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# Solid State Fermentation of Fresh Orange Pomaceby as Per Gillusniger for Production of Pectinase

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***Abstract:*** *Production of pectinases by Aspergillusniger was successfully carried out in solid state fermentation.In this study, orange pomace (one of the main source of pectin in the nature) was used as substrateand wild type of A.niger was isolated from a rotten lemon. The important parameters (such as temperature, moisture, C/N ratio) affectingonexo and endopectinases activities were optimized. The results indicated that the produced pectinases exhibit maximum activity in the range of 50-55℃ and maximum enzyme productivity occurred at moisture content of 70% (w/w) and C/N ratio of 10.Finally, activity of produced pectinases was evaluated by natural apple juice and it was confirmed that juice yield and clarity werestrictlyas a function of enzymatic hydrolysis activity.*

***Keywords:*** *Aspergillusniger, pectinase, pomace, solid state fermentation*

## 1. INTRODUCTION

Enzyme productionvia solid state Fermentation (SSF) was proposed as an alternative method to submerge fermentation (SmF). SSF processes are interesting for countries with abundance agricultural and industrial wastes. In Asian countries, application of SSF technology in industrial scale for enzyme production is considered as a reliable fermentation process. Nevertheless, there are still lots of challenges for implementation of SSF technology in large scale due to lack of suitable bioreactor and controlling units and accessories for SSF process which guarantee an acceptable production yield [1].

Pectinases are one of the most important groups of enzymes that can be obtained by SSF much effective than SmF [2]. Pectinases are responsible for the hydrolysis of pectinic chains found in the plant cell walls. Pectin is agalacturonic acid-rich polymer in primary cell wall and middle lamella of plant cells which plays an important role as a structural component. It is responsible for maintaining the integrity and safety of plant tissues [3, 4]. Pectin found and characterized in several fruits and vegetables [5-10].If pectin is hydrolyzed by pectinases; then the cell is ruptured and cell component is out.

Pectinases are widely used in fruit juice industry for the improvement of juice extraction yield of various fruits and accelerating clarification of extracted juices (see Fig.1)[11].Furthermore, it is used for oil extraction, degumming of plant fibers in pulp and paper industry and pretreatment of pectinic acid in wastewater [12].

Produced pectinases by Aspergillus species are commercially important for beverage industries. Several forms of pectinases were identified by Aspergillus species [13].In this study, A.niger was isolated from rotten lemon which seems well adapted to pectic substrate. According to previous works, it is confirmed that pectin induces pectinase production [2]. So it would be suitable and preferable to use pectin as substrate for pectinase production. Table 1 summarizes fruits and citruses peels which are the main sources of pectin in the nature. Citruses are plentiful and locally available in fruit markets (North of Iran). Furthermore, citrus peel can be used in its original form without any especial pretreatment in solid state fermentation.

**TABLE 1: Composition of Pectin in Different Fruits and Vegetables [14]**

| Fruit | Pectin content (%) | Fruit | Pectin content (%) |
|---|---|---|---|
| **Apple** | 0.5-1.6 | Blackcurrant | 0.8-1.1 |
| **Banana** | 0.7-1.2 | Cranberry | 0.8-1.2 |
| **Peaches** | 0.1-0.9 | Grape | 0.1-0.5 |
| **Strawberries** | 0.6-0.7 | Lemon | 1.8-2.2 |
| **Cherries** | 0.2-0.5 | Carrot | 1.2-1.5 |
| **Peas** | 0.9-1.4 | Mango | 0.3-0.5 |
| **Apricot** | 0.8-1.0 | Pineapple | 0.04-0.1 |
| **Oranges** | 0.5-3.5 | Plum | 0.7-0.9 |
| **Citrus peel** | 25-30 | Raspberry | 0.4-0.6 |
| **Blackberry** | 0.7-0.9 | | |

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Several effective parameters can influence the activity of obtained pectinase, the most important parameters are; temperature, pH, moisture content and C/N ratio. These parameters were extensively studied with different species of microorganisms as enzyme producers. In this work, we optimized these parameters and the effect of extracted enzyme at optimum condition was tested on the quality of apple juice.

## 2. MATERIAL AND METHOD

Microorganism isolation: Aspergillus niger was isolated from a rotten orange (Fig.2).The purified strain was cultured and maintained on a solid medium contained (in g per 100mL distilled water): pectin, 1; yeast, 0.5; peptone, 0.5; $MgSO_4$, 0.01; $K_2HPO_4$, 0.2 and agar, 1.5.

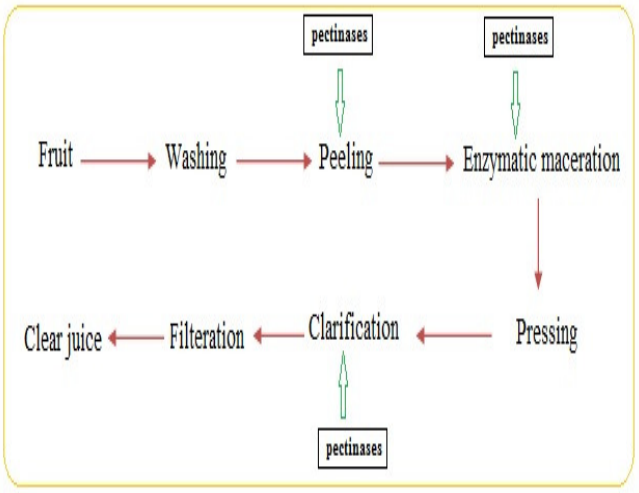


**Fig. 1. Fruit juice extraction process**

**Fig. 2. Isolated A.niger on plate agar**

Spore suspension preparation: Spore suspension was prepared by washing a 5-day incubated plate agar media with isotonic saline solution after sporulation and added to media to reach a final concentration of $4\times 10^7$ spores $g^{-1}$.

Substrate preparation: Fresh orange pomace with initial moisture content of 25-30% was obtained and grinded. Then ammonium sulfate and yeast extract were added as nitrogen sources. The media were optimized for nitrogen sources according to procedure reported by Mrudula et al. and obtained data were confirmed in our work. The moisture was fixed at 70% (w/w) and pH value set at 5[15].

Enzyme extraction: The enzyme was extracted by mixing acetate buffer (pH= 5) with cultured media after 96h incubation. The extracted solution was filtered and centrifuged at 4000 rpm for 20 min to separate suspended spores, and then it was maintained at 3℃ for further analysis.

### 2.1 ENZYME ASSAY

Endopectinase activity was assayed in a reaction mixture containing 1 mL of extract and 18 mL of 2% pectin in acetate buffer (0.1 M, pH 4.5) and reduction in viscosity was determined by Ostwald capillary viscometer (distilled water was used as reference). One unit of endopectinase activity was defined as the amount of enzyme required to reduce the viscosity by 50% in one minute.

Exopectinase activity was assayed in a reaction mixture containing 0.3 mL of suitable dilution of enzymatic extract and 0.7 mL acetate buffer (0.1 M, pH 4.5) and 1 mL of 0.9% pectin in acetate buffer. Reducing groups were determined by DNS method. One exopectinase unit was defined as the amount of enzyme that catalyses the formation of one µmol of galactronic acid per minute.

Evaluation of enzymatic activity on apple pulp: Apples were sliced into 5 mm cubes on a side. Equal amount of chopped apples (50 g) were put into two beakers at the same conditions. One mL of extracted pectinase was added to one beaker and 1 mL deactivated pectinase (incubate for five minutes heating in boiling water) was added to another beaker. Both beakers were kept at 50℃ for duration of 2 h and the produced juices were compared.

## 3. RESULT AND DISCUSSION

### 3.1 TEMPERATURE OPTIMIZATION

Exo and endo pectinases activities were measured at different temperature in the range of 30-70℃. Both Exo and Endo pectinase activity showed the highest activitybetween50-55℃ (Figs. 3 and 4).These results were confirmed with reported data by Arguelles et al. for exo and endopectinase activities [16].

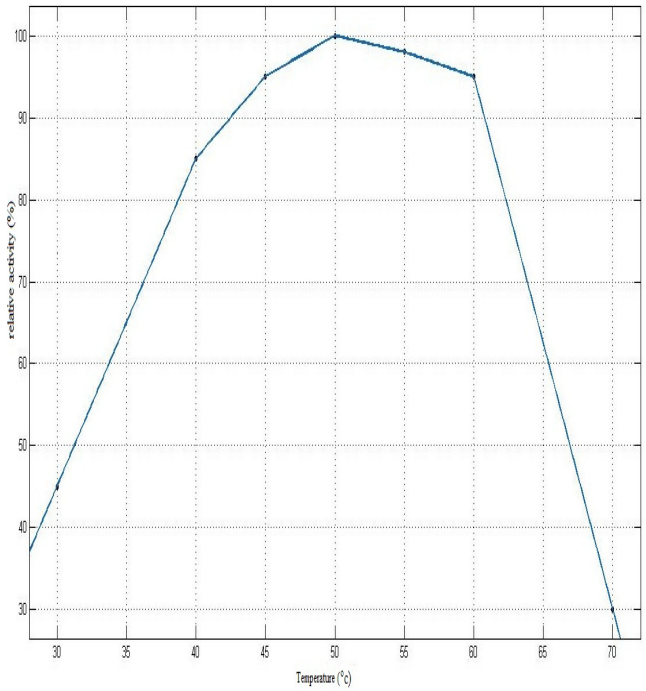


**Fig. 3. Effect of temperature on exopectinase activity**

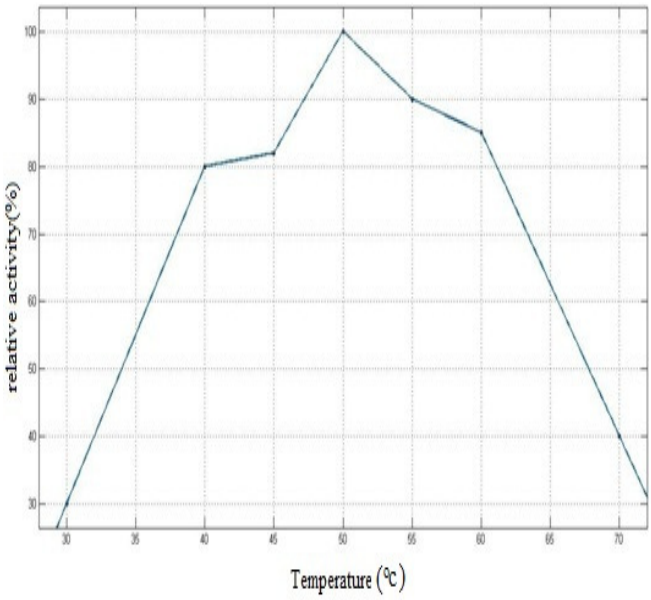


**Fig. 4. Effect of temperature on endopectinase activity**

### 3.2 MOISTURE OPTIMIZATION

Effect of moisture as an effective parameter in SSF was investigated. The results indicated that in the water content of 70% (w/w) the maximum activity was achieved.Figs. 4 and 5 exhibit the effect of different moisture on exo and endopectinase activities which indicate that moisture has great impact on exo and endopectinase activities.

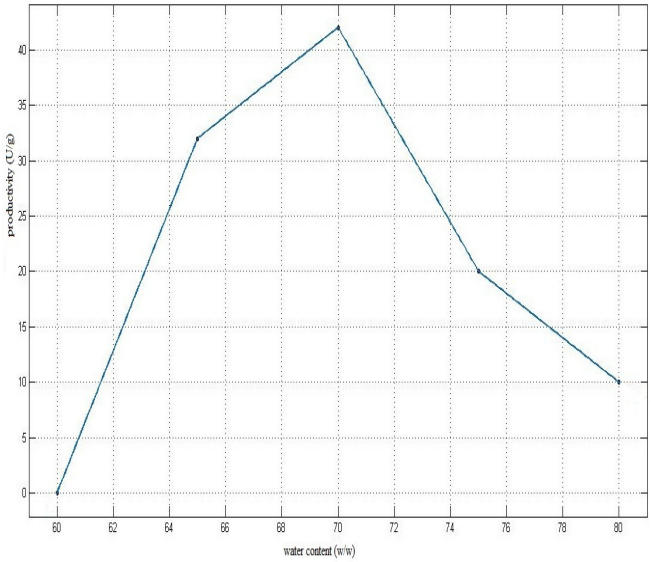


**Fig. 5. The effect of moisture on exopecinases activity**

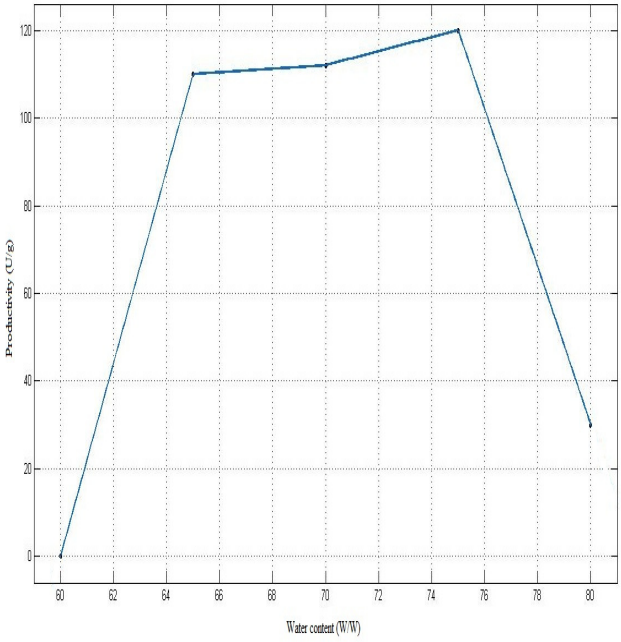


**Fig. 6. The effect of moisture on endopectinase activity**

### 3.3 C/N RATIO OPTIMIZATION

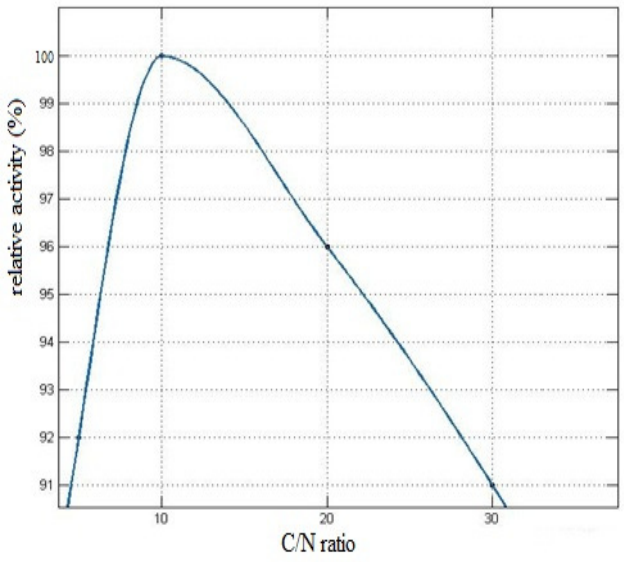


**Fig. 7. The effect of C/N ratio on exopectinase activity**

### 3.4 EVALUATION THE EFFECT OF OBTAINED ENZYME ON QUALITY OF APPLE JUICE

Drop of viscosity in apple juice due to enzymatic treatment:

Low viscosity of juice accelerates movement of the fluid in the heat exchanger so that pumping energy requirement decreases. In order to decrease viscosity of crude juice, usually enzymatic treatment is carried out. According to quantify, the effect of produced pectinases on juice viscosity, reduction in viscosity of 18mLof apple juice was investigated. The procedure is similar to method declared in endopectinase activity measurement. The results showed that with enzymatic treatment, the viscosity reduced about 7.2%.

Pectinases increase soluble Sugar concentration of apple juice:

Soluble sugar concentration increases due to action of pectinases on insoluble pectin which is suspended in apple juice. It was quantified by the described method for determination of exopectinase activity. The results indicated that suitable amount of enzyme can increase the soluble sugar by 7% (w/v), which implicate that apple juice would become sweater by the use of pectinases treatment.

Pectate formation:

Apple pectin is highly methylated. Pectin estrase is a kind of pectinases that strip methoxyl groups from pectin molecules as a result negative charge on pectin chains increases. In the presence of calcium ions calcium pectate is formed which is insoluble and gradually precipitate; finally, the clarified juice is produced[18]. In order to evaluate the effect of extracted pectinase on apple juice, 1 mL of enzyme mixed with 18 mL of apple juice and the mixture is filtered after one hour (similar procedure was carried out for the deactivated enzymatic extract). The obtained results in presence of pectinases revealed that pectate formation increased by 19% (Fig. 8).


**Fig. 8. Coagulated pectate of apple juice (after filtration) with and withoutenzyme treatment**

Improvement of apple juice extraction:

Enzymatic activity on natural apple was evaluated according to described method. The results showed that extraction of juice in the beaker with active enzyme was13mL more than another beaker.

## 4. CONCLUSIONS

In this study, attempt was made to produce pectinase via SSF using a pectinase producing strain of Aspergillusniger. Furthermore for maximum enzyme productivities, effective parameters such as temperature, moisture and C/N ratio were optimized. Obtained results showed a good compatibility with previous works. The produced pectinases exhibit significant activity in both artificial and natural pectinic environment.

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