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Characterization of α-amylases Isolated from Cyperus esculentus Seeds (tigernut): Biochemical Features, Kinetics and Thermal Inactivation Thermodynamics

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Abstract In this study, α-amylases from fresh and dried Cyperus esculentus seeds were isolated and characterized for biochemical and thermodynamic properties. The α-amylases (FTNE-α-amylase and DTNE-α-amylase) were optimally active at 60–70 ^{o}C, pH 7.0 (FTNE-α-amylase) and pH 6.0 (DTNE-α-amylase) with activation energies (Ea) of 3.56 and 6.81 kJ mol-1, respectively. Both enzymes retained above 70% of their hydrolytic activities after 1 h at pH 6.0–8.0. The enzyme activities were more enhanced in the presence of divalent metal ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Ba^{2+}, Co^{2+}, Pb^{2+}, and Hg^{2+}) than monovalent ions. SDS, cyclohexanol, ether and diethylamine inhibited them except EDTA. Using soluble starch as substrate, DTNE-α-amylase had higher Vmax, Kcat and lower Km than FTNE-α-amylase. Both enzymes expressed high substrate preferences for yam flour amidst other substrates with catalytic efficiencies (Kcat/Km) of 2.71 and 4.44 mL. mg respectively. The higher catalytic efficiency of DTNE-α-amylase was due to its transition state stabilization (ΔGE-S ¼ -0.90 kJ mol-1 and ΔGE-T ¼ - 2.22 kJ mol-1). Fourier Transformed-Infrared (FTIR) spectroscopy revealed changes in C–O stretching and -N-H bending in the substrate-bound enzymes. Thermal inactivation of the amylases followed first-order rate kinetics and was non-spontaneous (ΔG#>0). FTNE-α-amylase half-lives and D-values at 70 and 80 ^{o}C, and Z-value (256.41^{o}C), were however higher than in DTNE-α-amylase (Z-value ¼ 63.29 ^{o}C; t1/2 ¼ 22.43 min at 70^{o}C) indicating that FTNE-α-amylase is more thermally stable than DTNE-α-amylase and would be more industrially applicable as a thermostable starch-hydrolyzing enzyme.

Keywords: Cyperus esculentus seeds, α-amylase, Characterisation, Kinetics, Thermal stability, Thermodynamics