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# Pear Juice Clarification Using Polygalacturonase from *Beauveria bassiana*: Effects on Rheological, Antioxidant and Quality Properties

*Ayodeji Amobonye*[1], *Prashant Bhagwat*[1], *Faith Matiza Ruzengwe*[1,2], *Suren Singh*[1], *Santhosh Pillai*[1]*

[1]*Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, PO Box 1334, Durban, 4000, South Africa*
[2]*Department of Food Science and Technology, Chinhoyi University of Technology, P. Bag 7724, Chinhoyi, Zimbabwe*

**Key words:** antioxidant, *Beauveria bassiana*, juice clarification, enzyme, pear juice, polygalacturonase, rheology

Although pear is one of the most preferred fruits globally due to its high nutrient content, its juice products have not received equal consumer acceptability due to their undesirable haziness and turbidity. However, enzymatic treatment of juice has been noted to improve the overall acceptability of juices better than traditional decantation, filtration, and heating methods, which are either expensive or detrimental to the product quality. In this study, pear juice was effectively clarified using partially purified polygalacturonase from an entomopathogenic fungal endophyte *Beauveria bassiana* SAN01. The optimization of the juice clarification process led to 137% improvement in clarification compared to control, under optimal conditions of 39.37 U/mL enzyme load, 36.87°C temperature and 2.75 h treatment time. Results also showed that the polygalacturonase treatment resulted in a significant decrease in viscosity and an increase in the pseudoplasticity of the pear juice as the rheological data of the juice was observed to fit adequately into the power law model ($R^2$>0.9). Furthermore, the enzyme-assisted juice clarification was found to reduce browning index (-14.34%) and turbidity (-19.72%) while increasing the reducing sugar content (9.55%). Unlike some conventional juice treatments, the polygalacturonase treatment preserved the antioxidant potential and the total phenolic contents of the pear juice as no significant changes were observed after the treatment. This study thus demonstrates the efficacy of a fungal polygalacturonase in improving pear juice quality as well as the potential applicability of *B. bassiana* enzymes in the food industry.

## INTRODUCTION

Enzymes have continued to gain prominence in food processing over traditional chemical- and mechanical-based methods, as they are eco-friendly and have been shown to increase food nutritional value, with lesser energy input requirement [Kumar *et al.*, 2016]. Various carbohydrases have since been produced commercially for their applications in fruit juice processing, as the potential of novel enzyme sources in enhancing customer desirability is continually being investigated. These biocatalysts are used to improve the clarity of fruit products such as juices, as consumer preference for well-clarified fruit juices devoid of opalescence is gaining more popularity [Sharma *et al.*, 2017]. Furthermore, clarification of juice is critical as haze- and sediment-forming substances diminish fruit juice quality during storage [Dıblan & Özkan, 2021]. Pectinolytic enzymes, including pectinesterases, polygalacturonases, pectin lyases, and protopectinases, have been the most exploited enzymes in this regard [Ramadan, 2019]. This is because the turbidity of fruit products is mainly caused by pectin, the primary plant polysaccharide in fruit products; hence, the pectinolytic enzymes hydrolyze pectic substances, promoting the flocculation of suspended particles and enhancing clarification [Karmakar & De, 2019].

However, the presence of other polysaccharides including cellulose, hemicellulose, starch as well as other compounds, such as lignin, oxidized phenolics, polyphenols, proteins, tannins, and metals, have also been implicated in the turbidity and cloudiness of different juices [Narnoliya *et al.*, 2020; Ogando *et al.*, 2019]. Hence, eliminating these components from fruit juices before packaging or serving to consumers is critical [Narnoliya *et al.*, 2020]. Additionally, the action of pectinase on fruit juices releases sugars that enhance the sweetness of the products, minimizing the addition of extra sweeteners. However, besides improving the physical attributes of fruit juices, the maintenance or improvement of their nutritional content is just as important during juice processing. The major nutritional benefit of fruit juices is attributed to their vitamins and antioxidants; however, different processing methods have shown detrimental effects on these constituents, particularly a reduction in their contents. For example, heat processing has been shown to reduce the vitamin

* Corresponding Author:
E-mail: santhoshk@dut.ac.za (S. Pillai)

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contents of juice [Ordóñez-Santos & Martínez-Girón, 2020], while UV-radiation to decrease the antioxidant properties [Islam *et al.*, 2016]. However, despite different studies on the enzymatic clarification of fruit juices, the effects of these treatments on their nutritional contents have not been well demonstrated.

Pear is considered one of the most widely consumed fruits worldwide, and its popularity has been attributed to its high fiber, minerals, vitamins, and antioxidants [Salta *et al.*, 2010]. However, despite these remarkable qualities, especially the high nutrient content, it has been observed that pear juices are not as desirable as other fruit juices, especially apple and orange juices. This relatively low consumer acceptance of pear juice has been partly attributed to its haziness and insipid flavor, hence, the need for a more effective processing technology to meet consumer's preferences [Xie *et al.*, 2007].

*Beauveria bassiana,* though more widely known as an entomopathogenic fungal endophyte [Thaochan *et al.*, 2021], has also been shown to secrete different industrially important metabolites and enzymes including amylase, cellulase, chitinase, lipase and protease [Amobonye *et al.*, 2020]. The potential of the fungus to produce these biological macromolecules and its established safety for human use have thus prompted the application of many of its enzymes in chemical biotransformation [Shankar & Laxman, 2015]. We have previously demonstrated the potentials of partially purified *B. bassiana* endoglucanase and xylanase cocktail in biomass saccharification [Amobonye *et al.*, 2021b] as well as the potential of purified xylanase in wastepaper deinking [Amobonye *et al.*, 2021a]. Hence, this study investigated the enzymatic clarification of pear juice using partially purified polygalacturonase from *B. bassiana* SAN01. In this regard, the quality of pear juice was enhanced by statistically optimizing the enzymatic treatment using the central composite design of response surface methodology. Subsequently, the effects of the treatment on the rheological properties, color, total soluble solids, turbidity, and reducing sugar levels of the juice were evaluated. Furthermore, the total phenolic and flavonoid contents and antioxidant activities of the juice were selected as indicators of its functional quality after treatment. To the best of our knowledge, this is the first scientific attempt at clarifying pear juice after the extraction process using a microbial enzyme, as well as optimizing the process *via* a statistical approach.

## MATERIALS AND METHODS

### Chemicals and reagents

The wheat bran used in the study was obtained locally in Durban, South Africa. Activated charcoal, Folin-Ciocalteu reagent, gallic acid and 3,5-dinitrosalicylic acid (DNS) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). All other chemicals and reagents used were of analytical grade and were obtained from validated suppliers.

### Microorganism and enzyme production

The polygalacturonase used for the clarification of pear juice was produced from *B. bassiana* SAN01, which was isolated locally in its endophytic state from onion leaves [Amobonye *et al.*, 2021b]. The enzyme production was carried out under submerged fermentation conditions in a shaking incubator (120 rpm) using 1 mL of inoculum ($1\times10^7$ spores/mL). The fermentation conditions include an initial media pH of 6.0, incubation temperature of 30.42°C and wheat bran concentration of 47.2 g/L. The polygalacturonase activity was measured using 1% orange peel pectin, while the reducing sugars released were determined by the DNS method according to Bailey *et al.* [1992]. One unit (U) of polygalacturonase activity is defined as the amount of enzyme liberating 1 $\mu$mol of galacturonic acid per min, under standard assay conditions (40°C, 50 mM phosphate buffer, pH 5.5).

### Partial purification and decolorization of the enzyme

The crude enzyme was partially purified by ammonium sulphate precipitation (60%) for 24 h at 4°C and centrifuged at 10,000×*g* for 20 min at 4°C using a refrigerated centrifuge (Eppendorf 5810R, Hamburg, Germany). The precipitate was reconstituted in citrate buffer (50 mM, pH 5.5) and desalted by dialysis. Subsequently, the precipitated protein was decolorized using activated charcoal to obtain a clear enzyme. In this regard, 1% activated charcoal was added to the enzyme solution and incubated at 4°C for 30 min with gentle stirring. The mixture was then centrifuged at 5,000×*g* for 10 min to recover a clear supernatant [Kareem *et al.*, 2011]. Finally, the enzyme was concentrated by ultrafiltration using 10 kDa membrane centrifugal filter (Millipore Corp., Billerica, MA, USA) and passed through sterile syringe filters (0.2 mm) (Millipore Corp.) to remove microbial contaminants and other suspended solids.

### Juice processing

Pears (Forelle varieties) weighing ~170 g were purchased from a local shop in Durban, South Africa. The fruits were washed thoroughly with distilled water, deseeded manually, chopped, and extracted using a juicer. The extract was rapidly strained through a stainless-steel sieve (3 mm pore diameter) for the separation of the suspended matter. Subsequently, the extract was vacuum filtered using Whatman® Grade 3 filter paper, transferred to clean Schott bottles and pasteurized in a water bath (Memmert WB 22, Memmert GmbH + Co. KG, Schwabach, Germany) at 95°C for 1 min [Petruzzi *et al.*, 2017].

### Enzyme-assisted pear juice clarification

The Design Expert 11.0 (Stat-Ease, Minneapolis, USA) software was employed for the statistical optimization of the enzyme-assisted clarification of pear juice [Amobonye *et al.*, 2021b]. For each treatment, 50 mL of pear juice was subjected to different enzyme treatment conditions (Table 1). The three process parameters selected for the optimization of enzyme treatments by central composite design (CCD) as well as their ranges were identified from previous studies [Saxena *et al.*, 2014] and also based on preliminary experiments in our laboratory. These include the enzyme load, incubation time and temperature. Incubation was performed in a circulating water bath (IncuMax WB10C Water Bath, Amerex Instruments, Inc., Concord, CA, United States) with continuous agitation (50 rpm) while keeping the juice at its natural pH (pH 3.9). The partially purified polygalacturonase (40 U/mL)

TABLE 1. Coded and uncoded variables of the response surface design for the clarification of pear juice.

| Independent variables | Units | Levels | | | | |
|---|---|---|---|---|---|---|
| | | -1.68 | -1 | 0 | +1 | +1.68 |
| Enzyme load (A) | U/mL | 20 | 24.22 | 30 | 35.77 | 40 |
| Temperature (B) | °C | 30 | 34.23 | 40 | 45.78 | 50 |
| Time (C) | h | 1 | 1.42 | 2 | 2.58 | 3 |

was used in the optimization process and the enzyme treatment was terminated by heating the juice at 90°C for 1 min. The experimental design in the coded levels of process variables is summarized in Table 1. The optimization design was made of 15 combinations including 5 replicates of the center point and juice clarity was determined as the response.

**Rheological properties of pear juice**

Rheological properties of the juice samples were evaluated according to Domingues *et al.* [2011], using a rheometer (MCR102, Anton Paar GmbH, Graz, Austria), fitted with a parallel plate (50 mm diameter, 1.5 mm gap). The rheometer was equipped with a Peltier system (TEZ-15P-C) with a temperature accuracy of 0.01°C. For the steady flow studies, 2 mL of juice samples were placed between the plates. The shear stress (Pa) and viscosity were observed linearly increasing from 1 $s^{-1}$ shear rate to 300 $s^{-1}$; the data points were collected and analyzed using US200 Universal Software (Paar Physica, Anton Paar GmbH). The shear rate range used in this study is in line with many food processing applications such as pumping, grinding, in-pipe flow, mixing and stirring [Deshmukh *et al.*, 2015].

**Evaluation of fruit juice characteristics**

*Juice clarity*

The change in percent transmittance (Δ% T) was considered a measure of the juice clarity [Zhao *et al.*, 2019]. Initially, the absorbance of the juice samples was recorded at 660 nm with a UV–VIS spectrophotometer (Model UV-1201, Shimadzu Corp., Kyoto, Japan) with distilled water and heat-inactivated enzyme serving as the blank and the control respectively. Subsequently, the change in percent transmittance (Δ% T) was calculated according to the formula given below.

$$\text{Juice clarity } (\Delta\%\ T) = \frac{(\%T_s - \%T_b)\times 100}{\%T_c - \%T_b} \quad (1)$$

where: $\%T_s$ – percent transmittance of test sample; $\%T_b$ – percent transmittance of blank; $\%T_c$ – percent transmittance of control.

*Browning index and turbidity determination*

To determine the browning index, 5 mL of juice sample was mixed with 5 mL of 95% ethanol and centrifuged at 4,000×*g* for 10 min. The supernatant obtained was passed through a 0.45 $\mu$m membrane filter, and the absorbance was measured at 420 nm [Uçan *et al.*, 2016]. The turbidity of the juice was determined using a Turbidometer (Oxoid, Basingstoke, UK) and reported as nephelometric turbidity units (NTU) using hexamethylenetetramine solution as a standard [Pradhan *et al.*, 2020].

*Reducing sugars, total dissolved solids and titratable acidity*

Reducing sugars released after the enzymatic treatment of pear juice were determined using the DNS method [Bailey *et al.*, 1992]. Total soluble solids were measured using a digital refractometer with automatic temperature compensation (Atago Co., Ltd, Tokyo, Japan) and expressed as °Brix (%). The pH of the juice was measured using a benchtop pH-meter (Hanna Instruments, Woonsocket, RI, USA), while the titratable acidity was estimated by titrating 10 mL of diluted juice (1:10; juice: distilled water) with 0.1 N NaOH solution, using phenolphthalein as an indicator, and the acidity value was expressed in citric acid (0.0064 acid factor) equivalents [Santana *et al.*, 2021].

*Color measurement*

The color parameters (CIE $L^*$ $a^*$ $b^*$) of the raw and treated juice samples were measured using a ColorFlex EZ Spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA, USA), where $L$ value indicates lightness while $a^*$ denotes the red/green value ((+): red; (−): green) and $b^*$ the yellow/blue value ((+): yellow; (−): blue). Furthermore, the chroma ($C^*$) of the samples was calculated according to the formula (2) [Uçan *et al.*, 2016].

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

**Analysis of antioxidant activities and phenolic contents**

*DPPH radical scavenging activity*

Reaction mixtures containing the sample (100 mL) and DPPH$^\bullet$ (3.9 mL, 50 $\mu$M) in methanol were incubated in a water bath at 37°C for 30 min. The absorbance was measured at 517 nm using a Thermo Scientific GENESYS UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and the DPPH$^\bullet$ scavenging activity was calculated by the equation (3):

$$\text{DPPH radical scavenging activity } (\%) = \\ = [(Abs_0 - Abs_1)/Abs_0]\times 100 \quad (3)$$

where: $Abs_0$ and $Abs_1$ are the absorbance values of control (without juice) and test samples, respectively [Wang *et al.*, 2019].

*Total phenolic content*

The total phenolic content of juices was analyzed according to a modified method using Folin-Ciocalteu reagent. Each sample (0.1 mL) was mixed with 0.75 mL of diluted Folin-Ciocalteu reagent (10% *v/v*) and incubated for 5 min at room temperature and 0.75 mL of 2% sodium carbonate solution was added. After incubation for 15 min at room temperature, the absorbance of the solution was read at 750 nm. Gallic acid was used as the standard, and total phenolic content was expressed as mg gallic acid equivalents (GAE) per L of juice [Romero-González *et al.*, 2020].

*Total flavonoid content*

The total flavonoid content (TFC) of juices was estimated spectrophotometrically based on the aluminium chloride

colorimetric method [Cheng *et al.*, 2016]. Juice samples (0.5 mL) were added to 4.5 mL of an aluminium chloride reagent consisting of 10% aluminium chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 95% ethanol (1.5 mL) and distilled water (2.8 mL). The absorbance of the reaction mixture was measured at 415 nm after incubation at room temperature for 30 min. The TFC in each juice sample was calculated based on a standard calibration curve of quercetin and expressed as mg quercetin equivalents (QE) per L of juice. The aluminium chloride reagent mixture alone, without the addition of the sample, was used as a blank.

*Ferric reducing antioxidant power (FRAP)*

The FRAP assay was performed using a modified Wang *et al.* [2019] method. Briefly, a FRAP reagent was prepared from 300 mM acetate and glacial acetic acid buffer (pH 3.6), 20 mM ferric chloride and 10 mM 4,6-tripryridyl-s-triazine (TPTZ) made up in 40 mM HCl. All three solutions were mixed in the ratio 10:1:1, *v/v/v*. The FRAP assay was performed by incubating 1 mL of $dH_2O$, 25 $\mu$L of juice and 1 mL of the FRAP reagent at 37°C. The absorbance readings at time zero and after 40 min were recorded at 593 nm [Benzie & Strain, 1996]. Ferrous sulphate was used as the standard, and the FRAP was expressed in $\mu$mol of Fe (II) per mL of juice.

**Statistical analysis**

All the data presented are the mean ± standard deviation of triplicate values. The variation between groups was evaluated using the Duncan test and one-way analysis of variance (ANOVA) of the IBM SPSS v26 software package (IBM Corp., Armonk, NY, USA). A significance level of 5% was used in all the analyses.

## RESULTS AND DISCUSSION

**Optimization of enzyme-assisted pear juice treatment**

The efficacy of the *B. bassiana* SAN01 polygalacturonase in the clarification of pear juice was evaluated by response surface methodology (RSM) using a CCD design while measuring the juice clarity under different conditions as the response (Table 2). In the design of 15 experimental runs, pear juice clarity varied considerably from 25.46% and 100% with changing process conditions (Table 2). These results showed that the pear juice clarity was significantly improved by optimizing the enzyme load, incubation temperature and incubation period. In addition, close agreements were observed between all the experimental and predicted values in this study. It is posited that the polygalacturonase hydrolyzed the pectin into different monomers and oligomers, creating protein-sugar complexes which aggregated into larger particles and settled, resulting in improved clarity. Data from this study is corroborated by similar findings from previous investigations [Ahmed & Sohail, 2020; Sorrivas *et al.*, 2006].

The reliability of the model was confirmed from the F-value (41.06) and *p*-values as observed from the ANOVA (Table 3). The model, as well as some of the terms (A, B, C and AC), were found to be significant, judging from their *p*-values. However, exceptions were noticed in AB and BC with *p*-value greater than 0.05, which suggests that the interactive effects of the variables are not consequential. The model showed a high $R^2$ value of 0.9685, which demonstrates that the model can account for ~97% of the total variation and represent significant correlations among the selected variables.

TABLE 2. Central composite design (CCD) optimization of pear juice clarification and the response of the dependent variables.

| | Level | | | Clarity (%)* | |
|---|---|---|---|---|---|
| | A** | B | C | Actual | Predicted |
| 1 | +1.68 | 0 | 0 | 74.87 | 77.72 |
| 2 | 0 | -1.68 | 0 | 38.48 | 41.28 |
| 3 | 0 | +1.68 | 0 | 61.95 | 64.75 |
| 4 | 0 | 0 | -1.68 | 32.51 | 36.41 |
| 5 | 0 | 0 | +1.68 | 65.72 | 69.62 |
| 6 | +1 | -1 | +1 | 100.00 | 99.46 |
| 7 | 0 | 0 | 0 | 56.12 | 53.02 |
| 8 | -1.68 | 0 | 0 | 25.46 | 28.31 |
| 9 | 0 | 0 | 0 | 58.06 | 53.02 |
| 10 | -1 | -1 | -1 | 39.22 | 38.68 |
| 11 | 0 | 0 | 0 | 51.19 | 53.02 |
| 12 | +1 | +1 | -1 | 36.79 | 36.25 |
| 13 | 0 | 0 | 0 | 59.15 | 53.02 |
| 14 | -1 | +1 | +1 | 38.22 | 37.68 |
| 15 | 0 | 0 | 0 | 57.53 | 53.02 |

*Average of triplicate determinations. **Details of codes A, B & C are given in Table 1.

The interactions between the selected factors affecting the optimized clarification of pear juice were also represented in contour and response surface plots (Figure 1). The interaction between AB was moderately significant (*p*-value 0.0726), with increasing the dose of enzyme, a simultaneous increase in juice clarification was observed where 20 U/mL showed 28%, while 40 U/mL showed 78% juice clarification, keeping time at zero level (2 h). While with increasing temperature, the juice clarification moderately increased, showing 41% clarification at 30°C and 65% at 50°C, suggesting the inefficiency of the enzyme to work both at low and high temperature regimes (Figure 1a). The interaction between AC was most significant (*p*-value <0.0001), where both incubation time and enzyme load were found to interact considerably (Figure 1b). However, at an extreme lower enzyme dosage of 20 U/mL and incubation time of 1 h, the juice clarification was not effective. In contrast, a sharp increase in juice clarification was observed (39–100%) with a steady rise in both the factors, comprising enzyme load (24–36 U/mL) and incubation time (1.4–2.6 h), while keeping the temperature at 34°C. The interaction among BC was found to be statistically insignificant (*p*-value 0.8473), suggesting little interactions between incubation time and incubation temperature (Figure 1c), which is mainly because of the level of enzyme dosage which was studied at zero level (30 U/mL). It further

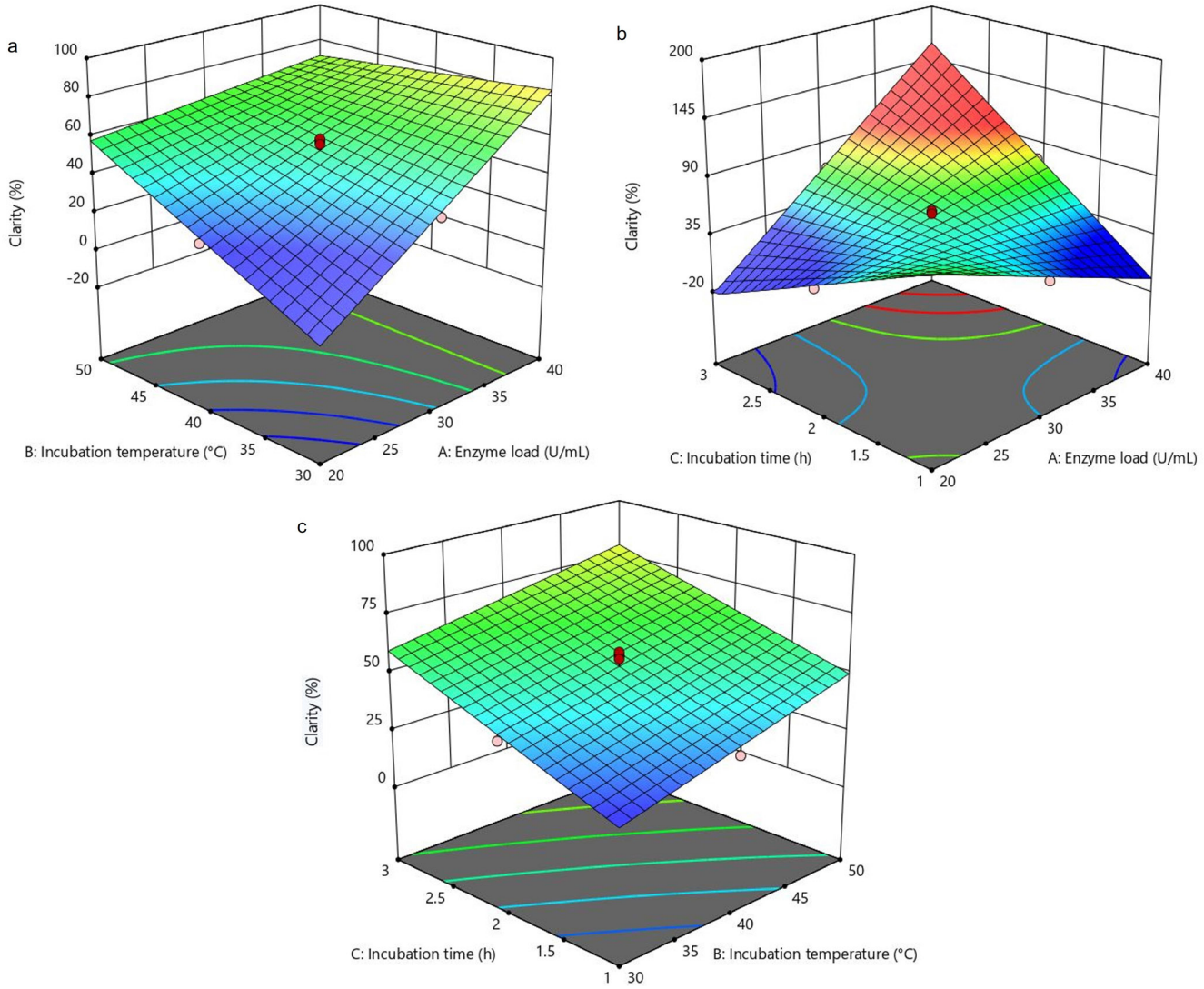


FIGURE 1. Response surface plots showing the interactions between the variables for enzymatic clarification of pear juice: (a) 3D plots of enzyme load – incubation temperature interaction; (b) 3D plots of enzyme load – incubation time interaction; (c) 3D plots of incubation time – incubation temperature interaction.

substantiates enzyme dose as the most critical factor, corroborated by its lowest *p*-value (<0.0001) (Table 3).

**Validation of the experimental model**

The adequacy of the model was further validated by conducting the enzyme treatment using derived optimal conditions. The conditions predicted for optimum pear juice clarification were enzyme load (39.37 U/mL), incubation temperature (36.87°C) and time (2 h 45 min) with a desirability of 0.927. As suggested by the model, the predicted value of maximum clarification, 140.65%, was in close agreement with the experimental value of 137±7.1%. Hence, the proximity between the predicted and experimental values points to the adequate prediction of the generated model. Thus, by applying the CCD designs, a 1.37-fold increase in clarity relative to the control was recorded.

**Effect of enzyme treatment on pear juice rheology**

The relationship between the shear stress and shear rate of the raw and enzyme-treated pear juice is shown

TABLE 3. Results of the analysis of variance (ANOVA) of quadratic models for pear juice clarification.

| Source | Sum of Squares | df | Mean Square | F-value | *p*-Value* |
|---|---|---|---|---|---|
| Model | 4926.40 | 6 | 821.07 | 41.06 | < 0.0001 |
| A** | 1220.67 | 1 | 1220.67 | 61.05 | < 0.0001 |
| B | 275.42 | 1 | 275.42 | 13.77 | 0.0059 |
| C | 551.45 | 1 | 551.45 | 27.58 | 0.0008 |
| AB | 85.41 | 1 | 85.41 | 4.27 | 0.0726 |
| AC | 1250.65 | 1 | 1250.65 | 62.54 | < 0.0001 |
| BC | 0.7909 | 1 | 0.7909 | 0.0396 | 0.8473 |
| Residual | 121.15 | 8 | 20.00 | | |
| Lack of Fit | 38.82 | 4 | 30.29 | 3.12 | 0.1481 |

$R^2$=0.9685, adjusted $R^2$=0.945, predicted $R^2$=0.9061, adequate precision (AP)=23.291.
*Significant at $p$<0.05. **Details of codes A, B & C are given in Table 1.

in Figure 2a. The rheograms of the raw and enzyme-clarified juice showed a monotonical increase in the shear stress with increasing shear rate. Subsequently, the Ostwald-de Waele model or power law equation was used to describe the relationships between the two variables as the recorded rate of increase of the shear stress was not directly linear to the shear rate increase [Sanchez *et al.*, 2009; Tavares *et al.*, 2007]:

$$\sigma = k \times \gamma^n \quad (4)$$

where: σ is the shear stress (Pa), $k$ is the consistency coefficient (Pa×s$^n$), γ is the shear rate (s$^{-1}$) and $n$ is the flow behavior index (-).

It was observed that flow behaviors of the raw and enzyme-treated juice samples fitted well within the power law model as shown by the $R^2>0.9$ (Table 4). Flow behavior index $n<1$ shows pseudoplasticity whilst $n=1$ shows Newtonian flow behavior. In this study, an increase in pseudoplasticity/shear thinning was observed after enzymatic treatment of the juice. In addition, the consistency coefficient, $k$, showed that the polygalacturonase treatment of pear juice decreased viscosity at any given shear rate (Table 4).

Similarly, a decrease in the viscosity values was observed in both samples with the increase in the shear rate (Figure 2b), and the treated juice was observed to be less viscous than the raw juice. Thus, it could be inferred that the observed decrease in the viscosity after enzymatic treatment is due to the hydrolytic action of the enzyme on the pectic materials present in the juice. This hydrolytic action of the enzyme results in reduced water holding capacity of the juice and subsequent release of free water into the system, reducing juice viscosity [Sharma *et al.*, 2017]. Similar flow behaviors have also been highlighted in previous clarification studies of various juices including apricot, banana, and sapodilla [Sharma *et al.*, 2017].

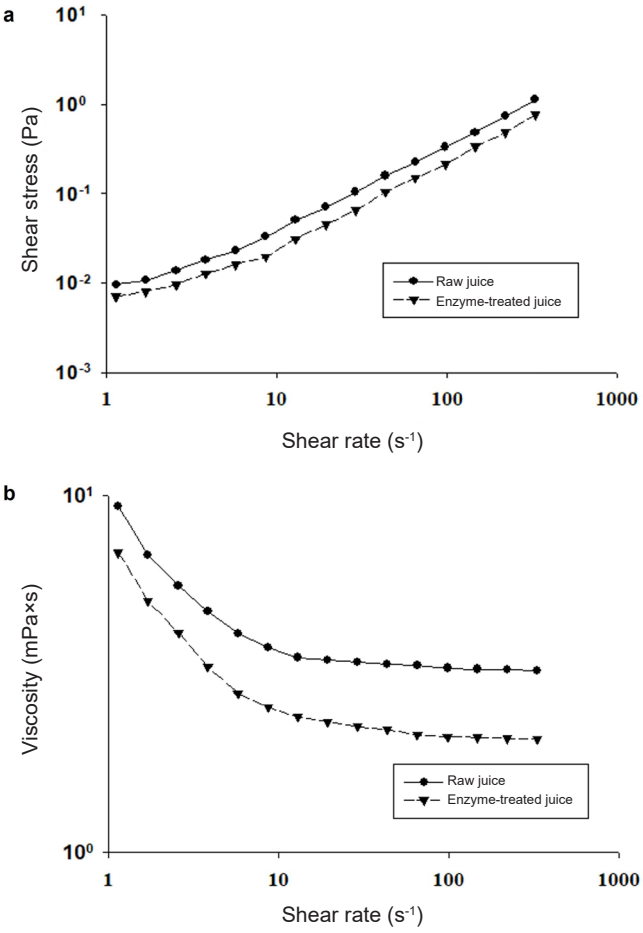


FIGURE 2. Effect of enzyme treatment on the (a) shear stress and (b) viscosity profile as a function of shear rate of pear juice.

TABLE 4. Power-law model coefficients of raw and enzyme-treated pear juice.

| | $k$ (Pa×s$^n$) | $n$ | $R^2$ |
|---|---|---|---|
| Raw juice | 0.0061±0.0002 | 0.89±0.03 | 0.9977 |
| Enzyme-treated juice | 0.0047±0.0001 | 0.87±0.03 | 0.9962 |

Results expressed as mean ± standard deviation; $k$: consistency coefficient, $n$: flow behavior index, $R^2$: coefficient of determination.

In practical terms, a reduction in the viscosity of fruit juice is considered vital as it prevents problems such as fouling of membrane surfaces encountered during the subsequent filtration process which often reduce juice quality and increase production cost. Furthermore, the reduction in viscosity and cluster formation resulting from the enzymatic clarification of juices further facilitates separation through centrifugation and filtration. Ultimately, these result in the final juice product exhibiting greater clarity, more concentrated flavor and color [Abdullah *et al.*, 2007; Sharma *et al.*, 2017]. In addition, many consumers have also shown preferences for juice products with relatively lower viscosity [Salehi, 2020].

**Effect of enzyme treatment on pear juice quality**

The color of food products is a major determining factor in customer preference. It has been observed that many consumers prefer brighter or lighter juices, hence, the color and the clarity of beverages are usually considered as standard quality indicators [Gonzalez Viejo *et al.*, 2019]. Browning, both enzymatic and non-enzymatic, has been noted for its detrimental effects on the nutritional and sensory appeal of fruit products. For example, in pear juice, the color has been noted to be one of the reasons for its non-preference by consumers [Xie *et al.*, 2007]. Furthermore, dark-colored fruit products are usually associated with deterioration, hence, a reduction in the dark color is desirable [Beveridge & Wrolstad, 1997; Singh *et al.*, 2021]. In this study, a reduction in browning (~14%) of pear juice was observed after the treatment with the fungal polygalacturonase (Table 5), indicating its effectives in improving the pear juice quality. The beneficial effect of polygalacturonase treatment is also demonstrated by the ~10% increase in reducing sugar content after processing (Table 5). This expected increase in reducing sugar content, which has also been observed in previous studies on juice clarification [Adedeji & Ezekiel, 2020; Yang *et al.*, 2019], is a result of the hydrolytic action of the glycosyl hydrolase on the pectin present in the raw juice. Furthermore, the increment in reducing sugar content is expected to enhance the sweetness and flavor of the juice products, thus, limiting the need for extra sweeteners during further processing.

TABLE 5. Effect of enzyme treatment on pear juice properties.

| | Raw juice | Enzyme--treated juice | Percent change |
|---|---|---|---|
| Browning index | 0.26±0.01 | 0.22±0.01 | -14.34* |
| Color $L^*$ | 14.23±0.34 | 17.53±0.41 | 23.19* |
| $a^*$ | -1.23±0.04 | -0.83±0.04 | 32.52* |
| $b^*$ | 3.71±0.11 | 6.27±0.26 | 69.00* |
| $C^*$ | 3.91±0.14 | 6.33±0.31 | 61.89* |
| pH | 3.93±0.04 | 3.79±0.03 | -3.56 |
| Reducing sugar content (mg/mL) | 75.5±2.3 | 82.6±2.7 | 9.55* |
| Titratable acidity (g/100 mL) | 0.28±0.01 | 0.27±0.01 | -3.57 |
| Total dissolved solids (Brix %) | 8.90±0.1 | 9.2±0.2 | 3.37 |
| Turbidity (NTU) | 1411±74 | 1132±35 | -19.72* |

Results expressed as mean ± standard deviation ($n$=3). *Significant at $p$<0.05.

TABLE 6. Effect of enzyme treatment on pear juice antioxidant properties.

| | Raw juice | Enzyme--treated juice | Percent change |
|---|---|---|---|
| DPPH· scavenging activity (%) | 66.0±2.2 | 68.2±3.0 | 3.23 |
| Total phenolic content (mg GAE/L) | 318.0±12.3 | 313.0±7.3 | -1.57 |
| Total flavonoid content (mg QE/L) | 118.6±3.4 | 136.8±4.7 | 15.35* |
| FRAP ($\mu$mol of Fe (II)/mL) | 133.1±5.0 | 132.7±6.7 | -0.34 |

Results expressed as mean ± standard deviation ($n$=3); GAE: gallic acid equivalents, QE: quercetin equivalents, FRAP: ferric reducing antioxidant power. *Significant at $p$<0.05.

It is also probable that the increased reducing sugar content observed in this study could be inhibitory to polyphenol oxidase and consequently resulted in reducing browning. Reducing sugars have been previously noted in this regard to inhibit the activity of polyphenol oxidase [Moon *et al.*, 2020; Nicoli *et al.*, 1991], an enzyme that has been implicated in enzymatic browning [Jiang *et al.*, 2016].

A significant improvement in the clarity of the juice was also inferred from the color change observed subsequent to the enzyme treatment as demonstrated by the ~23% increase in $L^*$ value (Table 5). The increase in $L^*$ value was also observed in a recent study on the application of pectinase in extracting juices from pears [Gani *et al.*, 2021]. Furthermore, there was an increase in the redness and yellowness of the enzyme-treated juice compared to the control, as deduced from the positive changes in the $a^*$ and $b^*$ values. Likewise, the increase in the color intensity and the brightness of the processed juice was also shown by the significant increase in the chroma value (61.89%) (Table 5).

Turbidity has been shown as a quality discriminant in fruit juice processing affecting food product stability [Salehi, 2020; Sharma *et al.*, 2017]. Turbidity in fruit juices results from unhydrolyzed complex carbohydrates, hence the hydrolysis of pectin has been identified as a significant step in this regard [Sorrivas *et al.*, 2006]. The efficacy of the *B. bassiana* polygalacturonase in the juice clarification process was highlighted by a ~20% reduction in its turbidity (Table 5). A similar trend in turbidity was also observed in the clarification of apple, grape and peach juices using polygalacturonase from *Coriolus versicolor* and *Penicillium notatum* [Amin *et al.*, 2017]. The reduction in turbidity and viscosity observed in this study after enzymatic treatment is also complemented by the observed increase in reducing sugar content. This could be comparable with a finding from Yang *et al.* [2019] where a linear relationship existed between complex sugars (substrates) clearance, and the reducing sugars (products) released.

The pH, titratable acidity, and total dissolved solids (Brix %) have been identified as important factors in the quality control of fruit juices, especially regarding storage stability [Adedeji & Ezekiel, 2020]. Minor fluctuations were observed between the pH of the control and the treated juice as only a 3.56% change was recorded (Table 5). Similar trends were observed for titratable acidity and total dissolved solids in previous enzymatic treatments of banana and strawberry juices, where the pH, titratable acidity and total dissolved solids were barely affected [Barman *et al.*, 2015; Sandri & Silveira, 2018]. The observed reduction in the pH and increase in titratable acidity is believed to be due to the release of galacturonic acid which is the hydrolytic product of pectin while the rise in Brix (%) could be due to the enzyme-facilitated degradation of the complex carbohydrate polymers in the juices [Bora *et al.*, 2017; Kyamuhangire *et al.*, 2002].

### Effect of *B. bassiana* polygalacturonase on antioxidant properties and phenolic contents

The increase in juice consumption has been ascribed to the reported health benefits of their antioxidants [Ern *et al.*, 2016]; hence, it is crucial to determine the effect of the enzymatic treatment on the antioxidant properties of fruit juices. Studies have shown the detrimental effects of various treatment processes, such as thermal, mechanical and irradiation, on the desirable antioxidant benefits of fruits and their products [Al-juhaimi *et al.*, 2018]. Though polygalacturonase treatment has been shown in a few studies to have negative effects on the DPPH radical scavenging activity of fruit juices, a slight increase in the scavenging activity (of 3.32%) was observed for pear juice after the polygalacturonase treatment (Table 6). Hence, it is hypothesized that the polygalacturonase enzyme was involved in the hydrolysis of the different linkages and complexes made between pear juice antioxidants and its pectin [Ribas-Agustí *et al.*, 2018].

Phenolics including flavonoids contribute the most to the antioxidant activities of fruits and their products. In this study, the total phenolic contents were not significantly affected after enzyme clarification; however, there was a significant increase (15.25%) in the total flavonoid content (Table 6). Pear juices have been shown to be very rich in phenolic compounds, including arbutin, chlorogenic acid, epicatechin, quercetin 3–glucoside, quercetin 3–galactoside and quercetin 3–rutinoside [Schieber *et al.*, 2001]. It is posited that

the hydrolytic action of the enzyme could release these compounds bound to the cell wall as well as cleave the internal glycosidic bonds of flavonoids into their aglycone moieties, thus, causing a significant increase in total flavonoids, and consequently an increase in the antioxidant activity, as shown previously by Mandalari *et al.* [2006]. Furthermore, it has also been noted that the enhancement of total antioxidative activity might be due to an increase in flavonoid aglycones [Gu *et al.*, 2019]. The FRAP assay is regarded as one of the best tests for determining the antioxidant capabilities of fruit juices [Sethi *et al.*, 2020]. The antioxidant activities, as measured by the FRAP method, were shown to be preserved in pear juice following *B. bassiana* polygalacturonase treatment as no significant change was observed. This recorded preservation of FRAP in processed juice relative to the untreated juice could be corroborated with previous studies such as in the enzyme-assisted processing of Indian plum juice [Koley *et al.*, 2011].

## CONCLUSIONS

Despite the popularity of the pear fruit, very little focus has been placed on its juice as a commercial product unlike other popular fruits, such as apples, oranges and pineapples. As earlier highlighted, this might partly be due to the undesirable cloudiness post-extraction. According to available literature, there are no research focused on the clarification of extracted pear juice, and particularly, no attempt has been made using microbial enzymes in that process. Thus, this study effectively utilized RSM to establish the optimum parameters for pear juice clarification using *B. bassiana* polygalacturonase. The optimal clarification conditions were validated using the conditions derived from the CCD model. The analysis of the quality characteristics and some biochemical properties has revealed that the polygalacturonase treatment had no detrimental effect on the most-desired physical and antioxidant properties of the juice. It was further observed that the enzyme-assisted treatment significantly enhanced many of the characteristics of the product, which are in line with customer and industrial preferences. Hence, results from this study have demonstrated, for the first time, the practical application of a novel fungal enzyme in improving some organoleptic properties of pear juice, especially the visual appeal. Like most fungi, *B. bassiana* has been shown to be acidophilic, thus, it is most probable that the polygalacturonase from this study will perform optimally at the acidic pH range, which is most suitable for its application in the juice and wine industries. However, further investigation into the enzyme's biochemical characteristics, its stability, and the effects of different immobilization methods on its activity will enhance its probability of being considered a commercial enzyme. Thus, in addition to the already established safety of the fungus and its products, findings from this study further underscore its applicability in many industrial processes, particularly in juice processing. Therefore, *B. bassiana* could be considered an important candidate for food processing enzymes, in addition to its well-known use in the agricultural industry.

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## CONFLICT OF INTERESTS

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

## ORCID IDs

A. Ayodeji https://orcid.org/0000-0002-3220-5858
P. Bhagwat https://orcid.org/0000-0001-5790-7824
S. Pillai https://orcid.org/0000-0003-3474-1555
F.M. Ruzengwe https://orcid.org/0000-0001-8589-7475
S. Singh https://orcid.org/0000-0003-1064-6297

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