reported that Solid-state fermentation with *A. niger* decreased the NFE content of sour cherry kernel (Gungor & Erener, 2020b). Similarly, NFE content of grape seed was reduced by *A. niger* strains (Altop et al., 2018). Similar results were observed from various studies on pineapple waste (Omwango et al., 2013) and apple pomace (Altop et al., 2023b). However, solid-state fermentation increased the NFE content of soybean husk (Ihtifazhuddin et al., 2016), maize shaft, cowpea shell, groundnut shell, and soybean hull (Amos et al., 2025). The difference between the results of the studies can be due to different *A. niger* strains and the substrates of the solid-state fermentation.

Conclusion

This study demonstrated that solid-state fermentation with different *A. niger* strains improve the nutritional composition of GCM. Solid-state fermentation increased crude protein, ether extract, and crude ash content and reduced crude fiber and NFE content of GCM. The results of this *in vitro* study demonstrated that solid-state fermentation is a useful feed processing method for improving the nutritional composition of agricultural residues and making feedstuff for livestock and poultry. However, *in vivo* trials need to be conducted to confirm the improvement in the nutritional composition of the GCM by solid-state fermentation

Declarations

Author Contribution Statement

EG: Conceptualization, data collection, investigation, formal analysis, methodology, writing original draft, review, and editing

Conflict of Interest

The author declares no conflict of interest.

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