ELSEVIER

Water Research 39 (2005) 975–980

WATER RESEARCH

www.elsevier.com/locate/watres

Detection of water soluble lectin and antioxidant component from *Moringa oleifera* seeds

A.F.S. Santos, A.C.C. Argolo, L.C.B.B. Coelho, P.M.G. Paiva*

Departamento de Bioquímica, CBB/UFPE, Av. Moraes Rego, S/N, Cidade Universitária, Recife-PE, 50670-420, Brazil

Received 9 December 2002; received in revised form 9 June 2004; accepted 23 December 2004

Abstract

Seed flour from *Moringa oleifera* is widely used as a natural coagulant for water treatment in developing countries. Extracts obtained by water soaking of *M. oleifera* intact seeds were investigated for the presence of lectin, trypsin inhibitor, tannin as well as antioxidant activity. A water soluble *M. oleifera* lectin (WSMoL) detected was mainly active with rabbit cells at pH 4.5; heat treatment, pH 7.0, fructose and porcine thyroglobulin abolished HA of WSMoL. Trypsin inhibitor or tannins were not detected; the antioxidant component (WSMoAC) reduced 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was slower than catechin and was thermostable. The extracts showed a primary glycopolypeptide band of Mw 20,000; the main native acidic protein showed hemagglutinating activity. WSMoL may be involved in seed coagulant properties.

Keywords: *Moringa oleifera*; Seeds; Lectin; Trypsin inhibitor; Tannin; Antioxidant activity

1. Introduction

The production of potable water involves coagulant use at a flocculation/coagulation stage to remove turbidity in the form of suspended and colloidal material (Diez et al., 2002); coagulants from *Moringa oleifera* seeds (Moringaceae family) have been recommended for water treatment in African and South Asian countries (Okuda et al., 1999). Natural biodegradable coagulants offer advantages to inorganic or synthetic organic polymers which are associated with human pathological processes (Okuda et al., 2001).

Food antioxidants are important for human nutrition by decreasing the oxidative damage to lipids, proteins and nucleic acids induced by free radicals (Soler-Rivas et al., 2000); however, some water soluble seed compounds are antinutritional factors (Hossain and Becker, 2002). Lectins constitute a heterogeneous protein group of non-immune origin with non-catalytic sites capable of recognizing and reversibly binding to cell surface carbohydrates. Lectins interact with glycoproteins in the mucosa of the intestinal wall and interfere with nutrient digestion and absorption resulting in reduced efficiency of nutrient utilization; also, reduction of amylase activity was demonstrated by interaction of lectin with either the enzyme itself or its substrate (Fish and Thompson, 1991). Long-term study of low lectin doses showed rat pancreas growth in vivo (Kelsall et al., 2002). Protease inhibitors disrupt enzymatic action by formation of stable complexes with trypsin and/or chymotrypsin. Inhibitors have been implicated in reducing protein digestibility in non-ruminants by inhibition of pancreatic proteolytic enzymes. Pancreas hypertrophy (Hanbury et al., 2000) and depression of animal growth rate (Vasconcelos et al., 2001) have been

*Corresponding author. Tel./fax: +55 081 32718354.
E-mail address: pmgpaiva@bol.com.br (P.M.G. Paiva).

0043-1354/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.watres.2004.12.016

associated to trypsin inhibitor in animal diet. Tannins are non-toxic polyphenols and poorly biodegradable, which inhibit growth of fungi, yeasts, bacteria and viruses; they form complexes with proteins which are frequently indigestible (Chung et al., 1998).

Evaluation of biological activity in water is an indication of treatment efficiency used for this purpose (Lindström-Seppä et al., 1998). In this work, lectin activity, trypsin inhibitors, tannins and antioxidant activity were investigated on water obtained after immersion of *M. oleifera* intact seeds.

2. Materials and methods

2.1. M. oleifera extract preparations

Mature seeds of *M. oleifera* were collected in Fortaleza city, State of Ceará, Brazil Northeast in dry season and stored at −20 °C. Three extracts (7.0 g l^{-1}) were prepared by soaking intact *M. oleifera* (Lam.) seeds, without coat removal, in distilled water. The extracts were obtained after 5 (T_5), 15 (T_{15}) and 37 h (T_{37}) at 28 °C, by collecting the supernatant waters and storing at −20 °C. Additionally, seed flour (7.0 g l^{-1}) was mixed with water by 5 h; the obtained water was filtered on paper (Toyo-Filter paper, Toyo Rashi Co., Japan) and used (T_5 flour) for lectin activity and protein determination.

2.2. Protein evaluation

The protein concentration was estimated according to Lowry et al. (1951) using bovine serum albumin (31–500 μg ml^{-1}) as standard; absorbance at 280 nm was also measured.

2.3. Hemagglutinating activity (HA)

Lectin activities of non-treated and heated (100 °C, 15 min) extracts were evaluated according to Correia and Coelho (1995) using glutaraldehyde-treated rabbit or human erythrocytes. The hemagglutinating activity was obtained by mixing a twofold serial dilution of the extracts (50 μl) in 0.15 M NaCl (pH 5.0) followed by the addition of a 2.5% (v/v) suspension of erythrocytes (50 μl), in microtiter plates (Kartell S.P.A., Italy). Titer was defined as the lowest sample concentration after 45 min which showed hemagglutination. A hemagglutinating inhibitory assay was performed by incubation (45 min) of T_5 with 200 mM monosaccharide solutions (D(+)-fructose and D(+)-glucose), trisaccharide (D(+)-raffinose) and porcine thyroglobulin glycoprotein (0.5 mg ml^{-1}), before rabbit erythrocyte suspension addition. The pH effect on HA, 10 mM citrate phosphate buffer containing 0.15 M NaCl (pH 4.5–6.0) and 10 mM sodium phosphate buffer containing 0.15 M NaCl (pH 6.5–7.5), were evaluated.

2.4. Trypsin inhibitory activity

The assay (Kakade et al., 1969) was made with bovine trypsin (10 μg ml^{-1} in 0.1 M Tris-HCl, pH 8.0; 20 μl); pre-incubation (37 °C, 10 min) of enzyme and extracts (30, 40 and 60 μl) was followed by addition (30 μl) of 4 mM *N*-α-benzoyl-DL-arginyl-ρ-nitroanilide (BAPA) dissolved in dimethyl sulfoxide and diluted with tris buffer. After 30 min at 37 °C, 10% (v/v) acetic acid was added (300 μl). The substrate hydrolysis was followed by measurement of absorbance at 405 nm, and the molar extinction coefficient for ρ-nitroanilide used for concentration calculation was 9100 (Oliva et al., 2000). The inhibitory activity evaluated the remaining hydrolytic activity towards BAPA.

2.5. Tannin assay

Tannin was detected with acidified vanillin reaction specific for estimating tannin content (Burns, 1971). The assay used 1 ml of methanolic solutions (2% v/v) of T_5 (1.42 mg), T_{15} (3.12 mg), and T_{37} (4.16 mg), as well as dilutions of catechin solution (100 mg 50 ml^{-1} of methanol) to obtain standard curve (0.078–1 mg).

2.6. Antioxidant activity

The free radical-scavenger capacity of non-treated and heated (100 °C, 15 min) aqueous extracts (50 mg ml^{-1}, 2 μl) and catechin standard (5 mg ml^{-1} of methanol, 2 μl) were determined according to Soler-Rivas et al. (2000) using dot-blots on thin layer chromatography (TLC) stained with a 0.4 mM 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) solution. The dilutions of applied samples were 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256. Positive results were observed on silica sheet which was yellow in color.

2.7. Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) for acidic and basic proteins were made according to Davis (1964) and Reisfeld et al. (1962), respectively. Also, detected protein bands were extracted by cutting the gel, followed by maceration in 0.15 M NaCl and HA evaluation. Denatured electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) and stained to both protein using Coomassie Brilliant Blue (Laemmli, 1970) and glycoprotein with Schiff's Reagent (Pharmacia Fine Chemicals, 1980).