Purification, Characterization of Lipoxygenase which produced by a local isolate of Aspergillus niger and using in improving the qualitative and rheological properteise of wheat flour

By

Nora Taha Yaseen AL-Temimy

Summary

Eighty three local fungal isolates were isolated from different resources (Peanuts , maize, rice, wheat, bread , local cheese of sheep ,milk local cream , Iranian cream, Roquefort cheese and soil). These isolates were purified and identified, it include 11 isolates of *Aspergillus flavus*, 13 *Aspergillus niger* , 8 *Aspergillus terreus* , 3 *Aspergillus parasaticus* ,3 *Alternaria spp.*,15 *Penicillium* spp., 7 *Fusarium* spp. , 5 *Trichoderma* spp.,11 *Rhizopus* spp. and 7 *Mucor* spp.

The ability of fungal isolates to produce aflatoxin were tested, and the isolates producing aflatoxin were removed *Aspergillus flavus*, *Aspergillus terreus,* and *Aspergillus parasaticus.* the best isolate which producing Lipoxygenase was *Aspergillus niger* isolated from the maize and selected   after   Primary and secondary screening.

The optimum conditions Lipoxygenase production were studied , The best media consist of (100 ml) was composed of 1gm Wheat germ as a carbon source , 0.3gm yeast extract as a nitrogen source and 100 ml of mineral solution, the initial pH was 6.5, inoculum volume was 10ml (10×10^6) spores /100ml , and incubated at 30℃ for 5 days. The enzyme was extracted by crushing the biomass with mortar, glass beads and phosphate buffer. Three steps were used to purify Lipoxygenase comprised the concentration by using ammonium sulphate with (30-90%) saturation , then ion exchange chromatography by using DEAE Sephadex A-50 with ingradient NaCl (0-1)N . Two peaks were appeared at washing step and four peaks were appeared at the elution step, only one has specific activity which was 592.14 unite/mg, this peak was chosen to the last step of purification, using gel filtration chromatography with Sephadex G-100. Only one peak showed specific activity 1069.78 unit/mg. The purity of enzyme was tested by gel electrophoresis using polyacrylamide, The enzyme is quiet purified through appearing one band .
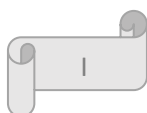
Summary

The characteristics of purified Lipoxygenase were studied as follow: The molecular weight was104 kD by using SDS- polyacrylamide gel electrophoresis, The optimum temperature and pH of enzyme activity were 35℃, 6.5 respectively. The thermo stability and optimum pH of stability were (0-45) ℃ and (6-7) respectively. The effect of some mineral ions on enzymatic activity, had been noticed that $CaCl_2$, KCl, $NiCl_2$, $MgCl_2$ and $MnCl_2$ increased the activity while the enzymatic activity was decreased when using $ZnCl_2$,$FeCl_3$, and $CuCl_2$. EDTA inhibited the enzyme activity. Studying of kinetic constants showed that Michaelis constant (k_m) and maximum velocity (v_{max}) of the enzyme were 0.2 mg/ml and 45 μm/ml/min respectively.The activation energy was 8 kcal/mole, while denaturation energy was 54 kcal/mole.

Chemical composition of wheat flour and physical properties were studied, as follow the moisture, Ash, protein, dry glutens and fat (14.80, 1, 12.1, 26.2, 1.15) % respectivally.

The effect of purified LOX on rheological properties of flour were studid using 5 treatment (20, 40, 60, 80) ppm. The pharinograph results showed improving the rheological properties according to the increasing of LOX concentration (20, 40, 60, 80)ppm, It was observed increase in water absorption, Development time and Stability as the concentration of the enzyme increased comparing with control. The best rheological properties of flour was sample 4 which treated with 80ppm LOX. The waterabsorption was 64.9%, Development time was 2.9min and Stability was 4.9min.

The effect of addition of purified LOX (20, 40, 60, 80) ppm\100gm flour on some flour properties such as (dough color, the volume and flavour of loaf) were studied. through sensory evaluation the fourth treatment (80ppm LOX \100gm flour) showed significant increasing at the level improbability **P ≤ 0.05**.