OPTIMIZATION OF TWO METHODS TO DETECT PLANT DERIVED INHIBITORS OF PROTEIN GLYCATION

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Glycated proteins are formed by a non enzymatic reaction between reducing sugars and protein in which unstable Schiff bases are formed followed by an Amadori production (fructosamine) to form advanced glycation end products (AGE). During later events of glycation, most AGEs form cross-links.

There have been several methods developed for the determination of fructosamine and protein cross-link formation. Of them Nitroblue tetrazolium (NBT) assay and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were optimized to detect fructosamine and protein cross-links respectively. D-glucose, D-fructose and D-ribose were used as model sugars and bovine serum albumin and lysozyme were used as proteins for glycation. Aminoguanidine (AG) was used as a standard inhibitor of glycation. Methanol extracts of *Kalanchoe pinnata* leaf, *Coriandrum Sativum* seed and *Murraya koenigii* leaf were used as potential inhibitors. Samples were incubated in sealed tubes at 37 °C for 3 weeks and were periodically checked for the amount of glycation products formed namely fructosamine and protein cross-links using NBT assay and SDS-PAGE respectively. Molecular weight markers were used for SDS-PAGE, simultaneously with the samples to quantify the approximate molecular weights of the high molecular products.

NBT assay was optimized to detect fructosamine formation. Sample volume, NBT reagent volume and incubation period were affected to the absorbance of the assay. Volume of 20 µl of sample and 400 µl of NBT reagent were used. Incubation time used was 15 minutes in the assay. Among the three sugars used, ribose showed the highest rate of fructosamine formation. Glucose showed a relatively slower rate of fructosamine formation in comparison to other two sugars. *K. pinnata*, *C. Sativum* and *M. koenigii* showed greater relative inhibition on fructosamine formation at the concentrations used compared to that of aminoguanidine. Fructosamine formation is seemingly declined with longer incubation periods.

SDS-PAGE method was optimized to observe the appearance of glycation related protein cross-linking and inhibition of cross-link formation. Gel percentage used was 12%. Molecular weight markers were used to determine the size of the cross-linked products. Appearance of dimer, trimer and tetramer was observed, which was dependent on the rate of glycation. Cross-link

formation increased with incubation period and increasing sugar concentration. Highest rate of appearance of protein cross-links were observed with ribose. Glucose showed a relatively slower rate of cross-link formation in comparison to other two sugars, showing basically the formation of dimer, but not the other higher molecular weight products. *K. pinnata*, *C. Sativum* and *M. koenigii* showed greater relative inhibition on cross-link formation (almost 100% compared to their test blanks) at the concentrations used compared to that of aminoguanidine. This study established the suitability of SDS-PAGE method to identify the effect of plant derived products on protein cross-linking for the first time.

In conclusion, we have optimized NBT assay and SDS-PAGE method to detect protein glycation products, fructosamine and protein cross-links respectively and inhibitors of formation of those glycation products. Further we have demonstrated the early glycation and protein cross-linking inhibitory potential of methanol extracts of *K. pinnata*, *C. Sativum* and *M. koenigii*.