The Effect of Pectinase, Cellulase, and Chitosan Combination in Lemon Juice Clarification *(Citrus limon)*

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Abstract. Lemon juice is a basic ingredient that contains dissolved suspensions that cause turbidity. To produce clearer lemon juice, clarification process can be done enzymatically or non-enzymatically. Enzymatic clarification was carried out using 0.2% pectinase produced by KJ 8 bacterial isolate and/or 0.5% cellulase produced by *Bacillus subtilis* Krakarya_1. Non-enzymatic clarification was carried out by adding 200 ppm chitosan. This research was conducted to determine the effect of the combination of pectinase, cellulase, and/or the addition of chitosan in lemon juice clarification.The experimental design used in this research was a one-factor Completely Randomized Design. The data were analyzed using ANOVA followed by level of 5%, Tukey's Honest Significant Difference test homogeneous data variance) or the Games-Howell test (non homogeneous data variance). The results showed that the use of pectinase cellulase and/or chitosan with/without combination affect to increase the transmittance value (0.233 ± 0.052 to 0.383 ± 0.041) and yield of lemon juice (71.375 ± 1.678 grams to 77.459 ± 1.397 grams) but decrease viscosity (0.717 ± 0.005 cP to 0.871 ± 0.007 cP), total soluble solids value (6.90 ± 0.089 °Brix to 7.93 ± 0.103 °Brix), and pH of lemon juice (2.40 ± 0.008 to 2.49 ± 0.008.).

1 Introduction

Lemon *(Citrus limon)* is classified into citrus fruits. Lemon has a sour taste because it is rich in citric acid. According to [1,2] lemons contain 4% to 4.38% citric acid, while Valencia oranges contain 0.22% - 0.98% citric acid. One part of lemon that is often used is the juice. Lemon juice is the basic ingredient of several popular drinks, one of which is lemonade. Also, lemon juice is used as a flavoring in lemon-flavored drinks such as soft drinks.

On the market, lemon juice is available in the form of cloudy juice and clear juice. As a drink, lemon juice that's consumed is cloudy. Clear lemon juice is produced in limited quantities. Usually, clear lemon juice is used as an acidity regulator in nectar and syrup [2, 6]

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In general, turbidity and haze in fruit juice are caused by the refraction of light by soluble suspensions in fruit juice. Particles causing turbidity of citrus fruits consist of colloidal suspensions of cell organelles, membranes, cell wall fragments, oil droplets, chromatophores, flavonoid crystals, and biopolymers such as pectin, cellulose, hemicellulose, and proteins. The particles that cause turbidity in citrus fruits contain approximately 52% protein, 4.5% pectin, 25% lipids, 2% hemicellulose, 1,5% cellulose, 5.7 nitrogen, 2% ash. The most important compounds in causing juice turbidity are pectin and other polysaccharides such as cellulose [7]. According to [10], lemons contain 0.63% pectin, calculated as anhydrogalacturonic acid. Although the content of pectin in citrus fruits is lower than other turbidity-causing components, pectin significantly affects the turbidity of citrus juice.

To make clearer juices, a clarification process is needed. The clarification process causes the degradation of the polysaccharide's semistable emulsions in the juice so that it gives a clearer appearance of fruit juice. Clarification can be done through enzymatic or non-enzymatic ways. Enzymatic clarification is also called depectinization [3]. In depectinization, dissolved pectin is degraded so that the haze-causing particles in the juice aggregate. In a suspension, pectin encloses protein particles. Pectin is negatively charged under acidic conditions. The pectinase enzyme degrades pectin so that pectin no longer encloses proteins. The positively charged protein binds to the negatively charged pectin, forming a larger-sized complex. This complex can later settle and then separated by filtration. For non-enzymatic clarification is done by using clarification aid. Commonly used clarification aid in fruit juice clarification includes gelatin, casein, bentonite, silica sol, polyvinyl pyrrolidone, and chitosan [8].

Besides pectin, an essential component that causes turbidity in citrus fruits is cellulose. Cellulose constitutes as much as 15% of the total haze-causing components. Based on preliminary research that has been done, lemon juice contains 0.371% cellulose and 0.234% pectin. Commercial pectinase enzymes usually consist of a mixture of various kinds of enzymes, one of which is a mixture of polygalacturonase enzymes with cellulase enzymes [10].

Because the presence of polysaccharide compounds in fruit juice affects the turbidity of the juice, research is needed to overcome this problem, especially in lemon juice. Clarified lemon juice using the commercial pectinase enzyme Rapidase Intense. From the study, it is known that the enzymatic treatment alone can not provide significant results on the turbidity reduction of lemon juice. In that study, the turbidity value of lemon juice changed from 1047.45 ± 22.36 NTU after extraction to 1045.21 ± 62.28 NTU after enzymatic treatment. Significant changes in turbidity were obtained after lemon juice was treated with clarifying aids such as bentonite, gelatin, and kieselsol. Another alternative is the use of chitosan. Chitosan is polycationic so that it can bind to pectin particles which are negatively charged in fruit juice.Compared the efficacies of bentonite, gelatin, and chitosan in clarifying juice. Based on the study, chitosan is best in reducing the turbidity of lemon juice. Therefore, this study uses chitosan as a clarifying aid in lemon juice clarification [3,8]

In addition to reducing turbidity, a clarification process is also carried out to assist the process of pressing the juice. The use of enzymes causes a decrease in the viscosity of fruit juice, which then facilitates the pressing process of the fruit so that the pressing process results in a higher yield of juice. Furthermore, the use of enzymes helps the filtration process where enzymes can increase fluxes and reduce membrane fouling. In the production of lemon juice itself, the clarification process is intended to help the process of juice pressing and juice filtration [9].

This study aimed to determine the effects of pectinase, cellulase, and/or chitosan with/without combination in lemon juice clarification. Parameters observed were transmittance, viscosity, pH, total soluble solids, and yield. These parameters were chosen because they can show the effects of enzymes and/or chitosan in lemon juice clarification. The juice clarification process can reduce turbidity/increase transmittance and increase yield; and reduce the value of viscosity, total soluble solids, and affect the pH value of juice. The pectinase enzyme used in this study was produced from the KJ 8 bacterial isolate. The KJ 8 bacterial isolate was isolated from a mixture of orange peel waste. This bacterial isolate was chosen because the enzymes produced were stable at pH 3-4 so that it was suitable for lemon juice clarification which was conducted in the pH range of 2-3 [3]. The cellulase enzyme used was produced from the *Bacillus subtilis* Krakarya_1 bacterial isolate which was isolated from vegetable waste. Krakarya_1 bacterial isolate was chosen because its enzyme is stable at pH 3 - 9 so that it is suitable to be applied to lemon juice clarification which is conducted at the pH of 2-3 [5]. In both studies, the use of enzymes increases the value of transmittance, decreases viscosity, affects pH, and decreases total soluble solids.

2 Materials and Methods

2.1 Production of partially purified enzyme [3,4,5]

The bacterial isolate used was the KJ 8 bacterial isolate that produced pectinase. For cellulases, the isolate used was the cellulase producing *Bacillus subtilis* Krakarya_1 bacterial isolate.

10% (v / v) bacterial inoculum stock was inoculated in 90% of liquid production media. Bacterial isolate culture was incubated in production media at 37°C until it reached the final logarithmic growth phase. For KJ 8 bacterial isolates, the end of the logarithmic phase was reached at the 8th hour while for *Bacillus subtilis* Krakarya_1 the final logarithmic phase was reached at the 10th hour. At the end of the logarithmic phase, a crude enzyme liquid was obtained.

The crude enzyme liquid was centrifuged at 4°C at a speed of 6000 rpm for 15 minutes. Centrifugation supernatant was precipitated with 10 - 90% ammonium sulfate fractionation to separate the enzyme from other proteins. For KJ 8, ammonium sulfate precipitation was carried out with 75% ammonium sulfate fraction, while for *Bacillus subtilis* Krakarya_1 S6, ammonium sulfate precipitation was carried out with 40% ammonium sulfate fraction. Pellets obtained from the fractionation were dialyzed with a cellophane membrane. The result of dialysis was a partially purified enzyme.

2.1.1 Enzyme Activity Measurement [3,4,5]

0,1 mL of the enzyme solution was mixed with 0,9 ml of the reagent medium (a mixture of 0.7% citrus pectin and 0.025 M sodium acetate buffer pH 4.8 for the pectinase enzyme; CMC for the cellulase enzyme). The mixture was then incubated at 37°C for 30 minutes. Next, the sample was added by 1 ml of DNS reagent and heated at 90°C for 10 minutes. The mixture was then cooled to room temperature. Then, the mixture was added with 0.5 ml of 40% K-Na-Tartrate and homogenized. After that, the samples were absorbed at a wavelength of 540 nm. Enzyme activity was measured by converting the absorbance value of D-galacturonic acid with the linear regression equation of the standard curve of 100 ppm D-galacturonic acid. The absorbance value obtained was then put into the equation obtained

from the standard curve of galacturonic acid. Enzyme activity was calculated using the formula :

$$Enzyme\ activity\left(\frac{U}{ml}\right) = \frac{X}{t\ x\ BM} \quad (1)$$

Note :
X : the amount of reducing sugar in the sample
t : incubation time
BM : standard molecular weight

As a standard, galacturonic acid was used in pectinase enzyme activity measurement, while glucose was used in cellulase enzyme activity measurement.

2.2 Production of Lemon Juice [4,6]

The lemon used in this research was Eureka lemon, yellow in color, ± 5 cm in diameter, weighing 50-80 grams, 7-12 cm long, thick and fleshy fruit, contains a lot of water, sour taste, and thick and smooth skin. This lemon is obtained from the Pasar Gedhe traditional market, Surakarta.

The lemon fruit was washed with running water to remove impurities that stick to the skin of the fruit. The lemon juice was then extracted using a press to extract the juice, while the solid was filtered using a filter cloth.

2.3 Lemon Juice Clarification [3,4,5,6,8]

Juice clarification was carried out enzymatically and/or using chitosan, with/without combination. For enzymatic treatment, 75 mL of lemon juice was treated by pectinase and/or cellulase with/without combination. The enzyme added was 0.2% (v/v) for the pectinase enzyme and/or 0.5% (v/v) for the cellulase enzyme. After the addition of the enzyme, incubation was carried out for 2 hours at 37^{o}C for the cellulase enzyme; or incubated for 2 hours at 50^{o}C for the pectinase enzyme and a combination of the pectinase and cellulase enzymes. At the end of the incubation period, enzyme deactivation was carried out by heating the sample in a 90^{o}C water bath for 1 minute. The sample was then cooled and centrifuged at 10.000 rpm for 10 minutes. The sample supernatant was then measured for the value of %transmittance, viscosity, pH, and total soluble solid, or continued with chitosan treatment.

For treatment with chitosan, 75 ml of fruit juice was added by 7.5 ml of 2% chitosan solution in distilled water. The mixture was then incubated at 25^{o}C with stirring slowly for 90 minutes. Chitosan treatment was always carried out after enzymatic treatment so that chitosan did not react with the enzymes.

The fruit juice samples that have been given enzymatic and/or chitosan treatment with/without a combination were measured for the transmittance value, viscosity, pH, total dissolved solids, and their clarification yield. In the study, the sample was repeated in triplicate and the test was repeated in duplicate. Data from the test results were analyzed using statistical tests.

2.3.1 Transmittance Measurement [3]

Transmittance was measured by using spectrophotometer at a wavelength of 660 nm. Aquades was used as a blank.

2.3.2 Viscosity Measurement [3]

Viscosity was determined using a falling ball viscometer. A ball with a certain density is immersed in a tube containing a liquid sample whose viscosity was to be calculated. The speed at which the ball falls over a certain distance was calculated. The formula for calculating the viscosity based on the time taken by the falling ball was as follows :

$$\eta = K(\rho_f - \rho)t \quad (2)$$

Note :
η : viscosity (cP)
K : viscometer constant
ρf : falling ball density (g/cm^3)
ρ : sample density (g/ml)
t : the time needed for the falling ball to go through the tube (second)

2.3.3 pH Measurement [3]

The pH of the clarified lemon juice was measured using a digital pH meter.

2.3.4 Total Soluble Solid Measurement [3]

The total soluble solid of clarified lemon juice was measured using a hand refractometer. The results is shown in ºBrix, according to what was indicated on the hand refractometer.

2.3.5 Clarification Yield Measurement [3]

The juice clarification yield was calculated by measuring the difference in initial fruit weights with the final weights produced.

2.4 Statistical Analysis

The data were analyzed for the homogeneity of variance using the Levene test. If the Levene test results showed that the data variance was homogeneous, the test was continued using the one way ANOVA (Analysis of Variance) statistical test with a significance level of 0.05%. If according to ANOVA results there were significantly different results, a Tukey's HSD (Honest Significant Difference) Post Hoc test was performed. If the Levene test results showed that the data variance was not homogeneous, the test was continued using Welch's ANOVA statistical test with a significance level of 0.05%. If according to Welch's ANOVA results there were significantly different results, a Games Howell Post Hoc test was performed.

3 Results and Discussion

3.1. Transmittance

Table 1. Transmittance of lemon juice

Sample	Transmittance (%T)
R	$0.200^{a} \pm 0.000$
P	$0.267^{ab} \pm 0.052$
S	$0.283^{b} \pm 0.041$
K	$0.233^{ab} \pm 0.052$
SK	$0.300^{b} \pm 0.000$
PS	$0.267^{ab} \pm 0.052$
PK	$0.267^{ab} \pm 0.052$
PSK	$0.383^{c} \pm 0.041$

Note : values were mean ±SD. Small letters in the same column show the statistical difference of each values at α=0.05

R	:	Control	SK	:	Cellulase-Chitosan
P	:	Pectinase	PS	:	Pectinase-Cellulase
S	:	Cellulase	PK	:	Pectinase-Chitosan
K	:	Chitosan	PSK	:	Pectinase-Cellulase-Chitosan

Table 1 shows the transmittance value of lemon juice treated using pectinase, cellulase, and/or chitosan with/without combination, and lemon juice without any treatment (control). Based on **Table 1**, it is known that the transmittance value of the treated lemon juice samples ranged from 0.233 ± 0.052 to 0.383 ± 0.041. The transmittance value of control lemon juice (sample R) was 0.200 ± 0.000. Among the treated lemon juice samples, the juice treated with chitosan (sample K) had the lowest transmittance value of 0.233 ± 0.052; while fruit juice treated with a combination of pectinase, cellulase, and chitosan (PSK sample) had the highest transmittance value of 0.383 ± 0.041. All treated samples have a higher transmittance value compared to sample R. Thus, it can be said that the use of enzymes and/or chitosan can increase the transmittance value of lemon juice.

Based on **Table 1**, it is known that among all samples treated, sample treated with cellulase enzymes (sample S), sample treated with a combination of cellulase and chitosan (SK sample), and PSK sample have significantly different transmittance values towards sample R. Although there are generally significant differences in the value of transmittance, both the S sample, SK sample, and PSK sample are still classified as cloudy juice.

Based on **Table 1**, it is known that generally, the transmittance value of lemon juice treated with pectinase with/without combination is higher than the sample R. However, the transmittance value is not statistically different. The results of this study are in accordance with a study by [5] in lemon juice. Treatment with commercial pectinase enzymes by Rapidase Intense, Novoferm 61, and Novozym 33095 as much as 40 µL / ml in lemon juice did not significantly reduce the turbidity of lemon juice. In the use of the pectinase enzyme Rapidase Intense, the turbidity value decreased slightly from 1047.45 ± 22.36 NTU after juice extraction to 1045.21 ± 62.28 NTU after enzymatic treatment. The turbidity disappears only after lemon juice is treated with microfiltration. According to [2] turbidity-causing components in citrus fruit juice ranged from 0.4 to 0.5 µm in size. Therefore, additional treatment is needed after treatment with enzymes in the processing of lemon juice. The need for microfiltration treatment is likely caused by the acidic nature of lemon juice. Lemon juice usually has a pH value below 2. Most pectinolytic enzymes work optimally at pH 3.8 – 4.5. The pH of lemon juice is too low, causing pectinolytic enzymes to work suboptimally. In this study, the pectinase enzyme used was the KJ 8 enzyme that works optimally at pH 4 and stable at pH 3 – 4 [4], while the pH of lemon juice control was 2.42 ± 0.013. Even though enzymatic treatment alone cannot produce clear lemon juice, enzymatic treatment helps the filtration process through the degradation of pectin and thus decreasing viscosity. In addition, a decrease in viscosity facilitates the process of pressing the fruit so that the pressing process results in a higher yield of juice [3].

Based on **Table 1**, generally speaking, treatment with cellulase enzymes with/without combination has a higher transmittance value compared to treatment with pectinase enzymes with/without combination. This is probably caused by the difference in the concentration of these enzymes. In this study, the concentration of the cellulase enzyme used was 0.5% while the concentration of the pectinase enzyme used was 0.2%. In the process of juice clarification, the mechanisms of the enzymes are as follows. For pectinase enzymes, pectinase degrades pectin so that pectin can bind to positively charged proteins, forming a complex. The complex is then separated from the juice so that the transmittance

value of the juice increases [4]. For cellulase enzymes, cellulase hydrolyzes the cell wall component which is one of the components responsible for juice turbidity. The hydrolysis of these cell wall components then causes an increase in the transmittance value of the juice [5]. Meanwhile, in the process of juice clarification, chitosan works by coagulating anionic components in fruit juices such as pectin and protein. The reaction that occurs is an electrostatic reaction where pectin and a negatively charged protein bind to the positively charged chitosan. The suspension formed from the reaction is then physically separated from the juice so that there is a decrease in turbidity/increase in transmittance. Chitosan performance in juice clarification is affected by chitosan concentration, processing temperature, and processing duration [6, 8].

Depending on the type of fruit, the turbidity of the juice may be a positive or negative parameter. Citrus fruits are usually sold in the form of cloudy juice. The making of citrus fruits clear juice is limited to lemons and limes where the juice is used as an acidifier and not for direct consumption. To make clear juice, firstly a depectinization is carried out to facilitate further processing. Turbidity in citrus fruits is considered to be lost if the transmittance value reaches 36%. Particles causing turbidity of citrus fruits consist of colloidal suspensions of cell organelles, membranes, cell wall fragments, oil droplets, chromatophores, flavonoid crystals, and biopolymers such as pectin, cellulose, hemicellulose, and proteins. The particles that cause turbidity in citrus fruits contain approximately 52% protein, 4.5% pectin, 25% lipids, 2% hemicellulose, 1.5% cellulose, 5.7 nitrogen, 2% ash (8,10].

3.2. Viscosity

Table 2. Viscosity of lemon juice

Sample	Viscosity (cP)
R	1.137^{f} ± 0.042
P	0.776^{d} ± 0.016
S	0.769^{cd} ± 0.020
K	0.871^{e} ± 0.007
SK	0.736^{abc} ± 0.016
PS	0.746^{bc} ± 0.008
PK	0.723^{ab} ± 0.017
PSK	0.717^{a} ± 0.005

Note : values were mean ±SD. Small letters in the same column show the statistical difference of each values at α=0.05

R	:	Control	SK	:	Cellulase-Chitosan
P	:	Pectinase	PS	:	Pectinase-Cellulase
S	:	Cellulase	PK	:	Pectinase-Chitosan
K	:	Chitosan	PSK	:	Pectinase-Cellulase-Chitosan

Table 2 shows measurements of the viscosity of lemon juice treated with pectinase, cellulase, and/or chitosan with/without combination, and lemon juice without any treatment (control). Based on Table 2, it is known that the viscosity value of clarified lemon juice ranges from 0.717 ± 0.005 cP to 0.871 ± 0.007 cP, while the viscosity value of the control lemon juice is 1.137 ± 0.042 cP. The viscosity value of control lemon juice (sample R) was the highest among all lemon juice samples. The viscosity value of lemon juice treated with chitosan (sample K) was the highest among all treated samples (0.871 ± 0.007 cP). The viscosity value of lemon juice treated with a combination of enzyme pectinase, cellulase, and chitosan (PSK sample) was the lowest among all treated samples (0.717 ± 0.05 cP). All

samples of clarified lemon juice have a statistically different viscosity value to the control lemon juice sample. In other words, it can be said that treatment with enzymes and/or chitosan with/without combination significantly reduces the viscosity of lemon juice. This study is in line with research by [5, 8, 9] in lemon juice. In those studies, the use of enzymes or chitosan significantly reduced the viscosity of fruit juice. The use of the commercial pectinase enzyme Rapidase Intense of 40 µL/ml reduced the viscosity value from 27.90 cP to 20.80 cP.

Based on **Table 2**, sample K had the highest viscosity value among the treated lemon juice samples. Chitosan interacts with pectin contained in fruit juice through electrostatic bonding reactions. This interaction causes a decrease in the viscosity value. On the other hand, chitosan has a fairly good water holding. This causes the viscosity value to decrease, but the decrease is not as drastic if chitosan is combined with pectinase enzymes and/or cellulase enzymes[8,9,11].

Based on **Table 2**, PSK sample had the lowest viscosity values among the treated lemon juice samples. The viscosity value of liquid food is mainly caused by the content of pectin. Treatment with the pectinase enzyme causes the pectin molecules in the juice to be degraded so that the pectin content decreases. The decrease in pectin content results in a decrease in the water holding capacity thereby reducing the value of viscosity due to the release of free water into the system.In addition to the pectinase enzyme, chitosan also interacts with pectin in fruit juice so that the juice viscosity value decreases [4,5].

In addition to pectin, the viscosity of fruit juice is also caused by cell wall component particles. According to [1] cellulase works by hydrolyzing cell wall components. Cellulase enzymes degrade cell wall components thereby reducing the viscosity of the juice.

The viscosity of liquid food is related to its total soluble solids content. A decrease in total soluble solids in the juice causes the juice to become runnier and its viscosity decreased. The decrease in total soluble solids is influenced by the degradation of sucrose into simpler compounds which causes a decrease in total soluble solids so that the juice becomes runnier and its viscosity decreases [3].

3.3. pH

Table 3. pH of lemon juice

Sample	pH
R	$2.42^{b} \pm 0.013$
P	$2.40^{a} \pm 0.008$
S	$2.44^{c} \pm 0.008$
K	$2.45^{c} \pm 0.008$
SK	$2.49^{e} \pm 0.008$
PS	$2.47^{d} \pm 0.008$
PK	$2.47^{d} \pm 0.015$
PSK	$2.48^{de} \pm 0.008$

Note : values were mean ±SD. Small letters in the same column show the statistical difference of each values at α=0.05

R	:	Control	SK	:	Cellulase-Chitosan
P	:	Pectinase	PS	:	Pectinase-Cellulase
S	:	Cellulase	PK	:	Pectinase-Chitosan
K	:	Chitosan	PSK	:	Pectinase-Cellulase-Chitosan

Table 3 shows the pH value of lemon juice treated with pectinase, cellulase, and/or chitosan with/without combination and untreated lemon juice (control). Based on **Table 3**, it is known that the pH value of the treated lemon juice ranges from 2.40 ± 0.008 to 2.49 ± 0.008. The control juice (sample R) has a pH value of 2.42 ± 0.013. Among the treated lemon juice, lemon juice treated with pectinase (sample P) had the lowest pH value (2.40 ± 0.008), while the lemon juice treated with a combination of cellulase and chitosan (sample SK) has the highest pH value (2.49 ± 0.008). Among all the treated lemon juice samples, only the P sample has a pH value lower than the R sample. This is because the pectinase enzyme produces galacturonic acid from pectin degradation so the pH value of the juice decreases [3]. In other words, it can be said that in general the treatment with enzymes and/or chitosan with/without combination significantly increases the pH value of lemon juice.

Based on **Table 3**, the pH value of sample P was the lowest among all treated samples (2.40 ± 0.008). The pectinase enzyme used in this study was the KJ 8 enzyme. Based on research conducted by [3] the KJ 8 enzyme belongs to the polygalacturonase enzyme group. Polygalacturonase enzyme catalyzes the hydrolysis of glycosidic bonds in pectin to produce acidic D-galacturonate. In addition to the release of D-galacturonate, treatment with enzymes also causes the release of carboxyl groups. The release of D-galacturonate and the carboxyl group causes a decrease in the pH value of fruit juice [9,10].

Based on **Table 3**, the pH values of SK sample were the highest among all treated samples (2.49 ± 0.008). Citric acid and malic acid are the two components responsible for the acidity of lemons [3]. Based on Table 3, the use of chitosan with/without combination increases the pH value of lemon juice. Chitosan binds with organic acids such as citric acid and malic acid through the flocculation process. The floc formed is then separated physically so that the organic acid decreases/the pH increases. Thus, this study is in line with the research of [8] that the use of chitosan in the clarification process decreases the total acidity value which then increases the pH value of the juice.

3.4. Total Soluble Solids

Table 4. Total soluble solids (TSS) of lemon juice

Sample	TSS (^{o}Brix)
R	7.73^{d} ± 0.103
P	7.03^{ab} ± 0.082
S	7.93^{d} ± 0.103
K	7.23^{bc} ± 0.151
SK	7.17^{b} ± 0.082
PS	7.40^{c} ± 0.179
PK	6.90^{a} ± 0.089
PSK	7.07^{ab} ± 0.103

Note : values were mean ±SD. Small letters in the same column show the statistical difference of each values at α=0.05

R	:	Control	SK	:	Cellulase-Chitosan
P	:	Pectinase	PS	:	Pectinase-Cellulase
S	:	Cellulase	PK	:	Pectinase-Chitosan
K	:	Chitosan	PSK	:	Pectinase-Cellulase-Chitosan

Table 4 shows the TSS value of lemon juice treated using pectinase, cellulase, and/or chitosan with/without combination, and untreated lemon juice (control). Based on **Table 4**, it is known that the TSS value of lemon juice treated ranged from 6.90 ± 0.089 to 7.93 ± 0.103 ºBrix. The value of TSS of lemon control juice was 7.73 ± 0.103 ºBrix. In general, the treated lemon juice has a lower TSS value compared to the control sample. Among the treated lemon juice samples, only those treated with cellulase enzyme (sample S) had higher TSS values than the control lemon juice (sample R) although the difference was not statistically significant. This higher TSS value is caused by cell tissue degradation by cellulase enzymes. This degradation releases the sugar component into the system so that the TSS value increases [3].

Based on **Table 4**, among all treated samples, sample S had the highest TSS value (7.93 ± 0.103), while lemon juice treated with a combination of pectinase and chitosan enzymes (PK sample) had the lowest TSS value (6.90 ± 0.089). In addition to sample S, all treated lemon juice samples have significantly lower TSS values than the R sample. In other words, it can be said that the use of enzymes and/or chitosan with/without combination generally can reduce the TSS value of fruit juice lemon significantly.

Based on **Table 4**, the PK sample has the lowest TSS value. According to research conducted by [8] the use of chitosan in lemon juice clarification significantly reduces the value of lemon protein. This is due to the polycationic nature of chitosan so that it can bind to the protein in lemon juice, forming a protein-chitosan complex which then can be separated physically from the juice so that the protein content in the juice decreases. Protein itself is one of the constituent components of TSS in fruits. The decrease in protein content then causes a decrease in the value of TSS lemon juice. Similar results also occur in research by [9] in acerola juice. In these studies, the use of chitosan caused a decrease in the TSS value of clarified fruit juice. Meanwhile, in this study, the use of the pectinase enzyme alone reduced the TSS value of lemon juice. These results are similar to the study by [3] in Garut orange. In that study, the use of the enzyme pectinase reduced the TSS value of Garut orange juice clarified from 9 ºBrix to 8 ºBrix after clarification. This decrease in TSS value was also found in a study by [10] in Valencia orange juice was clarified where the TSS value dropped from 11.09 ºBrix to 10.86 ºBrix and on lemon juice where the TSS value dropped from 8.87 ºBrix to 8.63 ºBrix.

Based on **Table 4**, even though the TSS value of PK sample is the lowest, the difference is not statistically significant for samples treated with solely pectinase enzyme (P sample) and sample treated with a combination of pectinase, cellulase, and chitosan (PSK sample). The PSK sample had a higher average TSS than the PK sample. This is caused by the use of cellulase enzymes in the PSK sample where the use of cellulase enzyme increases the TSS value of lemon juice.

According to [3] soluble solids in citrus fruit juice consist of high molecular weight polymeric materials such as protein, pectin, hemicellulose, and cellulose. The components of the dissolved material are also responsible for the turbidity of the juice. Based on **Table 1**, it is known that the use of enzymes and/or chitosan can reduce turbidity/increase the transmittance value of lemon juice. Meanwhile, based on **Table 4**, it is known that generally, the use of enzymes and/or chitosan with/without combination decreases the TSS value of lemon juice. Therefore, a decrease in TSS value is associated with a decrease in turbidity/increase in the transmittance value of lemon juice.

The decrease in viscosity is related to the decrease in the soluble solids content. The use of enzymes such as pectinase and cellulase can degrade dissolved solid components that are responsible for the viscosity of fruit juices [9]. Based on **Table 2**, the use of enzymes and/or chitosan reduces the viscosity of lemon juice. Meanwhile, according to **Table 4**, the use of enzymes and/or chitosan generally reduces TSS value of lemon juice.

According to [10] components measured as TSS in fruits include sucrose, reducing sugar, organic acids, and protein. Based on **Table 3**, it is known that the use of chitosan with/without combination generally increases the pH value of lemon juice. The increase in pH is caused by the reaction with organic acids in lemon juice so that organic acids decrease/the pH value rises. Meanwhile, based on **Table 4**, it is known that the use of chitosan with/without combination decreases the value of TSS. In other words, it can be said that the use of chitosan is associated with a decrease in the value of TSS and an increase in the pH value of lemon juice.

3.5. Yield

Table 5. Yield of lemon juice

Sample	Yield (gram)
R	$57.186^{a} \pm 0.951$
P	$72.199^{bc} \pm 1.992$
S	$75.770^{cd} \pm 0.184$
K	$71.375^{b} \pm 1.678$
SK	$76.215^{d} \pm 0.503$
PS	$77.459^{d} \pm 1.397$
PK	$73.356^{b} \pm 0.450$
PSK	$76.158^{d} \pm 0.591$

Note : values were mean ±SD. Small letters in the same column show the statistical difference of each values at α=0.05.

R	:	Control	SK	:	Cellulase-Chitosan
P	:	Pectinase	PS	:	Pectinase-Cellulase
S	:	Cellulase	PK	:	Pectinase-Chitosan
K	:	Chitosan	PSK	:	Pectinase-Cellulase-Chitosan

Table 5 shows the calculation of the yield of lemon juice treated with pectinase, cellulase, and/or chitosan with/without combination, and an untreated lemon juice (control). Based on **Table 5**, it is known that the yield of treated lemon juice ranges from 71.315 ± 1.678 grams to 76.215 ± 0.503 grams per 75 ml of fruit juice. The yield of control lemon juice (sample R) was 57.186 ± 0.951 gram per 75 ml of fruit juice. The yield of all the treated lemon juice was statistically higher than the yield of the control lemon juice. Therefore, it can be said that the use of pectinase, cellulase and/or chitosan with/without combination significantly increases the yield of lemon juice. In these studies, the use of enzymes significantly increased fruit juice yield. The use of pectinase, cellulase, and hemicellulase enzymes increased the yield of lychee juice from 60.14% to 79.21%. The use of crude pectinase enzyme by 0,5% increased the yield of orange juice from 73% to 86% [3,10].

Based on **Table 5**, lemon juice treated with chitosan (sample K) statistically has the lowest yield among all treated samples (71.375 ± 1.678 grams), while lemon juice treated with a combination of pectinase and cellulase enzymes (PS sample) had the highest yield among all treated samples (77.459 ± 1.397 grams). However, the difference in the yield values of PS sample with samples treated with cellulase and chitosan (SK sample) and sample treated with pectinase, cellulase, and chitosan (PSK sample) were not significantly different. The addition of pectinolytic enzymes facilitates the degradation of pectin. Pectin has good water binding capacity due to the presence of hydrophilic group (-OH) in pectin. Pectinolytic enzymes such as pectinase interact with the hydrophilic group, resulting in the

release of free water into the system. The release of free water increases the juice yield. The combination of pectinolytic enzymes with cellulases can increase fruit juice yield because cellulase enzymes degrade cell walls [10].

Other than increasing yield, the use of enzymes in juice clarification also decreases the viscosity of juice. The use of enzymes causes a decrease in the viscosity of fruit juice, which then facilitates the pressing process of the fruit so that the pressing process results in a higher yield of juice [9]. This happened in the current study. According to **Table 2**, enzymatic treatment significantly reduced the viscosity of lemon juice, while according to **Table 4.5**, treatment with enzymes significantly increased the yield of lemon juice. In other words, the decrease in viscosity is related to the increase in the yield of lemon juice.

Other than viscosity, the clarification yield is also related to soluble sugar content in the juice. The higher the soluble sugar content, the higher the fruit juice yield. This phenomenon occurs because enzymatic treatment degrades pectin and cell wall components, resulting in dissolved components such as acids and neutral sugars. However, according to **Table 4**, the use of enzymes and/or chitosan in general significantly reduces the TSS value of lemon juice, while according to **Table 5**, the yield actually increases significantly. In this study, the increase in the value of yield is related to a decrease in the value of dissolved sugar. The decrease in the value of dissolved sugar occurs due to the degradation of the soluble solid component by the enzyme, which then decreases the viscosity value of fruit juice thereby increasing the value of fruit juice yield [3]. These results are in accordance with a research by [3] in Garut orange and a research by [10] in Valencia orange juice.

4 Conclusions

The conclusions obtained were:

a. The use of pectinase, cellulase, and/or chitosan with/without combination affects all test parameters. Enzymatic and/or chitosan treatment with/without combination increases the transmittance value and the yield value of lemon juice. Enzymatic and/or chitosan treatment with/without combination decreases the viscosity, total soluble solids, and pH of lemon juice.
b. The transmittance of clarified lemon juice ranged from 0.233 ± 0.052 to 0.383 ± 0.041, while the control sample was 0.200 ± 0.000. The viscosity clarified lemon juice ranged from 0.717 ± 0.005 cP to 0.871 ± 0.007 cP, while the control sample was 1.137 ± 0.042 cP. The pH value of clarified lemon juice ranged from 2,40 ± 0,008 to 2,49 ± 0,008, while the control sample was 2.42 ± 0.013. Total soluble solid value of clarified lemon juice ranged from 6.90 ± 0.089 ^{o}Brix to 7.93 ± 0.103 ^{o}Brix, while the control sample was 7.73 ± 0.103 ^{o}Brix. The clarification yield of clarified lemon juice ranges from 71.375 ± 1.678 grams to 77.459 ± 1.397 grams, while the control sample was 57.186 ± 0.951 grams.

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