

Investigation of the Effect of Pectinase and Amyloglucosidase Enzyme Mixture on Clarification of Apple Juice

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Abstract Apple juice is the second most consumed fruit juice in the world and is popular among both adults and children with its unique taste. The raw apple juice obtained after juicing is treated with enzymes to remove suspended solids that clog filters, slow production and cause cloudiness in the juice, and hydrolyze the molecules that cause cloudiness. Enzymatic treatment can be applied as a hot method (1 to 2 hours at 40-55 °C) or a cold method (6 to 8 hours at 20-25 °C). In this study, it was aimed to determine the optimum temperature, optimum enzyme concentration and enzyme application time within the temperature range of the hot method, which is known to be more advantageous by using pectinase and aminoglycosidase enzyme mixture. The objective of enzymatic clarification of fruit juice is to provide a rapid, easy and economic filtration quality to fruit juices, to prevent subsequent turbidity, and to prevent gel formation during concentration by breaking down pectin. In this study, we examined the clarification of the juice obtained from Granny Smith apples grown in Kosovo with a commercially available pectinase and aminoglycosidase enzyme mixture. In our research, two enzymes responsible for the breakdown of pectin and starch, pectinase and amylase, were used to clarify apple juice. The most suitable enzyme mixture ratio and the working temperature of the

enzymes were optimized by mixing different ratios of pectinase and amylase enzymes. The viscosity, pH change, Total Acidity, density, turbidity test, brix, starch and pectin analysis of the apple juice clarified under the determined optimum conditions were performed. When the data obtained was examined, it was determined that the best results were obtained in apple juice incubated for 1 hour and 30 minutes at 55 °C with an enzyme concentration of 0.1/0.12 g/L and the percentage of clarity was 78.94%. The results showed that when pectinase and aminoglycosidase enzymes were used in combination for juice clarification, the clarity level in apple juice increased significantly. This can be applied to the clarification of other fruit juices.

Keywords Apple Juice, Enzyme, Pectinase, Amyloglucosidase, Clarification

1. Introduction

There are different types of apple juice that can be found in the grocery store: pure juice (not concentrated, 100% juice), concentrated juices, purified juice, cloudy juice, and others. On the other hand, there are beverages with fruit

juice content, cocktails and fruit juices with the least (10-20%) fruit juice content [1,2].

Raw apple juice obtained from the "Jonagold" variety (from pulp or by direct pressing) and after enzyme treatment showed 10% and 3% antioxidant activity, respectively, compared to the antioxidant activity of fresh apples [3].

Homogeneity and transparency are two key features of juices [4]. Clarification of juice is acceptable to meet international standards. Effective enzymatic treatment of fruit juice allows the juice to be more beneficial in quantity and shorter clarification process [5,6].

The enzymatic treatment of pectin by hydrolysis is affected by several parameters or variables, including incubation time, temperature, pH, and enzyme concentration [7,8,9]. In addition, the cost of the enzyme during the clarification process is important. A design and optimization of the clarification parameters is necessary to ensure that the liquor is clarified both from an economic point of view and from an environmental protection point of view. Optimization of parameters for pectinase treatment on sweet orange juice [8] and banana juice [9] has already been reported. There is also research on the depectinization of fruit juice treated with commercial fungal pectinases [10,11,12].

Fruit juice is a fruit with a pH range of 2.5-4.5, obtained by mechanical processing from fruits, which can be fermented but not fermented, and has the color, aroma and taste characteristics of the fruit it comes from. After juicing, raw apple juice is treated with enzymes to remove suspended solids that clog filters, slow production and cause cloudiness in the juice. The responsible enzymes hydrolyze pectin, hemicellulose and other polymers and colloids responsible for the high viscosity of the liquid. The enzymatic treatment can be performed using the warm method (1 to 2 h at 54 °C) or the cold method (6 to 8 h at 20 °C). From here, we also state that the importance of this article is to investigate the effect of two variables, temperature and time, on the activity of the enzyme. Through filtration, large particles, certain proteins and microorganisms are removed from apple juice [1]. This study analyzes the changes by treating eight apple juice samples with different enzyme concentrations at different temperatures and times.

The addition of enzymes causes the viscosity of the juice to decrease, which improves the squeezing of the pulp, extracts more juice without the need for complex pressing mechanisms, and is therefore more commercially effective [13]. Pectinases have numerous applications in the food industry, such as the processing of plant materials, the depectinization of fruit juices, and the maceration of fruits [14]. In this part of the research, where we discuss the two components of apple and the selection of enzymes, it is expected that the optimization of the concentration of these two enzymes will affect the physical properties and parameters of the liquid, such as turbidity and viscosity. The breakdown of pectin and starch by enzymes will allow

faster filtration of fruit juice and contribute to its clarity in later stages such as storage.

With the increasing demand for fruit juices, the production of residual fruit juice has also increased significantly. This leads to more research to develop better methods for processing the fruit. Consumer preference for completely clear, sparkling apple juice has made clear apple juice much more popular than cloudy apple juice. Clarification before concentrating is important in the fruit juice industry to reduce costs and increase shelf life. As an important step in the juice industry, the purpose of clarification is:

- To carry out the clarification process by removing the insoluble solids and pectic substances by breaking down pectin and starch with specific enzymes.
- To prevent turbidity after bottling.

The components found in apple juice are pectin, protein, starch and polyphenols, and all these components affect the contamination and clogging of the membrane during the filtration of the juice [15,16]. The use of pectinolytic enzymes for the pectinization step was effective in reducing membrane fouling [19]. For the degradation of pectin and starch, pectinase and amyloglucosidase were used at different concentrations to determine the optimum time and temperature at which pectinization would end faster. Depectinization improves the performance of membrane processes during juice filtration, and this has been shown for lemon and apple juices [17,18,19]. As a result, pectin-containing liquids are pectinized (treated with enzymes: pectinase and amyloglucosidase) to reduce contamination prior to purification, resulting in higher flow and lower energy consumption.

At the heart of our research is the optimization of factors such as the concentration of enzymes, the temperature and time at which the enzymatic treatment will be carried out, in order to reduce costs and increase efficiency.

Another aim of our research is to use commercial enzymes such as pectinase and amylase to break down pectin and starch in apple juice clarification (clarification), and to determine parameters such as incubation time, temperature and enzyme concentration to obtain maximum clarified juice.

In order to remove the turbidity caused by pectin in fruit juices, the effects on fruit juice were investigated by using minimum dose enzyme solutions. The effect of enzymes on the enzyme applied samples, and the effect of time and temperature on enzyme functioning were compared with an untreated apple juice sample and it was determined that the best quality product was obtained at which enzyme rate, temperature and time from this study.

2. Materials and Methods

2.1. Chemicals and Devices

Apples required for the study were collected in October

from apple plantations grown in the Prizren region. All of the chemicals used in the study were obtained from Merck-kGa and prepared analytically. Enzymes (pectinase and amyloglucosidase) used in the study were obtained from AB Novozyme Enzyme. The devices used in the study are detailed in Table 1.

Table 1. Equipment used

Device	Model	Company
Spectrophotometer	Genesis 10S UV – Vis Spectrophotometer	Thermo Scientific
Turbidimeter	2100AN IS turbidimeter	HACH
Deep freeze		Gorenje
pH meter	pH Meter PB-11	Sartorius mechatronics
Precision balance	Mettler Toledo	Development Production Testing
Automatic pipettes	Advanced pipette Plus Series Single – channel Adjustable Volume Volume : 10 – 100 μ L Volume : 100-1000 μ L	Hawach Scientific
Vortex	Led Digital Vortex Mixer Laboratory Equipment	ISOLAB Laborger äte GmbH
Water bath	Thermostat water bath HH-4	Wincom Company Ltd
Viscometer	DVE	Brookfield AMETEK
Refractometer	Refractometer "PAL- α "	Atago

Experimental studies were carried out in the laboratories of the UBT innovation center and Trakya University Arda Vocational School.

2.2. Apples

Granny Smith apples (Fig. 1a) harvested at the optimum

ripeness stage were used, and the apples were harvested in October on the fields of the company "FRUTEX" in the Republic of Kosovo and processed directly in the laboratory for current research.

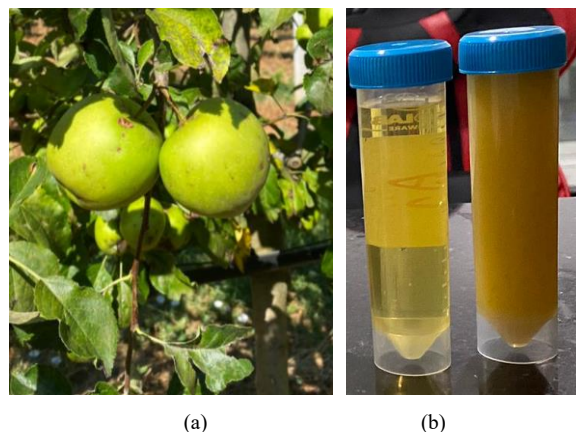


Figure 1. (a) Apple, Granny Smith and (b) clarified apple juice as part of the study and apple juice

2.3. Preparation of Apple Juice

To obtain apple juice, the fruits have gone through various stages of preparation and processing, which are schematically discussed in Figure 2.

Stage 1: Harvest and transport stage

Fruits were harvested in the fields of "FRUTEX" company, paying attention to maturity and harvesting methods. One of the most important factors affecting quality is the harvesting of the fruit during maturity. In order to avoid mechanical damage in the post-harvest period, the fruits are collected from the stem in a mature manner.

It is thought that during the period from harvest to preparation, delays in transportation and processing may cause direct losses (such as water loss and decomposition processes) and indirect losses that may cause a decrease or loss in taste/aroma, and nutritional quality. Therefore, care should be taken to transport the harvested fruit to the laboratory for processing as soon as possible.

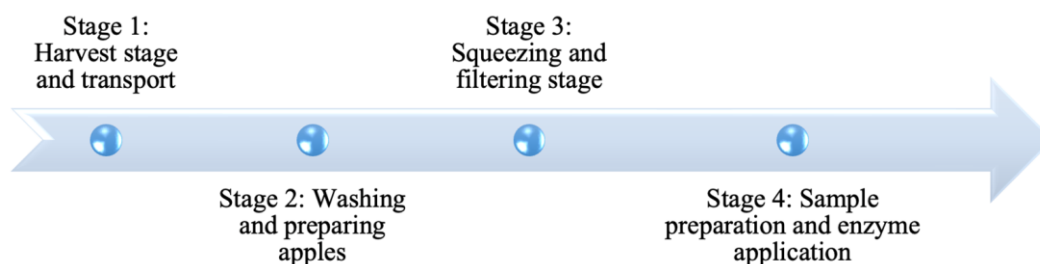


Figure 2. Apple processing stages

Stage 2: Washing and preparing the apples

The apples were washed using water purified by a water purification system, and then the peel and core of the apples were removed. The apples were cut into small cubes.

Stage 3: Squeezing and filtering stage

The apples cut into small cubes were mixed with a mixer without adding water. The resulting mass was then filtered and raw apple juice was obtained. The resulting juice was used in our research. 0.5% (w/v) ascorbic acid was added to the obtained apple juice to prevent enzymatic browning [20].

Stage 4: Sample preparation and enzyme application

The apple juice obtained after mechanical pressing and filtration processes was treated in 1 L amber bottles with the enzyme mixtures specified in Table 2, at the specified incubation time and temperature.

Table 2. Experimental design

(PEC/AMG) g/L	Incubation Time	Temperature (°C)	Code
0.005/0.0132	1 h 30 min	40	A1
	2 h	40	A2
	1 h 30 min	55	A3
	2 h	55	A4
0.01/0.0264	1 h 30 min	40	B1
	2 hour	40	B2
	1 hour 30 min	55	B3
	2 h	55	B4
0.05/0.06	1 h 30 min	40	C1
	2 h	40	C2
	1 h 30 min	55	C3
	2 h	55	C4
0.1/0.12	1 h 30 min	40	D1
	2 h	40	D2
	1 h 30 min	55	D3
	2 h	55	D4

PEC= Pectinase, AMG= Amyloglucosidase

In order to determine the effect of temperature on the clarification of apple juice with the enzyme mixture, the apple juices to which the enzyme mixture was added were incubated at 40 and 55 °C. A shaking water bath was used for incubation (120 rpm). The apple juices, which were subjected to enzyme treatment at the specified temperature, were heated at 90 °C for 5 minutes at the end of the incubation period to stop the enzyme activity.

As indicated in Table 2, physico-chemical analyses (pH, total acidity, turbidity, density, viscosity, Brix, clarity %) of the samples incubated at different times and temperatures were performed. The indicated physicochemical analyses were determined for enzyme-

treated apple juice and raw apple juice.

2.4. Determination of pH

pH measurement was made with PB-11 model pH meter of Sartorius company (n=5) by method AOAC, 2005 [21].

2.5. Determination of Total Acidity

In order to determine the titration acidity in apple juice samples, 5-10 mL was taken from the samples and 40 mL of distilled water was added to it. After adding 2-3 drops of 1% (v/v) phenolphthalein, it was titrated with 0.1 N NaOH solution until the first pink color was observed and the consumption was measured [22]. The % titration acidity was calculated in malic acid and was calculated according to the following equation;

$$\text{Titration acidity (\%)} = \frac{V \times N \times E}{m} \times 100 \quad (1)$$

V: Amount of NaOH consumed in titration (mL)

N: Normality of the NaOH solution used in the titration

E: Acid equivalent of 1 mL of 0.1 N NaOH

m: Actual amount of sample titrated (mL)

The actual sample amount was calculated according to the following equation;

$$\text{Actual sample amount} = \frac{\text{Amount of sample weighed}}{\text{Final diluted volume}} \times \text{Filtrate used for analysis} \quad (2)$$

2.6. Determination of Turbidity

Turbidity determination was measured with the 2100AN IS TURBIDIMETER device of Hach company by method AOAC, 2005 [21].

2.7. Determination of Density

Density determination was analyzed by pycnometer at 25 °C by method AOAC, 2005 [21].

2.8. Determination of Viscosity

The viscosity was analyzed on a calibrated Brookfield DVE viscometer, and the axis-spindle used was defined as 6.2. The viscosity analysis was carried out according to the method presented by Padma [23].

2.9. Determining the Brix Scale

In solutions containing dissolved substances, light is refracted as it passes from one medium to another with different densities. The refraction of light is characteristic of matter dissolved in water and is a measure of its concentration by method AOAC, 2005 [21]. The refractometer is first calibrated with water. Then, 1 drop of

the homogenized apple juice samples was made and read. The Brix degree determination of apple juice samples was made with a "Digital Hand Pocket Refractometer" type refractometer belonging to ATAGO company. The Brix determination was performed on all samples at room temperature before and after the addition of the enzyme solutions. The determination of the dry, soluble matter, expressed by the Brix scale, which corresponds to the percent by weight of sucrose in a solution and has the same refractive index as the product analyzed, was made by the refractometric method. The refractometric method is based on the relationship between the concentration of the solute and the refractive index of light. The sample to be analyzed was mixed thoroughly so that it was as homogeneous as possible, and the sample was also filtered when necessary. A few drops of liquid were placed on the prism of the refractometer (previously checked with distilled water) and the reading was taken. Analyzes were performed on liquid samples of apples treated with enzymes and on a controlled sample, which itself does not contain enzymes and therefore has not been subjected to enzymatic treatment.

2.10. Clarity Test (%)

The percentage of clarification of apple juice was determined by measuring the absorbance at 660 nm [32]. Clarification degree was calculated with the following equation;

$$\text{Clarity \%} = \frac{Abs_{\text{untreated sample}} - (Abs_{\text{control}} - Abs_{\text{sample}})}{Abs_{\text{untreated sample}}} \times 100 \quad (3)$$

$Abs_{\text{untreated sample}}$: untreated and unheated apple juice

Abs_{control} : apple juice without enzyme applied but heated to the specified temperature

Abs_{sample} : apple juice treated with the specified concentration of enzymes and incubated at the specified temperature

2.11. Pectin Test

With the pectin test, which involves mixing fruit juice with 96% (v/v) alcohol in a certain amount, pectic substances become insoluble or precipitate or become gelatinous [24]. 5 mL of fruit juice was taken into a test tube and 5 mL of 96% (v/v) ethyl alcohol was added and the tube was shaken vigorously. If the residue caused by pectin appeared in the tube left to itself within 1 minute, it means that the depectinization is not completed. In the 1:1 pectin test (1 mL of fruit juice in 1 mL of ethanol), the pectin chain containing more than 8-10 units of galacturonic acid becomes insoluble in 50% ethanol and precipitates as a gel. When the mass in the juice/alcohol mixture is uniformly cloudy and no separation is observed, it is understood that the juice tested is free of pectin. However, the pectin test can also be performed at 1:2 ratios

(1 mL fruit juice in 2 mL ethyl alcohol), even the shortest chains of pectic substances precipitated by about 66% at higher alcohol concentrations can be detected. After the process, the formation of sediment in the tubes was examined and the results were given as (+) and no sediment (-).

2.12. Starch Test (Iodine Test)

Some pome fruits such as apples, pears and quince contain around 1% starch before ripening. As maturation progresses, starch breaks down and finally disappears. For this reason, starch can be found in the juice obtained from unripe apples processed at the beginning of the season, and this may cause some problems. If the turbid apple juice taken from the press is filled into a bottle and waited, even a fine white residue may be collected at the bottom depending on the amount of starch present. While the raw juice is being passed through the separator, it is sometimes seen that the starch particles are collected in the separator as a hard mass. Starch that passes into fruit juice is pasted at the end of the heating applied during processing. Thus, it becomes dissolved in the juice and does not pose a problem since the amount is already low. Such apple juice, which is clearly bottled, becomes cloudy later on as a result of the retrogradation of starch." The juice, in which this phenomenon called "delayed clouding" occurs, becomes clear if heated, but becomes cloudy again when cooled. In order to prevent this cloudiness due to starch, starch must be broken down during clarification. Starch is broken down by adding amylase enzyme to fruit juice. Pectin and starch are broken down during the same process, namely during the depectinization stage. Starch degradation is monitored by the iodine test [24]. It is prepared by adding 2 g of potassium iodide and making it up to 100 mL with distilled water. 10 mL of fruit juice is filtered and 40 mL of 95% (v/v) ethyl alcohol is added and shaken. Afterwards, the sediment is separated by centrifugation. The toru is washed 2 times with 80% ethyl alcohol. The resulting sediment is dissolved by adding a small amount of water. Then, 1-2 drops of iodine solution are dripped. By examining the color formed, a conclusion is reached about the degradation level of starch. Blue indicates that the Starch is pasted but not broken; Violet, Starch has begun to break down; Brown, Formation of dextrin from starch; Red indicates that the Starch is broken down into dextrans; Orange indicates that glucose formation has started; Yellow indicates that glucose formation has been reached.

With the starch test, which involves mixing the iodine solution into the juice, the presence of starch in the juice can be detected by the presence of a blue color in the mixture. In the study, the starch test applied to the samples was performed at 10-minute intervals. The degradation of amidonite in apple juice samples treated with enzymes at different times and temperatures and in apple juice samples without enzyme treatment was observed with this test. The

results of the enzymatic treatment are given in Figure 7, the (+) sign indicates that the enzymatic process is continuing, that is, the starch is still present, the (-) sign indicates that the enzymatic process is finished and the starch is separated.

3. Results and Discussions

Physico-chemical analyses (pH, total acidity, turbidity, density, viscosity, Brix, clarity %) of the samples incubated with different concentrations of PEC/AMG enzyme mixture at different times and temperatures were determined for enzyme-treated apple juice and raw apple juice. The obtained results are given in Table 3 and Figures 3, 4, 5 and 6.

Plenty of quality apples are grown in the apple fields of the FRUTEX factory. When fruit purees are enzymatically processed, they provide 80% fruit juice yield. Purification of pectic substances by enzymatic hydrolysis depends on several variables such as the type and concentration of the enzyme, hydrolysis time and incubation temperature [25,26,27]. Commercial pectinase enzymes were used separately as single enzyme and multi-enzyme for clarification of apple juice under different enzymatic conditions such as incubation time, and temperature, but at constant enzyme concentration. It has been reported that using a mixture of hydrolytic enzymes such as pectinases and amyloglucosidases in the purification of fruit juices provides both clarification and shortening of the filtration time by approximately 50% [28].

In this study, Granny Smith apples were harvested, cleaned and mechanically ground before processing. Then, the apple juice obtained in turbid form was subjected to enzymatic treatment. Physico-chemical analyses were performed before and after the enzyme application using the instruments listed in Table 1 and comparisons were made.

While the pH value of apple juice was 3.53 before the addition of the enzyme mixture, it was determined that the pH value decreased after the treatment of apple juice with enzymes (Table 2). The pH of the enzyme mixture decreased with increasing temperature and increasing concentrations. At the same time, acidity values were measured in parallel with pH measurements in our study. It was determined that acidity values increased with increasing temperature and increasing concentration. It was also observed that the acidity level increased from 5.12 g/L to 5.76 g/L with the increase of the enzyme mixture concentration at 40 °C (Table 2). It was observed that the acidity level increased from 5.12 g/L to 6.08 g/L with

increasing concentrations of the enzyme mixture of apple juice processed during an incubation period of 1 hour and 30 minutes at 55 °C (Table 2).

Pectins are a family of plant cell wall-forming polysaccharides rich in covalently bound galacturonic acid [29]. Galacturonic acid makes up about 70% of pectin. The enzyme pectinase breaks down pectin, causing hydrolysis of galacturonic acids. Therefore, an increase in galacturonic acid molecules lowers the pH and increases the acidity level. Abbas, F. et al. [30] in their study on the effect of date pectinase enzyme in pectinase syrup found that the pH value decreased with the increase of the enzyme concentration applied, and the acidity degree increased in parallel. The acidity and acidity results obtained in this thesis study are similar to the results in the literature.

When the enzyme mixture was added to the apple juice, the result after 2 h of incubation at 40 °C showed a slight increase in the dry matter dissolved in the apple juice from 14.0 to 14.1 Brix (Table 2). However, after 1 hour and 30 min of incubation at 55 °C, the analysis of apple juice treated with the enzyme mixture by refractometer and expressed as Brix scale showed an increase from 14.0 to 14.4 Brix (Table 2). As a result of the study conducted by Kareem, S.O. et al. [31] on the application of pectinase enzyme on orange juice, a slight increase in Brix values was observed. The results obtained in Brix values obtained in this study are similar to the results in the literature. When the enzyme mixture was added to apple juice, it was observed that the density gradually increased with increasing concentration of the enzyme mixture after 2 h of incubation at 40 °C (Table 2). Higher density values were observed in apple juice incubated for 1 h and 30 min at 55 °C (Table 2).

In the analysis of turbidity of apple juice by adding enzyme mixture and incubating at 40 °C for 2 h, it was proven that the turbidity decreased by 33.6% with the increase of enzyme concentration (Table 2). Except for B3 sample, a 40% reduction in turbidity was determined as a result of incubation of the samples at 55 °C for 1 h and 30 min with an enzyme mixture.

As a result of the research conducted by Uzun, S. [32] by applying pectinase enzyme to apple juice, it was observed that the turbidity in apple juice was 4% by incubating this period for 20 min at 45 °C. However, it was determined that turbidity was reduced by 40% at 3% enzyme concentration incubated for 15 min at the same temperature. The results of turbidity values (nephelometric turbidity unit - NTU) obtained in this study are similar to the studies in the literature.

Table 3. Analysis results

(PEC/AMG) g/L	Incubation Time	Temp.(°C)	Code	pH	Total Acidity (%)	Brix	d (g/L)	Turbity (NTU)	Viscosity (cp)	Clarity (%)
0	-	-	0	3.53±0.02	5.12±0.02	14.0±0.1	1.051	309	30683	0
0.005/0.0132	1 h 30 min	40	A1	3.51±0.02	5.12±0.01	14.0±0.2	1.057	282	5289	64.56±0.34
	2 h	40	A2	3.52±0.02	5.12±0.01	14.0±0.2	1.064	279	4978	65.12±3.86
	1 h 30 min	55	A3	3.51±0.02	5.44±0.01	14.1±0.2	1.066	275	2897	69.72±2.42
	2 h	55	A4	3.51±0.02	5.42±0.02	14.1±0.1	1.063	273	3102	60.04±1.87
0.01/0.0264	1 h 30 min	40	B1	3.51±0.02	5.42±0.02	14.2±0.1	1.066	245	3567	62.98±1.23
	2 h	40	B2	3.51±0.02	5.44±0.01	14.1±0.1	1.058	237	3726	62.74±4.78
	1 h 30 min	55	B3	3.51±0.02	5.44±0.02	14.1±0.1	1.064	184	4376	62.23±3.12
	2 h	55	B4	3.50±0.02	5.53±0.02	14.0±0.1	1.065	181	4277	67.12±2.34
0.05/0.06	1 h 30 min	40	C1	3.51±0.02	5.51±0.02	14.2±0.1	1.068	224	3192	68.92±1.84
	2 h	40	C2	3.51±0.02	5.76±0.01	14.1±0.2	1.066	212	2987	62.14±3.32
	1 h 30 min	55	C3	3.50±0.02	6.08±0.01	14.2±0.1	1.068	195	2269	76.72±0.76
	2 h	55	C4	3.50±0.02	5.83±0.02	14.1±0.1	1.062	189	2189	70.87±1.98
0.1/0.12	1 h 30 min	40	D1	3.50±0.02	5.71±0.02	14.2±0.1	1.064	218	2165	72.34±1.12
	2 h	40	D2	3.49±0.01	5.76±0.01	14.1±0.1	1.062	205	2092	78.94±0.32
	1 h 30 min	55	D3	3.48±0.02	6.08±0.01	14.4±0.2	1.066	200	2021	72.14±0.94
	2 h	55	D4	3.42±0.01	5.01±0.01	14.2±0.1	1.060	201	2082	78.54±0.32

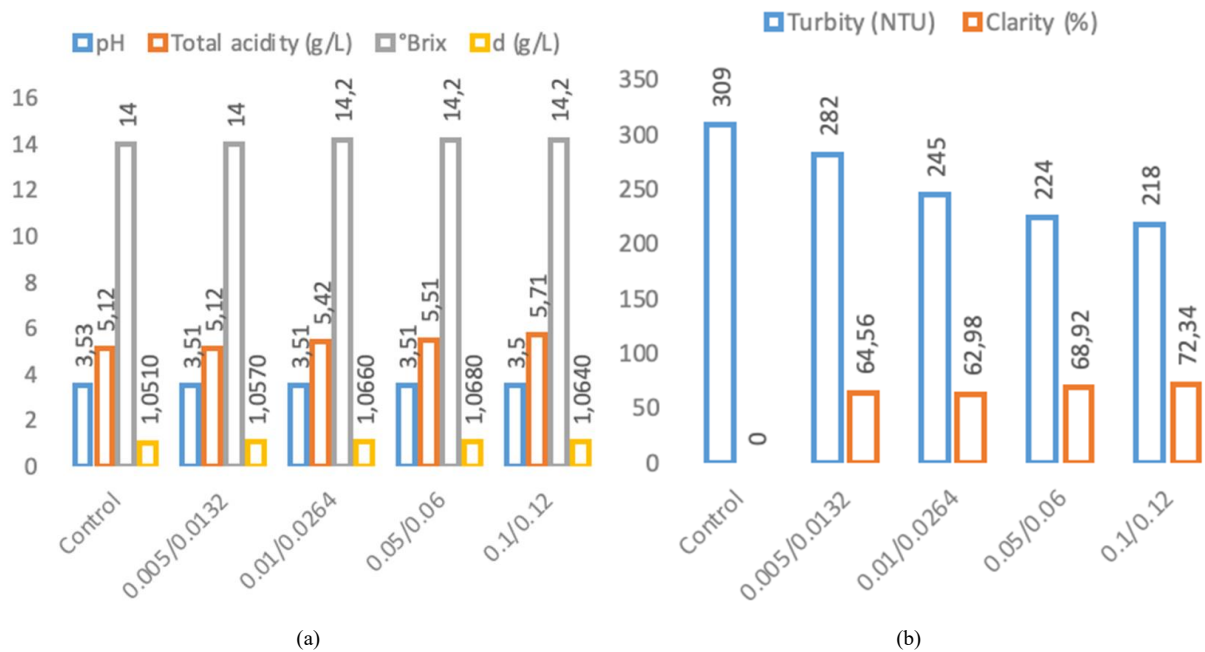


Figure 3. (a) Change in pH, total acidity, Brix, density depending on enzyme concentration, (b) Change in turbidity and clarity (%) (Incubation time: 1 h 30 min, temperature: 40 °C)

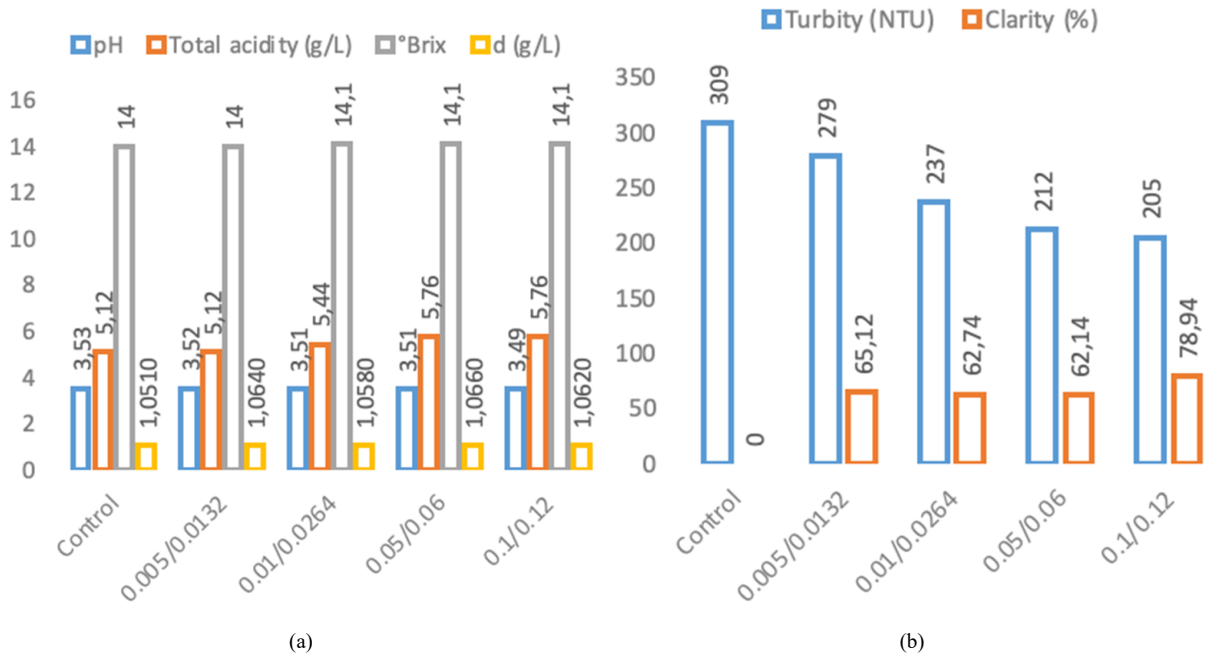


Figure 4. (a) Change in pH, total acidity, °Brix, density depending on enzyme concentration, (b) Change in turbidity and clarity (%) (Incubation time: 2 h, temperature: 40 °C)

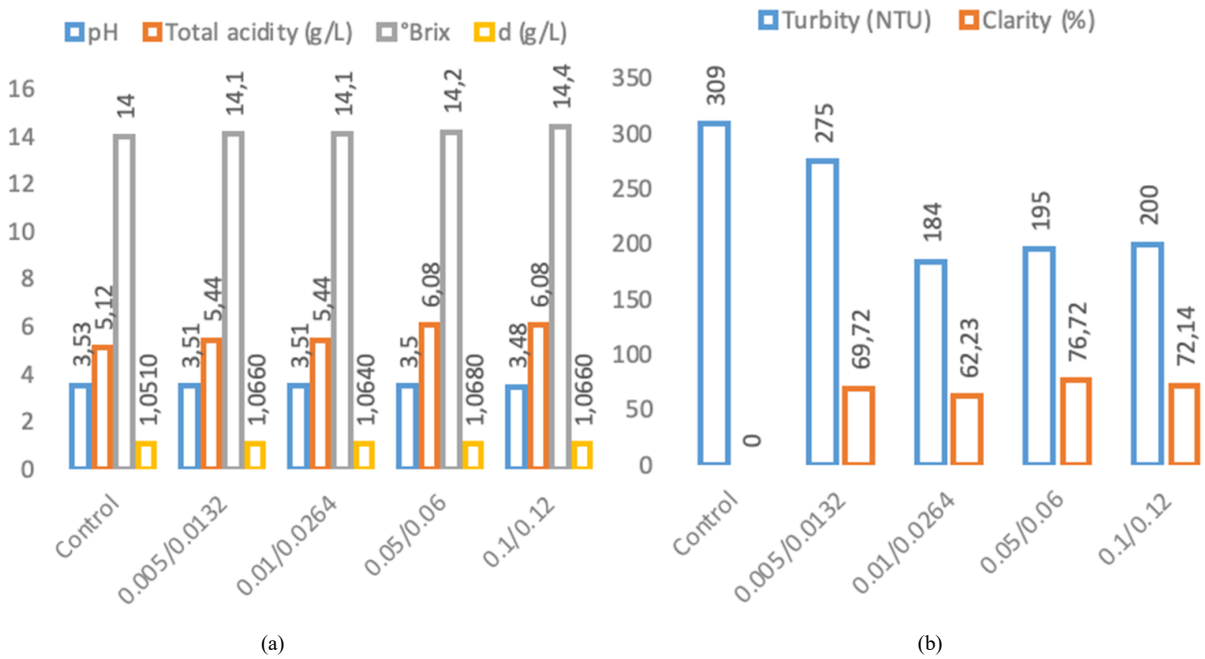


Figure 5. (a) Change in pH, total acidity, °Brix, density depending on enzyme concentration, (b) Change in turbidity and clarity (%) (Incubation time: 1 h 30 min, temperature: 55 °C)

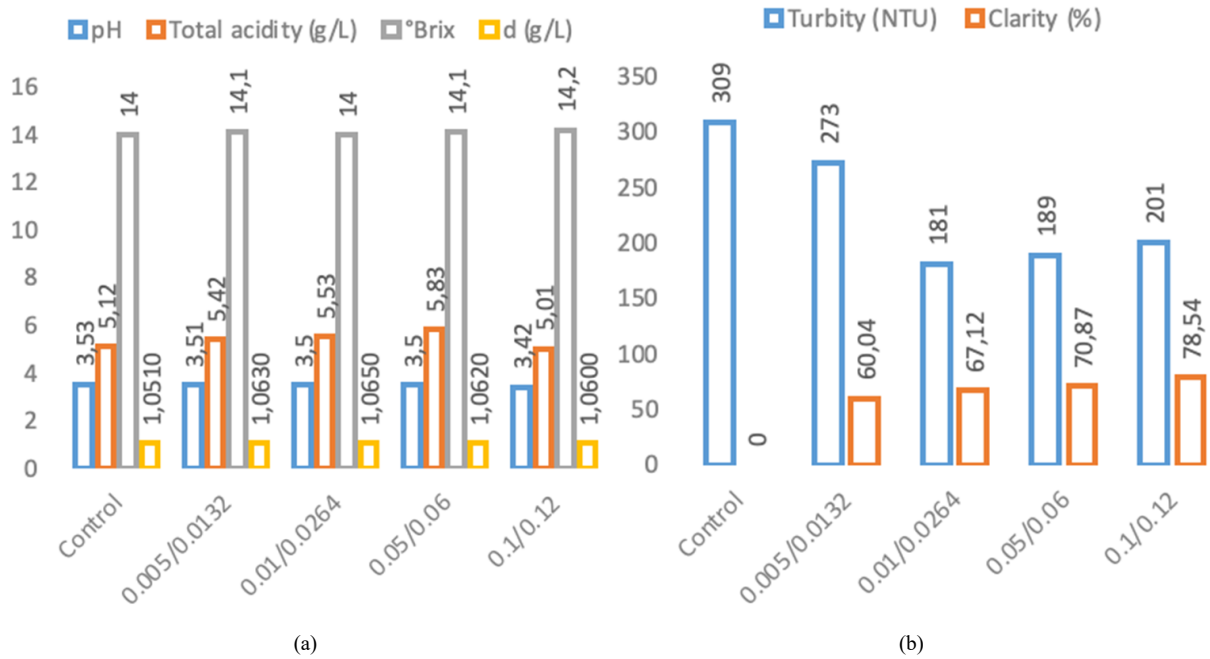


Figure 6. (a) Change in pH, total acidity, °Brix, density depending on enzyme concentration, (b) Change in turbidity and clarity (%) (Incubation time: 2 h, temperature: 55 °C)

Finally, the results obtained from the enzymatic treatment applied to the apple pulp show that the enzyme mixture of pectinase and amyloglycosidase has a significant effect on the viscosity of the apple pulp. It was determined that the viscosity before adding the enzyme solution to the apple pulp decreased from 30683 cp to 2000 cp. Therefore, it was determined that the viscosity decreased by approximately 90% (Table 2). In a scientific study conducted by Padma, P. et al. [23] on apple juice as a result of the application of pectinase enzyme solution, it was observed that the viscosity was reduced from 30000 cp to 400 cp. The viscosity results obtained in this study are similar to the results in the literature.

In the study, pectin test results applied to apple juice samples clarified by enzyme application at different incubation times and temperatures are given in Fig. According to the pectin test results, samples with sediment observed (+) and samples without sediment were indicated as (-). When the results are examined, it is seen that apple juice treated with enzyme for 1 h 30 min and 2 h at 40 °C did not form sediment in the samples treated with a high concentration of enzyme mixture. In these samples, it is seen that the pectin is completely broken down by the enzyme mixture. When the apple juice samples, which were treated with enzyme for 1 h 30 min and 2 h at 55 °C, are examined, it is observed that the pectin is completely degraded after the application of all enzyme concentrations (Fig. 7).

When the starch test (Iodine test) results are examined, it is seen that the starch test of apple juice samples treated with enzyme for 1 h 30 min and 2 h at 40 °C was negative only after high concentration enzyme mixture treatment.

When the results of the starch test applied to the samples prepared at 55 °C are examined, the test being negative for all enzyme concentrations indicates that the starch is completely broken down due to enzyme activity (Fig. 7).

Code	Pectin	Starch
A 1	+	+
A 2	+	+
A 3	-	-
A 4	-	-
B 1	+	+
B 2	+	+
B 3	-	-
B 4	-	-
C 1	-	-
C 2	-	-
C 3	-	-
C 4	-	-
D 1	-	-
D 2	-	-
D 3	-	-
D 4	-	-

Figure 7. Pectin and Starch test results of samples

4. Conclusions

The application of enzymatic solutions for liquid purification is a new approach in the liquid industry. This application was first used in Kosovo. In this study, standardized enzymatic purification conditions such as incubation time, temperature and enzyme concentration were investigated to increase the yield of purified apple juice as it is the most preferred juice by all consumers. Maximum cider yield can be achieved at 55 °C with an enzyme concentration of 0.05/0.11 g/L to 0.1/0.22 g/L for an incubation time of 1 h 30 min. A significant decrease in viscosity and a significant increase in apple juice quality were observed when enzymes were used together to remove turbidity from juice. We can say that the application of these applications during enzymatic treatment in the fruit juice industry will contribute significantly to better results.

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Competing Interest

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript. The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval

Not applicable.

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