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Kinetic Studies of *Aspergillus ficuum* Tannase Enzyme and its Application in Detannification of Grape Juice

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Abstract:

This investigation was carried out to study the characterization and kinetics of the partially purified *Aspergillusficuum*tannase enzyme for commercial scale application in the clarification and detannification of juices like grape. pH, temperature and optimum substrate concentration were determined for the tannase enzyme activity from *Aspergillusficuum*. Also, the Crude and partial purified enzyme were tested for detannification grape juice quality characteristics. Results evidenced that it was found that the optimum partial purified tannase parameters were: temperature 50 °C, PH 5.5 and substrate concentration 1.6% as a tannic acidwith 6.155 specific activities of partial purified tannase enzyme with 225.46% relative activity higher than its control. The tannase enzyme showed a higher temperature and lower pH optima than the soluble enzyme and showed 81.61% and 66.36 % tannin removal from grape juice by crude enzyme and partial purified enzyme treatment, respectively at 37 °C, which has not been reported earlier. Also, maximum velocity of reaction (Vmax) and Michaeli's constant (Km) values were 0.72 and 1.45 for partially purified tannase enzyme activity, respectively. Overall the quality of grape juice was better in partial pure tannase treated juice compared with untreated and crude enzyme treated juice. The present study exposed thatthis enzyme could demonstrate a valuable tool for a number of biotechnological applications. Also, the application of this enzyme on juice clarification also states that it can be employed in fruit juice processing for removal of tannin efficiently.

Keywords: Tannase, Enzyme, Kinetics, Grape, Juice, Tannin, Detannification.

Introduction

Tannins are plant ingredients, notify as the fourth most abundant group of these organisms of compounds , excel only on hemicellulose, lignin and cellulose. They are water soluble polyphenols with molecularmass ranging from0.3 to 5 kDa (Rana et al., 2004). It can be found in many plants and plant residues as Anacardiu-moccidentale (cashew), Vitisvinifera (grape), Malpighiaglabra (Barbados cherry), and Hancorniaspeciosa (mangaba fruit) (Soares et al., 2001 and Violante et al., 2009). Such residues, rich in tannins, can be excellent substrates for the production of tannase.

The main disadvantages of tannins are that their bitterness and ion chelating properties with iron that lead to anemia for prolonged usage.

Tannase or tannin acyl hydrolase (TAH) (EC 3.1.1.20), is an inducible enzyme that stimulate the hydrolysable tannins and hydrolysis of ester bonds in peptide such as tannic acid, liberation of gallic acid and glucose (Pinto et al., 2005; Costa et al., 2008). Tannase enzyme can be produced by microbes, plants and animals but microbial enzymes have the best stability in comparison to other resources as the fermentation technique

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provides more stable enzyme with cost effective manner (Raveendranet al., 2018). Tannase has several nutritional and industrial applications as in fruit juice clarification and coffeeflavored soft drinks manufacture and as analytical probefor determining structure of naturally occurring gallic acid esters (Seth and Chand, 2000). Also, tannase was used as detergent to clean up hard and acidic industrial effluent containing tannins (Belmareset al., 2004) and in high grade lather manufacture (Barthomeufet al., 1994). Such enzymes are naturally produced by ruminant animals, plants, and microorganisms such as filamentous fungi belonging to the genera *Aspergillus* and *Penicillium.* The genus Aspergillus is considered as the best producer, followed by Penicillium, both standing out as great decomposers of tannins (Sabu et al., 2005).

Tannase enzyme can used to reduce tannin contents in fruit juices (Rout and Banerjee, 2006). Grape fruit recognized since ancient times for its nutritional properties, immense medicinal and it is one of the rich sources of vitamin C. tannin content i.e. gallotannic acid in grape juice was high, has antioxidant properties and retards the oxidation of vitamin C and which upon hydrolysis produce gallic acid. The astringent taste of grape juice is due to tannins. On the other hand, reduces of grape juice acceptance as a juice. Hence enzymatic hydrolysis of tannin reduces its astringency with minimum loss of vitamin C into gallic acid in grape juice is useful since (Anita and Rita, 2009).

Currently, the whole grapes is similar to that of grape juice composition except that oils and crude fiber, which are mostly present in the seed. The main sugars present in grape juice is fructose and glucoseare . The grape juice quality depends on sugar content, acid content and flavour ingredients such as tannins, colour substances and volatile compounds. Determine the juice quality by changes that occur during growth and maturation. The organic principal acids of grape juice are citric, malic and tartaric acids are present. Aroma and flavour evolve during the ripening process. Colour in general the result of anthocyanin pigments lied in and near the skin in grape juice. The changes in the types of anthocyanin help to demonstrate why some grapes types are more suitable for juice processing and have better colour stability than others. Most of the researches dealing with the effects of pre-harvest changes on juice quality has used soluble solids percentage as the main index for quality. However, this is not the better method of quality evaluation. To evaluate juice quality, it is remarkable to estimate all main quality characteristics such as pH, acidity, flavour, colour with soluble solids percentage.

The objectives of this study were to evaluate the production of tannase enzyme from *Aspergillusficuum* from creative enzymes and to characterize the partial purified enzyme extract and apply it in the clarification and detannification of grape juice (*Vitisvinifera L.*). The main objectives of this study were to evaluate tannase production by isolation from *Aspergillusficuum* from creative enzymes, then optimize the production, and application of the latter in tannin removal from juice of grape, a nutrient rich tropical fruit without loss of its nutritional value.

Materials and Methods

Chemicals: Tannic acid was used as substrate for enzyme from WINI LAB,Tannase enzyme from *Aspergillusficuum* from creative enzymes and all other chemicals used were of analytical grade.

Acetone precipitation of tannase: Crude tannase enzyme powder was dissolved in 0.2 M acetate buffer in ratio 1:5 with shaking at 200rpm for one hour and culture filtrate used as a crude enzyme. For juice de-bettering, crude enzyme was partially purified with 60% acetone, the mixture was centrifuged at 5000 rpm for 10 minutes, the precipitate was dissolved in the minimum amount of distilled water to get partially purified tannase.

Tannase enzyme assay: Tannase assay was estimated by the modified colourimetric method of Sherief *et al*., (2011) based on estimation of violet colour produced when $FeSO_4$in presence of

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Rochelle salt (color reagent) react with gallic acid. The reaction mixture contained 0.5ml of tannic acid (2.0 % w/v in 0.2M sodium acetate buffer, pH 5.5) was added to 0.5ml of crude enzyme. This reaction mixture was incubated at 40°C for 30 min. The enzymatic reaction was terminated by adding 3.0 ml of precipitant solution (100 ml Quinine HCl (1.0%) mixed with 50 ml of 10.0 % Nacl). A control was prepared side by side using heat denatured enzyme. Then 1ml of the mixture was transferred in ependorfe tube and centrifuged at 9000 rpm in ultramicro-centrifuge for 15 min. 300ul of the supernatant is transferred to dry clean test tube and mixed well with 0.5ml of color reagent. The volume was completed to 5ml by adding distilled water. The developed color (reddish violet) was measured at 555 nm against boiled enzyme, using Spectro UV-VIS labomed. Inc. USA. One unit of enzyme (international units) was defined as the amount of enzyme able to release 1 ìmol gallic acid per minute of culture filtered under the standard assay conditions.

***Protein determination**:* Protein concentration was determined according to the method described by Lowry et al. (1951).

Characterization of partially purified tannase enzyme:

Effect of PH value of the reaction: The effect of different PH values ranging from4.5 to 6.5 on the activity of partially purified enzyme was investigated using 0.2M acetate buffer (4.5-5.5) and phosphate buffer (6-6.5), the reaction was conducted for 30 min at 40 °C.

Effect of temperature of the reaction: The reaction mixtures were incubated at different temperature range (40°C - 65°C). The activities of partially purified enzyme were measured and the optimum activity was chosen

Effect of substrate concentration on enzyme activity: To have maximum enzyme activity, it was subjected to different concentrations of tannic acid substrate from (1.6-2.4%).

Effect of gelatin on crude and partial pure enzyme: Gelatin was preswelled by dissolving one gram of gelatin in 1L of distilled water then heated at 60°C for 2 hours, then 1:1 volume of gelatin and enzyme (either crude or partial pure was mixed just before assay.

Kinetic constants of tannase enzyme: Kinetic constants of partially purified tannase enzyme was investigated using tannic acid concentration ranged from 1.6 to 2.4% was tested on the activity of the partially purified enzyme at 50°C and PH 5.5. 2,20-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) as a substrate, in 0.1 M sodium acetate buffer, pH 4. Km and Vmax were calculated according to Lineweaver and Burk (1934).

Fruit samples: Grape (*Vitisvinifera L.*) fruit grown in the Egypt was purchased from The Ministry of Agriculture in season 2018 and kept at 3-4°C until needed for chemical andtechnological studies.

Processing and extraction of grape juice: There are several options for juice extraction and subsequent treatment. Methods for commercial preparation of grape juice have undergone continuous change. In most commercial operations, the continuous pressing method is used. Hot pressing is appropriate for deeply pigmented grapes where maximum colour extraction is desired. Whereas, the immediate or cold press procedure is necessary to maintain the initial colour of light coloured grapes. For the preparation of juice, fruit grape (Vitisvinifera L.) was washed in running water. It was pulped with the aid of particle size sieve (strainer in stainless steel), in which it was screened through a 1.0 mm mesh screen) to remove the seeds. Then, pressed through three layers of cheesecloth to obtain clear grape juice. (Juice yield was determined in duplicate as g juice / 100g grape pulps).The extracted grape juice was stored at 4 °C for further analysis (Sabu et al., 2005).

Application of the crude enzyme and partial purified extract of grape juice for detannification: Tannin acyl hydrolase (TAH) (EC 3.1.1.20), or tannase enzyme 5,000 Units.

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This enzyme was derived from controlled fermentation's by selected strain of *Aspergillusniger*(SIGMA Chemical Co., St. Louis, MO, USA).Whereas, 200 g of grape pulp was stirred with 10% (v/w) of Tannase enzyme respectively. To clarify juice in vials Erlenmeyers 200mL grape juice were added and aliquots crude enzymatic extract (10mL), which contained 0.665 Unit /mg protein as a specific activity at pH 5.5 and 40°C and partial purified enzyme extract (10ml) , which contained 2.73 Unit/mg protein as a specific activity at pH 5.5 and 50°C. The grape juice without crude enzyme extract and partial purified enzyme extract was used how to control. The crude and partial purified enzyme treated pulp was incubated in a rotary shaker at 150 rpm at a temperature set at 37°C for 120 minutes and analyzed. Then, the crude and partial purified enzyme treated pulp was placed in a boiling water bath for 10 minutes to inactivate the crude and partial purified enzyme. The crude and partial purified enzyme treated pulp was then rapidly cooled by cold water to 25°C. Following the enzyme treatment, the crude and partial purified enzyme treated pulp was pressed through three layers of cheesecloth to obtain or extract clear grape juice, then tested and analyzed immediately.

Physical and chemical analysis:

Physico-chemical analysis: The pH of untreated and tannase enzyme treated grape juice samples were measured using a digital pH-meter (HANNA, HI 902 meter, Germany). The percent of Total Soluble Solids (TSS), expressed as °Brix (0-32), was determined with a Hand refractometer (ATAGO, Japan). Titratable acidity of grape juice samples was determined according to the method reported by Tung-Sun et al. (1995).10ml grape juice was centrifuged (HERMLE z323k, Germany)at 2000 rpm for 10minutes. Then, the supernatant was selected as a measure of juice clarity (turbidity) and juice with 450nm was classified as clarified (Amir-UZ-Zaman, 1985 and Tung-Sun, et al, (1995). Sedimentation was measured by the method of Krop and Pilnik, (1974). Browning was measured spectro photometrically (O.D 420nm) according to the method described by Stamp and Labuza, (1983).

Viscosity measurements: The viscosity measurements were carried out using a viscometers (VISCO:Cp, Brokfield, DV-111 Ultra, Made in USA) with thermostatic bath to control the working temperature 17.5 oC. The test sample (100ml) was heated to the desired temperature in water bath (accuracy +0.5), then transferred into measuring bowl, which was surrounding by temperature regulating vessel with spindle 3 and 250rpm. Results of viscosity were expressed in (cP) according to the method of Ibarz, et al, (1994).

Assay of pectinmethylesterase enzyme activity: Pectinmethylesterase enzyme (EC 3.1.1.11) activity was assayed according to the method of Arreola, et al, (1991) using a 0.1N NaOH solution.

Determination of condensed tannin: The condensed tannins in in the untreated and tannase enzyme treated grape juice were analyzed by butanol-HCl method described by Porter et al. (1986) and Vermerris and Nicholson, (2006).10ml grape juice was centrifuged (HERMLE z323k, Germany) at 2000 rpm for 10minutes. In brief, a sample was mixed with butanol-HCl reagent (77 mg of FeSO4·7H2O in 500 ml of HCl and *n*-butanol at a volume ratio of 2:3). Then, 0.5ml supernatant untreated and enzyme treated grape juice was added to 7ml Hcl-butanol reagent and vortex-cheker for 1min. The untreated and tannase enzyme treated grape juice samples was placed in a water bath at 95°C for 15 min., then measured spectrophotometrically by 4054 - UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 550nm. After mixing and allowing the mixture to stand for 30 min, the absorbance was read at 550nm. The concentration of tannins in the grape juice was expressed as ìg/ml or mg/L or g gallic tannic acid kg"1 using a standard curve obtained with known concentrations of gallic or tannic acid.

Colour characteristics: Colour of grape juice,

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crude enzyme treated juice and partial purified enzyme treated juice was measured using spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (International Commission on Illumination) as mentioned by Sapersand Douglas, (1987) and Hunter 1970. Colour of control and enzyme treated samples was measured using a HunterLabcolourimeter Hunter a*, b* and L*. Parameters were measured with a colour difference meter using a spectro-colourimeter (Tristimulus Colour Machine) with the CIE lab colour scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Colour Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16). The instrument (65°/0° geometry, D25 optical sensor, 10° observer) was calibrated using white and black reference tiles. The colour values were expressed as L* (lightness or brightness/ darkness), a* (redness/greenness) and b* (yellowness/blueness). The Hue (H)*, Chroma (C)* and Browning Index (BI) was calculated according to the method of Palou et al., (1999) as follows:

H* = tan-1 [b*/a*]……..…(1)
C* = square root of [a2* + b2*].....……..(2)
BI = [100 (x-0.31)] 10.72…….……(3)
Where:- X = (a*+ 1.75L*) / (5.645L*+ a* - 3.012b*)
Ä E = (Ä a2+ Ä b2 + Ä L2) 1/2 ……..……(4)
Where, all values were recorded as the mean of triplicate readings.

Non-enzymatic browning determination: Non-enzymatic browning was measured spectrophotometrically by 4054 - UV/Visible spectrophotometer, (LKB-Biochrom Comp., London, England), as absorbance at 420nm using ethanol as blank according to the method of Stamp and Labuza(1983) and Birk et al.,(1998).

Vitamin C determination: Vitamin C was analyzed using the A.O.A.C., (2006) method. The titrant was prepared with 50 mg of 2, 6-dichloroindophenol Na salt and 42 mg of sodium bicarbonate ($NaHCO_3$) in 50 mL of water. The solution was diluted to 200 mL with distilled water. The extracting solution was prepared with 15 g of metaphosphoric acid and 40 mL of acetic acid and then diluted to 500 mL with distilled water. Solutions were stored in amber bottles at 4°C. A 100 mL aliquot of control, crude enzyme treated and partial purified enzyme treated grape juice was added to 100 mL of the extracting solution and then filtered using a No.1 filter paper (Whatman, Maidstone, England). The solution was then titrated with the titrant until the solution turned bright pink for at least 5 s. A standard curve was created using pure ascorbic acid (Sigma Aldrich, St. Louis, MO). Vitamin C retention was calculated using equation (5).

$$\text{Retention (\%)} = \frac{\text{mg ascorbic aci /100mL juice after treatment}}{\text{mg ascorbic acid /100mL juice before treatment}} \times 1\,00 \quad (5)$$

Result and discussion

To achieve optimum tannase yield that used for juice debittering, tannase enzyme from *Aspergillusficuum* was partially purified by fractional precipitation with acetone and the precipitated enzyme fractions were tested for tannase and protein activity. Tannase was fractured into four fractions (20%-80%) followed by certification. Fractions with acetone give 41.5% recovered activity and 7.93% recovered protein. The results in table (1) showed that at 20% tannase activity was 4.2 U/mg protein that was 66% purification in comparison to its crude activity that was 6.31 U/mg protein. For fruit juice debittering, tannase was partially purified with 60% acetone concentration, as seen in table 1.

Characterization of partially purified tannase enzyme: Several properties of partially purified enzyme were determined, they include: optimum temperature, optimum pH, substrate concentration of the reaction, thermal stability and thermodynamic properties.

Effect of the reaction temperature: At pH 5.5 using 0.2M acetate buffer, tannase enzyme was subjected to temperature range from 45°C to 65°C. It was found that enzyme activity increased by

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153.37% and 156.74% at 50°C and 55°C respectively. Tannase enzyme was unstable upon remaining in 55°C with tannic acid for short period of time (from 5 to 20 minutes) that was the reason for selecting 50°C as the best temperature for further study, as seen in table 2.

Effect of PH value of the reaction: The effect of PH of the reaction on the enzyme activity was investigated using 0.2 M acetate buffer with PH range from 4.5 to 6.5 was tested. Optimum PH was at 5.5 with specific activity 2.73 U/mg protein as tannase enzyme is an acidic protein with an is electric point near PH 4, so higher PH range has negative effect on the enzyme activity, as seen in table 3.

Effect of substrate concentration on the activity of the partially purified enzyme:
Tannic acid concentration ranged from 1.6 to 2.4% was tested on the activity of the partially purified enzyme at 50°C and pH 5.5. It was found that best enzyme activity was at 1.6% tannic acid concentration with 225.46% higher than its control that was promising enzyme activity. This suggests that high concentration of tannic acid decrease enzyme activity, as seen in table 4.

Effect of gelatin on crude and partially purified enzyme: As the partially purified enzyme was unstable, it is recommended to increase activity

Table (1): Partial purification of tannase enzyme

Acetone concentration (%)	Protein content (mg)	Recovered protein (%)	Tannase activity (U)	Recovered activity (%)	Specific activity (U/mg protein)	Fold purification
0	698.82	100	217	100	6.31	1
20	3.34	0.478	14.03	6.465	4.2	0.666
40	27.34	3.91	30.97	14.27	1.13	0.18
60	10.16	1.45	18.1	8.34	1.78	0.28
80	14.56	2.08	26.97	12.43	1.85	0.29
Total	55.4	7.93	90.1	41.5	-	-

Table (2): Effect of temperature on the enzyme activity

Temperature	Specific activity (U/mg protein)	Relative activity (%)
40	1.78	100
45	2.56	143.82
50	2.73	153.37
55	2.79	156.74
60	1.26	70.79
65	0.3	16.85

Table (3): Effect of PH on enzyme activity

Temperature	Specific activity (U/mg protein)	Relative activity (%)
4.5	2.27	83.15
5	2.27	83.15
5.5	2.73	100
6	_	-
6.5	_	-

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and stability of the enzyme using carrier as gelatin for its application. It was noticed that activity of both crude and partially purified enzyme were 22.9 U and 29.88 U respectively with 186.05% and 188.06% higher enzyme activity than their controls that means that gelatin increase the enzyme activity. On the other hand, specific activity of both crude and partially purified enzyme was 0.416 U and 0.24 respectively which means that gelatin have high protein content, as seen in table 5.

Kinetic parameters (Km and Vmax values) of partially purified tannase enzyme: The kinetic parameters of the partially purified tannase enzyme were determined and calculated from Lineweaver and Burk plots. However, the Michaelis-Menten plot and Lineweaver-Burk plot are used for kinetic analyzes of data. While a plot of v as a function of CS yields a hyperbolic curve, the double reciprocal plot provides a straight line that is suitable for the estimation of the kinetic constants by linear regression (Alev Bayýndýrlý, 2010). The line relationship between 1/V vs. 1/[S] resulted in a straight line with 1/Vmax (intercept) and KM/Vmax (slope). This linear relationship was titled a Lineweaver-Burke. Plot 1/V vs. 1/[S] to obtain a straight line with a y-intercept = 1/Vmax and a slope =KM/Vmax. This plot is titled a Lineweaver-Burke plot. Then, maximum velocity of reaction (Vmax) and Michaeli's constant (Km) values were 0.72 and 1.45 for partially purified tannase enzyme activity, respectively. Vannelli*et al.* (2007) reported a PAL/TAL ratio ((Vmax/Km) Phe/(Vmax/Km)Tyr) of 0.8, while we observed a ratio of (Vmax/Km) tannase enzyme was 0.50. This implies that the enzyme is intrinsically a partially purified tannase enzyme.

Application of tannase enzyme for tannin removal from fresh grape juice: The tannin content of each untreated and treated juice sample was determined by the colorimetric method described by Porter *et al.* (1986) and Vermerris and Nicholson, (2006).

Similar study was conducted in this work showing that there were a slight increase in the studies using species of Aspergillus and Penicillium and Aspergillus to produce tannase (Aguilar et al. (2007), Belur and Mugeraya(2011), and Juliana e al., (2014)). The percentages of seeds, peel and juice in grape fruit were, 1, 36 and 63%, respectively.

In fresh grape juice, tannin content removed by tannase enzyme to 0.223 mg/L of tannin

Table (4): Effect of substrate concentration on enzyme activity

Relative activity (%)	Specific activity (U/mg protein)	Tannic acidconentration (%)
1.6	6.155	225.46
1.8	4.4	161.17
2	2.73	100
2.2	2.19	80.22
2.4	1.73	77.29

Table (5): Effect of gelatin on crude and partial pure enzyme

Partial pure /Crude	Tannase Activity (U)	Relative activity (%)	Specific activity (U/mg protien)	Relative specific activity (%)
Partial pure at 50°C	17.78	100	2.73	100
With gelatin	29.88	168.05	0.11	4
Crude at 40°C	12.177	100	0.665	100
With gelatin	22.9	188.06	0.416	62.56

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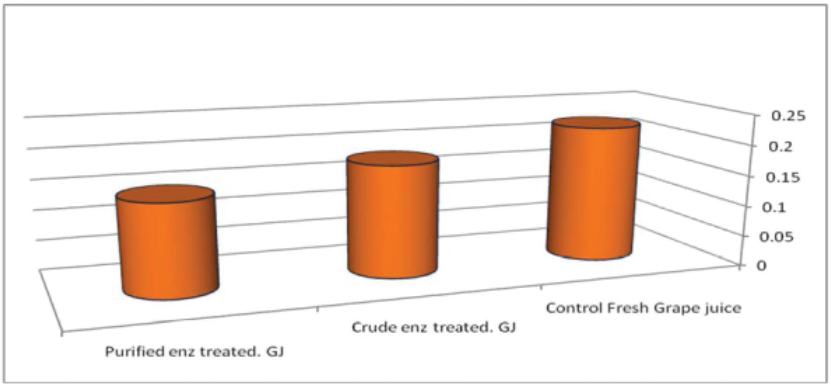

Fig (1): Effect of crude and partial purified tannase enzyme on tannin content (mg/L) of grape juice (GJ)

Table (6): Effect of tanninase enzyme on the characteristics of grape juice (GJ)

Characteristics (unit)	Untreated sample (C)	Crude Enzyme (CE)	Partial Purified Enzyme (PPE)
PH	3.99	4.14	3.96
TSS (g/Kg)	21	21	21
T. Acidity (%)*	0.61	0.59	0.65
TSS/TA (%)	34.54	35.43	32.51
Turbidity- 450nm	2.16	2.45	2.51
Vitamin C (%)	1.464	1.464	1.464
Viscosity (cP)	20.93	20.27	20.13
PME (unit/ml)	3.0	0.00	0.00

content with pH 3.99 at 37oC. It can be showed that maximum hydrolysis of 66.36 % took place after partial purified enzyme (PPE) (Figure 1). The tannin content in fresh grape juice was decrease from 0.223 ìg/ml or mg/L to 0.182 ìg/ml or mg/L by crude enzyme treatment and to 0.148 ìg/ml or mg/L by partieal purified enzyme treatment, as seen in Figure (1). The enzyme treatments were used for two successive runs resulting in 66.36 % and 81.61 % tannin hydrolysis after crude enzyme (CE) and partial purified enzyme (PPE) treated grape juice respectively. The results were encouraging considering the tannase enzyme treatment could be used successively with 81.61% and 66.36 % tannin removal by crude enzyme and partieal purified enzyme treatment, respectively. Enzymatic pre-treatments of some fruit juices were processed to reduce bitterness and haze have been founded by various authors (Chatterjee et al., 2004, Floribeth and Lastreto, 2004 and Mishra and Kar, 2003). There is no study of tannin content removal from grape juice which has a very strong astringency taste due to tannin content. Also, application of tannase enzyme in fruit juice processing were rare. However, results showed that the 33.64 % reduced in tannin content from grape juice by soluble tannase has been showed (Rout and Banerjee, 2006). Also, results showed that the tannin removal (66.36 %) was Better shown of the tannase enzyme used. No reduction in vitamin C content with the same content was reported after partial purified tannase

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enzyme (PPTE) pre-treatment of grape juice compared with untreated grape juice (Table 1) which was considerably very high compared to other conventional processing methods which involve non-enzymatic heat treatments and results in removal of vitamin C.

The results and discussion of the physico-chemical characteristics for unpre-treated and tannase enzyme pre-treated of grape juice was seen in table (6).

The pH of grape juice (GJ) was 4.14 in crude enzyme and 3.96 in the partial purified enzyme treated juice showing a increase in pH values in crude enzyme and a decrease in the partial purified enzyme treated juice. The increase and decrease in pH was directly related to the treated grape juice with crude enzyme and partial purified enzyme compared with 3.99 for untreated grape juice. TSS (g/Kg) of crude enzyme and partial purified enzyme treated juice were the same in the untreated GJ. Whereas, the TSS was obvious constant with untreated, crude enzyme and partial purified enzyme treated grape juice were 21. The major analytical measurement for grape juice quality was the TSS / acid ratio . The TSS / acid ratio of grape juice was increased by crude tannase enzyme pre-treatment 35.43 and decrease by partial purified tannase enzyme pre-treatment 32.51 of grape juice compared with 34.54 in unpre-treated grape juice. TSS / acid ratio was not so closely with flavour but shown to be correlated with sweetness (Guyer, et al, 1993).

Titratable acidity of crude tannase enzyme pre-treated juice was decrease to 0.59 and increase to 0.65 in partial purified tannase enzyme compared with 0.61 in unpre-treated grape juice sample (Table 6), which may resulting in degradation of pectin, enzymatic desterification and an increase of total acid. No sedimentation and no pectinmethylesterase enzyme activity (PME) in the crude and partial purified tannase enzyme pre-treated grape juice as compared to the unpre-treated grape juice (8%) and (3 unit/ml), respectively.

The crude and partial purified enzyme treated grape juices were free from sediments and consistently clear as indicated by increasing turbidity to (2.45) for crude enzyme treated GJ and to (2.51) for partial purified enzyme treated GJ, respectively, compared to control grape juice (2.16). The viscosity reduced to 20.27cP for crude enzyme treated GJ and to 20.13cP for partial purified en-

Table (7): Effect of tannase enzyme on color characteristics and browning of grape juice (GJ)

Color and browning measurements-unit	Untreated sample (C)	Crude Enzyme (CE)	Partial Purified Enzyme (PPE)
- L*-value	43.23	39.9	41.23
- a*-value	5.23	3.26	4.48
- b*-value	14.11	12.23	12.41
Hue angle*	69.66	75.07	70.15
Chroma**	15.05	12.66	13.19
∆ E***	51.68	54.22	53.08
Browning index (BI)	64.66	54.08	58.99
Browning (OD420nm)	7.56	6.76	7.22

*Hue angle = (tan -1 b/a)
** Chroma = sqaure of (a2+b2)
*** ∆E = (delta a2+ delta b2 + delta L2)1/2 where: a-ao, b-bo and L-Lo; subscript "o" indicates color of control or untreated sample.

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zyme treated GJ, while was 20.97cP in untreated Grape juice. Whereas, addition of crude tannase and partial purified tannase enzyme, especially partial purified tannase enzyme, not only improved juice filtration but also reduced the viscosity. Also, Dziezak, (1991) reported that the pectinase enzyme treatment (as a clarified pineapple juice treatment) reduce viscosity, increase extraction of colour, enhance clarification of juice and improved yields. These results were agreed with the results of Tung-Sun, et al, (1995).

Results in table 6, reported that the grape juice contained vitamin C (1.464mg /100 ml juice). It is of importance to mention that crude and partial purified tannase enzyme treated grape juice maintained vitamin C constant the same that of untreated GJ. However, vitamin C was 1.464 mg/ 100ml juice of untreated, crude and partial purified enzyme treated GJ, as seen in Table (6). Grape juice may suggested a good source for the content of vitamin C. These results were agreed with the results of Tung-Sun, et al, (1995) and Pong, et al, (1996). Where, Pong et al, (1996) used pectinase enzyme pre-treatment to clarify guava juice. This study showed reduced vitamin C content, increase in sugar and acid content in guava juice after pectinase enzyme pre-treatment with very small changes.

Effect of tannase enzyme on color characteristics and browning of grape juice (GJ):

Hunter color difference measurement values (L*, a*, b*, C*, BI and browning at R420nm (reflectance)) of crude (CE) and partial purified (PPE) enzyme treated PPJ were decreased, while increased in hue angle (H*) and ?E compared with untreated grape juice. This is probably due to release of caroteniods and anthocyanins as a result of crude (CE) and partial purified (PPE) enzyme addition. While, the addition of crude (CE) and partial purified (PPE) resulted in an decrease in the (L*, a*, b*, C*, BI and browning at R420nm (reflectance)-values but decrease in hue angle (H*) and ?E compared with untreated grape juice, as seen in Table (7).

Chroma of crude (CE) and partial purified (PPE) treated grape juice were higher than chroma of control grape juice. This reference that how a color diverge from gray color. Whereas, hue angles of crude (CE) and partial purified (PPE) treated grape juice were greater than hue angles of control juice, as seen in Table 7. These results suitable with the result of Saenz, et al, (1993).

While, ? E was increased by crude (CE) and partial purified (PPE) treated GJ and decreased by untreated GJ, as seen in table 7. This indicates that grape juice become green - blue but was not red because the hue angle was more positive, as seen in Table 7. Results showed that the crude (CE) and partial purified (PPE) treated grape juice was not redder and darker by Hunter Color results measures. These results are in invert with the results of Tung-Sun, et al, (1995) who established that the pectinase treated plum juice was darker and purple by Hunter Color results measured. The results showed that the browning (O.D 420nm) was decreased from 7.56in untreated GJ to 6.76 and 7.22 in crude (CE) and partial purified (PPE) treated GJ respectively, as seen in table 7. This indicates that the crude (CE) and partial purified (PPE) treatment prevented browning in grape juice compared to untreated grape juice.

Conclusion:

Kinetics of the partially purified tannase enzyme activity from Aspergillusficuum from creativeenzymeswas studied. It was found that the optimum partial purified tannase parameters were: temperature 50oC, PH 5.5 and substrate concentration 1.6% as a tannic acid. One of the best results was concerned at 1.6% substrate concentration with 6.155 specific activities of partial purified tannase enzyme with 225.46% relative activity higher than its control. The results found that the tannase enzyme could remove 81.61% and 66.36 % tannin content from grape juice by crude enzyme and partial purified enzyme treatment and results indicated lower pH and a higher temperature optimum than the soluble enzyme, respectively at 37oC. Also, maximum velocity of reaction

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(Vmax) and Michaeli's constant (Km) values were 0.72 and 1.45 for partially purified tannase enzyme activity, respectively. It indicated that this enzyme could demonstrate a valuable tool for a number of technological and biotechnological applications. Also, it can be applied for commercial scale operations and possible application in the clarification and detannification of juices like grape.

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