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Guar and Locust bean gum: Composition, total phenolic content, antioxidant and antinutritional characterisation

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ABSTRACT

Guar and locust bean gum are added to various food products because of their ability to modify the texture and rheology. No sufficient data is available about their bioactive roles except that these are physiologically similar to dietary fibre and may be used as low-energy fat replacers. Present study was undertaken to find out the nutraceutical and antinutritional properties of guar and locust bean gum. Antioxidant activity of gums was determined by ferrous ion chelating ability, reducing power, FRAP (ferric reducing antioxidant power) and ABTS (2,2-azinobis (3-ethylbenzothiazolin-6-sulfonate)) scavenging power. Determination of phytic acid, haemagglutinins, terpenoids, saponins and alkaloids was carried out to evaluate their antinutritional activity. Gums showed low antioxidant potential except ferrous ion chelating ability which was significant in both methanolic (65.08–66.82%) and ethanolic (36.46–55.40%) extracts. Gums also exhibited a high DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (11.67–29.78%). Bile acid binding capacity was high i.e., 49.83% (guar gum) and 54.42% (locust bean gum). Tannin and phytic acid content varied from 1.28 to 3.72 TAE/g and 0.098–0.165 mg/g, respectively. Haemagglutinin activity of gums was low (0.066–0.432 HU/mg protein) while the terpenoids, saponins and alkaloids were completely absent. Thus, gums may play beneficial bioactive roles in consumers in addition to conferring desirable organoleptic properties in different food products.

1. Introduction

Guar gum and locust bean gum are galactomannans consisting of β-(1 → 4) linked mannopyranosyl backbone attached to α-(l → 6) linked D-galactopyranosyl side chains (Estévez et al., 2004). The galactomannans are ground endosperm or extracted polysaccharides thereof, obtained from the seeds of Leguminosae. Guar gum, obtained from *Cyamposis tetragonoloba* L. and locust bean gum, obtained from *Ceretonia siliqua* have a closely related molecular structure. The mannose to galactose ratio in the molecule of guar gum is 2:1 and for locust bean gum is 4:1 (Mahungu & Meyland, 2008). This implies a higher degree of branching in guar gum as the branch points repeat after every two units of mannopyranosyl chain. Due to the differences in molecular structure, these exhibit variations in various physicochemical and rheological properties (Robinson, Ross-Murphy, & Morris, 1982).

Irrespective of these differences, both guar and locust bean gum find applications in different food and non food industries. The addition of seed gums in various food products is known to be useful in achieving desired texture and rheology. These are used as thickeners, stabilizers, emulsifiers, gelling agents and coffee whiteners. The baby foods, salad dressings, sauces and soups, icings, cake mixes, ice creams and cured meat foods are some other products in which seed gums are essentially used as ingredients (Kapoor, Pandey, Khanna, Dwiredi, & Singh, 1999; Reid & Edwards, 1995). However, with respect to their bioactive roles, sufficient data is not available except that these are physiologically similar to dietary fibre and may be used as low-energy fat replacers (Alcedo, 1999).

Plant based food products are usually characterised by the presence of some antioxidants and/or antinutritional factors. Antioxidants are involved in minimising the oxidative damage caused by reactive oxygen species (ROS). These prevent or reduce the risk of cardiovascular, neurodegenerative and aging-related diseases including various types of cancers (Krishnaiah, Sarbatly, & Bono, 2007). Many studies have reported vital roles of some plant polysaccharides in free radical scavenging and prevention of diseases appearing due to oxidative stress (Fan, Mazza, & Liao, 2010; Li, Wang, Wang, & Xiong, 2016; Tseng, Yang, & Mau, 2008). Similarly, various other phytochemicals in plant products have antinutritional effects either because of some negative physiological role or because they limit the bioavailability of some essential nutrients. An example to this is phytic acid which is a powerful chelating agent found mostly in legumes. It forms insoluble complexes with divalent cations and reduces their bioavailability (Weaver & Kanna, 2002). A reduced mineral bioavailability is also observed

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due to high polyphenole concentration in plant foods (Saharan, Khetarpaul, & Bishnoi, 2001). In addition, some other phytochemicals like haemagglutinins, associated with agglutination of RBCs; alkaloids, associated with toxicity to GI tract and saponins, associated with haemolytic activity are also reported as plant toxicants (Deshpande, 2002). The aim of present study was to determine and compare the proximate composition, antioxidant potential and antinutritional factors like tannins, phytic acid, haemagglutinin, terpenoid, saponin and alkaloid content of guar and locust bean gum.

2. Materials and methods

2.1. Materials

Pure gums of analytical grade were obtained from Himedia India. The powder was passed through 125 μm sieve to obtain uniform particle size. All chemicals used were of analytical grade.

2.2. Proximate composition

Moisture (925.10), protein (920.87), fat (920.85) and ash (923.03) contents were determined according to the methods of AOAC (1990). Crude fibre was determined using the fibre extractor (Velp Scientifica, FIWE, Raw Fibre Extractor).

2.3. Total phenolic content (TPC)

Gum samples (200 mg) were extracted at room temperature (25 ^{0}C) with 4 mL of acidified non-polar solvents i.e., HCl/water/methanol or ethanol in the volume ratio of 1:10:80 for 2 h. 200 μL of this extract was mixed with 2.5 mL of 10-fold diluted freshly prepared Folin-Ciocalteu reagent and allowed to react for 5 min. 1.5 mL of sodium carbonate solution (60 g/L) was added to the mixture after 5 min and the contents were incubated at room temperature for 90 min. The absorbance was read at 725 nm (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan). Total phenolic content of samples was determined using gallic acid standard curve and the results were expressed as mg of gallic acid equivalents per gram (GAE/g) of sample. The method was adopted from Gao, Wang, Oomah and Mazza (2002).

2.4. Ferrous ion chelating ability

Chelating ability of gums against ferrous ions was determined using the method of Dinis, Madeira and Almeidam (1994) with slight modifications. 0.5 mL of ultrasonicated (for 2 h) methanolic and ethanolic gum extract was mixed with 50 mL of ferrous chloride. To this was added 1.6 mL methanol/ethanol and, ferrozine (0.2 mL, 5 mM) after 5 min, with constant vortexing. The contents were incubated for 10 min at room temperature and absorbance was measured at 562 nm. The chelating activity was calculated as per the formula given below:

$$\text{Ferrous ion chelating activity}(\%) = 1 - \frac{\text{Absorbance of sample at 562 nm}}{\text{Absorbance of control at 562 nm}} \times 100$$

2.5. Reducing power

Gum sample (2 g) was extracted with 2 mL of 80% methanol/ethanol in polypropylene tubes by constant vortexing. The tubes were centrifuged (5810 R, Eppendorf, Hamburg, Germany) (3000 g, 10 min) and the supernatant was collected. 1 mL of this extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH-6.6) and 2.5 mL potassium ferricyanide. After incubation period of 20 min at 50 ^{0}C, trichloro-acetic acid solution (10% w/v) was added. The contents were centrifuged at 10,000 × *g* for 10 min 2.5 mL of this supernatant was mixed with 2.5 mL deionised water and 0.5 mL ferric chloride and the absorbance was recorded at 700 nm. The method was adopted from Zhao et al. (2008). Calculations were made by drawing an ascorbic acid standard curve and the results were expressed as ascorbic acid equivalents per gram (AAE/g) of flour.

2.6. Ferric reducing antioxidant power (FRAP)

The reducing power of gums against ferric ions was determined using the method of Stratil, Klejdus and Kubánhacek (2006) with slight modifications. FRAP value was obtained by mixing 100 μL of methanolic/ethanolic extract of gum samples with 900 μL warm (37 ^{0}C) FRAP solution. FRAP solution was prepared by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ solution (10 mM TPTZ in 40 mM HCl) in a proportion of 10:1:1, respectively. After vigorous shaking, the contents were incubated (37 ^{0}C, 40 min) and the absorbance (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan) was recorded at 595 nm. An ascorbic acid standard curve was prepared to calculate the antioxidant capacity of the samples. Results were expressed in mg of ascorbic acid equivalent per gram of sample on dry-weight basis (mg AAE/g dwb).

2.7. ABTS (2,2-azinobis(3-ethylbenzothiazolin-6-sulfonate) scavenging assay

The radical scavenging activity of the methanolic and ethanolic gum extracts against ABTS radical cation was measured using the method of Miller and Rice-Evans (1997) with some modifications. ABTS (19.2 mg) was dissolved in 5 mL of deionised water (Mili-Q) and 88 μL of 0.0378 g/mL potassium persulfate ($K_2S_2O_8$), followed by incubation in a dark room (25 ^{0}C, 16 h). It generated the $ABTS^+$ radical which was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. For estimation of ABTS radical scavenging ability, 30 μL of sample was mixed with 2970 μL of ABTS solution. After a 6 min equilibration period, absorbance was recorded at 734 nm and the calculations were made by drawing ascorbic acid standard curve. Results were expressed as μM ascorbic acid equivalents in mg/100 g (μM AAE in mg/100 g) of sample.

2.8. DPPH (1,1-diphenyl-2- picrylhydrazyl) radical scavenging assay

The DPPH radical scavenging ability of gum samples was determined using the modified method of Brand-Williams, Cuvelier and Berset (1995). Briefly 100 μL of the methanolic/ethanolic gum extract was reacted with 3.9 mL of a DPPH solution (6 × 10^5 mol/L). DPPH is reduced with an antioxidant due to which a decrease in absorbance is observed at 515 nm. Absorbance (A) (UV-Spectrophotometer, Model U-2900 2JI-0003, Hitachi, Japan) was read at 0 and 30 min using a methanol blank. % inhibition of DPPH radicals was measured as per the formula given below:

$$\%\ \text{inhibition} = [1 - (\text{Absorbance of sample at t} = 30/\text{Absorbance of control at t} = 0)] \times 100$$

2.9. Bile acid binding capacity

The bile acid-binding capacity of gums was determined according to the method of Doubilet (1936). 25 mg of gum sample was added to warm (37 ^{0}C) cholic acid solution with constant stirring for 2 h and was filtered through a 0.2-μm syringe filter. From the filtrate obtained, 1 mL was mixed with 1 mL of alcoholic furfural solution and 5 mL of 16 N sulphuric acid. The contents were placed in ice bath (5 min), then in hot water bath at 70 ^{0}C (8 min) and again in ice bath (2 min). Absorbance was recorded at 490 nm and bile acid binding capacity was calculated by the formula given below: