bacterium was isolated from fruit waste soil based on the potential degradation of the highly methylated pectin. According to the 16S rDNA sequence analysis, it was identified as Bacillus licheniformis BR1 which produces an extracellular polymethyl galacturonase PMG (EC 3.2.1.64) when cultured in medium containing citrus pectin0.1 %, peptone1%, yeast extract1%, pH 7.0. PMG production was optimized (6.22 U/ml) and the enzyme was purified to homogeneity in two steps column chromatography. The enzyme was a dimeric protein and exhibited an apparent molecular mass of 104 kDa by gel filtration chromatography and 56 kDa while running on SDS-PAGE. The enzyme exhibited maximum activity at 60 °C, pH 6 with 49% of total activity at 90 °C for 30 minutes and extends range of pH stability (5.0-9.0). Kmand Vmax of the PMG were determined 0.066 µmol/min and 2.51mg/ml respectively, using pectin as substrate. PMG activity significantly enhanced in the presence of 5 mM Ca2+, Cu2+, Mg2+, Mn2+ and Co2+, although Hg2+ and Fe2+ served as strong inhibitor. The enzyme was identified as a metal-dependent pectinase, which was inhibited by 5 mM of EDTA and showed suitable stability in the presence of 0.1% SDS and Tween 80. In overall, regarding to acidic properties and high operational stability of the purified pectinase, it seems that PMG can be an ideal functional substitute for applications in fruit juice industry, especially in citrus fruits extraction and clarification.

**Keywords:** Pectinase, Bacillus licheniformis, Purification, Polymethyl galacturonase, Thermostablity.

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**Abstract No.185**

## Effect of Chemical Modification on Thermal Stability, Structure and Function of Horseradish Peroxidase

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Biocatalysts are increasingly employed in chemical processing because of their selectivity as well as their potential as a greener alternative to chemical catalysis but, they are not usually tolerant to the presence of organic solvents, extremes of pH or high temperatures. Consequently, there is great interest in developing strategies to improve protein stability in desired reaction media. Compared with the strategies for obtaining stable enzymes, chemical modification is a simple and effective technique. Horseradish peroxidase (HRP) has achieved a prominent position in the pharmaceutical, chemical, and biotechnological industries therefore, methods improving the stability and functionality of HRP will clearly broaden the range of its present and future applications. In the present study, chemical modification of HRP was carried out with 2,3-dichloromaleic anhydride and 2,3-dimethylmaleic anhydride. Thermoinactivation, kinetic parameters and activation energy of catalysis and structural changes of HRP upon the modification were studied using spectroscopic techniques. The results indicated that 2,3-dichloromaleic anhydride increases thermal stability of HRP but 2,3-dimethylmaleic is not a stabilizer modifier for HRP. Catalytic efficiency and activation energy did not change remarkably following reaction of the enzyme with the carboxylic anhydrides. Fluoresnce measurements indicated that the intensity of protein emission decreases slightly upon the modification with both modifiers. A decrease in the distance between the heme and the tryptophan residue or decrease in compactness of the structure and therefore exposure of trp residue to solvent may lead to this change. On the whole, comparing the effect of 2,3-dichloromaleic with 2,3-dimethylmaleic implies that hydrophilization of the protein surface results in thermal stability of the protein.

**Keywords:** Horseradish Peroxidase, Thermal Stability, Chemical Modification, Spectroscopy.

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**Abstract No.186**

## Design and Construction of Antagonistic VEGF Variant for Inhibition of Angiogenesis

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Because of its central role in pathological angiogenesis, vascular endothelial growth factor (VEGF) has become a major target for anti-angiogenic therapies. We report here the construction of a heterodimeric antagonistic VEGF variant (HD-VEGF). In this antagonist, binding domains for the VEGF-receptor KDR/Flk-1 is present at one