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Effectiveness of Pectinolytic Treatment on Clarification of *Averrhoa carambola* Juice

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Averrhoacarambola is an underutilized fruit crop. Its juice is rich in soluble sugar, vitamins, minerals, dietary fibers, antioxidant compounds but low fat and cholesterol free contributing to our health benefits. It has is sweet, slightly acidic, succulent and juicy with attractive pulp and unique aroma. Its juice is cloudy with colloidal suspension, and green in colour. Its juice clarity is a determinant indicator for consumer preference. The cloudiness can be handled by enzymatic depectinization. The application of pectinolytic enzyme plays an important role in extraction and clarification of *Averrhoacarambola* juice. The main purpose of this study was to achieve the best quality of *Averrhoacarambola* fruit juice by application of pectinase treatment. This demonstration was performed at various pectinase ratios (0.075 %, 0.1%, 0.125 %, 0.15%, 0.175% v/v), incubation times (30, 45, 60, 75, 90 minutes), and incubation temperatures (30, 33, 37, 40, 43ºC). Optimal parameters were based on extraction yield (%), viscosity (cP), turbidity (NTU), soluble dry matter (ºBrix), titrable acidity (g/100 ml). Our results proved that pectinase concentration, incubation time and temperature had significant influence to yield, viscosity, turbidity, soluble dry matter with an exception of titrable acidity. The recommened parameters of enzymatic treatment to clarify *Averrhoacarambola* juice were 0.15% pectinase, 75 minutes at 37ºC of incubation.

Keywords: Averrhoacarambola, pectinase, clarification, yield, viscosity, turbidity, soluble dry matter, titrable acidity

INTRODUCTION

Averrhoa carambola is one of the most popular tropical fruits in Vietnam. It has different shapes from oblong to ellipsoid (Siti et al., 2008). It's quickly perishable during storage under ambient temperature (Elizabeth et al., 2017). It is a potential source of raw material for processing into fruit juice to enhance its economic value. *Averrhoa carambola* fruit juice is naturally cloudy by a huge amount of polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metals (Vaillant et al., 2001). Pectin is a methoxylatedgalacturonic acid polymer providing integrity and rigidity to the plant tissues (Praveen and Suneetha, 2016). Pectin has been categorized as either soluble or insoluble fibre. The high concentration of pectin leads to colloid formation. It's the main reason causing turbidity during fruit juice production. It's very difficult to filter this juice to an acceptable clarity by traditional filtration (Harsh et al., 2014). Removal of pectin is an essential step in fruit juice processing. Pectinase has been widely exploited in fruit industry for juice extraction and clarification (Sobini et al., 2018; Cocok et al., 2017). It can successfully degrade pectin through hydrolysis mechanism. The pectinase hydrolyzes pectin to form pectin-protein complexes to flocculate. Pectinase treatment increases extraction yield, reducing sugars, soluble dry matter, galacturonic acid and titrable acidity while lowering viscosity and pectin content of the juice (Harsh et al.,

2014). Higher clarity, more concentrated flavour and colour in *Averrhoacarambola* juice through using commercial enzyme could be noticed apparently (Kaur et al., 2004; Abdullah et al., 2007). Enzyme concentration, incubation temperature and incubation time are three important physicochemical factors affecting to enzyme activity (Lee et al., 2006; Sin et al., 2006; Aponso et al., 2017). Objective of our study focused on various variables such as pectinase concentrations, incubation times, and incubation temperatures to obtain the best quality of *Averrhoa carambola* fruit juice.

MATERIALS AND METHODS

Material

Averrhoa carambola fruitswere collected in SocTrang province, Vietnam. After collecting, they must be kept in dry cool place and quickly conveyed to laboratory for experiments. They were subjected to washing, peeling, de-seedling, blending, filtering and enzymatic treatment. Pectinase enzyme (Pectinex Ultra SP-L) was bought from Novozymes Switzerland.

Researching method

Averrhoa carambola juice was treated by different pectinase concentrations (0.075 %, 0.1%, 0.125 %, 0.15%, 0.175% v/v), incubation times (30, 45, 60, 75, 90 minutes), and incubation temperatures (30, 33, 37, 40, 43ºC). The treated juice was heated at 95ºC for 3 minutes to deactivate pectinase. The juice was then centrifugated at 4000 rpm for 5 minutes to collect supernatant. The supernatant was then filtered to get clear fruit juice. Optimal parameters were based on extraction yield (%), viscosity (cP), turbidity (NTU), soluble dry matter (ºBrix), titrable acidity (g/100 ml).

Physico-chemical analysis

Yield(%) was evaluated as percentage of the clarified juice achieved based on the initial fruit pulp.Turbidity (NTU) of juice was determined using a portable Turbidimeter. Viscosity (cP) of juice was determined using a Brookfield viscometer. Titratable acidity (g/100mL) was determined by titration with 0.1 NaOH and 1 mL of phenolphthalein indicator until pink color. Total soluble solid (ºBrix) was measured by refractometer.

Statistical analysis

The experiments were run in triplicate with three different lots of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTSAND DISCUSSION

Yield (%) as well as soluble dry matter (ºBrix) of *Averrhoa carambola* juice increased when increasing pectinase concentration 0.15%, incubation time 75 minutes at 37ºC (see table 1-3). Increase in pectinase concentration and incubation time might decrease the turbidity of the *Averrhoa carambola* juice. The turbidity of the *Averrhoa carambola* juice decreased dramatically when pectinase concentration and incubation time increased (see table 1 and 2). The lowest turbidity was noticed at incubation temperature 37ºC (table 3). The viscosity of *Averrhoa carambola* juice decreased as the pectinase concentration and incubation time increasing as depicted in table 1 and 2. Optimal incubation temperature was recoreded at 37ºC (table 3).

Table 1: Effect of pectinase concentrations to physico-chemical quality of *Averrhoa carambola* juice

Pectinase (%)	0.075	0.100	0.125	**0.150**	0.175
Yield (%)	42.38±0.01^{c}	53.21±0.00^{b}	54.62±0.02^{ab}	56.01±0.03^{a}	56.12±0.01^{a}
Turbidity (NTU)	97±1^{a}	56±2^{b}	40±0^{bc}	28±3^{c}	26±1^{c}
Viscosity (cP)	1.96±0.03^{a}	1.90±0.01^{ab}	1.82±0.00^{b}	1.71±0.02^{bc}	1.58±0.03^{c}
Soluble dry matter (ºBrix)	24.17±0.03^{c}	24.54±0.00^{bc}	24.97±0.02^{b}	25.43±0.01^{ab}	25.79±0.00^{a}
Titratable acidity (g/100mL)	1.05±0.02^{a}	1.04±0.01^{a}	1.03±0.00^{a}	1.02±0.03^{a}	1.03±0.00^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2: Effect of incubation time (minutes) to physico-chemical quality of *Averrhoa carambola* juice

Incubation time (minutes)	30	45	60	**75**	90
Yield (%)	56.01±0.03^{c}	57.36±0.02^{bc}	58.72±0.01^{b}	59.45±0.02^{ab}	60.27±0.03^{a}
Turbidity (NTU)	28±3^{a}	24±1^{ab}	21±2^{b}	15±1^{bc}	12±0^{c}
Viscosity (cP)	1.71±0.02^{a}	1.62±0.03^{ab}	1.54±0.01^{b}	1.46±0.00^{bc}	1.40±0.01^{c}
Soluble dry matter (ºBrix)	25.43±0.01^{c}	26.02±0.02^{bc}	26.43±0.03^{b}	26.84±0.02^{ab}	26.97±0.01^{a}
Titratable acidity (g/100mL)	1.02±0.03^{a}	1.04±0.00^{a}	1.05±0.01^{a}	1.05±0.01^{a}	1.06±0.02^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3: Effect of incubation temperature (ºC) to physico-chemical quality of *Averrhoa carambola* juice

Incubation temperature (ºC)	30	33	**37**	40	43
Yield (%)	59.45±0.02^{c}	60.72±0.03^{bc}	62.54±0.02^{a}	61.93±0.01^{ab}	61.34±0.00^{b}
Turbidity (NTU)	15±1^{a}	11±2^{b}	8±1^{c}	9±2^{bc}	13±1^{ab}
Viscosity (cP)	1.46±0.00^{a}	1.32±0.01^{b}	1.21±0.03^{c}	1.27±0.03^{bc}	1.39±0.02^{ab}
Soluble dry matter (ºBrix)	26.84±0.02^{c}	27.13±0.00^{bc}	29.07±0.02^{a}	28.69±0.01^{ab}	27.93±0.00^{b}
Titratable acidity (g/100mL)	1.05±0.01^{a}	1.02±0.03^{a}	1.00±0.00^{a}	1.04±0.00^{a}	1.07±0.03^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Under enzymatic treatment, the hydrolysis of pectin caused a reduction of water holding capacity. Free water was released to the juice and its viscosity was reduced (Siti et al. 2008). *Averrhoacarambola* juice with lower viscosity was highly preferable in the enzymatic clarification (Sin et al., 2006). Pectinase decomposed pectin resulting to lower viscosity and cluster formation, therefore facilitating the suspended removeal centrifugation or filtration (Ivana et al., 2011). There was not significant difference of titratable acidity (g/100mL) in spite of pectinase concentration, incubation time and temperature (table 1-3). Our data were similar to other findings. Abdullah et al. (2007) reported that 0.10% of pectinase at 30°C for 20 min revealed significant modification to the clarified juice. In another report, Siti et al. (2008) concluded that 0.01% pectinase at 30 minutes of in 30ºC were optimal for clarifying *Averrhoacarambola* juice.

CONCLUSION

A limitation on the consumption of exotic *Averrhoacarambola* fruit is owing to its perishable nature. It's normally processed into fruit juice as an instant drink. The high concentration of pectin creates colloid formation which is responsible for the main problems during the processing of clear *Averrhoacarambola* fruit juice. Pectin degradation of *Averrhoacarambola* fruit juice through the application of pectinase has been proven as an efficient alternative to minimize turbidity. The resulting juice will have lower viscosity and minimal amount of pectin suitable for further filtration. In this research, we have successfully demonstrated that the cloudiness of *Averrhoacarambola* juice could be effectively removed by enzymatic depectinization.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Minh Phuoc Nguyen arranged the experiments and also wrote the manuscript.

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