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ORIGINAL ARTICLE

A safe potential juice clarifying pectinase from *Trichoderma viride* EF-8 utilizing Egyptian onion skins

Abdel-Mohsen S. Ismail [a], **Heba I. Abo-Elmagd** [b], **Manal M. Housseiny** [b,*]

[a] *Department of Chemistry of Natural and Microbial Products, National Research Centre, Dokki, Cairo, Egypt*
[b] *Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Roxy, Cairo, Egypt*

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Abstract The production of a notable, safe and highly active pectinase by the local fungal strain *Trichoderma viride* EF-8 utilizing the abundant pigmented Egyptian onion (*Allium cepa* L.) skins (6.5%, w/v) was achieved in 4 days submerged fermentation (SMF) cultures, at temperature and pH of 30 °C and 4.0, respectively. The indigenously produced pectinase was partially purified by 50% batch ethanol precipitation and its general properties were studied following the standard procedures. The lyophilized enzyme preparation was free of any ochra or aflatoxins. The optimum conditions for the partially purified enzyme form were 2 mg/mL and 1% (w/v) enzyme protein and substrate (citrus pectin) concentrations, reaction pH and temperature of 7.0 and 40 °C, respectively. The results presented the low cost onion skins waste as the major substrate for the fungal pectinase production and its subsequent use in perfect fruit (apple, lemon and orange) juices clarification with remarkable stability during and after this process, which certainly enhance fruit juices processing in the tropics.

1. Introduction

Recently, the controlled biological degradation of the wastes for the production of valuable compounds, such as proteins, polysaccharides, hormones, enzymes and others for medical and industrial uses became the main target of many scientists. Thus, the bioconversion of plant biomass to fermentable sugars utilizing a wide range of microbial enzymes such as cellulases, xylanases, pectinases and inulinases leading to the production of biofuel and many other industrial and medical compounds, which is unchallenged and represents the most plentiful processes worldwide for the exploitation of huge agricultural wastes [15,24].

The hydrolysis of pectin backbone is obtained by the synergistic action of several enzymes, including pectin methylesterase, EC 3.1.11.1; endo-polygalacturonase, EC

* Corresponding author at: Faculty of Education, Ain Shams University, Biological and Geological Sciences Department, Roxy, Cairo 11757, Egypt. Tel.: +20 01062072725.
E-mail address: manal_maher2@yahoo.com (M.M. Housseiny).

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3.2.1.15; exopolygalacturonase, EC 3.2.1.67; pectate lyase, EC 4.2.2.2; exo-pectate lyase, EC 4.2.2.9 and endopectin lyase, EC 4.2.2.10 [8,31].

Pectinases are of significant importance in the current biotechnological area with their all embracing applications in fruit juice extraction and clarification, scouring of cotton, degumming of plant fibers, waste water treatment, vegetable oil extraction, tea and coffee fermentations, paper bleaching, poultry fodders (as additives) and in the alcoholic beverage and food industries [12,13]. Preparations containing pectin-degrading enzymes used in the food industry are mainly of fungal origin because fungi are potent producers of pectic enzymes and it is really encouraging that the optimal pH of the fungal enzymes is usually very close to the pH of many fruit juices, which ranges from 3.0 to 5.5 [16].

It is well known that pectic substances are responsible for the consistency and the turbidity appearance in fruit juices, so that the presence of pectic substances in fruit juices that suspend toward insoluble (pulp) particles leads to many problems in the clarification of fruit juices. The addition of pectinases, which results in the rapid drop in viscosity as well as the flocculation of the micelles present, allows these particles to be easily separated by sedimentation or filtration [14].

Since Pharaonica era, Egypt has expansively cultured onion (*Allium cepa*) plant and nowadays Egypt became one of the biggest onion producers around the world, where five Egyptian onion species at least are cultivated in both upper and Lower Egypt. Huge and increasing amounts of the pigmented onion skins are discarded annually either during export or from manufacturing processes. These vast amounts of pigmented onion skins are not utilized in any way causing very hard environmental impacts, especially after burning.

The skin on the onion with a variety of useful functions; was utilized to dye the silk material. This is not only much beneficial to the environmental protection and economic aspect by making use of the disposed skins as waste but also contributable to the development of biocompatible fabric materials [31].

The present study aimed to the perfect utilization of the pigmented onion skins excluded from different Egyptian food industry factories for pectinase production by the local fungal isolate *Trichoderma viride* applying eco-friendly technology. Moreover, the optimum conditions for the perfect pectinase action were established and the application of the partially purified enzyme form in different juices clarification was successfully carried out.

2. Materials

2.1. Microorganisms and culture conditions

Six fungal isolates were screened for pectic enzymes production. The fungal isolates screened, *Aspergillus niger, Penicillium loliense, Trichoderma harzianum, Trichoderma longibrachiatum, T. viride* and *Ulocladium botrytis*. These fungal species were isolated from soil in Cairo governorate and identified in Taxonomy Department, Ain-Shams University, Cairo City, Egypt. These isolates were maintained as single spore on potato-dextrose-agar (PDA) medium, subcultured on PDA slopes and incubated at 30°C for 7 days.

2.2. Onion skins and fruit samples collection

Egyptian pigmented onion (*A. cepa* L.) skins were collected from market in Cairo governorate. Three different fruits at commercial maturity (apple, *Malus domestica* Boek, Var Anna; orange, *Citrus sinensis* and lemon, *Citrus limon*) were purchased from the market. Fruits were carefully selected for uniformity in maturity, size, color and absence of physical damage. The fruits were carefully washed with tap water, cut into halves and juiced with juice extractors (Multipress automatic Braun MP80, Kronberg, Germany). Ascorbic acid was added to the pressed juice in order to avoid undesirable enzymatic browning.

2.3. Media

The following media were used and had the following composition (g/L):

2.3.1. Fungal maintenance and subculturing (PDA) medium

Potato slices, 500; dextrose, 10; agar, 20 and distilled H_2O.

2.3.2. Fungal (growth enhancement) medium

This was according to [11] and has the following composition: peptone, 5; yeast extract, 1; glucose, 10; $MgSO_4{\cdot}7H_2O$, 0.5; KH_2PO_4, 0.5 and distilled H_2O.

2.3.3. Pectinase production medium

This was adaptation of the medium formulated by Abdel-Fattah et al. [1] as follows: Egyptian onion skins, 65; $MgSO_4{\cdot}7H_2O$, 0.5; KNO_3, 2.5 and distilled H_2O.

3. Methods

3.1. Fungal inoculum preparation

The fungal inoculum was prepared by fungal strain cultivation in the above mentioned growth enhancement medium and the culture was incubated in bench-top shaker at 30°C for 48 h. Fungal inoculum pellets (5 mL) were used for fungal culture inoculation, transferred to 250 mL Erlenmeyer flask containing 50 mL pectinase production medium, incubated in bench-top shaker (180 rpm) at 30 °C and lasted for 7 days. The content of each flask was gathered up and thoroughly mixed with 10 mL cooled sterilized distilled water, then the mixture was filtered through a double-layer cotton cloth and centrifuged at 5000*g* for 10 min. The enzyme activity and protein content was determined in the supernatant.

3.2. Protein content estimation

The protein content of any enzyme preparation was determined by the method of Lowery et al. [20].

3.3. Pectinase activity assay

This was performed according to the method of Silva et al. [30]. Pectinase activity was evaluated by mixing 0.2 mL of the enzyme preparation and 0.8 mL of citrus pectin solution

(0.5%, w/v citrus pectin in 0.05 M acetate buffer, pH 5.0). Reaction mixtures were incubated at 50 °C for 10 min and the released reducing sugars were determined by Somogyi [32]. One unit of pectinase activity was defined as the amount of enzyme releasing 1 μmol of reducing sugars (as D-anhydrogalacturonic acid) per min reaction.

3.4. Outer onion skins pretreatments

Physical (Water-soaking, crushing, boiling for 5, 10 and 15 min and autoclaving), chemical treatments (treatment overnight with 1N-NaOH or 1N-HCl) and physico-chemical treatment (unpigmentation of 1 g?? crushed pigmented skins with 1.5 g sodium chlorite dissolved in 155 mL distilled water and few drops of glacial acetic acid, GAA) were applied on the pigmented onion skins. After any pretreatment process, the skins were washed several times with distilled water before use in the production medium.

3.5. Optimization of fungal pectinase production

The optimization of fungal pectinase production medium was carried out by modification of the physical parameters, such as the incubation period (4–7 days), the incubation temperature (20–45 °C) and initial pH (3–9).

3.6. Pectinase partial purification

This was performed by sequential or batch precipitation of the lyophilized culture filtrate either by ammonium sulphate or organic solvents (ethanol or acetone).

3.6.1. Ammonium sulphate fractional precipitation

All the purification steps were carried out at 4°C to prevent the enzymes denaturation. The lyophilized culture filtrate was used as an enzyme source. Ammonium sulphate (50% and 70%, saturation) was added slowly to enzyme solution with gentle stirring, then kept at 0–4°C at least for 6 h and the precipitate was collected by centrifugation at 5000*g* for 20 min, then dissolved in 0.2 M-acetate buffer, pH 5.5 and centrifuged. Finally, the supernatant was dialyzed against 25 mM-acetate buffer (pH 5.5) at 4 °C till the removal of all sulphate ions, lyophilized and assayed for its protein content and pectinase activity.

3.6.2. Acetone or ethanol sequential precipitation

Acetone or ethanol sequential precipitation was carried out using the chilled acetone or ethanol (0 °C) as a precipitating agent. Acetone or ethanol with concentrations of (50%, 70% and 90%, v/v) and (32%, 40%, 45%, 50% and 70%, v/v), respectively were subsequently added slowly to the lyophilized enzyme solution and incubated in ice–salt bath with continuous stirring. The precipitated enzyme was collected, washed and dried. The subsequent acetone or ethanol fractions were obtained by the addition of the higher concentrations, then washed several times, dried and assayed for their protein content and pectinase activity.

3.6.3. Ethanol batch precipitation

Batch precipitation was performed by the addition of ethanol with concentration of 50%, v/v, to the crude enzyme solution slowly with continuous stirring and the precipitated enzyme fraction was same treated as previously mentioned.

3.7. Study of the partially purified pectinase properties

The general properties of the batch 50% ethanol were studied to determine the optimum conditions for the fungal enzyme action, those included the effect of the enzyme protein concentration, the substrate (citrus pectin) concentration, the reaction pH and the reaction temperature on the enzyme activity.

3.8. Culture toxicity tests

Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals *in vivo* lethality to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay [6] was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95% considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins [5].

3.9. Juices clarification process

Lemon, orange and apple fruits were purchased from local market at Cairo/Egypt. The fruits were rinsed with running water grated and well mixed using a lab mixer for 2–3 min until homogeneity. Each extracted juice was filtered through cheese cloth and stored at 5 °C until use in the clarification process.

3.9.1. Juice clarification and the enzyme efficiency test

Enzyme efficiency tests were carried out on the 50% bulk ethanol pectinase fraction, which was separately mixed with the fresh juice of orange, apple or lemon, applying the optimum reaction conditions, previously specified. Similar mixtures were prepared with the boiled juice, where the petinase activity is only due to the enzyme fraction and the difference between the activities in the fresh juice–enzyme and in the boiled juice–enzyme mixtures represents the fresh juice containing pectinase activity. At the end of the incubation period, the residual pectinases activity was estimated in the usual manner.

4. Results

Study of the Egyptian outer pigmented onion skins composition showed that these skins were carbohydrate polymers (holocellulose and pectin) – rich with low moisture and ash contents (Table 1). Six fungal species were tested in shaken cultures containing pigmented onion skins as the sole carbon source, at different culture ages for their pectinase productivity (Table 2). The results showed *T. viride* potency in four days shaken cultures at 30 °C for pectinase production, consequently, it was selected for effective pectinase production.

Some preliminary effective pretreatments of the pigmented onion skins included physical, chemical and physico-chemical treatments revealed that the physico-chemical pretreatment by skins milling, then sodium chlorite and GAA treatment

Table 1 % composition of the native Egyptian onion skins.

Component(x)	Pectin	Hemicellulose	α-Cellulose	Pigments	Moisture	Others
Percentage (%)	23.3	14	41.1	5.1	3	13.5

Table 2 Screening of some filamentous fungi utilizing Egyptian pigmented onion skins as a sole carbon source for extracellular pectinase enzymes production in shaken cultures.

Fungal isolates	Pectinase activity (U/mg CF*)	
	4 days	7 days
Aspergillus niger	0.095 ± 0.15^{a}	0.052 ± 0.25^{a}
Penicillium loliense	0.079 ± 0.19^{b}	0.073 ± 0.20^{a}
Trichoderma harzianum	0.073 ± 0.16^{a}	0.050 ± 0.16^{b}
T. longibrachiatum	0.066 ± 0.14^{a}	0.059 ± 0.18^{b}
T. viride	0.334 ± 0.20^{b}	0.214 ± 0.13^{b}
Ulocladium botrytis	0.186 ± 0.15^{a}	0.052 ± 0.15^{b}

Values are expressed as (mean ± SD, n = 3).
The same letter within the same column are not significant ($P \leqslant 0.05$).
* CF, in this and the following tables = culture filtrate.

Table 3 Production of extracellular pectinases by *T. viride* utilizing different pretreated Egyptian onion skins on equal carbon basis.

Skins pretreatment	Pectinase activity (U/mg CF)
Control (citrus pectin)	0.379 ± 0.22^{a}
None (native skins)	0.335 ± 0.24^{a}
H_2O soaking*	0.322 ± 0.27^{a}
Crushing	0.457 ± 0.25^{a}
Crushing + H_2O soaking*	0.320 ± 0.30^{b}
Boiling in H_2O for	
(5 min)	0.296 ± 0.35^{b}
(10 min)	0.303 ± 0.33^{b}
(20 min)	0.309 ± 0.26^{a}
Autoclaving (1.5 atm., 120 °C, 15 min)	0.268 ± 0.31^{b}
1 N–HCl soaking*	0.329 ± 0.30^{b}
1 N–NaOH soaking*	0.398 ± 0.24^{a}
Unpigmentation (sodium chlorite + GAA)	0.486 ± 0.21^{a}

Values are expressed as (mean ± SD, $n = 3$).
The same letter within the same column are not significant ($P \leqslant 0.05$).
* Overnight.

was the most appropriate for maximal pectinase productivity (Table 3).

The initial acidic pH 4.0 of the basal production medium and the incubation temperature 30 °C were the optimum for pectinase productivity by *T. viride* EF-8 and led to the maximal yield in four days shaken cultures (Table 4).

From the purification steps performed (sequential and batch precipitation), it is obvious that ethanol with a concentration of 50%, v/v, was the most appropriate and precipitated out the most active pectinase enzyme more than the other precipitants (Table 5 and 6).

It was obvious from Table 7 that the general properties of the partially purified pectinase produced by *T. viride* exhibited its maximal activity at pH 7.5, applying 0.02-M acetate buffer, pH 5.0 and displayed excellent activities within a very wide range of temperatures (30–60 °C) with the maximal activity at 50 °C. Moreover, the results demonstrated a parallel relationship existed between the enzyme concentration and pectinase activity up to the concentration of 2 mg/mL. The results indicated also that the substrate concentration above 1.5% (w/v) was a limiting factor for pectinase activity.

Table 4 Optimization of pectinase enzymes productivity by *T. viride* in 4 days shaken cultures.

Parameter		Protein content of CF (mg/mL)	Pectinase activity (U/mg CF)
Initial pH	3	0.32 ± 0.14^{a}	0.380 ± 0.26^{a}
	4	0.11 ± 0.20^{b}	0.400 ± 0.24^{a}
	5	0.18 ± 0.22^{b}	0.332 ± 0.25^{a}
	6	0.35 ± 0.17^{a}	0.300 ± 0.33^{b}
	7	0.23 ± 0.23^{b}	0.278 ± 0.20^{a}
	8	0.23 ± 0.26^{b}	0.256 ± 0.35^{b}
	9	0.11 ± 0.23^{b}	0.123 ± 0.36^{b}
Incubation temperature (°C)	20	0.13 ± 0.25^{a}	0.259 ± 0.35^{a}
	25	0.11 ± 0.28^{a}	0.321 ± 0.30^{a}
	30	0.02 ± 0.30^{b}	0.397 ± 0.31^{a}
	35	0.07 ± 0.33^{b}	0.345 ± 0.28^{b}
	40	0.14 ± 0.24^{a}	0.304 ± 0.36^{a}
	45	0.21 ± 0.20^{a}	0.234 ± 0.25^{b}

Values are expressed as (mean ± SD, $n = 3$).
The same letter within the same column are not significant ($P \leqslant 0.05$).

Enzyme efficiency tests (Table 8) of the partially purified *T. viride* EF-8 pectinase mixed with the fresh or boiled fruit juices, applying the optimum conditions earlier specified for the enzyme activity, distinctly revealed that with any of the boiled juices, neither the juice components nor juice environment inhibited the enzyme activity, furthermore, it is noticed that there was an increase in the partially purified effectiveness and this was recorded after mixing with the fresh juice within 180 min comparing with the control and this may be attributed to the activity of pectic enzymes already presented in the fresh juice and participated in the whole pectinase activity. This asserted a good evidence for an excellent applicability of the partially purified pectinase preparation in fruit juice clarification process.

5. Discussion

The utilization of microbial enzymes has found broad technological application in different industrial processes and the majority of the industrial enzymes are of microbial origin [3]. In the present study, six recommended fungal isolates were tested for their pectinase production suitable for clarification of different fruit juices. The results revealed the superiority

Table 5 Sequential purification of the crude fungal pectinases by different precipitating agents.

Precipitating agent	Saturation or concentration (%)	Total protein content of fraction (mg)	Protein recovery (%)	Total pectinase activity (U/fraction)	Specific activity (U/mg protein)	Purification fold	Recovered activity (%)
Crude (air dried)	None	12.0	100.00	24.3	2.02	1.00	100.00
Ammonium sulphate	50	2.5	20.83	7.0	2.8	1.39	28.81
	70	3.5	29.17	7.3	2.08	1.03	30.04
Ethyl alcohol	32	4.5	37.50	6.3	1.40	0.69	25.92
	40	4.0	33.33	6.5	1.62	0.80	26.75
	45	5.0	41.67	11.7	2.34	1.16	48.15
	50	6.0	50.00	18.2	3.03	1.50	74.91
	70	2.0	16.67	2.0	1.00	0.50	8.23
Acetone	50	4.0	33.33	6.7	1.68	0.83	27.57
	70	1.0	8.33	1.3	1.3	0.64	5.35
	90	0.5	4.17	0.250	0.50	0.25	1.03

Table 6 Batch (bulk) purification of the crude fungal pectinases by ethyl alcohol.

Precipitating agent	Concentration (%)	Total protein content of fraction (mg)	Protein recovery (%)	Total pectinase activity (U/fraction)	Specific activity (U/mg protein)	Purification fold	Recovered activity (%)
Crude (air dried)	None	12.0	100.00	24.3	2.02	1.00	100.00
Ethyl alcohol	40	3.5	29.17	9.3	2.65	1.31	38.27
	45	4.7	39.16	15.4	3.28	1.62	63.37
	50	5.2	43.33	20.6	3.96	1.80	84.7
	70	1.7	14.17	4.2	2.47	1.22	17.28

Table 7 General properties of the different fungal pectinase forms.

Parameter	Enzyme form	Pectinase specific activity (U/mg protein)	
		Crude	50% bulk ethanol enzyme fraction
Enzyme protein concentration (mg/mL)	**1**	1.60±0.03 [a]	2.40±0.10 [a]
	2	2.90±0.01 [a]	3.20±0.11 [b]
	3	2.60±0.11 [b]	3.01±0.09 [c]
	4	2.10±0.10 [b]	2.50±0.08 [c]
	5	1.45±0.09 [b]	1.98±0.10 [a]
Substrate (Citrus pectin) concentration (%, w/v)	**0.25**	1.78±0.05 [a]	2.02±0.12 [a]
	0.50	2.21±0.02 [b]	2.45±0.04 [b]
	1.0	2.47±0.01 [b]	3.14±0.11 [a]
	1.5	2.74±0.05 [a]	2.90±0.06 [c]
	2.0	2.00±0.08 [c]	2.78±0.04 [b]
Reaction pH value,			
Citrate buffer (0.3 M)	**5**	2.10±0.07 [a]	2.31±0.11 [a]
	6.5	2.18±0.05 [b]	2.45±0.09 [b]
	7	2.40±0.05 [b]	3.43±0.04 [c]
	7.5	2.80±0.04 [c]	3.00±0.06 [c]
	8	2.45±0.03 [c]	3.04±0.09 [b]
Glycine-NaOH buffer (0.2 M)	**8.5**	2.14±0.06 [a]	2.56±0.11 [a]
Reaction temperature (°C)	**30**	2.26±0.04 [a]	2.59±0.10 [a]
	40	2.48±0.05 [b]	3.15±0.12 [b]
	50	2.60±0.04 [a]	2.78±0.14 [b]
	60	2.23±0.05 [b]	2.63±0.10 [a]

Values are expressed as (mean ± SD, $n = 3$).
The same letter within the same column are not significant ($P \leqslant 0.05$).

Table 8 Efficiency of 50 % bulk ethanol pectinase* fraction mixed with different fruit juice.

Mixing period (min)	Juice specimen	Enzyme efficiency (%) with		
		Fresh juice	Boiled juice	Difference**
20	Lemon	106.3 ± 0.35[a]	102.2 ± 0.36[a]	4.1
	Orange	108.1 ± 0.33[a]	104.3 ± 0.29[b]	3.8
	Apple	107.6 ± 0.25[b]	103.6 ± 0.25[b]	4.0
30	Lemon	112.4 ± 0.22[a]	107.5 ± 0.20[a]	4.9
	Orange	117.7 ± 0.25[a]	111.3 ± 0.26[b]	6.4
	Apple	115.1 ± 0.30[b]	112.0 ± 0.29[b]	3.1
60	Lemon	123.6 ± 0.25[a]	117.8 ± 0.32[a]	5.8
	Orange	121.8 ± 0.19[b]	118.2 ± 0.35[a]	3.6
	Apple	126.5 ± 0.18[b]	119.3 ± 0.25[b]	7.2
120	Lemon	93.1 ± 0.28[a]	89.6 ± 0.26[a]	3.5
	Orange	89.4 ± 0.25[a]	84.4 ± 0.31[b]	5.0
	Apple	84.8 ± 0.15[b]	81.3 ± 0.35[b]	3.5
150	Lemon	79.1 ± 0.30[a]	73.5 ± 0.24[a]	5.6
	Orange	82.0 ± 0.20[b]	76.5 ± 0.25 [a]	5.5
	Apple	77.1 ± 0.27[a]	72.9 ± 0.30[b]	4.2
180	Lemon	69.4 ± 0.25[a]	62.0 ± 0.25[a]	7.4
	Orange	73.1 ± 0.29[b]	67.3 ± 0.19[b]	5.8
	Apple	63.1 ± 0.30[b]	59.0 ± 0.22[a]	4.1

Values are expressed as (mean ± SD, $n = 3$).
The same letter within the same column are not significant ($P \leqslant 0.05$).
* Compared with control and carried out under optimum activity conditions.
** Represents the original activity in the fresh juice.

of *T. viride* EF-8 as a potent producer for pectic enzymes. The ability to synthesize pectinases is wide spread among many microbial groups but molds (e.g. *T. harzianum; A. niger, Rhizopus*) are preferred because as much as 90% of the enzyme can be extracted into the culture medium [4]. In this respect, [25] reported the production of pectinase enzymes by *T. harzianum*.

Various waste pretreatments and production systems providing technical and economic feasibility to harness the renewable materials, while at the same time cleaning up the environment have been highlighted. Physico-chemical and biological pretreatment processes appear to be the mostly preferred methods [23]. Higher yields of pectinases were obtained when the pigmented onion skins were physico-chemically pretreated compared with native ones pointing out the inhibitory effects of both their pigments and crystallinity on the secreted enzymes action and this was earlier reported by [10] where he pointed out that the good softness and poor crystallinity of the grated orange peels strongly pronounced them for multienzyme complexes production through their fungal biodegradation.

Regarding the fermentation conditions optimization, the decrease in the enzyme yield after 4 days may be due to the variation in pH of the medium towards the acidity resulted by the formation of citric and acetic acids. In this respect, [26] obtained maximum pectinlyase activity by *Penicillium griseoroseum* after 65 h, while [9] mentioned that *A. foetidus* grown in apple pulp produced maximal pectinlyase activities after 96 hours. The effect of the higher acidic pHs was substantiated with the hereinafter enzyme properties.

The incubation temperature was also found to influence pectinase production. The incubation temperature has a profound effect on the enzyme yield and the duration of enzyme phase synthesis. Most of the fungi for pectinase production investigated showed optimum growth in the range of 45–60 °C [7,27].

It is worthy to note that 2 mg/mL enzyme protein concentration was the most favorable and led to the maximal activity. Respecting the effect of substrate (citrus pectin) on the enzyme activity, the pectin concentration of 1.0–1.5% (w/v) was the most appropriate for both the crude and the partially purified enzyme forms. In this respect, [2,30] reported that 0.8–1.0% (w/v) pectin was the most appropriate for other fungal pectinases.

It is worthy to mention that the fraction precipitated at batch 50% ethanol was unique in being the richest in its pectinase activity. Many investigators used ammonium sulphate, ethanol and acetone for the precipitation of pectic and other enzymes [1].

Enzyme activity is markedly affected by pH because substrate binding and catalysis are often dependent on charge distribution on both substrate and, in particular, enzyme molecules [15]. In this manner, [29] reported that polygalacturonase (PG) from *Penicillium viridicatum*, maintained 90% of its activity after incubation in pHs from 5.0 to 8.5. Also, the PG from *Thermoascus aurantiacus* retained more than 90% of its activity when incubated in pH between 7.0 and 8.0 [21]. With regard to temperature effect on pectinase activity, [33] reported that PG was almost inactivated at a temperature above 60 °C for 5 min. The endo-PG from *Aspergillus japonicas* lost more than 50 % of its activity during 5 min at 50 °C [28]. Lehninger et al. [18] reported that every enzyme is optimally active and stable up to a certain temperature and gets denatured at higher temperatures and the activity of the enzyme gradually increased with increasing assay pH up to 7.5 and thereafter declined. This is a well established fact that each enzyme has a characteristic pH optimum for its activity [18].

The turbidity of the fresh juice is mainly caused by the presence of the pectic fine particles. Application of pectinases leads to the pectin particles degradation and consequently reduce the juice turbidity [17,19]. Moreover, the pectic substances possess a high water holding capacity and develop a cohesive network structure. Also, the degradation of pectin by pectinases leads to a reduction in viscosity [17,19]. Pectin in fruit juices may exist with various degrees of esterification depending upon fruit variety and ripening [22].

Conclusively, this research establishes a simple and created technology for cheap and perfect exploitation of considerable plant waste, the outer pigmented onion skins discarded through all the onion processes, representing a double interest goal for all onion producing countries, the first is the production of an applicable and cheap enzyme preparation and the second is the face of hard environmental pollution by agricultural wastes burning.

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