Assessment of different enzymes on clarification of pineapple juice

A Thesis

submitted to the

UTTAR BANGA KRISHI VISWAVIDYALAYA

in partial
fulfillment of the requirements for the award of the degree of

MASTER OF SCIENCE (HORTICULTURE)
in
POST-HARVEST MANAGEMENT

by
NALANDA ACHARYA
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DEPARTMENT OF POMOLOGY AND POST HARVEST TECHNOLOGY
FACULTY OF HORTICULTURE
UTTAR BANGA KRISHI VISWAVIDYALAYA
PUNDIBARI, COOCHBEHAR, WEST BENGAL

2024

Dedicated to my late grandmother

Junu Chettri

I'll look up to you , as I go by !

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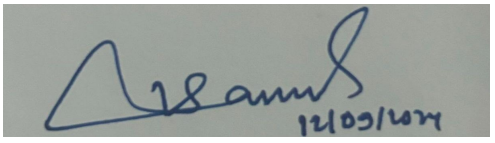
CERTIFICATE-I

This is to certify that the work incorporated in the thesis, entitled as "Assessment of different enzymes for clarification of pineapple juice" submitted by Ms. Nalanda Acharya in the partial fulfillment of the requirement of the Degree of Master of Science (Horticulture) in Post-Harvest Management of Uttar Banga Krishi Viswavidyalaya (UBKV), is a faithful and bona-fide research work carried out under my supervision and guidance. The results of the thesis have not been submitted for any other degree or diploma. Thesis has been checked against plagiarism and the similarity index has been achieved as 8% which is below the maximum tolerance range as per stipulation of this university .The assistance and help received during the course of investigation have been duly acknowledged.



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CERTIFICATE-II

This is to certify that the thesis entitled "Assessment of different enzymes for clarification of pineapple juice" submitted by Ms. Nalanda Acharya in partial to Uttar Banga Krishi Viswavidyalaya, Pundibari in partial fulfillment of the requirement of the degree in **Master of Science (Horticulture) in Post-Harvest Management** has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner. The committee recommends the thesis be accepted for the award of the degree in **Master of Science (Horticulture) in Post-Harvest Management.**

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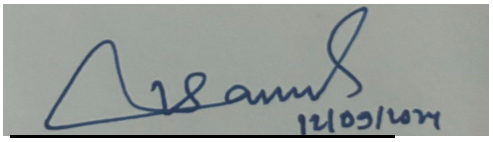
ABSTRACT

The demand of fruit juices is increasing rapidly as a result of increasing health awareness among the people. Most of the juice extraction processes are not producing satisfactory quantity and quality of juices. The experiment was carried out with thirteen treatments and four replications for each in completely randomized design and was conducted at the Departmental laboratory of Pomology & Postharvest Technology, Directorate of Research and in the Central Instrumentation Centre, Uttar Banga Krishi Vishwavidyalaya, Pundibari, Cooch Behar, West Bengal during 2023 to 2024. Only 33 percentage of pineapple comprises the pulp while remaining 67 percentage of the fruit is effectively wasted. Given the scarcity of resources and the high global population in this day, it is only smart to use existing resources to the fullest extent possible. Hence the study "Assessment of different enzymes for production of clarified pineapple juice" was taken up to develop processing of clarified pineapple juice with the incorporation of the peel in the extraction process. Pineapple pulp along with peel was treated with pectinase enzymes at various concentrations (0.05 percentage and 0.075 percentage) and incubation period (3 and 4 hours), cellulase and hemicellulase enzyme at various concentrations (0.01 percentage and 0.015 percentage) and incubation period (3and 4 hours) and a comparison of the treatments with control has been conducted where there was no addition of enzymes. The effect of enzymatic conditions on total soluble solids(ºB), juice recovery, titratable acidity, reducing sugar, total sugar, L a* b* Hunter colour values, sedimentation, hue angle, chroma value, colour difference(ΔE), transmission and consistency of processed juice were studied. Results of the study suggest that treating pineapple juice samples with pectinase enzyme where 0.05 percentage concentration with

3 hours incubation is best for obtaining highest juice recovery percentage of 86.88 and 0.075 percentage concentration with 3 hours incubation is best for obtaining b* value of 28.84 and 0.075 percentage concentration with 4 hours incubation has shown to have highest acidity and total sugar percentage of 1.36 and 13.98 respectively. The effect of cellulase enzyme shows that concentration of 0.015 percentage with 4 hours incubation has obtained the highest result of 36.07 for L value, 9.51 percentage for reducing sugar and 10.90 for colour difference(ΔE),0.015 percentage concentration of cellulase with 3 hours incubation has obtained the maximum result of -1.50 for a*value, 41.42 percentage for chroma value and in terms of sedimentation percentage the maximum value of 11.93 percent is seen in control, 0.01 percentage concentration with 4 hours incubation has obtained the highest result of 15.16 (ºB) for total soluble solids. The hemicellulase enzyme with concentration of 0.015 percentage with incubation period of 4 hours has the best result of 8.58 percent for hue angle and 28.85 percent for transmission and 0.015 percentage concentration with 3 hours incubation has shown the maximum effect of 20.46 percent on consistency of the juice.

Keywords: enzyme clarification, pineapple juice, pineapple peel, pectinase, cellulase, hemicellulase.



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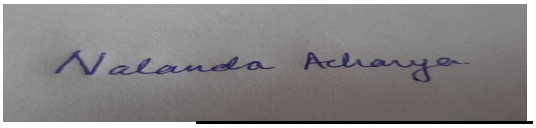

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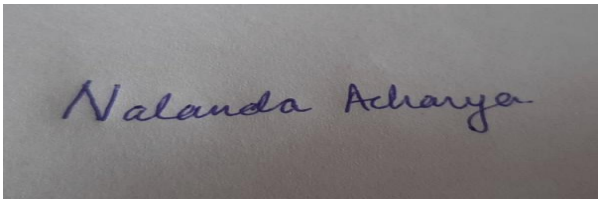

Nalanda Acharya

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ABBREVATION

%	Per cent
°C	Degree Celsius
am	Ante meridiem
CD	Critical difference
cm	Centimeter
et al.,	Co-workers
g	Gram
i.e.,	That is
kg	Kilogram
m	Meter
mm	Millimeter
MT	Metric tones
ns	Nonsignificant
S. Em (±)	Standard error of mean
TSS	Total soluble solids
µL	Microlitre

CHAPTER-1

INTRODUCTION

INTRODUCTION

Pineapple (*Ananas comosus*) of the Bromeliaceae family is a fruit crop that is widely grown for commercial purposes worldwide. Because of its superior flavour and taste, it is called the "queen of fruits" (Baruwa, 2013). Pineapples can be stored and eaten or served raw, cooked, or juiced. This fruit is seasonal and extremely perishable. Mature fruit has 14 percentage sugar, good levels of citric acid, malic acid, vitamins A, and B, as well as bromelin, an enzyme that breaks down proteins (Joy, 2010). The pineapple is a delightful tropical fruit that offers a wealth of health advantages, remarkable juiciness, and a lively tropical flavour. Pineapples are rich in minerals, water, crude fibre, vitamin C, Ca, K and other nutrients that are beneficial for the digestive system, and helps maintain a healthy weight, and supports balanced nutrition (FAO STAT 2011).

Pineapple is a monocotyledonous perennial plant that grows well in neutral to slightly acidic soil with a pH of 4.5 to 6.5. It also grows well in sandy loams, clay soils, and acidic loams. In terms of climate, pineapple grows best in hot, muggy climates with bright days and cool nights (Hossain, 2016). Right behind citrus fruits and bananas, it is currently the third most consumed fruit. FAO estimated in 2022 that nearly 28 million tons of pineapple are produced annually worldwide, with China, the Philippines, Costa Rica, Brazil, and Indonesia being the top producers.

Smooth Cayenne, Queen, and MD2 are the most widely grown pineapple varieties and they account for about 80 percentage of the world's pineapple commerce (Hossain, 2016). India comes in fifth place worldwide, in terms of pineapple output and West Bengal, Assam, Kerala, and Karnataka are the top producers. In the year 2022-2023, the country's total area and production for pineapple were 109.00 ha and 1856.000 MT respectively (Anon., 2023)

Pineapple is used to make a wide range of goods, such as dried pineapple, pasteurized pineapple juice, concentrate, and canned pineapple slices (Khalid *et al.,* 2016) However, fresh pineapple juice is the most well-liked of all these items due to its flavour, aroma, and many useful qualities (Rattanathanalerk *et al.,* 2005)

Pineapple juice consists of minerals, vitamins, and polyphenols in abundant amount (Elkins *et al.,*1997). It also contains high quantities of antioxidants, including phenolic compounds and vitamin C (Hossain and Rahman, 2011). In addition to imparting flavor, color, and oxidative stability to fruits and vegetables, phenolic compounds have also

been connected to inhibitory effects on hydrolytic enzymes, anti-inflammatory effects, and antioxidant activity through the scavenging of free radicals in human cells (Naczk and Shahidi, 2004).

Production of any fruit juice beverage is basically done by pressing or extracting the natural liquid found in fruits and vegetables. It contains plant bioactive and micronutrients in addition to free sugars. Pressing the pulp of fresh pineapple segments yields pineapple juice which contains plenty of vitamins and minerals and Vitamin C content in fresh pineapple juice that ranges from 9.2 to 93.8 mg per 100 ml (Kabasakalis, 2000). Pineapple juice contains potassium, magnesium, phosphorus, iron, and manganese among other key minerals and it can be found as concentrates, blended juice, or transparent juice with other fruits.

Today's methods for fruit juice production have made the use of enzymes a clear requirement. Production of a clear and aesthetically pleasing final product, acceleration of the extraction of juice from raw materials, and efficiency boost processing (pressing, solid settling or removal of enzymes) are the primary purpose of producing any fruit juice. Enzymes have been utilized for as long as humans have processed food.

In this day and age, pectinases, cellulases, and hemi-cellulases are the enzymes that are used for the improvement of fruit and vegetable juice pressing, extraction, and clarification. Enzymes in the food industry have three fundamental uses and they are to regulate food quality, employ them as food additives and to alter the physico-chemical characteristics of both the food and some of the food additives.

The weight composition of the different pineapple pieces comprises of pulp (33 percentage), peel (41 percentage), core (6 percentage) and crown (20 percentage) (Medina and García, 1999) This implies that just 33 percentage is available to the juice and canning industries (assuming full use). But if the juice industry could use the 41 percentage from the peel, it might generate a significant profit while also lowering the quantity of waste generated therefore the current experiment has got to do with the extraction of pineapple juice with its peel intact i.e., without having the peel removed.

The juice extracted out of the fruit from which the peel has not been removed may not be as aesthetic to look at as compared to the juice extracted after removal of its peel but if the nutritional value, overall quality standard and ultimate health benefits derived from the juice is not found to be compromised, then the fruit processing industries may economically be benefitted owing to the elimination of the step of peel removal which in turn reduces time requirement, labour cost and peel related waste materials.

Considering the above, the research proposal entitled “Assessment of different enzymes on clarification of pineapple juice’’ is undertaken with the following objective:

1. To evaluate the enzyme assisted juice clarification process from unpeeled pineapple on quantity and quality of extracted juice.
2. To find out the concentration of different enzymes and incubation time for the production of clarified pineapple juice.

CHAPTER-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Several researchers have reported the assessment of various enzymes in the process of juice clarification and a brief review of some of those research works about the present study is discussed as follows:

2.1 Importance of Pineapple

According to Afzal F (2019), he stated that Pineapple which is scientifically called *Ananas Comosus* of the Bromeliaceae family is derived from the Tupi word 'nanas' meaning 'excellent fruit'. This fruit is very delicious and has excellent flavor and nutritive value. Though it has different regional names such as Keehom (Manipur), Ananus (Marathi), Annasahannu (Kannada), Anasipazham (Tamil), Kaitachchakka (Malayalam), etc. it is popularly known as Ananas in almost all parts of India. It is also rich in vitamin C and also a good source of other vitamins i.e. A & B. It contains a special enzyme called 'Bromelin' which helps in the digestion of protein.

As per GOI (2014), around 23.61 million tonnes of pineapple are produced globally at the moment by 85 nations. Thailand accounts for around 11.22 percent of the global pineapple production, making it the most productive country in the world. In the international rankings of countries that produce pineapples, India came in at number seven in 2013–14.

According to Ndungu (2014), The smooth Cayenne cultivar is widely grown in numerous tropical nations, including Hawaii, the Philippines, Australia, South Africa, Puerto Rico, Kenya, Mexico, Cuba, and Formosa. He also added that there are hundreds of types and out of all, most popular ones are Smooth Cayenne and Queen. In the last ten years, there has been a development of a new variety which is called and it now accounts for 80 percentage of the world's pineapple commerce.

According to (Debnath *et al.,* 2012). Ascorbic acid is found in pineapple juice, which is also a potent source of vitamin C. Ascorbic acid, also known as vitamin C, is a potent antioxidant that also helps the body absorb iron, which helps fight off viral and bacterial infections. Half a cup of pineapple juice provides half of an adult's daily necessary vitamin C intake. Manganese, a trace mineral essential for bone formation and the synthesis and activation of particular enzymes, is one of the many essential minerals found in pineapples. It lowers blood pressure, regulates heart rhythm, and aids in the absorption of iron.

According to research by Kader *et al.,* (2010), the Honey Queen pineapple variety performs better than the Giant Kew type in terms of sweetness and nutritional value. The Giant Kew cultivar had 3.68 percentage total sugar, 1.75 percentage non-reducing sugar, and 6 percentage total soluble solids (TSS). On the other hand, the Honey Queen variety has a TSS of 10 percentage, a 4.84 percentage total sugar content, and a 1.59 percentage non-reducing sugar content. While Honey Queen has a higher concentration of all the minerals, Giant Kew has a higher level of vitamin C. The Honey Queen contains more calcium than the Giant Kew.

Global production began around the fifteenth century, according to Medina *et al.,* (2005). Pineapples were found throughout the world's tropical regions even in Europe. The most popular type, Smooth Cayenne (Cayena Lisa), was originally brought to Europe from French Guyana. The world's tropical regions are the primary locations for pineapple cultivation. Over 2.1 million acres are planted with fruit, and it is grown in over 82 countries. She added that commercial pineapple canning did not begin in Hawaii until the late 1800s. The majority of the remaining fruit (50 %) is supplied by these nations, who are also significant producers including Nigeria, Kenya, Indonesia, Mexico & Costa Rica.

Dull *et al*. (1971) state that the edible portion of pineapples has been the subject of the majority of research, and that the fruit's moisture content varies from 81.2 to 86.2 percentage. It also contains 13–19 percentage total solids, the main ones being fructose, glucose, and sucrose. Carbohydrates make up up to 85 percentage of total solids, while fiber makes up only 2-3 percentage. The most common organic acid in it is citric acid. The pulp has a low content of nitrogenous materials, lipids (0.1 percentage), and ash 25 percentage to 30 percentage of nitrogenous materials

2.2 Significance of fruit juices

According to Lozano *et al.,*(2006) juice is primarily made up of water, soluble solids (such as sugars and organic acids), chemicals that give juice its aroma and flavour, vitamins, minerals, pectic substances, pigments, and to a very minor extent, proteins and lipids. Fruits lose acidity and starch as they ripen, while their sugar content rises. Fruit's cellular juice is extracted, either by pressing or diffusion, to produce juices, which are goods for immediate consumption. Bhat (2000) asserts that the manufacturing of fruit and vegetable juices is significant for both business & public health reasons. Thus, marketing of their juices helps to ensure that a broad variety of customers have access to healthy components derived from fruits and vegetables throughout the year. Fruit and vegetable juice manufacturing calls for techniques for extraction, clarity, and stabilization.

2.3 Use of enzymes in juice clarification

Camara *et al.,* (2022) studied the pectinase-clearing capacity of three strains of *Yarrowia lipolytica* (Tias J0-6; YA J3-1; Buy J2-1) that were found for fermentation of cocoa juice. Fruit juices were subjected to enzymatic treatments to clarify them, with the free enzymatic treatment juice serving as a reference. The clarified juices' antioxidant activity, as well as their physicochemical and phytochemical properties, were analyzed. The Tias J0-6 strain was found to have the best clarifying activity on pineapple and orange juices out of the three strains that were tested. The clarified juices showed declining viscosity and pH levels and rising clarity values. For the clarified pineapple and orange juices, pectinase of Tias J0-6 permitted improving clarity and decreasing viscosity

Santana *et al.,* (2020) stated that crude extract containing cellulase from Pseudozyma sp. produced by liquid fermentation was applied to tangerine juice. By varying the temperature and duration of incubation, crude extract was used to examine the thermal stability of cellulase. The findings demonstrated that the Pseudozyma sp. cellulase is thermostable at 60, 70, and 90 °C and retained 98 percentage, 88 percentage, and 80 percentage of activity, respectively, over a one-hour incubation period. Through adjusting the enzyme extract concentration (percentage, v v-1) and the shaker duration (minutes) at 150 rpm and 50°C, the ideal conditions for clarity were confirmed. Tangerine juice's viscosity was reduced by 65 percentage when the ideal conditions for clarity were reached in the 80th minute at a 1.25 percentage enzymatic extract concentration (v v-1). The examination of the tangerine juice's physical and chemical characteristics upon clarification revealed that the enzyme extract enhanced the procedure that led to the juice's clarification. Given that this technology can be applied in the citrus juice industry, the results are encouraging.

As stated by Cerreti *et al.,* (2016) pectinases were applied, and the fruit juice's clarity and yield increased while its nutrients, color, and flavor were retained. The effects of pectinolytic and/or proteolytic clarifying treatment on haze-causing compounds and turbidity in pomegranate juice were investigated in this study. The combination of pectinase and protease enzymes together showed a strong and synergistic effect, yielding the greatest results in terms of juice turbidity levels and possible haze generation. The data indicated that although pectinolytic and proteolytic treatments did not modify the total amount of pectins, proteins and phenols, they affected the haze forming activity of turbidity- causing molecules.

According to Jori *et al.,* (2015) to prepare clarified blended juice, different amounts of

cellulase (0.15-0.75 percentage) and pectinase (0.2-0.6 percentage) were added throughout the course of a one-hour treatment period at variable temperatures (35-55ºC). The pulp was treated with 0.34 percentage cellulase and 0.5 percentage pectinase enzymes in combination at 45.5ºC for one hour in order to reach the ideal conditions for clarified blended pulp with 81.92 percentage clarity and 88.53 percentage yield.

Arsad *et al.,*(2015) studied to ascertain the impact of various enzymatic treatments on the processing of sugar palm (*Arenga pinnata*) fruit juice. Novozymes Cellulase and Pectinex Ultra SP-L, were used to treat sugar palm fruit purees both separately and in combination. The enzymes were added at a concentration of 0.05 percentage (w/w), and the mixture was incubated for 60 minutes at 45°C. The outcomes demonstrated a substantial decrease in the juices treated with enzymes relative to the untreated juice in terms of proximate content, including crude fiber, crude protein, and carbohydrates. Additionally, the enzyme treatment boosted the yield, TSS, sugar content, L value, and promoted juice clarity while lowering the ascorbic acid and viscosity of the juice.

Kumar and Sharma (2015) studied that in order to improve the pineapple (*Ananas comosus*) mill juice's quality in terms of filtering rate, clarity, relative viscosity, and percentage overrun, the mill juice was degumming using both commercial and crude enzymes in the current study. The effects of temperature (35–55 C), time (210–540 min), and concentrations of cellulase (25–125 mg/100 ml), pectinase (25–150 mg/100 ml), and hemi-cellulase (25–150 mg/100 ml) on the filtration rate, clarity, relative viscosity, and percentage overrun of the pineapple mill juice were examined According to the study, the following parameters for the enzymatic treatment were recommended: temperature 46 °C, time 455 min, concentrations of hemi-cellulase (78 mg/100 ml), pectinase (141 mg/100 ml), and cellulase (124 mg/100 ml). The mill juice samples were subjected to the same time and temperature parameters for the crude enzyme treatment. A principal component analysis was used to examine the differences between samples treated with commercial and raw enzymes. According to the findings, the commercial enzymes were not as effective as the crude enzyme in degumming pineapple mill juice.

Kohli *et al.,* (2015) state that pectinases are produced during the ripening process of fruits, and they function by rupturing glycosidic linkages to convert polygalacturonic acid to monogalacturonic acid. During this process, fruit cell walls become softer and the fruit's production of juice increases. While alkaline pectinases have economic and environmentally favourable commercial applications, acidic pectinases are mostly used in fruit juice processing (i.e., extraction and clarity). Industrial applications have made

use of pectinolytic bacteria to produce pectinases, which are environmentally benign enzymes that mineralize environmental pectic materials. The ability of pectinase to withstand harsh conditions during infection is attributed to its distinct catalytic machinery. Research on the pectin degradation pathway has led to an understanding of the roles played by various pectinases in the subsequent stages of substrate digestion in various cellular compartments. There is no doubt that the pH affects how an enzyme behaves. Fruit juice extraction and clarifying are two applications of acidic pectinases, particularly in the food processing industry.

Pectins also add to the viscosity and turbidity of fruit juice, according to Giacobbe *et al.,* (2014). When fruits with high pectin content are mechanically crushed, the result is a highly viscous fruit juice that stays attached to the fruit pulp. Pressing or other mechanical processes are not easy to use to obtain this fruit juice. Because pectin is involved in the crosslinking of cellulose and hemicellulose fibers, pectinases aid in improving cellulases' ability to reach their substrates.

According to Tapre and Jain (2014), pectinolytic enzymes are crucial enzymes in the food business, particularly when processing fruit juice since they are necessary to achieve stability and clarity and the production of different kinds of juices has increased as a result of the usage of enzymes in the juice business. One of the major categories of enzymes utilized in the fruit processing business is pectinolytic and these enzymes convert complex plant tissue polysaccharides into simpler compounds like galacturonic acids. The production of different kinds of juices has increased as a result of the usage of enzymes in the juice business. By breaking down the pectin and enabling the suspended particles to settle, enzymatic processing not only makes the juice transparent, but it also gets rid of undesired color, aroma, and stability changes.

According to Ucan *et al.,* (2014) lemon juice from the Interdonato type was subjected to various enzyme treatments at specified concentrations throughout the depectinization process. After the enzyme treatment, the total pectin concentration of lemon juices rapidly reduced and was no longer detectable after filtration. Following pulp separation, viscosity values dropped; the biggest drop was seen with the filtration.

Sharma *et al.,* (2014) studied that the enzymatic degradation of the biomaterial in clarification depends on the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations. The use of enzymes like cellulases, pectinases, and amylases alone, or in combination, can give better juice yields with superior quality fruit juices and he concluded that pectinase

enzyme can give maximum juice yield i.e., 92.4 percentage at 360 minutes incubation time, 37 C incubation temperature and 5 mg/100gm of enzyme concentration. Whereas the combination of two enzymes i.e., pectin methyl esterase (PME) and polygalacturonase (PG) at 120 minutes of incubation time, 50^{o}C of incubation temperature and 0.05mg/100 gm of enzymatic concentration can give the maximum yield of 96.8 percentage in case of plum fruits.

Amulu *et al.,* (2014) studied the quality of combined pineapple and pawpaw blended fruit juice, along with the effects of temperature and enzyme content. The effects of process factors on quality revealed that the ideal ranges for temperature and enzyme concentration were 50–70 °C and 0.12–0.18 w/v, respectively, for superior blended juice quality. Better quality was found in a blend of 60 percentage pineapple juice and 40 percentage pawpaw juice. This demonstrated how well pawpaw and pineapple juices may combine to create a premium drink.

Kaur and Sharma (2013) In order to maximize the output and clarity of carrot juice, a combination of crude pectolytic and cellulolytic enzymes was used as a pretreatment before the juice was extracted. At first, the enzymatic treatment resulted in considerably increased acidity, total soluble solids, and β-carotene content when compared to the control. The enzymatically treated juice samples had lower pH, viscosity, and pectin content. While decreasing sugar concentration and pH increased after storage, β-carotene, pectin content, and total acidity decreased. The total color difference and browning index (BI) rose as a result of color parameters L- and a- decreasing and b- slightly increasing. At both temperatures, the L-, a-, b-value, BI, and β-carotene were described using zero- and first-order kinetic models. Juice treated with enzymes was shown to be more palatable after three months at 5±1°C in the refrigerator, according to sensory analysis.

The effects of enzymatic treatment on pineapple (*Ananas comosus*) juice output, viscosity, and clarity were investigated by Kumar *et al.,* (2012).The incubation temperature (47°C),incubation duration (446 min), and enzyme concentration (0.14mL/50g of pulp) were the ideal parameters for the enzymatic treatment. In terms of improving the quality and recovery of pineapple fruit juice, the crude enzymes were competitive with the commercial enzymes. Principal component analysis was used to make the comparison in an optimal setting.

Sandri *et al.,* (2011) stated that evaluation of the effectiveness of commercial and laboratory-produced fungal pectinolytic preparations used in the clarification of apple, butia palm fruit, blueberry, and grape juices. In order to compare two crude enzymatic

extracts produced by *Aspergillus niger* T0005007-2 (TE1) and *Aspergillus oryzae* IPT 301 (TE2) to the commercial preparations Pectinex Clear and Pectinex BE Colour, which are used for the clarification of clear (apple, butia palm fruit) and dark (blueberry and grape) juices, respectively, the extracts were tested in solid-state and submerse cultures. Reactions with pectinases total activity at 1 U/mL of fruit juice were carried out for 30 and 60 minutes at 30 and 50 degrees Celsius. While there was no obvious correlation between temperature increase and increased clarity, time increased led to better clarification. The apple and blueberry juices clarified similarly when the crude preparation TE1 was used, as opposed to the commercial products. However, TE1 showed the best results for butia palm and grape juice, indicating the commercial application potential of *A. niger* T0005007-2 enzymes.

Tochi *et al.,* (2009) studied that the two commercial enzyme preparations (derived from *Aspergillus niger*) were utilized separately or in combination in order to extract pineapple juice in two steps. Determining how the enzyme preparations affected the extraction process in comparison to the control, measurements were made of the percentage of juice recovered, soluble sugars, total phenolics, titratable acidity, viscosity, and turbidity of the recovered juice. A five-b point hedonic scale was used to judge the acceptability of the ready-to-serve pineapple juice (RTS).

Shah and Nath (2007) treated Litchi pulp with varied enzyme doses for 30 to 150 minutes at 45°C. Before being microfiltered, pineapple juice was treated enzymatically for 60 minutes at 30°C using 0.03 percentage (v/v) of two enzymatic preparations ; Celluclast 1.5 L and Pectinex SP-L.Additionally, they discovered that the percentage of recovered pineapple juice, particulates, and sensory attributes were all affected by pectinase and pectinase/hemicellulases enzyme preparations throughout a 30-minute period at temperatures of 35, 37.5, and 40°C. Enzymatic treatment resulted in a considerable decrease in perceived viscosity and a rise in juice yield, clarity, and TSS.

According to Kareem and Adebowale (2007) orange juice was clarified using the crude extract containing pectinase. Ascorbic acids, total soluble solids, pH, turbidity, viscosity, and total titratable acidity of the cleared juice were measured. While the turbidity and viscosity of the juice decrease as the quantity of pectinase enzyme increases, the optimal yield of juice (97 percentage) was achieved at 1 percentage pectinase enzyme concentration. The juice treated with pectinase enzyme did not exhibit any significant changes in pH or total titratable acidity. The concentration of pectinase enzyme increases with ascorbic acid and total soluble solids. The pectinase enzyme concentrations on the

orange juice's yield, viscosity, turbidity, and total titratable acid showed significant differences but there was no significant change in the pH, ascorbic acid, or total soluble solids. Citrus peel was shown to be a substrate for pectinase production in the results, and using this enzyme to clarify orange juice could improve fruit juice processing in tropical climates.

According to research by Sin *et al.,* (2006), the enzymatic treatment increases the juice's clarity. Part of the positively charged protein beneath is exposed at a faster rate of clearing due to an increase in enzymatic concentration. As a result, there is less electrostatic repulsion between cloud particles, which leads to the accumulation of bigger particles and ultimate settle-out. It was discovered that when the concentration of the enzyme increased, the absorbance value dropped. At the maximum dose of enzyme, the absorbance value for clarity was lowest. A clearer juice was indicated by lower absorption values. By exposing a portion of the positively charged protein underneath, an increase in enzyme concentration may speed up the process of clarity. This is because it will lessen the electrostatic repulsion that causes cloud particles to clump together to form larger particles and finally settle out.

Mantovani *et al.,* (2005) stated that the four specific fungal strains' culture filtrates were used to test the pectic enzyme activity. All fungi produced pectin lyase (PL), although the strain *Penicillium expansum* F16 did not exhibit pectinesterase activity in culture filtrates. A rise in transmittance at 660 nm indicated that the apple juice had been well-clarified as a result of the use of crude enzymes.

Santin *et al.,* (2004) added that cloudiness, mostly caused by the presence of pectin is one of the main issues faced during the manufacturing of fruit juices. Plant polymers and cell detritus are molecular structures that resemble fibers and can be linked to pectin. With the exception of enzymatic de-pectinization, it is challenging to eliminate the cloudiness they produce. Enzymes have the potential to increase yield and facilitate the clarification of many juices. More juice may be extracted per ton of fruit because enzymes break down pectin or cell walls. An enhanced juice yield and better color extraction can occasionally be achieved by adding an enzyme complex, producing a premium product.

According to Chadha *et al.,* (2003), the yield of juice obtained from the juice extraction techniques can be enhanced by combining them with different pre-treatments, such as enzymatic, hot, and cold extraction. The effects of temperature, incubation duration, pectolytic and cellulolytic enzyme concentrations, and their ratios, on the enzymatic hydrolysis of carrot mash were investigated. The carrot mash was treated at 3.5–55°C for

50–90 minutes, with an enzyme protein content of 0.2–0.4 mg/kg. Pectolytic and cellulolytic enzymes were combined in a 3:7–7:3 ratio. The findings indicated that every parameter had a substantial ($P<0.01$) impact on the amount of pectin, crude fiber, and juice output. Incubation time and enzyme ratio had less of an impact than incubation temperature and enzyme concentration. Juice yield rose by five to ten percent. Enzymatic hydrolysis caused the juice's viscosity to drop while its pH, color index, and total solids all increased.

According to Vaillant *et al.,* (2001), after partial enzymatic liquefaction, six tropical fruit juices (mango, pineapple, naranjilla, Castilla's blackberry, passion fruit, and tangerine) were subjected to microfiltration using a mineral tubular membrane with a nominal pore diameter of 0.2 lm. It was discovered that there is a volumetric reduction ratio (VRR) for passion fruit juice at which the overall costs of manufacturing clarified juice are at their lowest.

According to a study by Sreenath *et al.,* (1994) the primary goals of the dual extraction of pineapple pulp and residue were the recovery of soluble solids, particulate matter, and reduced acidity juice. When compared to the untreated samples, the viscosity of the pineapple juice treated with a pooled enzyme was reduced. The breakdown of pulp residue as a result of celluclast or pectinex treatment stayed relatively constant. Similar to untreated samples, the RTS juice made from pineapple pulp or residue treated with celluclast or pectinex was deemed satisfactory on a five-point Likert scale.

Dzogbefia *et al.,* (2006) studied for six days, yeast was cultured in papaya juice with 1 percentage pectin added in the lab to manufacture the enzyme. The amount of free-run juice recovered in each treatment was compared with a control sample. Measured weights of papaya mash were mixed with known amounts of enzyme preparation for varied reaction times. The flow rate of free-run juice increased quickly after 200 g of papaya mash was treated with various dosages of the pectic enzyme extract. When initial rates were assessed, the mash treated with 32 mg of total protein extract and a 30-min reaction period was the best for a maximum rate of juice flow (25 ml/min). The treated samples yielded a flow rate that was more than twice that of the untreated samples when juice flow was observed over a period of six minutes.

According to Granada *et al.,* (2001), pectinolytic enzymes were employed for the purpose of extraction by cold pressing the clarified blackberry juices (Rubus spp. L.) of three distinct cultivars: Guarani, Tupi, and Brazos to increase yield by comparing the extracted juice with the juice that did not contain enzymes. Following clarity, the juices' chemical

and organoleptic properties were examined and discovered that the use of the enzymatic preparation significantly increased juice extraction for the three cultivars and for the control. Study revealed that the use of the enzyme was rather effective, resulting in increase in extraction

According to Sreenath & Santhanam (1992), a commercial pectinase derived from *Aspergillus niger* that contained several polysaccharidess was able to breakdown the grape mash and clarify the white grape juice to a degree of 98–99 percentage. This was accomplished without adjusting the mash pH by optimizing the grape mash treatment with 0.048 percentage of enzyme at 27–30°C for 30 rains. Following pectinolytic juice clearing, there was a reduction in juice viscosity and total phenols. This commercial preparation has an advantage over the other enzymes since it perfectly removes turbidity, reduces juice viscosity, and breaks down the protopectin haze complex. However, the origin, kind, and purity of the enzymes as well as fruit varietals, ripening, and haze components all affect how effective pectinolytic clarifying is.

2.4 Impact of enzyme concentration and incubation time on clarification

Ghorband and Joshi (2023) found out how the quantity of enzymes, duration of incubation, and temperature affected the extract yield, TSS, ascorbic acid, and viscosity of the juice from dragon fruit (*Hylocereus polyrhizus*). Three distinct concentration ranges, as well as incubation times and temperatures—38–380 IU, 90–150 min., and 30–60 °C were used for pectinase-assisted enzymatic extraction. The clarified juice samples were subjected to analyses to extract yield, TSS, ascorbic acid, and viscosity. It was discovered that 380 IU of enzyme concentration, 45 °C of incubation temperature, and 120 minutes of incubation duration were the ideal parameters for pectinase enzyme treatment for dragon fruit juice. While TSS and total phenol increased only little, the results demonstrated a considerable improvement in extract yield.

Kumar and Singh (2019) studied the commercial enzymes pectinase, cellulase, and hemicellulase were added to guava juice at varying concentrations, and the mixture was incubated for 30-150 minutes at 55°C. The guava juice was treated simultaneously with all three enzymes at compromised process parameters (Pectinase 1.00 percentage, Cellulase 0.50 percentage, and Hemicellulase 0.80 percentage for 90 minutes at 55 0C). The result was clarified juice with a turbidity of 18 NTU and a yield of 62. The range of variables for enzymatic treatment conditions (enzyme concentration: 0.20-1.40 percentage w/w, 0.20-0.80 percentage w/w, and 0.20-1.00 percentage w/w for pectinase, cellulase, and hemicellulase, respectively), as well as the incubation time (30-150 min)

and temperature (55 ºC) were determined based on previous individual experiments, the results of which were extremely similar to those of previous experiments, which had yielded optimum values of 0.96 percentage, 0.57 percentage, and 0.77 percentage for reported in earlier studies, which provided the ideal values for the enzyme concentrations of pectinase, cellulase, and hemicellulase, respectively, as well as the ideal values for an incubation duration of 99 minutes at a temperature of 55 ºC. In these circumstances, the juice yielded 64.7 percentage and had a turbidity value of 17 NTU.

According to Kumar *et al.* (2014) studies that at various temperatures (37–75 °C), the activities of free and immobilized xylanase were measured. But when the temperature rose, the immobilized enzyme's activity increased up to a maximum before declining.

According to Abdullah *et al.* (2007), the effects of the enzymatic treatment conditions - incubation duration, temperature, and enzyme concentration on physical attributes like turbidity, clarity, viscosity, and color were simultaneously analyzed using response surface methodology (RSM). Pectinase enzyme was added to carambola fruit juice and incubated for several amounts of time (20–100 min), at varied temperatures (30–50 C), and at different enzyme concentrations (0.01–0.10 v/v percentage). The effects of these three factors on turbidity, clarity, viscosity, and color were assessed as independent variables. The majority of the dependent variables were significantly impacted by the enzyme concentration, suggesting that this was the most significant factor influencing the features of the carambola fruit juice.

According to Roldan *et al.* (2006) the study examined the impact of Trichoderma hydrolytic enzymes, namely pectinase, cellulase, chitinase, and/or glucanase, on the clarification of juice, fermentation process, and the ultimate characteristic of young wine. Additionally, the super extraction and health stage of Palomino fino grape were examined. The system used to collect the juice (frozen or fresh grape juices) and the grape sanitary stage (healthy or contaminated) determined how the enzymatic treatment affected the clarity of the juice. The application of enzymes in contaminated juice had the maximum efficacy. The enzymatic preparations had no effect on the kinetics of fermentation, although they did cause a decrease in turbidity. Furthermore, regardless of the use of enzymes, the greatest variations in wine characteristics were seen when comparing wines from juices that were subjected to various conditions (healthy or diseased, frozen or fresh). Wines containing higher alcohol content and more acidity, such as methanol, propanol, and isobutanol, were produced from supraextracted juices.

When Riu-Aumatell *et al.,* (2005) assessed the volatile content of pear, apricot, and peach

juices, they found that the flavor was improved by the enzymatic process. Due to the samples' high polysaccharide content, which may have enhanced the preservation of volatile molecules, peaches had the lowest concentration of volatile molecules. The polysaccharides segregated by molecular weight and the amount of soluble polysaccharides were altered by the enzymes used for fruit juice extraction or clarifying. This would point to variations in the fruit juice matrix, which might be connected to the noted shifts in the volatile profile. The terpenes and norisoprenoids in apricot juice were improved by the enzymes as varietal ingredients.

According to Cinar *et al.* (2005) using varying quantities of cellulase and pectinase combinations, carotenoid pigments were extracted enzymatically from orange peel, sweet potato, and carrot. The study examined the impact of extraction time and enzyme concentrations on the color output of carotenoid pigments. It was investigated how long the extraction process took, how much cellulase and pectinase was added, and how much color was produced. When the extraction period is increased for the same level of color yield, the high cost of enzymes can be significantly decreased. It was found that by prolonging the extraction period, the same color yield could be obtained at lower enzyme concentrations, which would cut the cost of enzymes.

In order to extract the juice, Al-Hooti *et al.* (2002) mixed date fruit pulp with three times as much water before adding an enzyme. The tamer fruits of two commercial types, Birhi and Safri, was used to produce date syrup in a laboratory setting for use in other culinary products. A high total sugar content was discovered in both types. Out of all the extraction methods used to produce date syrup, the application of pectinase/cellulase enzymes resulted in the most recovery of total soluble solids as opposed to a control group that did not have these enzymes. The Birhi variety of date syrup was found to have lower L* a* b* color values than the Safri type, indicating lighter color for the former. This was observed for both concentrated and diluted date syrup. The findings suggest that pectinase/cellulase enzymes may be used to extract rich date syrup from milder fruits for use in the creation of culinary products.

In their study, Chen *et al.,* (2001) treated litchi fruit with a composite enzyme solution containing pectinase, cellulase, papain, and a-amylase, and then investigated the effects of particular enzymes on the nutritional profile, stability, and transmission of the resulting juice. The juice treated with enzymes exhibited 50 percentage greater transmittance and stability than the untreated juice and also the enzymes treated juice showed better stability 168 hours after treatment. The results showed that after enzyme treatment, high

nutritional contents, stability, and transmission were achieved with good sensory quality. The litchi juice's soluble solids, total sugars, reducing sugars, total acids, and amino acids were all markedly elevated by the intervention.

According to Brazil *et al.,* (1995) pink guavas were mashed, and the pulp was then treated with 600 ppm of a pectic enzyme for 120 minutes at 45°C. When the pulp was compressed, an average juice yield of 84.70 percentage was obtained. After fining agents were added and filtration was performed, the hazy, pink squeezed juice turned clear and light yellow in color. This clear juice was kept fresh using the hot-pack technique. A number of significant physical and chemical changes in the juice were measured during the extraction and clarity process, including changes in total soluble solids (Brix), acidity, viscosity, total phenolic content, color, turbidity, and ascorbic acid retention.

2.5 Effect of enzyme on juice recovery:

Singh *et al.,* (2012) reported that utilizing macerating enzymes to extract juice from different fruits resulted in a higher juice recovery rate. To get the highest possible production and volume of different fruit juices, the enzymatic process should be adjusted in terms of incubation temperature, duration, and enzyme concentration.

When compared to the control group that did not receive enzymatic treatment, the yield of mixed juice and puree from pomace produced by the enzymatic processing of apples increased significantly from 81.8 percentage to a value ranging from 92.3 to 95.3 percentage (Oszmianski *et al.*, 2009).

When extracting juice from kiwi fruit, the use of different combinations of pectinase, cellulase, and amylase considerably enhanced the yield of juice. The combination of pectinase (0.05 g/kg), amylase (0.025 g/kg), and cellulases (0.025 g/kg) had the best results, with a juice of 78.46 percentage as compared to 58.44 percentage of the control sample. Juice recovery from pineapples treated with pectinase and cellulase in a 1:1 ratio at 0.025 percentage concentration was 74.75 percentage (Sreenath *et al*., 1994).

Based on their research, Vaidya *et al.,* (2009) found that kiwi fruit pulp was macerated for two hours at 50 °C using a combination of enzymes (pectinase 0.025g/kg + amylase 0.025g/kg + mash enzyme 0.05g/kg), which made it easier to extract the juice. In contrast to the control (58.44 percentage), the treatment increased juice recovery (78.46 percentage) while having no effect on the clarified juice's TSS, titratable acidity, pH, lowering, or total sugar content. The juice's high concentration of ascorbic acid and high acidity were its most notable features. However, these content decreased after clarifying

because the enzyme combinations in this investigation were utilized for two hours, high yield and clear juice were observed.

Wang *et al.,* (2008) studied the blackberry enzymatic maceration using eight distinct pectinolytic enzyme preparations. When macerated blackberries were treated with enzyme preparations, juice yields increased significantly; however, there was no discernible variation in yield between the different enzymes. Due to varying enzyme treatments, the juices' levels of anthocyanins and polyphenols as well as their clarity differed widely. Compared to juices prepared with other enzyme preparations, juice made with Klerzyme 150 exhibited higher anthocyanin content and improved clarity.

According to Lee *et al.,* (2006) Pectinase was added to banana juice at different enzyme concentrations (0.01–0.1 percentage), treatment durations (30–120 min), and temperature (30–50°C). For turbidity, viscosity, clarity, and filterability, the coefficient of determination, or R2, values, were higher than 0.900. Filterability, clarity, and viscosity were shown to decrease with increasing time and/or concentration of enzyme treatment, whereas turbidity and viscosity increased. The ideal parameters for clarifying banana juice were 0.084 percentage enzyme concentration, 43.2C incubation temperature, and 80-minute incubation duration, as determined by response surface and contour plots (Rai *et al.,* 2004).

Kashyap *et al.,* (2001) studied that the pectinolytic enzymes aid in the pressing or juice extraction process of apple juice, contributing to the sedimentation, filtration, or centrifugation of flocculent precipitates. The concentration of the enzyme, pH, temperature, and length of time the enzyme is in contact with the pulp all influence how clear apple juice becomes. Overall, the concentration of the enzyme utilized at a constant temperature between 5 and 50 °C, with a treatment time of 2 to 16 hours, determines how long it takes to produce clarity.

According to Joshi *et al.,* (1991), an optimal mixture of several enzymes is said to improve juice recovery, TSS, clarity, and reduce turbidity and viscosity. Pectinases are widely used in many contemporary fruit and vegetable juice production techniques, but combinations of cellulytic and pectolytic enzymes are also being used extensively to improve pulp liquefaction and produce better yields of juice with higher soluble solids contents. These enzymes are thought to disrupt the compartmentalization of cellular contents. The fruit processing business extensively uses combinations of pectinolytic and cellulolytic enzymes to boost the extraction yield, lowering sugars, soluble dry matter, and titrable acidity of products from some fruits, like peaches, plums, and apricots.

2.6 Enzyme treatment effect on total soluble solids:

Yusof and Ibrahim (1994) studied that the pectinase enzyme was added to the soursop juice extraction process at concentrations of 0 to 0-1 percentage for one to three hours of incubation. The results showed that using the enzyme was advantageous because it produced 41 percentage more juice. Significant reductions in viscosity and turbidity were observed, along with increases in acidity and Brix. The findings of the sensory examination show that the extracted juice was of higher quality than a manufactured good.

2.7 Enzyme treatment effect on the juice colour:

Sin *et al.,* (2006) in their study found out that color is an important sensory criterion for fruit juices and that the enzyme clarification should be conducted under moderate temperatures because the temperature of 40–60°C facilitated enzymatic clarification in their experiment with sapodilla juice. A higher temperature increased the enzymatic treatment rate in the clarification process.

2.8 Enzyme treatment effect on Reducing Sugar:

As studied by Landbo *et al.,* (2007) elderberry juice was subjected to various enzymatic maceration treatments to determine their effects on juice yield, turbidity, and phenol yield (total phenols and total anthocyanins). The juice output was greatly enhanced by the higher reaction temperature, longer maceration time, and increased dose of the pectinolytic enzyme. When compared to pressing without prior enzymatic treatment, turbidity levels were typically reduced by 30 percentage with enzymatic prepress treatment. *Aspergillus niger* preparation Pectinex BE Color produced marginally higher juice and phenol yields as well as lower turbidity levels than the other enzyme preparations tested, according to an analysis of the responses obtained after the ideal enzymatic treatment with five different pectinolytic enzyme preparations.

Chapter-3

Materials and Methods

MATERIALS AND METHODS

The methodologies adopted for this dissertation work entitled "Assessment of different enzymes on clarification of pineapple juice" have been detailed in this chapter in line with the hypothesis postulated

3.1. Location/Place of Work:

The experiment was conducted on ''Assessment of different enzymes on clarification of Pineapple (*Ananus comosus*) juice'' in the laboratory of the Department of Pomology and Post-Harvest Technology, Faculty of Horticulture, Directorate of Research and Central Instrumentation Centre Laboratory of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal during the year 2023-2024. The University is located at 26°19'86" N latitude and 89°23'5" E longitude, under the sub-Himalayan Terai agro-climatic zone of West Bengal (measured with GPS Garmin-72) at 43 metres above sea level.

3.2. Experimental Design and Treatment Applications

Fresh fruits of kew variety was obtained and purchased from a local market in Pundibari, Cooch Behar, West Bengal. Enzymes, Pectinase ex. *Aspergillus niger* 3.5U/mg and Cellulase ex. *Aspergillus niger* 13000CMC U/g was purchased from SRL and Hemi-cellulase from 0.3- 3.0 unit/mg was purchased from Sigma Aldrich. The enzymes were stored at -20 °C until used.

3.3. Preparation of the fruits and Juice extraction process

The fruit along with the crown section was washed thoroughly under running tap water several times and left to dry until completely moisture free. Once the fruit was completely dried, the weight of the fruit was taken after the removal of the crown section. In the next step, the fruit was sliced into four parts and weight of each of the part was recorded. Next, each of the four pieces were chopped into further smaller parts and the weight was taken and recorded. Then after, the chopped pieces were grinded with the help of a mixer grinder after adding recommended doses of enzymes into the mass of the chopped pieces placed in the grinder. The chopped pieces were grinded and mixed with the enzymes thoroughly. The enzyme added macerated pulp from each of the four pieces was left to incubate at room temperature. Using a mesh cloth, the juice thus obtained was strained and poured into a beaker and the weight of the strained juice was recorded. The entire

process was repeated with each of the original four pieces of the fruit. The juice was then taken up for analysis at the laboratory.

- Design of experiment = Completely Randomized Design
- Number of Treatment = 13
- Number of Replication = 4
- Duration of experiment = December 2023 to March 2024

Table 3.1: Treatment details

Treatment	Enzyme	Enzyme Dosage (%)	Incubation Time
T_0	No enzyme	-	
T_1	Pectinase	0.05	180 minutes
T_2	Pectinase	0.05	240 minutes
T_3	Pectinase	0.075	180 minutes
T_4	Pectinase	0.075	240 minutes
T_5	Cellulase	0.01	180 minutes
T_6	Cellulase	0.01	240 minutes
T_7	Cellulase	0.015	180 minutes
T_8	Cellulase	0.015	240 minutes
T_9	Hemi-cellulase	0.01	180 minutes
T_{10}	Hemi-cellulase	0.01	240 minutes
T_{11}	Hemi-cellulase	0.015	180 minutes
T_{12}	Hemi-cellulase	0.015	240 minutes

3.4. Observation Recorded

3.4.2. Physical Parameters

3.4.1.1. Juice Recovery Percentage

Juice recovery percentage was calculated using the following formula

$$\text{Juice Recovery Percentage} = \frac{\text{Weight of juice extracted}}{\text{Weight of fruit pieces taken for juice extrction}} \times 100$$

3.4.1.2. Sedimentation Percentage

An amount of 20 g of enzyme extracted pineapple was kept undisturbed for 24 hours to filter through a filter paper. After the duration of 24 hours, the weight of the juice will be taken in a beaker and it will be kept undisturbed at room temperature for 24

hours. After 24 hours, the clear juice from the top will be decanted and the residue settled at the bottom will be weighed to calculate the sediment.

$$\text{Sedimentation Percentage} = \frac{\text{Weight of sediment deposited}}{\text{weight of juice taken for the test}} \times 100$$

3.4.1.3. Juice Clarity in terms of Transmittance

Enzymatic treatment leads to an increase in the clarity of juice. Juice clarity was determined in terms of absorbance and transmittance at 660 nm using a UV visible spectrophotometer. An increase in enzymatic concentration increases the rate of clarification by exposing part of the positively charged protein beneath thus reducing electrostatic repulsion between cloud particles which causes these particles to aggregate into larger particles and eventually settle out (Sin *et al.,* 2006). Clarity showed the lowest absorbance values at the highest enzyme concentration, where lower absorbance indicates a clearer juice is being produced. It was also observed that the absorbance values decreased with increasing incubation time at fixed temperature. In general, the time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature (Kilara, 1982). The data has been recorded for the juice with and without the addition of the enzyme to understand the changes taken place in the clarity of the juice.

3.4.1.4. Juice consistency

Consistency is an important consideration in the evaluation of the quality of fruit juice. Consistency can also be employed in the definition of these products as a means of distinguishing one class of products from another on the basis of their pulp content and other fluid characteristics. In this experiment, consistency is tested using a smooth glass surface of length 15 cm .10 microliters of the juice is dropped at an angle of 45 degrees, using a micro pipette. The time taken for the fruit juice to travel from the point it was dropped to the bottom was recorded for all the samples before and after addition of the enzymes.

3.4.1.5. Hunter Colour Parameter

The Hunter parameters are used to describe the visual color change of fruit juice (Avila *et al.,* 1999). The “L” value represents the light-dark spectrum, “*a**” value is for the green-red spectrum and “*b**” value represents the blue-yellow spectrum (Ranganna, 1986). Hunter Colour parameter was measured using Ultrascan Pro from Hunter ColourLab Associates. From the obtained value the following values were estimated

$$Hue\ Angle = \tan^{-1}\frac{b^*}{a^*}$$

$$Chroma\ Value = \sqrt{a^2 + b^2}$$

$$Colour\ Difference\ (\Delta E) = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}$$

3.4.2. Physico-Chemical Parameters

3.4.2.1. Total Soluble Solids (°brix)

Total soluble solids is used as a measure of sugar accumulation in fruits and this gives an overview of the maturity of the raw material used in processing the juice. The total soluble solids (TSS) of the fruit samples were determined by a hand-held refractometer and the results are expressed in °Brix (°B). A small drop of fruit juice was placed over the prism surface and the reading was observed through the eyepiece. The average of TSS was obtained from a direct reading of the instrument (Ranganna,1986).

3.4.2.2. Titratable Acidity (%)

Fruit juice contain a number of fairly simple organic acids which are readily neutralized by strong bases and thus maybe titrated against standard bases such as Sodium hydroxide. Enzyme added juice was taken as sample and it was diluted to 100ml with distilled water. Using a standard solution of 0.1 N sodium hydroxide, the sample was then titrated to the endpoint. The endpoint was determined using the phenolphthalein indicator. 1 ml of phenolphthalein indicator was added to the sample and titrated to the faint pink endpoint. The volume of 0.1N sodium hydroxide used is recorded.

The formula used for titratable acidity (%):

$$\frac{Titrate\ x\ normality\ of\ NaOH x\ volume\ made\ up\ x\ equivalent\ weight\ of\ acid\ x\ 100}{volume\ of\ sample\ x\ weight\ of\ sample\ x\ 1000}$$

$$= \frac{0.1 \quad 100 \quad 70 \quad 100}{10 \ \ 10 \ \ 1000}$$

3.4.2.3. Reducing Sugar (%)

The enzyme added fruit juice sample was taken. 100ml of volumetric flask was taken and volume was made up to 100 ml. The sample was then taken in the burette. Then 100 ml of conical flask was taken, added 2 ml of Fehling's solution A and 2 ml of Fehling's solution B and the volume was made up to 50, then added 1-2 drops

of methylene blue indicator into it. Brick red color given the endpoint would be determined by titration of the sample against Fehling's solution in the conical flask (Ranganna, 1986).

The formula used for reducing sugar (%):

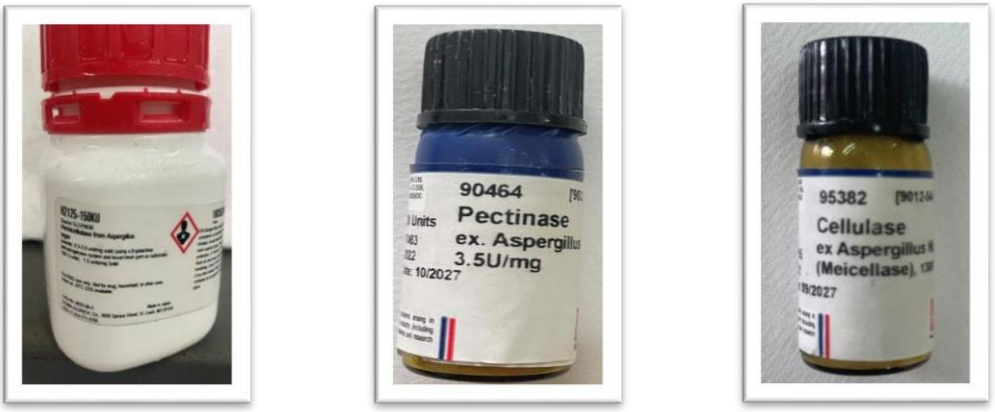
$$= \frac{Fehlings\ factor\ x\ dilution\ x\ 100}{titre\ x\ weight\ of\ sample} = \frac{0.05 \quad 100 \quad 100}{10}$$

3.4.2.4. Total Sugar (%)

Total sugars was determined by Lane and Eynon (Ranganna, 1986). Enzyme added fruit juice sample was taken and used to determine the total sugars. 100ml of volumetric flask was taken and the volume was made up to 100 ml. Then 20 ml of juice was taken in a volumetric flask and 1-2 ml of conc. HCl was added to it. On the next day, a drop of phenolphthalein indicator was added to it. The sample was taken in the burette. After that, 100 ml of conical flask was taken, 2 ml of Fehling's solution A and 2 ml of Fehling's solution B and a volume made up to 50 ml, then added 1-2 drops of methylene blue indicator into it. Titration was done by continuous heating of the flask and pouring the sample from the burette drop by drop. Finally, the brick red color is given as the endpoint (Ranganna, 1986).

The formula used for total sugar:

$$= \frac{Fehlings\ factor\ x\ dilution\ x\ 100}{titre\ x\ weight\ of\ sample} = \frac{0.05 \quad 100 \quad 100}{20}$$



[A] [B] [C]

Plate 1: Enzymes used for clarification [A]: Hemi-cellulase; [B]: Pectinase; [C]: Cellulase

Plate 2: Different observations recorded in the laboratory

CHAPTER-4

RESULTS AND DISCUSSION

RESULT AND DISCUSSION

The results obtained from the present study "Assessment of different enzymes on clarification of pineapple (*Ananus comosus)* is presented systematically in this chapter. Efforts have been made to analyze performance of each treatment in light of data generated for different parameters through standard statistical tools. Comparison of treatments were done for physical qualities of extracted juice and then for physic-chemical parameters.

4.1 Physical Parameters

The physical parameters like Juice recovery percentage, sedimentation percentage, clarity in terms of transmittance, consistency and the Hunter colour parameters of the enzyme clarified juice are presented in this sub-section-

4.1.1. Effect of enzyme treatments on percentage recovery of juice

Table: 4.1 provides the values of percentage (percentage) juice recovery under different enzyme treated samples along with control where no enzyme was used. The assumption for recording this observation was that addition of enzyme for certain incubation period shall help in extracting the soluble components of the cellular content from the macerated fruit tissues.

The data obtained has established the assumption as true. It is clearly evident that the percentage juice recovery was higher over control in all treatments where enzymes were added. Among the enzyme treated samples the T_1 where 0.05 percentage of pectinase enzyme was added and incubated for 3 hours had the highest juice recovery percentage with mean value of 86.89 percentage. The treatment T_4, where the samples were incubated for 4 hours with 0.075 percentage of pectinase enzyme, with value of 86.10 percentage was found to be as good as T_1.

All other treatments where pectinase and cellulase were added (T_2, T_3, T_4, T_5, T_6, T_7 and T_8) were statistically at par with T_1 and T_4. However, the treatments where hemi-cellulase were added (T_9, T_{10}, T_{11} and T_{12}) were found to be significantly different from T_1 and T_4. With clear evidence, it could be established that amongst the sub-set of cellulase treated samples, T_7 (where 0.015 percentage of cellulase enzyme was added and incubated for 3 hours) had the highest juice recovery percentage with mean value of 84.20 percentage as compared to other cellulase treated samples (T_5, T_6 and T_8). Among the treatments where hemi-cellulase enzyme were added, T_9 where 0.01 percentage hemi-cellulase were added

and incubated for 3 hours showed better percentage juice recovery than the other treatments (T_{10}, T_{11}, T_{12}).

When enzymes like pectinases are added to the juice samples, the enzymes tend to separate the cells which are glued together by the compound pectin. The addition of the enzyme pectinase leads to dissolution of the compound pectin which further leads to separation of the cells. Hence the separation of the cells makes it easier for the juice to be extracted which in turn increases the percentage of the juice recovery.

These findings were supported by Oszmianski *et al*. (2009) where he observed that the yield of mixed juice obtained in the enzymatic processing of apples ranged from 92.3 to 95.3 percentage, and increased significantly when compared to the control without the enzymatic treatment (81.8 percentage). Similar findings by Sreenath *et al*. (1994) stated that pectinase and cellulase treatment in combination at 1:1 ratio at 0.025 percentage concentration resulted in juice recovery of 74.75 percentage from pineapple.

TABLE 4.1: Effect of different treatments on physical parameters of enzyme clarified juice

Treatment	Juice Recovery (%)	Sedimentation percentage	Percentage Change in Transmission (Clarity)	Percentage Change in Consistency
Control	60.81 ± 2.35^{f}	11.933 ± 1.625^{a}	-	-
T_1	86.89 ± 5.75^{a}	5.830 ± 1.384^{bc}	20.562 ± 8.682^{ab}	11.218 ± 2.661^{d}
T_2	83.77 ± 14.5^{ab}	6.341 ± 0.977^{bc}	28.322 ± 5.967^{a}	16.177 ± 1.746^{abcd}
T_3	81.37 ± 4.63^{abc}	6.886 ± 0.551^{bc}	22.315 ± 5.889^{ab}	15.407 ± 3.321^{abcd}
T_4	86.10 ± 8.70^{ab}	6.470 ± 0.390^{bc}	18.995 ± 3.034^{ab}	17.080 ± 4.710^{abc}
T_5	77.98 ± 5.84^{abcd}	7.269 ± 2.142^{bc}	16.077 ± 5.717^{b}	16.504 ± 1.407^{abc}
T_6	77.31 ± 2.42^{abcd}	7.644 ± 2.398^{bc}	23.186 ± 7.923^{ab}	15.437 ± 3.763^{abcd}
T_7	84.20 ± 1.83^{ab}	8.298 ± 1.208^{b}	20.397 ± 5.583^{ab}	15.261 ± 1.901^{abcd}
T_8	78.50 ± 2.87^{abcd}	7.333 ± 3.531^{bc}	19.116 ± 9.021^{ab}	17.575 ± 5.352^{ab}
T_9	74.70 ± 10.1^{bcd}	5.057 ± 1.148^{c}	22.076 ± 4.107^{ab}	15.964 ± 2.063^{abcd}
T_{10}	72.08 ± 4.53^{cde}	6.048 ± 1.849^{bc}	20.802 ± 7.958^{ab}	11.979 ± 1.647^{cd}
T_{11}	68.60 ± 7.13^{def}	5.880 ± 0.518^{bc}	20.760 ± 12.078^{ab}	20.465 ± 7.621^{a}
T_{12}	63.84 ± 7.56^{ef}	5.763 ± 1.657^{bc}	28.853 ± 6.369^{a}	14.901 ± 2.436^{bcd}
SE(m)	3.474	0.855	3.621	1.841
SE(d)	4.913	1.209	5.121	2.604
C. D	9.937	2.444	NS	NS

Data presented are mean ± SD (n=4)

4.1.2. Effect of enzyme treatment on the Sedimentation percentage of clarified Juice

Table 4.1 provides the effects of enzyme on the sedimentation of the juice extracted, with different treatments along with the control where no enzyme was added. The recording of this observation was done to determine, the role and effect of enzymes on the sedimentation percentage of the juice extracted.

The sedimentation percentage, from the various treatments lies within the range of 5.057-8.298 percentage. The sedimentation percentage, observed to be the most significant was 11.933 percentage in control where no enzymes were used. The second amongst the enzymatically treated juice samples shows 8.298 percentage from the treatment T_7 where 0.015 percentage cellulase was added and incubated for 3 hours and the lowest significant value was 5.057 percentage observed from the treatment T_9 where 0.01 percentage hemi-cellulase was added and incubated for 3 hours.

For pectinase enzyme treatment, the sedimentation percentage observed to be the highest was 6.886 percentage from the treatment T_3 where 0.075 percentage enzyme was added for 3 hours incubation and the lowest observed was 5.830 percentage from T_1 where 0.05 percentage enzyme was added and incubation period was 3 hours.

The sedimentation percentage recorded for the cellulase enzyme treated juice was observed. The highest was recorded to have a mean value of 8.298 percentage observed in T_7 where 0.015 percentage enzyme was used and incubated for 3 hours and the lowest was 7.269 percentage from the treatment T_5 where 0.01 percentage enzyme was incubated for 3 hours.

The recorded data for the sedimentation percentage of hemi-cellulase treated juice was recorded to be within the range of 5.057-6.048. The highest value was 6.048 recorded from T_{10} which had 0.01 percentage enzyme with 4 hours incubation followed by value of 5.880 for T_{11} which had 0.015 percentage enzyme for 3 hours incubation followed by a value of 5.763 for T_{12} which had 0.015 percentage enzyme for 4 hours incubation and a value of 5.057 for T_9 which had 0.01 percentage enzyme for 3 hours incubation.

With the addition of cellulase enzyme, the cell walls break down into smaller pieces and the breakage of the cell walls facilitate the larger particles to settle down. The lighter particles remain in the state of suspension and they don't settle down because of the reduction in their weight due to the action of the enzyme. The larger particles that settle

down are then calculated to obtain the sedimentation percentage of the enzyme treated juice sample.

The sedimentation percentage, given in **Table: 4.1**, from the various treatments lies within the range of 5.057-8.298 %. The sedimentation percentage, observed to be the most significant was 8.298% from the treatment T_7 where 0.015% cellulase was added and incubated for 3 hours. Similar works conducted in elderberry, by Landbo *et al*. (2007) was done where they observed that with the utilization of enzymes the sedimentation percentage reduction was 30% lower than those of samples produced without enzyme addition

4.1.3. Effect of enzyme treatment on the clarity (in terms of transmission percentage) of clarified Juice

Table:4.1, shows values of the effect of percentage change in transmission under different treatments. The treatment T_{12} where 0.015 percentage hemi-cellulase was added and incubated for 4 hours shows the maximum change in transmission with mean value of 28.85 percentage. The treatment T_2 where 0.05 percentage pectinase added and incubate for 4 hours has recorded to be as good as treatment T_{12} with mean value of 28.32 percentage.

For the enzymatic treatments of cellulase (T_5, T_6, T_7 and T_8) the treatment T_6 where 0.01 percentage enzyme was added and incubated for 4 hours has shown the highest change in transmission with mean value of 23.18 percentage while the lowest change in transmission for the enzyme cellulase was recorded for treatment T_5 which is also the lowest change in transmission for all treatments.

With mean value of 16.0 percentage the treatment T_5 where 0.01 percentage cellulase enzyme was added and incubated for 3 hours shows the minimum change in transmission among all the treatments.

Cellulase breaks down the cell wall and the cell membrane when they are added to the juice samples. The enzyme dissolves the cell wall making juice extraction easier along with the juice comes other components like sugar, mitochondria and vacuole. All of these components that remain within the cells are also disrupted and released during the process of extraction of the juice. The cell organelles are compartmentalized through membrane mostly composed of lipoprotein. The enzyme breaks down the membranes and facilitates release of the soluble content of the cells into extracted juice. The suspended material after juice extraction are also solubilize by the disintegrating enzymes. Therefore, when

the enzymes break the particles into smaller ones, the light can easily pass through without inference increasing the percentage transmission and clarity because of the addition of the enzymes. The light can now pass through because the path of light is not interfered by larger particles.

4.1.4. Effect of enzyme treatment on consistency of clarified Juice

Data presented in **Table: 4.1**, shows effect of enzyme on change in consistency under all treatments. It is evident from the data that the treatment T_{11} where 0.015 percentage hemi-cellulase enzyme was added and incubated for 3 hours has maximum change in consistency with mean value of 20.46 percentage.

The treatment T_8 where 0.015 percentage cellulase was added and incubate for 4 hours has revealed to have the highest change in consistency after treatment T_{11} with mean value of 17.57 percentage.

Among the treatments for pectinases the range of change in consistency varied from 11.21 percentage to 17.08 percentage in which the treatment T_4 is the highest recorded whereas the treatment T_1 has recorded the lowest.

A network of molecules is present in fruits and fruit juices, and the sugar components are trapped within this network. The thick viscosity of the fluids is caused by the trapped sugar molecules. The network of molecules breaks down when the enzymes are added, causing the molecules to break apart into smaller fragments. The sugar molecules can no longer be trapped due to the network's disruption. As a result, the components become free soluble components and the consistency becomes thinner. It then behaves like an ideal solution by increasing the consistency.

4.1.5. Effect of enzyme treatment on Hunter colour parameters of clarified Juice

Table 4.2, provides the recorded information pertaining to the color of the processed juice samples treated with enzymes (pectinase, cellulase & hemi-cellulase). The control which is free from enzyme treatment was also recorded. Sin *et al*. (2006) found out that color is an important sensory criterion for fruit juices and that the enzyme clarification should be conducted under moderate temperatures because the temperature of 40–60°C facilitated enzymatic clarification. In the above table **(Table: 4.2)**, 'L' values denotes the lightness of the extracted juice, 'a' value denotes the different color where, a positive value of 'a' symbolizes red color and a negative value of "a" symbolizes an inclination towards the color green and the 'b' value denotes the different color where, a positive value symbolizes yellow and a negative value of 'b' symbolizes an inclination towards

the color blue.

4.1.5.1. L* Value of the clarified juice

L-values denotes the lightness of the extracted juice with different treatments along with the control where no enzyme was added. The presumption for recording this observation was to determine, the addition of the enzymes and the incubation period helps influence the texture of the processed juice.

The data recorded showed that the color of the juice also changes with the addition of the enzymes. Among the enzyme treated samples, the L value for T_8 (0.015% cellulase incubated for 4 hours) with a mean value of 36.074 was observed to show the most significant texture (lightness), and the least significant was observed from T_5 (0.01% cellulase incubated for 3 hours) which recorded to have the value of 33.641. The treatments with the enzyme cellulase recorded to be significantly greater.

TABLE 4.2: Effect of different enzyme treatments on Hunter colour parameters of clarified juice

Treatment	L Value of Processed Juice	a* Value of Processed Juice	b* Value of Processed Juice
Control	21.238 ± 3.365^{c}	-2.927 ± 0.585^{c}	28.552 ± 1.378^{ab}
T1	31.483 ± 5.536^{ab}	-1.837 ± 0.659^{ab}	22.328 ± 3.644^{bc}
T2	26.489 ± 7.607^{bc}	-2.074 ± 0.759^{ab}	19.444 ± 4.715^{c}
T3	30.827 ± 6.742^{ab}	-1.509 ± 0.326^{ab}	28.843 ± 3.236^{a}
T4	28.577 ± 7.624^{abc}	-1.968 ± 0.913^{ab}	21.529 ± 8.754^{c}
T5	33.641 ± 1.158^{ab}	-1.794 ± 0.663^{ab}	18.546 ± 4.531^{c}
T6	35.191 ± 0.895^{a}	-1.436 ± 0.438^{a}	20.893 ± 4.312^{c}
T7	34.271 ± 4.020^{a}	-1.406 ± 0.301^{a}	22.721 ± 2.077^{abc}
T8	36.074 ± 1.726^{a}	-1.993 ± 0.339^{ab}	22.583 ± 3.888^{abc}
T9	30.710 ± 3.533^{ab}	-1.528 ± 0.294^{ab}	25.134 ± 3.222^{abc}
T10	29.826 ± 3.505^{abc}	-1.716 ± 0.432^{ab}	21.889 ± 0.430^{c}
T11	29.490 ± 1.922^{abc}	-2.437 ± 0.518^{ab}	23.106 ± 0.801^{abc}
T12	31.677 ± 3.212^{ab}	-1.918 ± 0.663^{ab}	20.468 ± 2.481^{c}
SE(m)	2.228	0.281	1.963
SE(d)	3.150	0.398	2.776
CD	6.373	0.804	5.615

The juice treated with the enzyme pectinase shows a minimal effect on the L value (color) of the juice extracted. From the observation recorded, the data presented in Table 4.2, shows that in the pectinase treated juices, the maximum color (lightness) or L value was

observed in T_1 (0.05% with 3 hour incubation) with a value of 31.483.In T_2 (0.05% pectinase incubated for 4 hours) least value of 26.489 was observed.

Among the treatments recorded for cellulase (T_5,T_6,T_7,T_8) the highest was observed in T_8 (0.015% cellulase was incubated for 3 hours) with a mean value of 36.07 and the least significant value was observed in T_5(0.01% cellulase incubated for 4 hours) with a value of 33.64

The next significant data was observed from the juice samples treated with hemi-cellulase enzyme (T_9 ,T_{10}, T_{11}, T_{12}) The highest value of 31.677 was recorded for T_{12} where 0.015% enzyme was added and incubated for 4 hours and the lowest value recorded was for T_{11} (0.015% enzyme added and incubated for 3 hours) which was 29.490

The result according, to Table: 4.2, recorded the L value of the juice, which also changes with the addition of the enzymes. Among the enzyme treated samples, T_8 (0.015% cellulase incubated for 4 hours) with a mean value of 36.074 was observed to show the most significant texture (lightness). The L* value is a measurement for lightness and if the value is high, it indicated that the juices are clarified. Findings by Kothari *et al*. (2013) supports the findings. They stated that apple juice clarification up to 30% was observed after 4 hours of incubation with pectinase and cellulase enzyme. The enzyme mixture gave up to 50% juice clarification. Visually, a mixture of these enzymes treated apple juice exhibit maximum clearance of juice.

4.1.5.2. '*a**' Value of the clarified juice

The Table 4.2, shows the color between the different enzyme treated pineapple juice samples, along with the control (no enzyme was given). The idea for taking the observation was to understand the effects of the enzyme treatment on the various processed juice samples taken under this study. The study is evident that the texture were higher than that of the control in all the treatments.

The above observation of the value 'a' shows that treatments T_2 (0.05% pectinase incubated for 4 hours) and T_{11} (0.015% hemi-cellulase incubated for 3 hours) showed a significant inclination towards a green color. The highest observation recorded for green color was from the treatment, T_{11} with value of -2.437 treated with the enzyme hemi-cellulase, and the lowest observation was recorded in T_7 (0.015% cellulase incubated for 3 hours) with value of -1.406.

For pectinase enzyme treated processed juice, for the 'a' value was the highest observation recorded was from T_1 (0.05% enzyme incubated for 3 hours) with a mean

value of -1.837 and the least significant value was from T_2 (0.05% pectinase incubated for 4 hours) which is -2.074

Similarly, the most significant 'a' value recorded for the samples treated with the enzyme cellulase was from T_8 (0.015% enzyme incubated for 4 hours) with a mean value of -1.993 and the least significant value was recorded from also from the samples treated with the enzyme cellulase which was recorded in the treatment T_7 (0.015% enzyme incubated for 3 hours) with value of -1.406

For the 'a' value of the juice samples treated with the enzyme hemi-cellulase the most significant observation was from T_{11} where 0.015% enzyme was added and incubated for 4 hours obtained a mean value -2.437 and the least significant value from T_9 where 0.01% enzyme was added and incubate for 3 hours to have a value of -1.528

4.1.5.3. '*b**' Value of the clarified juice

From the pertaining data of Table 4.2, the 'b*' value of all the enzyme treated juices were observed to have an inclination towards yellow color. The most significant observation for the treatments of the various processed juice samples (b*value) were recorded, the highest observation was recorded from T_3 where 0.075% pectinase was added and incubated for 3 hours. T_3 has recorded to have a mean value of 28.843 and the least significant value obtained is 18.546 from the treatment T_5 which was treated with 0.01% cellulase with 3 hours incubation.

The highest observation for the treatment of pectinase for the 'b*' value was 28.843 which was observed in T_3 where 0.075% enzyme was added for 3 hours incubation and the lowest was 19.444 which was recorded from T_2 where 0.05% enzyme was added and incubated for 4 hours.

Similarly, the cellulase treated enzyme juice was recorded and the highest value was observed to be at par between T_7 (22.721) where 0.015% enzyme was added and incubated for 3 hours and T_8 (22.583) where 0.015% enzyme was added and incubated for 4 hours and the lowest value recorded was 18.546 from the treatment T_5 where 0.01% enzyme incubated for 3 hours for the 'b*' value.

The juice treated with hemi-cellulase was also presented in Table 4.2, where the highest 'b*' value was 25.134 recorded from T_9 where 0.01% enzyme was added and incubated for 3 hours and the least significant value was 20.468 recorded from T_{12} where 0.015% enzyme was added for incubation period of 4 hours.

Similar work done by Saud *et al*. (2002) where the CIE L*a*b* color values were found

to be affected significantly by different treatments as well as by the variety. The diluted syrup was found to possess lighter color (higher Lightness value) for both the treatments for the Birhi variety than the Safri. Their results indicate the possibility of employing pectinase or cellulase enzymes to produce concentrated date syrup from tamer fruits for use in food product development.

4.1.5.4. Change in Hue angle of the clarified juice

The Table: 4.3, provides the values of percentage change in hue value in processed juice under various different treatments where the enzymes were used. The data obtained shows that the treatment, T_{12} where 0.015 percentage of hemi-cellulase enzyme was added and incubated for 4 hours had the highest percentage change in hue value with mean value of 8.588 percentage among other treatments of hemi-cellulase enzyme (T_9,T_{10} and T_{11}).

The treatment T_{11} where 0.015 percentage of enzyme hemi-cellulase incubated for 3 hours showed second highest change in hue value after T_{12} with mean value of 6.16 percentage.

TABLE 4.3: Effect of different enzyme treatments on change in colour characteristics of clarified juice

Treatment	Change (percentage) in Hue Value in processed juice	Change (percentage) in Chroma value in processed juice	Color Difference (ΔE) after processing of Juice
T_1	4.813 ± 3.066^{bcd}	41.075 ± 27.633^{a}	6.561 ± 3.971^{a}
T_2	2.276 ± 1.150^{cde}	32.539 ± 15.030^{a}	7.114 ± 3.016^{a}
T_3	2.267 ± 1.390^{cde}	35.042 ± 12.310^{a}	10.325 ± 1.921^{a}
T_4	3.698 ± 0.885^{bcde}	40.463 ± 30.583^{a}	8.062 ± 7.725^{a}
T_5	1.844 ± 1.196^{e}	26.746 ± 10.036^{a}	6.955 ± 3.873^{a}
T_6	2.271 ± 1.284^{cde}	25.065 ± 10.186^{a}	7.865 ± 3.961^{a}
T_7	3.057 ± 1.70^{cde}	41.427 ± 30.793^{a}	9.373 ± 1.577^{a}
T_8	2.060 ± 2.383^{de}	33.454 ± 19.527^{a}	10.902 ± 5.785^{a}
T_9	3.954 ± 2.962^{bcde}	30.767 ± 6.807^{a}	7.644 ± 1.246^{a}
T_{10}	5.004 ± 2.314^{bc}	33.336 ± 10.842^{a}	8.606 ± 2.550^{a}
T_{11}	6.169 ± 2.452^{ab}	39.176 ± 21.523^{a}	7.708 ± 4.144^{a}
T_{12}	8.588 ± 1.733^{a}	34.259 ± 6.363^{a}	8.309 ± 2.559^{a}
SE(m)	1.002	9.441	1.972
SE(d)	1.418	13.351	2.789
CD	2.875	NS	NS

Treatment, T_1 where 0.05 percentage pectinase were added and incubated for 3 hours showed highest change in hue value with mean value of 4.81 percentage among other

treatments where pectinase enzyme was added.

The enzyme cellulase was added at 0.01 percentage and incubated for 3 hours for the treatment T_5, which showed the lowest change in hue value as compared to the other treatments where the enzyme cellulase was added (T_5,T_6,T_7 and T_8) .

The treatments where cellulase and pectinase were found to be significantly different than T_1. In case of pectinase enzyme, the treatment T_1 (0.05percentage incubated for 3 hours) showed highest hue value while T_3 (0.075 percentage incubated for 3 hours) showed lowest change in hue value. In case of cellulase enzyme, the treatment T_7 (0.015 percentage incubated for 3 hours) showed the highest change in the hue value while the treatment T_5(0.01 percentage incubated for 3 hours) showed the lowest change in hue value. The data obtained shows that in case of hemi-cellulase enzyme, the treatment T_{12} (0.015 percentage incubated for 4 hours) showed the highest change in the hue value compared to all the other treatments.

4.1.5.5. Change (percentage) in chroma value in processed juice

The data presented in Table 4.3, provides the value of percentage change in chroma value in processed juice under various treatments where the enzymes pectinase, cellulase and hemi-cellulase were added. It is clear from the table that the treatment T_7 where cellulase enzyme of 0.015 percentage was incubated for 3 hours has recorded the highest change in chroma value in processed juice with mean value of 41.42 percentage. All other treatments where pectinase and hemi-cellulase were added (T_1, T_2, T_3, T_4, T_5, T_6, T_8, T_9, T_{10}, T_{11}, T_{12}) are significantly at par with T_7.

The treatment T_1 where pectinase of 0.05 percentage was added and incubate for 3 hours has also recorded to be as good as T_7 with mean value of 41.07 percentage. Among the different treatments of hemi-cellulase enzyme (T_9,T_{10},T_{11} and T_{12}), the treatment T_{11} where 0.015 percentage of enzyme was added and incubated for 3 hours has recorded to have the highest change in chroma value with mean value of 39.17 percentage.

While the highest change in chroma value was been recorded for the enzyme cellulase of treatment T_7, the lowest change in chroma value is also of the enzyme cellulase of treatment T_6 where 0.01 percentage of the enzyme was added and incubated for 4 hours.

It is further clear from the data obtained that the treatment of enzymes did not interfere with the chroma value. The changes were not significant in any of the enzyme treatments. It can be concluded that the chroma value has remain unchanged with the addition of the enzymes in the juice samples.

4.1.5.6. Effect of enzyme on Color difference (ΔE) after processing of juice

The data presented in the Table:4.3., revealed differences in the effect of enzyme on color difference after processing of juice for all treatments. The data obtained shows that the treatment T_8 where 0.015 percentage of cellulase enzyme was added and incubated for 4 hours has shown the maximum color difference after the enzymatic treatment with mean value of 10.90 percentage.

With clear evidence it was established that the treatment T_1 where 0.05 percentage pectinase enzyme was added and incubated for 3 hours has shown the lowest effect on colour difference after enzymatic treatment with mean value of 6.56 percentage. For the treatments with hemi-cellulase enzyme (T_9,T_{10},T_{11} and T_{12}), the treatment T_{10} where 0.01 percentage of enzyme was added and incubated for 4 hours showed highest color difference with mean value of 8.60 percentage while the lowest effect of the enzyme hemi-cellulase was obtained for the treatment T_9 where 0.01 percentage enzyme was added and incubated for 3 hours with mean value of 7.64 percentage. However, the table reveals that all the treatments are non-significant.

The data acquired further demonstrate that the enzyme treatment had no effect on the color difference. No matter whatever enzyme treatment was used, there were no appreciable changes. Conclusion: Since the enzymes were added to the juice samples, the color difference has not changed.

4.2 Physico-Chemical Parameters

The physico-Chemical parameters like TSS, Acidity, Reducing and Total sugar of the enzyme clarified juice are presented in this sub-section-

4.2.1 Effect of enzyme treatment on Total Soluble solids of clarified Juice

The TSS of the enzyme treated pineapple juice samples has been recorded and given in **Table: 4.4.** The assumption of recording this observation was to determine the TSS of the juice extracted with addition of the enzymes and the incubation period. It is clearly evident that the TSS value is higher as compared to the control in all the treatments of the extracted juice.

The values of TSS of different treated enzyme was observed within the ranges of 14.481-15.163. The highest significant value was a mean value of 15.163 °B recorded from the treatment T_6 where 0.01 percentage cellulase was used and incubated for 4 hours and the least significant value was a mean value of 14.481 percentage observed from T_{10} using hemi-cellulase of 0.01 percentage for 4 hours incubation.

The enzyme treated juice for pectinase was recorded maximum value in T_4 (14.813 percentage), whereas T_1 and T_3 are at par with each other with a value of 14.800 percentage and the lowest value obtained was from T2 (14.569 percentage).

Similarly, the juice treated with cellulase was also recorded. The highest value was from T_6 (15.163 percentage), followed by T_5 and T_7 which are at par with each other with a value of 15.144 percentage and the lowest was recorded by T_8 (15.125 percentage).

The TSS of the enzyme treated juice with hemi-cellulase was also observed. The highest value was from T_{12} (14.631percentage) and the lowest was recorded from T_{10} with a mean value of 14.481 percentage.

The data obtained from the table shows similarities in the treatments T_5, T_6, T_7 and T_8. It is also clearly evident that the treatments T_1, T_2, T_3, T_4, T_{10}, T_{11} and T_{12} along with control T_0 are similar to each other

TABLE 4.4: Effect of different enzyme treatments on physico-chemical characteristics of clarified juice

Treatment	Physico-chemical parameters of enzyme clarified Juice			
	TSS (°B)	Titratable Acidity (percentage)	Reducing Sugar (percentage)	Total Sugar (percentage)
Control	12.482 ± 1.075^{c}	1.357 ± 0.154^{a}	7.183 ± 0.632^{c}	9.771 ± 1.384^{d}
T_1	14.800 ± 0.029^{b}	1.173 ± 0.273^{ab}	8.950 ± 0.126^{ab}	12.977 ± 0.268^{abc}
T_2	14.569 ± 0.480^{b}	1.225 ± 0.254^{ab}	8.902 ± 0.195^{ab}	13.122 ± 0.353^{abc}
T_3	14.800 ± 0.020^{b}	1.221 ± 0.168^{ab}	9.126 ± 0.222^{ab}	13.163 ± 0.310^{abc}
T_4	14.813 ± 0.014^{b}	1.365 ± 0.146^{a}	9.182 ± 0.301^{ab}	13.987 ± 0.563^{a}
T_5	15.144 ± 0.031^{a}	1.089 ± 0.072^{ab}	9.083 ± 0.651^{ab}	13.400 ± 0.352^{a}
T_6	15.163 ± 0.014^{a}	1.085 ± 0.055^{ab}	9.176 ± 0.738^{ab}	13.351 ± 0.228^{ab}
T_7	15.144 ± 0.013^{a}	1.194 ± 0.033^{ab}	9.487 ± 0.505^{a}	13.428 ± 1.095^{a}
T_8	15.125 ± 0.020^{a}	1.308 ± 0.153^{a}	9.517 ± 0.708^{a}	13.737 ± 0.358^{a}
T_9	14.575 ± 0.102^{b}	1.173 ± 0.129^{ab}	8.396 ± 0.339^{a}	11.547 ± 1.344^{c}
T_{10}	14.481 ± 0.306^{b}	0.993 ± 0.200^{b}	8.961 ± 0.787^{ab}	11.546 ± 1.521^{c}
T_{11}	14.569 ± 0.075^{b}	1.216 ± 0.093^{ab}	9.214 ± 0.783^{ab}	11.725 ± 2.047^{bc}
T_{12}	14.631 ± 0.145^{b}	1.173 ± 0.211^{ab}	9.070 ± 0.620^{ab}	11.527 ± 1.275^{c}
SE(m)	0.100	0.083	0.279	0.518
SE(d)	0.142	0.117	0.395	0.733
C. D	0.288	NS	0.798	1.482

Starch is present in fruits and fruit juices, and when an enzyme is added, the starch is transformed into soluble sugar, increasing the amount of sugar and its overall soluble

solids content.

The values of TSS, given in the above result (Table 4.1) of different treated enzyme was observed within the ranges of 14.481- 15.163. The highest significant value was a mean value of 15.163 percentage recorded from the treatment T_6 where 0.01 percentage cellulase was used and incubated for 4 hours.

The above result can be supported by the work done by Yusof and Ibrahim (1994), where they found that the use of enzyme for soursop at various enzyme levels significantly increased the soluble solids content from 6.8 to 7.3° Brix within the 1 hour of incubation. Similarly, pectinase treated apricot, pear, mayhaw, banana had a higher brix levels as compared to untreated juices Joshi *et al*. (2011)

Sreenath *et al*. (1994) used pectinase and cellulases enzymes for extraction of pineapple juice at enzymatic concentration of 0.025 percentage. The TSS of the final pooled juice was around 12 °Brix.

4.2.2 Effect of enzyme treatment on titratable acidity (%) of clarified Juice

The effects of the enzyme treated pineapple juice on the titratable acidity was recorded. The observation was taken and recorded in Table 4.4, the observation was taken to determine the utilization of enzymes on the influences it has on the juice extracted. The titratable acidity observed from the various treatments lies within the range of 0.993-1.365 percentage, where the highest was a mean value of 1.365 percentage recorded from T_4 which has a combination of 0.075 percentage enzyme with 4 hours incubation whereas the least significant value was a mean value of 0.993 percentage recorded from T_{10} using hemi-cellulase as the enzyme with 0.01 percentage concentration with 4 hours incubation.

The acidity (percentage) observed in the pectinase treated juice, was 1.365 percentage which recorded highest in T_4 where 0.075 percentage enzyme was incubated for 4 hours and the lowest value was 1.173 percentage observed in T_1 where 0.05 percentage enzyme was used for 3 hours incubation.

The acidity recorded for the treatment with cellulase, was recorded highest in T_8 (1.308 percentage), followed by T_7 (1.194 percentage), whereas T_5 and T_6 are statistically at par with each other.

The acidity, similarly for the processed juice treated with hemi-cellulase was also recorded. The highest observation was recorded in T_{11} (1.216 percentage), and the lowest value was recorded from T_{10} with an average value of 0.993 percentage, treatments T_9 and T_{12}, from the recorded data are statistically at par with each other.

The above result, presented in Table 4.4 showed that the acidity (percentage), lies within the ranges of 0.993-1.365 percentage, where the highest was a mean value of 1.365 percentage recorded from T_4 which has a combination of 0.075 percentage enzyme with 4 hours incubation period. Findings by Yusof and Ibrahim (1994) found that the total titratable acidity for enzymatically extracted juice from soursop increased significantly from 0.41 percentage to 0.49 percentage for the 1, 2 and 3 h of incubation at the 0.025 percentage enzyme concentration but not at 0.05 percentage, 0.075 percentage and 0.1 percentage concentrations.

4.2.3 Effect of enzyme treatment on Reducing Sugar (%) of clarified Juice

The Table 4.4, provides the effects of enzyme on the reducing sugar of the processed juice extracted, with different treatments along with the control where no enzyme was added. The recording of this observation was done to determine the influence of the enzymes on the reducing sugar. It is evident that the percentage of reducing sugar were higher over the control.

Among, the enzyme treated samples the range of reducing sugar (percentage) lies within 8.396-9.517 percentage. The highest significant value was 9.517 percentage recorded in T_8 in which cellulase was use as the enzyme with concentration of 0.015 percentage and 4 hours incubation and the least significant value was 8.396 percentage in T_9 in which hemi-cellulase was the enzyme used with 0.01 percentage concentration with 3 hours incubation.

The observation of reducing sugar in the enzyme treated juice (pectinase) was highest in T_4 (9.182 percentage), and the lowest value observed was from T_2 with a mean value of 8.902 percentage.

The samples treated with cellulase enzyme was also recorded for their reducing sugar, the highest value was recorded in T_8 (9.517 percentage), and the lowest value was observed from T_5 (9.083 percentage).

The processed juices were also treated with hemi-cellulase, the reducing sugar recorded was highest in T_{11} with a mean value of 9.214 percentage, and the lowest value was from T_9 with an average value of 8.396 percentage.

The addition of the enzymes to the fruit juices accelerated the chemical reactions which in turn leads to the reduction of sugar content in the fruit juices.

The reducing sugar as given in Table 4.4, was observed to be within the ranges of 8.396-9.517 %. The highest significant value was 9.517% recorded in T_8 in which cellulase was

use as the enzyme with concentration of 0.015 percentage and 4 hours incubation. Similar findings conducted in sugar palm by Arsad *et al*. (2015) where enzymatic treatments increased the amounts of natural sugars in the juices by partly degrading the existing sucrose. Fang *et al*. (1986) also observed that the concentration of reducing sugars increased over time.The increase in the reducing sugars concentration was higher in the juices pasteurized at higher temperatures on the processing of sugar palm fruit juice.

4.2.4 Effect of enzyme treatment on total sugar (%) of clarified Juice

The effects of enzyme on the total sugar present in the processed juices are given in **Table 4.4,** with different treatments. The control was also added (free from enzyme).

It is true that the percentage of total sugars were higher than the control. The recording of this observation, was to determine the total sugar percentage with the presence of enzyme addition.

The range of total sugar (percentage) from the various treatments lies within 11.527-13.987 percentage. The highest significant value was observed from T_4 with an average value of 13.987 percentage, using the enzyme pecyinase with 0.075 percentage with 4 hours incubation and the least significant value was observed from T_{12} with an average value of 11.527 percentage, using hemi-cellulase as the enzyme with 0.015 percentage concentration with 4 hours incubation.

The enzyme treatment for pectinase with regards to the total sugar, was recorded and the highest value was in T_4 (13.987 percentage), and the lowest value was recorded from T_1 with a mean value of 12.977 percentage. Similarly, the enzyme treatment (cellulase) for the total sugars was recorded highest in T_8 with a mean value of 13.737 percentage, and the lowest value from T_6 with a mean value of 13.400 percentage.

The enzyme treatment (hemi-cellulase) was also recorded where the highest observation was in T_{11} (11.725 percentage), and the lowest value was recorded from T_{12} with mean value of 11.527 percentage.

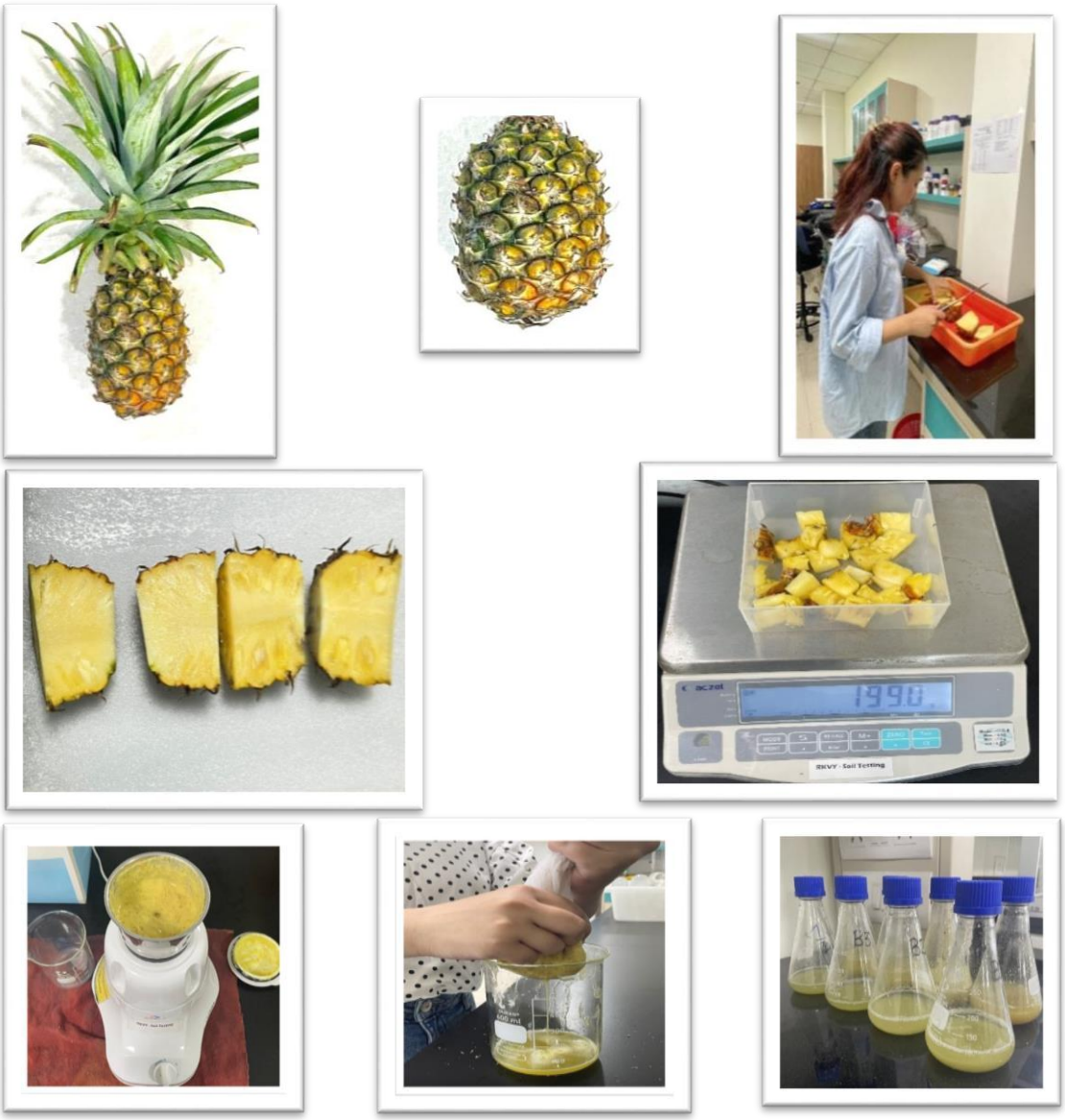
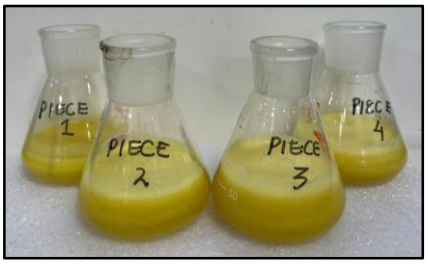
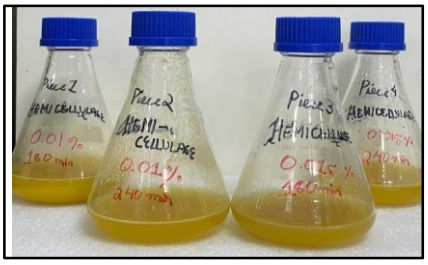


Plate 3: Process of juice extraction



Without enzyme addition

Pectinase treated



Hemi-Cellulase treated

Cellulase treated

Plate 4: Enzyme extracted juice

CHAPTER-5

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Keeping in view the juice extraction processes in most of the cases being inadequate to fulfil the increasing demand of the healthier fruit juices amongst the modern day well informed consumers, this present study has been carried out with twelve treatments and four replications at the laboratory under Pomology and Post-harvest Technology, Directorate of Research and Central Instrumentation Centre of the Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal , India during the year 2023 to 2024 where samples of pineapple juice are subjected to different enzyme treatment combinations such as **T_0**: Control (no enzyme); **T_1**: Pectinase- 0.05 percentage concentration and 3 hours incubation period; **T_2**: Pectinase- 0.05 percentage concentration and 4 hours incubation; **T_3:** Pectinase- 0.075 percentage concentration and 3 hours incubation period; **T_4**: Pectinase- 0.075 percentage concentration and 4 hours incubation ;**T_5**: Cellulase- 0.01 percentage concentration and 3 hours incubation; **T_6**: Cellulase- 0.01 percentage concentration and 4 hours incubation ; **T_7**: Cellulase-0.015 percentage concentration and 3 hours incubation ; **T_8**: Cellulase-0.015 percentage concentration and 4 hours incubation; **T_9**:Hemicellulase-0.01 percentage concentration and 3 hours incubation; **T_{10}**: Hemicellulase-0.01 percentage concentration and 4 hours incubation; **T_{11}**:Hemicellulase-0.015 percentage concentration and 3 hours incubation;**T_{12}**:Hemicellulase-0.015 percentage concentration and 4 hours incubation .

Parameters such as juice recovery, titratable acidity, reducing sugar, total soluble solids content, total sugar, L a* b* value, sedimentation, hue angle, chroma value, colour differentiation(ΔE), transmission and consistency of the processed juice has been studied in the experiment among the twelve combinations of treatments and control.

Among the enzyme treated samples, T_1 where 0.05 percentage of pectinase enzyme was added and incubated for 3 hours shows the highest juice recovery percentage with mean value of 86.89 percentage. The treatment T_4, where the samples were incubated for 4 hours with 0.075 percentage of pectinase enzyme, with value of 86.10 percentage was found to be as good as T_1. The lowest juice recovery percentage T_0 (control) with the percentage recovery of 60.80

The values of TSS of different enzyme treated samples are observed within the ranges of 11.86-15.16 and the highest significant value is a mean value of 15.163°B recorded from the treatment T_6 where 0.01 percentage cellulase is used and incubated for 4 hours and

the least significant value is 11.86 observed in T_0 where no enzymes is used. The second lowest value after T_0 is 14.481 percentage observed from T_{10} where hemi-cellulase of concentration 0.01 percentage for 4 hours incubation.

The titratable acidity observed from the various treatments lies within the range of 0.993-1.365 percentage, where the highest value is 1.365 percentage recorded from T_4 which has a combination of 0.075 percentage of pectinase enzyme with 4 hours incubation whereas the least significant is 0.993 percentage recorded from T_{10} using hemi-cellulase as the enzyme with 0.01 percentage concentration with 4 hours incubation.

Among the enzyme treated samples the range of reducing sugar (percentage) lies within 7.18-9.51 percentage. The highest significant value is 9.51 percentage recorded in T_8 in which cellulase with concentration of 0.015 percentage and 4 hours incubation and the least significant value is 7.18 percentage recorded in T_0 in which no enzyme was used. Second lowest value after T_0 is recorded in T_9 with a value of 8.39 percent.

The range of total sugar (percentage) from the various treatments lies within 9.771-13.987 percentage. The highest significant value is observed from T_4 with an average value of 13.987 percentage, using the enzyme cellulase with 0.015 percentage with 4 hours incubation and the least significant value is 9.771 observed from T_0. In the treatment T_{12}, value of 11.527 percentage, using hemi-cellulase as the enzyme with 0.015 percentage concentration with 4 hours incubation shows second lowest value after T_0.

The data recorded for the L value showed that among the enzyme treated samples, the L value for T_8 (0.015 percentage cellulase incubated for 4 hours) with a mean value of 36.07 was observed to show the most significant texture (lightness), and the least significant was observed from T_0 which recorded to have the value of 23.03

The ‘a*’ value shows that treatments T_2 (0.05 percentage pectinase incubated for 4 hours) and T_{11} (0.015 percentage hemi-cellulase incubated for 3 hours) showed a significant inclination towards a green colour. The highest observation recorded for green colour was from the treatment, T_{11} with value of -2.437 treated with the enzyme hemi-cellulase, and the lowest observation was recorded in T_7 (0.015 percentage cellulase incubated for 3 hours) with value of -1.406.

The highest observation for the treatment of pectinase for the ‘b*’ value was 28.84 which was observed in T_3 where 0.075 percentage enzyme was added for 3 hours incubation and the lowest was 18.54 which was recorded from T_5 where cellulase enzyme (0.01 percentage) was added and incubated for 4 hours.

The sedimentation percentage, from the various treatments lies within the range of 5.057-11.93 percentage. The sedimentation percentage, observed to be the most significant was 11.93 percentage from the treatment T_0 where no enzyme was added and and the lowest significant value was 5.05 percentage observed from the treatment T_9 where 0.01 percentage hemi-cellulase was added and incubated for 3 hours. The treatment T_{12} (0.015 percentage of hemi-cellulase with 4 hours of incubation) with value of 5.763 is shown to be as good as T_9.

The data obtained for hue value shows that the treatment, T_{12} where 0.015 percentage of hemi-cellulase enzyme was added and incubated for 4 hours had the highest percentage change in hue value with mean value of 8.58 percentage while the treatment T_5 (0.01 percentage incubated for 3 hours) showed the lowest change in hue value of 1.84 percent.

While the highest change in chroma value was been recorded for the enzyme cellulase of treatment T_7, the lowest change in chroma value is also of the enzyme cellulase of treatment T_6 where 0.01 percentage of the enzyme was added and incubated for 4 hours. However, from the data obtained it shows that the treatment of enzymes did not interfere with the chroma value. The changes were not significant in any of the enzyme treatments hence it can be concluded that the chroma value has remain unchanged with the addition of the enzymes in the juice samples.

In terms of colour difference, the treatment T_8 where 0.015 percentage of cellulase enzyme was added and incubated for 4 hours has shown the maximum colour difference after the enzymatic treatment with mean value of 10.90 percentage and the lowest is shown in T_1 where 0.05 percentage pectinase concentration and incubation of 3 hours. The data acquired further demonstrate that the enzyme treatment had no effect on the colour difference. No matter whatever enzyme treatment was used, there were no appreciable changes hence it is conclusive that the enzymes were added to the juice samples, the colour difference has not changed.

The effect of percentage change in transmission under different treatments show that the treatment T_{12} where 0.015 percentage hemi-cellulase was added and incubated for 4 hours shows the maximum change in transmission with mean value of 28.85 percentage while the lowest transmission recorded in T_5 with value of 16.07 percentage.

For the parameter of consistency, the treatment T_{11} where 0.015 percentage hemi-cellulase enzyme was added and incubated for 3 hours has maximum change in consistency with mean value of 20.46 percentage and the treatment T_8 where 0.015 percentage cellulase was added and incubate for 4 hours has revealed to have the highest

change in consistency after treatment T_{11} with mean value of 17.57 percentage. The lowest recorded value for change in percentage of consistency is shown in T_1 (0.05 percentage of pectinase with 3 hours incubation) with value of 11.21 percentage.

CHAPTER-6

FUTURE SCOPE OF RESEARCH

FUTURE SCOPE OF RESEARCH

The present study sufficiently provides evidence that the enzymes pectinase, cellulase and hemi-cellulase can be used to treat raw pineapple juice for clarification since these enzymes of pineapple positively influence the juice of pineapple in respect of juice recovery , L a* b* value, sedimentation percentage, TSS, titratable acidity, total sugar, hue value, transmission and consistency. This study also shows the beneficial effect of individual enzymes at different concentration and incubation time. Therefore, it is paramount that the present investigation should be stretched to address the following underlying aspects related to my work in which further studies of research can be conducted:

1) Combined effect of pectinase, cellulase and hemi-cellulase for clarification of pineapple juice could be examined to harness the beneficial effects of all the enzymes.

2) The concentration and incubation time need to be standardized during application of combinations of all the enzymes for clarification of pineapple juice.

3) Additionally other macerating enzymes such as ligninase, xylanase and protease can be used for obtaining maximum yield of juice.

4) These enzymes could also be evaluated for extraction of juices in other fruit crops.

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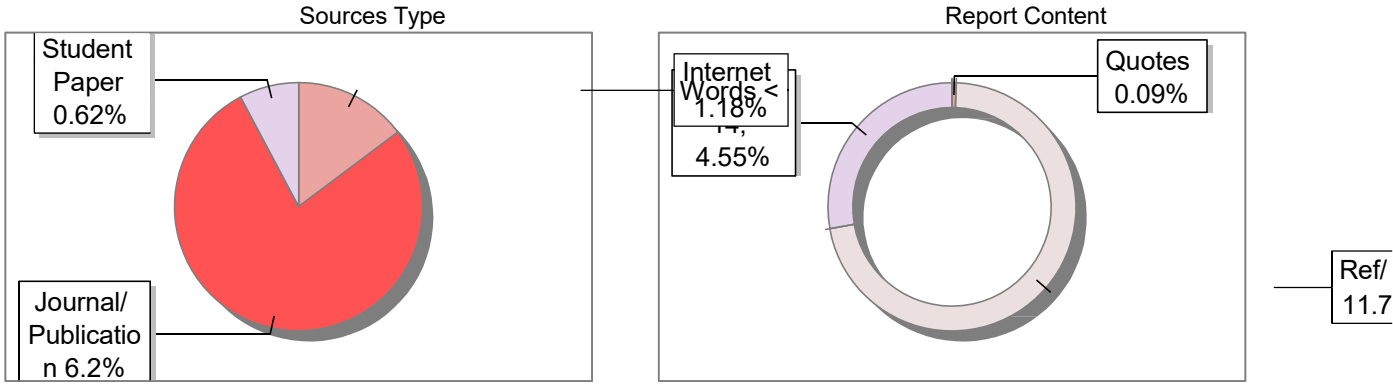

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