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Original article

Utilization of agro-industrial orange peel and sugar beet pulp wastes for fungal endo- polygalacturonase production

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ABSTRACT

The pectinase enzymes are involved in several industrial applications, and industrial waste is one of the largest environmental pollutants, so this study aims to Endo-polygalacturonase (*endo*-PG) producing using *Aspergillus niger* AUMC 4156, *Penicillium oxalicum* AUMC 4153 and *P. variotii* AUMC 4149 by using some agro-industrial wastes (dried orange peel and sugar beet pulp) as a sole raw carbon source for degradation these waste in the process of urban wastes disposal. The fermentation process was carried out as a submerged culture technique under both shaken and static culture conditions. *A. niger* AUMC 4156 was the most promising producer of *endo*-PG under static conditions while *P. oxalicum* AUMC 4153 was the highest producer of *endo*-PG under shaken conditions. Sugar beet pulp proved to be the most preferable to orange peel as the only source of carbon in both shaken and static cultures. The medium that encompassing orange peel as a single carbon source afforded the highest protein content with all tested fungal strains in stirred and static cultures in comparison with sugar beet pulp. The highest activity of *endo*-polygalacuronase that produced using *A. niger* AUMC 4156 and *P. oxalicum* AUMC 4153 was achieved by using sugar beet pulp at 3% concentration under static cultures, meanwhile maximal enzyme activity produced by both fungal strains required 2% sugar beet pulp under shaken cultures. Sugar beet pulp showed promised potential as a good inducer for *endo*-polygalacturoase production, and enzymes production depended on fungal strains, culture medium, and submerged fermentation conditions.

1. Introduction

Currently, the essential exploitation of the wastes food and agricultural industries that contribute to environmental contamination is the degradation of the waste biologically under controlling using the microorganisms aimed at the production of useful products such as vitamins, polypeptides, proteins, oligosaccharides, polysaccharides, enzymes, hormones, and others.

Pectic compounds are copious in the plant biomass composition, their levels are between 4 and 30% in the pulp of beet and the peel of citrus fruit. Pectins are complex acidic polysaccharides composed of the heterogeneous group with high molecular weight that are contains mostly D- galacturonic acid (Antranikian, 1992). The hydrolyzing enzymes of pectic constituents are generally called pectic enzymes, pectinases, or pectinolytic enzymes, which comprise pectinesterase, polygalacturonase, pectin lyase, and pectate lyase based on their action mode. Polygalacturonases (PGases) are hydrolytic depolymerases with endo (EC: 3.2.1.15) and exo (EC: 3.2.1.67) activities (Alkorta et al., 1998; Kashyab et al., 2001). The pectinase complex enzymes, which are of the utmost importance, are polygalacturonase, pectin lyase, pectin esterase and pectate lyase. The pectin Hydrolases as Polygalacturonases catalysis α-1,4-glycosidic linkage hydrolysis in pectic acid (Palagiri et al., 2019).

The synthesis of pectinase enzymes ability is common among most groups of microbes, but fungi are preferred for industrial purposes that are because approximately 90% of the produced enzyme may be secreted into the media culture (Solis et al., 1990). Among

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fungi, *Aspergillus niger*, *Penicillium,* and *Rhizopus* have many advantages as enzyme producers since they are recognized as Generally Regarded As Safe (GRAS) strains and yield extracellular products that can be recuperated easily from fermented medium (Blanco et al., 1999). The *Aspergillus* and *Penicillium* strains mainly were utilized for exo- and *endo*-PGase production studies (Favela-Torres et al., 2006). The *A. niger* strain is considered one of the utmost essential fungi in biotechnology applications. It is a safe fungus for production (Schuster et al., 2002). *P. oxalicum* produced high levels of polygalacturonase, pectinesterase, and pectin lyase (Jing et al., 2008). Both solid-state fermentation (SSF) and Submerged fermentation (SMF) methods were extensively utilized in the production of PGase using various microorganisms types (Favela-Torres et al., 2006).

Pectinases possess common applications in several industries such as food technology and fabric production (Henriksson et al., 1999), it is completely suitable for the maceration of plant tissues, degumming, natural fibers removing and wastewater treatment (Baracat-Pereira et al., 1993). Pectin esterase, pectin lyase and polygalacturonase have been used in the extraction of fruit juice, clarification and depectinization processes however the combination of cellulose, pectinases and hemicellulase are applied in cell walls devastation and juice extraction from fruit (Voragen et al., 1986).

Because of the growth of pectinase, decreasing applications the cost of its production has become currently among the utmost significant objectives. Therefore, the selection of nitrogen and carbon sources of low cost become a useful consideration for the producers. Several findings have stated that numerous waste materials resulting from agricultural manufacturing comprising of pectin, like the sugar beet pulp (SBP), the pulps of (citrus pellets, lemon, and henequen), orange peel (OP), wheat bran, apple pomace, and additional relative resources were utilized in the pectinase production inducting as carbon source using numerous microbes (El-Sheekh et al., 2009; Heerd et al., 2014; Ismail, 1996; Said et al., 1991). These enzymes not only provide a viable alternative economically, nevertheless, they are also environmental friends (Viikari et al., 2001).

Sugar beet pulp (SBP) a by-product of the table sugar industry is of low cost, it exists in great amounts. About 271.6 million tons of the sugar beets were produced in 2011 globally (FAO, 2011), and it arrived to 284.4 Mt and 285.08 Mt in 2019 and 2021 respectively (OECD/FAO, 2021) which resulted after the extraction of sucrose in the manufacture of around 68 million tons of the wet SBP or about 17 million tons of the dehydrated biomass (Foster et al., 2001). Pulp of sugar beet is principally contained 28.7 pectins, 9.0 proteins, 17.5 hemicellulose, 20 cellulose, and 4.4 lignin (% of dray matter) (Jacob, 2009). Approximately 30.10 million tons of orange manufacturing will treat to income the juice of orange, essential oils, and additional by-products (FAO, 2003), and about 75.54 Mt of orange were produced worldwide in 2018 (Ritchie and Roser, 2020). Today, orange juice is considered one of the greatest consumed drinks (Martín et al., 2010). Subsequently, a great proportion of the citrus fruit is used for the production of the juice and marmalade. Also, almost 50–60% of the citrus fruit amount is altered to waste (Wilkins et al., 2007). Dry orange peel OP is rich in hemicellulose, pectin, and cellulose (Ismail, 1996). Orange peel mainly consists of hemicellulose (10%), cellulose (13.6%) (Ververis et al., 2007). Therefore, the present study was aimed at utilization of dried OP (a by-product of orange juice extraction) and dried SBP (sugar beet by-product industries) as a sole carbon source through its microbial biodegradation by *A. niger* AUMC 4156, *P. oxalicum* AUMC 4153, and *P. variotii* AUMC 4149 for the low-cost production of *endo*-PG (E.C. 3.2.1.15) enzyme and aids to resolve discarding problems of these by-products. Also, the comparison between shaken and static submerged fermentation conditions was investigated for optimizing the production of *endo*-PG by the tested fungal strains.

2. Materials and methods

2.1. Microorganisms

A. niger AUMC 4156, *P. oxalicum* AUMC 4153 and *Paecilomyces variotii* AUMC 4149, known as highly pectinase–producing strains, were isolated from infected and decayed citrus fruits and identified at Assiut University Mycological Center (AUMC) as previously described by (Al-Mowallad, 2008).

2.2. Culturing media

The stock cultures of fungal strains were maintained on potato dextrose agar medium slant in a refrigerator at 5℃ by periodic subculturing. Fungal spore suspensions used as inocula were prepared by using fungal growth enhancement agar medium as described by Ismail (1996), its composition was (g/L): glucose, 5.0; yeast extract, 10; peptone, 0.5; KH_2PO_4, 1.0; $MgSO_4$, 1.0 and agar, 20. Slants tubes incubated at 30℃ for 4 days. Spores were harvest from the slants tubes by using sterile saline solution (0.9% w/v). The spore suspensions were adjusted to a final concentration of approximately $3x10^8$ spores per ml, the number of the spore was estimated by the direct microscopic count method by the haemocytometer.

2.3. Wastes

2.3.1. Orange peel powder

Sweet OP was collected by unpeeling the fresh sweet orange fruits that were obtained from the local market of the fruit of Assiut city, Assiut, Egypt. The peels were washed and sliced into small species, then they were arid using a hot air oven at 50 °C to reach final moisture lower than 10–11% for 24 h. The dried peels were powdered by Lab. grinder (diameter of sieves 1.5 mm).

2.3.2. Sugar beet pulp powder

The sugar beet manufacturing by-product was obtained from Delta sugar Company, Kafr El-sheikh, Egypt. It was arid at 50 °C to reach final moisture lower than 10–11% for 24 h. The dried small particles of pulp were powdered by Lab. grinder (diameter of sieves 1.5 mm).

2.4. Chemicals

Polygalacturonic acid from orange (Sigma P3889, Germany), was used as a substrate for the determination of *endo*-polygalacturonase activity. Folin ciocalteu′s phenol reagent (2 N), (Sigma-Aldrich F9252, Germany) was used for the estimation of protein content. Bovine serum albumin, (Sigma A2153, Germany), used for the preparation of standard curve for calculation of protein content.

2.5. Chemical analysis procedures

Gross chemical composition of dried OP and SBP measured according to the references methods of the Association of Official Analytical Chemists (AOAC, 1990).

2.6. Cultivation of fungi and endo-PG production by submerged fermentation

The fermentation broth medium used for fungal growth and the production of enzyme was prepared according to Paterson et al.

(1989), with replacement of pure pectin with OP powder or SBP powder (30 g/L). The medium composition was (OP powder or SBP powder, 30; $(NH_4)_2HPO_4$, 2; $NH_4H_2PO_4$, 0.9; $MgSO_4$, 0.1 and KCl, 0.5 g/L). Medium pH adjusted to 5,6 and 7 (optimum pH for pectinase production by *A. niger* AUMC 4156, *Paecilomyces variotii* AUMC 4149, and *P. oxalicum* AUMC 4153, respectively (Al-Mowallad, 2008). The Cultivation process was made in a 250 ml Erlenmeyer flask; each flask contains 100 ml of sterile fermentation broth media, which then was inoculated with the addition of 1 ml of fungal spore suspension that containing about $3x10^8$ spores per ml. Then, the flasks then were incubated for a period of 5 days at 30C under both static and shaken (150 rpm) submerged fermentation. All experiments were accomplished in triplicates. At the incubation period end, each flask contents were filtered by Whatman No.1 filter paper. The culture filtrates served as the crude enzyme source.

2.7. Assay for endo-polygalacturonase activity (endo-PG)

The activity of Endo-polygalacturonase (EC: 3.2.1.15) in culture filtrate was measured viscometrically by measuring reduction in viscosity (as a result of enzyme action on the substrate) as described by Ghildyal et al. (1981). With certain slight modifications as follows:

One milliliter of culture filtrate was mixed with ten ml of 1% (w/v) Polygalacturonic acid from orange (dissolved in 0.05 M of the buffer of sodium acetate, pH 5.0). The mixture was then incubated for 20 min at 45C using a controlled water bath. The viscosity reduction was followed by using of Ostwald Viscometer (Sarvamangala and Dayanand, 2006). The *endo*-PG activity was expressed as the ratio of the viscosity reduction of 1% buffered polygalacturonic acid solution upon the enzyme action under assay conditions (Couri and Farias, 1995; Ismail, 1996).

2.8. The influence of different concentrations of waste on endo polygalacturonase production

Different concentrations of SBP waste (1–5% w/v) were studies for optimizing their levels for supporting high *endo*-polygalacturonase production.

2.9. Estimation of protein content

Protein concentration of the culture filtrates was determined using of the method of (Lowry et al., 1951), bovine serum albumin was used as standard.

2.10. Statistical analysis

All trials were accomplished in triplicates, the means and standard deviations St.D of the results were calculated. All data were statistically analyzed by analysis of variance (ANOVA) using one-way and Duncan's multiple pot hoc tests by means of the SPSS V. 21 (SPSS Inc., Chicago, IL, USA). The differences were indicated statistically significant at $p \leq 0.05$ level.

3. Results

3.1. Gross chemical composition of dried orange peel and sugar beet pulp

The OP and SBP wastes, which were used separately as a single carbon source for *endo*-polygalacturonase enzyme production, were analyzed to determine their chemical composition. The results are given in Table 1 showed that pectin content was high in both wastes but the highest level of pectin content was detected in SBP (36.3%) in comparison with that in OP (20.4%). While highest total carbohydrates and reducing sugar amount were obtained in OP (20.9 and 17.8%, respectively) comparing with SBP (3.2 and 1.3%, respectively). The high pectin concentration in these wastes could be very useful for pectinase enzymes production.

Table 1
Gross chemical composition of the dried orange peel and sugar beet pulp (g/100 g on a dry weight basis).

Constituents	*Sugar beet pulp%*	*Orange peel %*
Moisture	12.00 ± 2.21^{c}	10.00 ± 1.53^{b}
Total sugars	3.2 ± 0.75^{e}	20.9 ± 2.5^{a}
Reducing sugars	1.3 ± 0.27^{f}	17.8 ± 1.20^{a}
Pectin	36.3 ± 2.18^{a}	20.4 ± 1.44^{a}
Protein	9.8 ± 1.42^{c}	8.3 ± 0.62^{b}
Fat	2.3 ± 0.53^{f}	1.6 ± 0.07^{d}
Ash	5.6 ± 0.94^{d}	4.3 ± 0.58^{c}
Crude fiber	16.6 ± 1.42^{b}	9.5 ± 1.10^{b}

Difference letters in the same column indicate the significant differences at $p \leq 0.05$.

In the SBP, there were significant differences were appeared between pectin, crude fiber, moisture, protein, ash, total sugar, fat and reducing sugar content respectively at $p \leq 0.05$ level. While in OP total sugar, pectin and reducing sugar were higher contents they differed significantly from that of moisture, crude fiber, protein, ash, and fat respectively at $p \leq 0.05$.

3.2. Suitability of orange peel and sugar beet pulp for endo-polygalacturonase production using static and shaken submerged fermentations

Data presented in Table 2 showed that the synthesis of *endo*-PG by all of the tested fungal isolates depended on the presence of their substrates (OP or SBP) in the culture medium used, the fungal strain and submerged cultures technique (static or shaken). The synthesis of *endo*-PG occurred in all tested fungal strains using both agro-industrial wastes. *A. niger* AUMC 4156 resulted in highly active *endo*-PG in static cultures while shaken cultures were more suitable for *P. oxalicum* AUMC 4153. The highest levels of *endo*-PG activity was discovered in *A. niger* AUMC 4156 culture filtrate under static conditions with both SBP and OP. Endo- PG activity amounted to 54, 52, and 48% by *A. niger* AUMC 4156, *P. oxalicum* AUMC 4153 and *Paecilomyces variotii* AUMC 4149, respectively. The statistical results showed that Endo-PG activity from *A. niger* was significantly increased than that of *P. oxalicum* and *P. variotii* respectively in the static cultures for both SBP and OP media at $p \leq 0.05$ level. While in the shaken culture *P. oxalicum* was the highest followed by *A. niger* and then *P. variotii* which lowered significantly comparing with both fungi strains at $p \leq 0.05$ level. On the other hand, the Endo-PG activity from both *A. niger* and *P. variotii* high in the static SBP followed by the static OP, shaken SBP and shaken OP respectively with significant differences at $p \leq 0.05$. While for *P. oxalicum* the shaken SBP showed significant differences in compare with that of all other media cultures at the same level.

The results in Table 3 demonstrated that the use of OP as the single source of carbon caused in utmost protein amount with all of the tested fungal strains in stirred and static culture filtrates in comparison with SBP, which permitted the creation of the highest specific *endo*-PG activities. On other hand, the protein content of the culture filtrates was low in stirred condition comparing with the static condition. The highest protein content levels of *A. niger* AUMC 4156, *P. oxalicum* AUMC 4153 and *P. variotii* AUMC 4149 culture filtrates were (4.74, 4.45, and 4.98 mg/ml, respectively) using OP under static conditions. Also, it was observed that the produc-

Table 2
Endo-PG activity (% reduction in viscosity of 1% buffered polygalacturonic acid solution) in various shaken and static fungal cultures*.

Fungal strains	Endo- polygalacturonase activity (% reduction in viscosity)			
	Sugar beet pulp 3%(w/v)		Orange peel 3% (w/v)	
	Static	Shaken	Static	Shaken
***A. niger* AUMC 4156**	54.44 ± 1.4^{a1}	51.11 ± 2.5^{b3}	52.22 ± 1.63^{a2}	48.89 ± 1.4^{b4}
***P. variotii* AUMC 4149**	42.65 ± 2.31^{c1}	5.88 ± 1.86^{c3}	14.06 ± 1.14^{c2}	2.94 ± 0.41^{c4}
***P. oxalicum* AUMC 4153**	50.22 ± 2.74^{b2}	52.94 ± 2.0^{a1}	49.26 ± 2.53^{b2}	50.00 ± 1.3^{a2}

*Fermentation conditions: 5 days at 30C, shaken (150 rpm). Difference letters in the same column indicate significant differences at $p \leq 0.05$. Difference numbers in the same raw indicate significant differences at $p \leq 0.05$.

Table 3
Protein content (mg/ml) of the culture filtrates of static and shaken fungal cultures*.

Fungal strains	*Protein content (mg protein/ml)*			
	Sugar beet pulp 3% (w/v)		Orange peel 3% (w/v)	
	Static	Shaken	Static	Shaken
***Aspergillus niger* AUMC 4156**	3.72 ± 0.42^{a}	1.66 ± 0.09^{b}	4.74 ± 0.6^{a}	2.57 ± 0.43^{b}
***Paecilomyces variotii* AUMC 4149**	2.82 ± 0.9^{b}	2.94 ± 0.07^{a}	4.45 ± 0.74^{b}	3.75 ± 0.71^{a}
***Penicillium oxalicum* AUMC 4153**	3.98 ± 0.82^{a}	2.29 ± 0.3^{a}	4.98 ± 0.42^{a}	3.40 ± 0.20^{a}

*Fermentation conditions: 5 days at 30C, shaken (150 rpm). Difference letters in the same column indicate significant differences at $p \leq 0.05$.

tion of extracellular enzyme activities wasn't reliable with the concentration of the extracellular protein found in the culture filtrates.

The statistical analysis results showed that protein contents that produced by both *P. oxalicum* AUMC 4153 and *A. niger* AUMC 4156 and in both static SBP and OP media was higher with significant differences than that by *P. variotii* AUMC 4149 in the same media at level $p \leq 0.05$, while in the shaken SBP and OP media the protein of *A. niger* AUMC 4156 was decreased significantly that that of both other fungal strains at level $p \leq 0.05$.

3.3. The impact of different concentrations of sugar beet pulp

The obtained results of different SBP concentrations (1–5%) on the production of *endo*-PG by *A. niger* AUMC 4156 and *P. oxalicum* AUMC 4153 are summarized in Tables 4 and 5. The *endo*-PG activity increased gradually with increasing the added concentration of SBP reaching maximum at 3% for *A. niger* AUMC 4156 and *P. oxalicum* AUMC 4153 under static conditions. The maximum activity of endo- polygalacturonase took place at the concentration of 2% SBP for *A. niger* AUMC 4156 and *P. oxalicum* AUMC 4153 in stirred submerged fermentation.

It found that there are significant differences between the different SBP concentrations on *endo*-PG production by *A. niger* using both static conditions at $p \leq 0.05$ level, the highest significant differences of *endo*-PG production in the static condition was shown in the concentration 3% followed by 4%, 2%, 5%, and the concentration 1%, respectively. Also, the *endo*-PG production in the shaken condition both 2% and 3% concentrations were increased significantly, followed by 1%, 4%, and 5% respectively at the same significant level. For comparing between the static and shaken growing conditions using the same concentration, the *endo*-PG production by *A. niger* was high significant differences at $p \leq 0.05$ level was appeared for the shaken media in the low concentrations 1% and 2%, while the static media was surpassed significantly in the high concentrations 3%, 4%, and 5%. The protein production by *A. niger* was increased significantly when SBP concentration increasing in both static and shaken conditions, and protein contents of the static condition were showed a highly significant difference in increasing in all concentrations compared with that of the shaken condition at $p \leq 0.05$ level.

Results in Tables 4 and 5 also revealed that further increasing of sugar beet pulp concentration in the fermentation medium can cause a reduction in *endo*-PG yield by *A. niger* AUMC 4156 and *P. oxalicum* AUMC 4153, probably due to carbohydrate catabolic repression in the cultures, which was maybe higher under shaken culture than static culture, this could be as a result of agitation action. So maximum *endo*-PG activity (52%) was detected at low concentration (2%) of SBP while minimum *endo*-PG activity (44%) was observed at high concentration (5%) by both fungal strain tested.

The effect of different concentrations of SBP on *endo*-PG production by *P. oxalicum* AUMC 4153 using both static and shaken conditions, explored significant differences between the used concentration in the static condition, it was high in 3% followed

Table 4
Effect of different concentrations of sugar beet pulp on *endo*-PG production by *Aspergillus niger* AUMC 4156 using both static and shaken conditions*.

Sugar beet pulp powder Concentration % (w/v)	Endo- polygalacturonase activity (% reduction in viscosity)			
	Static		Shaken	
	(% reduction in viscosity)	Protein content (mg/ml)	(% reduction in viscosity)	Protein content (mg/ml)
1%	43.33 ± 2.19^{d2}	1.38 ± 0.04^{e1}	48.89 ± 1.54^{b1}	0.80 ± 0.05^{e2}
2%	47.78 ± 1.73^{c}	2.99 ± 0.08^{d1}	52.22 ± 1.97^{a1}	1.29 ± 0.05^{d2}
3%	54.77 ± 3.03^{a1}	3.75 ± 0.04^{c1}	51.00 ± 2.59^{a2}	1.63 ± 0.03^{c2}
4%	51.11 ± 2.27^{b1}	4.43 ± 0.06^{b1}	46.66 ± 2.12^{c2}	2.94 ± 0.07^{b2}
5%	46.67 ± 2.26^{c1}	4.77 ± 0.03^{a1}	44.45 ± 2.06^{d2}	3.50 ± 0.04^{a2}

*Fermentation conditions: 5 days at 30C, shaken (150 rpm). Difference letters in the same column indicate significant differences at $p \leq 0.05$. Difference numbers in the same raw indicate significant differences at $p \leq 0.05$.

Table 5
Effect of different concentrations of sugar beet pulp on *endo*-PG production by *Penicillium oxalicum* AUMC 4153 using both static and shaken conditions*.

Sugar beet pulp powder Concentration % (w/v)	Endo- polygalacturonase activity (% reduction in viscosity)			
	Static		Shaken	
	(% reduction in viscosity)	Protein content (mg/ml)	(% reduction in viscosity)	Protein content (mg/ml)
1%	36.76 ± 1.07^{d2}	1.48 ± 0.05^{d1}	48.53 ± 2.04^{b1}	0.96 ± 0.01^{e2}
2%	42.65 ± 2.53^{c2}	3.24 ± 0.02^{c1}	53.47 ± 2.16^{a1}	1.92 ± 0.02^{d2}
3%	50.73 ± 1.91^{a2}	3.95 ± 0.04^{b1}	52.94 ± 2.13^{a1}	2.33 ± 0.01^{c2}
4%	45.59 ± 1.42^{b}	4.42 ± 0.06^{a1}	45.59 ± 1.87^{c}	2.58 ± 0.03^{b2}
5%	41.18 ± 2.06^{c2}	4.67 ± 0.05^{a1}	43.38 ± 1.33^{d1}	2.90 ± 0.02^{a2}

*Fermentation conditions: 5 days at 30C, shaken (150 rpm). Difference letters in the same column indicate significant differences at $p \leq 0.05$. Difference numbers in the same raw indicate significant differences at $p \leq 0.05$.

by 4%, 2%, 5%, and 1% respectively at $p \leq 0.05$ level. Moreover, in the shaken condition both 2% and 3% were the highest then 1%, 4%, and 5% respectively. The shaken media reveal significant differences in the concentrations 1, 2, 3, and 5%, from that of the static media while no differences are shown in the concentration 4%. In addition, protein production was augmented significantly by the elevation of media concentrations in both conditions, and the static media was exceeded in its protein contents in all concentrations comparing with that of the shaken media.

4. Discussion

4.1. Gross chemical composition of dried orange peel and sugar beet pulp

The results of this study are in good agreement with those reported by Xue et al. (1992), who reported that the chemical structure of SBP (% on a dry weight basis) was as follows: pectin, 28.7; protein, 9.0; fat, 1.2; ash, 5.1; cellulose, 20.0; hemicellulose, 17.5 and lignin, 4.4. Also, Bhattacharya and Sleiman (1971); Castle (1972); (Kelly, 1983); and Mansfield et al. (1994) found that the chemical structure of the dried SBP was as follows: dray matter, 83.8–92.5; protein, 9.3–10.7; crude fiber, 18.4–22.4 and ash, 3.25–6.67 (% on a dry weight basis). The dry OP contains 25%–30% (dry weight) pectins (Aravantinos-Zafiris et al., 1994).

In agreement with this study results, it reported that OP waste contains moisture 40.7%, fat 1.85%, pectin 7%, lignin 6.4%, crude fiber 7.8%, total sugar 14.08%, non-reducing sugar 3.70%, reducing sugars (10.70%) and ash 7.39% (Ahmed et al., 2016). Similar results have reported by Ali et al. (2010), it showed that the chemical composition of sweet OP (% on a dry weight basis) was as follows: pectin, 12.8; total sugar, 16.5; reducing sugar, 12.4; crude protein, 4.2; crude fat, 1.5; total ash,2.1 and crude fiber, 8.6. And Mansour (1996), who found that the chemical contents of OP powder (% on a dry weight basis) was as follows: pectin, 22.5; total nitrogen, 0.41; fat, 2 and total carbon, 54.0. While Nassar et al. (2008) found that, the chemical composition of OP powder (% of dry weight) was 9.46 moisture, 5.15 protein, 4.35 fat, 9.21 sugar, and 2.6 ash.

Chemical analysis of the dry pigmented and grated OP exposed that, both samples were pectin rich (21.5 and 25.6%) respectively (Ismail, 1996). In accordance with the present study results, Citrus peels were reported to be consist of nearly 24% of pectin substances on basis of α-D-galacturonic acid (Yapo et al., 2007).

4.2. Suitability of orange peel and sugar beet pulp for endo-polygalacturonase production using static and shaken submerged fermentations

The production of *endo*-PG by *P. oxalicum* AUMC 4153 under shaken conditions was higher than that under static conditions using SBP as a sole carbon source while using OP resulted in similar *endo*-PG activity under both static and shaken conditions. These results are similar to those reported by Ismail (1996) who found that the arrangement of enzyme activities production during the incubation is depended on the strain of the used fungi, the age of the culture and the used media culture. In addition, found highly active *endo*-pectinase was produced by some strains in the shaken cultures while static cultures were found to be the most suitable for the other strains, *A. niger* 2 produced higher activities of *endo*-PG in shaken submerged fermentation than in static condition using grated OP while production was high in static condition using pigmented OP. *P. oxalicum* 7 produced higher activities of *endo*-PG in static condition than shaken condition using both media. On the other hand, among 6 of the tested fungal isolates, *A. niger* A-20 was demonstrated to be the greatest potent and produced highly active *endo*-PG after five days using OP as the single source of carbon in stirred cultures. Also, Fawole and Odunfa (2003) reported the creation of polygalacturonase by *A. niger* under static culture was higher than that under agitated culture using apple pectin as an individual carbon source. A previous study showed an optimum production of pectinase by *A. niger* on the OPs at 50 °C, pH 5, 96th hour (Mrudula and Anitharaj, 2011). It was reported that *A.niger* exhibited incredible potential for the pectinase synthesis (Ahmed et al., 2016). It found that the best cellulase and pectinase producing fungal isolated from decaying OPs and soil were *Aspergillus flavus, Aspergillus oryzae,* and *Penicillium atrovenetum*, chosen for use on enzyme production from OPs in a solid-state fermentation (Adeleke et al., 2012).

The data from this study also designate that, the production of *endo*-PG by *A. niger* AUMC 4156 and *P. oxalicum* AUMC 4153 grown on SBP were higher than that obtained on OP using both shaken and static culture conditions because that the significant difference in pectin content in both raw materials (SBP and OP). Since the pectin amount of the beet pulp is elevated, therefore, it can be used for the production of pectinolytic enzymes by microorganisms without adding pectinaceous substances as inducers (Jacob, 2009). This obtained result also agrees with that obtained with Heerd et al. (2014) who reported that the uppermost polygalacturonase activity was obtained by *A. sojae* using SBP in comparison with OP and apple pomace. Also, Bia et al. (2004) noted that production of *A. niger* CGMCCO455 endo- pectinase and exo- polygalacturonase reaches its maximum after 96 h at 30C using SBP as carbon source. The newly isolated fungus *Penicillium. oxalicum* (CGMCC 0907) grew well at 30 °C for 72 h and secreted of *endo*-pectinases and *exo*-polygalacturonase using SBP (Zhang and Bai, 2003).

The results of the present study were confirmed by Ismail (1996) who concluded that the contents of protein in the filtrates culture fundamentally depended on the used culture medium. The medium that containing OP as a singular source of carbon gave the uppermost protein quantity by each of the studied fungal strains. This might be recognized by the biosynthesis of numerous

enzymes. It appeared that no reliable association has occurred between either the mycelial growth of fungi or the protein level of the culture filtrate and every examined enzyme activity. This conclusion was also confirmed by (El-Sheekh et al., 2009), who reported that adding some additives comprised of certain industrial wastes (sugar cane molasses, beet molasses and wheat bran) to the basal medium lead to different *endo*-PG activities and protein content levels of *Aspergillus carneus* NRC1 culture filtrates. Also, Hadj-Taieb et al. (2006) found that adding milled OP, gruel (a by-product from wheat manufactory) to the basal liquid medium as carbon sources lead to different endo and *exo*-PG activities and protein content levels of CT1 mutant of *Penicillium occitanis* culture filtrates under shaken submerged fermentation (150 rpm) for 5 days at 30C. They reported that production of *endo*-PG and *exo*-PG was abundantly advanced on gruel in comparison to the OP that led to higher protein content.

4.3. The impact of different concentrations of sugar beet pulp

This results of the impact of different concentrations of SBP in this study are in line with that obtained by Naidu and Panda (1998) have stated that high carbon sources concentration inhibits the synthesis of enzymes, due to increasing the amount of reducing sugar in the fermentation medium (as a result of polysaccharides hydrolyzation by pectinase enzymes). Also, Fawole and Odunfa (2003) found that catabolic repression was enhanced by agitation in the culture of *A. niger*. This may be the reason for the reduction of *endo*-PG activity when increasing the concentration of SBP as a sole carbon source in a fermentation medium.

Similar results were observed by Puchart et al. (1999) reported that *Thermomyces lanuginosus* showed the best production of *endo*-PG during growth on SBP as a sole carbon source at concentration 2% in shaken submerged fermentation. And in close agreement with that observed by Anuradha et al. (2010) who indicated that production of polygalacturonase by *Aspergillus awamori* MTCC 9166 was high using raw pectin sources like Carrot peel, Jack fruit rind, Beetroot peel, and OP at concentration 1% in stirred submerged fermentation (200 rpm) for 5 days at 27C.

5. Conclusion

Sugar beet pulp proved to be a good inducer for *endo*-polygalacturoase production. The total protein content of the culture filtrates depended on the media culture that used and fermentation conditions (static or shaken). The static culture was more suitable for Endo- polygalacturonase production in SBP and OP by both *A. niger* and *P. variotii*, while shaken culture was the best for Endo- polygalacturonase production in SBP and OP by *P. oxalicum*. Production of enzymes depended on fungal strains, culture medium and submerged fermentation conditions (static or shaken). For the production of commercial enzymes must be tested all type of fermentation conditions, to know which is the best for producing the enzymes and specific activity of the enzyme, and additional studies are recommended to optimizing enzymes production and extraction methods from different industrial wastes. The results of is study can be used in several food and pharmaceutical and other industrial applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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