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UTILIZATION OF AGRICULTURAL BY PRODUCTS FOR THE PRODUCTION OF LIPASE BY *ASPERGILLUS NIGER* BY SUBMERGED FERMENTATION

Javaid Iqbal* and Muhammad Yaqub**

Wheat bran and other cereal brans were used for the production of lipase by *Aspergillus niger* by submerged fermentation. The opimum pH, incubation time and wheat bran concentration were 7.0, 96 hours and 1% respectively. Addition of olive oil and other lipid materials stimulated the enzyme production significantly. Starch and sucrose as carbon sources and ammonium nitrate and soybean meal as nitrogen sources slightly affected the enzyme production. Wheat bran seemed to be the best substrate for lipase biosynthesis by *Aspergillus niger*.

INTRODUCTION

Production of lipase by submerged fermention using chemically defined media has been reported earlier (Mamoru *et al.* 1965; Juichiro *et al.* 1966; Kundu and Pal, 1978; Sviridenko *et al.* 1978). Wheat bran provides all the nutrients necessary for mould growth for the production of enzyme. Pakistan being an agricultural country have abundant supply of this raw material.

Attempts were made to explore the possibilities for the exploitation of brans of various cereal brans, wheat bran in particular, for the enzyme production in conical flasks. Effect of pH, incubation time, concentration of wheat bran, lipid materials, carbohydrates and various nitrogen sources were studied.

MATERIALS AND METHODS

1. Isolation of Lipase Producing Mould Cultures

Strains producing lipolytic enzyme were isolated from the soil. The isolated strains were identified as *Aspergillus niger* following the method of Raper and Fennel (1957). These cultures were grown on the Czapak's dox medium containing olive oil. Stock cultures were preserved in soil containing olive oil (1%) and sucrose (1%).

* Food Technology & Fermentation Division, PCSIR Laboratories, Lahore.
** Department of Chemistry, University of Agriculture, Faisalabad.

2. Inoculum Preparation

Vegetative inoculum (24 hr old) was used throughout the present studies. The spores from 5-7 days old cultures were wetted with 5 ml. 0.05 % Monoxal 0.T (diacetyl ester of sodium sulphosuccinic acid). Spore suspension (2 ml) was poured aseptically into 250 ml. conical flask containing g/100 ml of the medium NH_4NO_3 0.1, $MgSO_4 . 7H_20$ 0.04, K_2HPO_4 0.2 and sucrose 1.0. The flask was placed on a rotary shaker (120 r.p.m.) for 24 hours at $30 \pm 2°C$

3. Fermentation Procedure

Wheat bran, was purchased from the local market. Wheat bran (2.5 g) was suspended in 250 ml distilled water contained in cotton plugged one litre conical flasks, and adjusted to pH 7.0 These flasks were autoclaved 121°C for 15 minutes. After cooling, these flasks were inoculated with 2 % vegetative inoculum eseptically Flasks were placed on the rotary shaker for 96 hours at $30 \pm 2°C$.

After 96 hours incubation, flasks were taken off and centrifuged in order to obtain supernatent fermented broth. Enzyme was precipitated with alcohol and the precipitate were dissolved in 100 ml 0.2 M phosphate buffer pH 6.5.

4. Assay of Lipase

The lipase activity was determined by the method described by the Kundu and Pal (1970). Lipase unit was defined as that amount of activity which required 1 ml. of 0.05 N KOH to neutralize the liberated fatty acids under the assay conditions.

RESULTS AND DISCUSSION

1. Screening of Cultures

15 isolated strains of *Aspergillus niger* were screened to select the highest amount of lipase producing strain by surface culture method (Kundu and Pal, 1970). Table-1 indicates that strain No. 6 gave better results and was selected for further studies as *Aspergillus niger*.

2. Rate of Lipase Formation

The fermentation medium was incubated upto 144 hours at 35°C. The enzyme activity was determined at various time intervals. The optimum incubation period was found to be 96 hours.

3. Effect of pH

The initial pH of the fermentation medium was adjusted to various values with 0.1 N HCl or NaOH. pH of the medium during fermentation was kept constant by adding sterile 0.1 N NaOH at regular intervals under aseptic conditions. Fermentation was carried out at 35°C for 96 hours. Lipase production was maximum at pH 7.0. The enzyme formation was greatly affected at extreme levels of pH. Pal and Kundu (1978) reported the same pH (7.0) for maximum enzyme production while Valdehra and Harmon (1969) reported the pH range 7.5 - 9.0.

4. Effect of Wheat Bran Concentration on the Production of Lipase by Aspergillus Niger

Various amount of wheat bran (1–5 %) were employed as substrate for the production of lipase. Table-2 showed that the enzyme formation was maximum when 1% wheat bran was used and it remained about the same at 2 and 3 % of wheat bran concentrration. There was a non-significant difference among the means of three levels of wheat bran i. e. (1, 2 and 3 %). For further investigations 1% wheat bran was used keeping in view the better yield and economic factor.

5. Effect of Addition of Rice Bran, Maize Bran, or Gram Bran on the production of Lipase by Aspergillus Niger

Table-3 showed the effect of addition of different agricultural by-products, such as rice bran, maize bran or gram bran to the fermentation medium for the synthesis of lipase. The enzyme production was decreased by adding such cereal brans except rice bran. Wheat bran was found to be the best substrace for biosynthesis of lipase by *A. niger* in shake culture.

6. Effect of Inorganic Nitrogen sources on the production of Lipase

The nitrogen sources play a significant role in the enzyme formation. The effect of different inorganic nitrogen sources were studied (Table–4). The amount of inorganic nitrogen sources were added on the basis of 0.1% nitrogen to the fermentation medium. The analysis of variance shows that Ammonium nitrate gave a significant yield of enzyme, while other nitrogen sources suppressed the enzyme formation. Fukumoto *et al* (1964) used combined $NaNO_3$ and poly peptone. Troflmenko *et al* (1975) recommended $(NH_4)_2SO_4$ in combination with corn as nitrogen source. For the present strain NH_4NO_3 0.1% proved to be the best nitrogen source for the production of lipase.

7. Effect of Organic Nitrogen sources on the production of Lipase by Aspergillus Niger

The effect of organic nitrogen sources on lipase synthesis was also investigated. The organic nitrogen sources were added to provide 0.1% nitrogen to the fermentation medium.

Among all organic nitrogen sources, soybean meal slightly increased the lipase production as compared with control culture (Table-5). Analysis of variance shows a significant difference between medium containing soybean meal and control. Mieko *et al* (1966) recommended soybean meal as nitrogen source. Szell *et al* (1975) obtained maximum yield by supplementing the medium with soybean meal and corn steep liquor.

It was quite clear that soybean meal is a better nitrogen source for the production of lipase by *Aspergillus niger*.

8. Effect of Carbohydrates on the production of Lipase by Aspergillus Niger

The effect of different carbon sources on lipase formation was studied. It was found that sucrose and starch induced the enzyme synthesis, while glucose, fructose, lactose and molasses checked the production of enzyme (Table-6). It is evident from the analysis of variance that there was a significant difference in case of strach and sucrose. Kundu and Pal (1978) reported sucrose and Juichiro *et al* (1966) recommended glucose as an essential constituent in the medium. The present strain gave good yield of enzyme when sucrose or starch were added to the fermentation medium.

9. Effect of Lipid materials on the production of Lipase by Aspergillus Niger

The lipid materials act as an inducer for the enzyme synthesis. Addition of different lipid materials to the fermentation medium increased the lipase production (Table-7). Butter fat and mustard oil caused moderate enzyme production, whereas coconut and peanut oil did not increase the anzyme yield. Addition of 1% olive oil to the fermentation medium proved to the best inducer for this strain. Atve and Markov (1976) also reported higher yield of anzyme with the addition of olive oil.

Table 1. *Screening of Cultures*

Strain No.	Lipase Activity Units/5 ml.
1	2.6
2	3.6
3	4.5
4	3.4
5	10.0
6	20.3
7	10.0
8	12.4
9	11.3
10	3.2
11	6.5
12	2.1
13	7.3
14	2.9
15	13.6

Critical difference = Cd_1 S.E. × 0.05t = 0.632 × 2.145 = 1.356
Cd_2 S.E. × 0.81t = 0.632 × 2.624 = 1.658

*Enzyme activity was determined after 48 hours incubation at 30°C.

Table 2. *Effect of Wheat Bran Concentration*

Concentration of wheat bran %	Lipase activity u/5 ml
1	13.2
2	12.2
3	10.3
4	7.4
5	3.3

Cd_1 = 2.535
Cd_2 = 4.203

Fermentation medium (pH 7.0) was incubated at 35°C for 96 hours.

Table 3. *Effect of addition of rice bran, maize bran, or gram bran on the production of lipase by A. niger*

Cereal brans 1%	Enzyme activity u/5 ml
Rice bran	10.4
Maize bran	8.5
Gram bran	5.9
Wheat bran (control)	11.9

$Cd_1 = 1.479$

$Cd_2 = 2.716$

Fermentation medium (pH 6.5) was incubated at 05°C for 96 hours.

Table 4. *Effect of inorganic nitrogen sources on the production of Lipase by A. niger*

Nitrogen source 0.1%	Lipase activity u/5 ml
Sodium nitrate	5.6
Ammonium nitrate	13.2
Ammonium chloride	5.2
Ammonium sulphate	6.1
Di-ammonium hydrogen phosphate	3.9
Control	9.3

(Critical difference) $Cd_1 = 0.727$
" " $Cd_2 = 1.141$
*pH of the medium was adjusted to 6.5
**Samples were analyzed after 96 hours incubated at 35°C

Table 5. *Effect of organic nitrogen sources on the Production of lipase by Aspergillus niger*

Niterogen sources 0.1%	Enzyme activity u/5 ml
Urea	4.6
Peptone	3.5
Corn steep liquor	2.6
Penicillium waste mycelium	2.4
Cotton seed meal	9.6
Peanut meal	4.9
Soybean meal	11.6
Control (Wheat bran)	9.7

(Critical difference) $Cd_1 = 0.669$
" " $Cd_2 = 0.990$

'** were analyzed after 96 hours incubated at 35°C.

Table 6. *Effect of Carbohydratas on the production of lipase by A. niger*

Carbon sources 1 %	Enzyme activity u/5 ml
Starch	12.4
Sucrose	16.4
Glucose	6.7
Fructoss	6.7
Lactose	6.1
Molasses	6.0
Nil (Control)	11.3

(Critical difference) $Cd_1 = 1.189$
" " $Cd_2 = 1.801$
*Samples were analysed after 96 hours incubated at 35°C.
**pH of the medium was adjusted at 6.5.

Table 7. *Effect of lipid materials on the production of lipase by A. niger*

Lipid materials 1%	Lipase enxyme actizity u/5 ml
Mustard oil	10.7
Peanut oil	9.9
Coconut oil	6.7
Butter fat	14.9
Olive oil	18.8
Contrcl	9.5

(Critical difference) $Cd_1 = 1.293$
" " $Cd_2 = 2.028$
*Samples were analyzed after 96 hours incubated at 35°C.
**pH of the medium adjusted to 6.5.

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