Food Chemistry 173 (2015) 920–926

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antinutritional factors and functionality of protein-rich fractions of industrial guar meal as affected by heat processing

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ARTICLE INFO

Article history:
Received 3 May 2014
Received in revised form 29 September 2014
Accepted 14 October 2014
Available online 28 October 2014

Keywords:
Antinutritional factors
Trypsin inhibitor
Heat treatments
Colour measurement
FTIR spectra
Industrial guar meal

ABSTRACT

Proximate composition analysis and antinutritional factor composition of different fractions of industrial guar meal: raw churi (IRC), heated churi (IHC), final churi (IFC) and guar korma (IGK) were studied and compared. Protein content was found to be very high in IGK (52.7%) when compared to the churi fractions (32–33%) and the trypsin inhibitor activities were found to be negligible in all the fractions (0.58–1.8 mg/g). Single fraction (IGK) was selected for further studies, based on the protein content. The antinutritional factors of selected fractions were significantly reduced by different heat treatments. Heat treatments significantly increased the water absorbing capacity of IGK, but reduced the nitrogen solubility, emulsifying and foaming capacity. Highest L^* value was observed for boiled IGK, highest a^* and b^* values for roasted IGK, during colour measurement. FTIR spectral analysis revealed the presence several aromatic groups in IGK and slight modifications in the molecular structure during heat treatments.

1. Introduction

Guar seed is a oval-to-round-shaped dicotyledonous seed with light pale to brown colour (Vishwakarma, Shivhare, & Nanda, 2012), commonly known as cluster bean. It is an industrially important legume, as guar gum of high export value is extracted from it. India is the largest producer of guar bean in the world, which accounts almost 80% of the total production. India exported around 4 lakh metric tons of guar products in 2012–2013 (APEDA). Guar seed has three parts: the seed coat (14–17%), the endosperm (35–42%), and the germ (43–47%). Endosperm is the part from where the industrially important guar gum is extracted, which forms a viscous gel in cold water and is a polymer of β-galactomannan. Guar gum extraction results in the production of protein-rich byproducts, Churi and Korma (guar meal), which are the germ and hull portions of the seed (Sharma & Gummagolmath, 2012). Guar gum is used in pharmaceuticals, as well as in oil well drilling, paper making and even explosives (Shahbazi, 2012). It has found applications in the food Industry also, where it is used to produce emulsifiers, food additives, food thickeners and gelling agent (Sharma & Gummagolmath, 2012; Vishwakarma et al., 2012).

Even though guar meal is rich in protein, it is not used as a feed ingredient, even in poultry diets, because of the presence of several antinutritional factors, e.g. trypsin inhibitor, saponins, polyphenols and β-galactomannan gum residue (Hassan, Al-Yousef, & Bailey, 2013). Several researchers were of the opinion that trypsin inhibitor is the primary antinutritional factor that limits the use of guar meal in feed (Couch, Bakshi, Ferguson, Smith, & Creger, 1967; Lee, Bailey, & Cartwright, 2003) but, according to the recent reports of Lee et al., 2003, guar meal contains negligible amounts of trypsin inhibitor, and can be used as an effective complement to soybean meal in poultry feed as it is protein-rich (Lee et al., 2003). β-Galactomannan gum residue acts as a growth depressing agent in poultry, but this effect can be overcome by the inclusion of certain enzymes, such as pectinase and cellulase as they are capable of hydrolysing the galactomannan gum (Gheisari, Shavakhi Zavareh, Toghyani, Bahadoran, & Toghyani, 2011). Saponin and polyphenols also can be possible antinutritional factors in guar meal. Saponin content in the feed is found to decrease the palatability of the feed, and to inhibit the feed intake (Hassan et al., 2013). They can increase the permeability of intestinal villi, which in turn results in the increased absorption of nutrients which are not normally absorbed (Francis, Kerem, Makkar, & Becker, 2002).

Several feeding trials in poultry have been conducted with the dietary inclusion of guar meal, with and without the inclusion of hydrolysing enzymes to increase the dietary inclusion of guar meal as a high protein supplement. Inclusion of a guar germ fraction (at a 7.5% level) in broiler diet does not significantly affect the feed

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http://dx.doi.org/10.1016/j.foodchem.2014.10.071
0308-8146/

conversion (Lee et al., 2003). Addition of β-mannanase to a germ fraction increased its upper feeding level from 2.5% to 5% for broiler chickens (Lee, Connor-Appleton, Bailey, & Cartwright, 2005). Inclusion of guar meal in the broiler diets, based on the incremental programme, is also found to increase the feeding level (Gheisari et al., 2011).

The current work is carried out to ameliorate the levels of antinutritional factors present in guar meal by some conventional heat treatments so as to increase the use of guar meal as a protein-rich dietary source in poultry. The functional properties that are crucial for any substance to be used as a food ingredient, before and after treatments, are also considered.

2. Materials and methods

2.1. Chemicals and reagents

BAPNA (benzoyl-DL-arginine-p-nitroanilide) and trypsin were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Sample

Four industrial guar meal fractions were supplied by Jai Bharat Gum & Chemicals Pvt. Ltd. Haryana, India. The fractions are guar raw churi (germ portion of the guar seed), guar heating churi (heated germ fraction during gum extraction process), guar final churi (germ portion after gum extraction process), and guar korma (combination of germ and hull).

2.3. Proximate composition analysis

Moisture, ash, fat and crude fibre content of the different industrial guar meal fractions were determined by the standard methods of the Association of Official Analytical Chemists (2005). Protein content of the samples were determined by the automatic Kjeldahl method, using the Kjeldahl digester and distillation unit (Gerhardt, Vapodest 30S, Germany). The carbohydrate content is expressed as the weight difference, using moisture, ash, crude fibre, fat and crude protein content data. All the analyses were done in triplicate and the final values are expressed as their means.

2.4. Antinutritional factors

2.4.1. Trypsin inhibitory activity

Trypsin inhibitor activity was determined, following the methodology described by Smith, Van Megen, Twaalfhoven, and Hitchcock (1980), using BAPNA (benzoyl-DL-arginine-p-nitroanilide) as substrate (0.92 mM in 0.05 M Tris buffer/0.02 M $CaCl_2$, pH 8.2). One gramme of finely ground sample was extracted in 50 ml of 0.01 N NaOH for 3 h; the pH was maintained within the range 8.5 to 9.0. One ml of the extract and 2 ml of trypsin solution 0.002% (Type I, of bovine pancreas, SIGMA) in 0.001 M HCl were mixed with 1 ml of water; the reaction began when adding 5 ml of substrate at 37 °C. After 10 min, the reaction was stopped by the addition of 1 ml of 30% acetic acid. The reaction mixture was filtered through filter paper (Whatman No. 3) and the absorbance read at 410 nm. The activity was interpreted as the increment of 0.01 units of absorbance at 410 nm for 10 ml of reaction mixture. Trypsin inhibitor activity is expressed in terms of mg trypsin inhibited per g of dry sample.

2.4.2. Phytate

The samples were extracted with 2.4% HCl. To 3 ml of extract, one ml of Wade's reagent was added, and the mixture was centrifuged at 6000 rpm for 10 min. The absorbance was measured at 500 nm. The phytate concentration was calculated from the difference between the absorbance of the control and the sample (Vaintraub & Lapteva, 1988).

2.4.3. Tannin

One gramme of seed meal was boiled with 80 ml of water for 30 min, then filtered and made up to 100 ml. Portions of sample (10–50 μg) were taken and 0.5 ml of 50% Folin–Ciocalteu reagent and 1 ml of saturated sodium carbonate were added. The reactants were mixed well and incubated for 30 min at ambient temperature. The colour developed was measured at 760 nm against a reagent blank. The concentration was determined against a standard calibration curve for tannic acid, and expressed as tannic acid equivalents (Ranganna, 1986).

2.4.4. Saponin

One gramme of sample was treated with methanol to extract the saponin from the sample. Quantification of total saponin was carried out according to the method of Hiai, Oura, and Nakajima (1976). To 250 μl of saponin solution, an equivalent amount of methanolic vanillin was added. Sulphuric acid was added to the resulting solution and kept at 60 °C. After 10 min of incubation it was rapidly cooled by keeping in an ice bath. Absorbance was measured at 540 nm against a reagent blank. Saponin concentration was expressed as diosgenin equivalents.

2.4.5. Hemagglutination assay

Hemagglutination assay was carried out according to the method of Lis and Sharon (1972) with some modifications. Rabbit blood was collected in Alsevere's solution and centrifuged at 3000 rpm for 5 min and the blood cells were collected. The collected cells were washed 3 times, using 0.9% saline, by centrifugation under the above conditions. The cells were suspended at 4% (v/v) in 10 mM phosphate buffered 0.9% saline, pH 7.4 (PBS). 1 volume of 1% trypsin was added to this and incubated at 37 °C for 1.0 h. The red blood cells were washed four times with 0.9% saline, and the cells were resuspended in 0.9% saline at 2% concentration (v/v). Protein samples were serially diluted in a microtitre plate, using 0.9% saline, and incubated at 37 °C for 1.0 h. Then, 0.1 ml of trypsinized red blood cells were added to each well and incubated again at 37 °C for 1.0 h for hemagglutination to occur. The plates were then tilted about 45 degrees to check the agglutination. The samples with red blood cells moved in a tear drop fashion were considered negative. Soy lectin was used as a positive control for the experiment.

2.5. Processing

2.5.1. Autoclaving

50 g of the sample were autoclaved at a temperature 121 °C for 15 min. After cooling, the sample was dried in an oven at 40 °C.

2.5.2. Boiling

50 g of sample were boiled in 500 ml of tap water in a beaker for 30 min, maintaining the volume of water constant. After cooling, the water was drained from the beaker. The content in the beaker was washed with tap water two times, so as to remove its bitter smell. The sample was then dried in an oven at 40 °C.

2.5.3. Roasting

The samples were roasted at 150 °C for 15 min, and cooled in desiccators.