

**EFFECTS OF VISCOZYME, TANNASE AND  
PROTEASE ENZYMES ON CATECHIN AND  
CAFFEINE PROFILES OF COLD-WATER-  
SOLUBLE INSTANT BLACK TEA**

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## ABSTRACT

Cold-water instability of tea infusion due to the development of haze and the formation of tea cream is a serious issue in manufacturing cold-water-soluble instant black tea. Different types of enzyme treatments have been invented with the aim of reducing tea cream formation. However, effects of these enzyme treatments on the catechin and caffeine contents of cold-water-soluble instant black tea have not yet been studied in detail. This research was conducted to study the effects of treatment with different combination of viscozyme, tannase and protease enzymes on the catechin and caffeine contents of cold water soluble instant black tea. A black tea extract was obtained by brewing a black tea sample with boiling water (at black tea: water ratio of 1:6) for 10 minutes followed by filtering through a nylon cloth filter. After that the black tea extract was allowed to cool to room temperature. Then the samples of black tea extracts were separately treated with previously optimized level (0.3%, wt/wt based on tea solid in the extract) of viscozyme, tannase and protease enzymes in different combinations at 40°C for 40 minutes. After that samples were heated up to 90°C and allowed to cool to room temperature. Then these samples were centrifuged at 3500 rpm for 10 minutes and supernatants were obtained. Catechins and caffeine contents of supernatants were determined by High Performance Liquid Chromatography on a phenyl-hexyl column (5  $\mu\text{m}$ , 25 $\times$ 0.5 cm) using a two-solvent gradient elution over a period of 45 min with a flow rate of 1 mL min<sup>-1</sup> while maintaining the column temperature at 35°C $\pm$ 0.5°C and UV detection at 278 nm. Two mobile phases were 9% (v/v) acetonitrile with 2% (v/v) acetic acid and 20  $\mu\text{g ml}^{-1}$  EDTA (A) and 80% (v/v) acetonitrile with 2% (v/v) acetic acid and 20  $\mu\text{g ml}^{-1}$  EDTA (B). This experiment was repeated thrice. Data were subjected to Analysis of Variance and mean separation by performing Duncan Multiple Range Test ( $p < 0.05$ ) using SAS software. Samples treated with combination of tannase and

viscozyme contained significantly higher amount of gallic acid ( $306.7 \pm 35.1 \mu\text{g ml}^{-1}$ ) than the other samples. Further, this sample contained significantly higher amount of Epigallocatechin ( $72.5 \pm 5.7 \mu\text{g ml}^{-1}$ ) and Epicatechin ( $89.2 \pm 8.4 \mu\text{g ml}^{-1}$ ) than the control and samples treated without tannase. As a result of degallation of gallated catechins, samples treated with tannase contained no or significantly less amount of Epigallocatechin gallate and Epicatechin gallate. Further, samples treated with tannase contained significantly higher amounts of total catechins and caffeine than the other samples. Viscozyme is known to improve the clarity of tea infusion. Findings of this study have proven that tannase treatment can improve the content of constituents such as catechins and caffeine that contribute to the organoleptic properties of tea infusion. Therefore, it can be concluded that treatment with combination of viscozyme and tannase will improve the physicochemical and organoleptic properties of cold-water-soluble instant black tea.

*Keywords:* Cold-water- soluble instant black tea, viscozyme, tannase, protease, catechins