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Effect of fermentation with *Aspergillus oryzae* on the phytochemical and nutritional content of cereals

Efecto de la fermentación con *Aspergillus oryzae* sobre el contenido fitoquímico y nutricional de cereales

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ABSTRACT

The aim of this study was the fermentation of cereal grains with *Aspergillus oryzae* at different times (0, 3, 5 and 7 days) to increase the total phenolic, protein and condensed tannins content. Therefore, four cereal grains were used: corn, oat, barley and sorghum. Cereal grains were subjected to a solid-state fermentation with de *Aspergillus oryzae*. Higher increases of protein in corn, oat and sorghum were observed at 5 and 7 days of fermentation. In relation to phenolic content, the highest content was shown at 7 days of fermentation in all grains. Otherwise, condensed tannins showed higher contents in corn and sorghum at 5 days of fermentation; whereas, oat reached higher contents at 7 days. No changes were observed in barley among 3 and 7 days of fermentation time. In conclusion, solid state fermentation increased phytochemicals and nutritional contents by changing fermentation time.
Keywords: state-solid fermentation, filamentous fungi, bioactive compounds, phenolics.

RESUMEN

El objetivo del presente trabajo fue fermentar granos de cereales con *Aspergillus oryzae* a diferentes tiempos (0, 3, 5 y 7 días) para incrementar su contenido fenólico total, proteico y taninos condensados. Para esto, se utilizaron granos de cuatro cereales: maíz, avena, cebada y sorgo. Los granos se sometieron a una fermentación en estado sólido con una cepa de *Aspergillus oryzae.* Los mayores incrementos de proteína cruda en maíz, sorgo y avena fueron observados a los 5 y 7 días de fermentación. En relación al contenido fenólico, se mostró el mayor contenido a los 7 días en todos los granos. Por otro lado, el contenido de taninos condensados mostró un mayor incremento en maíz y sorgo a los 5 días de fermentación, mientras que en avena se alcanzó a los 7 días. La cebada no mostró diferencias de 3 a 7 días. En conclusión, la fermentación en estado sólido incrementó el contenido fitoquímico y nutricional de cereales, al manipular el tiempo de fermentación.
Palabras clave: fermentación en estado sólido, hongos filamentosos, compuestos bioactivos, fenoles.

INTRODUCTION

Cereals are an important source of food for humans and animals, as they provide energy, protein, and a variety of bioactive substances (Xiao *et al*., 2015[a]; Borras & Torres, 2016). These bioactive compounds, called phytochemicals or secondary metabolites, are organic molecules that do not participate directly in primary metabolism but exhibit various biological activities (Camacho-Escobar *et al*., 2020). Phenolic compounds are the most abundant group of secondary metabolites in plants; their basic structure consists of a hydroxyl group attached to an aromatic ring, and from this structure a variety of compounds originate, such as phenolic acids, coumarins, lignins, tannins, and flavonoids (Sánchez, 2022). Phenols found in cereals can be soluble, insoluble, or bound. Among the soluble phenols are free, glycosylated, and esterified phenols, which are found in greater quantities in the outer layers of the grains, such as the pericarp, testa, and aleurone cells (Cabrera-Soto *et al*., 2009). Most insoluble phenols form covalent bonds with cell wall components such as pectin, cellulose, and structural proteins (Shahidi & Yeo, 2016). In cereals, the most abundant phenols are derivatives of benzoic acid, cinnamic acids, and flavonoids (Balli *et al*., 2019). These exhibit various biological properties such as antioxidant, anti-inflammatory, and antimicrobial properties, among others (Torres-León *et al*., 2019). The bioactivity of phenolic compounds depends on their bioaccessibility, which translates into their release from the food matrix so that they can be available (Gutierrez-Gijalva *et al*., 2016).

Solid-state fermentation (SSF) is a technique that has been used in Eastern countries since ancient times to produce a variety of traditional foods (Xu *et al*.,2018). It is defined as an efficient and economical process in which microorganisms grow on solid substrates to transform and enrich them nutritionally or to produce various secondary metabolites under reduced moisture levels (Liu *et al*., 2022). Microorganisms such as fungi, yeasts, and lactic acid bacteria are used in the fermentation process, but the most commonly used are filamentous fungi, which are capable of adapting to different environments with low water activity requirements.

Currently, this process is applied to increase the bioaccessibility of nutrients and phenolic compounds (Gebru & Sbhatu, 2020). In previous studies, a variety of cereals were fermented in a solid state to improve their phenolic content (Cia *et al*., 2012; Bhanja Dey & Kuhad, 2014[a]; Bhanja Dey & Kuhad, 2014[b]; Xiao *et al*., 2015[a]; Xu *et al*., 2018; Gebru & Sbhatu, 2020).

Based on the above, the objective of this study was to ferment grains from four cereals at different times with *Aspergillus oryzae* to increase their phenolic and protein content.

MATERIAL AND METHODS

Raw material

CAFIME variety corn grains from the spring-summer 2022 cycle were used, which were provided by INIFAP-Durango (Francisco I. Madero experimental field located in the Municipality of Pánuco de Coronado, Durango), while oat, barley, and sorghum grains were purchased at the local market. All grains were cleaned and stored at room temperature until later use.

Obtaining and preserving the strain

The fungus *Aspergillus oryzae* strain 2094 was provided by the strain collection of the Durango Institute of Technology-TecNM. Potato dextrose agar (PDA) was used as a preservation medium at a temperature of 30 °C. The inoculum was prepared from a 13-day-old culture by suspending spores in distilled water at a concentration of 1 x 10^6 spores/mL.

Solid-state fermentation (SSF)

SSF was performed using the method described by Bhanja Dey & Kuhad (2014[a]). Two hundred grams of each grain (corn, oats, barley, and sorghum) were weighed and placed in 1 L glass jars, 200 mL of distilled water was added, and they were sterilized at 121 °C for 15 min. Once the process was complete, they were left to cool to room temperature. The sterilized grains were then inoculated with 20 mL of the spore suspension (prepared beforehand). The incubation time was 0, 3, 5, and 7 days, respectively, at a temperature of 30°C. Once the fermentation process was complete, fermented grains were sterilized again and left to dry at 55 °C for a period of 72 hours. They were then ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) to a particle size of 1 mm and stored in Ziploc bags in a dark, dry place at room temperature for later analysis.

Chemical analyses

The crude protein (CP) content of the fermented grains was determined according to AOAC (1990). Phenolic compounds were determined according to the methodology proposed by Heimler *et al*. (2005). One gram of sample was macerated with 90 mL of 70% ethanol (adjusted to pH 2.0) and left overnight at room temperature. The mixture was then filtered and degreased with petroleum ether. The degreased extract was subsequently evaporated at room temperature and redissolved in 70% ethanol (pH 2.0) to a final volume of 2 mL. To determine total phenols (TP), 125 µl of phenolic extract was taken, 0.5 mL of deionized water and 125 µl of Folin-Ciocalteu reagent were added. The

mixture was left to stand for 6 min, and then 1.25 mL of 7% sodium carbonate (Na_2CO_3) was added. The final volume was adjusted to 3 mL with deionized water. The mixture was left to stand for 90 min, and after this time, the absorbance was measured at 760 nm, using water as a blank. The amount of total phenols was expressed as gallic acid equivalents (µg EAG/g meal). To determine condensed tannins (CT), 3 mL of 4% vanillin solution in methanol and 1.5 mL of concentrated hydrochloric acid were added to 50 µl of the extract. The mixture was allowed to stand for 15 min and the absorption was measured at 500 nm, using methanol as a blank. The amount of CT was expressed as catechin equivalents (µg CE/g meal).

Statistical analysis

The data obtained for crude protein, total phenols, and condensed tannins were analyzed using one-way ANOVA, and the means were compared using Tukey's test ($p < 0.05$), using the SAS statistical package version 9.0 (SAS Inc., Cary, NC, USA). All treatments were performed in triplicate, and the values were expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

Crude protein (CP)

Table 1 shows the results of the crude protein content of cereal grains fermented at different times. Corn and oats reached the highest CP levels on day 5 of fermentation ($p < 0.05$), while barley and sorghum reached the highest levels on day 3 of fermentation ($p < 0.05$). Several studies with different microorganisms have demonstrated an increase in protein during fermentation. (Chen *et al*., 2013 & Chen *et al*., 2014) observed an increase in CP in soybean meal from 50.46 to 58.99 and 9.2 %, respectively, when fermented with *Aspergillus oryzae* for 0, 25, and 36 hours. Similarly, Xiao *et al*. (2015[b]) observed that the fermentation of chickpea meal with *Cordyceps militaris* increased the CP content from 22.13 to 26.43 %. In addition, Xiao *et al*. (2018) worked with red bean meal fermented with *Cordyceps militaris* and observed a significant increase of 9.3% (23.61 to 25.81%) with the fermentation process. Chen *et al*. (2021) fermented soybean meal with *Bacillus velezensis* bacteria for 24 hours and then with *Lactobacillus plantarum* for 48 hours; the results showed an increase inC P from 47.28 to 51.08 % at 24 hours, while at 48 hours a maximum of 52.36 % was obtained. Similarly, Sánchez-García *et al*. (2022) worked with lentil and quinoa seeds and their meals, fermenting them with the macrofungus *Pleurotus ostreatus* at different times. They obtained a significant increase in total protein content between 7 and 26%. In addition, they observed that particle size and substrate influenced protein content. Similarly, Cubillos-Orjuela *et al*. (2024) fermented a food product that

included a percentage of cereal chaff (10 and 20 %) with lactic acid bacteria, and found that including 20% cereal chaff and fermenting for 48 hours resulted in a 2.2 % increase in crude protein.

Table 1. Crude protein content (% CP) in grains fermented at different times

Fermentation time (days)	Corn	Oats	Barley	Sorghum
Control	11.11 ± 0.30^{cd}	15.33 ± 0.04^{c}	11.44 ± 0.22^{ab}	13.27 ± 0.26^{ab}
0	10.89 ± 0.11^{d}	16.05 ± 0.18^{bc}	12.65 ± 0.45^{a}	12.65 ± 0.45^{b}
3	11.80 ± 0.38^{bc}	16.09 ± 0.16^{bc}	11.64 ± 0.60^{ab}	13.57 ± 0.10^{a}
5	12.64 ± 0.25^{a}	16.47 ± 0.47^{ab}	9.93 ± 0.74^{c}	13.96 ± 0.05^{a}
7	11.86 ± 0.15^{b}	17.02 ± 0.46^{a}	11.12 ± 0.27^{bc}	13.97 ± 0.48^{a}

Values expressed as means ± standard deviation. $^{a-d}$ Different letters in the same column indicate statistical differences ($p < 0.05$)

Total phenols

Table 2 shows results of the total phenolic content in cereal grains fermented with *Aspergillus oryzae* at different times. In general, it can be seen that the phenolic content of all grains increased as the fermentation time increased. The highest values ($p < 0.05$) were observed in corn after 5 days of fermentation, while in oats and barley, the highest phenolic content ($p < 0.05$) was observed after 7 days of fermentation. In sorghum, the phenolic content was similar from 3 to 7 days of fermentation ($p > 0.05$). The increase in oats, barley, and sorghum at 7 days of fermentation was 248 %, 117 %, and 57 %, respectively. Corn showed an increase of 59 % after 5 days of fermentation. According to Saharan *et al*. (2017), the increase in phenolic content during fermentation is related to the production of enzymes by the fungus, such as α-amylase, β-glucosidase, and xylanase, which have the ability to hydrolyze carbohydrates, degrade the cell wall, and release phenolic compounds. The improvement in phenolic content in cereals fermented with filamentous fungi was observed by other authors, Cai *et al*. (2012) fermented oatmeal with *Aspergillus oryzae* var. effuses, *Aspergillus oryzae*, and *Aspergillus niger* for 3 days at 25 °C and reported that the fungus *Aspergillus oryzae* var. effuses increased the phenolic content in fermented meals to a greater degree. For their part, Bhanja Dey & Kuhad, (2014[a]) fermented whole wheat, brown rice, oats, and corn grains with *Aspergillus oryzae* NCIM 1212, *Rhizopus oligosporus* NCIM 1215, *Aspergillus awamori* MTCC No. 548, and *Rhizopus oryzae* RCK2012 at 30 °C for 3 days. The results showed that the phenolic content in all grains fermented with *Aspergillus oryzae* NCIM 1212 was higher than with the other fungi. Similarly, Bhanja Dey & Kuhad (2014[b]) fermented wheat grains with *Rhizopus oryzae* RCK2012 and found that fermentation increased the phenolic content in aqueous extracts by 377%. Abd Razak *et al. (*2015) studied the effect of

fermentation with *Rhizopus oligosporus* and *Monascus purpureus* (individually and mixed) on the phenolic content of rice bran. These authors found that the phenolic content of aqueous and methanolic extracts from fermented samples was higher than that of unfermented samples, with the exception of the aqueous extract from the sample fermented with *Monascus purpureus*. It was also observed that the mixture of fungi in the aqueous extracts produced a higher phenolic content than individually, while in the methanolic extracts the phenolic content was similar in both forms. In turn, Sandhu *et al.* (2016) fermented six varieties of wheat with *Aspergillus awamorinakazawa* at 30 °C for 6 days. The results showed that fermentation significantly increased the phenolic content on the fourth day in all six varieties. Additionally, Saharan *et al*. (2017) evaluated wheat, rice, oat, corn, and sorghum grains fermented with *Aspergillus oryzae* (MTCC 3107) for 6 days at 30 °C. The highest phenolic content values were found in wheat and oats on the fourth day of fermentation, while in rice, sorghum, and corn, the maximum increase was observed on the fifth day. Sánchez-Magaña *et al*. (2019) investigated the effect of fermentation time on the free, bound, and total phenolic content in raw, cooked, and fermented corn meals at different times with *Rhizopus oligosporus* NRRL 2710. They found that fermentation increased the free phenolic content after 48 hours, with the maximum value (227.75 mg gallic acid/100 g sample) occurring at 108 hours. The bound and total phenolic content began to increase after 72 hours, with a maximum value (993.44 mg gallic acid/100 g sample) at 108 hours. According to previous studies, it is important to consider several factors that influence the phenolic content of cereals, such as species, variety, microorganism, fermentation time, extraction method, and analysis method.

Table 2. Total phenolic content (µg EAG/g meal) in grains fermented at different times

Fermentation time (days)	Corn	Oats	Barley	Sorghum
Control	456.03 ± 23.34^{c}	342.76 ± 7.75^{d}	338.98 ± 15.56^{d}	703.73 ± 31.58^{b}
0	513.03 ± 32.10^{c}	382.28 ± 19.63^{d}	381.14 ± 3.40^{d}	752.87 ± 4.98^{b}
3	622.95 ± 24.76^{b}	748.6 ± 86.66^{c}	487.44 ± 6.71^{c}	923.68 ± 122.63^{ab}
5	727.09 ± 13.96^{a}	1068.53 ± 20.58^{b}	586.7 ± 19.70^{b}	1069.7 ± 118.56^{a}
7	690.95 ± 17.93^{a}	1194.07 ± 33.89^{a}	736.08 ± 49.49^{a}	1107.38 ± 119.64^{a}

$^{a-d}$ Different letters in the same column indicate statistical differences ($p < 0.05$)

Condensed tannins (CT)

Table 3 shows the condensed tannin content in fermented cereal grains. Significant differences ($p < 0.05$) were found as fermentation time progressed. This behavior was more pronounced in corn, as the CT content increased as fermentation time increased

relative to time 0. Sorghum recorded the highest CT content (202.11 µg CE/g meal) after 5 days of fermentation, while corn reached its maximum (97.19 µg CE/g meal) after 7 days ($p < 0.05$). Espitia-Hernández *et al*. (2022) reported results opposite to those of the present study with sorghum; they worked with two varieties of sorghum (red and black) fermented in a solid state with *Aspergillus oryzae* and *Aspergillus niger* for 96 h. Red sorghum fermented with *Aspergillus oryzae* showed no significant differences ($p > 0.05$), while with *Aspergillus niger* there was an increase after 12 hours, with the highest value at 72 hours (76.07 mg EC/100 g sample). Regarding black sorghum fermented with *Aspergillus oryzae*, condensed tannins decreased after 24 hours ($p < 0.05$); on the contrary, with *Aspergillus niger*, the CT content increased after 36 hours, and the highest value was obtained at 84 hours (73.20 mg EC/100 g sample). A previous study with lactic acid bacteria and yeasts was conducted by Terefe *et al*. (2021), who evaluated the effect of solid-state fermentation with *Lactobacillus plantarum* and *Saccharomyces cerevisiae* and their co-culture on the condensed tannin content in corn meal at 0, 12, 24, 36, and 48 h. CT content decreased with all strains as the fermentation time increased; the lowest values were found with co-culture at 48 h (12.3% CE). Published information on the effect of solid-state fermentation on the condensed tannin content in cereals is very limited. However, studies have been conducted with other species, as reported by Dhull *et al.* (2020), who evaluated the effect of solid-state fermentation of three lentil varieties (HM-1, LL-931, and Sapna) with *Aspergillus awamori* on condensed tannin content. The results for the three varieties indicated an increase in CT as the fermentation time increased, with the tannin content maximizing at 6 days in all three varieties (3.16, 4.52, and 4.30 mg CE/g dry basis, respectively).

Altop *et al.* (2018) worked with olive leaves to ferment them in a solid state with four strains of *Aspergillus niger* (F1, F2, F3, and F4) and analyze their effect on condensed tannin content. Fermentation increased the condensed tannin content in olive leaves with all strains, and the highest TC value was found with strain F2 (11.44%). Similarly, Duhan *et al*. (2021) fermented peanut cake (residue obtained after peanut oil extraction) in a solid state with *Aspergillus oryzae* for 6 days to determine its effect on condensed tannin content. The results clearly demonstrated that fermentation increased the TC content, reaching its maximum value (245 µg/g) after 6 days of fermentation. The above indicates that the type of cereal, variety, species, microorganism, and strains play a very important role during SSP in order to release condensed tannins from the cell wall.

Table 3. Condensed tannin content (µg CE/g meal) in grains fermented at different times

Fermentation time (days)	Corn	Oats	Barley	Sorghum
Control	76.04 ± 3.46^{a}	76.11 ± 8.68^{a}	40.29 ± 5.63^{b}	130.99 ± 5.77^{c}
0	37.43 ± 2.94^{c}	43.02 ± 8.47^{b}	77.02 ± 2.76^{a}	156.37 ± 2.82^{b}
3	59.03 ± 3.43^{b}	76.16 ± 8.63^{a}	40.19 ± 5.76^{b}	145.19 ± 2.69^{b}
5	63.04 ± 11.37^{ab}	73.97 ± 11.28^{a}	43.02 ± 2.99^{b}	202.11 ± 3.26^{a}
7	51.48 ± 5.28^{bc}	97.19 ± 5.96^{a}	48.68 ± 2.65^{b}	151.23 ± 8.50^{b}

Values expressed as means ± standard deviation. $^{a-c}$ Different letters in the same column indicate statistical differences ($p < 0.05$)

CONCLUSIONS

Solid-state fermentation with *Aspergillus oryzae* significantly increased the crude protein content and total phenolic content in oat, barley, corn, and sorghum grains after 3 days of fermentation. Based on the results, it is considered that the best fermentation time to increase the nutritional and phytochemical content in corn and sorghum is 5 days of fermentation, while for oats and barley it is 7 days of fermentation. SSP is an efficient, simple, and economical technique that can be used to enrich the nutritional and phytochemical components of cereal grains.

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Errata Erratum

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