

Clarification of orange juice by crude fungal pectinase from citrus peel

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ABSTRACT

Fungal pectinase enzyme was produced by *Rhizopus oryzae* on a solid culture containing citrus peel of orange (35% w/v). The crude extract with maximum pectinase activity of 1,360 u/ml was used to clarify orange juice. The yield, turbidity and viscosity as well as pH, total soluble solids, ascorbic acids and total titratable acidity of the clarified juice were determined. The optimum yield (97%) of juice was obtained at 1% pectinase enzyme concentration, while the turbidity and viscosity decreases with increasing concentration of pectinase enzyme. There were no marked changes in the pH and total titratable acidity of the pectinase

enzyme treated juice. Ascorbic acid and total soluble solids increase with increasing pectinase enzyme concentration. There were significant differences ($p < 0.05$) in the pectinase enzyme concentrations on the yield, viscosity, turbidity and total titratable acid of the orange juice while no significant ($P > 0.05$) difference was found in the pH, ascorbic acid and total soluble solids. The results presented citrus peel as substrate for pectinase production and its subsequent use in the clarification of orange juice could enhance fruit juice processing in the tropics.

Key words: Orange juice, pectinase, clarification

INTRODUCTION

Orange (*Citrus sinensis*) belongs to Citrus fruits and believed to have originated from Asia (Beaven *et al*, 1972). Citrus fruits and juices serve as primary sources of our daily requirement of Vitamin C. In addition, supplementary nutritional value is obtained from the amino acids, inorganic salt, carbohydrates and probably other still unidentified factors found in the edible pigment (Beaven *et al*, 1972). Tropical fruit juices have become important in recent years due to the overall increase in “natural fruit” juice consumption as an alternative to the traditional caffeine-containing beverages such as coffee, tea, or carbonated soft drinks. By incorporating tropical fruits into fruit juice blends, food technologists have been able to exploit their exotic flavours without adding artificial flavour.

The demand for and acceptance of citrus fruit in the daily diet of human is based largely on their nutritional value, flavour, aroma and other aesthetic characteristics such as colour, texture and cloudiness (Braddock, 1981). A significant number of consumers do not relish orange juice in hazy or cloudy condition. Therefore, different methods have been used to clarify orange juice such as centrifugation, sedimentation and filtration, none of which has actually met brilliant and polish colour desired by most consumers (Baker and Bruemer, 1972; Braddock, 1981). Consequently, attention is being shifted to the use of pectic enzymes for better clarification.

Pectinases are a group of enzyme that degrade pectin-containing substances into smaller fractions thus resulting into viscosity reduction, less gel formation and high degree of

juice concentration (Screenath *et al*, 1987). Several attempts have been made on the production of pectinase by fungi using citrus peels, sugar cane molasses and other agricultural wastes as substrates (Hart *et al*, 1991, Kareem and Akpan, 2004). However, reports on the use of enzymes in tropical fruit juice processing are limited. This study is an attempt to merge the utilization of citrus peels for pectinase production by *Rhizopus oryzae* strain and the subsequent utilization of the enzyme for clarifying orange juice.

MATERIALS AND METHODS

Microorganism

Strains of *Rhizopus oryzae* obtained from the environment were screened for pectinolytic activity using the method of Onyeocha and Ogbona (1983). They were grown on a culture medium containing yeast extract 1g/l, agar 15g/l, and pectin 5g/l (pH 5). The plates were incubated at 30°C for 24 hrs and then flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide. The clear zones shown around the fungal clones against the opaque background indicated pectolytic enzyme activity and their sizes were measured accordingly. The pectinase positive strains were stored on potato dextrose agar slants at 4°C and sub-cultured twice a month.

Pectinase Production

The solid culture containing 35g dry citrus peel, 0.8g urea, and 3.2g ammonium sulphate. The mixture was moistened with 20ml distilled water and autoclaved at 121°C for 20 min. After cooling to ambient temperature, it was inoculated with spore suspension (1ml) of *R. oryzae* and incubated at 30°C for 72h. The fermented material was mixed with phosphate buffer (pH 5.0) at ratio 1:4 w/v and kept at 4°C overnight. The crude extract was partially clarified using Imarsil 1% (w/v) as described by

Kareem and Akpan (2003). The supernatant was used for the treatment of orange juice.

Enzyme Assay

The pectinase activity was determined by measuring the reducing sugar produced by 3,5 dinitrosalicylic acid method (Muller, 1959) using galacturonic acid as standard. One exo-pectinase unit is defined as the amount of enzyme that liberated one micromolecule of galacturonic acid equivalent per minute

Extraction of Orange Juice

Mature ripened orange fruits (*Citrus sinensis*) were obtained from a major market in Abeokuta, Nigeria. The oranges were sorted, washed and peeled. The juice was extracted using a domestic juice extractor.

Enzyme Treatment of Extracted Juice

The extracted juice was pasteurized at 85°C for 3 min to inactivate the natural fruit enzymes or microbes present and then cooled down to 40°C before the addition of pectinase enzyme. Varying concentration of enzyme (0, 0.25, 0.5 0.75 and 1%) was added. The samples were incubated at 40°C for 1h. After incubation, the samples were treated at 85°C for 3 min to inactivate the enzyme.

METHODS

Yield

The clarified juice was filtered using Whatman No 4 filter paper. The volume of fruit juice obtained from each sample was measured using 200ml volumetric flask and the weight of juice recorded.

Determination of Turbidity and Viscosity

Turbidity of clarified juice was measured using a Turbidimeter model 2010 (HACH). The viscosity measurement was made using a Brookfield Viscometer (Model LV) with 600ml beaker and viscosity reading was taken using spindle No 2 rotated at 30rpm.

Distilled water) with 0.1 N NaOH. The acidity value was expressed as citric acid. The pH value was determined using pH meter (Jenway Ltd)

Determination of Ascorbic Acid and Total Soluble Sugar

Ascorbic acid content of the clarified juice was determined by 2,6, dichlorophenol indophenol dye titration method (Rangana, 1977). The total solid content was determined using the Abbey Refractometer. Total sugar was determined colorimetrically using the method of Dubois *et al*, (1986).

Statistical Analysis

Datas were analyzed using Analysis of Variance (ANOVA) and means separated using Duncan's Multiple Range Test, using SPSS statistical package (SPSS 2002, version 10.0).

RESULTS AND DISCUSSION

Five strains of *Rhizopus oryzae* were screened for pectinase production. The results presented in table 1 showed that *Rhizopus oligosporus* (Rx-02) gave the widest zones of hydrolysis (8mm) on pectin-agar medium against the opaque background which is an indication of the ability to degrade pectin into galacturonic acid. The *Rhizopus oligosporus* (Rx-02) gave the highest pectinase activity when cultured on citrus peel and was successfully used to clarify orange juice. The result of the effect of different enzyme concentration on the yield of orange juice is shown in table 2. The yield varied from 73- 97% with untreated juice having the lowest and juice treated with 1% enzyme concentration had the highest yield. The observation may be due to degradation of pectic substances which lead to increase in the yield of juice (Gerthartz, 1990). There is significant difference ($P<0.05$) in the yield of orange juice clarified with different concentration of pectinase enzyme (Table 2).

Fig 1 showed that untreated juice was highly turbid (184 NTU), indicating the presence of suspended colloidal particles, which was offset upon enzyme treatment. The turbidity decreased with increase in levels of enzymes used with 2% enzyme concentration giving the least turbidity of 36 NTU. There is significant difference ($p<0.05$) in the turbidity of enzyme treated orange juice (Table 2). The result of viscosity showed about 51% reduction in viscosity in the treated juice compared to untreated juice (Table 2). Loss in viscosity has been reported to facilitate the filtration process and increase the efficiency of the juice concentration process (Screenath *et al*, 1987; Kareem, 1998). There is significant difference ($P<0.05$) in the viscosity of orange juice clarified with different concentration of pectinase enzyme (Table 2).

The pH values of enzyme clarified orange is shown in Table 3. The pH decreases from 3.8 in untreated juice to 3.54 in juice treated with 2.0% enzyme concentration. The pH of the juice decreases with increasing levels of enzyme concentration (Fig 2). A further increase in enzyme concentration above 1% did not significantly decrease the pH value. According to Baker and Bruemmer (1972), a decrease in pH value from 4.5 to 3.0 would increase the shelf life about 3 times. In this experiment, the slight decrease in pH is expected to give an improved quality in treated juice. The total titratable acidity increases from 0.038g/100g in untreated juice to 0.047g/100g in juice treated with 0.5% enzyme. However, above 0.5% enzyme concentration the total titratable acidity decreases (Fig 2). The dominant acid in orange juice is citric acid, which increases 4 times after the fruit was harvested (Paull and Chen, 1983). It is an important factor in determining the quality of fruit juice.

The result of Brix analysis on enzyme treated orange juice indicated a slight increase in soluble solids content from 6.0 to 6.2^o Brix (Table 2). Arthey and Ashurst (1996) had earlier reported that the Brix content of orange should be within the range of 4^o – 9^o Brix. Generally, the variations in enzyme concentration used did not significantly ($p>0.05$) affect the Brix value.

The ascorbic acid content of orange juice is normally 53.2 mg/100g (Siong *et al*, 1988), but in this experiment it varied from 48.30 mg/100g as the enzyme level is increased. This may be due to partial loss of nutrient during pasteurization. A similar report by Yusof and Ibrahim, (1994) indicated that the enzyme treatment did not seem to increase the ascorbic acid content.

CONCLUSION

The pectinase produced by *R. oryzae* degrades polysaccharide materials in the orange juice into smaller fractions thus facilitating filtration; reduction in viscosity and turbidity. It may be deduced that the use of citrus peel as substrate for pectinase production would promote local production of food enzymes. The subsequent application of the fungal pectinase in the clarification of orange juice would enhance the production of clarified fruit juice in the tropics.

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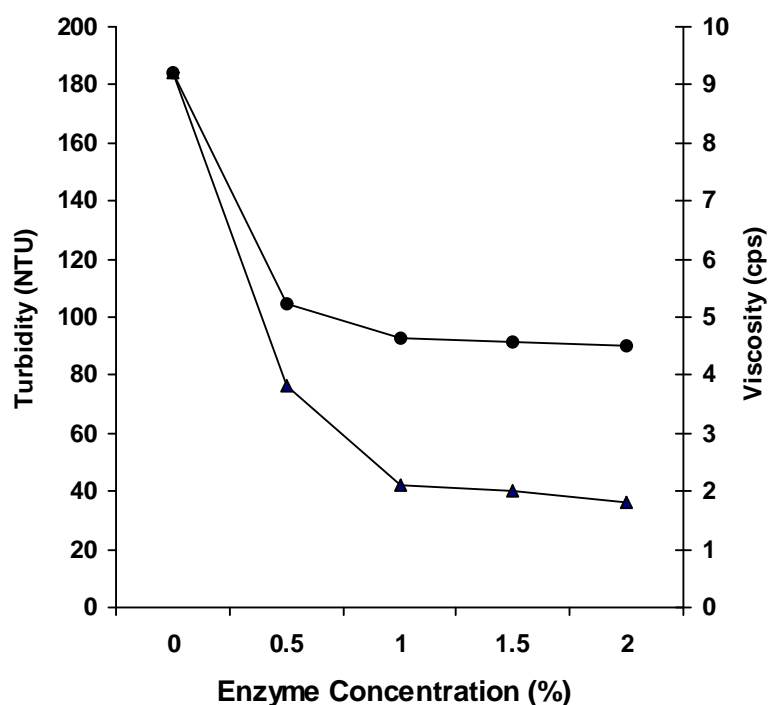


Fig 1: Effect of different pectinase enzyme concentrations on turbidity and viscosity of orange juice

—▲— turbidity —●— viscosity

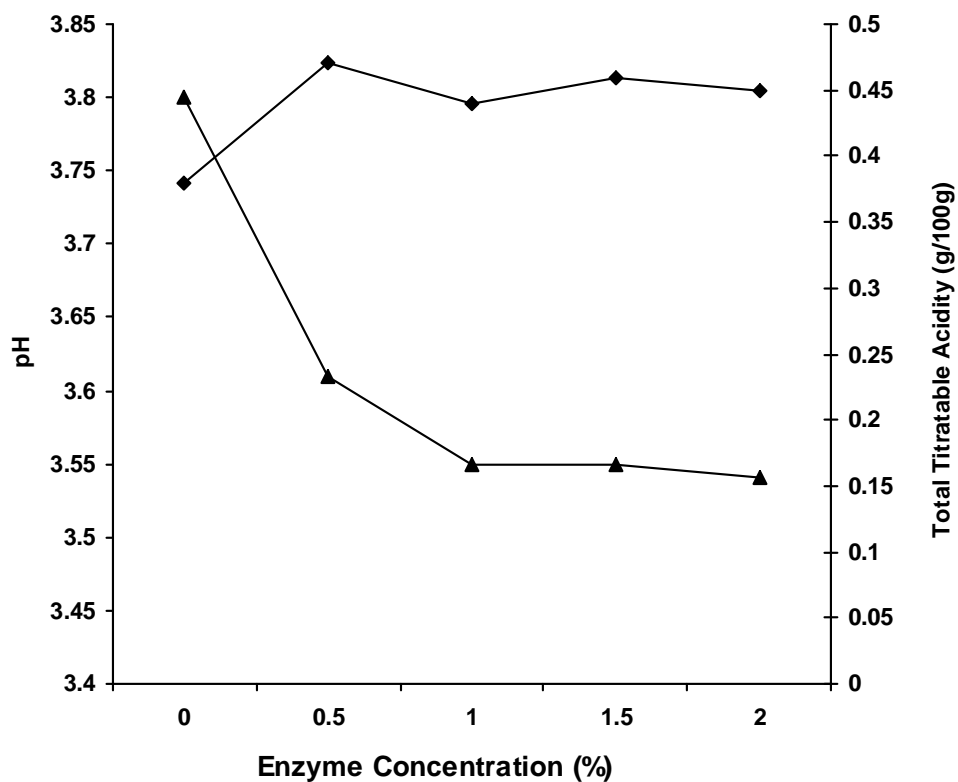


Fig 2 : Effect of different pectinase enzyme concentrations on pH and total titratable acidity of orange juice

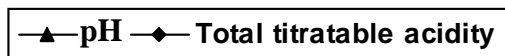


Table 1: Pectinase production and activity of *Rhizopus oryzae* strains

Strains	Width of clear zones on pectin agar (mm)	Pectinase activity (U/ml)
Rx-01	6	1,040
Rx-02	8	1,360
Rx-03	4	625
Rx-04	6	1,200

Table 2: Effect of different Pectinase concentrations on yield, total soluble solids, viscosity and turbidity of orange juice

Enzyme	Yield (%)	Total Soluble Solids ^{ns} (°Brix)	Viscosity ^{ns} (cps)	Turbidity (NTU)
Concentration (%)				
0.0	73 ^a ± 0.5	6.42 ± 0.02	9.2 ± 0.2	184 ^c ± 0.05
0.5	86 ^b ± 0.4	6.54 ± 0.04	5.24 ± 0.1	76 ^b ± 0.04
1.0	97 ^c ± 0.5	6.60 ± 0.07	4.63 ± 0.3	42 ^a ± 0.03
1.5	92 ^d ± 0.2	6.74 ± 0.04	4.58 ± 0.1	40 ^a ± 0.02
2.0	89 ^c ± 0.8	6.84 ± 0.04	4.51 ± 0.2	36 ^a ± 0.04

± Standard Deviation, n = 3.

^{ns} Not Significantly Different ($p > 0.05$).

Mean values having different superscript within column are significantly different ($p < 0.05$), n = 3

Table 3: Effect of different pectinase Concentrations on pH, ascorbic acid and total titratable acidity of orange juice

Enzyme Concentration (%)	pH ^{ns}	Ascorbic Acid ^{ns} (mg/100g)	Total Titratable Acidity (TTA)
0.0	3.80 ± 0.1	48.01 ± 1.0	0.38 ^a ± 0.02
0.5	3.61 ± .01	48.30 ± 0.3	0.47 ^b ± 0.01
1.0	3.55 ± 1.0	48.31 ± 1.0	0.44 ^b ± 0.04
1.5	3.55 ± 0.5	48.33 ± 0.4	0.46 ^b ± 0.03
2.0	3.54 ± 1.0	48.59 ± 1.0	0.45 ^b ± 0.03

± Standard Deviation, n = 3

^{ns} Not Significantly Different ($p > 0.05$).

Mean values having different superscript within column are significantly different ($p < 0.05$), n = 3