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Determination of crude saponin and total flavonoids content in guar meal

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ABSTRACT

Saponins and flavonoids have received considerable attention due to their beneficial effects on animal and human health. In the present study, the crude saponin was extracted from guar [*Cyamopsis tetragonoloba* (L.) Taub] meal and the total flavonoid content in guar meal was determined by two different spectroscopic methods; oxidation of flavonoids by ceric sulfate [Ce (IV)] and complexed by aluminum chloride using quercetin as flavonoid standard. Results obtained from this study showed that the average concentration of crude saponin (saponin rich guar meal extract) was 6.2 ± 0.7% dry matter of guar meal. However, the average total flavonoid contents determined in guar meal were 0.28 ± 0.01 mg and 0.21 ± 0.01 mg/g for Ce (IV) method and aluminum chloride method, respectively. Results obtained in this study can be useful to nutraceutical, pharmaceutical utilization and can provide additional information about crude saponin and flavonoid concentration in guar meal especially for populations consuming guar bean.

Keywords: Aluminum chloride, ceric sulfate [Ce (IV)], flavoinoid, guar meal, quersetin, saponin.

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INTRODUCTION

Legume saponin and flavonoids are plant secondary metabolites widely distributed in the plant kingdom (Basu and Rastogi, 1967; Curl et al., 1986; Fenwick et al., 1991; Croteau et al., 2000, Hassan et al., 2010) among them guar. Determining the crude saponin and the total flavonoids content in legume plants is attracting attention for human and animal nutritionists worldwide due to their beneficial effects on human and animal health.

Saponins are among several plant compounds which have beneficial effects. Among the various biological effects of saponins are antibacterial and antiprotozoal (Mahato et al., 1982; Sen et al., 1998, Avato et al., 2006) and anticancer (Ma et al., 2007) activities.

Flavonoids have many beneficial effects on human and animal health such as anti-aging (Hadnick, *et al*., 1998), antioxidant (Croteau et al., 2000; Wildman, 2001; Winkel-Shirley, 2001, 2002), antibacterial and antifungal activities (Croteau et al., 2000; Wildman, 2001; Winkel-Shirley, 2001, 2002; Hassan et al., 2010), anticancer, anti-cardiovascular disease and anti-inflammatory (Hadnick, et al., 1998; Middleton et al., 2000; Nijveldt et al., 2001).

Guar, *Cyamopsis tetragonoloba* L. (syn. *C. psoraloides*) or cluster bean, is a drought-tolerant summer annual legume native to India and Pakistan (Rahman and Shafivr, 1967; Patel and McGinnis, 1985). Guar contains many important nutrients and phytochemicals such as saponin and flavonoids and is well-known traditional plant used in folklore medicine (Mukhtar *et al*., 2006). Many researchers have shown the relationship between legume consumption and health benefits, such as protection from cardiovascular disease, breast cancer, colon cancer, other cancers and diabetes (Kushi et al., 1999; Mathers, 2002; Messina, 1999), anti-inflammatory (Khare, 2004), arthritis (Katewa et al., 2004), anti-oxidant and laxatives effect. More than 6000 flavonoids have been identified in plants (Harborne and Williams, 2000). Although guar seeds have been used in pharmaceuticals, nutraceuticals and industrials, the composition and content of flavonoids in guar meal are

unknown (Morris, 2004). To better understand the association of flavonoid intake and health outcomes, estimation of the flavonoids content in food plants, an intense area of research is required (Dauchet et al., 2006; Chun et al., 2007).

Guar meal is a by-product produced by isolating the guar gum from guar bean containing saponin (Curl et al., 1986; Hassan et al., 2010), 33 to 47.5% crude protein (Bakshi, 1966; Ambegaokar et al., 1969) and can be used as a feed ingredient in animal and poultry nutrition (Lee et al., 2003a, b, 2005; Gutierrez et al., 2007; Hassan et al., 2008).

The developed indirect spectrophotometric method is based on the redox reaction between flavonoids and Ce (IV) at room temperature. This method is based on the oxidation of flavonoid with Ce (IV). Ce (IV) is a non-fluorescent oxidizing agent. Quercetin was used to construct the calibration curve. Ce (IV) is a strong oxidizing agent capable of reacting quantitatively with flavonoids. Chang et al. (2002) reported that the total flavonoid content determined by aluminum chloride method may represent the real content of total flavonoids. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids (Duke, 2002).

To date, few researches have conducted to evaluate the crude saponin and the total flavonoid contents in guar meal. Although various studies have been reported on soybean, little research has been conducted on other legumes such as guar meal. Therefore, this study was conducted to determine the concentration of the crude saponin in guar meal and determine the total flavonoid in guar meal by using two different spectroscopic methods; the oxidation of flavonoid by Ce (IV) method and complexed with aluminum chloride method.

MATERIALS AND METHODS

Chemicals and raw materials

Commercial fine powdered guar meal was purchased from Rama Industries, Manufacturer and Exporter of Guar Gum Splits and Powder, The Government Recognized Export House, Gujarat, India and stored at room temperature until analysis. For total flavonoid determination, a standard curve of quercetin was established using reagents. Quercetin, aluminum chloride and ceric sulfate [Ce (IV)] were purchased from Sigma Aldrich Chemical Co. (St., Louis, MO, USA).

Isolation of Saponin-Rich Guar Extract

A total of 5 samples from guar meal were used in this study. Guar meal was extracted by refluxing 25 g of guar meal with 250 ml of ethanol: water, 1:1 (v: v) for 3 h in a simple reflux apparatus. Refluxed extracts were cooled and filtered through 150 µm (Watman No. 2) then 125 µm pore size (Watman No. 114) filter papers. Ethanol was removed from the filtered extract by evaporating under reduced pressure in a roto-evaporator (Buchi, Rinco Instrument Co., Inc., Greenville, IL, Switzerland, model 310391) until two-thirds of the initial volume was removed. The remaining aqueous extract was partitioned with n-butanol, 1:1 (v: v) overnight at room temperature using a separatory funnel. The upper n-butanol extract was collected in a glass flask and the lower aqueous extract was collected and further partitioned with n-butanol two more times to increase the yield of crude saponin extract. The butanol extracts were pooled and evaporated to remove the butanol or dryness under vacuum at temperature below 50°C using the roto-evaporator procedure described above. A minimum volume of distilled water was added to the dry n-butanol extract and the resulting material was freeze-dried and weighed.

Extraction of flavonoids from guar meal

A total of 5 samples from guar meal were used in this study. About 1 ± 0.0001 g of dried powder of guar meal was first dissolved or extracted with 10 ml of ethanol (80%). After centrifugation at 1,000 × g for 10 min, the supernatant was collected and the precipitate was then extracted again with 5 ml of ethanol (80%) twice. Finally, the supernatant was combined with previous supernatant and adjusted to 25 ml with ethanol (80%) and stored in an amber bottle.

Preparation of quercetin standard solution

For total flavonoid determination, a standard calibration curve of quercetin was applied using quercetin. Stock quercetin solution was prepared by dissolving 100 mg quercetin in 100 ml ethanol, then the standard solutions of quercetin was prepared by serial dilution using ethanol.

Determining total flavonoid content

Ceric sulfate [Ce (IV)] method

A total of 5 samples from guar meal were used in this study. Different serial quercetin concentration solutions ranged from 1.5 to 7.5 µg/ml were prepared in 10 ml volumetric flasks. For determining the total flavonoid content in the ethanol extract, 1 ml of ethanol extract was mixed with 0.3 ml of 0.025 M Ce (IV) solution and the mixture was diluted to 10 ml with distilled water. After mixing or shaking for a few minutes, the solution was allowed to stand for 15 min at room temperature. The absorbance of the solution due to formed Ce(III) (as a result of Ce (IV) oxidation of flavonoids) was measured at 320 nm wavelength using distilled water as blank using UV-visible spectrophotometer (Shimadzu, 1700) with UV-probe software connected to computer. The absorption of Ce (IV) in absence or presence flavonoid reacted solutions was recorded. The difference absorption due to Ce (IV) between the blank (containing the same amount of Ce (IV)) and flavonoid reacted solutions was recorded.

Aluminum chloride colorimetric method

A total of 5 samples from guar meal were used in this study. The total flavonoid content was determined using aluminum chloride colorimetric method according to Nabavi et al. (2008). Quercetin was used to make the standard calibration curve. The standard calibration curve was prepared by dissolving 10 mg of quercetin in 80% ethanol and then diluted to 25, 50 and 100 mg/ml. The diluted standard quercetin solutions (0.5 ml) were separately mixed with

1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After mixing, the solution was incubated for 30 min at room temperature. In preliminary experiments, the wavelength scans of the complexes of 15 standards with aluminum chloride showed that the complexes formed by flavonols with extra ortho-dihydroxyl groups, such as quercetin had maximum absorbance at 415 to 440 nm. In compromise, therefore, the wavelength 415 nm was chosen for absorbance measurement. Generally, the aluminum chloride complexes of compounds with more functional groups were absorbed stronger at 415 nm and showed the absorption maximum at longer wavelength. The absorbance of the reaction mixtures were measured at 415 nm wavelength with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of ethanol extracts or flavonoid standard solutions were reacted with aluminum chloride for determination of flavonoid content as described above.

RESULTS AND DISCUSSION

In the present study, the crude saponin was extracted and the concentration of crude saponin was found to be about 6.2 ± 0.7% dry matter of guar meal. Previous studies reported that the concentration of crude saponin in the guar meal was 4.8 ± 0.6% (Hassan et al., 2010) and 13% dry matter guar meal (Curl et al., 1986).

Several studies reported that saponin concentrations are affected by plant species and plant variety (Shiraiwa et al., 1991a, b), degree of maturity, growing environment, agronomic factors such as climate and soil, cultivation year, location grown, season (Oleszek, 1996), and extraction method (Tschesche et al., 1969; Fenwick and Oakenfull, 1983; Onning et al., 1993).

Saponins frequently are isolated by boiling in methanol (Massiot et al., 1991; Oleszek et al., 1992), ethanol (Levy et al., 1989; Oleszek et al., 1990) and n-butanol (Massiot et al., 1991, 1992). In this experiment, guar meal was extracted by ethanol: distilled water (1:1, v: v) and the resulted extract was partionated with n-butanol (1:1, v: v) to elute crude guar saponin. Khalil and El-Adawy (1994) extracted saponins from peas, beans and soybean seeds by refluxing seed samples using four different methods. They extracted with ethanol: distilled water (1:1) for 2.5 h in a water bath at 95°C, pure methanol in a Soxhlet apparatus for 50 h, distilled water for 5 h in a boiling water bath, and phosphate buffered saline at pH 7.3 while shaking for 2 h. Extraction with n-butanol efficiently isolates monodesmosidic and short-sugar-chain bisdesmosidic saponins, but results in partial or total loss of long-chain bisdesmosidic and trisdesmoside saponins (Oleszek, 1996).

Our results showed that flavonoid contents of five guar meal samples determined by aluminum chloride colorimetric method were generally lower than those determined by Ce (IV) colorimetric method. The average total flavonoid contents determined in the guar meal were 0.28 ± 0.01 mg and 0.21 ± 0.01 mg quersetin/g for Ce (IV) method and aluminum chloride method, respectively. Determining the total flavonoids by using aluminum chloride method is based upon the formation of stable complex between aluminum chloride and keto and hydroxyl groups of flavones and flavonoids. However, using Ce (IV) method is based on the oxidation reaction between Ce (IV) and flavonoids.

In the present study, the average of total flavonoid content in the guar meal as quercetin, the major type of flavonoid reported, was lower than those reported in the other legume seeds such soybean. Wang and Morris (2007) observed that the average of the total flavonoid content in soybean seeds was 0.450 mg/100 g. Kobeasy et al. (2011) reported that total flavonoid content was 0.83 ± 0.01 mg quercetin/g guar bean. However, Kaushal and Bhatia (2006) found that guar beans contain a range of 0.13 to 0.23% total flavonoids. No significant differences in the concentration of total flavonoids between guar and soybean seeds (16.975 mg/100 g in soybean versus 17.034 mg/100 g in guar) were detected (Wang and Morris, 2007).

Some research found extensive variability in flavonoid content among seeds of 36 guar accessions (Wang and Morris, 2007). Yang et al. (2008) reported that the total flavonoid content was ranged from 0 to 254 mg/100 g fresh weight of edible plants species. They found that about 75% of samples were found to contain flavonoids more than 0.5 mg/100 g with the mean average of 33 ± 48 mg/100 g. Prati et al. (2007) determined that total flavonoid content and composition in forage and grain legume crops and they found that excluding soybean, only 7 out of 77 grain legume accessions exhibited a total flavonoid content higher than 0.1 mg/g fresh seed weight with mean average content of 0.33 ± 0.31 mg/g fresh seed weight, while in 38 out of 47 forage legume, the mean total flavonoid content was 1.49 ± 0.89 mg/g fresh seed weight.

Conclusion

Results obtained indicated that guar meal not only contains an amount of crude saponin but also contain an amount of total flavonoids. We suggest that both methods used in this study can be conducted to determine the total flavonoids content in guar meal. This is results might be very important for nutraceutical and pharmaceutical application especially for the population that consume diets containing guar meal. Finally, results obtained in this study can provide additional information about the total flavonoid content of guar meal.

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