ISSN 1330-9862 *original scientific paper*
(FTB-1680)

Pectin Esterase Production from Apple Pomace in Solid-State and Submerged Fermentations

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Received: November 11, 2005
Accepted: March 14, 2006

Summary

Comparison of the production of pectin methylesterase (PME) by *Aspergillus niger* from apple pomace in solid-state (SSF) and submerged fermentation (SmF) was made for higher PME production. pH value of 4.0, incubation temperature of 25 °C and incubation period of 96 h were found optimum in both SSF and SmF, while optimum dilution levels were 1:3 and 1:6, respectively. Ammonium sulphate in SSF and diammonium hydrogen phosphate in SmF as nitrogen sources at the rate of 0.2 % gave the highest yield of PME. Sodium chloride at the rate of 0.5 % in SSF and manganese sulphate at the rate of 2 % in SmF as additives gave the highest yield of PME. Overall, the SSF gave 2.3 times higher PME activity than SmF, using optimized parameters of fermentation.

Key words: apple pomace, pectin methylesterase, solid-state fermentation, submerged fermentation, *Aspergillus niger*

Introduction

Apple is grown worldwide including India (*1*) and around 30 % of the total yield is converted into different products like juice, concentrate, canned slices, wine, cider, *etc*. (*2*). Apple pomace is a leftover residue after juice extraction containing peel, seeds and remaining solid parts representing about 25–35 % of the quantity of the processed apples (*3,4*). Apple pomace is a rich source of pectin besides other nutrients, hence it can serve as an important natural substrate for pectinase production.

Pectinase enzymes include pectin methylesterase (pectin esterase) and depolymerising enzyme (polygalacturonase and lyases). Pectin esterase hydrolyses the pectin to methanol and polygalacturonic acid (*5*) and the enzyme polygalacturonase further hydrolyses the polygalacturonic acid into monogalacturonic acid by breaking the glycosidic linkage (*6*). Pectinases find extensive applications in fruit processing industries including clarification of fruit juices, wines, extraction of fruit juice, in the manufacturing of pectin free starch, or curing of coffee (*7,8*).

Pectinase is produced by several fungi including *Aspergillus* sp., *Botrytis cinerea*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Rhizopus stolonifer*, *Trichoderma* sp., *Neurospora crassa, etc.*, but *Aspergillus* is the major source (*9*). Among pectinases, pectin esterase (E.C. 3.131.11) is the first enzyme of pectin hydrolysis that catalyses the hydrolysis of methylated carboxylic ester group in pectin into pectic acid and methanol. It is used in the treatment of certain foodstuffs and can be produced by a wide variety of plants and microorganisms (*10*).

Enzymes like amylase, xylanase and cellulase have been produced from apple pomace as a substrate (*11*) but information on pectinase production from apple pomace is scanty especially with respect to optimum pro-

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duction conditions. However, the production of pectinase from apple pomace is promising. It has several advantages like easy availability of cheaper raw material and easier processing of the substrate. Being economical, it may lead to reduction in cost of the enzyme and may prove as an efficient method of pomace utilization.

Materials and Methods

Raw material

Partially dried apple pomace (10–11 % moisture) was procured from Himachal Pradesh Horticultural Produce, Processing and Marketing Corporation, Processing unit at Parwanoo, Himachal Pradesh, India. It was further mechanically dehydrated at (60±1) °C until the moisture content of dried apple pomace reached (4±1) %, which generally took 3–4 h. Dried apple pomace was ground into fine powder and packed in polyethylene pouches for further studies.

Inoculum

Strain No. UHF 121 was selected out of 10 strains of *Aspergillus niger* and employed to conduct both the solid-state and submerged fermentations. Inoculum was prepared by suspending one loop of inoculating needle (30-mm diameter full of spores of 5-day-old culture) in 2 mL of distilled water.

Effect of different dilution levels

To find out optimum dilution level for maximum pectin esterase production in solid-state fermentation (SSF), 250-mL conical flasks, each containing 25 g of apple pomace powder, were supplemented with different quantities of water (1:3, 1:4, 1:5 and 1:6) and were sterilized at 121 °C for 15 min as per standard procedure. After sterilization, the flasks were cooled, inoculated with 2 mL of spore suspension/inoculum and incubated at 28 °C for 36 h. For submerged fermentation (SmF), 25 g of apple pomace powder was diluted with water in the ratio of 1:6, 1:7, 1:8 and 1:9, and boiled for half an hour. Apple pomace extract (APE) was taken out, then sterilized, cooled, inoculated and incubated as for SSF. The enzyme extracts were kept in a refrigerator until they were used for enzyme assay. The apple pomace to water ratio in preparation of APE that gave the highest pectin esterase production was selected for further studies.

Effect of pH

For both fermentations, the initial pH values of the medium obtained after dilution with water were adjusted to 3.5, 4.0, 4.5 and 5.0, using 0.1 M HCl or NaOH solution. After inoculation, all flasks were incubated at 28 °C for 96 h.

Effect of incubation temperature

For both fermentations, a range of temperature (22, 25, 28 and 31 °C) was used to incubate the presterilized and inoculated flasks containing apple pomace in optimum dilution ratio for 96 h.

Effect of incubation periods

Flasks containing diluted, sterilized and inoculated (described earlier) apple pomace were incubated at a temperature of 28 °C for 24, 48, 72, 96, 120 and 168 h. The optimum incubation period was selected for both types of fermentations.

Effect of different nitrogen sources

For both SSF and SmF, diammonium hydrogen phosphate (DAHP), ammonium sulphate, urea and peptone at the rate of 0.1 and 0.2 % each were added in 250-mL conical flasks containing 25 g of apple pomace powder moistened with 75 mL of distilled water and were sterilized as described earlier. The flasks were cooled, inoculated with 2 mL of spore suspension and incubated at 28 °C for 36 h.

Effect of different additives

The effect of different additives ($MgSO_4$, $MnSO_4$, NaCl and $FeCl_3$, which were used at concentrations of 0.5, 1.0, 1.5 and 2.0 % each) on pectin esterase production was studied in both fermentations. The measured quantity of additives was added to the medium obtained after the dilution of apple pomace with water (1:3). After sterilization, the flasks were inoculated as described for nitrogen source.

Assay of pectin esterase activity

The method described by Ranganna (*12*) was used for the assay of pectin esterase. To extract the enzyme, 0.25 M NaCl solution was added at the rate of 40 mL/100 g of medium to each flask, which were allowed to stand for one hour with intermittent stirring. The mixture was then filtered through a muslin cloth and the extract thus obtained was referred to as the enzyme extract. The PME was measured by the addition of 2 mL of 1.5 M NaCl to 10 mL of 1 % pectin solution. A few drops of Hinton's indicator were then added and it was titrated to pH=7.5 with 0.02 M NaOH. The mixture was transferred to a constant temperature water bath maintained at 30 °C. When the pectin solution had obtained the temperature of the bath, enzyme sample and water were added to adjust the volume to 20 mL. Immediately, time and volume of alkali required to maintain the pH at a constant value were recorded and the results were expressed in pectin methylesterase units (PME U/g of the expression for milliequivalents of ester hydrolyzed per minute per gram of enzyme) as given below:

$$\text{Activity of pectin methylesterase/(U/g)} = \frac{V(\text{in mL})\text{ of 0.02 M NaOH consumed}\cdot 1\text{ min}\cdot 3.1\cdot\text{dilution}}{V(\text{in mL})\text{ of enzyme preparation}\cdot\text{total time of determination in min}}$$

Results and Discussion

The results (Fig. 1) show that maximum PME production took place at dilution level of 1:3 for SSF and 1:6 for SmF, but decreased subsequently with the increase in dilution levels in both cases. At each level of dilution,

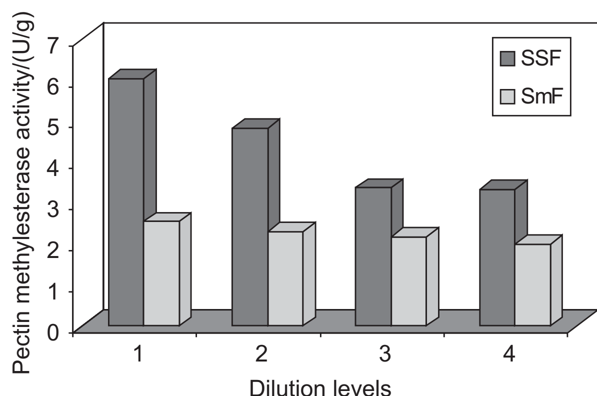

Fig. 1. Comparison of dilution levels on pectin methylesterase activity in SSF and SmF
Dilution levels for SSF: 1=1:3, 2=1:4, 3=1:5, 4=1:6
Dilution levels for SmF: 1=1:6, 2=1:7, 3=1:8, 4=1:9

SSF produced an enzyme with more PME activity and it was about 2.3 times greater at a dilution level of 1:3 compared to SmF. Interestingly, with the increase in dilution levels, the enzyme production was reduced rather drastically in the SSF but no such effect was seen in SmF. Apparently, the conditions of fermentation had more effect than the dilution level only.

The differences in PME production in SSF and SmF were also clear at different pH values (Fig. 2). Although optimum dilution levels were different for SSF and SmF, optimum pH in both cases was the same (pH=4.0). This may be due to the fact that the optimum pH for the production of PME is more related to the optimum conditions required for the growth of specific microorganism

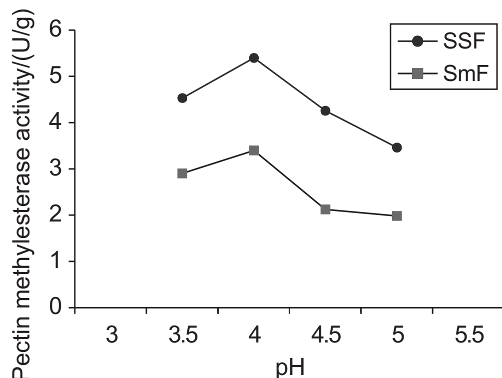

Fig. 2. Effect of pH on pectin methylesterase activity in SSF and SmF

employed to conduct the fermentation than other factors, so it may have remained in a particular range for the same microorganism, irrespective of the type of fermentation. However, even here the PME production was higher in SSF than SmF at all pH values. Data in Fig. 3 clearly indicate that the PME activity was again higher in SSF than SmF, irrespective of the incubation temperature. Although maximum PME production was recorded at 25 °C in both fermentations, a decrease in PME production was observed both at higher and lower temperature than 25 °C. As stated earlier for pH, the optimum incubation temperature is specific and related to

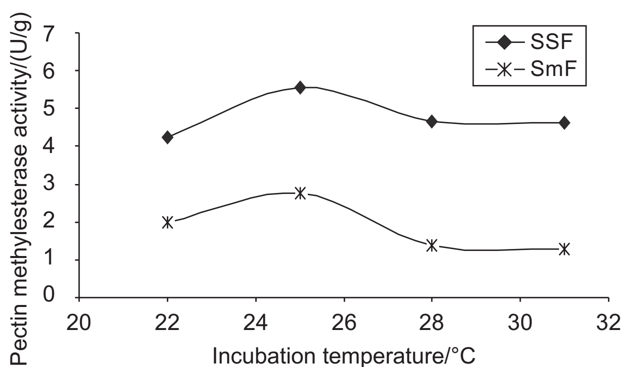

Fig. 3. Effect of incubation temperature on pectin methylesterase activity in SSF and SmF

the producer organism, hence similar results in SSF and SmF were obtained. The results of incubation periods (Fig. 4) show that with the increase in the incubation period in both fermentations, the PME activity increased. The lowest PME activity was observed at 24 h of incubation, while the maximum was recorded at 96 h of incubation period and thereafter, only negligible increase in PME production took place, irrespective of SSF or

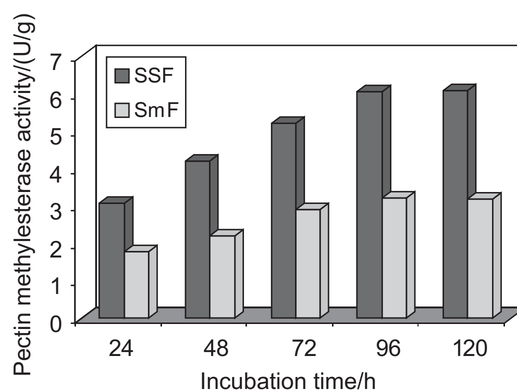

Fig. 4. Effect of incubation time on pectin methylesterase activity in SSF and SmF

SmF. But here too, SSF proved superior to SmF in all incubation periods. The trends obtained, irrespective of the influence of the type of nitrogen source (Table 1) on PME, showed that SSF exhibited higher PME production than SmF. Amongst the various nitrogen sources used in SSF, maximum PME activity was found when apple pomace medium was supplemented with ammonium sulphate, closely followed by DAHP. However, in SmF maximum PME activity was observed in DAHP-supplemented medium. Out of different additives employed in SSF, maximum PME activity was observed in NaCl-supplemented medium (Table 1). An overall view of the results obtained so far from these experiments clearly exhibited that the SSF produced maximum PME activity in apple pomace medium supplemented with different additives. However, in submerged fermentation, $MnSO_4$ gave the highest PME production. Although the exact reason for increasing the PME production in SSF by the use of NaCl is not clear, it seems to enhance the stability of the enzyme against inactivation. In case of

Table 1. Effect of nitrogen sources and additives on pectin methylesterase activity (units) in SSF and SmF

Nitrogen source	Fermentation	
	Solid-state	**Submerged**
Peptone	5.40	1.80
Diammonium hydrogen phosphate	6.35	2.96
Ammonium sulphate	6.46	2.30
Urea	5.77	2.44
CD (P≥0.05)	0.105	0.07
Additive		
Magnesium sulphate	6.22	2.48
Manganese sulphate	5.54	2.96
Sodium chloride	6.75	2.14
CD (P≥0.05)	0.420	0.477

SmF (where dilution needed was higher than in SSF), Mn content may have become critical or limited so its supplementation might have enhanced the enzyme production in SmF but not in SSF.

Solid-state culture conditions are reportedly more suitable than liquid for the growth of filamentous fungi (*13*) and might be the reason behind our findings of higher enzyme production in SSF than SmF. Higher productivities of endo- and exo-pectinase and pectin lyase were obtained with SSF than SmF, using *A. niger* as a strain (*14*). Also, solid substrates not only supply the nutrient to the microbial cultures growing on it, but they also serve as an anchorage for the cells (*15*), and low moisture content of fermenting medium in SSF might have prevented the bacterial contamination (*16–18*). It has also been reported earlier that the regulatory mechanism of pectin methylesterase production by *A. niger* in SSF and SmF is different, *i.e.* SSF pectinase synthesis is less affected by the catabolic repression than SmF (*19*). Therefore, our results are in agreement with these views and are desirable too.

Conclusions

An overview of the results obtained show that simple SSF of apple pomace was found suitable to produce low bulk, high value product, *i.e.* pectinase, by giving 2.3 times higher PME activity than SmF using optimized parameters of fermentation. Thus, production of pectinase from apple pomace can become a potential tool to prevent environmental pollution as well as the pectinase enzyme itself can find a number of applications in the food processing industry.

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