Optimization and purification of cellulase produced by *Penicillium decumbens* and its application

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Background and objectives
Cellulases are an enzyme group based on catalytic action. They include endocellulase, exocellulase, beta glucosidase, cellulose phosphorylases, and oxidative cellulases. This work was aimed at the production of cellulase by fungal strains from *Penicillium* sp. Selection of the best organism that gives the highest productivity of the enzyme, examination of the cellulase production under the optimum conditions, purification of cellulase, identification by high-performance liquid chromatography, and its application in clarification and yield increase of apple juice were also studied.

Materials and methods
Three strains of *Penicillium* sp. were examined using the method of Congo red for cellulase production. The factors affecting cellulase production by fungus *Penicillium decumbens* were identified. Cellulase produced by *P. decumbens* was purified using ammonium sulfate precipitate (80% saturation) followed by ion exchange chromatography by Sephadex G-200. High-performance liquid chromatography technique was used to measure the purity of cellulase produced from *P. decumbens*. The cellulase enzyme was used to increase the yield of apple juice and apple juice clarification, as examples of its application in the food industry.

Results and conclusion
The *P. decumbens* colony proved to have the largest decolorization zone, and the cellulase produced was a large amount (21.5 U/ml). The highest activity of cellulase was seen in the media containing 50% carboxymethylcellulose and 50% dates molasses waste (Dibs) as carbon source after incubation for 6 days, and the optimum pH and temperature for the production cellulase were pH 4.0 at 30°C. Utilization of 80% ammonium sulfate gave pure enzyme cellulase (38.25 U/ml) and has a high degree of specific activity (25.5 U/mg protein). Cellulase activity of 42 U/ml and the degree of specific activity of 46.6 U/mg protein, with a 4.3-fold purification of cellulase, with 42% recovery from the crude cellulase, was obtained with Sephadex G-200. The results revealed an increase in the quantity of produced apple juice treated by cellulase enzyme.

Keywords:
application, cellulase, *Penicillium*, production, purification

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Introduction

Cellulase (E.C 3.2.1.4) is an enzyme that hydrolyzes cellulose by breaking down the 1, 4 β-D glycoside bond in cellulose to beta-glucose. Cellulose is an important carbon source. Cellulose consists of *D*-glucose units that are linked with *β*-1, 4-glycosidic bonds; it has two general forms in biomass: crystalline and amorphous. To produce cellulases, we can use inexpensive media such as agriculture and food industries for growing microorganisms during production of cellulases, which are an economic and available source. Cellulase is produced by a large number of microorganisms, such as bacteria, fungi, and a few actinomyces [1,2]. The cellulase enzyme production from certain fungi has been reported by Zaldivar and colleagues [3–8]. Fungi are an important microorganism that produce cellulase, and *Aspergillus*, *Trichoderma*, and *Penicillium* sp. are the ones that produce the highest amount of cellulase, as showed by Madhavan and Mangalanayaki [9]. Studies on the optimization of fungal cellulase production are reported by Remaz [10] and Sethi and Gupta [11]. Cellulases enzyme are used in commercial and medical applications, with high purity and maximum yield. Various laboratories have attempted to purify cellulase, and there is no method that is satisfactory to purify the enzyme. The clarification of juice can be performed by the complete removal of all suspended solids. These suspended solids are polysaccharides (cellulose, hemicellulose, pectin,

lignin, and starch). The enzymatic process is accredited to offer a number of advantages over mechanical–thermal comminution of fruit pulps. The application of enzyme facilitates juice extraction process and improves the quality of the finished product. The enzymes lead to release of cell contents. Consequently, high yield of juice can be obtained [12,13]. During the processing of pulpy juices through microfiltration, the fouling materials are formed, which consist of pectin, cellulose, lignin, and hemicellulose [14]. Retained particles on the membrane surface and in the filter greatly reduce the performance of filtration. Juice clarity is vital for the uniformity of end product to have a pleasing hedonic response. Treatment by enzymes is considered to be the single largest processing aid contributor. The treatment of grape juice by commercial carbohydrate preparation resulted in 98–99% clarity and 25–30% mash degradation. The enzymatic treatment showed best results at enzyme level of 0.048%, temperature of 27–30°C, and 30 min incubation time, whereas the mash pH was kept unchanged. The treatment caused a reduction in total phenols and juice viscosity by 32 and 25%, respectively [15].

Materials and methods

Microorganisms

The fungal strains of *Penicillium* used in this study were brought from the Egyptian Microbial Culture Collection (EMCC) at the Microbial Research Centre (Cairo, MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Three Fungal strains of *Penicillium*. *Penicillium brasilianum* (EMCC Number: 555T), *Penicillium occitanis* (EMCC Number: 888T), and *Penicillium decumbens* (EMCC Number: 999T) were maintained on potato dextrose agar in Petri dishes, incubated at 28±1°C for seven days, and stored at 4°C.

Substrates

Dates molasses waste (Dibs) was obtained from local dates factories in the Qassim region, Kingdom of Saudi Arabia. It was dried in an oven below 45°C and then blended in a blender to a powder for subsequent use in the fermentation medium, and then stored in the refrigerator at 4°C.

Qualitative screening of cellulase-producing fungi

Identification of cellulase-producing fungi was done using Congo red staining method according to Farkas *et al.* [16]. Medium used for detection of cellulase activity was carboxylmethylcellulose agar (CMC agar), which contained the following (g/l): $NaNO_3$, 2.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; CMC–Na salt, 10 g; and agar, 20 g. The pH of the medium was adjusted to 6.0 [17]. Cellulase-producing fungi were screened on selective CMC agar. Plates were spot inoculated with spore suspension of pure culture and incubated at 28°C. After 5 days, 10 ml of 1% Congo red staining solution was added to the plates for 15 min. Then, the Congo red staining solution was discarded, and 10 ml of NaOH (1 N) was added to the plates for 15 min. Then it was removed, and the staining of the plates was measured by noticing the formation of yellow zones around the fungal spore-inoculated wells. The diameter of the clear zone of decolorization around each colony was measured.

Cellulase production

The cultures that made a zone of hydrolysis around its colonies were inoculated in 250-ml Erlenmeyer conical flask containing 50 ml of CMC medium containing the following (g/l): $(NH_4)2SO_4$ 1.4 g; KH_2PO_4 2.0 g; urea 1.3 g; $CaCl_2$ 0.3 g; $MgSO_4.7H_2O$ 0.3 g; $FeSO_4.7H_2O$ 0.005 g; $MnSO_4.H_2O$ 0.0016 g; $ZnSO_4.7H_2O$ 0.0014 g; $CoCl_2$ 0.002 g; peptone 1.0 g; CMC–Na salt 10 g; and tween 80 (1%) 2.0 ml. The pH of the medium was adjusted to 6.0 [18]. The inoculated flasks were incubated on a rotary incubator shaker at 150 rpm for 7 days at 28±1°C. The mycelium of each isolate was collected by centrifugation at 4000–5000 rpm for 15 min at 4°C. The cell-free supernatant was used as a crude enzyme for further determinations.

Cellulase assay

Cellulase activity was determined according to the procedure of Mandels *et al.* [19], using the dinitrosalicylic acid reagent, 1 ml of 0.05 M sodium citrate buffer (pH 4.8), and 0.5 ml of enzyme solution. The mixture was incubated in water bath at 50°C for 60 min. At the end of the incubation period, tubes were removed from the water bath, and the reaction was stopped by addition of 3 ml of 3, 5-dinitrosalicylic acid reagent per tube. The tubes were incubated for 5 min in a boiling water bath for color development and were cooled rapidly. The reaction mixture was measured at 550 nm in an ultraviolet visible spectrophotometer. The concentration of glucose released by enzyme was determined by comparing against a standard curve constructed similarly with known concentration of glucose. One unit of enzyme activity was defined as the amount of enzyme required to releasing 1 µ mole of glucose per ml per min. under the aforementioned assay conditions and was expressed as U/ml.

Optimization of culture conditions for cellulase production

The physicochemical parameters, such as the incubation period, initial pH, temperature, and substrate in submerged fermentation, affecting the production of cellulase were optimized by changing one parameter at a time to enhance cellulase production.

Effect of different concentrations of substrate

Carboxymethylcellulose in fermentation medium was replaced by 10, 20, 30, 40, 50, 60, 70, and 80% dates molasses waste (Dibs) as a carbon source.

Effect of incubation period

To study the effect of incubation period, the fungal culture was inoculated in CMC medium and incubated for different incubation time 3, 4, 5, 6, 7, 8, and 9 days at 28°C on an incubator shaker at 150 rpm under submerged conditions.

Effect of temperature

To find the optimum temperature for growth and cellulase production, the fungal culture was cultivated in CMC medium. Incubation was at different temperatures (25, 28, 30, 32, and 35°C) for 6 days on an incubator shaker at 150 rpm. The fermentation media pH was adjusted to 6.0.

Effect of initial pH

The effect of the pH value was investigated by cultivation of the selected fungal isolate in CMC media. Media were adjusted to different pH values (4.0, 5.0, 6.0, and 7.0) with NaOH (1 N) or HCl (1 N). Media were inoculated and incubated at 30°C for 6 days under shaking at 150 rpm.

Preparation of crude enzyme

The fungal strain selected in this study was allowed to grow on CMC fermentation medium under all previous studied optimal conditions (at 30°C, on rotary incubator shaker at 150 rpm for 6 days). At the end of the incubation period, the obtained extract was filtrated by centrifuged at 5000 rpm for 30 min at 4°C, to obtain the cell-free filtrate as a crude cellulase enzyme. Cellulase activity and protein content were determined.

Purification of cellulase enzyme

Cellulase enzyme was partially purified by 80% ammonium sulfate followed by ion exchange chromatography by Sephadex G-200. Ammonium sulfate (80% saturation) was added to the crude enzyme at 4°C in an ice bath. The precipitate was collected by centrifugation at 10 000 rpm for 30 min at 4°C, and the precipitate having most of the enzyme activity was suspended in 20 mmol/l sodium phosphate buffer, pH 6.0. For desalting, the suspension was loaded onto Sephadex G-25 gel filtration column at a flow rate of 2 ml/min. Then suspension was centrifuged at 10 000 rpm for 30 min at 4°C to get a clear supernatant. Cellulase activity and protein content were determined. Sephadex G-200 ion exchange column was equilibrated with 0.02 M sodium phosphate buffer (pH=6.0). Fractions containing enzyme activity were concentrated through a freeze dryer and dissolved in a minimum volume of 20 mmol/l sodium phosphate buffer, pH 6.0, and applied onto a column at a flow rate of 15 ml/h and eluted using the same buffer. The fraction were collected and analyzed for cellulase activity and protein content. Fractions containing cellulase enzyme were pooled and concentrated using ammonium sulfate precipitation.

Determination of protein content

Total protein contents of cell-free supernatant and purified samples were assayed by the method of Bradford [61] using an established calibration curve, with bovine serum albumin as a standard.

Analysis of cellulase using high-performance liquid chromatography

To test for purity of the enzyme, the homogeneity of the purified cellulase was detected using high-performance liquid chromatography (HPLC) techniques according to the method described by Abdel-Salam *et al.* [20] for lactoferrin protein detection using HPLC. Cellulase enzyme standard (Cat: S25241; Fisher Science Education) was dissolved in HPLC mobile phase with known concentration, and 20 µl was injected at the same condition as the cellulase enzyme produced in this study. Cellulase enzyme was detected by comparison of peak areas and retention time of unknown samples with those of corresponding cellulase enzyme standard solutions using Agilent HPLC-1100 with Agilent HPLC-1100 quaternary and capillary pump and G1377A1100 series microwell plate autosampler. The detection was carried out using Agilent Diode-Array Detector at 280-nm wavelength using RP-C18 column.

Enzyme applications

Application of cellulase on increased yield of apple juice

Imported red apple fruits were obtained from local market, Burida, Qassim region, Kingdom of Saudi Arabia. The yield was determined according to Srivastava and Tyagi [21] as follows: apples were chopped into cubes that are roughly 5 mm on a side. Approximately 10 g weight was add into each beaker,

along with 24 ml of water was added to the first beaker, 24 ml of cellulase enzyme was added to the second beaker, and 24 ml of diluted cellulase preparation was added to the third beaker. Beakers were put into water bath at 40°C for 2 h. After removing the beakers from the water bath, the apple pieces in each were stirred. The paper coffee filters in funnels were used to filter the juice from the apple preparations into 100-ml graduated cylinders. Volume of juice obtained was record after filtration. Increase yield of apple juice was recorded.

Effect of cellulase on clarification of apple juice

It was carried out according to the method of Kanmani *et al.* [22], as follows: three test tubes were used: one as a control and others as tests. In the first tube, 1 ml of water was added to 5 ml of cloudy apple juice; in the second tube, 1 ml of purified cellulase enzyme was added to 5 ml of cloudy apple juice; and in the third tube, 1 ml of commercial cellulase enzyme was added to 5 ml of cloudy apple juice. The content of the tube was stirred well to mix the enzyme throughout the juice and kept in boiling water bath at 50°C, and clarification of the apple juice was observed. Then, test for starch and pectin was carried out to test the effectiveness of the enzyme treatment. All analyses were carried out in triplicate. Starch, pectin, and stability tests were carried out according to Kanmani *et al.* [22].

Results and discussion

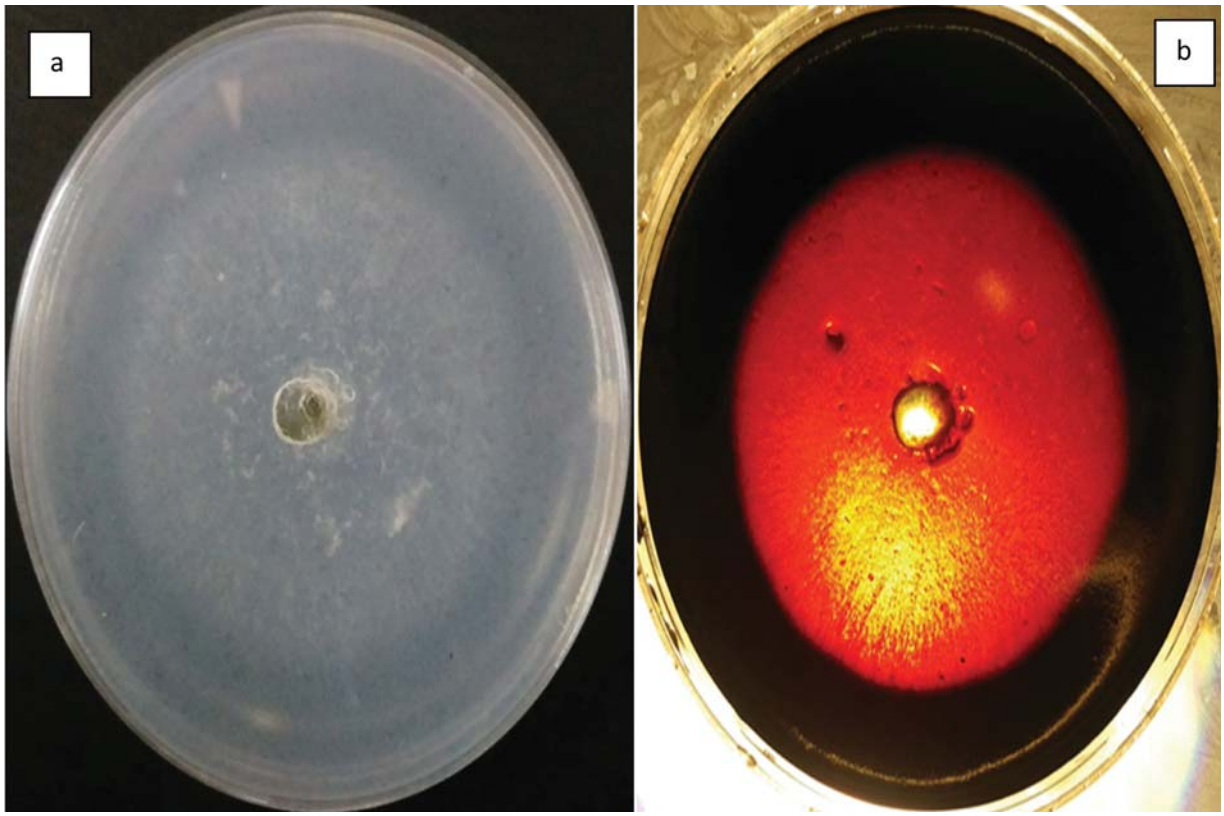
Identification and screening of cellulase-producing fungi

Results presented in Fig. 1a show the zones of cellulase hydrolysis in CMC agar plates without Congo red. After 5 days, the isolates produced around its growth a clear zone on agar plates after adding 1% Congo red (Fig. 1b). This indicates cellulase production, and then, the diameter of zone was measured around the colony.

Results in Table 1 show that all of the tested fungi produced zones of cellulase hydrolysis in CMC agar within 5 days. Of the three fungal strains, *P. decumbens* colony showed the largest decolorization zone (67 mm), whereas both *P. brasilianum* and *P. occitanis* produced zone diameters of 55 and 45 mm, respectively. The fungus *P. decumbens* was selected for cellulase production to provide a high quantitative in comparison with cellulolytic activity.

The fungal cultures of *Aspergillus* sp. and *Penicillium* sp. were screened for their cellulolytic activity, and all the fungal strains produced zones of hydrolysis in CMC

Figure 1



Zones of hydrolysis in CMC agar without Congo red (a) and with Congo red (b). CMC, carboxylmethylcellulose.

agar during 3 days, and among the four fungal isolates, *Aspergillus sp.* (isolate 3) was detected to produce maximum zone of hydrolysis (42 mm) of carboxymethylcellulose, whereas *Aspergillus* sp. (isolates 1 and 4) produced zone diameters of 27 and 40 mm, respectively. *Penicillium* sp. (isolate 2) produced zone diameters of 23 mm [23]. The clear zone that appeared around the growing colony after adding Congo red was a conclusive evidence that fungi produced cellulose [24].

Selection of fungal strains for cellulase production

The results in Fig. 2 show that all tested strains produced cellulase enzyme. *P. decumbens* proved to have higher cellulase-producing potential (21.5 U/ml), followed by *P. occitanis* (17.9 U/ml), whereas *P. brasilianum* produced the least amount of cellulase (14.3 U/ml). Many studies reported that *Penicillium* species secrete cellulase enzyme and high β-glucosidase activity [25–27]. Strains like *P. brasilianum*, *P. occitanis*, *and P. decumbens* are capable of secreting a complex of cellulase enzymes, which could have practical application in the enzymatic hydrolysis of cellulose. These strains have the capability to give high levels of extracellular cellulases [28–30]. *Aspergillus* sp. (isolate 3) produced high level of CMCase (64 U/ml). CMCase of 43.32 U/ml was produced by *Penicillium* sp. (isolate 2), which showed its effectiveness next to isolate 3; when compared with cellulases activity of isolate 3, *Aspergillus* sp. isolates 1 and 4 exhibited lower activities of CMCase (5.55–5.66 U/ml) [23]. The extracellular filtrate of *Penicillium pinophilum* MS20 produced CMCase of 9.61 U/ml [31]. Cellulase activity from *Aspergillus niger* was 0.54 U/ml [5], whereas the cellulase activity in *Trichoderma viride* was 33.8 U/ml [32]. From the obtained results in Fig. 2, the strain *P. decumbens* was employed in the subsequent experiments.

Table 1 The diameter of hydrolytic zone and colony diameter

Fungal strains	Zone of hydrolysis on CMC agar (mm)
Penicillium brasilianum	55
Penicillium occitanis	45
Penicillium decumbens	67

The results were average of three replications. CMC, carboxyl methyl cellulose.

Optimization conditions for production of cellulase

Optimization of the conditions of the culture for producing cellulase by selecting the best environmental conditions and nutritional is important to increase the yield of the produced cellulase.

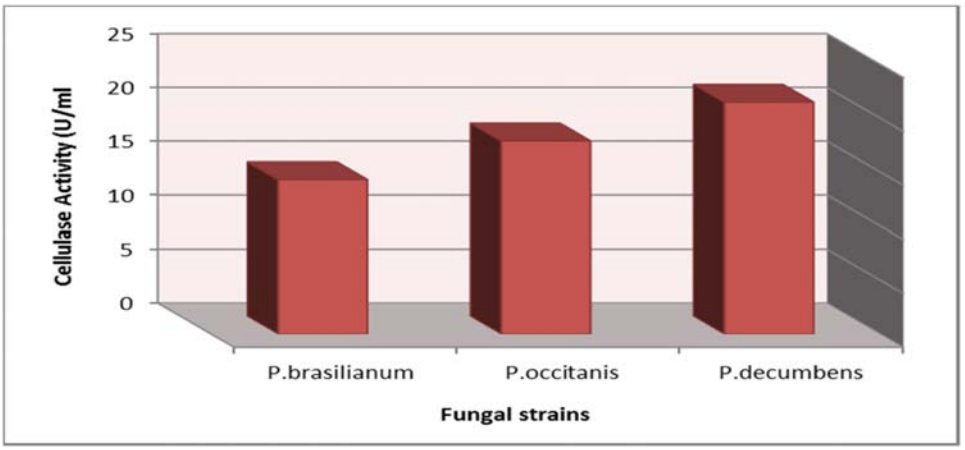
Effect of different concentration of substrate

Figure 3 shows that great amounts of cellulase (21.69 U/ml) were produced in the medium replaced by 50% of dates molasses wastes (Dibs) instead of carboxymethylcellulose as a carbon source. The best replacement ratio was 50%. The reason for this is because the dates molasses waste (Dibs) provides the required ratio of nutrients, and it gives the best chance of the fungus to grow and thus the synthesis of cellulase. This was confirmed by Aqidi *et al.* [33] when the fungus *Aspergillus oryzae* was grown using the seeds of dates powder. Cellulase enzyme was highest in the culture filtrate of *A. niger* in the presence of carboxymethylcellulose [6].

Effect of incubation period

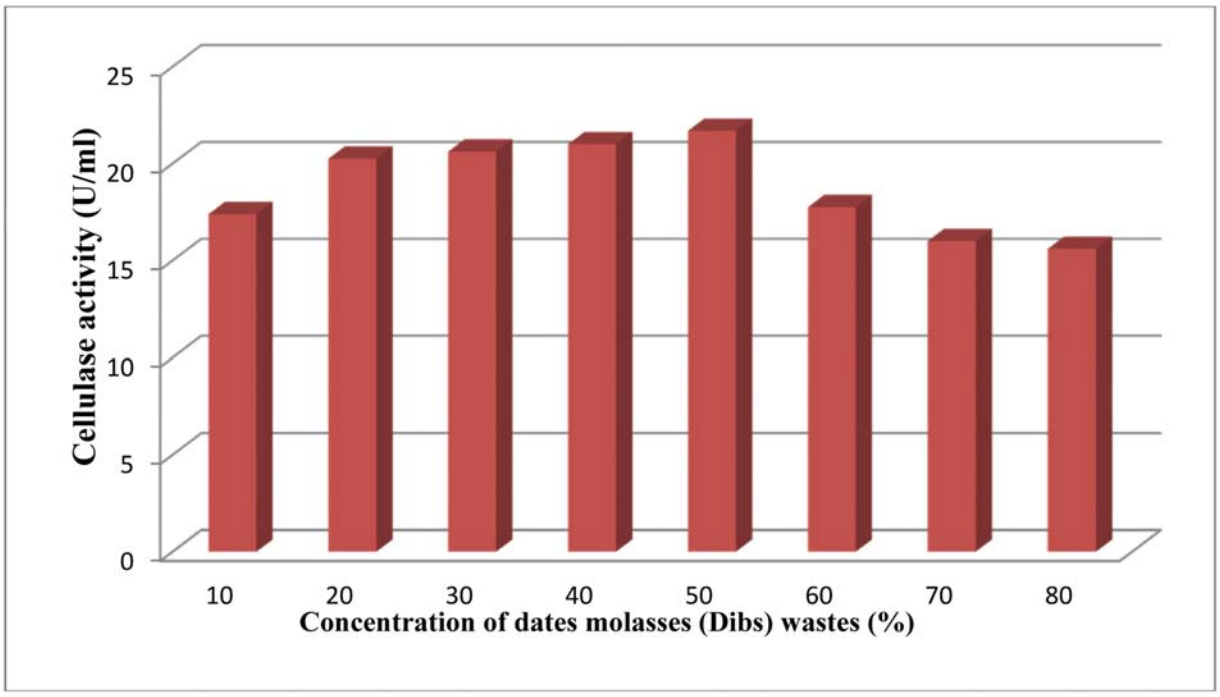
The results in Fig. 4 show that *P. decumbens* was capable of producing cellulase in different tested

Figure 2



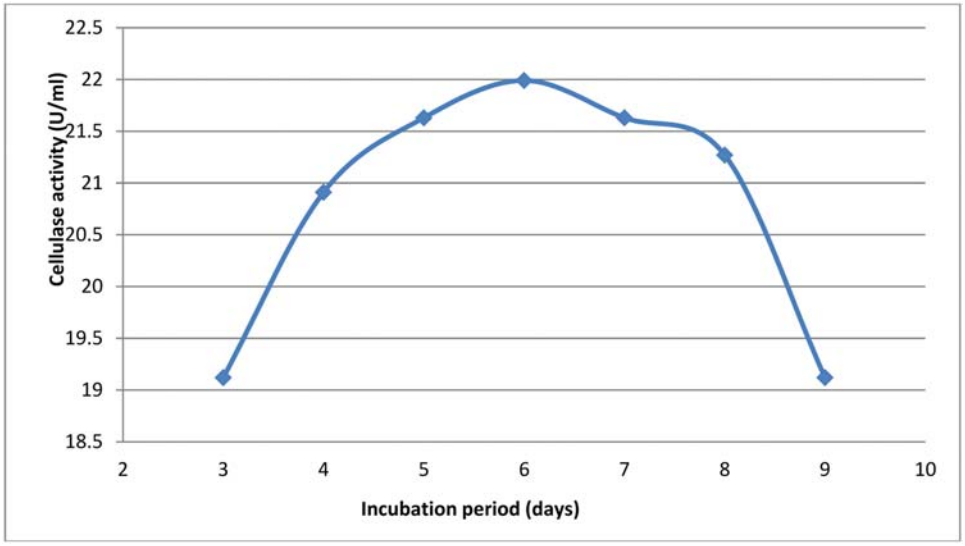
Cellulase production by fungal strains from *Penicillium* sp.

Figure 3



Cellulase production by *Penicillium decumbens* at different concentration of dates molasses.

Figure 4

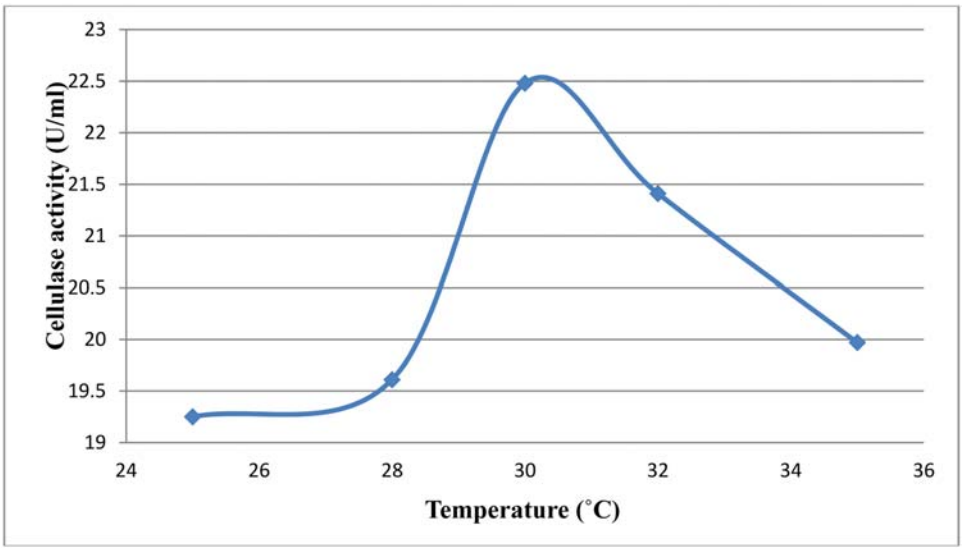


The production of cellulase by *Penicillium decumbens* at different incubation periods.

incubation periods. *P. decumbens* gave the highest amount of cellulase activity (21.99 U/ml) on the sixth day of the incubation period.

It could be detected from the present results in Fig. 4 that incubation for 6 days was optimum for cellulase production. These results disagree with those of Duenas *et al.* [34] and Khan *et al.* [35], who produced cellulase after incubation time of 4 days. Maximum cellulase production after incubation for 3 days was reported by Fadel [36]. However, maximum cellulase activity appeared after 12 h of inoculated by *Aspergillus flavus was reported by Ojumu et al* [37]. The maximum level of cellulase production was observed after 6 days of incubation, as shown in Fig. 4, which is probably owing to the stopping of the growth and release of cellulase into the medium during the later growth phase of *P. decumbens*. The decrease in enzyme activity upon prolonged incubation may be owing to irreversible adsorption to substrate or due to feedback inhibition or denaturation of

Figure 5



Cellulase enzyme production by *Penicillium decumbens* at different temperatures.

the enzymes, resulting from the variation of pH and the cellular metabolism during fermentation [38]. However, after fermentation for 3 days, the highest activity could be obtained. This is because of the consumption of the nutrients and production of other components in the fermentation medium [39].

Effect of temperature

Results in Fig. 5 show that cellulase production increased as incubation temperature increased until reached maximum (22.48 U/ml) at 30°C and then decreased. The obtained results are in agreement of Olutiola [40], Atif *et al.* [41], Deswal *et al.* [42], and Saro *et al.* [43], as they found the maximum temperature for cellulase activity was 30°C. As the temperature increased, there was a reduction in the enzyme production, because the high temperature can change membrane composition and can cause protein catabolism and inhibition of growth of fungi.

These results disagree with those of Murugan *et al.* [44], which showed maximum cellulase production at 35°C. The maximum growth and cellulase activity by *Trichoderma* species were at 25–35°C [45]. However, Narasimha *et al.* [5] produced cellulase from *A. niger* at 28°C. On the contrary, maximum growth and cellulase activity by *Aspergillus fumigates* at temperature between 30°C and 45°C have been reported [46].

Effect of initial pH

Results presented in Fig. 6 show that the cellulase production was optimum (23.97 U/ml) by *P. decumbens* at an optimal pH of 4.0. More or less than this point decreased the cellulase production.

These results were consistent with Fadel [36], as he mentioned pH 4.5 as the optimal level for the highest production of cellulase by *A. niger*. Two major activities were observed at pH 4.5 and 7.5 [47]. The range of pH 6.0–7.0 was the best for production of cellulase by *A. niger*. The optimum pH value of other cellulolytic organisms varied from acidic condition [48]. The pH of 3.5 was optimum for cellulase production from *Trichoderma reesei* [49]. *T. reesei* produced higher yields of cellulase at pH 7 [50]. However, pH 5–6 gave high activity of cellulase [51]. Change in pH of medium reduces the activity of cellulases because of the microorganism needs acidic pH to grow and produce the cellulase [52].

Partial purification of cellulase

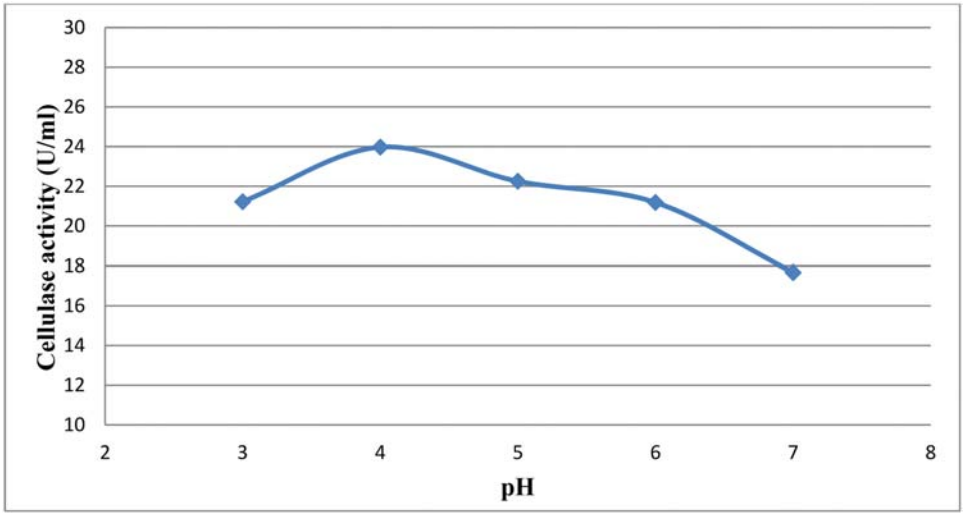
Precipitation with ammonium sulfate 80% saturation showed high cellulolytic activity (38.25 U/ml) and specific activity (25.5 U/mg protein) compared with crude cellulase (23.27 U/ml), as reported in Table 2. The enzyme after precipitation with 80% ammonium sulfate was almost 2.91 folds, and the yield was 67%. Cellulase produced from *T. reesei* was purified by ammonium sulfate, which achieved 2.41 folds, with a yield of 59.51% [53].

Purification of cellulase

Ion exchange chromatography on Sephadex G-200

Cellulase activity was determined in the fractions of single protein peak after Sephadex G-200 chromatography. Data in Fig. 7 indicate that most of the cellulolytic activity was determined at fraction 11–23. The highest activity of cellulase was in the fraction 18.

Figure 6



Cellulase enzyme production by *Penicillium decumbens* at different tested pH values.

Table 2 Purification steps for cellulase produced by *Penicillium decumbens*

Purification steps	Volume (ml)	Enzyme activity (U/ml)	Total units	Protein content (mg/ml)	Total protein	Specific activity (U/mg) protein	Purification fold	Recovery %
Cell-free supernatant	300	23.27	6981	2	600	11.64	1	100
Pellet after 80% ammonium sulfate	120	38.25	4590	1.5	180	25.5	2.91	67
Sephadex G-200 column	70	42	2940	0.9	63	46.6	4.3	42

The purification results, which are summarized in Table 2, record that the Sephadex G-200 chromatography resulted in 4.3-fold purification of cellulase with 42% recovery from the crude enzyme. The specific activity of the enzyme was found to be 46.6 U/mg protein. There are many research studies that have purified the enzyme, which showed 46.5 U/mg protein specific activity, 18.6% enzyme recovery, and 12.6-fold purification during purification of cellulase from *Rhizopus oryzae* [54]. The specific activity of 24.61 U/mg protein, 4.51 purification fold, and 13.46% enzyme recovery during the purification of cellulase were reported by Kaur et al [55], and 6.5 U/mg specific activity, 2.4% enzyme recovery, and 13-fold enzyme purification during their research work were reported by Oyekola [56]. On the contrary, a much higher value for purification (408 folds) after a four-step purification of cellulase from *Mucor circinelloides* NRRL 26519 was reported by Saha [57].

Analysis of cellulase using high-performance liquid chromatography

HPLC technique was used in measuring purity of cellulase enzyme produced from *P. decumbens*. The obtained results illustrated in Figs 8 and 9 show that seven peaks were detected when cellulase enzyme samples isolated from *P. decumbens* sample were subjected to HPLC. The chromatographic data are similar with the findings reported by Chang *et al.* [58], as they found that cellobiase and endoglucanase appear together after separation by ion exchange chromatograph; separated cellulase under similar conditions found three peaks, and all of them exhibiting mixed activity [59]. The present investigation indicates that the HPLC separation of cellulase mixtures and the determination of the enzyme activity of the peaks could be a valuable method for identification of cellulase complex.

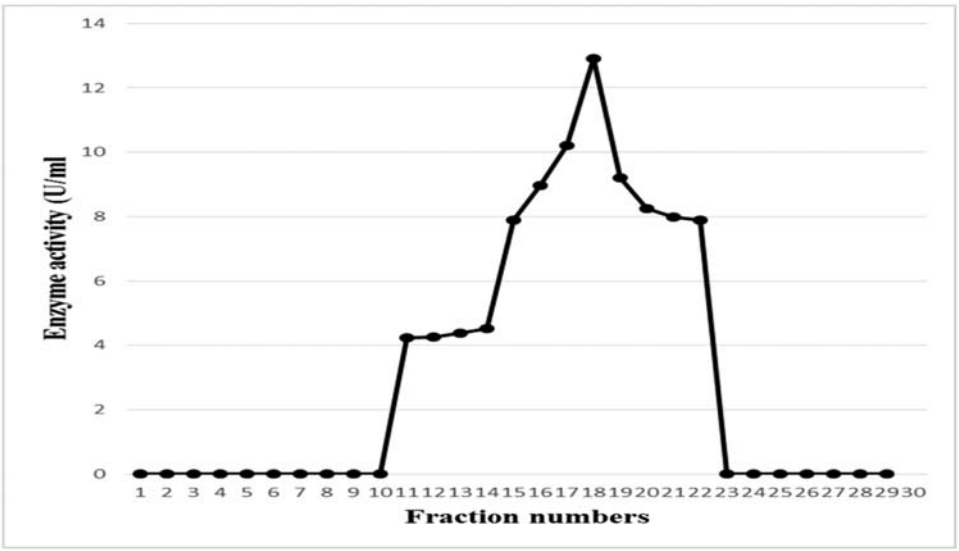
Enzyme applications

Clarification of apple juice using cellulase enzyme

Cellulase is one of the most widely used enzymes in clarifying apple juice. The cell wall of the apple fruit is made up of pectin. In order to produce clear apple juice, it is absolutely necessary to remove pectin and starch from it. Using cellulase enzyme under specific conditions helps to clarify apple juice.

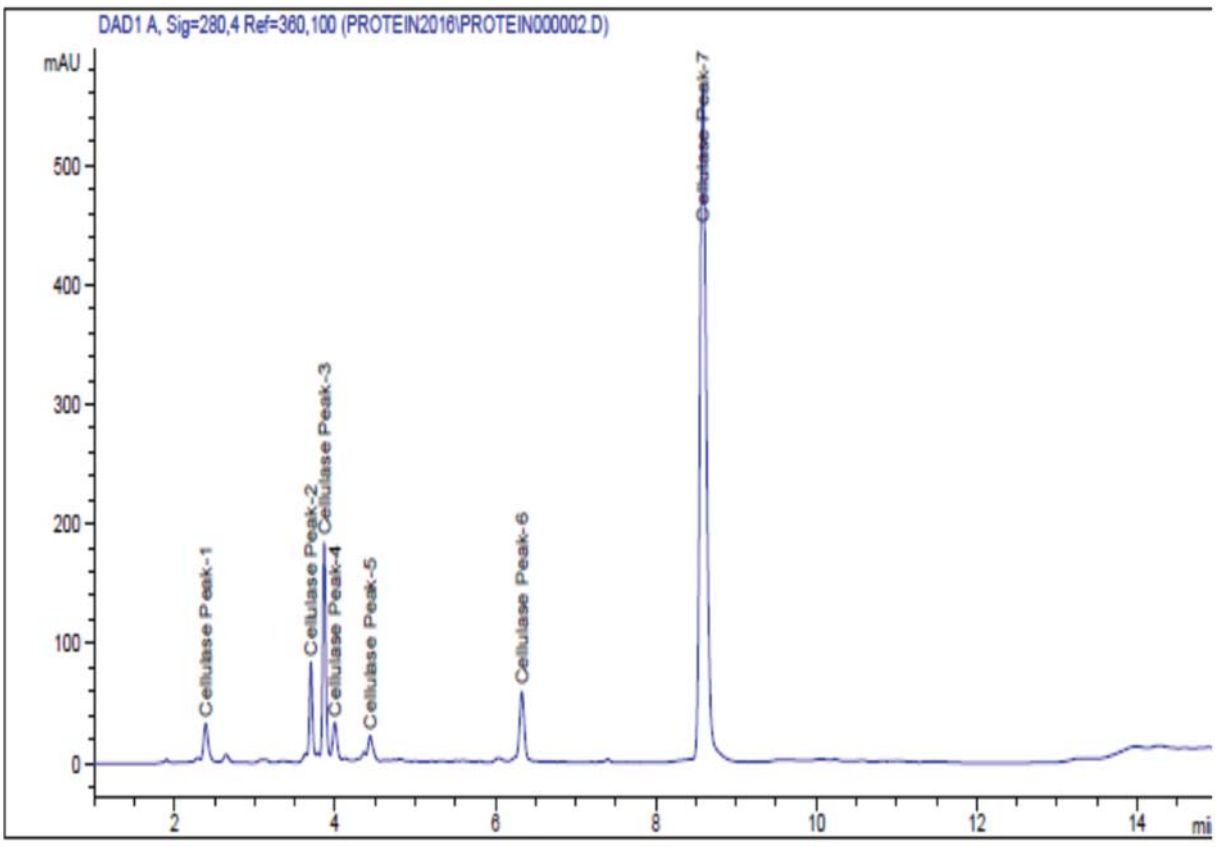
Results in Table 3 show that the clarification of the apple juice using cellulase enzyme provides a clear apple juice by the removal of pectin. In pectin test, results

Figure 7



Fractionation of *Penicillium decumbens* cellulase enzyme by Sephadex G-200.

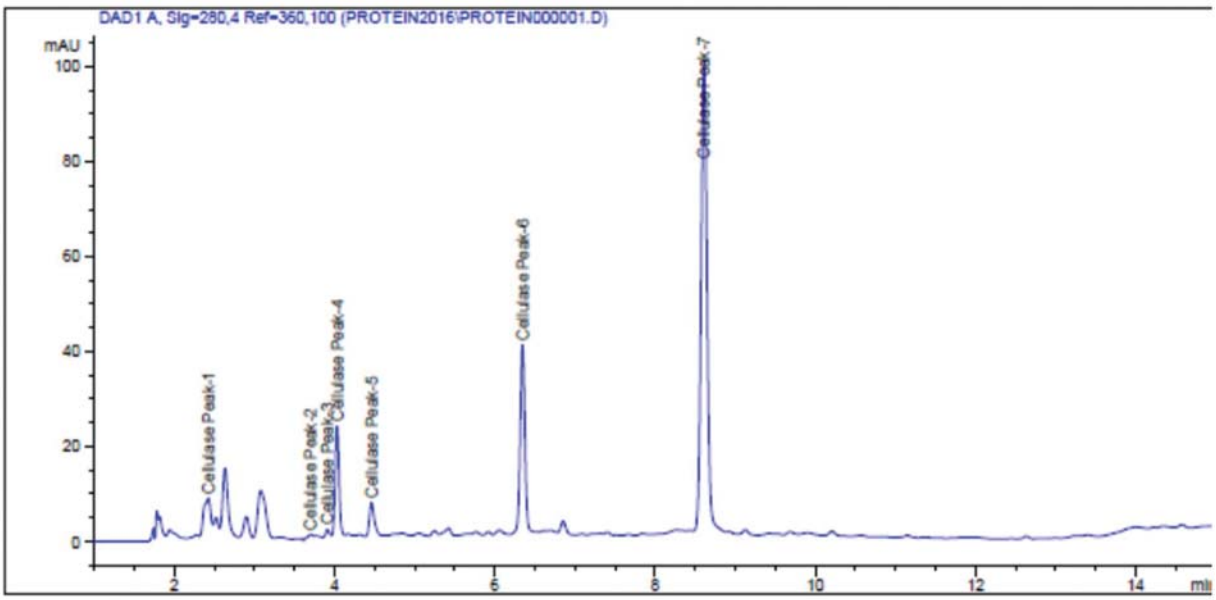
Figure 8



Chromatogram of cellulase standard.

showed that, no flocculation was apparent after 15 min, which indicates that the pectin is broken down as a result of the presence of cellulase. In the starch test, it showed negative results by forming yellow color, which indicates the absence of starch after the addition of iodine solution. In the stability test, results showed no haze formation and the juice was suitable for concentrating. These results are in agreement with those of Kanmani *et al.* [22], who obtained a clear apple juice using cellulase produced and purified from *Bacillus pumilus*, and both starch and pectin test showed negative results.

Figure 9



Chromatogram of cellulase isolated from *Penicillium decumbens.*

Table 3 Pectin and starch tests of apple juice as affected by cellulase enzymes

Treatments	Pectin test	Starch test
Water	+	+
Produced cellulase enzyme	–	–
Commercial cellulase enzyme	–	–

Table 4 Yield of apple juice as affected by cellulase enzymes (from 10 g fruit)

Treatments	Juice volume (ml) after incubation	Yield increase %
Water	14	–
Produced cellulase enzyme	20	43
Commercial cellulase enzyme	20	43

Yield of apple juice as affected by cellulose treatment

Data reported in Table 4 show the effect of adding produced cellulase on the yield of apple juice compared with commercial cellulose. From the results in Table 4, it could be observed that cellulase increased the amount of produced apple juice. In addition, maximum volume was obtained by the cellulase enzyme produced and purified from *P. decumbens* and commercial cellulase enzyme. The maximum activity of cellulase was at the temperature 40°C, and it enhanced the yield of apple juice up to 20 ml/10 g.

There was a significant increase in apple juice when cellulase enzyme was added. Approximately 43% increase was detected as a result of produced or commercial cellulase treatment. Cellulase can hydrolyze the cellulose in the cell wall, thereby releasing more juice. Cellulose maximum volume obtained at pH 4 and at temperature 50°C was 22–26 ml/50 g [21]. If a fruit juice is treated with macerating enzymes (cellulases and pectinases), in addition to other benefits, an increase in bivalent cations like calcium and magnesium has been observed as a result of increased enzymatic degradation of the cell wall [60].

Conclusion

A fungal strain of *Penicillium* was selected, which has a high ability to enzyme production. *P. decumbens* proved to have higher cellulase-producing potential. The optimal environmental factors were determined for cellulase production. The purified enzyme was recovered, with the final yield of 42% with 4.3-fold purification using ion exchange chromatography on Sephadex G-200. The obtained results revealed that the cellulase produced using *P. decumbens* under investigated conditions can be used as an effective tool for increasing the yield of apple juice, as well as to clarify it successfully.

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Conflicts of interest

None declared.

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