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Analysis of the effect dosage of the enzyme pretreatment on different physicochemical characteristics of black tea extract

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Abstract
The objective of the present study was to investigate the effect of enzyme pretreatment on tea extract from Black tea samples using Pectinex Ultra SP.L, and Viscozyme L enzymes. The effect of enzyme dosage was evaluated by analyzing the yield, color and turbidity of the tea extract obtained by carrying out an enzyme pretreatment with three different dosages. The antioxidant activity and Total polyphenol content of the tea extract were measured based on the DPPH radicals scavenging assay and Folin-ciocalteus (ISO 14502-1) method respectively. From the results of the experiment it was determined that there is no significant difference ($p>0.05$) in extractable solid yield and colour when the dosage was changed but there is a significant difference ($p<0.05$) turbidity when the dosage was changed. There is no significant difference ($p>0.05$) in the antioxidant activity and Total polyphenol content in enzyme treated tea extract and the control sample.

Keywords: Antioxidant activity, Black tea, enzyme pretreatment, Pectinex Ultra SP.L, Viscozyme L

1. Introduction
Now tea is an extremely popular beverage around the world and can be served hot or ice cold [1]. Black Tea is fully oxidized during processing steps which accounts for its stronger flavor as compared to the other types of teas which are comparatively less oxidized [2]. It is manufactured as a fermented tea product followed by withering of the tea leaves [3]. Among three types of tea, black tea is the most popular tea produced and consumed preferentially [4]. Enzymes are ideal for fruit juices due to their efficiency of specific action, high purification, and standardization. Enzymes are either an integrated part of the juice or added to it, providing a number of advantages in the process, such as high substrate specificity, high reaction rate under mild operational costs and investment, and fast and continuous readily controlled reaction with generally low operational costs and investment and increasing the yield and production of various types of juices [5]. Viscozyme L is a commercially available enzyme mixture containing a wide range of carbohydrate activities including cellulase, hemicellulase, xylanase, arabanase and glucanase activities capable of effectively hydrolyzing plant cell wall polysaccharides [6]. The enzyme preparation is produced from a selected strain of the Aspergillus group. The combination of cell Wall digesting enzymes such as Celluclast and viscozyme has a pH of about 4.5. Pectinex Ultra SPL is natural enzyme preparations produced by the fungus *Aspergillus*. Pectin enzymes constitute a unique group of enzymes that catalyze the degradation of pectin polymersin plant cell walls. Parameters such as time, pH, and temperature and enzyme concentration influence enzymatic activity co-operatively. Hence this offer possibility to control the process [7]. In this study the variation of different physical characters when enzymes are used in extraction of black tea are analyzed.

2. Materials and methods

Table 1: Factor and Levels

Factor	Level		
	Low (-1)	**Center (0)**	**High (1)**
Enzyme Dosage % (W/W)	0.02	0.1	0.5
Temperature	40^0C	40^0C	40^0C

2.1 Preparation of Tea Extract
Exactly, 100.0g of Tea was weighted and 1000ml of boiling water was taken into a beaker. Then 2.0g of citric acid was measured and it was dissolved in hot water. The hot water was allowed to cool down to 65^0C and 100.0g of Tea was added into it. They were kept for 30min and filtered the tea residues and tea infusions were taken.

2.2 Preparation tea samples with enzyme treatment
Firstly the PH (3.0-5.0) of the tea extraction was adjusted. Exactly 200.0g of tea extract was weighted in to food grade beaker. Exactly 0.02, 0.10, and 0.50 of enzymes (Viscozyme L and Pectinex Ultra Sp-L separately tested) dosages (%w/w) were weighed and added to each 200.0g of tea extraction. The extraction containing enzymes was incubated in water baths at 30^0C, for 30 minutes. Then samples were kept on a hot plate in 90^0C for 5 min, to deactivate enzymes. Then samples were fast cooled by using ice bags. Concentrate was centrifuged for 15 minutes. Tea cream was separated from the tea extract and clear tea extract was taken as an above procedure. After that the clear tea concentrates brix were measured by using brix

meter and final weight of tea concentration was taken separately.

2.3 Measurement of responsible variable for study of both enzyme treated samples and non enzyme treated samples.

2.3.1 Determination of Extractable Solids Yield (ESY) in Extract

Extractable solids yield (ESY) was calculated from the TSS content in the extract and the amount of tea used for the extraction [8] before calculation ESY few assumptions should be considered.

2.3.1.1 Tea Solid

The brix values of extracts were taken using a brix meter at room temperature. But added citric acid was given an additional 0.2 Brix value for Brix meter reading. So it was cut down from the Brix meter reading.

Additional brix reading because of Citric acid =

$$\frac{\text{added weight of citric acid} \times 100}{1000\text{ml of water}}$$

$$\frac{2.0\text{g} \times 100}{1000\text{ml of water}}$$

$$= 0.2$$

Total tea solid content in tea infusion samples were calculated from Brix reading multiplied by final weight of the samples after centrifuge process.

Tea solid content =

$$\frac{\text{Brix reding} - 0.2 \times \text{final weight of tea sample}}{100}$$

2.3.1.2 Calculation of Extractable Solid Yield Percentage

Extractable solids yield (ESY) percentage was calculated from the tea solid content in the extract and the amount of raw tea used for the extraction

$$\text{ESY\%} = \frac{\text{Total tea solid content} \times 100}{\text{Amount of raw tea}}$$

2.3.2 Determined Colour of the Tea Extract

The colour was measured for samples at 620nm at room temperature using the colour meter.

2.3.3 Determined Turbidity of the Tea Extract

Turbidity was measured with a turbidity meter and expressed in Nephelometric Turbidity Units (NTU). It is the measurement of optical clarity based on a light scattering technique.

2.3.4 Total Polyphenol Content

The total polyphenol content was determined according to ISO 14502-1 method. (Folin-Ciocalteu method for tea)

2.3.5 Antioxidant Activity

Using the DPPH method, the procedure followed the method of [9] with some modifications

3. Results and discussion

3.1 Determination of Extractable Solids Yield (ESY), Colour and Turbidity in Extract

Table 2: Extractable Solids Yield (ESY), Colour and Turbidity in Extracts with different enzyme dosages.

Combination			Responsible variable of the Pectinex			Responsible variable of the Viscozyme		
Dosage	Temp.	Time	Colour	Turbidity (NTU)	ESY%	Colour	Turbidity (NTU)	ESY%
0.02	40	30	0.36±0	1.67±.22	18.55±.94	0.35±.01	1.71±.16	18.17±.7
0.1	40	30	0.36±.01	1.79±.28	18.23±.07	0.34±.01	1.61±.12	18.41±.28
0.5	40	30	0.34±0	1.78±.02	18.27±.39	0.35±.03	2.47±.49	17.98±.03

According to table 2, the dosage of viscozyme and pectinex enzymes do not significantly effect on the ESY% at (P>0.05) level. But it can be seen that, ESY is inversely proportional to the dosage of the both enzyme treatments because when the dosage is increased the ESY has been slightly reduced. When the turbidity is concerned as shown in Table 2, at the default α of 0.05, the effects on the turbidity of the both enzyme treatments is not significant at ($p < 0.05$) level, however turbidity is directly proportional to the dosage of the both enzyme treatment. According to the result turbidity is increased significantly between 0.02-0.10%w/w of dosage rather than 0.1-0.5%w/w of dosage for Pectinex enzyme treatments but for Viscozyme enzyme treatment turbidity is increased significantly between 0.1-0.5 %w/w of dosage rather than 0.02-0.1%w/w. When colour is concerned it can be seen that the dosage of Pectinex enzyme have a significant effect on the colour in dosages of 0.1% and 0.5%.Viscozyme enzyme have a significant effect on the colour in dosages of 0.02% and 0.1%.

3.2 The Anti Oxidant Activity (AOA)% and Total phenolic content(Tpc)% at different enzyme treatment and reference

Table 3: The Anti Oxidant Activity (AOA) % and Total phenolic content (Tpc) % at different enzyme treatment and reference

	AOA %mg/100ml of extract (% GAE)	Tpc% mg/100ml of tea extract (%GAE)
Viscozyme	226.75±32.74%	353.92±41.42%
Pectinex	198.63±16.58%	340.87±32.96%
Reference	189.46±7.82%	309.57±30.41%

According to table 3 the ascending order of Tpc and AOA have been recorded as reference, Pectinex treated, and

viscozyme treated tea extracts. The highest AOA in Viscozyme treated tea extract variety was recorded as a mean of 226.75mg Galic acid equivalents per 100ml of tea extract. AOA of Pectinex treated tea extract was recorded as a mean of 198.63mg Gallic acid equivalents per 100ml of tea extract and for the reference sample, the lowest was recorded mean of 189.46 mg Galic acid equivalents per 100ml of tea extract. The highest Tpc in Viscozyme treated tea extract variety was recorded as a mean of 353.92mg Galic acid equivalents per 100ml of tea extract. Tpc of Pectinex treated tea extract was recorded as a mean of 340.87mg Gallic acid equivalents per 100ml of tea extract and for the reference sample, the lowest was recorded mean of 309.57mg Galic acid equivalents per 100ml of tea extract.

4. Conclusion

It can be concluded that different enzyme dosages have a significant effect on some of the qualities of black tea extracts such as turbidity but not for extractable solid yield and colour and the tea extract treated with viscozyme enzyme contained the highest anti oxidant activity and polyphenolic content compared to the extract treated with pectinex enzyme and the non treated extract.

5. Reference

1. Damiani E, *et al*. Journal of Food Composition and Analysis Antioxidant activity of different white teas: Comparison of hot and cold tea infusions §', Journal of Food Composition and Analysis. Elsevier Inc. 2014; 33(1):59-66. doi: 10.1016/j.jfca.2013.09.010.
2. Aditi J, *et al*. Tea and human health: The dark shadows, Toxicology Letters. Elsevier Ireland Ltd, 2013, pp. 1–6. doi: 10.1016/j.toxlet.2013.04.010.
3. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. Journal of Nutrition, 2003; 133, 32755-32845.
4. Weerawatanakorn M, *et al*. Chemistry and health beneficial effects of oolong tea and theasinensins, Food Science and Human Wellness. Beijing Academy of Food Sciences. 2015; 4(4):133–146. doi: 10.1016/j.fshw.2015. 10.002.
5. Nagar VV. Mini Review Pectinases : Enzymes for fruit processing industry. 2014; 21(2):447-453.
6. Chen N, *et al*. Development of defatted soy flour based bio-adhesives using Viscozyme L, Industrial Crops & Products. Elsevier B.V., 2015; 76, pp. 198-203. doi: 10.1016/j.indcrop.2015.04.008.
7. Enzim KK, Ekstrak H. Effects of Enzyme Concentration, Temperature, pH and Time on the Degree of Hydrolysis of Protein Extract from Viscera of Tuna (Euthynnus affinis) by Using Alcalase. 2013; 42(3):279-287.
8. Chandini SK, *et al*. Enzymatic treatment to improve the quality of black tea extracts', Food Chemistry. Elsevier Ltd. 2011; 127(3):1039-1045. doi: 10.1016/j.foodchem. 2011.01.078.
9. Brand-Williams W, Cuvelier M, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food Science and Technology, 1995; 28:25-30.