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Dyeing of Wool Fibres with Synthetic Dyes and Effect of Proteolytic Enzymes

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In spite of protein and protein fragments (casein, peptone, *etc.*) are used widespread as a substrate for the proteolytic enzymes, the substrate prepared from dyes that adsorbed on appropriate material, such as wool and cotton are also used for enzymes activity determination. The object of this work is to develop the substrates which are easily and economically obtainable and also environmental safer, for the frequently used proteolytic enzymes such as subtilisin carlsberg, trypsin, chymotrypsin and protease type XVI and if it is possible to prepare the specific substrate at least for one of this enzymes. For this aim, wool was dyed with synthetic dyes such as procion yellow, procion red and procion blue. The results indicate that the most appropriate complex was found to be wool-procion yellow and the most unappropriate complex was found to be wool-procion blue complex.

Key Words: Proteolytic activity, Dyed-wool, Natural dyes, Insoluble substrates.

INTRODUCTION

Many methods have been developed for the quantitative measurement of proteolytic activity[1,2]. A very old and widely used method is based on the use of insoluble dye-stained proteins as substrates. These substrates are usually prepared by immobilization or adsorption of reactive dyes to insoluble proteins, such as collagen, keratin, elastin or fibrin. Examples of these substrates are *e.g.*, indigo carmine-stained fibrin, congo red-dyed hide powder[3] or azocoll[3,4].

A number of methods are available for measuring the activity of proteolytic enzymes[5]. Synthetic substrates radioactively labelled materials and natural proteins with choromophoric of fluorogenic groups attached have been utilized, along with the measurement of amino acids relased during digestion[6].

Azocoll, an insoluble protein-dye conjugate, has been widely used for the assay of proteolytic enzymes[7-11].

Soluble proteins can be converted to an insoluble form by cross-linking with a suitable bifunctional reagent and reactive dye is then attached to this insoluble protein matrix[12]. Spectrophotometric assays are the most popular techniques for the determination of proteolytic activity. Both low-molecular-weight synthetic chromogenic peptides and dye-stained soluble and insoluble proteins are usually used as substrates[13].

In a previous work, the authors reported the development of proteolytic assay by using wool-synthetic dyes complexes.

EXPERIMENTAL

Preparation of wool: The wool was washed with ether (100 mL) for remove fatty acids then washed with 80 % ethanol for removal of soaps. The wool was dried in air then washed with warm distilled water to remove persipration. Additionally washed with 1 % HCl to remove Mg and Ca soaps. The above procedure was repeated two times. Finally, the wool was washed with diluted NH_3 to remove hydrogen ion and then washed with double distilled water many times than dried in air.

Dyeing of wool with synthetic dyes: 0.5 g wool was added in 0.03 g/100 mL dye-water solution then 0.5 g NaCl was added and mixture was stirred at 80ºC for 1 h. The wool washed with double distilled water and dried in air. Finally the dried wool was washed with soap solution (25 mL), double distilled water (50 mL) and then dried in air.

Determination of optimum pH: Enzyme activity was measured at different pH's (pH: 7; 7.5; 8) 0.1 M phosphate buffer; pH: 8.5 0.1 M Tris-HCl buffer, pH: 9; 9.5; 10; 10.5 0.1 M carbonate/bicarbonate buffer).

Determination of incubation time and agitation speed: 2 mg wool-dye complexes was added to the 3 mL of buffer solution which was added previously determined as optimum pH. The mixture was vortexed for 5 min, then 50 mL enzyme (0.335 U) was added to the mixture.

The solution was incubated at 37ºC (10, 20, 30, 40, 50, 60, 70 and 80 min) for the determination of incubation time and agitated at 40, 60, 80, 120 and 160 rpm for the determination of agitation speed. The reaction was stopped with 0.6 M, 1 mL TCA and was kept for 10 min then centrifuged for 10 min at 4500 rpm and filtered. The absorbance of filtered was read on UV-visible spectrophotometer. The wavelengths were used all experiment conditions for $\lambda_{wool\text{-}p\text{-}red}$: 536 nm, $\lambda_{wool\text{-}p\text{-}blue}$: 616 nm and $\lambda_{wool\text{-}p\text{-}yellow}$: 410 nm.

RESULTS AND DISCUSSION

These wool-synthetic dye complexes were hydrolyzed by different protease, such as subtilisin carlsberg, protease type XVI, trypsin and chymotrypsin. The pH dependence of proteases was studied in the pH range of 6-10.5. Optimum pH's were found to be pH: 9 for subtilisin calsberg and protease type XVI, pH:8 for trypsin and chymotrypsin with used wool-synthetic dye complexes, respectively. The results are shown in Figs. 1 to 4. The activity of each enzymes was done at these pH's. Figs. 5 to 8 show appropriate incubation time for each enzyme. 50 min was found better for used enzymes. We have observed non-linearity in the prolonged time. Effect of agitation speed is given in Figs. 9 to 12. Agitation speed greater than 120 rpm caused little increase in the reaction rates, expect for wool-p.red with subtilisin carlsberg, protease type XVI and trypsin.

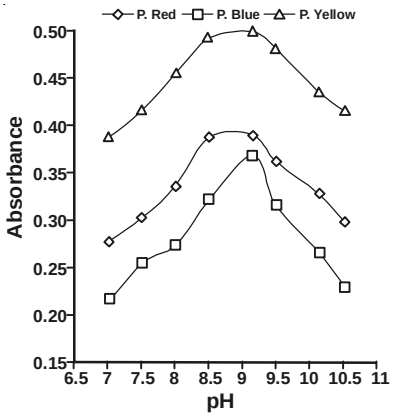

Fig. 1. Effect of pH on subtilisin carlsberg activity

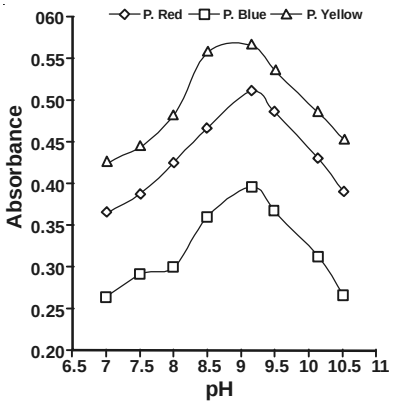

Fig. 2. Effect of pH on protease type XVI activity

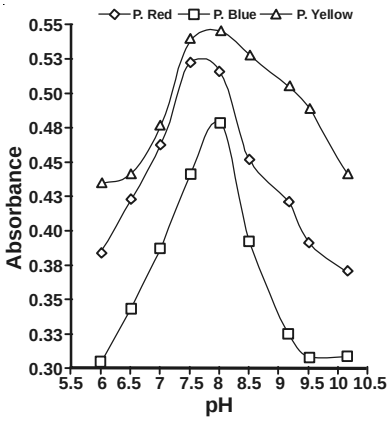

Fig. 3. Effect of pH on trypsin activity

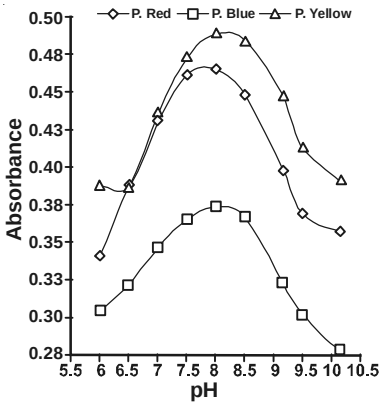

Fig. 4. Effect of pH on chymotrpsin activity

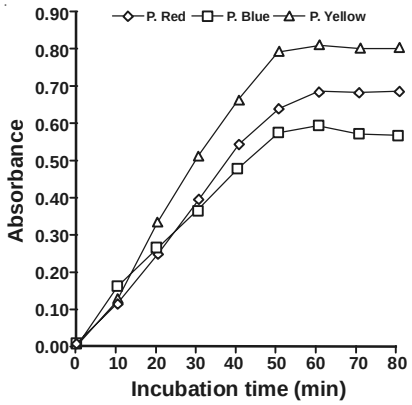

Fig. 5. Effect of incubation time on subtilisin carlsberg activity

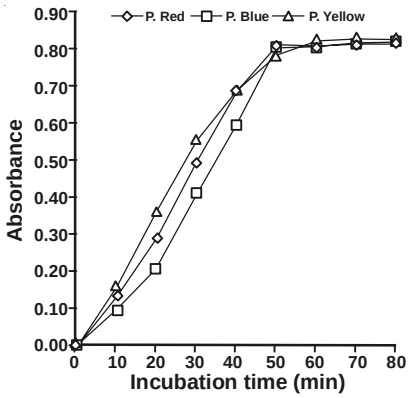

Fig. 6. Effect of incubation time on protease type XVI activity

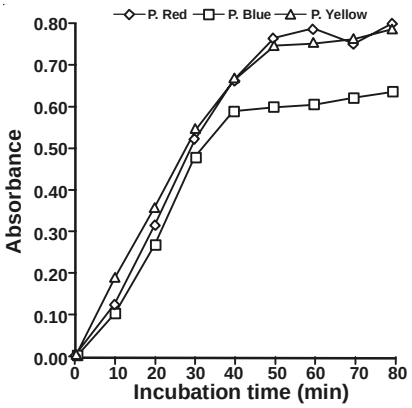

Fig. 7. Effect of incubation time on trypsin activity

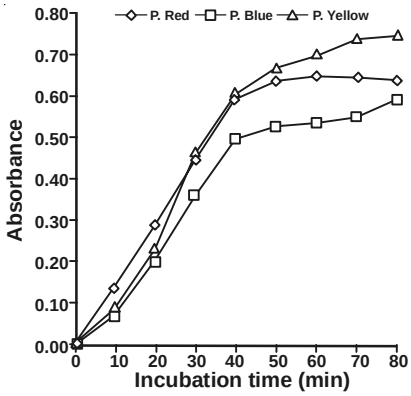

Fig. 8. Effect of incubation time on chymotrpsin activity

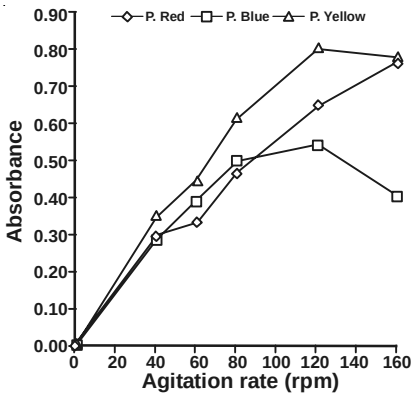

Fig. 9. Effect of agitation rate on subtilisin carlsberg activity

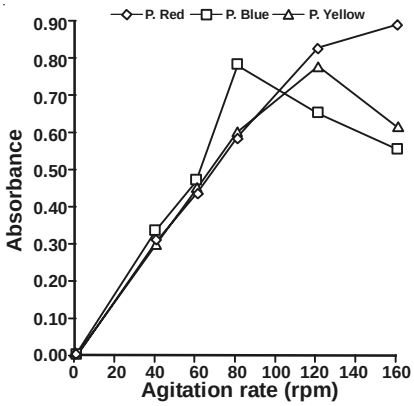

Fig. 10. Effect of agitation rate on protease type XVI activity

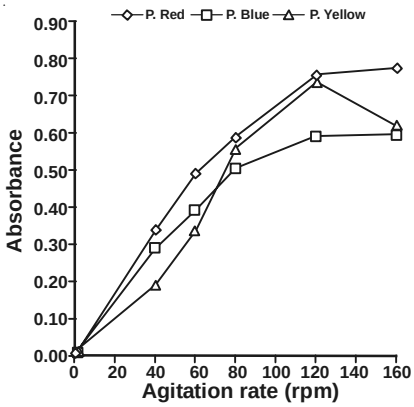

Fig. 11. Effect of agitation rate on trypsin activity

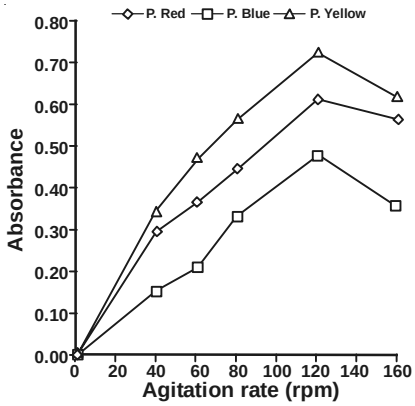

Fig. 12. Effect of agitation rate on chymotrpsin activity

Due to the simplicity of preparation these substrates could be successfully used in biochemistry and biotechnology researchs.

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