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Studies on karkade (*Hibiscus sabdariffa*): protease inhibitors, phytate, *in vitro* protein digestibility and gossypol content

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Karkade defatted flour extract had chymotrypsin inhibitor activity almost half that of trypsin (21.8 and 40.5 enzyme inhibitor activity units/mg protein, respectively). The protein isolate had a weak chymotrypsin inhibitor activity compared to that of tryprin (4.9–28.3 enzyme inhibitor activity units/mg protein, respectively). The trypsin inhibitor activity of the protein isolate was 70% of that of the defatted flour extracts. Extraction of trypsin inhibitor by citrate buffer (pH 4.6) gave a higher specific activity of trypsin than by phosphate buffer (pH 7.6), Tris buffer (pH 9.0) or distilled water. Heating the extract in boiling water for 10 min destroyed 66.1% of the trypsin inhibitor activity. Karkade protein isolate had lower free and total gossypol than did the whole seed and defatted flour. The *in vitro* protein digestibility of karkade defatted flour and protein isolate was lower than that of casein. Phytic acid was higher in karkade defatted flour than in soybean defatted flour. Copyright ©1996 Elsevier Science Ltd.

INTRODUCTION

The value of plant protein in supplying the protein needs in developing countries has been recognized in recent years. The seeds of *Hibiscus* are excellent sources of proteins (25.2%) and oil (21%) (Abu-Tarboush, 1996; Al-Wandawi *et al.*, 1984). However, *Hibiscus* seeds, like other seeds and legumes, contain antinutritional factors which may reduce their nutritional value or their protein digestibility. The importance of antinutritional factors is expected to increase in the years ahead along with increased use of proteins of plant origin (Esen, 1982). Protease inhibitors are among the most important antinutritional factors encountered in seeds. Trypsin inhibitors have been isolated in pure crystalline form from different plant sources (Kunitz, 1947; Roy & Rao, 1971). The presence of trypsin inhibitors in soybeans and other legumes was reported by Roy & Bhat (1974). Heat-inactivation of trypsin inhibitor in green soybeans was studied by Collins & Beaty (1980).

Gossypol is a constituent of cotton seeds (Murti & Achaya, 1975) and is toxic to monogastric animals (Berardi & Goldblatt, 1980). Schmidt & Wells (1990) reported the presence of gossypol in okra seeds and in seeds of three other plants in the Hibiscadeae tribe.

Mohamed *et al.* (1986) reported that soybeans and other oil seeds contain phytic acid which influences the bioavailability of trace minerals.

The purpose of this study was to assess the levels of trypsin and chymotrypsin inhibitors, levels of phytate, *in vitro* protein digestibility and gossypol content of karkade seed defatted flour and protein isolate. The effect of the type of buffers in the extraction of trypsin inhibitor and its thermal stability were also investigated.

MATERIALS AND METHODS

Preparation of defatted flour and protein isolate

Seeds of *H. sabdariffa* were obtained from the western region of Sudan. Seeds were cleaned by removing dust and plant debris. They were ground in a Waring blender to a flour consistency. Seeds were milled to pass through a 0.8 mm sieve using an ultra-centrifugal mill. The resultant flour was defatted with *n*-hexane at room temperature (22°C). The oil-free flour was desolventized in open air at room temperature. The defatted flour was further milled to pass through a 0.355 mm sieve.

The method of El-Tinay *et al.* (1988*a*) for protein extraction and the method of El-Tinay *et al.* (1988*b*) for protein coagulation at the isoelectric point were used for preparation of protein isolates.

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