Industrial Engineering Journal
ISSN: 0970-2555
Volume : 52, Issue 6, June : 2023

Production of Protease from *Aspergillus flavus* isolated from Agro Industrial waste of Bilaspur in Solid State Fermentation

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Abstract:
Wastes from the agro-industrial sector are an important source for enzyme and microbial production. Ten agro-industrial wastes, including Wheat Bran, Soya Oil Cake, Maize Bran, Green Gram Hull, Rice Bran, Black Gram bran, Batari bran, Bajra, Jawar and Kodo were used as substrates for Aspergillus flavus to produce protease. Estimates for protease enzyme after every 24 hours of incubation, which shows that all ten agricultural industrial wastes produce the most protease after 7 days of incubation. During present investigation, it was discovered that all the substrate studied supported development and the synthesis of enzymes. After 72 hours of incubation, wheat bran, as opposed to other substrates, allowed Aspergillus flavus to produce the most enzymes.

Index Term: **Protease, fermentation, temperature,** ***Aspergillus flavus.***

I. Introduction

Recent advancements in industrial biotechnology have led to the use of previously unknown microorganisms and the development of improved methods for enzyme production, which have increased enzyme yields and made it possible to develop an appropriate industrial process (Sumantha et al., 2006). Recycling agro-industrial waste into soils helps reduce environmental contamination caused by these waste products in developing nations. But little is understood about their chemistry and how it affects soil fertility (Wakene Negassa et al., 2011). Approximately 998 million tones of agricultural waste are produced worldwide each year. Only 15% of the waste is used as animal feed, mostly as a supplement, plant fertilizer, or soil conditioner, with the remaining waste being disposed in landfills, which has negative environmental effects, including global warming (Cauto and Sanroman, 2005). One must transform waste into value-added products in order to reduce waste. With the addition of nitrogen sources, microbes can transform agro-industrial waste, which contains fat, crude fiber, carbohydrates, and protein, into usable agricultural and industrial goods. In solid state fermentation, they are regarded as the most suitable substrates for the synthesis of enzymes.

II. Materials & Method

Ten agricultural wastes such as Wheat Bran, Soya Oil Cake, Maize Bran, Green Gram Hull, Rice Bran, Black Gram bran, Batari bran, Bajra, Jawar and Kodo were used as substrates for *Aspergillus flavus* to produce protease. All ten types of agro-industrial waste were gathered locally (Table -1).

III. Preparation of the Substrate and Solid-State Fermentation

As indicated by Tunga et al. (1998) and Ellaiah (2003), ten commercially available protein-rich agro-based substrates were utilized as solid substrates, and their effects on the synthesis of proteases were investigated. All the substrates were of animal feed grade and were inexpensive and readily available. All of the agricultural industrial waste used in this study was homogenized into a powder, about 2 mm in size, and then sieved using 20–40 mesh screens to produce particles with a diameter of 0.42–0.85 mm. The tests for SSF were carried out in a 150 ml Erlenmeyer flask with 10 gm of substrate and 50% moisture. It was properly sterilized and spore- suspension from a 7-day-old culture of A. flavus. The first seven days of incubation were spent at ambient temperature (25–30^{o}C).

S. No.	Local name	Substrates	Scientific names
1.	Wheat	Bran	*Triticum aestivum*

2.	Soya oil cake	Bran	*Glycine max*
3.	Maize	Bran	*Zea mays*
4.	Green Gram	Hull	*Cicer arietinum*
5.	Rice	Bran	*Oryza sativa*
6	Black Gram	Hull	*Vigna mungo*
7	Batari	Bran	*Pisum arvense*
8	Bajra	Grain	*Pennisetum americanum*
9	Jawar	Grain	*Sorghum bicdor*
10.	Kodo	Grain	*Paspalam scrobiculatum*

Table 1: 10 Agro-based Substrate for Solid Substrate Fermentation

IV. Enzyme Estimation

A test tube containing 5 ml of phosphate buffer was filled with 1 g of the fermented substrate from the Petri plate after every 24 hours of the solid-state fermentation process, which took place throughout a 7-day incubation period. To eliminate all particulates, the contents were homogenized and centrifuged at 2000 rpm for 30 minutes. Enzyme estimate was done on the culture filtrate that had been filtered via Whatmann filter paper. According to the advice of Keay and Wrildi, (1970), protease activity was measured. As previously noted, the protocol was utilized to create the standard graph.

S. No.	**Substrate**	**Relative Enzyme Activity U/g DMS**					
		24h	**48h**	**72h**	**96h**	**120h**	**144h**
1.	Wheat bran	15.30 ± 3.40	225.30 ± 4.51	305.30 ± 9.80	296.00 ± 10.15	274.00 ± 12.85	215.70 ± 6.51
2.	Defat soya oil cake	11.00 ± 5.00	163.00 ± 7.23	275.00 ± 5.51	278.70 ± 10.79	272.00 ± 6.66	180.70 ± 8.50
3..	Maize bran	12.70 ± 5.51	115.70 ± 8.74	133.70 ± 2.52	137.70 ± 6.03	139.30 ± 5.51	108.70 ± 6.00
4.	Green gram bran	9.00 ± 4.00	55.70 ± 4.16	86.70 ± 4.16	98.00 ± 10.58	106.70 ± 7.09	95.00 ± 6.56
5.	Rice bran	8.30 ± 3.79	30.70 ± 4.04	75.60 ± 2.52	94.40 ± 4.04	115.00 ± 6.08	81.30 ± 6.51
6.	Black Gram bran	11.70 ± 3.06	48.70 ± 3.51	55.30 ± 8.02	79.60 ± 6.11	95.00 ± 7.55	87.70 ± 8.33
7.	Batari bran	7.70 ± 2.52	33.70 ± 5.69	41.30 ± 4.73	74.60 ± 3.51	86.00 ± 1.00	71.70 ± 4.04
8.	Bajra	11.30 ± 3.51	192.70 ± 5.13	201.30 ± 6.03	196.70 ± 5.03	199.70 ± 6.43	166.00 ± 4.58
9.	Jawar	13.70 ± 3.79	165.70 ± 4.04	173.00 ± 6.08	170.00 ± 5.29	167.30 ± 1.53	105.60 ± 6.03
10.	Kodo	10.40 ± 3.06	125.00 ± 5.57	146.00 ± 6.08	140.30 ± 6.03	141.30 ± 5.86	131.00 ± 3.61

Table 2: Time course of protease production by by *Aspergillus flavus* on different solid substrates

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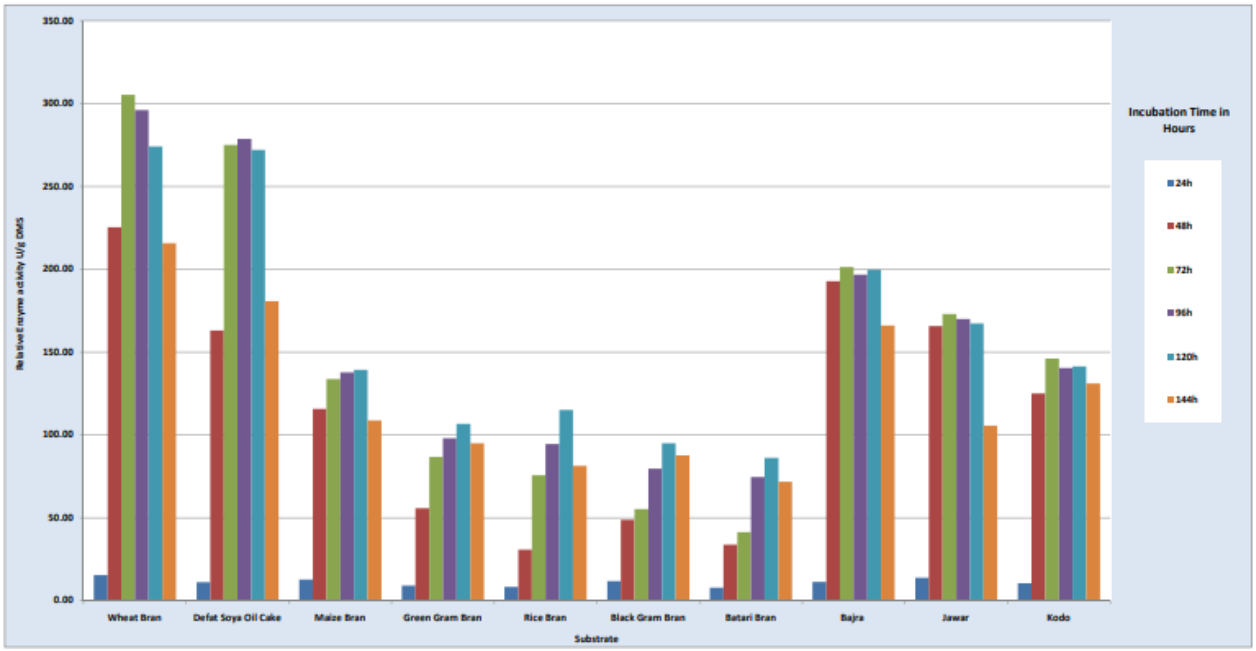

Graph 1: Utilization of agro industrial waste for production of protease by *Aspergillus flavus* in solid state fermentation

V. Results and Discussion

After every 24 hours of incubation, the protease enzyme was calculated. Results are shown in Table 2 and graph 1. It was discovered throughout the current experiment that *Aspergillus flavus* was able to thrive and produce enzymes on every substrate examined. After 72 hours of incubation, wheat bran assisted *Aspergillus flavus* in producing the greatest amount of enzyme liberation i.e. 305.30± 9.80 u/g DMS. Defatted soya cake came next, and after 72 hours of incubation, it produced proteases 90.5% more effectively than wheat bran. Table -2 shows the competency of substrates in descending order. The composition of the fermentation medium, its N and C content, and the preparation procedure all affect how much protease is produced (Dixit and Verma, 1993). Wheat bran similar ability may be seen in its adequate nutritional balance, ability to stay loose in a moist condition after autoclaving, and higher surface area that easily supports both inter- and intra-individual mass transfer. After incubating *Thermoas thermophilum* for six days, Megalla et al. (1990) succeeded in producing protease. Protease activity was discovered on the fifth day of incubation in the study conducted by Lazim et al., (2009). As opposed to *Aspergillus oryzae*, where Battaglino et al. (1991) reported that the maximum protease activity (15.80 Ug-1) was reached on the ninth day of incubation. Similar to this, Malathi and Chakraborty (1991) used SSF at 30°C to produce the most protease activity from *Aspergillus flavus* between the fifth and seventh day of incubation.

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