EVALUATION OF EINKORN BRAN AS SUBSTRATE FOR SYNTHESIS OF ALPHA AMYLASE FROM *PENICILLIUM HERQUEI* IN SOLID STATE FERMENTATION PROCESS

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ABSTRACT

Solid State Fermentation (SSF) is a process defined as microorganisms growing on a solid substrate in the absence of water. It is similar to natural microbial processes such as composting and ensiling. This technique has been known for a long time and is used widely in the Far East. It can be also used in industrial applications such as Roquefort cheese, antibiotics, organic acids, plant growing agents, food additive materials and enzyme production. In this study, α-amylase production was done in SSF process from *Penicillium herquei*. Agricultural by-products such as wheat (*Triticum aestivum* L.) bran and einkorn (*Triticum monococcum* L.) bran have been tested for enzyme production, individually and as a mixture. The highest enzyme activity (602 U/gds±5.6) was recorded in the SSF process where einkorn bran was used as the substrate. The process parameters such as initial moisture level and substrate amounts were optimized to determine their effects on enzyme synthesis from *P. herquei*.

Keywords: Solid State Fermentation, einkorn, *Penicillium herquei*, amylase

INTRODUCTION

Many industrially important reactions usually undergo more easily in warm, biological conditions. This is why the usage of enzymes in the industry is inevitability. Worldwide, the industrial enzyme market is worth nearly 1.4 billion USD and with an annual 10% market expansion as well as 4-5% selling increase, it is one of the most widespread consumption areas. 75% of industrial enzyme productions fall under food, detergent and starch industries (Cowan, 1996).

In the industry, the most used enzyme for starch hydrolysis is α-amylase. α-amylase (1,4-α-D-glucanohydrolase; EC 3.2.1.1) hydrolyses the α-1,4 bonds in the starch. α-amylase is an internally working enzyme. It randomly hydrolyses the internal bonds in starch, and paves the way of forming branched and linear oligosaccharides (Crabb and Mitchinson, 1997).

This enzyme is used for making sugar syrups obtained from starch made out of glycose, maltose and some higher oligosaccharides (Hagihara et al., 2001). It is also widely used in the liquefaction, paper, food, pharmaceutical and sugar industries. Low cost media is required for the production of α-amylase to meet the demands of these industries (Haq et al., 2003). Fungal α-amylases are made using different fermentation techniques. The production of these α-amylases was investigated by submerged fermentation (SmF) and solid substrate fermentation (SSF) (Ramachandran et al., 2004).

To produce the enzymes used in the industry, liquid suspensions called SmF are used. However, in the 99% of environments where known microorganisms survive, free water does not exist (Hölker et al., 2004). Productions using the SmF method, may not allow for natural environments of microorganisms and negatively affect their metabolically efficiency. SSF is an enzyme production technique alternative to SmF. SSF is defined as growing microorganisms on humidity solid materials in the absence of water (Perez-Guerra et al., 2003). In this fermentation system the water amount very low compared to SmF. This is why the environments where the microorganisms adapt to can be made. One of the most important reasons why SSF is used against SmF is because of the abundance of materials used in the nature and the cheapness of the procedure. These substrates are not suitable for SmF (Hölker et al., 2004). Side products of farming are generally best substrates used in SSF. These substrates are used in producing enzyme making microorganisms. Some of the agricultural by-products are wheat bran, rice bran, corn bran, wheat hay, rice hay, rice shell, sawdust, banana peels, tea refuse, the refuse made by producing starch from cassava (*Manihot esculenta*), palm tree factory refuse, wheat flour, corn flour and starch (Pandey et al., 1999).

Siyez (*Triticum monococcum* L.), einkorn wheat, is among the rare agricultural culture legacies. Einkorn wheat depending on regional and cultural differences, is used in both food and animal feed. In the Kastamonu province (Turkey), it is being grown in an 8 km^2 area; however most of it is being used as animal feed. The rest is consumed by mixing it with bulgur and different produce (https://kastamonu.tarim.gov.tr/Belgeler/).

In this study, the main goal was to test the einkorn bran as a substrate for α-amylase production from Penicillium herquei *in SSF process.*

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MATERIAL AND METHODS

The isolation and storage of *Penicillium herquei*

The *P. herquei* used in the study is a microfungi isolated from air and received from Trakya University Arda *Vocational* School (Turkey) micro fungus collection. Stock fungus cultures were passaged to Potato Dextrose Agar (PDA) mediums once a month. The fungi cultures were stored in +4°C to use in later experiments.

Substrates

Einkorn bran (EB) and wheat bran (WB) are used as substrate. These were obtained from Alatarla village (Germeç), Taşköprü (Kastamonu, Turkey) and Yayla Flour Factory (Kırklareli, Turkey).

Inoculum preparation

A volume of 7 mL of sterile distilled water was transferred to a sporulated (7days old) PDA slant culture. The spores were dislodged using the inoculation needle under aseptic conditions and the suspension, with appropriate dilution, was used as inoculum. A volume of 1 mL of spore suspension contained about 1×10^6 spores.

Solid State Fermentation

The SSF process was carried out in 250 mL Erlenmeyer flasks containing 5 g of substrates (EB and WB). Acetate buffer (0.1 M, pH 5.0) was used to adjust the moisture content from 35 to 95%. After autoclaving at 121°C, 20 min., flasks were cooled and inoculated with 1 mL inoculum level. After incubation at 30°C for 7 days, 50 mL 0.1 M acetate buffer (pH 5.0) was added to the fermented Erlenmeyer flasks. The mixture was shaken for an hour at 30°C (150rpm/min). The slurry was squeezed through muslin cloth. The extract was filtered through Whatman No. 1 filter paper and the filtrate was used as the crude enzyme.

Enzyme assay

Soluble starch (0.8%) was dissolved in boiling 0.1 M acetate buffer (pH 5.0) and then cooled to 30°C. Fresh iodine reactant was prepared by adding 1.0 mL stock solution (5.0% KI in 0.5 I_2) to 5.0 mL 5M HCl containing 500 mL water. For analysis, the 0.1 enzyme and 0.2 soluble starch combinations were incubated at 30°C for 5 minutes. The reaction was stopped by adding 5.0 mL of iodine reactant. The absorbance was measured at 620 nm against at a blank (Abou-Zeid, 1997). One of the units of α-amylase activity was determined to be the amount of enzyme that hydrolyses 0.1 mg starch in a minute. The enzyme productivity was given as the U/g of the solid substrate (U/gds). All of the data are the mean of three repetitions.

The effect of substrate amount on the amylase synthesis of *P. herquei*

2.5, 5.0, 7.5 and 10.0 g weighed EB was placed in 250 mL Erlenmeyer flask. The final substrate moisture content of the mediums was adjusted to 65 % (w/v) with acetate buffer (pH 5.0; 0.1 M). They have been autoclaved at 121°C for 20 min. Mediums were inoculated with 1 mL ($1x10^6$ spore/mL). The flasks were incubated at 30°C for 7 days.

The effect of initial moisture levels on the amylase synthesis of *P. herquei*

EB was used as substrate. The initial moisture contents (35, 45, 55, 65, 75, 85 and 95%) of solid substrates (before autoclaving) were adjusted with acetate buffer (pH 5.0; 0.1M), autoclaved at 121°C for 20 min. Mediums were inoculated with 1 mL ($1x10^6$ spore/mL). The flasks were incubated at 30°C for 7 days.

Dry weight determination

Dry weight of the samples was determined by drying them in a hot air oven at 80°C for 24h.

RESULTS AND DISCUSSION

Polysaccharides (cellulose, hemicellulose, pectin and starch), lignin and protein in the polymeric form in nature are metabolized by different microorganisms as energy source. These substrates do not dissolve in water, absorb water on their matrix, and provide the moisture required in the SSF system for the metabolic activity and growth of the microorganism. While fungi micelles process into the substrate particles for

nutrients, bacteria and yeast cultures grow on the surface of substrate fibers and particles. Filamentous fungi can grow in SSF cultures in the absence of free water. But single-celled organisms, such as bacteria and yeasts, always require some free water in their environment (Nigam and Singh, 1994). For this reason, production with SSF is more suitable for fungi. There are many important factors affecting the SSF process. Some of these are suitable substrate choice, initial moisture levels and substrate amount.

In this study, EB and WB agricultural by-products for the production of α-amylase from *P. herquei* were tested as substrates. Among them, EB was first evaluated in the SSF process for the production of α-amylase. In addition, the effects of EB and WB mixtures on the production of α-amylase at different ratios (1:4; 2:3; 3:2 and 4:1, w/w) were investigated. Among these, EB was found to be the best substrate for the production of α-amylase from *P. herquei* (602 U/gds±5.6) (Table 1). In the studies; It was determined that the protein content of einkorn wheat varies between 14.1-25.2/100 g, which is higher than other wheat types (e.g. 10-12/100 g in bread wheat). It has been reported that einkorn wheat is rich in mineral substances and phosphorus is an important part of them. In addition, it has been reported that mineral content such as calcium, manganese, sulfur, zinc, iron, copper and selenium is more than bread wheat and most of the mineral elements are in bran fraction (https://kastamonu.tarim.gov.tr/Belgeler/).

Table 1. Wheat and einkorn bran as substrates to produce α-amylase from *P. herquei*

SUBSTRATE	ACTIVITY (U/gds)
Einkorn bran	602 U/gds±5.6
Wheat bran	292 U/gds±6.1
Einkorn bran+Wheat bran (1/4)	520 U/gds±5.0
Einkorn bran+Wheat bran (2/3)	558 U/gds±4.5
Einkorn bran+Wheat bran (3/2)	574 U/gds±4.1
Einkorn bran+Wheat bran (4/1)	594 U/gds±6.1

The α-amylase activity was the highest in the SSF process, where 5.0 g of the EB was used as a substrate (0.558±8.0) (Table 2). This amount of substrate optimized the enzyme production. Further increase in substrate concentration will not increase the yield and this increase will not affect the growth of the organism.

Table 2. Effect of the amount of substrate on the amylase synthesis of *Penicillium herquei*

SUBSTRATE AMOUNT (g)	ENZYME ACTIVITY (U/gds)
2.5	452±5.5
5.0	558±8.0
7.5	332±7.5
10.0	232±2.3

SSF cultures were moistened with acetate buffer (pH 5.0; 0.1 M) at 35, 45, 55, 65, 75, 85 and 95% (w/v) to optimize the initial moisture level. Thanks to the enzyme activity measurements at the end of the production, the best initial moisture content was determined to be 55%. The maximum yield was 518±8.9 U/gds (Table 3). The initial moisture content in SSF cultures is one of the most important factors as it affects the enzyme release and biosynthesis. It is believed that high moisture content causes a decrease in porous structure of wheat bran and thus reduced oxygen transfer and low moisture content decreases the solubility of nutrients in the substrate (Ellaiah et al., 2002). The optimum moisture content for growth and substrate use is between 30% and 80%. This situation varies according to the substrate and organism used for production. For example; in the production of *Aspergillus niger* on starchy substrates such as cassava and wheat bran, the initial moisture level is lower than that of coffee palp and sugar cane (Raimbault, 1998). In the SSF cultures prepared by using corn cob leaf, wheat bran, wheat straw and rice straw as the substrate to produce the enzyme from *Penicillium chrysogenum*, it was stated that the initial moisture content was between 55% and 75% (Balkan and Ertan, 2007). This is probably due to the water-holding capacity of the substrates. In the SSF cultures using wheat barn as a substrate, the initial moisture levels for *Aspergillus sp.* (Ellaiah et al., 2002), *Aspergillus oryzae* (Francis et al., 2002), and *Bacillus subtilis* (Baysal et al., 2003) were reported as 80, 70 and 30 % respectively.

Table 3. In the SSF cultures using einkorn bran as a substrate, the effect of initial moisture level on amylase synthesis of *Penicillium herquei*

MOISTURE (% w/v)	ENZYME ACTIVITY (U/gds)

35	280±0.64
45	306±1.5
55	518±8.9
65	482±5.2
75	436±8.0
85	282±1.9
95	254±1.1

CONCLUSION

According to the results of this study, it was concluded that EB as a substrate can be used for the production of α-amylase from *P. herquei* in the SSF process. This substrate is suitable for the industrial production of this enzyme. However, this study is a completely laboratory-scale study and should be further developed for a large-scale SSF. There are some studies that have been made to add significant added value to the agriculture and food industry of einkorn wheat. In addition to these studies, it is thought that this study will make a contribution.

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