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RESEARCH ARTICLE

Synergistic Effect of Pectinolytic and Proteolytic Enzymatic Clarification on Cantalopue Juice

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Abstract

Cantaloupe is consumed worldwide thanks to its sweetness, juicy taste, pleasing flavor, and nutritional value. It's highly perishable under ambient condition if fresh consumption cannot move quickly. It's urgent to process this therapeutic fruit in industrial scale. In fact, it can be processed into different products including functional juice. Clarity in fruit juices is desirable to make it useful in international standard**.** Objective of this study focused on the effectiveness of various processing variables affecting to cantaloupe juice turbidity and clarity. In our research, a mixture of pectinase and protease in different concentration (0.03%:0.07%, 0.04%: 0.06%, 0.05%:0.05%, 0.06%:0.04%, 0.07%:0.03%), holding temperature (26, 28, 30, 32, 34^{o}C), holding time (45, 60, 75, 90, 105 min), pH (4.3, 4.5, 4.7, 4.9, 5.1), agitation (250, 500, 750, 1000, 1250 rpm) were examined. Carotenoid (µg/g), flavonoid (g/L), turbidity (NTU) and viscosity (cP) were key indicators to determine the optimal treatment. Our results showed cantaloupe fruit juice would have the best carotenoid, flavonoid, clarity as well as viscosity by treatment of pectinase: protease ratio concentration 0.06%: 0.04%, temperature 20^{o}C in 75 min at pH 4.7 with agitation 1000 rpm. Synergistic effect of the combined usage of pectinase and protease enzymes was clearly demonstrated. From this finding, the added value of cantaloupe juice would be improved in storage as well as commercial distribution without any worry about haze cloud.

Keywords: *Cantaloupe, Juice, Pectinase, Protease, Carotenoid, Flavonoid, Turbidity, Viscosity.*

Introduction

Conventionally, immediate turbidity is presumed to be caused by pectin, while turbidity development during cold storage (haze formation) is assumed to be due to protein–phenol interactions [1, 2]. Pectinase enzyme has been utilized to depectinize various fruit juices including apple, orange, mosambi, banana, lemon, mango, pineapple, grape, guava, papaya and date syrup [3]. It flocculates pectin by making its complex with proteins, thereby, the resulting juice exhibits lesser viscosity which ultimately enhances the filtration, flavor and color. Partial hydrolysis of the negatively charged coat leads to the exposure of positively charged surface and attraction between unlike charged particles leads to flock formation [4].The rate of enzymatic hydrolysis depends on several physical-chemical factors such as incubation time, temperature and enzyme concentration [5]. The pectinase concentration has an important effect on the linear and the quadratic effects of clarity because it is dependent on the enzyme. Enzymatic reaction rate is increased by the temperature, as well as the clarification rate, as for the enzyme until it is below the denaturation temperature [6].Gelatin is used as fining agent during juice processing. Gelatin forms gelatin-tannin complexes thereby adding to clarification. Juice containing both gelatin and pectinase enzyme was about 1.5- to 2-times more clarified in same time of incubation as compared to that containing enzyme alone [4]. The addition of pectinases to the juice reduces its viscosity, improving the press ability of the pulp, disintegrating the jelly structure and increasing the fruit juice yields [7].Enzymatic degradation of fruit juice depends upon the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations [8]. Cantalpoue (*Cucumis melo* L.), belongs to the Cucurbitaceae family, and it is well recognized for culinary and medicinal purposes [9]. The cantaloupe is well recognized by its net-like slightly ribbed, gray-to-green or light brown skin. Cantaloupe is an excellent source of phytonutrients such as carotenoid, flavonoid, vitamin C, lutein, beta-cryptoxanthin and zeaxanthin, microelements and other bioactive compounds contributing to variety of pharmaceutical properties such as analgesic, anti-inflammatory, antioxidant, antiulcer, anticancer, antimicrobial, diuretic, and anti-diabetic, hepato-protective [10, 17]. Cantaloupe can be processed into various forms such as juice, wine, fresh-cut, yogurt, etc [18, 21]. There were not many studies mentioned to combination of pectinase and protease for clarification of fruit juice. A significant and synergistic effect of the combined use of pectinase and protease enzymes in terms of turbidity and potential haze formation of the pomegranate juice was revealed [22].

One research developed a tailored pectinolytic and proteolytic enzymatic clarification process for pomegranate juice [23].Therefore, objective of this our study penetrated on the effectiveness of various processing variables such as pectinase and protease ratio concentration, holding temperature, holding time, pH, agitation that were able to affect to some physico-chemical charateristics of cantaloupe fruit juice.

Materials and Method

Material

Cantaloupe fruits were collected from Vinh Long province, Vietnam. After collecting, they must be quickly conveyed to laboratory for experiments. They were subjected to washing and treatment. The fruits were peeled and pulped by a juice extractor and seeds were discarded. Pulp (25 g) was mixed with 50 mL distilled water and pectinase enzyme in a 100 mL Erlenmeyer flask, placed in an incubator. Different parameters of pectinase:protease enzyme concentration (0.03%:0.07%, 0.04%: 0.06%, 0.05%:0.05%, 0.06%:0.04%, 0.07%:0.03%), holding temperature (26, 28, 30, 32, 34^{o}C), holding time (45, 60, 75, 90, 105 min), pH (4.3, 4.5, 4.7, 4.9, 5.1), agitation (250, 500, 750, 1000, 1250 rpm) were examined. At the end each treatment, pectinase and protease enzyme were deactivated at 95^{0}C in 2.5 minutes. Clear fruit juice was collected by filtration and ready for testing. Pectinase and protease were all purchased from Sigma Aldrich.

Researching Procedure

Effect of Pectinase: Protease Ratio during Pectinolytic and Proteolytic Enzymatic Juice Clarification

Raw cantaloupe fruit juice samples were treated with different pectinase: protease ratio concentration (0.03%:0.07%, 0.04%: 0.06%, 0.05%:0.05%, 0.06%:0.04%, 0.07%:0.03%), at 36^{o}C within 45 min at pH 4.3. At the end of treatment, all samples were pasteurized at 95^{0}C in 2.5 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Carotenoid (µg/g), flavonoid (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of Holding Temperature during Pectinolytic and Proteolytic Enzymatic Juice Clarification

Raw cantaloupe fruit juice samples were incubated with different temperature (26, 28, 30, 32, 34^{o}C) with 0.06% pectinase:0.04% protease within 45 min at pH 4.2. At the end of treatment, all samples were pasteurized at 95^{0}C in 2.5 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Carotenoid (µg/g), flavonoid (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of Holding Time during Pectinolytic and Proteolytic Enzymatic Juice Clarification

Raw cantaloupe fruit juice samples were incubated in different duration (45, 60, 75, 90, 105 min) with 0.06% pectinase: 0.04% protease in 30^{o}C at pH 4.3. At the end of treatment, all samples were pasteurized at 95^{0}C in 2.5 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Carotenoid (µg/g), flavonoid (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of pH during Pectinolytic and Proteolytic Enzymatic Juice Clarification

Raw cantaloupe fruit juice samples were incubated in different pH values (4.3, 4.5, 4.7, 4.9, 5.1) with 0.06% pectinase: 0.04%

protease at 30°C within 75 min. At the end of treatment, all samples were pasteurized at 95°C in 2.5 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Carotenoid (µg/g), flavonoid (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Effect of Agitation during Pectinolytic and Proteolytic Enzymatic Juice Clarification

Raw cantaloupe fruit juice samples were incubated in different agitation speed (250, 500, 750, 1000, 1250 rpm) with 0.06% pectinase: 0.04% protease at 30°C within 75 min in pH 4.7. At the end of treatment, all samples were pasteurized at 95°C in 2.5 minutes to deactivate pectinase. Clear fruit juice was collected by filtration and ready for testing. Carotenoid (µg/g), flavonoid (g/L), turbidity (NTU) and viscosity (cP) were important indicators so they were chosen to identify the optimal treatment.

Physico-chemical, Sensory and Statistical analysis

Carotenoid content (µg/g) was analyzed by HPLC. Flavonoid (g/L) was quantified using aluminum chloride colorimetric method. Turbidity (expressed as Nephelometric Turbidity Units, NTU) was measured using a turbid-meter. Viscosity (cP) was analyzed by viscometer. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Statgraphics Centurion XVI.

Result & Discussion

Effect of Pectinase: Protease Ratio during Pectinolytic and Proteolytic Enzymatic Juice Clarification

The advantage of using two enzymes in combination is revealed by means of synergistic enzymatic activities (pectinolytic and proteolytic). This is due to the pectinolytic activity that degrades the pectin chains, thus exposing positively-charged proteins. Secondly, proteolytic activity breaks down haze-active proteins, thus preventing new protein-phenol interaction [23]. In our research, raw cantaloupe fruit juice was treated by different pectinase: protease ratio concentration (0.03%:0.07%, 0.04%: 0.06%, 0.05%:0.05%, 0.06%:0.04%, 0.07%:0.03%).

Our result showed that the optimal pectinase: protease ratio concentration should be 0.06%:0.04% to achieve the best quality of cantaloupe fruit juice (see Table 1).

Table 1: Effect of pectinase concentration (%) to quality of cantaloupe fruit juice

Pectinase: Protease ratio concentration	0.03%: 0.07%	0.04%: 0.06%	0.05%: 0.05%	0.06%: 0.04%	0.07%: 0.03%
Carotenoid (µg/g)	68.43±0.01[c]	74.12±0.03[bc]	77.49±0.00[ab]	82.04±0.03[a]	76.25±0.00[b]
Flavonoid (g/L)	3.75±0.03[c]	4.33±0.01[bc]	4.97±0.02[ab]	5.46±0.00[a]	4.67±0.01[b]
Turbidity (NTU)	2.15±0.02[a]	2.06±0.02[b]	1.97±0.00[bc]	1.73±0.01[c]	2.10±0.03[ab]
Viscosity (cP)	2.13±0.00[a]	2.05±0.00[ab]	1.94±0.01[bc]	1.82±0.02[c]	1.99±0.01[b]

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%)

In one report, apple juice was clarified by fungal pectinolytic enzyme and gelatin, the most effective clarification was achieved with 15 IU/ml of enzyme preparation, in presence of 0.01% gelatin, at 45°C in 6 hrs of holding time [4]. In another research, enzymatic clarifications of pressed cherry juice were evaluated by supplementation of pectinase and protease. The protease treatment resulted in significant reduction of immediate turbidity, but had low clarification impact during the subsequent cold storage. In contrast, pectinase addition exerted a weak effect on immediate turbidity reduction, but effectively decreased the turbidity development during storage [2]. One research developed a tailored pectinolytic and proteolytic enzymatic clarification process for pomegranate juice. The optimum enzymatic treatment conditions were: temperature 25-30°C, time 100-110 min, protease-pectinase complex enzyme amount 0.22-0.25 g/100 g of pomegranate juice [23].

Effect of Holding Temperature in Pectinolytic and Proteolytic Enzymatic Juice Clarification

Chill haze is a reversible turbidity and it is soluble when the liquid returns to room temperature [23]. In our research, raw cantaloupe fruit juice was treated by pectinase: protease ratio concentration 0.06%:0.04% in different holding temperature (26, 28, 30, 32, 34°C). Our result showed that the optimal pectinase: protease treatment holding temperature should be conducted at

30°C to achieve the best quality of cantaloupe fruit juice (see Table 2).

Table 2: Effect of holding temperature (°C) in complex enzymatic entreatment to quality of cantaloupe fruit juice

Holding temperature (°C)	26	28	30	32	34
Carotenoid (µg/g)	82.04±0.03[bc]	85.27±0.00[ab]	87.55±0.03[a]	84.92±0.02[b]	81.84±0.02[c]
Flavonoid (g/L)	5.46±0.00[c]	5.63±0.03[b]	5.86±0.01[a]	5.74±0.03[ab]	5.59±0.01[bc]
Turbidity (NTU)	1.73±0.01[a]	1.66±0.02[ab]	1.32±0.00[c]	1.49±0.01[bc]	1.63±0.03[b]
Viscosity (cP)	1.82±0.02[a]	1.54±0.00[bc]	1.40±0.02[c]	1.59±0.00[b]	1.71±0.02[ab]

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%)

Effect of Holding Time in Pectinolytic and Proteolytic Enzymatic Juice Clarification

Holding time had a direct impact on the activity of enzyme. In our research, raw cantaloupe fruit juice was treated by pectinase: protease ratio concentration 0.06%:0.04% at holding temperature 30°C by different time (45, 60, 75, 90, 105 min). Our result showed that the optimal pectinase: protease treatment holding time should be last for 75 min to achieve the best quality of cantaloupe fruit juice (see Table 3).

Table 3: Effect of time (min) in complex enzymatic entreatment to quality of cantaloupe fruit juice

Holding time of treatment	45	60	75	90	105
Carotenoid (µg/g)	87.55±0.03[b]	88.13±0.03[ab]	89.24±0.04[a]	89.27±0.02[a]	89.28±0.01[a]
Flavonoid (g/L)	5.86±0.01[b]	5.93±0.02[ab]	5.98±0.04[ab]	6.00±0.00[a]	6.00±0.02[a]
Turbidity (NTU)	1.32±0.00[a]	1.26±0.01[ab]	1.19±0.01[b]	1.08±0.01[bc]	1.06±0.03[c]
Viscosity (cP)	1.40±0.02[a]	1.31±0.00[ab]	1.25±0.02[b]	1.19±0.03[bc]	1.17±0.00[c]

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%)

Effect of pH in pectinolytic and proteolytic enzymatic Juice Clarification

In our research, raw cantaloupe fruit juice was treated by pectinase: protease ratio concentration 0.06%:0.04% at holding temperature 30°C within 75 min by different pH values (4.3, 4.5, 4.7, 4.9, 5.1). Our result showed that the optimal pectinase: protease treatment holding time should be emphazied at pH 4.7 to achieve the best quality of cantaloupe fruit juice (see Table 4).

Table 4: Effect of pH in complex enzymatic entreatment to quality of cantaloupe fruit juice

pH of treatment	4.3	4.5	4.7	4.9	5.1
Carotenoid (µg/g)	89.24±0.04[b]	91.12±0.02[ab]	91.46±0.02[a]	88.49±0.03[c]	87.34±0.01[d]
Flavonoid (g/L)	5.98±0.04[c]	6.13±0.03[bc]	6.48±0.01[a]	6.25±0.02[ab]	6.18±0.00[b]
Turbidity (NTU)	1.19±0.01[a]	1.12±0.01[b]	1.04±0.03[c]	1.09±0.03[bc]	1.17±0.00[ab]
Viscosity (cP)	1.25±0.02[a]	1.19±0.00[b]	1.13±0.04[c]	1.16±0.00[bc]	1.23±0.04[ab]

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%)

The decrease in pH was directly related to increasing pectinase and protease concentration [24].

Effect of Agitation during Pectinolytic and Proteolytic Enzymatic Juice Clarification

Raw cantaloupe fruit juice samples were incubated in different agitation speed (250, 500, 750, 1000, 1250 rpm) with 0.06% pectinase: 0.04% protease at 30°C within 75 min in pH 4.7. Our result showed that the agitation was adequate at 1000 rpm to achieve the best quality of cantaloupe fruit juice (see Table 5).

Table 5: Effect of agitation in complex enzymatic entreatment to quality of cantaloupe fruit juice

Agitation (rpm)	250	500	750	1000	1250
Carotenoid (µg/g)	91.46±0.02[b]	92.36±0.01[ab]	92.43±0.02[a]	92.46±0.01[a]	92.47±0.02[a]
Flavonoid (g/L)	6.48±0.01[b]	6.57±0.03[ab]	6.94±0.01[a]	6.97±0.01[a]	6.99±0.01[a]
Turbidity (NTU)	1.04±0.03[a]	0.98±0.00[ab]	0.93±0.03[b]	0.92±0.02[b]	0.92±0.02[b]
Viscosity (cP)	1.13±0.04[a]	1.09±0.02[ab]	1.02±0.00[b]	1.02±0.00[b]	1.01±0.03[b]

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%)

The addition of enzyme complex can result in improved yield for the juice and in a better extraction for colour, with the outcome being a high-end product [25].

Conclusion

Cantaloupe is a round melon enclosing moderately sweet orange flesh with good nutritional attributes. Production of juice from cantaloupe fruit could help reduce the level of post-harvest loss and increases variety of juices. Enzymatic treatment for juice extraction offers a number of advantages over mechanical-thermal process of fruit juice. The use of complex enzymes can enhance yield and help in the clarification of this fruit juice.

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