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Optimizing and investigating the kinetics of black carrot juice clarification process using pectinase enzyme

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ABSTRACT

This study aims to optimize the activity and investigate the kinetics of the pectinase enzyme (EC 3.2.1.15) in clarifying black carrot juice. For this purpose, the effect of independent factors, including different concentrations of pectinase enzyme (5,6, and 7 gr/hl), brix (4, 4.75, 5.5, 6.25, 7, and 7.75 ◦Brix), time (0.5, 3, and 5.5 h), and temperatures (45, 50, and 55 ◦C) were investigated for the clarification and characteristics of black carrot juice. For this purpose, optimal conditions were determined based on the maximum amount of color, the minimum amount of turbidity and the maximum amount of anthocyanin and the Box–Behnken statistical design. The greatest effect of the pectinase enzyme occurred in the first fifteen minutes. The change graph was linear in this period and followed an almost constant trend. The rate of turbidity decrease after this period followed a downward trend and decreased very slightly but did not stop. Also, the greatest effect of the pectinase enzyme in increasing color occurred in the first twelve minutes. In this period, the graph was linear and then the change trend was very slow. Based on the results, the effect of temperature, time and enzyme concentration on turbidity reduction was significant ($p \leq 0.05$) and also the effect of temperature and time on turbidity reduction was exponential or quadratic. The effect of process time on color changes was not significant, but the effect of time and enzyme concentration on the increase of color was significant and quadratic, and increasing the temperature from 45 to 50 ◦C, the color intensity increased, and increasing the temperature to 55 ◦C, the color intensity decreased. The effect of all three studied factors on the amount of anthocyanin was significant and the amount of anthocyanin increased with increasing temperature, time and enzyme concentration. The optimal point of clarification was obtained at a concentration of 7 g/hl, a time of 5.5 h and a temperature of 55 ◦C. The general conclusion showed that the optimal conditions obtained had a positive effect on the qualitative characteristics of the product.

1.Introduction

Fruit juice is essentially an unfermented but fermentable liquid extracted from the edible parts of mature fruits [1]. According to Codex definition, “fruit juices that are unfermented and intended for direct consumption are obtained from the mechanical processing of fresh, ripe, or sound fruits, which are preserved solely by physical means” [2].

Fruit juices are a rich source of polyphenolic compounds as well as carotenoids, and many people are aware of their importance in daily dietary intake [3]. The high financial turnover has driven the fruit juice industry towards producing high-quality products that are nutritious and minimally processed to retain their health benefits [4]. Since the clear appearance of fruit juice is a determining factor for consumers, the fruit juice industry invests in methods that optimize this characteristic [5]. Typically, clear fruit juice is prepared as a ready-to-drink beverage. In contrast, concentrated juice is reconstituted for beverage consumption or can be used as a flavoring agent in many products such as ice cream and jams. Cloudy juice obtained from pressing fruits is a colloidal suspension consisting of a solution of pectin, sugars, and tannins [4]. To obtain clear juice, these suspended particles must be removed. This process, known as clarification, is recognized as one of the most important unit operations in juice processing [6]. Enzymatic treatment also allows for the effective use of clarifying agents to aid in the removal of cloudiness. The addition of fining or clarifying agents is considered to modify the clarity, color, flavor, or stability of juices. They are categorized based on their general nature into (i) earth materials (bentonite, kaolin), (ii) proteins (gelatin, casein, albumin), (iii) polysaccharides (agar), (iv) carbon, (v) synthetic polymers (PVPP, nylon), (vi) silicon dioxide (kieselguhr), and (vii) others, including metal chelators, enzymes, etc. [7].

Pectinases are a group of multiple enzymes that share a common substrate, namely pectin. Due to the structural heterogeneity of pectin, they can affect different parts of pectin. Thus, they are classified into various groups such as protopectinases, polygalacturonases, pectin lyases, and pectate lyases [8]. Pectins are a generic name for a mixture of very different compounds containing pectic acid as a main component [9]. Pectinases destabilize protein-pectin complexes by hydrolyzing pectic compounds. These complexes have a positively charged protein core surrounded by negatively charged pectin molecules. Partial hydrolysis of pectin molecules exposes positively charged surfaces, leading to the instability of the complexes. Consequently, the electrostatic repulsion between particles decreases, and the complexes bind to each other, forming heavier particles. Simultaneously, sedimentation occurs, following Stokes' law [10]. Clarification of fruit juice using gelatin and bentonite is a common industrial method [7]. These are absorbent compounds used as clarifiers, and when added to fruit juice, they form a flocculent precipitate [11]. Bentonite is a natural mineral based on clay belonging to the montmorillonite group (hydrated aluminum silicates), which swells in water and then becomes gelatinous [12]. In addition

to its surface adsorption properties, bentonite contributes to the clarification of juice by imparting a negative charge [11]. The effects of gelatin and bentonite are respectively directed towards the absorption of phenolic and protein compounds [14]. Gelatin is a proteinaceous absorbent compound that causes the sedimentation of negatively charged particles such as polyphenols and pectic compounds (secondary turbidity factors) [11]. Low turbidity and high retention of anthocyanins are considered key indicators for clarifying anthocyanin-rich juices such as strawberry juice and pomegranate juice [15].

Color is an important indicator in evaluating the quality of foods. The color differences in fruits and vegetables throughout the seasons and the negative effects of processing and storage often necessitate the commercial use of colors to preserve consumer-preferred hues. However, in recent decades, the safety of food colorings has been a highly controversial topic, with almost all opinions against synthetic colors. Due to legal actions and consumer concerns regarding the use of artificial additives, there has been significant interest in food colors derived from natural sources [16]. In recent years, black carrots have gained attention for their extraordinary color and the presence of health-promoting phenolic compounds [17]. The structural configuration of the phenolic compounds in black carrots indicates the presence of a unique aromatic ring that forms phenolic acids. Similarly, chlorogenic acids, which are derivatives of hydroxycinnamic acids resulting from the esterification of cinnamic acids (caffeic, ferulic, and p-coumaric acids), are also found in carrots [18]. Additionally, black carrots contain a relatively higher content of flavonoids compared to orange or red carrots. These flavonoids are significant due to their biochemical and medicinal roles as antioxidants, anti-inflammatory agents, anti-atherosclerotic, anti-platelet aggregation, anti-tumor, and anti-microbial properties [19]. Likewise, the presence of numerous bioactive compounds such as carotenoids, polyacetylenes, falcarindiol, falcarindiol-3-acetate, and anthocyanins (cyanidin, delphinidin, petunidin, poncetin, malvidin, and pelargonidin) is noteworthy [20]. Unlike other phenolic compounds, anthocyanins increase during the ripening of fruits and remain stable after reaching a certain level [21]. The anthocyanins present in black carrots are more stable over a wider pH range and at higher temperatures due to their higher acylated forms compared to other anthocyanins found in fruits or vegetables [22]. These characteristics enable the food industry to utilize the anthocyanins from black carrots as a natural colorant instead of carmine and other synthetic colors [23].

The aim of this study is to examine the effects of various factors on the pectinase enzyme activity in clarifying black carrot juice, improve the production process by identifying enzymatic kinetics, and assess the impact of the processes employed on product quality.

2. Materials and Methods

2.1 Materials Used

The raw materials included black carrots from Dezful farms, pectinex enzyme XXL from Novozymes, citric acid from Jovin-Iran, gelatin from Erbi Gel - Germany, bentonite from Barite Flat - Iran, medical ethanol from

Pakdis Orumiyeh - Iran, filter paper from Schneicher Schull - Germany, and 37% hydrochloric acid from Merck - Germany.

2.2 Sample Preparation

Initially, to prepare the mash, the black carrots were cut into small pieces using a regular or fine grater and mixed with a small amount of acidified water (citric acid with 99.3% purity). The amount of acidified water was adjusted so that the acidity of the black carrot juice after pressing fell within the range of 2.2-3.2 g/l. The acidified mash was heated for 1-2 minutes at a temperature of 55-60 ◦C. The heated mash was then pressed to extract the juice (using a manual juicer). To measure the turbidity and color values during clarification, an appropriate amount of distilled water was placed in a water bath, and its temperature was set between 45 and 55 ◦C.

2.3 Experimental Design

Based on a Box-Benken design, the effects of three factors—pectinase enzyme concentration, time, and temperature—on three quality factors were investigated. The effects of different concentrations of pectinase enzyme (5, 6, and 7 g/hl) at various times (0.5, 3, and 5.5 hours) and temperatures (45, 50, and 55 ◦C) on turbidity, color, and anthocyanin content were examined. The concentrations of gelatin (7 g/hl) and bentonite (150 g/hl) were kept constant in all cases. Three different water baths were used in this design to also examine the effects of varying conditions on the process and minimize error. Thus, different concentrations of the enzyme were repeated under the same and different temperatures and times in different water baths.

3-2-1 Measurement of Turbidity

First, the temperature of the water bath was set to the desired level. After adding various amounts of the enzyme to the juice and confirming the negative pectinase test using acid alcohol (1 mL of 37% hydrochloric acid and 99 mL of 96% medical ethanol), gelatin (7 g/hl), and bentonite (150 g/hl) were added (as per Table 1). After the specified time had elapsed, the black carrot juice was filtered using filter paper, and its turbidity was measured. A HACH Model 2100 turbidity meter was used for turbidity measurement.

3-2-2 Measurement of Color

Initially, the water bath temperature was set to the desired level. After adding various amounts of the enzyme to the juice and confirming the negative pectinase test using acid alcohol (1 mL of 37% hydrochloric acid and 99 mL of 96% medical ethanol), gelatin (7 g/hl), and bentonite (150 g/hl) were added (as per Table 1). After the specified time had elapsed, the black carrot juice was filtered, its Brix was reduced to 0.25, and its color was analyzed using a spectrophotometer at wavelengths of 430 nm and 520 nm. For color measurement, a UV/Vis spectrophotometer (Model 7310 Stone, Staffs, UK) was used. The Brix of the juice was measured using an Atago Rx-7000a refractometer from Tokyo, Japan.

Table 1: The exprimental design matrix

Run	A:Temperatur (◦C)	B:Time (h)	C: Enzyme concentration (g.hl)	NTU	Colour	Anthocyanins (mg/100 gr)
1	45	5.5	6	5.80	1.019	25.52
2	45	3	5	8/88	1.001	12.20
3	50	0.5	5	12.20	1.008	10.88
4	50	3	6	4.40	1.025	29.75
5	45	0.5	6	11.80	1.01	10.25
6	45	3	7	7.20	1.005	22.54
7	55	3	7	4.95	1.002	54.27
8	50	0.5	7	7.70	1.018	14.56
9	50	3	6	4.90	1.022	28.90
10	50	3	6	5.20	1.017	26.20
11	55	5.5	6	2.40	0.994	50.20
12	50	5.5	7	2.90	1.022	35.60
13	50	5.5	5	4.22	1.014	28.25
14	55	3	5	5.80	0.985	42.50
15	55	0.5	6	10.80	0.997	17.70

3-2-3 Measurement of Anthocyanins

First, the water bath temperature was set. After adding various amounts of the enzyme to the juice and confirming the negative pectinase test, gelatin (7 g/hl) and bentonite (150 g/hl) were added (as per Table 1). After the specified time elapsed, the black carrot juice was filtered, and the clear juice was reserved for subsequent steps. For anthocyanin measurement, a buffer was prepared first. A pH 0.1 buffer (potassium chloride, 0.025 molar) was made by weighing 1.86 grams of KCl in a beaker and adding distilled water up to about 980 mL. The pH was adjusted to 0.1 (±0.05) with HCl (approximately 3.6 mL). The sample was transferred to a one-liter volumetric flask and diluted with distilled water to volume. A pH 4.5 buffer (sodium acetate, 0.4 molar) was prepared by weighing 54.43 grams of CH3CO2Na·3H2O in a beaker and adding distilled water up to about 960 mL. The pH was adjusted to (±0.05) 4.5 with HCl (approximately 20 mL). This was then transferred to a one-liter volumetric flask and diluted with distilled water to volume. A suitable dilution factor was determined by diluting the test sample with the pH 0.1 buffer until the absorbance at 520 nm fell within the linear range of the spectrophotometer. Using this dilution factor, two dilutions of the test sample were prepared, one with pH 0.1 buffer and the other with pH 4.5 buffer, and then incubated at room temperature for 20 minutes. The samples were then placed in the refrigerator at 4 ◦C. For the clarified black carrot juice samples, a dilution was prepared at each stage, and their temperature was reduced to 4 ◦C before centrifugation at 7000 RPM for 15 minutes using a Hettich Rotina/s centrifuge. The supernatant was then collected, and absorbance was read at 520 nm and 700 nm [16].

To calculate the total anthocyanin (TA), the following formula was used:

$$TA = \frac{A \times V}{M}$$

$A = B - C$

B = Absorption difference of the sample at pH 1 at wavelength (A520 nm − A700 nm)

C = Absorption difference of the sample at pH 4.5 at wavelength (A520 nm − A700 nm)

V = Sample volume (ml)

M = Sample weight (grams)

TA= Total anthocyanin

2-4 Kinetic Study of Enzyme Activity

The changes in NTU and color were examined with three concentrations of pectinase enzyme and six different Brix levels according to Tables 2, 3, and 4. The Lineweaver-Burk plot was then used to investigate the kinetic activity of the enzyme.

2-4-1 Measurement of Turbidity

Black carrot juice was poured into six beakers, each containing 100 mL with different Brix levels, and the water bath temperature was set to 50 ◦C. Then, according to Tables 2, 3, and 4, different concentrations of the enzyme were added. After confirming the negative pectinase test using acid alcohol (1 mL of 37% hydrochloric acid and 99 mL of 96% medical ethanol), constant amounts of gelatin (7 g/hl) and bentonite (150 g/hl) were added. The black carrot juice was filtered every three minutes using filter paper, and its turbidity was measured with a turbidity meter, and the results were recorded. This process continued until the 36th minute. A HACH Model 2100 turbidity meter was used for turbidity measurement. The Brix of the juice was measured using an Atago Rx-7000a refractometer from Tokyo, Japan.

2-4-2 Measurement of Color

Black carrot juice was poured into six beakers, each containing 100 mL with different Brix levels, and the water bath temperature was set to 50 ◦C. Then, according to Tables 2, 3, and 4, different concentrations of the enzyme were added. After confirming the negative pectinase test using acid alcohol (1 mL of 37% hydrochloric acid and 99 mL of 96% medical ethanol), constant amounts of gelatin (7 g/hl) and bentonite (150 g/hl) were added. The black carrot juice was filtered every three minutes using filter paper, its Brix was reduced to 0.25, and its color was analyzed using a spectrophotometer at wavelengths of 430 nm and 520 nm. This process continued until the 36th minute. For color measurement, a UV/Vis spectrophotometer (Model 7310 Stone, Staffs, UK) was used. The Brix of the juice was measured using an Atago Rx-7000a refractometer from Tokyo, Japan.

Table 2: Changes in colour and NTU at the concentration of 7 g/hl

Time / min	BX=7.75		BX=7.00		BX=6.25		BX=5.5		BX=4.75		BX=4.00	
	Colour1	NTU	Colour2	NTU	Colour3	NTU	Colour4	NTU	Colour5	NTU	Colour6	NTU
0	0.956	33	0.956	30	0.956	26	0.956	23	0.956	20	0.956	18
3	0.981	30	0.985	28	0.988	22	0.992	20	0.996	17	1	14
6	0.994	22	0.997	19	0.999	16	1.003	14	1.007	12	1.012	10.2
9	1.009	14.8	1.011	13.7	1.014	11	1.017	9.2	1.02	8.5	1.024	7.9
12	1.017	8.8	1.018	6.2	1.022	5.7	1.025	5.2	1.028	4.7	1.03	3.9
15	1.022	3.01	1.024	2.97	1.026	2.92	1.029	2.88	1.031	2.81	1.033	2.7
18	1.023	2.97	1.025	2.95	1.027	2.9	1.03	2.86	1.033	2.79	1.034	2.68
21	1.025	2.94	1.027	2.91	1.029	2.87	1.032	2.83	1.034	2.77	1.036	2.66
24	1.027	2.9	1.029	2.88	1.03	2.84	1.034	2.8	1.036	2.75	1.038	2.65
27	1.028	2.88	1.031	2.85	1.032	2.8	1.036	2.77	1.038	2.73	1.039	2.63
30	1.03	2.85	1.033	2.83	1.034	2.77	1.038	2.73	1.04	2.71	1.041	2.61
33	1.031	2.81	1.034	2.77	1.035	2.68	1.039	2.62	1.041	2.58	1.043	2.52
36	1.033	2.75	1.035	2.7	1.037	2.57	1.041	2.51	1.43	2.45	1.044	2.38

Table 3: Changes in colour and NTU in the concentration of 6 g/hl pectinase enzyme

Time / min	BX=7.75		BX=7.00		BX=6.25		BX=5.5		BX=4.75		BX=4.00	
	Colour	NTU	Colour	NTU	Colour	NTU	Colour	NTU	Colour	NTU	Colour	NTU
0	0.956	33	0.956	30	0.956	26	0.956	23	0.956	22	0.956	21
3	0.966	30	0.967	27	0.97	23	0.973	19	0.974	17	0.976	17
6	0.975	25	0.976	22	0.981	17	0.986	14	0.987	13	0.989	13
9	0.984	20	0.985	16	0.991	11	0.996	9	0.998	9	1	8.8
12	0.992	16	0.993	11	1.001	8	1.006	7	1.008	6	1.011	8.1
15	1	13	1	8	1.008	6	1.014	6	1.016	5.5	1.019	5.1
18	1.007	11	1.007	6	1.016	5.5	1.022	5.5	1.024	5	1.026	4.6
21	1.013	9	1.014	5.5	1.024	5	1.024	5	1.026	4.5	1.028	4.4
24	1.016	7.3	1.017	5	1.028	4.7	1.025	4.5	1.028	4.2	1.03	4.1
27	1.019	6.1	1.02	4.7	1.024	4.4	1.026	4.2	1.03	3.9	1.031	3.8
30	1.021	5.1	1.023	4.4	1.027	4.1	1.028	3.9	1.032	3.6	1.032	3.5
33	1.023	4.3	1.025	4.1	1.03	3.8	1.03	3.6	1.034	3.3	1.035	3.2
36	1.025	4	1.027	3.8	1.031	3.6	1.032	3.3	1.036	3.2	1.038	3.1

Table 4: Changes in colour and NTU at a concentration of 5 g/hl of pectinase enzyme

Time / min	BX=7.75		BX=7.00		BX=6.25		BX=5.5		BX=4.75		BX=4.00	
	Colour	NTU	Colour	NTU	Colour	NTU	Colour	NTU	Colour	NTU	Colour	NTU
0	0.956	30	0.956	27	0.956	24	0.956	23	0.956	22	0.956	21
3	0.963	27	0.965	24	0.966	20	0.968	19	0.969	17	0.969	16
6	0.97	23	0.973	20	0.974	16	0.976	15	0.977	13	0.977	12
9	0.975	19	0.979	16	0.982	12	0.984	11	0.985	9	0.985	8
12	0.98	16	0.984	12	0.989	8	0.99	7	0.992	8	0.993	7
15	0.985	14	0.989	10.2	0.994	6.9	0.996	6.5	0.998	7	1	6
18	0.989	12	0.994	8.7	0.999	6	1.001	6	1.004	6.5	1.006	5.4
21	0.993	10.2	0.999	7.8	1.004	5.2	1.006	5.5	1.008	5	1.011	5
24	0.997	8.7	1.004	7	1.008	5	1.011	5.1	1.012	4.7	1.015	4.6
27	1	7.2	1.008	6.2	1.012	4.8	1.014	4.7	1.016	4.5	1.019	4.2
30	1.003	6.3	1.011	5.6	1.016	4.6	1.017	4.5	1.02	4.3	1.023	4.1
33	1.006	5.5	1.014	5	1.019	4.4	1.02	4.3	1.023	4.1	1.026	4
36	1.008	4.9	1.016	4.7	1.022	4.3	1.023	4.1	1.026	4	1.029	3.9

2-5 Statistical Analysis

For quantitative characteristics, descriptive statistics were used with mean and standard deviation, and one-way ANOVA at a significance level of 0.05 was used to compare the means of the test results. Data analysis was performed using Excel software.

3- Results and Discussion

3-1 Examination of Turbidity Changes

According to Figure (A)1, the effects of time and enzyme concentration on turbidity reduction are significant with $P < 0.05$. As time and enzyme concentration increase, turbidity decreases. The F-value indicates that the effect of time is significantly greater than that of enzyme concentration, suggesting that increasing time has a much more substantial impact on turbidity reduction compared to enzyme concentration. As shown in Figure (B)2, temperature has a significant effect on turbidity reduction as a quadratic factor due to $P < 0.05$. The graph indicates that the effect of temperature in relation to enzyme concentration is exponential. The lowest turbidity was achieved at a concentration of 6 g/hl of pectinase enzyme at a temperature of 55 ◦C after 5.5 hours. After analyzing the

data, the predictive equation for estimating turbidity was determined as follows:

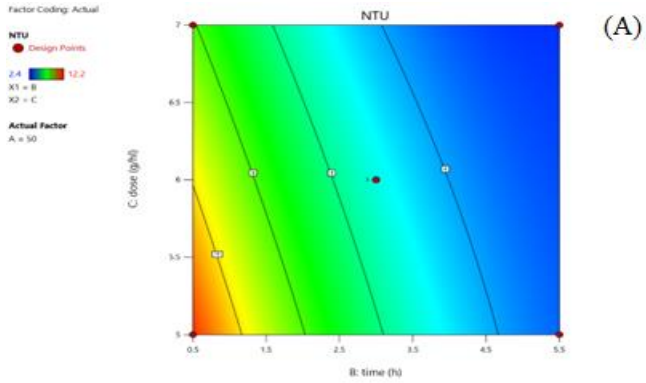
Turbidity:172.77-5.37 Te - 4.63 time-1.99 dose +.318 time*dose +.54Te^2 +.227$time^2$

Overall, the model used to predict data on juice turbidity was meaningful with:

R^2 =0.966

Adgusted R^2 =0.940

In general, turbidity indicates the presence of suspended colloidal solid particles in fruit juice samples, and the addition of pectinases leads to the degradation of pectin, resulting in reduced turbidity [24]. Wang et al. (2023) demonstrated that increasing the amount of pectinase enzyme enhances the rate of enzymatic reaction and decreases turbidity. However, when the amount of pectinase enzyme continued to increase, the improvement effect on the turbidity of sticky rice tea was no longer significant due to the reduction of substrate (pectin) [24]. Golazilmaz and Sonur Gunay (2023) showed that as the amount of enzyme used increased, turbidity decreased due to the enzymatic degradation of polysaccharides [25]. Diman et al. (2011) optimized the xylanase enzyme derived from Bacillus stearothermophilus for clarifying pineapple juice using RSM. At the end of the enzymatic process, it was found that turbidity decreased by 34.35%. Idris-Arjeh, Mir Khalil Pirouzi Fard, and Sajad Pirsah (2018) reported a turbidity reduction in sugar beet syrup due to pectinase enzyme treatment [10].



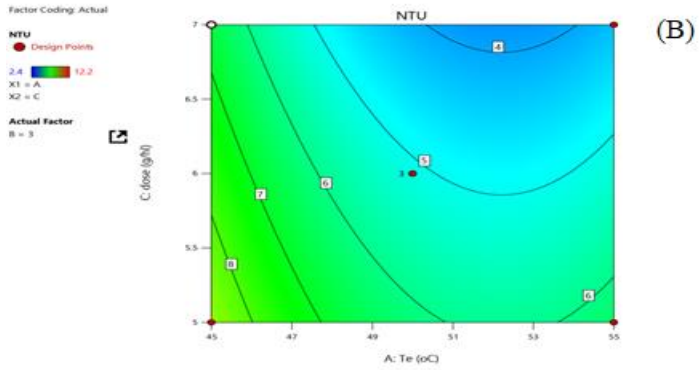


Figure 1: (A) Changes of NTU with enzyme concentration and time, (B) NTUchanges with enzyme concentration and temperature

3-2 investigation of Color Changes

According to Figure 3, the incubation time has no effect on the increase or decrease of color. Temperature and enzyme concentration are significant in increasing color, with $P < 0.05$, and exhibit a quadratic effect such that as temperature increases from 45 to 50 ◦C, color increases, but with further increases up to 55 ◦C, color decreases. Temperature has a greater impact on color increase due to a higher F-value and lower P-value compared to enzyme concentration.

The highest color was achieved at a concentration of 6 g/hl and at a temperature of 50 ◦C after three hours.

After analyzing the data, the predictive equation for estimating color was determined as follows:

Colour: -0.84 +0.06 Te +0.08dose -0.00067 Te^2 - 0.006$dose^2$

Overall, the model used for predicting the color of fruit juices was significant with:

R^2=0.9

Adgusted R^2 =0.860

Nurjana Ay and Noor Azia (2011) investigated the effects of different concentrations of pectinase enzyme on various factors in durian juice. They showed that as the enzyme concentration increased, color significantly increased [27]. Heating creates opportunities for oxidative reactions that lead to the degradation of pigments. The increase and decrease in color align with previous studies, indicating that color increased from 45 to 55 ◦C and then decreased with further increases in temperature.

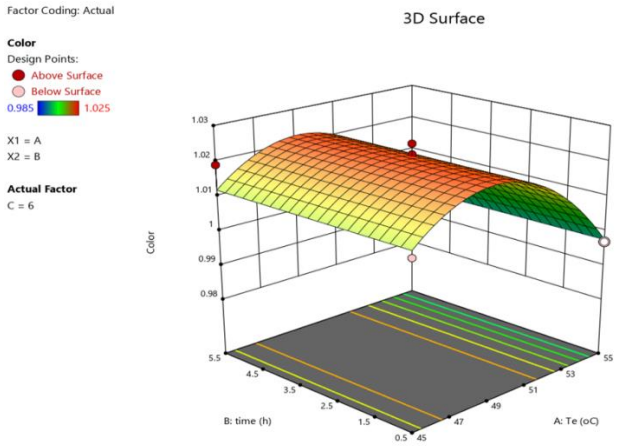


Figure 2: Color relationship with time and temperature

3-3 Investigation of Anthocyanin Changes

According to Figure 3 (A, B, C), the effects of all three factors—temperature, time, and enzyme concentration—are significant in the changes of anthocyanins with $p \leq 0.05$. The relationship of all three factors with anthocyanins is linear; as each of these

factors increases, anthocyanins also increase. Based on the F-value, enzyme concentration has the least effect, while temperature has the greatest impact on anthocyanin changes. The highest number of anthocyanins was obtained at a temperature of 55 ◦C, a time of three hours, and with a concentration of 7 g/hl of pectinase enzyme. After analyzing the data, the predictive equation for estimating anthocyanins was determined as follows:

Anthocyanin(mg/100gr)= -128.194 +2.35Te+4.30time+4.14dose

Overall, the model used for predicting the anthocyanin content in fruit juices was significant with:

R^2 =0.826

Adjusted R^2 =0.778

Orhan Derli's Bouquet et al (2023) observed a 12% reduction in monomeric anthocyanins after pectin degradation in strawberry juice [15]. Conversely, Turkilmaz et al. (2012) noted an increase in anthocyanin content in black carrot juice after pectin degradation [16]. This may be due to the difference between the pectin and protein ratios in black carrot juice and strawberry juice, as well as the different anthocyanin stabilities in both juices. The ratio of pectin (14.3 g/kg) [26] to protein (11.4 g/kg) [29] in black carrot juice is 1.2, while this ratio is 209 for strawberry juice. Therefore, only a small portion of the free pectin can bind to the proteins in strawberry juice, as the protein content is much lower. The remaining free pectin, with a negative charge, can interact with positively charged monomeric anthocyanins in strawberry juice, potentially leading to pigment formation that increases color density and stability. Since black carrots contain pectin, pectin degradation is first carried out to break down pectin and form protein-pectin flocs that can be easily separated from the juice. Due to the low pectin content in black carrot juice, pectin can bind to monomeric anthocyanins in lower amounts [30]. Thus, the changes in anthocyanin content in black carrot juice after pectin degradation may primarily result from the release of monomeric anthocyanins due to cell wall destruction, rather than copigmentation with pectin. The overall increase in anthocyanin content with enzymatic treatment and its relative reduction during clarification with gelatin and bentonite leads to a decrease in the rate of increase in anthocyanin content compared to the effects of increasing temperature and time [16].

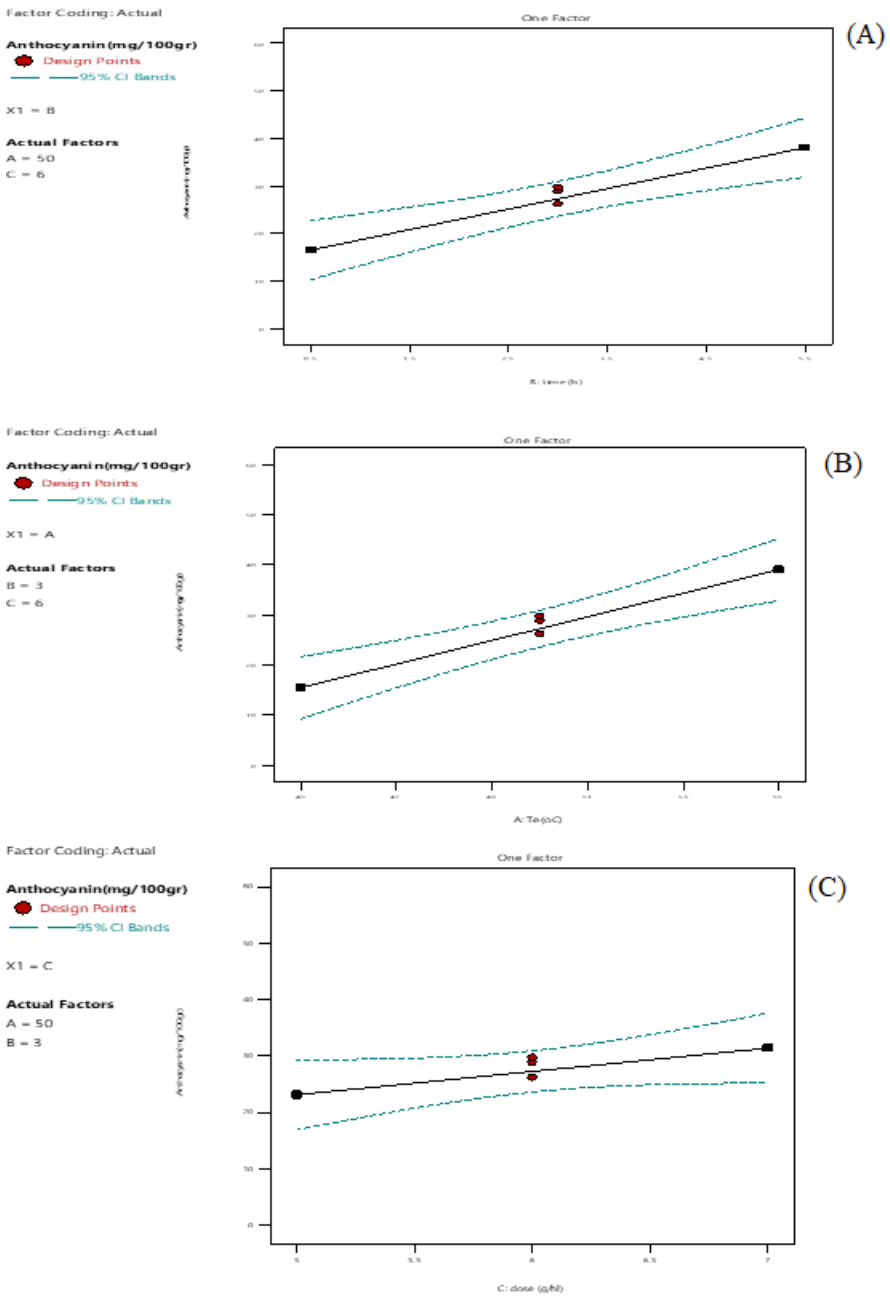


Figure 3(A):Anthocyanin changes with time (B) Anthocyanin changes with temperature (C) Anthocyanin changes with enzyme concentration

3-4 Kinetics of Enzyme Activity

According to the Lineweaver-Burk model, the maximum reaction rate (Vmax) was calculated to be 766.16, and the Km constant was 581.69 from the obtained data. Based on the numbers in the table below, the turbidity changes can be plotted at a concentration of 7 g/hl of pectinase enzyme.

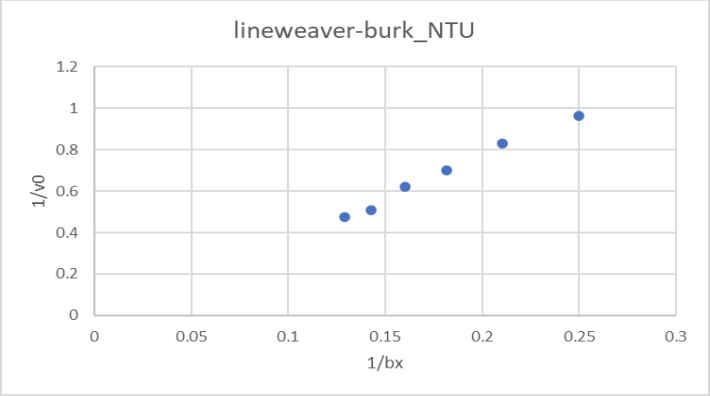


Figure 4: The NTU changes according to the concentration of 7 g/hl of pectinase enzyme

As seen in Figure 4, the greatest effect of pectinase enzyme on turbidity occurs in the first 15 minutes. During this period, the turbidity changes are linear, and afterwards, the trend stabilizes. The reduction in turbidity slows down significantly after the initial 15 minutes, although it does not stop.

According to the Lineweaver-Burk model, with Vmax calculated as 0.00436 and Km constant as 1.022 from the obtained data, the color changes can be plotted at a concentration of 7 g/hl of pectinase enzyme

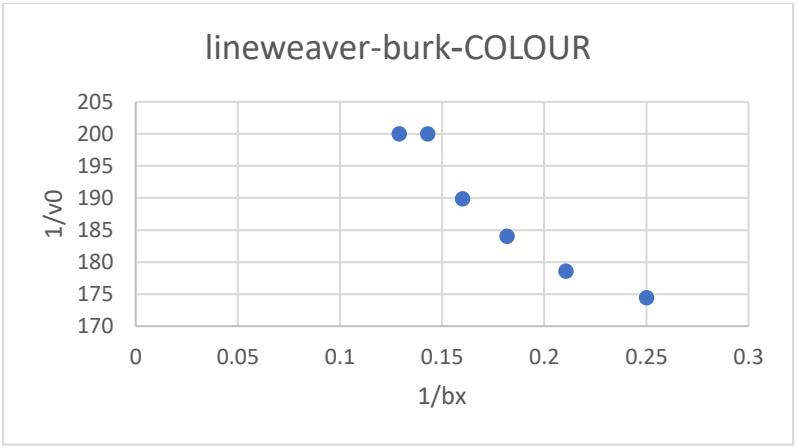


Figure 5: The colour changes according to the concentration of 7 g/hl of pectinase enzyme

Figure 5 shows that the greatest effect of pectinase enzyme on color increase occurs in the first twelve minutes. During this period, the color increase is almost linear, and after that, the color increases at a stable and very slow rate, but does not stop.

According to the Lineweaver-Burk model, with Vmax calculated as 2.9339 and Km constant as 7.2865 from the obtained data, the turbidity changes can be plotted at a concentration of 6 g/hl of pectinase enzyme.

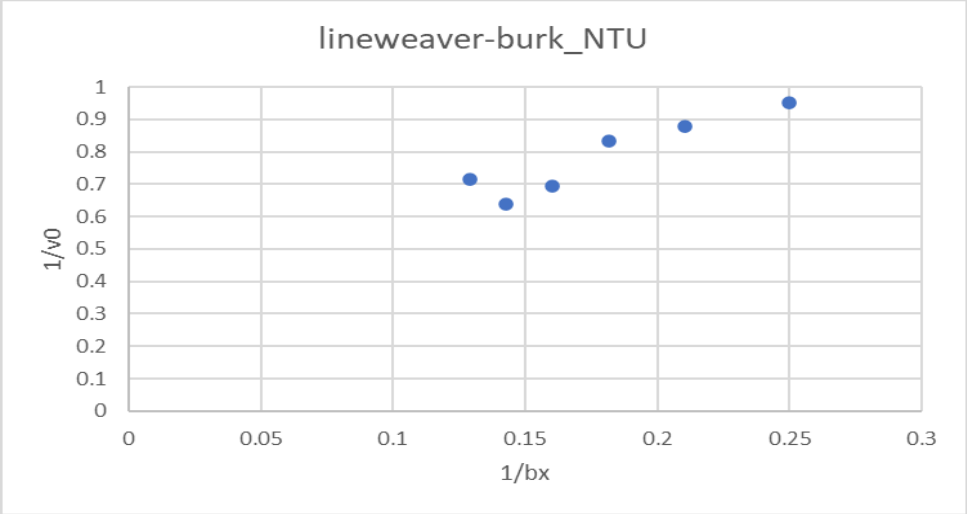


Figure 6: Kinetics of changes in NTU concentration of 6 g/hl of pectinase enzyme

Figure 6 indicates that at a concentration of 6 g/hl of pectinase enzyme, the changes in turbidity are not regular and linear and do not follow a consistent trend.

According to the Lineweaver-Burk model, with Vmax calculated as 0.00232 and Km constant as 2.875 from the obtained data, the color changes can be plotted at a concentration of 6 g/hl of pectinase enzyme.

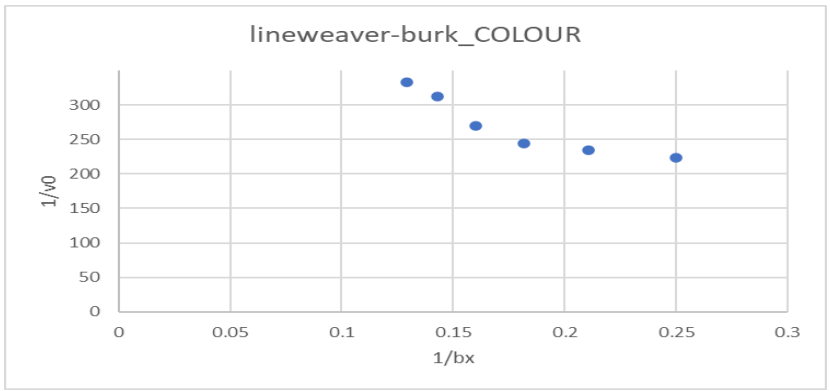


Figure 7: Kinetics of colour changes with a concentration of 6 g/hl of pectinase enzyme

According to Figure 7, the most significant color changes occur in the first 12 minutes, with the rate of increase being initially slow and then accelerating over time.

Using the Lineweaver-Burk model, with Vmax calculated as 1.4591 and the Km constant as 1.775 from the obtained data, the turbidity changes can be plotted at a concentration of 5 g/hl of pectinase enzyme.

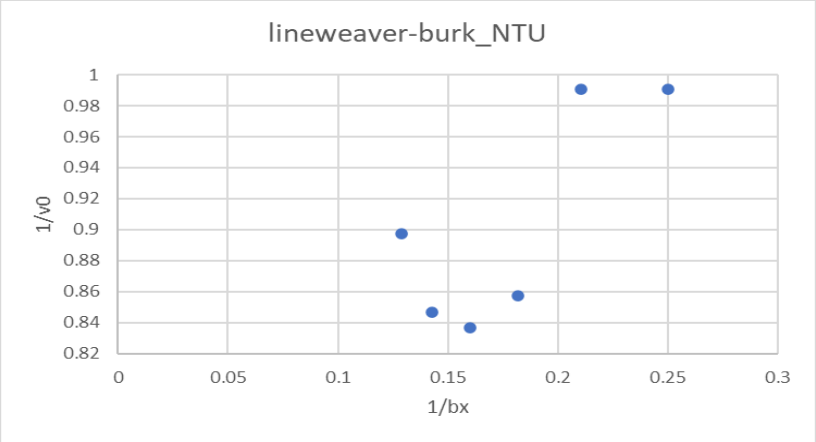


Figure 8: Kinetics of NTU changes with a concentration of 5 g/hl of pectinase enzyme

Investigation of Figure 8 indicates that at this enzyme concentration, the turbidity reduction trend is highly irregular and does not follow a specific formula.

3-5 Optimization of the Clarification Process

According to the graphs, the optimal point of the process is approximately at a concentration of 7 g/hl of pectinase enzyme, at a temperature of 55 ◦C, and for a duration of 5.5 hours. The desirability factor is 0.963, which is a suitable value. At this point, the anthocyanin content is nearly at its maximum, while the turbidity is close to the minimum obtained in this study.

Table 5: The optimal values obtained

Temperature	time	dose	NTU	Color	Anthocyanin (mg/100gr)	Desirability
54.99	5.49	6.99	3.051	0.99	53.97	0.96

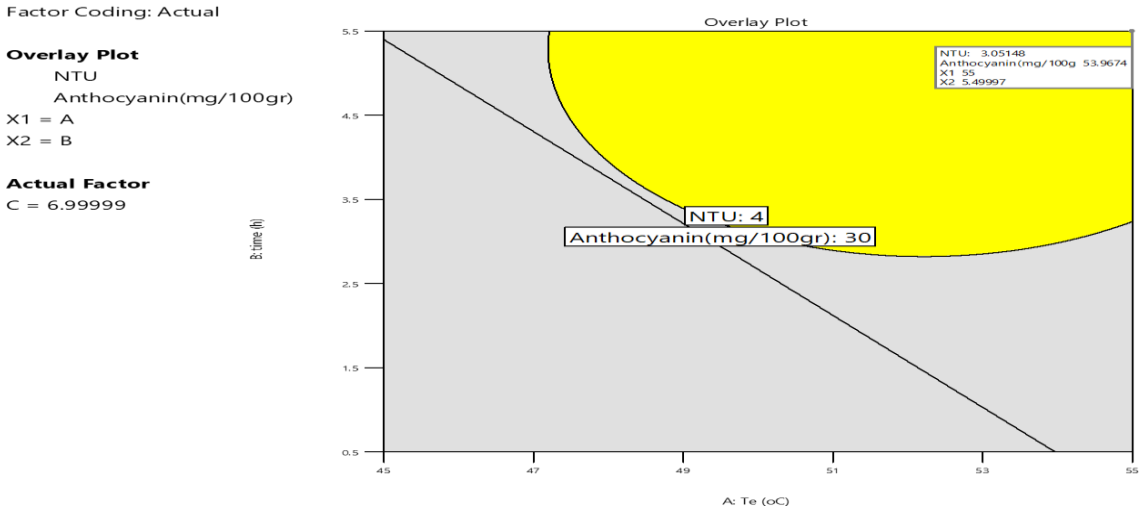


Figure 9: The optimal range obtained

4.Conclusion

Fruit juices must undergo enzymatic clarification processes and the use of processing aids to be appealing and attractive to consumers. However, this process should be designed to consider not only commercial attractiveness but also nutritional aspects and consumption factors. The optimal range for usage must be determined, ensuring that important quality factors such as anthocyanins, turbidity, and color are at their best possible levels. In this study, various amounts of pectinase enzyme at different temperatures and times were examined to determine the optimal usage point. Ultimately, the optimal clarification point was found to be at a concentration of 7 g/hl, a time of 5.5 hours, and a temperature of 55 ◦C. The overall conclusion indicates that the optimal conditions obtained positively impact the quality characteristics of the product.

5-Reference

[1] Amiri, S., & Rajabi, M. (2021). *An overview of the application of natural antimicrobial compounds from plant, animal and microbial origin in foods*. Journal of food science and technology (Iran), **18**(119), 143-156.

[2] Chen, M.-F., 2009. Attitude toward organic foods among Taiwanese as related to health consciousness, environmental attitudes, and the mediating effects of a healthy lifestyle. Br. Food J. 111 (2), 165-178.

[3] Alizadeh, S., Pirsa, S., & Amiri, S. (2023). Development of a colorimetric sensor based on nanofiber cellulose film modified with ninhydrin to measure the formalin index of fruit juice. *International Journal of Biological Macromolecules*, *253*, 127035.

[4] Pirsa, S., Banafshechin, E., Amiri, S., Rahimirad, A., & Ghafarzadeh, J. (2021). *Detection of fraud of palm, sunflower, and corn oil in butter using HPLC profile of tocopherols and tocotrienols by response surface method*. Journal of the Iranian Chemical Society, **18**, 1167-1177.

[5] Sandri, I. G., Fontana, R. C., Barfknecht, D. M., & da Silveira, M. M. (2011). Clarification of fruit juices by fungal pectinases. *LWT-food Science and Technology*, *44*(10), 2217-2222.

[6] Tajchakavit, S., J.I. Boye and R. Couture, "Effect of processing on post-bottling haze formation in apple juice", Food Research Internat., 34, 415-424 (2001).

[7] Benitez, E. I., & Lozano, J. E. (2007). Effect of gelatin on apple juice turbidity. Latin American applied research, 37(4), 261-266

[8] Nighojkar, A., Patidar, M. K., & Nighojkar, S. (2019). Pectinases: production and applications for fruit juice beverages. In *Processing and sustainability of beverages* (pp. 235-273). Woodhead Publishing.

[9] Kashyap, D. R., Vohra, P. K., Chopra, S., & Tewari, R. (2001). Applications of pectinases in the

commercial sector: a review. *Bioresource technology*, *77*(3), 215-227.

[10] Arjeh, E., Pirouzifard, M. K., & Pirsa, S. (2018). Enzymatic pre-treatment of raw sugar beet juice using pectinase to improve the conventional purification process. Innovative Food Technologies, 6(1), 45-54.

[11] Arjeh, E., Pirouzifard, M., & Pirsa, S. (2019). Purification of beet molasses using bentonite and gelatin: process evaluation and optimization. Journal of food science and technology (Iran), 16(88), 289-301

[12] Lambri, M., Dordoni, R., Silva, A., & De Faveri, D. M. (2012). Comparing the impact of bentonite addition for both must clarification and wine fining on the chemical profile of wine from Chambave Muscat grapes. *International journal of food science & technology*, *47*(1), 1-

[13] Akshi Aziz, Summer 1999, Transparency of Juice, Mirkhalil Victory Fard, Urmia, University Jihad Publications, 178

[14] Gökmen, V., & Çetinkaya, Ö. (2007). Effect of pretreatment with gelatin and bentonite on permeate flux and fouling layer resistance during apple juice ultrafiltration. *Journal of food engineering*, *80*(1), 300-305.

[15] Dereli, B. O., Türkyılmaz, M., & Özkan, M. (2023). Clarification of pomegranate and strawberry juices: Effects of various clarification agents on turbidity, anthocyanins, colour, phenolics and antioxidant activity. *Food Chemistry*, *413*, 135672.

[16] Türkyılmaz, M., & Özkan, M. (2012). Kinetics of anthocyanin degradation and polymeric colourformation in black carrot juice concentrates during storage. International journal of food science & technology, 47(11), 2273-2281.

[17] Amiri, S., Rezazad Bari, L., Malekzadeh, S., Amiri, S., Mostashari, P., & Ahmadi Gheshlagh, P. (2022). Effect of Aloe vera gel-based active coating incorporated with catechin nanoemulsion and calcium chloride on postharvest quality of fresh strawberry fruit. *Journal of Food Processing and Preservation*, *46*(10), e15960.

[18] Chatatikun, M., & Chiabchalard, A. (2013). Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (Daucus carota Linn.) root crude extracts. Journal of Chemical and Pharmaceutical Research, 5(4), 97-102.

[19] Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. Pharmacological research, 55(3), 207-216.

[20] Akhtar, S., Rauf, A., Imran, M., Qamar, M., Riaz, M., & Mubarak, M. S. (2017). Black carrot (Daucus carota L.), dietary and health promoting perspectives of its polyphenols: A review. *Trends in Food Science & Technology*, *66*, 36-47.

[21] Rezazad Bari, L., & Amiri, S. (2022). The Application of Packaging Containing Titanium Dioxide Nanoparticles on the Physicochemical and Biochemical Characteristics of Grape Fruit During Cold Storage. *Packaging Science and Art*, *12*(48), 1-10.

[22] Witrowa-Rajchert, D., Bawoł, A., Czapski, J., & Kidoń, M. (2009). Studies on drying of purple carrot roots. Drying Technology, 27(12), 1325-1331.

[23] Kırca, A., Özkan, M., & Cemeroğlu, B. (2007). Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food chemistry*, *101*(1), 212-218.

[24] Wang, Q., Liu, K. Y., Zhang, Q., An, J. S., Xie, Z. Z., Chen, Z., ... & Li, R. Y. (2023). Optimisation of clarification process of glutinous rice tea wine, and its antioxidant activity. *International Food Research Journal*, *30*(1), 205-215.

[25] Ozyilmaz, G., & Gunay, E. (2023). Clarification of apple, grape and pear juices by co-immobilized amylase, pectinase and cellulase. *Food Chemistry*, *398*, 133900

[26] Dhiman, S. S., Garg, G., Sharma, J., & Mahajan, R. (2011). Characterization of statistically produced xylanase for enrichment of fruit juice clarification process. *New Biotechnology*, *28*(6), 746-755.

[27] Norjana, I., & Noor Aziah, A. A. (2011). Quality attributes of durian (Durio zibethinus Murr) juice after pectinase enzyme treatment. *International Food Research Journal*, *18*(3).

[28] Yusuf, E., Tkacz, K., Turkiewicz, I. P., Wojdyło, A., & Nowicka, P. (2021). Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. *European Food Research and Technology*, *247*, 3053-3062.

[29] Saleem, M. Q., Akhtar, S., Imran, M., Riaz, M., Rauf, A., Mubarak, M. S., ... & Hassanien, M. F. (2018). Antibacterial and anticancer characteristics of black carrot (Daucus Carota) extracts. *J. Med. Spice Plants*, *22*, 40-44.

[30] Legentil, A., Guichard, I., Piffaut, B., & Haluk, J. P. (1995). Characterization of strawberry pectin extracted by chemical means. *LWT-Food Science and Technology*, *28*(6), 569-576.

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مقاله علمی_پژوهشی

بهینه‌سازی و بررسی سینتیک فرآیند شفاف‌سازی آب‌هویج سیاه با استفاده از آنزیم پکتیناز

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چکیده

هدف ازاین‌مطالعه، بهینه‌سازی فعالیت و بررسی سینتیک آنزیم پکتیناز (EC 3.2.1.15) درشفاف‌سازی آب‌هویج‌سیاه می‌باشد. بدین منظورتاثیر فاکتورهای مستقل، شامل غلظت‌های مختلف آنزیم پکتیناز (gr/hl ۶،۷، ۵)، بریکس (۷/۷۵، ۷ ، ۶/۲۵ ، ۵/۵ ، ۴/۷۵ ، ۴ درجه بریکس)، زمان (۵/۵، ۳ ،۰/۵ ساعت) و دما (۵۵، ۵۰، ۴۵ درجه سانتیگراد) برشفاف‌سازی و ویژگی‌های آب‌هویج‌سیاه بررسی شد. بدین منظور براساس بیشینه مقدار رنگ، کمینه مقدار کدورت وبیشینه مقدار آنتوسیانین وبا طرح آماری باکس-بنکن[1] شرایط بهینه تعیین گردید. بیشترین تاثیرآنزیم پکتینازدر پانزده دقیقه ابتدایی صورت گرفت. در این بازه نمودار تغییرات بصورت خطی بود و بعد ازآن تقریبا روند ثابتی را طی کرد و میزان کاهش کدورت بعد از این مدت روند نزولی را طی کرد و بسیار ناچیز کاهش پیدا کرد ولی متوقف نشد. نیز بیشترین تاثیرآنزیم پکتیناز در افزایش رنگ در دوازده دقیقه ابتدایی صورت گرفت. در این مدت نمودار بصورت خطی بود و بعد از آن روند تغییرات بسیار کُند بود. براساس نتایج، تاثیر دما، زمان و غلظت آنزیم برکاهش کدورت معنی‌دار بوده ($p\leq0.05$) و همچنین اثر دما و زمان برکاهش کدورت بصورت نمایی بود. اثر زمان فرآیند بر تغییرات رنگ معنی‌دار نبود ولی اثر زمان و غلظت آنزیم برافزایش رنگ معنی‌دارو بصورت درجه دو بود و با افزایش دما از۴۵به۵۰درجه سانتیگراد، شدت رنگ افزایش داشت وبا افزایش بیشتر دما تا۵۵درجه سانتیگراد، شدت رنگ کاهش یافت. تاثیر هرسه فاکتور مورد مطالعه برمیزان آنتوسیانین معنی‌داربود و با افزایش دما، زمان و غلظت آنزیم، مقدار آنتوسیانین افزایش داشت. نقطه بهینه شفاف‌سازی در مقدار غلظت ۷g/hl، زمان۵/۵ساعت و دمای۵۵درجه سانتیگراد بدست آمد. نتیجه-گیری کلی نشان داد شرایط بهینه بدست آمده، تاثیر مثبت بر ویژگی‌های کیفی محصول را نشان داد.

[1]-Box–Behnken design