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Rapid and mass screening method for galactomannan content in fenugreek seeds

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Abstract

Present study describe a method of quantitative analysis of galactomannan content in fenugreek whole seed. This method is determined by the criteria of simplicity, accuracy, low cost and ability to be applied to a large number of samples. This method is based on phenol-sulphuric method of total carbohydrate analysis and a rapid method for determination of gum in guar.

Key words : fenugreek phenol-sulphuric method, galactomannan, gum

Fenugreek (*Trigonella foenum-graecum* L.) is a medicinal plant with potential applications in the natural health product industry. Fenugreek has also been used for over two thousand years as a medicinal plant in various parts of the world (Srinivasan, 10) and may be regarded as the oldest medicinal plant in human history. Fenugreek, perhaps, is best known for presence of the distinctive, pungent aromatic compounds in the seed (Max, 8) that impart flavour, colour and aroma to foods, making it a highly desirable supplement for use in culinary applications.

Galactomannan (mucilage or gum) in fenugreek acts as a thickener or stabilizer in foods such as soups, sauces and ice-cream (Seghal *et al.,* 9). Currently, the food industry utilizes locust bean gum and guar gum as emulsifiers, viscosity-builders, thickeners and stabilizers. Galactomannan represents the major polysaccharide found in fenugreek seeds and accounts for approximately 17 – 30 % of the dry seed weight (Kochhar *et al.,* 6). It is an integral component of the cell wall which is found concentrated around the seed coat. Galactomannans are structurally composed of a 1→4 beta-D-mannosyl backbone substituted by a single galactose unit α-linked at the C-6 oxygen (Bhaumick, 1). Fenugreek galactomannans are unique relative to other commonly used galactomannans such as those found in guar and locust beans. They contain a galactose to mannose ratio of 1:1. This high degree of galactose substitution renders the molecule relatively more soluble compared to galactomannans from guar or locust bean, which has a galactose to mannose ratio of 1:2 and 1:4, respectively (Brummer *et al.,* 2). The soluble nature of galactomannan fiber from fenugreek has been linked to numerous human health benefits, mainly in the reduction of plasma glucose levels which has an antidiabetic effect (Madar and Shomer, 7). Hannan *et al*., (5) also have demonstrated that the soluble dietary fiber (SDF) portion of fenugreek can significantly improve glucose homeostasis in type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption. They have also suggested that the SDF fraction may enhance insulin action in type 2 diabetes as indicated by the improvement of oral glucose tolerance in these test subjects. Presence of galactomannan in fenugreek seed is recognized as the principal source of soluble dietary fiber in the plant. Dietary fiber is known to have the potential to reduce risk of cardiovascular disease and to protect against some cancers through the reduction of low-density lipoprotein (LDL) and total cholesterol.

The choice of methods to provide analyses is determined by the criteria of simplicity, accuracy, low cost and ability to be applied to a large number of samples. In addition, it was thought important to choose or design methods that could be used manually for small as well as large samples (25-100). This communication describes techniques that have been adopted for the routine analysis of galactomannan to determine galactomannan content in nineteen fenugreek genotypes.

Different genotypes of fenugreek were procured from seed bank of NRCSS, Ajmer. A modified method was used for estimation of galactomannan in fenugreek seeds (Das *et al.,* 3). 100 mg of fenugrek powder was taken in a conical flask, add 40 ml of 0.1 ml $HgCl_2$ solution (0.1 M), autoclave for one hour at 15 PSI, cool and make up to 100 ml with 0.01 M $HgCl_2$ solution. Entire solution was centrifuge for 5 minutes at 5000 rpm. One millilter of the supernatant was taken and 2.0 ml of 2 % phenol was added. Five millilitre of concentrate sulphuric acid (G.R.)

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was added in this solution and kept for one hour to cool the solution. Absorbance was then recorded at *490 nm* from UV-Vis Spectrophotometer (Lab India).

Standard curve was prepared by taking 10.0 mg mannose and 10.0 mg galactose in a volumetric flask and total volume made to 100 ml with distilled water. This was used as a stock solution which was equivalent to 200 ppm of galactomannan. Ten milliliter of this solution was diluted to 100 ml to give a working standard solution 30 ppm or 30 mg ml^{-1}. Different concentration (12, 18, 24, 30, 42, 48, 54 and 60 ppm) of standards were taken. Galactomannan content expressed in percent.

Total carbohydrate: Total carbohydrate was analysed with modified method described by Dubois *et al.*, (4). Initial step for hydrolysing of polysaccharides was done by method described as in galactomannan method.

As result indicated in table-1 shows that the galactomannan ranging between 25.50 to 28.19 percent in fenugreek seeds of different genotypes. Maximum galactomannan observed in LAM Selection (28.21%) followed by Hisar mukta (28.19 %). While, minimum galactomannan was observed in CO-1 (25.50 %) followed by RMt-1 (26.13%).

Table-1: Total galactomannan and carbohydrate content in different genotypes of fenugreek seeds.

Genotype	Galactomannan (Soluble fiber) (%)	Total carbohydrate (%)
CO-1	25.50	53.50
AFg-4	27.45	57.90
Raj. Kranti	26.36	56.78
RMT-1	26.13	54.23
AM-2	27.44	57.34
Hisar Madhvi	26.75	56.42
AFg-3	27.86	54.33
GM-2	26.27	53.12
Azad Methi	28.18	56.23
RMt-305	27.26	57.01
Hisar Mukta	28.19	58.26
RMt-303	27.50	58.12
Pant Ragini	26.40	55.12
LAM Selection	28.21	58.21
RMT- 143	27.25	57.00
Hisar Sugandha	27.26	56.66
Hisar sonali	26.71	58.01
AM- 1	26.42	58.54
RMT 351	27.90	58.12
SD±	0.7900	1.718

Total carbohydrate content was observed 58.55 % in AM-1 followed by Hisar Mukta (58.26%). Total carbohydrates represents soluble fibre, insoluble fibres and rest of all carbohydrates and sugars.

Generally galactomannan analysis done by chromatographic methods. These method are more sensitive in nature as compare to colorimetric method. But HPLC method is more expensive and takes more time than colorimetric methods as well as it required a more skills. The results in present study are in lieu of results obtained by previous workers (Kochhar *et al.,* 6). The present colorimetric method is also suitable for mass screening of galactomannan in fenugreek. In present method phenol sulphuric method of total carbohydrate analysis is slightly modified with in view of the ability of fenugreek galactomannan having galactose to mannose ratio of 1:1, Hence, the standard curve prepare with the galactose and mannose sugar with the ratio of 1:1 (Burmer, 2). This method for determination of galactomannan content in fenugreek is simple, accuracy, low cost and ability to be applied to a large number of samples.

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