ELISA ANALYSIS OF SOYBEAN TRYPsin INHIBITORS IN PROCESSED FOODS

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ABSTRACT

Soybean proteins are widely used in human foods in a variety of forms, including infant formulas, flour, protein concentrates, protein isolates, soy sauces, textured soy fibers, and tofu. The presence of inhibitors of digestive enzymes in soy proteins impairs the nutritional quality and possibly the safety of soybeans and other legumes. Processing, based on the use of heat or fractionation of protein isolates, does not completely inactivate or remove these inhibitors, so that residual amounts of inhibitors are consumed by animals and humans. New monoclonal antibody-based immunoassays can measure low levels of the soybean Kunitz trypsin inhibitor (KTI) and the Bowman-Birk trypsin and chymotrypsin inhibitor (BBI) in processed foods. The enzyme-linked immunosorbent assay (ELISA) was used to measure the inhibitor content of soy concentrates, isolates, and flours, both heated and unheated; a commercial soy infant formula; KTI and BBI with rearranged disulfide bonds; browning products derived from heat-treatment of KTI with glucose and starch; and KTI exposed to high pH. The results indicate that even low inhibitor isolates contain significant amounts of specific inhibitors. Thus, infants on soy formula consume about 10 mg of KTI plus BBI per day. The immunoassays complement the established enzymatic assays of trypsin and chymotrypsin inhibitors, and have advantages in (a) measuring low levels of inhibitors in processed foods; and (b) differentiating between the Kunitz and Bowman-Birk inhibitors. The significance of our findings for food safety are discussed.
INTRODUCTION

Active protease inhibitors can limit the digestibility and bioavailability of soy protein. In addition, at least in the rat, consumption of dietary protease inhibitors can result in development of pancreatic adenomas (see reviews by Gallaher and Schneeman, 1986; Gumbmann et al., 1986; Grant, 1990). However, some inhibitors also have potentially beneficial effects. They have been shown to inhibit carcinogenesis in both in vitro and in vivo systems (Yavelow et al., 1983; Troll et al., 1986; St. Clair et al., 1990), and to stimulate human T cells (Richard et al., 1989).

In assessing the possible significance of protease inhibitors in human nutrition, Morgan et al. (1986) note that one group of humans, namely, infants fed soybean-containing formulas because of allergy to cow's milk, may be at risk. These authors also note that although it is possible that the period of continuous exposure to protease inhibitors is too short for any significant effect on the pancreas to occur, it would nevertheless be desirable to eliminate all trypsin inhibitor activity in these products.

In order to assess the impact of food processing on the nutritional quality and healthfulness of products containing soy protease inhibitors, it is necessary to measure these specific inhibitors accurately. In soy products, one complication is the occurrence of multiple soybean protease inhibitors, including the Kunitz trypsin inhibitor (KTI) and the double-headed Bowman-Birk trypsin and chymotrypsin inhibitor (BBI). Both KTI and BBI exist as several isoforms, which are derived from different genes or are produced by proteolysis; and other inhibitors are also present (Laskowski, 1986; Tan-Wilson and Wilson, 1987). It is therefore impossible to establish the exact protease inhibitor composition of a sample through enzymatic assay, especially in samples with low residual inhibitor activity, such as toasted soy flours or soy protein isolates. We have found that immunoassays using monoclonal antibodies offer the specificity and sensitivity necessary to analyze complex, processed food samples (Brandon et al., 1987b, 1988, 1989, 1990). These methods could be used to assess improved food processing strategies for optimizing the content of protease inhibitors in soy foods (Brandon et al., 1986a, 1987a; Friedman et al., 1989, 1990). In addition, we have found the immunoassays useful for screening soybean germplasm (Friedman et al., 1990). Finally, they can also be used, in model systems, to study the effects of processing conditions on food proteins.

METHODS AND MATERIALS

Antibodies and assays. Rabbit polyclonal antibodies and mouse monoclonal antibodies to KTI were described in detail previously (Brandon et al., 1986b, 1987b, 1988; Brandon and Bates, 1988). Antibodies to BBI were described by Brandon et al. (1989). The assays were described in detail in the above references, but will be summarized briefly here. In inhibition ELISA's, assays were conducted in plastic ELISA