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The Effect of Heat Treatment of Amaranth  
on Trypsin and Chymotrypsin Inhibitory Activity and  
in Vitro Digestability

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## 5. SUMMARY

The nutritional value and digestability of amaranth species *A. hypochondriacus* K343 was studied. Analysis of chemical composition in percent dry matter showed 16.3% protein, 4.5% fat, 70% carbohydrates, 4.4% fiber and 12% moisture. Trypsin and chymotrypsin inhibitor contents (TI and CI) were low, 0.017 TIU/mg and 0.022 CIU/mg amaranth. These values were similar to those found in literature. Inhibitor extraction resulted in high losses of activity following dialysis with a MW cut-off of 3500. Inhibitors showed high heat stability. After exposure of amaranth flour to 100° C for 6 hours 50 % CI activity was retained, 10% CI residual inhibitory activity remained following 30 min autoclaving. TI appeared to be more heat sensitive than CI. The effectiveness of heat exposure on inhibitor inactivation was drastically reduced when large batches of coarse flour were treated. This may have been caused by decreased heat accessibility and increased flour particle size.

Protein of heat treated samples was extracted, yielding mainly albumins. Protein extracts were subjected to in vitro digestion with pepsin, pancreatin and pepsin+pancreatin, simulating gastrointestinal treatment of proteins to assess the influence of heat treatments on digestion. Digestion samples and controls were evaluated by SDS-PAGE. Undigested protein extract showed 15 bands with molecular weights (MW) ranging from 15 to 69 kD. No-enzyme controls showed fewer bands of lower MW probably due to denaturation by basic and acidic pH. No differences were found between controls of the various heat treatments.

Electrophoretic patterns resulting from pepsin, pancreatin and successive pepsin+pancreatin digestion varied considerably due to different specificities of the enzymes. Pepsin digestion resulted in low MW subunits, successive pepsin+pancreatin digestion produced middle to low MW bands, while pancreatin digestion bands ranged from high to low MW. Compared to their no-enzyme controls, greatest changes were observed following successive pepsin+pancreatin

digestion, with high MW-bands disappearing and low MW material accumulating. These results indicate that successive digestion was most efficient, followed by pepsin digestion, while pancreatic digestion was the least efficient. Differences in the digestability of amaranth proteins following various heat treatments were observed. Heating at 60° C for 24 hours did not influence pancreatic digestion whereas the autoclaved samples seemed less accessible to pancreatic digestion. Aliquots withdrawn at intervals during incubation might reveal differences in the initial rates of protein hydrolysis. In contrast with globulins and glutelins, albumins are known to be digested well when raw, while their digestability decreases after heat treatment.

The present results indicate that TI and CI may not be responsible for amaranth's low digestability. The significantly improved nutritional value of amaranth after heat treatment can not be sufficiently explained by TI and CI destruction, as they require drastic treatment before inactivation. The digestability of amaranth albumin by pancreatin in vitro was decreased by heat treatment. The influence of heat treatment on the digestability of amaranth globulins and glutelins as well as of whole amaranth flour might shed new light on the reasons behind amaranth's low digestability.