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Investigating the antifungal effect of Iranian chestnut flour on *Penicillium expansum* and *Aspergillus niger*

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ABSTRACT:

Numerous studies have demonstrated the antimicrobial properties of the Iranian chestnut. This fruit can be used as a natural preservative to increase the shelf life of food. *Penicillium expansum* and *Aspergillus niger* are considered as important molds in food spoilage, including bread, fruit and vegetables. The purpose of this study is to evaluate the anti-fungal effects of Iranian chestnut flour on *Penicillium expansum* and *Aspergillus niger*. For this purpose, Sabouraud dextrose agar was used by adding 0.5 and 1 % of the chestnut flour and wheat flour along with a control (without flour). Then, the fungal suspension containing the spores of *P. expansum* and *A. niger* was cultured and the results were recorded. The results showed that *A. niger* was extremely sensitive to the chestnut flour as there was no evidence of growth in the presence of 0.5% chestnut flour. However, the growth of *P. expansum* was well in 0.5% chestnut flour, but its growth was greatly reduced by increasing the chestnut flour in the medium to 1%. The results show that Iranian chestnut flour contains strong amounts of anti-fungal compounds and so it was able to control the growth of *P. expansum* and *A. niger*. Thus, it could be suggested as a natural preservative to control food spoilage and bakery products.

Keywords:
Chestnut, *Penicillium expansum*, *Aspergillus niger*, Antifungal compound.

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INTRODUCTION

Molds lead to fatal disease in humans and animals by the production of fungal spoilage in the food. Hence, the addition of preservatives is effective as they decrease the microbial agents and increase the shelf life of the food. Chestnut is regarded as a natural preservative (Khaledi, 2013).

The scientific name of Iranian chestnut is *Quercus branti* belonging to Beech family. There are 500 different kinds of chestnut in the form of tree and shrubbery in different areas of north hemisphere which grows naturally in Mediterranean weather in moderate and mountainous jungles. The total area of chestnut jungles in Iran was estimated to be more than 4.5 million hectares (Azkia and Yosefi, 2005). This large area of the chestnut jungles lead to the production many tons of fruit per year. *Quercus* is used very limitedly in the preparation of animal feed and in the industry for tannins production, but a very large volume is wasted in the forest (Ghahfarokhi *et al.*, 2012). It has many nutritional, therapeutic, pharmaceutical and industrial properties (Panah, 2012). It contains 11% fat, 11.5% fiber, 18.5% protein, 59- 85% carbohydrates and 2630 kilocalories/kilogram of energy. Minerals and vitamins such as A, B and C are the other constituents of this fruit. It is placed in protein concentrates category in food products categorization. Hence, this fruit has a high nutritional value and digestibility (Rajablou, 2009; Saffarzadeh *et al.,* 1999).

The *Penicillium* fungus is widespread in the nature and form one of the important molds in the food industry. The *Penicillium expansum* is considered as the main mold in bread preparation and also causes green spots on the meat and the soft rot of the fruit due to green and blue spores (Frazier and Vestehuf, 2010). It also causes green-blue molded rot of citrus and blue rot in apples, grapes, pears and nucleated fruits. *P. expansum* produces a mycotoxin called as patulin (Jey *et al*., 2011). Most *Aspergillus* species spoil food and some of the species are involved in the preparation of food. *Aspergillus niger* is widespread and certain species are used in the production of different types of enzymes such as 'citric and Glokonik' on a commercial scale (Frazier and Vestehuf, 2010). It is the main reason for the spoilage of bread and causes black rot in peaches, citrus, plum and fig (Jey *et al.,* 2011).

Karimi *et al.* (2010) studied the deterrence effect of chestnut extract and the results show that the hydro-alcoholic extract of the chestnut fruit has very low toxicity and it has a good deterrence effect on the replication of the herpes simplex virus type A in cell culture. Ebrahimi *et al.* (2010) studied the comparative effect of antimicrobial activity of different compounds of Iranian chestnut against *Escherichia coli* bacteria. The results show that Iranian chestnut has compounds with antibacterial properties. Sakar *et al*. (2005) proved that the antimicrobial activity of *Quercus aucheri* leaves against *Staphylococcus* and *Escherichia coli* bacteria. Nagesh *et al*. (2012) stated that the Gall chestnut methanol extract has high antibacterial effect against *Enterococcus faecalis*. Tayel *et al.* (2013) also investigated the production of anti-fungal cotton textiles with Gall chestnut extract. The results show that the species of *Quercus infectoria* could be suggested as a powerful antifungal agent in the preparation of disinfectants. Thus, the purpose of this study was to investigate the antifungal effects of Iranian chestnut flour on *Aspergillus niger* and *Penicillium expansum* and thus presenting it as a natural preservative to increase the shelf life of food.

MATERIALS AND METHODS

The preparation of chestnut flour

Iranian chestnut fruits were used for the production of chestnut flour. Chestnut was collected in fall during 2013 from the chestnut jungles of the province Lordegan located in Chaharmal-Bakhtiyari in the area of Zagros. Then, the hard and outer shell of the

chestnut were separated and dried in the sun. After that, the soft and inner shell of the fruit were removed and they were transported to the mill for grinding. After grinding, the resulting flour was poured into the bags and it was kept in a place away from heat, moisture and other factors of contamination (Khaledi *et al*., 2013).

Microorganisms

The *Penicillium expansum* mold (ATCC 42710) and *Aspergillus niger* (ATCC 16404) used in this study were grown in the slope culture media inside the twisty pipe from the Pasteur Institute of Tehran. Sabouraud Dextrose Agar medium (SDA), was used to culture it further.

The preparation of fungal suspension

The samples were serially diluted till 10^{-6} dilution in sterile physiological saline. 1ml from 10^{-5} and 10^{-6} dilution were pour plated on SDA and it was kept inside the incubator at 25-30^{0}C. After 2 to 4 days, the colonies were counted by using colony counter device. The tube corresponding to10-100 microbes per ml were selected for further treatment. All the tubes were placed in the refrigerator throughout the period. The operation was performed separately for each mold (Mortezavi *et al*., 2009). Sterile condition was maintained using 70% alcohol.

Preparing the medium containing microbial suspension and flour

SDA culture medium was used after adding a certain amount of wheat flour and chestnut flour. The medium was prepared by adding 0.5% and 1% of each flour and poured onto petriplates after autoclaving.

From microbial suspension containing 10-100 microbes per ml, 1.0 ml were distributed to each treatment containing three replicas for each. Then, the plates were incubated at 25-30^{0}C. After that, the growth of colonies were counted daily for 4 days by using colony counter device and the results were recorded. A control was maintained by using SDA medium without flour and the results were recorded (Khaledi *et al*., 2013). It should be noted that the microbial suspension used in this study contained 20 spores of *A. niger* and *P. expansum* molds in per ml.

Data analysis

In the present study, all the treatments were performed in triplicate and the results of all the tests were analyzed by comparing the means using a factorial model by the Duncan test at 95% using SPSS software version 19. All the charts were drawn using Excel software version 2007.

RESULTS

According to Table 1, *P. expansum* and *A. niger* molds showed high sensitivity to the pulp of the chestnut flour, whereas they grow well on the pulp of the wheat flour.

The results indicated that the *P. expansum* mold could grow well on the wheat flour. Their growth and activity was the same on the wheat flour and SDA medium. Hence, there was no significant difference between the fungal growth in these environments. The results of the replicates are shown clearly on the Figure 1 and 2.

It is clearly seen from the Figure 1 that the *P. expansum* mold could grow well on the medium

Table 1. Comparing the means of the results of pulp production by the microbial suspension

Treatment	Chestnut flour and *Penicillium*	Chestnut flour and *Aspergillus*	Wheat flour and *Penicillium*	Wheat flour and *Aspergillus*
Day				
1	4 ^{ab}	5 ^{abc}	1 ^{d}	19 ^{f}
2	6 ^{ab}	7/33 ^{bcd}	19 ^{e}	28/67 ^{f}
3	6 ^{ab}	7/33 ^{bcd}	19 ^{e}	28/67 ^{f}

In each column, the means at least in one common letter has no significant difference at 5% probability level based on Duncan test

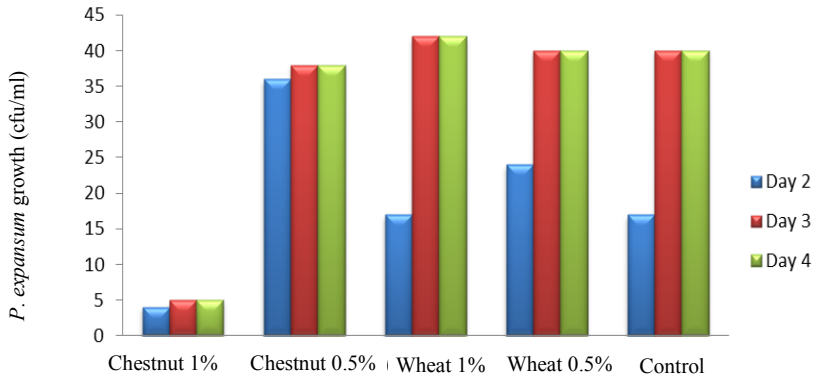

Figure 1. *P. expansum* growth at different flours (replicate 1)

containing 0.5% chestnut flour but its growth was highly reduced by increasing the chestnut flour to 1% and its activity was greatly reduced compared to the control one. The sensitivity of the *P. expansum* is dependent on the chestnut flour because, the sensitivity was increased by increasing the amount of the flour in the medium. The results also show that the *A. niger* mold could grow well on the environments prepared with wheat flour. In general, its growth and activity was the same as in wheat flour and the control. Hence, there was no significant difference between the growth of the fungus in these environments. But *A. niger* mold showed a high sensitivity to the chestnut flour and its activity was greatly reduced in the presence of 0.5% chestnut flour. The findings for the replicates are clearly shown in Figure 3 and 4.

DISCUSSION

The analysis of test results revealed that *P. expansum* and *A niger* are sensitive to chestnut flour in the pulp; so their growth and activity were greatly reduced. It can be attributed to the presence of antimicrobial compounds in the chestnut. The antimicrobial activity of the chestnut could be due to the tannins present in it, as these trees are important due to the tannins found in different parts of them. Tannins have different properties including their antimicrobial effects. Tannins can be toxic to bacteria, microbes, fungi and even viruses since they prevent their growth by depositing microbial proteins. They also prevent protein availability to the microbes or play their role through the mechanism of trapping iron, hydrogen bonding and enzymes. Tannins are also capable of preventing enzyme proliferation by releasing reverse transcriptase enzyme in

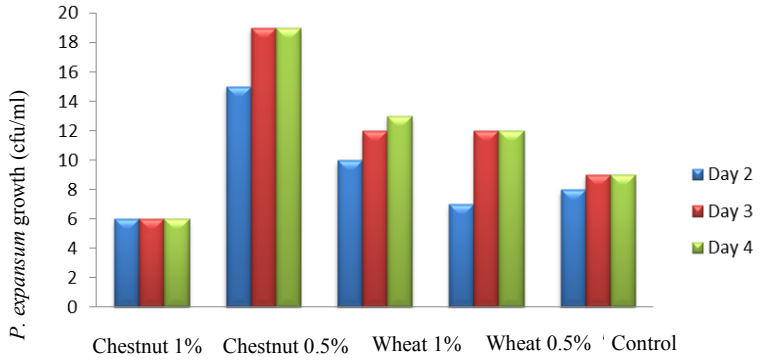

Figure 2. Growth of *P. expansum* at different flours (replicate 2)

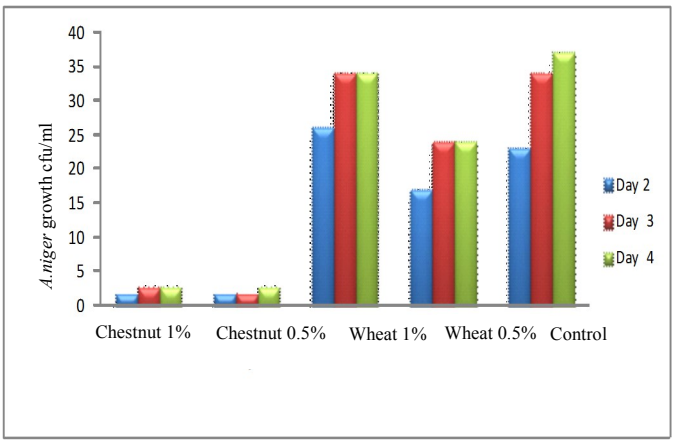

Figure 3. Growth of *A. niger* in chestnut and wheat flour (replicate 1)

human viruses (Nair *et al*., 2007). Nezhad and Azari (2003) measured the amount of Tannins in 10 Iranian chestnut species. *Quercus blengri* species contain the highest amount of tannins (9.7%) among all the species. This amount varied in other species from 3.2 to 7.5%. Besides, Panahi *et al.* (2013) studied the inhibitory effect of ethanol extract of the *Quercus cortex* on the yeast *Candida albicans*. The results showed that the ethanol extract of the pair chestnut contains metabolites that has an inhibitory effect on the *Candida albicans* (Panahi *et al*., 2013). Sarmamy *et al*. (2011) also studied the antifungal activity of the gall chestnut and pomegranate extract against *A. niger* and *Penicillium* molds. The results showed that all the concentrations used against both fungal extracts were effective (Sarmamy *et al*., 2011). Sharifi *et al*. (2011) investigated the effects of hydro-alcoholic extract of pair *Quercus* on the *Saprolegnia* mold. The results showed that the pair chestnut extract has antifungal properties against *Saprolegnia* fungi. Hence, the hydro-alcoholic extract of the pair *Quercus* could prevent from fungal growth and it has antifungal effects which was on par with the anti-fungal agent malachite green used in medicine. Therefore, the results and findings of the mentioned study was in line with the results of the current study as it show that the Iranian chestnut has effective anti-fungal substances.

CONCLUSION

It could be claimed with regard to all the test results and findings that the Iranian chestnut affects the growth of *Aspergillus niger* and *Penicillium expansum*.

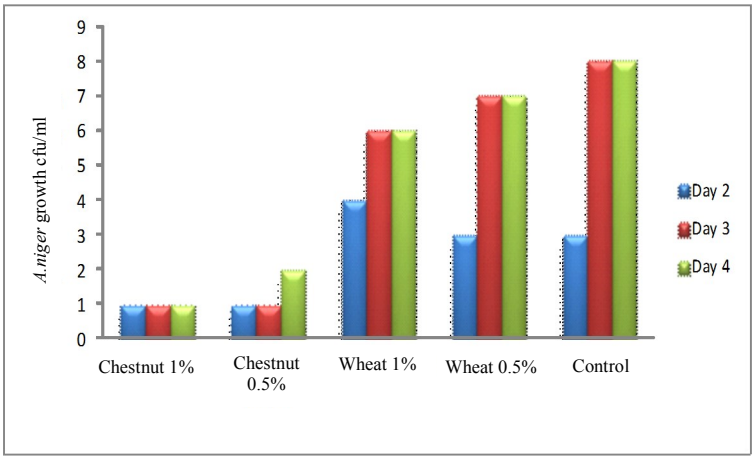

Figure 4. Growth of *A. niger* in chestnut and wheat flour (replicate 2)

Thus, this study show that the Iranian chestnut has strong anti-fungal compounds and it could be suggested as a natural preservative to control food spoilage including bakery products, fruit and vegetables.

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