

APPLICATION OF CRUDE PECTINASES PRODUCED BY *ASPERGILLUS SOJAE* (ATCC 20235) AS A PROCESSING AID FOR BANANA JUICE EXTRACTION

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Received: May 7, 2016

Accepted: Jul. 3, 2016

ABSTRACT: Crude pectinases produced by genetically modified *Aspergillus sojae* (ATCC 20235) namely PGzyme was used for extraction and clarification of banana juice comparing with two commercially available pectinase (*Fructozym* and *Flavourzyme*). Therefore, endopolygalacturonase (Endo-PG), exopolygalactouronase (Exo-PG) and polymethylgalacturonase (PMG) activities in each enzyme preparation were estimated. The results revealed that *Fructozym* is more effective to extract and clarify of banana juice rather than of PGzyme. While, *Flavourzyme* has no marked effect on the extraction or clarification of the banana juice. Physicochemical characteristics of the produced juices by the studied different enzymes were also discussed.

Key words: Pectinases, banana juice, physicochemical characteristics

INTRODUCTION

Fungal pectinases are widely utilized in the industrial sector where they represent more than 25% of the total commercial enzymes which are used in food processing (Moussa-Ayoub *et al.*, 2011; Sagu *et al.*, 2014). Fungal pectinases are produced at the commercial scale (Naidu and Panda, 1998) and broadly used in the field of food processing (Grassin and Fauquembergue, 1996; Kashyap *et al.*, 2001). Pectinases have many applications in extraction of juice from soft fruits, clarification of fruit juices and wine, maceration of plant tissue, liquefaction and oil extraction (Mutlu *et al.*, 1999; Kashyap *et al.*, 2001; Jayani *et al.*, 2005). They are preferred rather than other chemical methods due to high affinity of enzymes to their substrates and generate less toxicity (Hoondal *et al.*, 2002). Crude pectinase (PGzyme) is mainly a group of pectinolytic enzymes that are produced by the fermentation process of *Aspergillus sojae* growing on agro wastes (Naidu and Panda, 1998; Pandey *et al.*, 2000). The product of this fermentation has obtained as GRAS (Generally Recognized As Safe)

status, which has approved its use in the food industry (Heerd *et al.*, 2012). Endopolygalacturonases (Endo-PG) [EC3.2.1.15], Exopolygalacturonase (Exo-PG) [EC 3.2.1.67] and polymethylgalacturonase (Exo- PMG) [EC 3.2.1.82] are hydrolyzed the glycosidic bonds while pectin lyase (EC 4.2.2.10) belongs to trans elimination cleavage enzyme. Pectinmethylestrase (PME) [EC 3.1. 1. 11] is a degrading enzyme which de-esterifies methyl ester linkage in pectic substances (Jayani, *et al.*, 2005). Recently increases attention has been focused on the utilization of polycarbohydrates hydrolysis enzymes in order to overcome the loss of juice yield and improve the juice quality (Kulkarni *et al.*, 2010; Vijayanand *et al.*, 2010; Vishal *et al.*, 2015). Banana (*Musa spp.*) is one of the most consumed fruit around the world as fresh. The fruits are rich in carbohydrates and minerals especially potassium and magnesium (Kyamuhangire *et al.*, 2002). Over-ripe fruits that are normally rejected by the consumers and unmarketable (Emerald and Sreenarayanan, 1999) could be processed as banana juice

under good manufacturing practices (Mohapatra *et al.*, 2011). The juice has special flavor that can use in various foods and drinks (Shahadan and Abdullah, 1995). The presence of large amounts of polysaccharide in banana cells has critical effect on the juice yield extraction (Lee *et al.*, 2006).

This work was carried out to report the use of crude enzyme preparation namely PGzyme produced by solid state fermentation from genetically modified *Aspergillus sojae* (ATCC 20235) as a processing aid for banana juice extraction and clarification.

MATERIALS AND METHODS

Materials:

Enzymes: Two kinds of fungal commercial enzymes namely Fructozym[®]P was obtained from (Erbaslöh Geisenheim AG- Germany) and Flavourzyme[®] 500 MG purchased from Novozymes (Swiss land). Crude pectinases PGzyme was produced by genetic modified *Aspergillus sojae* (3UV-ATCC 20235) on solid-state fermentation systems using sugar beet pellets and wheat bran as fermentation medium. The enzyme was harvested after 6 days of fermentation by adding tap water, shaking and centrifugation according to Heerd, (2013). Banana (*Musa spp.*) fruits were purchased from the local market of Bremen City (Germany) at May 2015 and kept at room temperature until reach to stage 4 of rippling. All chemicals used are analytical grade.

Methods

Protein assay

Total protein content of enzyme (mg/ml) was calorimetrically determined according to Bradford (1976) with slight modification from Thermo Scientific using bovine serum albumin BSA (2mg/ml) as a standard protein.

Determination of pectolytic enzyme activity

Endo-polygalacturonase (Endo-PG) activity was determined calorimetrically using Ruthenium red at 535 nm as described by Ortiz *et al.* (2014). Mean Exo-polygalacturonase (Exo-PG) activity was determined using 3,5-dinitro salicylic acid (DNS) according to Miller, (1959) with slight modification by Silva *et al.* (2005). Exo-polymethylgalacturonase (Exo-PMG) was determined via the method described by Heerd *et al.* (2012).

Banana juice extraction and clarification

The extraction and clarification of banana fruits by selected enzymes were performed at 40°C for 3 hrs by adding (10 U enzyme preparation /mL) according to the method described by Tadakittisarn *et al.* (2007).

Physical analysis of banana juice

Physical characteristics of banana juice including clarity by measuring the juice transmittance at 670 nm (Johnson *et al.*, 1995), browning index (BI) according to Youn *et al.* (2004), total soluble solids (TSS) and refractive index (RI) using Abbe refractometer (Al-Hooti *et al.*, 2002), pH value using pH-meter (HI 2211 pH/ORP meter HANNA) at 25°C according to AOAC (1995), viscosity using Ostwald viscometer at 25°C and turbidity as Nephelometer Turbidity Unit (NTU) using nephelometer (a Hack turbid meter, Germany) following the method of Byaruagaba-Bazarike *et al.* (2012) were performed.

Determination of reducing sugar in banana juice

Reducing sugars (RS) using 3,5-dinitrosalicylic acid (DNS) was determined as described by Negrulescu *et al.* (2012) with some modification. A microplate reader (Tecan Deutschland GmbH) was performed in triplicates. Standard curve was carried out parallel under the same conditions.

Statistical analysis

Data are represented means and standard deviation (M±SD) for three replicates. Analysis of variance (ANOVA) was applied between means and were further tested using the Dunc's according to SAS (2004).

RESULTS AND DISCUSSION

Protein content and enzyme activity

Results of Table (1) display that Fructozym contains the highest (p<0.05) protein content (2.08mg/ml) among the three studied enzymes. On the other hand, PGzyme had the lowest protein (0.27mg/ml). The same trend is also shown for Endo-PG activity, since Endo-PG activity of 1.0% Flavourzyme is 107.1 U/ml which represents the medium activity among PGzyme (42.2 U/ml) and Fructozym (133.9 U/ml). Although, Endo-PG activity of Fructozym significantly (p< 0.05) higher than that of PGzyme but its Exo-PG activity (281.0 U/ml) was lower than that of PGzyme (336.0 U/ml). At the same time, no significant (p< 0.05) difference between PMG activities of both enzymes was detected. Nevertheless, Flavourzyme had moderate activity between PGzyme and Fructozym in Endo-PG (107.1 U/mL) while a lower activity (50.0 U/mL) was detected in Exo-PG and Exo-PMG (9.3 U/mL). These

variations may be related to the fungus strain, production conditions and method of enzyme separation (Heerd, 2013). It is well known that Endo-PG catalyses random hydrolysis of α-1→4 glycosidic linkages in pectin chain as results viscosity of pectin solutions are decreased and insoluble pectin turns to soluble one. While Exo-PG catalyses hydrolysis in a sequential fashion of α-1→4 glycosidic from the non-reducing end in pectin split galacturonic acid and/or monosaccharide which leads to increase the reducing power of the pectin solutions (Kashyap *et al.*, 2001; Jayani *et al.*, 2005).The PMG's are group of enzymes hydrolyzed the glycosidic bonds in the esterified pectin (Jayani *et al.*, 2005).

Specific activity (SA)

Specific activity (SA as units/mg protein) is the number of enzyme units per ml divided by the concentration of protein in mg/ml. It is an important measure for enzyme purity. Although calculating SA value of the enzyme depends mainly on the enzyme activity, they are usually greatly differing from each other's depending on the definition of the enzyme unit. Thus, enzyme batches that are below the expected SA value may contain impurities or inactivated enzyme molecules.

Table 1: Protein content (mg/ml) and activity (U/ml) of enzymes preparation

Enzyme pattern	PGzyme	1% Flavourzyme	Fructozym
Protein (mg/mL)	0.27 ± 0.06c	0.59 ± 0.15b	2.08 ± 0.19a
Endo-PG (U/mL)	42.2±4.9c	107.1 ± 2.22b	133.9 ±0.02a
Exo-PG (U/mL)	336 ± 0.18a	51 ± 0.09c	281 ± 0.09b
Exo-PMG (U/mL)	21.65 ±2.04a	9.27 ± 0.58b	22.31 ±1.23a

M±SD = means and standard deviation of three successful trails
 In a row, means followed by the same letter are not significantly different at 5% level

The SA is inversely proportional to the amount of the protein in enzyme preparation. Whenever, protein content increased in the enzyme solution, the SA decreased. Therefore, as the protein content in the enzyme preparations increased, SA of Endo-PG, Exo-PG and Exo-PMG decreased (Fig. 1 A, B and C).

Effect of enzymes treatment on characteristics of banana Juice

Physicochemical properties including browning index (BI) at 420 nm (expressing color), turbidity, refractive index (RI), viscosity, reducing sugar (RS) and pH of juice are playing an important role on the quality and acceptance of consumer.

Yield and viscosity

The yield of banana juice is significantly ($p < 0.05$) increased due to the use of the pectinolytic enzyme preparations. Where, the yield and viscosity of the juice extracted without enzyme (control) is (48.2%) significantly ($p < 0.05$) increases to 60.5 % and 72.8 % when extracted with PGzyme and Fructozym, respectively. On the other hand, viscosity of the juice decreases due to the use of enzymes. The viscosity of control juice is 7.62 cp decreases to 1.48 cp and 1.38 cp for juice extracted by PGzyme and Fructozym, respectively (Fig. 2). The decrement of viscosity may be related to the degradation of pectin and pectic substances in banana pulp led to reduce water holding capacity of the macromolecules and the free-water release to the system causing lower viscosity and extra yield (Kashyap *et al.*, 2001; Lee *et al.*, 2006).

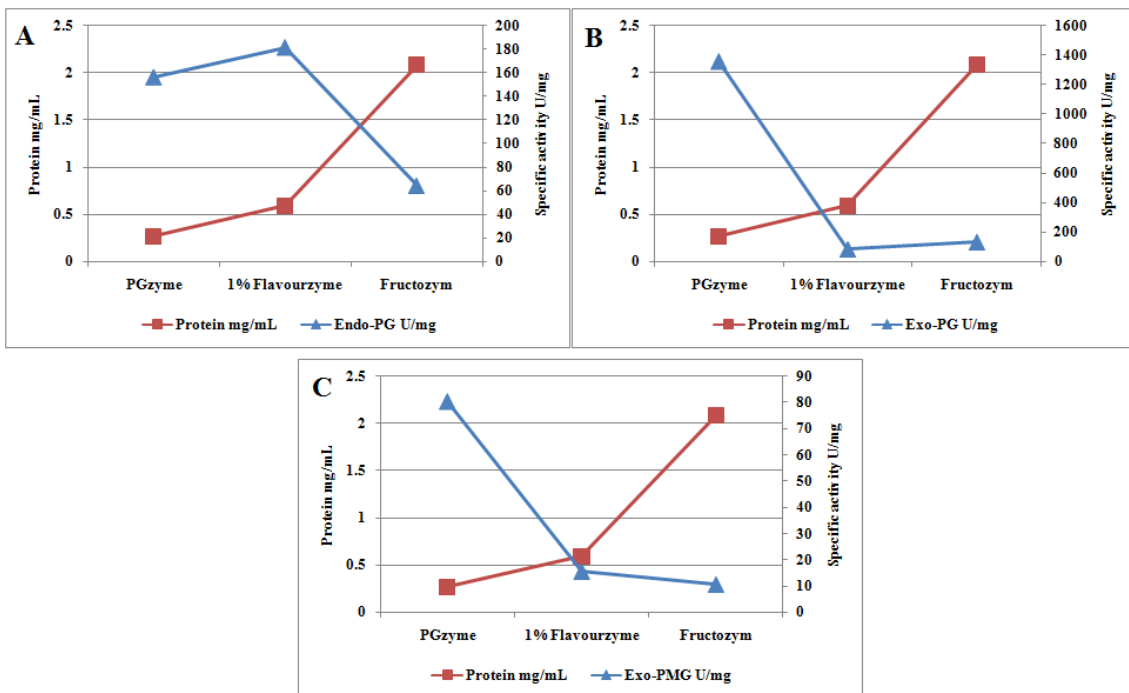


Figure (1): Specific activity (U/mg) comparing with protein content (mg/mL), A: Endopolygalacturonase, B: Exopolygalacturonase and C: Exopolymethylgalacturonase

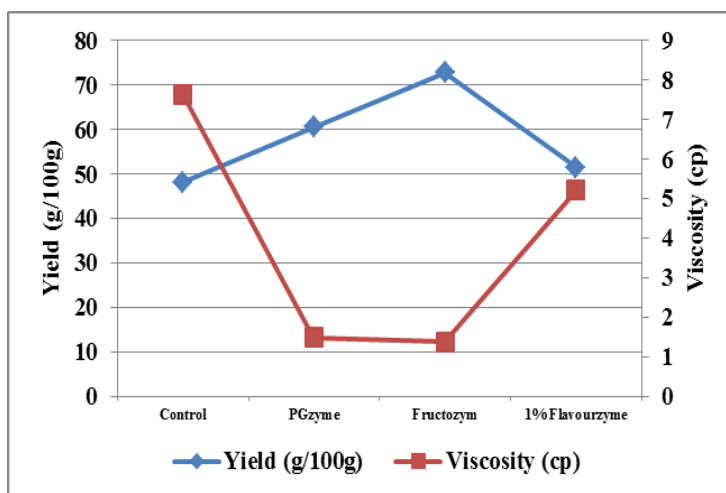


Figure (2): Effect of enzymes treatment on the yield and viscosity of banana juice

This implies that Fructozym and PGzyme are more effectively on hydrolysis of pectic substances in banana pulp than that of Flavourzyme where there are no significant ($p > 0.05$) differences between the yield and viscosity of control juice and that juice extracted by Flavourzyme. As for Fructozym and PGzyme, they have high amounts of Endo-PG and Exo-PMG, which they could degrading pectin in middle lamella and cell walls converting the insoluble pectic substances in solid state to soluble one and free-run juice release (Kyamuhangire *et al.*, 2002). As a result, increases the juice yield and depresses its viscosity.

Clarity and Turbidity

The clarity of juice is an important index for the degree of juice clarification (Sin *et al.*, 2006). The clarity is dramatically increased in the juice extracted by Fructozym (98.6%) and PGzyme (97.4%) comparing with that of Flavourzyme (25.5%) and control (19.5%) (Fig 3). The presence of other enzymes may increase the rate of clarification owing to reduce electrostatic repulsion between the charged protein particles, which caused clouds, leading to aggregate the dispersed protein particles into larger particles and eventually precipitate (Sin *et al.*, 2006).

Contrary to the increase of clarity due to the presence of enzyme, turbidity decreases. Where, the turbidity of the control is 781.3 NTU which significantly ($p < 0.05$) drops to 12.00 and 61.3NTU for juice extracted by Fructozym and PGzyme, respectively. The turbidity of juice extracted by Fructozym is the best one. This may be attributed to the differential concentration of Endo-PG and Exo-PMG in Fructozym and PGzyme preparations. Where they have higher amounts of Endo-PG and Exo-PMG, consequently they could convert insoluble pectin into soluble pectin leading to augment the clarity and decrease the turbidity of the juice (Byaruagaba-Bazirake *et al.*, 2012; Kyamuhangire *et al.*, 2002).

Consequently, banana fruits which are treated with Fructozym or PGzyme preparation produce higher clarity and lower turbidity juice than that treated with Flavourzyme (Fig. 4).

Browning index (BI) and reducing sugar (RS) content of banana juice

The BI is the first impression for the juice quality. Where the obscure juice would mean that the product was deteriorated (Abdullah *et al.*, 2007). As shown in Fig. (5) BI of control sample is 1.915 units

decreased to 0.186 and 0.267 units for Fructozym and PGzyme, respectively. The BI of juice extracted by Fructozym is 0.186 significantly ($p < 0.05$) lower than that of juice extracted by PGzyme (0.267). No markedly difference is recorded between control sample and juice extracted by Flavourzyme. At the same time, reducing sugar (RS) increases due to the enzyme treatment. The highest concentration of RS is 113.21mg/ml

detected in the juice extracted by Fructozym followed by PGzyme (44.07 mg/ml). The increment in the reducing sugar could be correlated with the exo-polysaccharidases especially Exo-PG (Lanzarini and Pifferi, 1989). The variations in the RI and RS could be related to the different concentrations of carbohydrate hydrolases such as cellulases, hemicellulases and pectinases in the commercial and PGzyme preparations.

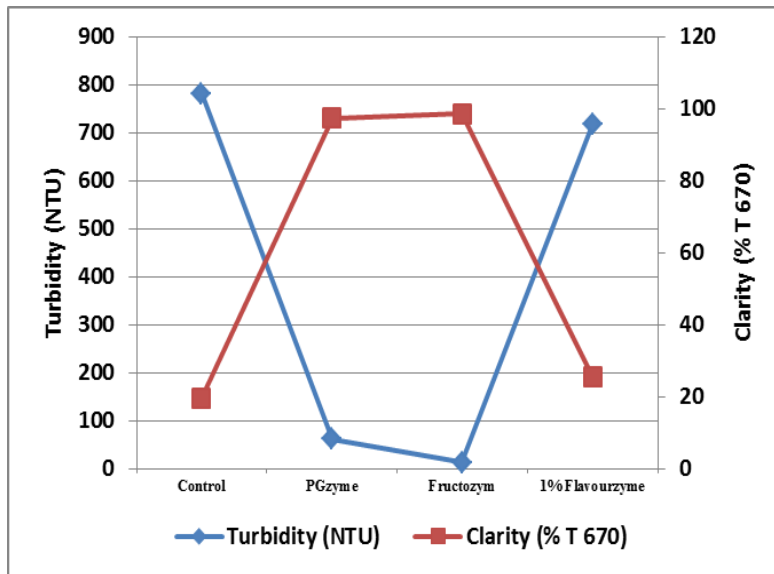


Figure (3): Turbidity and clarity of banana juice as affected by enzymes treatment

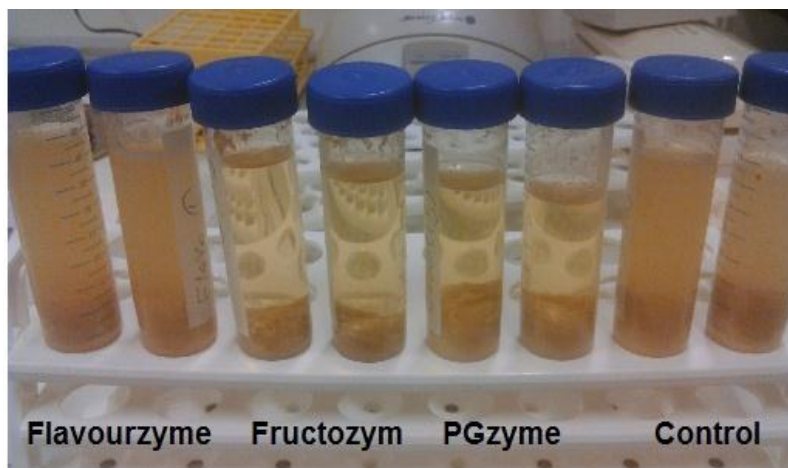


Figure (4): Clarity and turbidity of banana juice as affected with different enzymes treatment

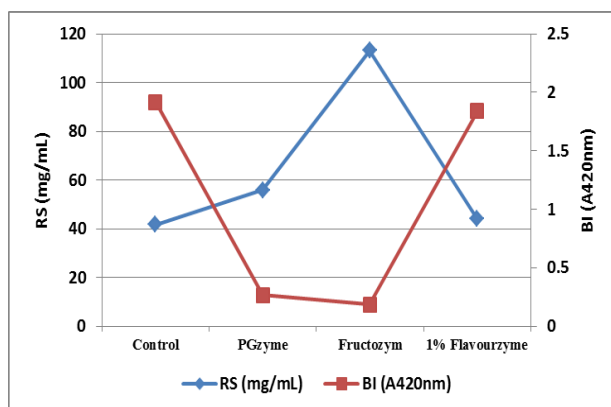


Figure (5): Browning index (BI) and reducing sugars content (RS) of banana juice as affected by enzymes treatment

These results are in full agreement with those reported in the literature before (Mabesa *et al.*, 1989; Kyamuhangire *et al.*, 2002; Fundira *et al.*, 2002).

Refractive index (RI) and total soluble solids (TSS)

Reflective index (RI) of banana juice tends to slightly decline as a function of the enzyme treatment. On the other hand, TSS content increases. The significantly highest concentration of TSS is (17.5°Brix) in the juice extracted by Fructozym followed by PGzyme (16.25°Brix) (Fig. 6). While, the lowest RI is (1.351) in banana juice extracted by PGzyme followed by that of Fructozym (1.353). This means the long chain of pectin and other polysaccharides falls short in length as a result of breakdown the glycosidic linkages in the backbone of pectin and other polysaccharides. Such results are obtained by Byaruagaba-Bazirake *et al.* (2012).

The pH of banana juice

The pH values of banana juice extracted by both Fructozym (4.43) and PGzyme (4.67) are significantly ($p > 0.05$) lower than those found in control (4.84) and juice extracted by Flavourzyme (4.82) (Table 2). But no significantly ($p > 0.05$) differences are found between pH's banana juices extracted by Fructozym (4.43) or PGzyme (4.67). This may be related to the activity of Exo-PG and Exo-PMG in the enzymes preparation (Fructozym and PGzyme) which split

galacturonic acid and release in the juice leading to increase its acidity. As a results, the pH of the juice decreases.

Generally, the variations in the juice yield, viscosity, Browning index, Reducing sugars content, reflective index and total soluble solids of banana juice refractive index, total soluble solid could be related to the different concentrations of carbohydrate hydrolases such as cellulases, hemicellulases and pectinases in the commercial and PGzyme enzyme preparations. Expecting that the increment in the reducing sugar could be correlated with the exo-polysaccharidases especially Exo-PG (Lanzarini and Pifferi, 1989). These results are in the line of Tadakittisarn *et al.* (2007) and Byaruagaba-Bazirake *et al.* (2012). Therefore, the high TSS of juice extracted by enzyme preparation may be explained by the degradation of pectin, cellulose and hemicelluloses in the cell wall, causing release of some neutral sugars and leading to increment of °Brix degree. Dorreich (1993) reported that such °Brix increase can be as high as 10%. In this respect Lanzarini and Pifferi (1989) pointed out the use of pectinase can considerably increase the soluble solids and reduce the viscosity of the fruit pulp. It is clear from the previous data that the quality of banana juice extracted with the aid of enzyme preparations (Fructozym and/or PGzyme) is improved.

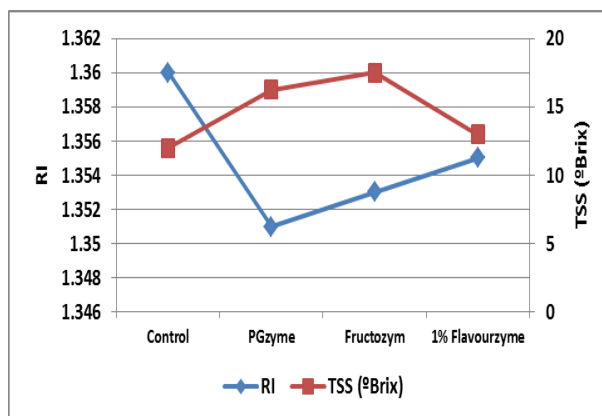


Figure (6): Refractive index (RI) and total soluble solid content (TSS) of banana juice as affected by enzymes treatment

Table 2: pH of banana juice as affected by enzymes treatment

Characteristic	Control	PGzyme	Fructozym	1% Flavourzyme
pH	4.84 ± 0.01a	4.67 ± 0.01b	4.43 ± 0.01c	4.82 ± 0.01a

M±SD = means and standard deviation of three successful trails

In a row, means followed by the same letter are not significantly different at 5% level

In conclusion, utilization of PGzyme and /or Fructozym preparations could be an excellent and promising tool to extract and clarify banana juice from pulp fruits at commercial scale.

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استخدام إنزيمات البكتينيز الخام PGzyme الناتجة من فطر *Aspergillus sojae* (ATCC 20235) و المعدل وراثيا في استخلاص و ترويق عصير الموز

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المخلص العربى

تهدف الدراسة لأستخدم أنزيم PGzyme الخام المنتج من فطر *Aspergillus sojae* (ATCC 20235) المعدل وراثيا في استخلاص و ترويق عصير الموز من الثمار الناضجة للموز مقارنة بنوعين من الأنزيمات البكتينية التجارية و التي تسمى Fructozym و Flavourzyme. لذلك، تم تقدير نشاط انزيمات (Endo-PG) endopolygalacturonase و (Exo-PG) exopolygalactouronase و (PMG) polymethylgalacturonase لكل مستحضر انزيمي. وقد بينت النتائج أن Fructozym هو أكثر فعالية في استخلاص و ترويق عصير الموز عن PGzyme في حين أن المستحضر الأنزيمي Flavourzyme كان ليس له تأثير ملحوظ في استخلاص و ترويق عصير الموز. كما تمت مناقشة الخصائص الفيزيائية و الكيماوية لعصير الموز الناتج من استخدام المستحضرات الأنزيمية المختلفة و التي شملتها الدراسة.

