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# *Neosartorya glabra* polygalacturonase produced from fruit peels as inducers has the potential for application in passion fruit and apple juices

## *Poligalacturonase de Neosartorya glabra produzida a partir de cascas de frutas como indutores tem potencial para aplicação em sucos de maracujá e maçã*

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## Summary

Polygalacturonases are enzymes with the biotechnological potential for use in fruit juice clarification and for the enhancement of filtration efficiency. The aim of this work was to assess the production of polygalacturonase by the fungus *Neosartorya glabra* by means of solid-state and submerged fermentation using fruit peel residues as the carbon source, and also apply the enzyme in the clarification and decrease in viscosity of passion fruit and apple juices. The highest polygalacturonase (4.52 U/g/h) production was obtained by means of submerged fermentation in Vogel´s medium (1964) containing orange peel – Bahia variety (*Citrus sinensis*), at a concentration of 1.5% (w/v, dried mass) at 30-35°C for 72 h. The polygalacturonase of the crude extract presented optimal activity at 60°C and pH 5.5. The enzyme retained around 90% of the initial activity after 180 minutes at 40°C, and 50% of the initial activity after 150 minutes at 50°C. The enzyme was shown to be stable at acid pH values (3.0-6.5) after 120 minutes at 25°C. All these favourable enzymatic properties make the polygalacturonase attractive for potential uses in the industry of pectin-rich fruit juices, since the application of the crude extract to passion fruit (*Passiflora edulis*) juice caused an 80% reduction in viscosity and 75% decrease in light absorbance. In the processing of apple pulp juice (*Malus domestica*), there was a 50% reduction in viscosity and 78% decrease in light absorbance.

**Keywords:** *Neosartorya glabra; pectin; polygalacturonase; peel; viscosity.*

## Resumo

Poligalacturonases são enzimas com potencial biotecnológico para aplicação na clarificação de sucos de frutas e para aumento da eficiência da filtragem. O objetivo deste trabalho foi avaliar a produção de poligalacturonase pelo fungo *Neosartorya glabra*, por fermentação submersa e em estado sólido, usando resíduos de casca de frutas como fonte de carbono, além de avaliar também a aplicação desta enzima na clarificação e na diminuição de viscosidade de sucos de maracujá e maçã. A maior produção de poligalacturonase (4,52 U/g/h) foi obtida por fermentação submersa em meio de Vogel (1964) contendo cascas de laranja (*Citrus sinensis*), variedade Bahia, na concentração 1,5% (m/v, massa seca), a 30-35°C por 72 h. A poligalacturonase do extrato bruto apresentou atividade ótima a 50-60°C e pH 5,5. A enzima reteve cerca de 90% da atividade inicial após 180 minutos, a 40°C, e 50% da atividade inicial após 150 minutos, a 50°C. A enzima mostrou-se estável em pH ácido (3,0 a 6,5) após 120 minutos, a 25°C. Todas estas propriedades enzimáticas fazem desta poligalacturonase uma alternativa atrativa para a aplicação na indústria de sucos de frutas ricas em pectina, uma vez que a aplicação do extrato enzimático bruto no processamento de suco de polpa de maracujá (*Passiflora edulis*) causou uma redução da viscosidade de 80% e uma diminuição da absorbância da luz de 75%. No processamento de suco de polpa de maçã (*Malus domestica*), houve uma redução da viscosidade de 50% e uma diminuição da absorbância da luz de 78%.

**Palavras-chave:** *Neosartorya glabra; pectina; poligalacturonase; casca; viscosidade.*

## 1 Introduction

Pectinases are enzymes with important industrial applications (IRSHAD et al., 2014), representing approximately 25% of the commercial enzyme production (MAKKY; YUSOFF, 2015). These enzymes can be divided into protopectinase, de-esterifying and depolymerizing pectinases according to their mode of attack of the pectin backbone. Polygalacturonases (PG) are the depolymerizing pectinase most used in industries and also the most studied (NEVADITA et al., 2013). PG hydrolyses the α-1,4-glycosidic linkages between the galacturonic acid residues in pectin, an acidic polysaccharide complex that occurs mainly in the middle lamella of higher plants (RIDLEY et al., 2001; ZENI et al., 2011; TU et al., 2013).

Pectinases are secreted in high amounts by some fungal species and are used in biotechnological applications, for example, in the liquefaction and extraction of plant tissues, the degumming of natural fibres, the reduction of viscosity, increase in yield and clarification of fruit juices (UENOJO; PASTORE, 2007). In addition, the study of fruit peels as a cheap carbon source for pectinase production is a growing segment in the biotechnological industry (BATOOL et al., 2013).

There are a great variety of fruit juices on the market, such as apple and passion fruit juices, which are commonly consumed and well-established products.

The culture of passion fruit has grown over the last 30 years on the tropical fruit market, on account of the fast economic return and distribution throughout the year (MELETTI, 2011). The most cultivated species in the world is the yellow passion fruit species (*Passiflora edulis* f. flavicarpa). Brazil is the world's largest producer and consumer of this fruit, accounting for 50-60% of the total world production (OLIVEIRA et al., 2016). In 2013, the world apple production was approximately 80 million tons according to FAOSTAT (2016), and the productivity of the apple in Brazil is around 15-30 tons/ha (SEBRAE, 2016). Apples and passion fruits are rich sources of pectin. Apple pulp contains from 0.6 to 1.5% pectin while passion fruit pulp contains 0.5% pectin (CANTERI, 2011). Thus the application of polygalacturonases to viscous and jellied fruit juices is an alternative to make them more transparent and homogeneous (SIN et al., 2006; ONGARATTO; VIOTTO, 2016), since these enzymes can break down the pectin present in the cell wall, resulting in a reduction in the solids content, facilitating filtration and increasing the overall juice production. The aim of this work was to describe the production of PG by *Neosartorya glabra* with fruit peel as the carbon source, and analyse the effect of crude PG on the apple and passion fruit juices from the viscosity and light absorbance.

## 2 Material and methods

### 2.1 Microorganism

*Neosartorya glabra* was isolated from the State Forest of Bebedouro in Bebedouro city, São Paulo State, Brazil. This is a conservation area, managed by the *Instituto Florestal do Estado de São Paulo* (IFESP), and the forest has 99.41 hectares, an altitude of 570 meters, warm weather (17.2-22.7°C) and a vegetation consisting of pines and eucalyptus (IF, 2016).

*Neosartorya glabra* was identified and preserved by the URM Culture Collection at the Federal University of Pernambuco (Brazil). Stock cultures are being preserved under the register number of 7294 and the fungus was maintained in the laboratory on PDA medium slants (Himedia) at 4°C.

### 2.2 PG production

PG production was evaluated in solid-state fermentation (SSF) and submerged fermentation (SbmF). SbmF was carried out by inoculating 1 mL of spore solution ($8 \times 10^7$ spores) into 125 mL Erlenmeyer flasks previously sterilized at 127°C and 1.5 atm for 15 minutes, containing 25 mL of Vogel's minimal medium (VOGEL, 1964). For the preparation of the Vogel minimum culture medium, the stock solution was diluted 50-fold with distilled water, and a 0.01% biotin solution and 1% carbon source added as described below. The stock solution contained 125 g $Na_3$citrate. $2H_20$; 250 g anhydrous $KH_2PO_4$; 100 g anhydrous $NH_4NO_3$; 10 g $MgSO_4$. $7H_20$; 5 g $CaCl_2$. $2H_2O$; 5 mL trace element solution; and diluted to 1L with distilled water. An aliquot of 2 mL chloroform was added as a preservative. The stock solution was stored at room temperature. The solution of trace elements was composed of 5 g citric acid. $1H_2O$; 5 g $ZnSO_4$. $7H_2O$; 1 g $Fe(NH_4)_2(SO_4)_2$. $6H_2O$; 0.25 g $CuSO_4$. $5H_2O$; 0.05 g $MnSO_4$. $H_2O$; 0.05 g anhydrous $H_3BO_3$; 0.05 g $Na_2MoO_4$. $2H_2O$, and 100 mL distilled water. The media were supplemented with carbon sources: 1% (w/v) of fruit peel (residues), such as apple (*Malus domestica* var. Fuji), passion fruit (*Passiflora edulis* var. Yellow), banana (*Musa genus* var. Silver), pear (*Pyrus communis* var. Williams), guava (*Psidium guajava* var. Red), orange (*Citrus sinensis* var. Bahia, Lima, Pear and Rubi); lemon (*Citrus aurantifolia* var. Tahiti, Crave and Sicilian) and tangerine (*Citrus reticulate*) peels.

The production of PG in SbmF was also evaluated from mixtures of tangerine and orange peels (Pear and Bahia varieties); and from mixtures of lemon peels (Tahiti and Sicilian varieties). All fruit peels used in SbmF were obtained after drying at 50°C for 12 h and grinding into fine particles (1 mm). The cultures were incubated at 30°C (static condition) for 96 h, vacuum filtered using a Büchner funnel and Whatman nº 1 filter paper, and the cell-free filtrates obtained used to determine the extracellular PG activity. SSF was carried out by inoculating 1 mL of

spore solution (6.8 x $10^7$ spores) into 125 mL Erlenmeyer flasks previously sterilized at 127°C and 1.5 atm for 15 minutes, containing 2 g of dried fruit peels (apple, passion fruit, banana, pear, guava, orange (var. Bahia) and lemon (var. Tahiti) as the carbon source and 3 mL of distilled water. The flasks were incubated at 30°C in an incubator (static conditions) with 70% of humidity for 96 h. The fruit peels used in SSF were obtained by drying at 50°C and retaining on a 0.5 mm screen. After cultivation, the culture media were suspended in 30 mL of distilled water with agitation (100 rpm) for 30 min at 4°C. Subsequently, the cultures were vacuum-filtered using a Büchner funnel and Whatman nº 1 filter paper. The cell-free filtrates obtained were used to determine the extracellular PG activity.

### 2.3 Effect of cultivation temperature and time on PG production

The experiment to determine the effect of cultivation temperature was carried out using SbmF after inoculating the fungus into Vogel's medium containing orange peel (var. Bahia) as the carbon source (1%). The cultures were incubated (static conditions) at 25, 30, 35 and 40°C for 96 h and the PG activity then determined.

The time-course of PG production was also carried out in Vogel's medium (using orange peel from the Bahia variety as the carbon source). The cultures were incubated under static conditions at 35ºC for 7 days. Samples were withdrawn every 24 h, vacuum-filtered, and the extracellular PG activity determined. The mycelium obtained after filtration was triturated with glass beads in a porcelain mortar and suspended in 30 mL of 100 mM sodium acetate buffer, pH 5.0. It was then centrifuged at 13,000 x *g* for 15 min at 4°C to remove cell debris. The supernatant obtained was used to determine intracellular PG activity.

### 2.4 Effect of the carbon source concentration on PG production

The experiment was carried out using SbmF by inoculating the fungus into Vogel's medium containing orange peel (var. Bahia) as the carbon source. The culture media were supplemented with different concentrations (0.25-4.0% (w/v)) of dried orange peel and incubated under static conditions at 35ºC for 72 h.

### 2.5 Extraction and quantification of pectin in the fruit peels

The pectin contents of the apple, passion fruit, banana, guava, orange and lemon peels were analysed according to Carvalho et al. (2006) with modifications. One gram of dry and crushed peels was boiled with 200 mL of distilled water for one hour. The material was filtered and the volume completed to 500 mL. An aliquot of 100 mL was withdrawn, and 300 mL of distilled water and 10 mL of 1M sodium hydroxide added with continuous stirring, after which it was held overnight without stirring. A volume of 50 mL of 1M acetic acid was then added, and after 5 minutes, 50 mL of 2M calcium chloride was added. The solution was boiled for 1 minute, maintained without stirring for 90 minutes and then filtered through Whatman filter paper (3-micron porosity) and dried.

The % pectin was estimated as follows in Equation 1:

$$\frac{\left(\begin{array}{l}\text{Filter paper with the pectin} - \\ \text{previously weighed filter paper}\end{array}\right) \times 100 \times 5\ (\text{dilution})}{\text{Sample weight}} \quad (1)$$

### 2.6 PG activity

The PG activities were determined by the method of Miller (1959), using 3,5 dinitrosalicylic acid (DNS). The assay was carried out with 50 µL of the enzyme and 50 µL of 1% (w/v) substrate: sodium polypectate from Sigma-Aldrich in 100 mM sodium acetate buffer, pH 5.0. The samples were incubated at 60°C for 10 min and 100 µL of DNS then added to the assay. The absorbance was measured at 540 nm. The results were obtained using a curve of monogalacturonic acid (0-1 mg/mL) as the standard. One unit of enzymatic activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar per minute under the assay conditions.

### 2.7 Effect of temperature on PG activity

The effect of temperature on PG activity was analysed using the extracellular crude extract from *N. glabra* cultivated on orange peel (var. Bahia). The assays were carried out with the enzyme and 1% (w/v) substrate, and incubation at the temperatures of 30 to 70°C with 10°C intervals.

Thermal stability was determined by a prior incubation of the enzyme at 40, 50 and 60°C for up to 24 h, at pH 5.5. Samples were withdrawn after 10, 20, 40, 60, 120, 150, 180 min and 24 h of incubation, and the residual activities measured as described above.

### 2.8 Effect of pH on PG activity

The effect of pH on the PG activity was also analysed using the extracellular crude extracts from *N. glabra* cultivated on orange peel (var. Bahia). The assays were carried out using the enzyme and 1% (w/v) substrate, with incubation in 100 mM sodium acetate buffer, from pH 4.0 to 8.5, with 0.5 pH unit intervals. The pH stability was determined by incubating the enzyme in 100 mM McIlvaine buffer (citrate-phosphate) (1:1v/v) from pH 3.0 to 7.5 for 120 minutes at 25ºC. After incubation, the residual activities were measured as described above. The control was that of time 0 (zero), when the residual activity was considered to be 100%.

### 2.9 The effect of enzyme application on fruit juice processing

The extracellular crude extract obtained from the cultivation of *N. glabra* was used as the source of PG. Apple (var. Fuji) and passion fruit (var. Yellow) pulps were used to analyse the action of PG on the fruit juices. The methodology of application was an optimized version of that reported by Kashyap et al. (2001). The selected fruits were washed, cut and triturated. The sliced apples were maintained in contact with oxygen for 15 min to avoid the inhibition of pectinase by polyphenols. Twenty grams of sliced and triturated fruits were placed into 125-mL Erlenmeyer flasks and 4.5 U of crude enzyme per gram of fruit added. The control (untreated pulp) was prepared under the same conditions, but using a boiled enzyme extract. The flasks were incubated at 37°C for 5 h with agitation. The fruit juices were then extracted by filtration and the viscosity and turbidity of the extracts studied. The viscosity was analysed using an Ostwald's viscometer. The percentage of decrease in viscosity (A) was calculated according to Roboz et al. (1952), through the Equation 2:

$$A = (Vo - Vt / Vo - Vs) \times 100 \quad (2)$$

*Vo* is the flow time (seconds) of fruit pulp treated with inactivated enzyme (control);

*Vt* is the flow time (seconds) of fruit pulp treated with active enzyme;

*Vs* is the flow time (seconds) of the inactivated enzyme plus water

The difference in turbidity between the treated and untreated fruit juice samples was analysed from the light absorbance in a spectrophotometer at different wavelengths (250-570 nm, with 10 nm intervals) using a glass cuvette. The untreated sample (control) was the fruit pulp treated with inactivated enzyme.

## 3 Results and discussion

### 3.1 The effect of the carbon sources on PG production

The effect of the fruit peels as PG inducers was analysed using SbmF (Figure 1A) and SSF (Figure 1B). Glucose was used as the control (repression conditions). Using SbmF (Figure 1A) orange peel was the best PG inducer (4.3 U/mL), followed by lemon peel, which had a production corresponding to 92% of the activity observed with orange peel. The worst PG inducer was banana peel under the conditions analysed. Using SSF (Figure 1B), guava peel was the best PG inducer (1.2 U/mL), with a PG production 16.5% greater than the second-best residue, apple peel.

Table 1 expresses the enzyme productivity (U/gram of dried peel per hour of fermentation) using SbmF and SSF. SbmF showed better enzyme productivity and has the advantages of easy recovery of the extracellular enzymes,

**Table 1.** Comparison between SbmF and SSF in the production of PG using different carbon sources.

| Carbon Source (peel) | SbmF (U/g per hour) | SSF (U/g per hour) |
|---|---|---|
| Glucose (control) | 0.21 | - |
| Banana | 0.44 | 0.03 |
| Guava | 1.29 | 0.18 |
| Orange (Bahia) | 4.52 | 0.06 |
| Lemon (Tahiti) | 4.25 | 0.11 |
| Apple | 2.10 | 0.15 |
| Passion Fruit | 2.89 | 0.07 |
| Pear | 2.70 | 0.09 |

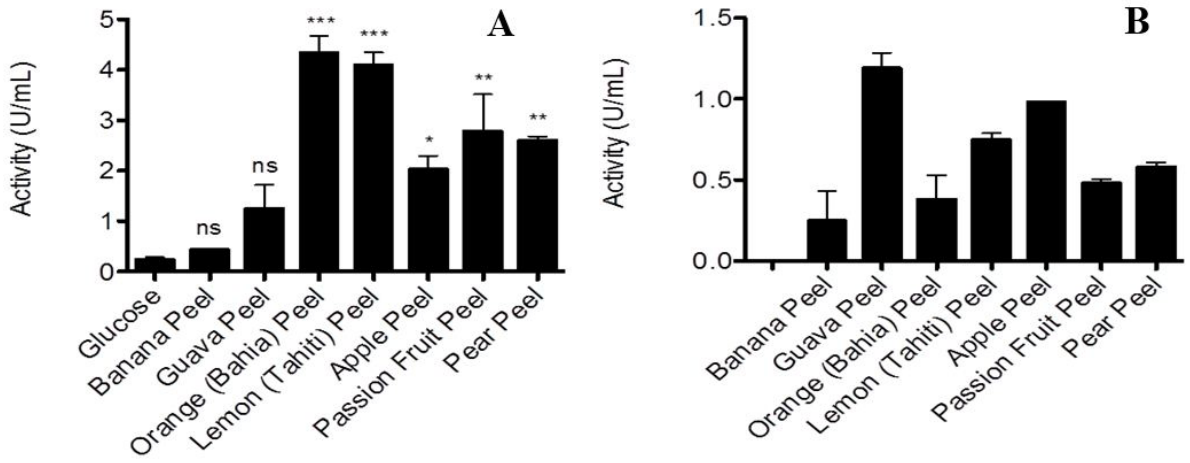


**Figure 1.** The effect of the carbon sources on PG production. (A) shows the PG activities using SbmF and (B) using SSF. The microorganism was cultivated in Vogel's medium and incubated under static conditions at 30°C for 96 h. Symbols: (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ versus Glucose according to the Student Newman Kwels test (SNK); ns - not significant.

mycelia and spores. SbmF is a process of a homogeneous nature with easy control of the parameters, such as temperature and agitation (FERNANDÉZ, 2009), and more than 75% of industrial enzymes are produced using SbmF (SUBRAMANIYAN; VIMALA, 2012). Camargo et al. (2005) reported the production of 3.6 U/mL of PG by *Aspergillus sp.* in a medium supplemented with orange (pulp, peel and seeds) as the carbon source. Maller et al. (2011) and Mrudula and Anitharaj (2011) observed that orange peel was the best inducer of pectinase production by *A. niveus* and PG by *A. niger*, respectively.

### 3.2 Effect of different varieties of orange and lemon peels on PG production

Since orange (Bahia variety) and lemon (Tahiti variety) peels were the sources that stood out as inducers of PG production using SbmF, a study using tangerine and other varieties of orange and lemon peels was carried out (Figure 2). Orange peel (Bahia variety) remained as the best residue for PG production by the fungus *N. glabra*, but orange (var. Pear), tangerine and lemon (var. Tahiti) peels were also shown to be good inducers, presenting 96%, 86% and 90% of the production observed with the Bahia orange peel, respectively.

Tables 2 and 3 illustrate the influence of mixtures of peels of different oranges and the peels of distinct lemons on PG production from *N. glabra*, respectively. It can be seen that Bahia orange peel remained as the best inducer of PG production even when using mixtures of other orange peels and tangerine (Table 2), whereas lemon peel (var. Tahiti) was the best PG inducer when compared to the mixtures with lemon peel (var. Sicilian) (Table 3).

Analysing Tables 2 and 3 and Figures 1 and 2, it can be seen that orange peel (var. Bahia) was the best PG inducer. Quantitative analyses for the determination of pectin in the dry peels used in this work showed that banana, guava, apple, lemon, passion fruit and orange peels have 19%, 20%, 15.5%, 15%, 16% and 17% of pectin, respectively. The values are close and probably other parameters influenced the PG synthesis, such as: the availability of inducers, structural differences of the pectin contents – branching degree, phenol groups, polygalacturonase-inhibiting protein, and the presence of fungicides, pesticides, ions, detergents, urea, 2-mercaptoethanol, vitamins, phosphorus and others (KAUR et al., 2004; TAI et al., 2013; KANT et al., 2013; SCHWAN-ESTRADA et al., 2000). According to Al-Saadi et al. (2009), orange peels contain alkaloids, saponins, terpenes, resins, flavonoids, tannins, phenols and sugars. The orange peels contain 23.8% of sugar, 4%

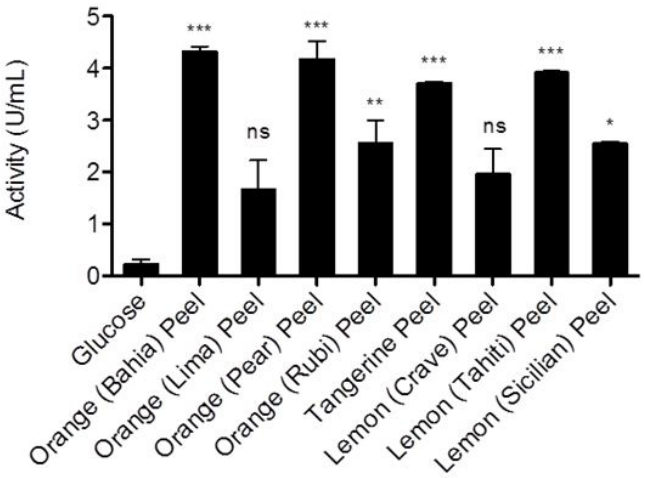


**Figure 2.** Effect of tangerine peel and different varieties of orange and lemon peels as carbon sources for PG production using SbmF. The microorganism was cultivated in Vogel's medium in a bacteriological incubator at 30°C for 96 h. Symbols: (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ versus Glucose according to the Student Newman Kwels test (SNK); ns - not significant.

**Table 2.** Influence of the mixture of some varieties of orange peel on PG production.

| Mixture | Bahia Orange Peel (%) | Pear Orange Peel (%) | Tangerine Peel (%) | Enzymatic Activity (U/mL) |
|---|---|---|---|---|
| 1 | 1.00 | - | - | 4.21 ± 0.32 |
| 2 | - | 1.00 | - | 4.08 ± 0.4 |
| 3 | - | - | 1.00 | 3.48 ± 0.2 |
| 4 | 0.50 | 0.50 | - | 2.77 ± 0.29 |
| 5 | 0.50 | - | 0.50 | 2.25 ± 0.18 |
| 6 | - | 0.50 | 0.50 | 1.91 ± 0.01 |
| 7 | 0.75 | 0.25 | - | 2.67 ± 0.03 |
| 8 | 0.25 | 0.75 | - | 2.81 ± 0.28 |
| 9 | 0.75 | - | 0.25 | 2.32 ± 0.13 |
| 10 | 0.25 | - | 0.75 | 2.48 ± 0.46 |
| 11 | - | 0.75 | 0.25 | 2.85 ± 0.45 |
| 12 | - | 0.25 | 0.75 | 2.45 ± 0.28 |
| 13 | 0.33 | 0.33 | 0.33 | 2.71 ± 0.37 |

The microorganism was cultivated in Vogel's medium and incubated under static conditions at 30°C for 96 h.

of protein, 11.86% of water and 5.34% of ash. In addition, according to Canteri et al. (2012), orange peel has a high pectin content (3.5-5.5% of pectin per fresh-fruit weight), which is a good inducer of PG production. Furthermore, this result is very interesting, since orange juice is one of the most widely consumed beverages nowadays and consequently the amount of waste (mostly peel) generated is large.

The cultivation of orange has become an important economic sector in the United States (Florida and California), Brazil, Mexico, Pakistan, China, India, Iran and the Mediterranean countries. Approximately 50-60% of the processed fruit is transformed into citrus waste (peel, seeds and membrane residues), which must be processed in order to avoid the disposal of this residue in the soil resulting in further environmental problems (MARTÍN et al., 2010). Thus orange peel can be used as an alternative and viable pectinase inducer.

### 3.3 Effect of cultivation temperature and time-course on PG production

*N. glabra* was cultivated at several temperatures for the analysis of the optimal cultivation temperature. The highest PG activity occurred in the range from 30 to 35°C (Figure 3A) and 35°C was selected as the optimal temperature.

Using SbmF for the cultivation of *N. glabra* in Vogel's culture medium supplemented with 1% (w/v) of orange peel (*Citrus sinensis* var. Bahia), higher extracellular PG activity (3.2 U/mL) was obtained after 72 hours of fermentation under static conditions at 35°C. The PG activity remained approximately constant after 168 h of fermentation (Figure 3B). The significant secretion of extracellular PG facilitates its recovery from the fermentation medium, being more beneficial for the industry. The cultivation time may be dependent on the amount of nitrogen and carbon source available in the medium, since short periods of incubation may not result in the maximum production of the metabolite of interest. On the other hand, cultures incubated for long periods may lead to depletion of nutrients, cell death of the fungus and degradation of the enzymes (PELCZAR et al., 1996). Maciel et al. (2013) observed that the highest PG production by *A. nigri* occurred between 48 and 96 h and Patil et al. (2012) observed that 72 h was the optimal cultivation time for exo-PG production by *Paecilomyces variotii*.

### 3.4 Effect of the carbon source concentration on PG production

In the study of the effect of the carbon source concentration on the submerged fermentation of *N. glabra* under static conditions at 35°C, higher PG activity (4U/mL) was obtained in Vogel's culture medium supplemented

**Table 3.** Influence of the mixture of two varieties of lemon peel on PG production.

| Mixture | Tahiti Lemon Peel (%) | Sicilian Lemon Peel (%) | Enzymatic Activity (U/mL) |
|---|---|---|---|
| 1 | 1 | - | 3.78 ± 0.25 |
| 2 | - | 1 | 3.28 ± 0.43 |
| 3 | 0.5 | 0.5 | 2.69 ± 0.26 |
| 4 | 0.75 | 0.25 | 2.58 ± 0.30 |
| 5 | 0.25 | 0.75 | 3.08 ± 0.25 |

The microorganism was cultivated in Vogel's medium and incubated under static conditions at 30°C for 96 h.

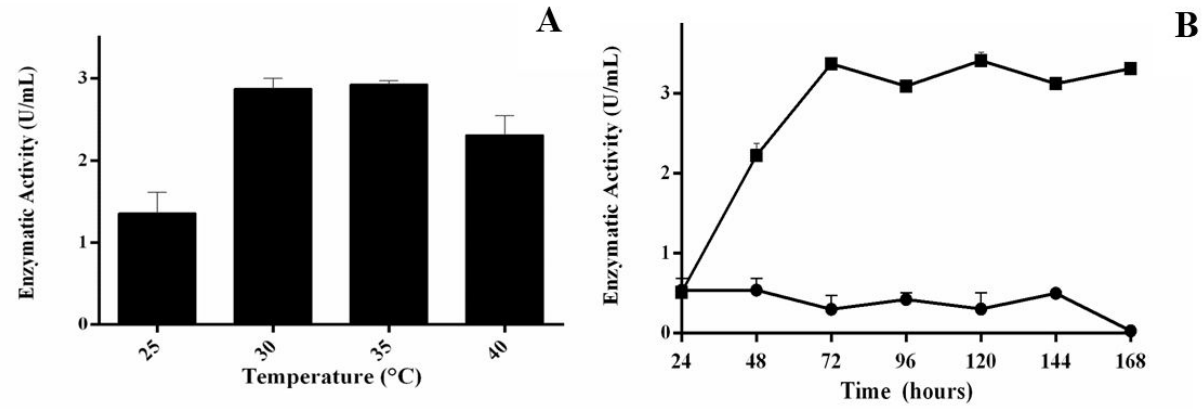


**Figure 3.** The effect of cultivation temperature (A) and time (B) on PG activity. Symbols: (■) extracellular and (□) intracellular PG activities. The microorganism was cultivated in Vogel's medium (SbmF) using orange peel (var. Bahia) as the carbon source and incubating under static conditions.

with 1.5% (w/v) orange peel (*Citrus sinensis* var. Bahia) after 72h (Figure 4). Camargo et al. (2005) observed the best production of the enzyme by *Aspergillus sp*. using 2% (w/v) orange bagasse in the cultivation medium.

The carbon source concentration is an important parameter to be determined in the optimization of fungal cultivations, because it is a precursor of the carbon chains for the synthesis of all the cell components. Low concentrations may be insufficient for inducing good enzyme levels and very high concentrations may act as inhibitors, since the excess (mainly complex substrates) can contain other sugars (monosaccharides), which have a higher affinity for the membrane transporters and are more easily captured by the fungus than is pectin, thereby decreasing the secretion of pectinase (KUBICEK, 2013). The inhibitory effect was observed with concentrations above 3% (w/v) of orange peel and could be correlated with an excess of substrate, such as fruit processing residues and other compounds that lead to the death of the fungus, such as pesticides, very common in fruit peels (SIDDIQUI et al., 2012).

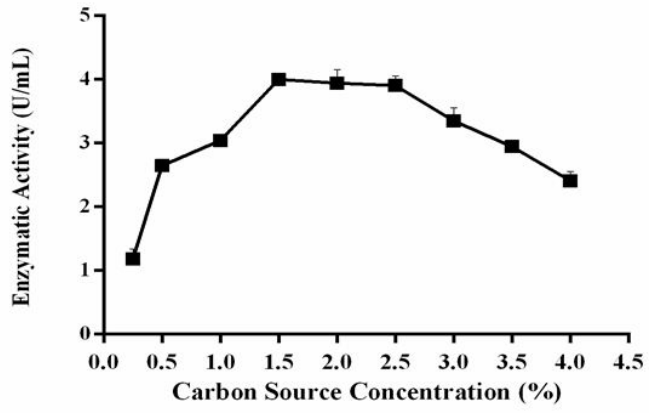


**Figure 4.** The effect of the carbon source concentration on PG production. The microorganism was cultivated in Vogel's medium (SbmF) using orange peel (var. Bahia) as the carbon source and incubated under static conditions at 35°C for 72 h.

### 3.5 Effect of temperature on PG activity

The crude PG from *Neosartorya glabra* presented optimal activity at 50-60°C (Figure 5A) suggesting good applicability in processes involving high temperatures. Siddiqui et al. (2012) reported the optimal temperature as 55°C for the PG from *Rhizomucor pusillus*. Kashyap et al. (2001) observed maximum PG activity from *A. niger* at 50°C and Maller et al. (2011) reported the optimal temperature as 55°C for the PG activity from *A. niveus*.

The PG from *N. glabra* retained more than 90% of its initial activity after 180 min at 40°C. At 50°C, the enzyme presented a $t_{50}$ of 150 min and at 60°C it was not stable (Figure 5B).

### 3.6 Effect of pH on PG activity

The crude PG from *N. glabra* presented optimal activity at pH 5.5 (Figure 6A). This result implies in applications in acidic processes, such as in the citrus juice industry. The PG from *N. glabra* showed stability at acid pH values when incubated in 100 mM McIlvaine buffer for 120 minutes at 25°C. The incubation in buffer at pH 3.0 resulted in an increase in residual activity of more than 20% (Figure 6B) when compared to the control (non incubated enzyme). It has been reported that most pectic enzymes show stability under acidic pH conditions (GUMMADI; KUMAR, 2006). An acid solution presents a lot of free $H^+$, which facilitates protonation of the $COOH^-$ (glutamic acid and aspartic acid) and $NH^{2-}$ (lysine, histidine and arginine) groups of the enzymes, directly influencing bonding to the substrate (SILVA; SILVA, 2010).

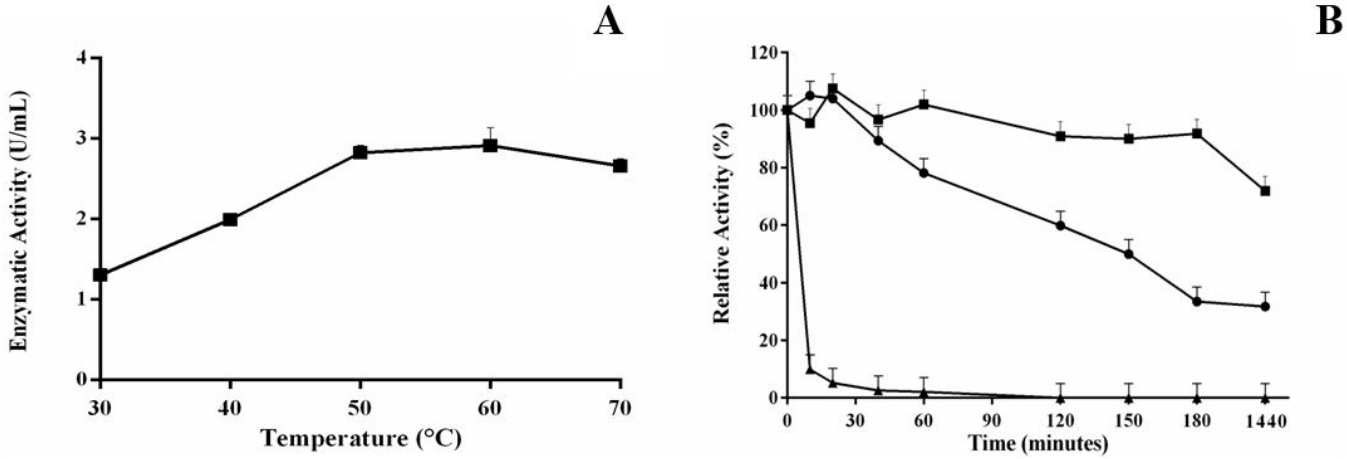


**Figure 5.** The effect of temperature on PG activity (A), thermostability of PG at pH 5.5 (B). Symbols: thermal stability carried out at 40°C (■), 50°C (●) and 60°C (▲).

### 3.7 The application of the enzymatic extract in fruit juice processing

The effect of the crude PG produced by *N. glabra* in reducing the viscosity of passion fruit and apple pulps was analysed. The treatment of passion fruit pulp with crude PG from *N. glabra* decreased the viscosity by 80%, whereas treated apple pulps showed a 50% reduction in viscosity in relation to the untreated sample. In the juice industry, the pectin polymer present in the fruit pulp can interact with water molecules in an acidic medium and form a gel, increasing the viscosity. On account of this, it is common practice to apply pectinases to lower the viscosity and the water-binding capacity of the pectin, for easier juice extraction (NAKKEERAN et al., 2011; REHMAN et al., 2013). These results are in agreement with Domingues et al. (2014), who analysed the reduction in viscosity of passion fruit juice samples using the enzymatic complex Pectinex 3XL from Novozymes, and verified that the enzymatic treatment was efficient in reducing the viscosity. Laorko et al. (2010) also used the enzymatic treatment of apple juices before micro- and ultrafiltration tests. Patil et al. (2012) tested an exo-PG produced by *Paecilomyces variotii* in the treatment of fruit juices, and observed viscosity decreases in orange, apple, grape, banana and guava juices. Tu et al. (2013) reported a 17.6% viscosity reduction in papaya juice with the application of an endo-PG.

The turbidity of the treated and untreated pulps was analysed via light absorbance at different wavelengths in a spectrophotometer. The juices treated with crude PG showed different values for absorbance at the different wavelengths as compared to the untreated juice (control). Treated passion fruit juice showed a higher difference in absorbance between 430-500 nm (Figure 7A) and at these wavelengths the light absorbance of the treated samples was shown to decrease by 75%. For apple samples, the

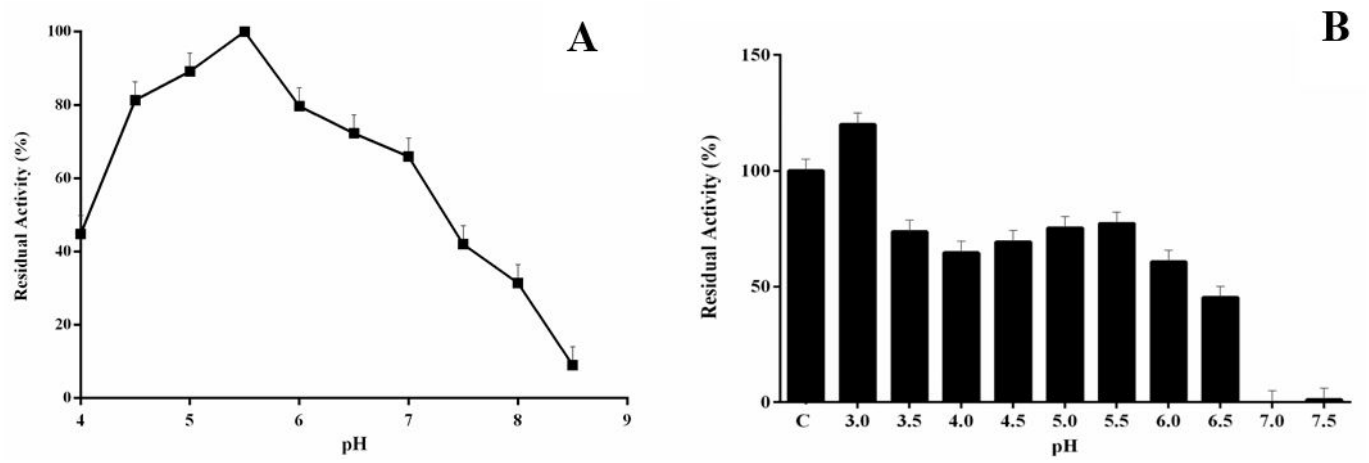


**Figure 6.** The effect of pH on PG activity (A), pH stability of PG at 25°C for 120 minutes (B). C= control.

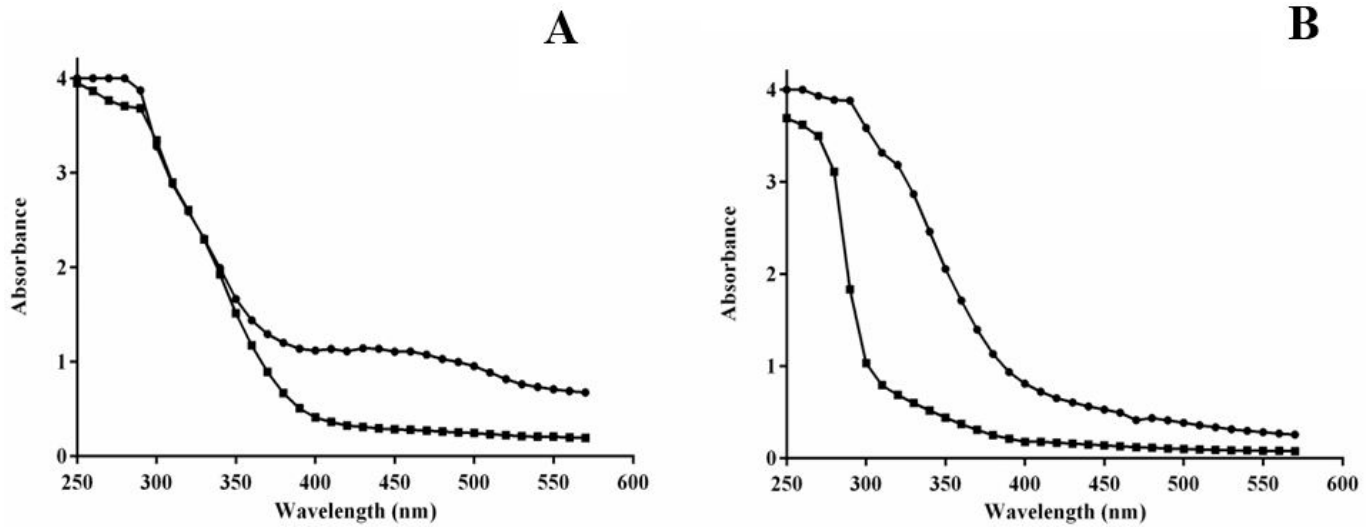


**Figure 7.** Estimation of the turbidity of the juices. The values for absorbance of treated and untreated passion fruit juice samples (A), and apple juice samples (B). Symbols: untreated samples – control (●), treated samples (■).

highest difference in absorbance was observed between 320-360 nm (Figure 7B) and at these wavelengths the light absorbance of the treated samples was shown to decrease by 78%. These data suggest the range of wavelengths that could be used to estimate the hydrolysis caused by the enzyme action and the decrease in light absorbance after enzyme treatment, which is an indicator of the decrease in turbidity.

## 4 Conclusion

The use of residues from fruit juice processing as a carbon source, especially orange peel (var. Bahia), was favourable for PG production by *N. glabra*. This result was very interesting since the use of low-cost substrates is required in industrial processes. Optimal PG production conditions were obtained with 1.5% dried and ground orange peel (Bahia) as the carbon source when using SbmF, cultivating the microorganism in Vogel's minimal medium and incubating at 35°C for 72 h. The PG of the crude extract presented optimal activity at 50-60°C and pH 5.5 The enzyme was highly stable at 40°C for 180 minutes and retained 50% of the initial activity after 150 minutes at 50°C. The PG was stable in the acidic range after 120 minutes of treatment at 25°C. The enzyme extract obtained under the optimized condition was used in the extraction of fruit juices and in the application tests, where it showed a reduction in viscosity of 80% and a decrease in light absorbance of 75% with the passion fruit pulps, and a reduction in viscosity of 50% and decrease in light absorbance of 78% with the apple pulps. This work contributes to the improvement of knowledge on the influence of physical-chemical factors on the production of polygalacturonase by *Neosartorya glabra* and its success in the application to passion fruit and apple pulps.

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