G1 exhibited a significant improvement in digestibility of globulin G1.

The practical applications of this study include the maintenance of the fresh quality of beans stored for up to two years by the combined effect of refrigeration and hypobaric storage conditions. Another practical application is the potential improvement of bean protein digestibility, by soaking fresh or cooked beans in a solution of pineapple flesh or shell tissues prior to cooking. Both of these applications may potentially improve legume protein quality and availability in developing tropical countries.

A Molecular Approach to Reduce Flatulence in Beans: Purification of Galactinol Synthase from *Phaseolus vulgaris*

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The ultimate goal of this project is to reduce flatulence in beans by blocking the biosynthesis of flatulence-causing galactose oligosaccharides (GO) in the seed through genetic engineering. Our initial objective is to purify galactinol synthase (GS), a key enzyme in GO biosynthesis and subsequently clone its gene.

We have purified GS from kidney bean and obtained a partial sequence of the protein. The purification scheme included the following steps: extraction of proteins from kidney bean cotyledon, fractionation with ammonium sulfate at 25-40% saturation, chromatography on DEAE ion exchange column, chromatography on Affi-gel Blue Gel column and finally chromatography on UDP-hexanolamine agarose column.

Diagnostic SDS-PAGE (sodium dodecyl sulfate- polyacrylamide gel electrophoresis) of the final purification product from the UDP-hexanolamine column showed three protein bands by silver staining in the active fractions: 43kD, 41kD and 38kD, while the adjacent inactive fraction contained 43/41kD proteins only. We concluded that the 38kD protein is GS. The 38 kD band protein was sequenced and the N-terminus had the following sequence: NKVINVPAGFGYELYNRNRINR-LGPKAN (single letter amino acid code). This enzyme bound specifically to UDP-galactose and myoinositol (K_m=0.4mM and K_m=4.5mM, respectively). DTT and $MnCl_2$ were required for GS activity.

Meanwhile, we also purified to homogeneity GS from zucchini leaf using the same scheme as that used for kidney bean and had the N-terminus sequenced. It is a 36kD protein with a partial N-terminus sequence of PAATETAIE which is identical to the sequence of GS from zucchini leaf found by a Du Pont research group.

We shall use the protein sequences from kidney bean and zucchini leaf to clone the cDNA for kidney bean GS using polymerase chain reaction (PCR). PCR products will be sequenced and used as probes to screen a kidney bean seed cDNA library.