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Enzymatic pectinase application in extraction and purification of juice turbidity from red rose apple pulp (*Syzygium malaccensis*)

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Abstract
The rose apple is widely cultivated in Mekong delta, Vietnam. The fruit has the shape and size of a small pear. Unripe rose apples have a bright green color. If ripe, the skin is rose-pink and waxy. The fruit is crispy, has a wooly texture and tastes like an apple. The liquid to flesh ratio of the wax apple is comparable to a watermelon. It's remarkably refreshing and juicy. We investigate the effectiveness of pectinases from two agents: Pectinex Ultra SP-L for extraction and Pectinex 3XL for clarification of rose apple juice from its pulp. Optimal parameters are as follows: Pectinex Ultra SP-L ratio 0.04%, temperature 30 ^{o}C in 60 minutes for rose apple pulp extraction; Pectinex 3XL ratio 0.03%, temperature 40 ^{o}C in 80 minutes for rose apple juice clarification.

Keywords: *rose apple juice, pectinase, extraction, clarification*

1. Introduction
The use of enzymes in juice industry has contributed in increasing the yield and production of various types of juices. Enzymatic processing makes the juice not only clear by breaking down the pectin and allowing the suspended particles to settle down, but also eliminate undesirable changes in colour, bouquet and stability. Pectic enzymes are also helpful in other processes such as in the manufacture of fruit purees, wine clarification, deskinning of orange segments (Tapre et al., 2014). The rose apple (*Syzygium malaccensis*, (L.) Merrill & Perry), family Myrtaceae, is native to Southeast Asia. Different parts of the plant such as seeds, bark, fruit and leaves have been used in traditional medicine (Savitha, Padmavathy and Sundhararajan, 2011). This plant has become well adapted to Vietnam, particularly in the Mekong river delta. Fruits measure 4-7 cm of diameter. The fruits have a high percentage of pulp, containing an enticing aroma and sweet taste, slightly acidic, and these are most often used for juice, jellies and jams (Guedes et al., 2004).

Fig 1: Rose apple (*Syzygium malaccensis)*

According to Costa et al. (2006) the fruit rose apple contains vitamins A, B1, B12, along with calcium, iron and phosphorus. The according Augusta et al. (2010) rose apple presents a peel of high-fiber content 9.34 g.100 g-1, lipids 4.51 g.100 g-1, carbohydrate 59.25 g.100 g-1 and 8.62 g.100 g-1 protein, contributing an energy value of 312.07 g kcal.100-1 . Regarding the amounts of vitamin C (292.59 mg.100g-1) and anthocyanins (300.54 mg. 100g-1) are high and, as in most of the fruits of the Myrtaceae concentrated in greater proportion the bark. Pectinase is an enzyme that breaks down pectin. Pectin is one of the compounds found in plant cell walls; it is in the plate (middle lamella) that is the first part of the wall to be formed during cytokinesis, following cell division.

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Pectinases are one of the upcoming enzymes of fruit industry. These enzymes break down complex polysaccharides of plant tissues into simpler molecules like galacturonic acids. The role of acidic pectinases in bringing down the cloudiness and bitterness of fruit juices is well established (Hassan K. Sreenath et al., 1994; Tung-Sun Chang et al., 1994; Bitange Nipa Tochi et al., 2009; Manuel Pinelo et al., 2010; K. Mehraj Pasha et al., 2013, Shefali Srivastava and Sudhir Kumar Tyagi t al., 2013; Robin et al., 2013).

Pectins contribute to fruit juice viscosity and turbidity. A mixture of pectinases and amylases is used to clarify fruit juices. It decreases filtration time up to 50% (Blanco et al., 1999). Treatment of fruit pulps with pectinase also showed an increase in fruit juice volume from banana, grapes and apples (Kaur and Kumar, 2004). Pectinases in combination with other enzymes, viz., cellulases, arabinases and xylanases, have been used to increase the pressing efficiency of the fruits for juice extraction (Gailing et al., 2000). Vacuum infusion of pectinases has a commercial application to soften the peel of citrus fruits for removal. This technique may expand in future to replace hand cutting for the production of canned segments (Baker and Wicker, 1996). Infusion of free stone peaches with pectin methylesterase and calcium results in four times firmer fruits. This may be applied to pickle processing where excessive softening may occur during fermentation and storage (Baker and Wicker, 1996).

Apple juice is manufactured as natural, unfiltered and unclarified, juice containing a high percentage of pulp; as a hazy juice that has been centrifuged to remove coarse particles but not filtered; and finally as filtered clear and amber colored juice prepared by enzymatic treatment (Kilara,1982). Although pectinases that can depolymerize highly esterified pectin are the major types of enzymes used in apple juice processing, they are by no means the only enzymes used for this purpose. A combination of pectinases and cellulases has been reported to give a juice yield up to 100% (Alkorta et al., 1998). Another potential contributor to the haziness is starch. Unripe apples may contain up to 15% starch. This can be broken down using an amylase which is active at the pH of apple juice and added at the same time as the pectinases.

Grape juice is not consumed in large amounts because it is too sweet (about 200 g l-1 sugars) or too acid (up to 10 g l-1 tartaric acid). It is mixed with other fruit juices such as apple and the technology of grape juice processing has been reviewed (Pederson, 1980; Luh and Kean, 1975). With the high pectin content (5±10 g l-1) grapes are difficult to crush and to press. They are de-stemmed, crushed and heated to 60°C or 80°C to release color (in case of black grapes) from the skins and destroy the endogenous polyphenoloxidase. Then enzymes such as Cytolase PCL5 (Gist -Brocades) or Ultrazyme (Novo Nordisk) are added at approximately 50 g ton-1 to macerate the berries and increase the yield. The free running juice is separated from the solids by different kinds of filters (rotary vacuum, earth) and/ or by centrifugation. The filtered juice is cooled to 0°C to prevent fermentation and then depectinized, at approximately 200 ppm over about two weeks. Pectinases, mainly PG and hemicellulases like arabino-galactanases, allow insoluble particles to flocculate. At the same time, cold storage of the juice encourages removal of tartarate and reduces the total acidity to acceptable levels. The juice is then filtered with diatomaceous earth and concentrated, pasteurized and bottled.

The production of clear juices and concentrates from strawberries, raspberries or blackberries requires enzymatic depectinization. The juices from these fruits contain high amounts of pectin that remains as colloidally dissolved residue' making the juices viscous (Will and Dietrich, 1992). The clarification, filtration, and concentration of these juices therefore become difficult. These residual pectins and hemicelluloses also bind to phenolic substances and protein during the processing and storage and result in the formation of irreversible complexes that enzymes cannot breakdown. An additional problem is contamination of strawberries and raspberries by B. cinerea (Grassin and Fauquembergue, 1996). This fungus secretes a b-1, 3±1, 6-linked glucan in the berries,which forms gum and hence reduces the filter ability and the clarity of the juice. These glucans can be hydrolized by b-glucanases.

Purpose of our study is to investigate the enzymatic pectinase application in extraction and purification of juice turbidity from rose apple pulp (*Syzygium malaccensis*).

2. Material & Method

2.1 Material

2.1.1 Rose apple fruits

Rose apple fruits are purchased in Tra Vinh local market, Vietnam. Then we wash them in clean water; remove their core before grinding into pulp readry for extraction and clarification. All all fruits used during the experiments are kept in 8-10 ^{o}C in clean dry cool place.

2.1.2 Enzyme reagents

We use two pectinase enzyme reagents: Pectinex Ultra SPL Novozymes for extraction and Pectinex 3XL Novozymes for clarification. All of them are originated from Denmark.

2.1.3 Yeast

Saccharomyces cerevisiae is supplied from Pasteur Institute, HCM City, Vietnam

2.2 Research content

2.2.1 Optimal conditions for pectinases in juice extraction and clarification

-Effect of time, temperature and enzyme concentration to juice extraction by Pextinex Ultra SP-L

- Effect of time, temperature and enzyme concentration to juice clarification by Pextinex 3XL

2.2.2 Criteria in raw juice extracted by enzyme

- Transparence of juice extract
- Total soluble solid, TSS (0Bx)
- Total acidity (g/l)
- Sensory

2.2.3 Criteria in rose apple juice beverage

- Ethanol (% v/v)
- Residual sugar (g/l)
- Total acidity (g/l)
- Sensory

2.3 Experiment arrangement

2.3.1 Effect of time, temperature and enzyme concentration to juice extraction by Pextinex Ultra SP-L

2.3.1.1 Effect of enzyme supplementation

Enzyme *Pextinex Ultra SP-L* are supplemented with different ratio: CT1: 0%; CT2: 0.02%; CT3: 0.03%; CT4: 0.04%; CT5: 0.05%; CT6: 0.06%.

2.3.1.2 Effect of temperature to enzyme activity

We investigate different temperature levels: CT1: 20 ^{0}C; CT2: 25 ^{o}C; CT3: 30 ^{o}C; CT4: 35 ^{o}C; CT5: 40 ^{o}C.

2.3.1.3 Effect of time to enzyme activity
We investigate different time levels: CT1: 30 minutes; CT2: 40 minutes; CT3: 50 minutes; CT4: 60 minutes; CT5: 70 minutes.

2.3.2 Effect of time, temperature and enzyme concentration to juice clarification by Pextinex 3XL
2.3.2.1 Effect of enzyme supplementation
Enzyme *Pextinex Ultra SP-L* are supplemented with different ratio: CT1: 0%; CT2: 0.01%; CT3: 0.02%; CT4: 0.03%; CT5: 0.04%.

2.3.2.2 Effect of temperature to enzyme activity
We investigate different temperature levels: CT1: 30 ^{0}C; CT2: 35 ^{o}C; CT3: 40 ^{o}C; CT4: 45 ^{o}C; CT5: 50 ^{o}C.

2.3.2.3 Effect of time to enzyme activity
We investigate different time levels: CT1: 20 minutes; CT2: 40 minutes; CT3: 60 minutes; CT4: 80 minutes; CT5: 100 minutes.

2.4 Research method
Determination of soluble dry matter by refractometer
Determination of total acidity by neutralization
Determination of juice transparency by the transparency degree (%) by UV-VIS at wave length 420, 520 and 620 nm
Determination of tannin by $KMNO_4$ titration
Determination of pectin by Cancium pectate method
Determination of reduced sugar by acid dinitrosalicylic
Determination of Vitamin C by iodua titration
Determination of extraction recovery by comparision of filtrate weight and product weight
Determination of color by UV-VIS at wave length 520 nm
Sensory evaluation by TCVN 3215-79

2.5 Statistical analyses
Use Microsoft Excel 2003 at 95% confidence level.

3. Result & Discussion
3.1 Raw rose apple quality

Table 1: Raw rose apple quality

Criteria	Value
Moisture (%)	82.5±1.2
Soluble dry matter (^{o}Brix)	12.5±0.1
Reduced sugar (g/100g)	7.36±0.02
Total acidity (g/100g)	10.18±1.1
Organic acid (%)	0.41±0.13
Vitamin C (mg/l)	1.82±0.01
Tanin (%)	0.122±0.002
Juice pH	4.0±0.001
Pectine (%)	0.40±0.001

From table 1 above, soluble dry matter in rose apples is 17.5%; sugar 10.18g/100g; total acidity 0.41%; vitamin C 1.82 mg/l. Pectin content (0.4%) in this juice is quite high which hinders the extraction and clarification.

3.2 Effect of enzyme ratio, temperature and time to rose apple juice extraction recovery by enzyme PECTINEX ULTRA SP-L
3.2.1 Effect of enzyme ratio to rose anpple juice extraction recovery
We investigate enzyme activity from 0.02% to 0.06%

Table 2: Effect of enzyme ratio application to rose apple juice extraction

Sample	Enzyme ratio (%)	Recovery (%)	TSS (^{o}Brix)
1 (control)	0	75.2^a	12.5
2	0.02	80.2^b	12.7
3	0.03	82.5^c	13.0
4	0.04	85.8^d	14.1
5	0.05	85.9^d	14.2
6	0.06	86.0^d	14.3

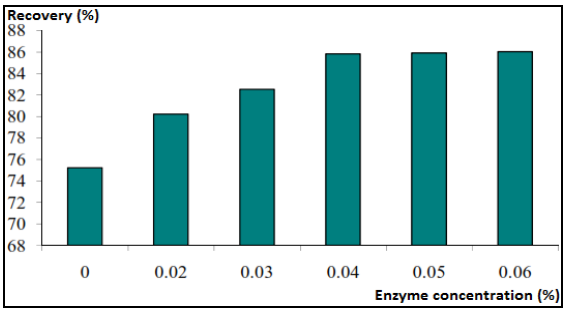

Fig 2: Effect of enzyme ratio (Pectinex Ultra SP-L) to juice recovery

At 0.04%, juice recovery is highest at 85.8% with soluble dry matter 14.1^{o}Bx. SO we choose this value for further experiments.

3.2.2 Effect of temperature to enzyme activity (Pectinex Ultra SP-L)

Table 3: Effect of temperature to enzyme activity (Pectinex Ultra SP-L)

Sample	Temperature (°C)	Juice recovery (%)	TSS (°Brix)
1	20	81.4[b]	13.4
2	25	82.5[c]	13.5
3	30	85.5[d]	14.1
4	35	80.1[a]	13.1
5	40	80.0[a]	13.0

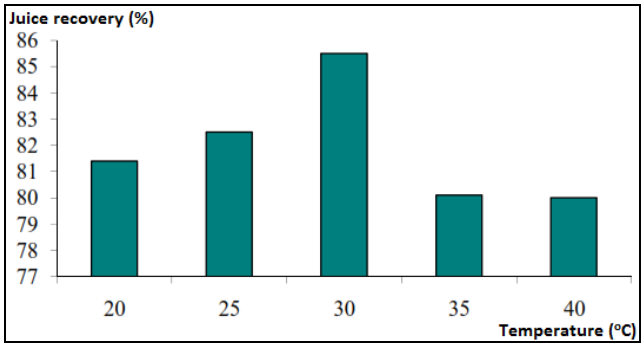

Fig 3: Effect of temperature (Pectinex Ultra SP-L) to juice recovery

At 30°C, we get the highest juice recovery 85.5% with soluble dry matter 14.1 °Brix. We choose this temperature for further experiments.

3.2.3 Effect of treatment time to juice recovery

Table 4: Effect of treatment time to juice recovery

Sample	Treatment time (minutes)	Juice recovery (%)	TSS (oBrix)
1	30	81.2[a]	13.1
2	40	82.4[a]	13.4
3	50	83.2[b]	13.9
4	60	85.7[c]	14.2
5	70	85.8[c]	13.3

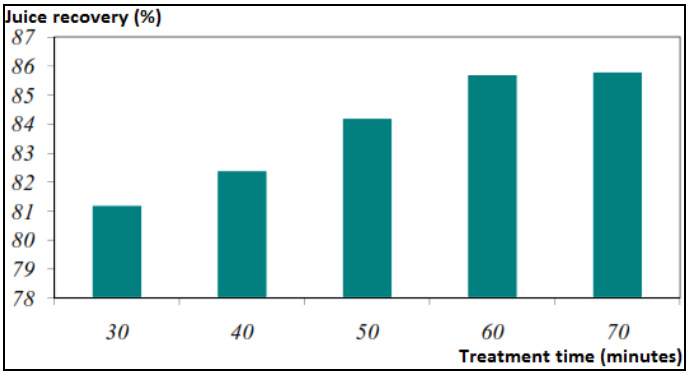

Fig 4: Effect of treatment time (Pectinex Ultra SP-L) to juice recovery

At the treatment time 60 minutes, we get the highest soluble dry matter 14.2 °Brix, juice recovery 85.7%. So we choose this value for further experiments. When we compare enzyme treatment (Pectinex Ultra SP-L, 0.04% enzyme, temperature 30 °C, time 60 minutes) and control (non enzyme treatment).

Table 5: Effect of enzyme treatment and non-enzyme treatment to juice recovery

Sample	Recovery	TSS (°Brix)
Non-enzymatic treatment	75.2	12.5
Enzymatic treatment	85.6	14.1

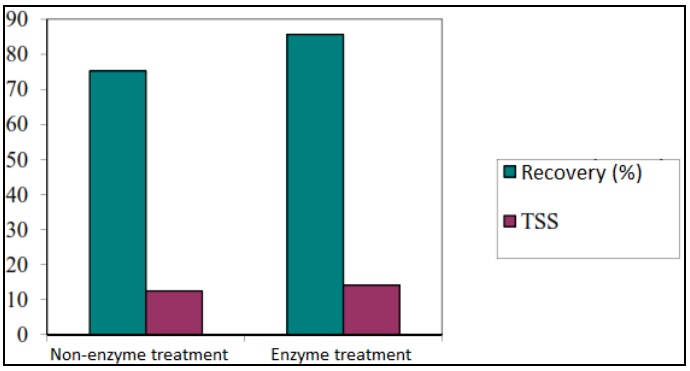

Fig 5: Effect of enzyme treatment and non-enzyme treatment to juice recovery

From table 5 and figure 5, using appropriate enzyme Pectinex Ultra SP-L increases rose apple juice recovery 10.4% (from 75.2% to 85.6%). TSS also increases 1.6% from 12.5 ^{o}Brix to 14.1 ^{o}Brix.

3.3 Effect of enzyme ratio, temperature and time to rose apple juice purification capability by enzyme PECTINEX 3XL

3.3.1 Effect of enzyme ratio (Pectinex 3XL) to juice transparence

We investigate different enzyme ratio from 0% (control) to 0.04%. Results are as follows:

Table 6: Effect of enzyme ratio (Pectinex 3XL) to juice transparency

Sample	Enzyme ratio (%)	Transparence, T (%)
1 (control)	0	67.2[a]
2	0.01	75.4[b]
3	0.02	80.7[c]
4	0.03	90.2[d]
5	0.04	90.3[d]

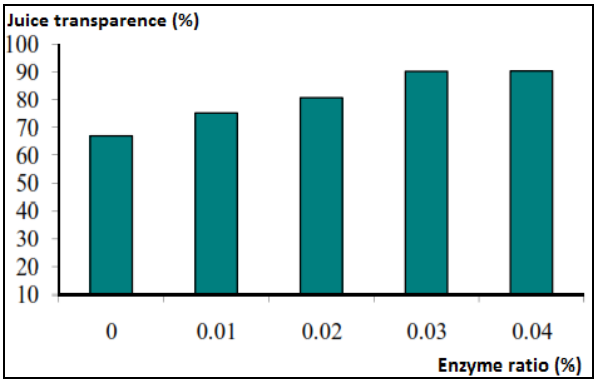

Fig 6: Effect of enzyme ratio (Pectinex 3XL) to juice transparence

In table 6 and figure 6, Pectinex 3XL application has positively impacted juice transparency compared to non-enzyme treatment samples, especially at 0.03% enzyme. This value is not significantly different to 0.04% treated enzyme so we choose 0.03% enzyme for further experiments.

3.3.2 Effect of temperature to to juice transparen by enzyme Pectinex 3XL

We investigate the effect of different treatment temperatures (from 30oC to 50oC) to juice transparency capability.

Table 7: Effect of temperature to to juice transparence by enzyme Pectinex 3XL

Sample	Temperature (^{o}C)	Transparence, T (%)
1 (control)	30	83.7[a]
2	35	86.8[b]
3	40	89.6[c]
4	45	86.4[d]
5	50	86.3[d]

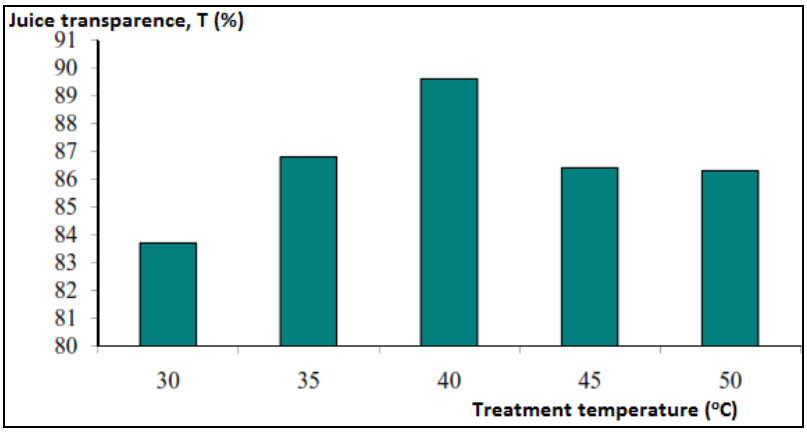

Fig 7: Effect of temperature to juice transparency by enzyme Pectinex 3XL

When increasing temperature from 30°C to 40°C, juice transparence also increase from 83.7% to 89.6%, especially at 40oC with the optimal transparency (89.6%). If continue increasing temperature, juice transparency will be reduced owing protein denaturation. So 40oC is selected for juice clarification by enzyme Pectinex 3XL.

3.3.3 Effect to treatment time to juice transparency by enzyme Pectinex 3XL

We investigate different treatment times from 20 minutes to 100 minutes, Clarification results are as follows:

Table 8: Effect to treatment time to juice transparency by enzyme Pectinex 3XL

Sample	Treatment time (minutes)	Transparence, T (%)
1 (control)	20	83.6[a]
2	40	85.4[b]
3	60	85.5[c]
4	80	89.8[d]
5	100	89.7[d]

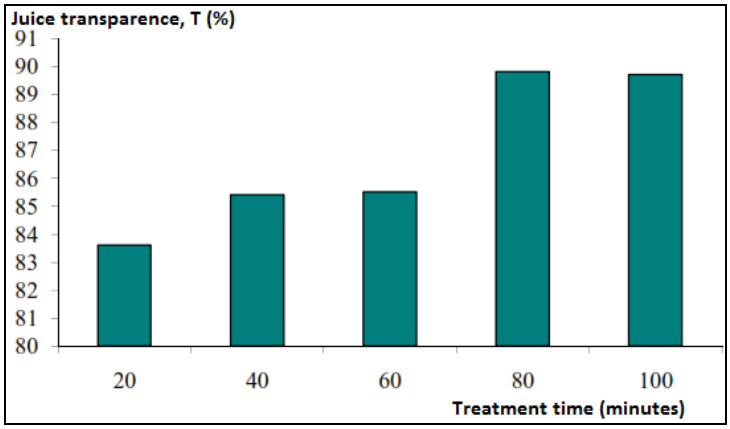

Fig 8: Effect to treatment time to juice transparency by enzyme Pectinex 3XL

The longer treatment time is, the more juice clarification effectiveness we get. At 80 minutes the highest juice transparency 89.8% is noticed. If continue increasing treatment time to 100 minutes, there is not significantly different in juice transparence. After finding optimal parameters affected to enzyme activity Pectinex 3XL, we demonstrate a comparison between Pectinex treatment and non-enzymatic treatment to juice traspararence. Results are as follows.

Table 9: Effec of enzyme Pectinex 3XL treatment to juice transparence

Sample	Transparence, T (%)	Turbidity
Non-enzymatic treatment	60.1	5.152.
Enzymatic treatment	89.8	6.815

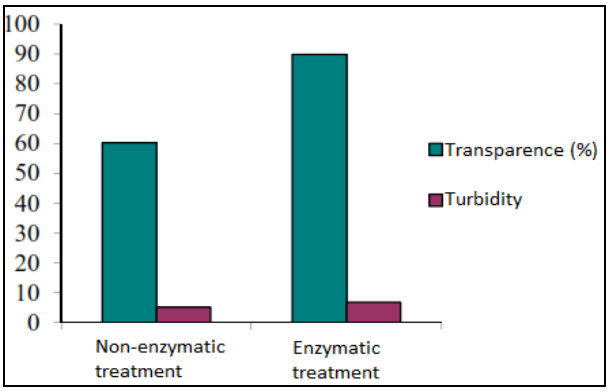

Fig 9: Effect of enzyme Pectinex 3XL to juice transparence

From table 9 and figure 9, juice transparence in the enzymatic treatment increases 29.7%, for enzymatic treatment 89.8% higher than non-enzymatic treatment 60.1%.

4. Conclusion

Increased awareness in health issue leads to increasing in consumption of fruit juices and other natural products as an alternative to the traditional caffeine containing beverages such as coffee, tea or carbonated soft drinks. Pectinolytic enzymes are one of the important groups of enzymes used in fruit processing industry. The pectinase degrades polysaccharide materials in the rose apple juice into smaller fractions like galacturonic acids thus facilitating extraction. The subsequent application of the pectinase in the clarification of rose apple juice would enhance the production of clarified fruit juice in the tropics.

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