

Original Article

STUDIES ON PREBIOTIC FOOD ADDITIVE (INULIN) IN INDIAN DIETARY FIBRE SOURCES -
GARLIC (*ALLIUM SATIVUM*), WHEAT (*TRITICUM SPP.*), OAT (*AVENA SATIVA*) AND DALIA
(BULGUR)

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ABSTRACT

Objective: In the present investigation inulin has been extracted from dietary fibre rich Indian food stuffs, namely, garlic, wheat, oat and dalia. Inulin in the raw food stuff and in the extract has been assessed qualitatively and quantitatively.

Methods: Inulin has been extracted from each food source using a combination of lab-scale chemical processes and unit operations. Qualitative assessment of inulin in different food samples and their extracts has been done using FTIR and TLC and quantitative assessment has been done using high performance liquid chromatography (HPLC) and also through combination of TLC and spectro-photometry.

Results: The concentration (on dry weight basis) of inulin in natural prebiotic sources has been determined to be 16.60%, 13.07%, 8.94%, 14.95% for garlic, wheat, oat and dalia respectively. The extraction of inulin from garlic, wheat, oat and dalia was possible up to the extent of 99.46%, 77.94%, 53.31% and 89.15% respectively.

Conclusion: It may be concluded that all the food samples, investigated under the study, may serve as potential sources for extraction of prebiotic inulin. The present extraction procedure may be escalated to commercial scale for the production of inulin particularly from garlic for which the efficiency is as high as 99.46%.

Keywords: Dietary fibre, Prebiotic, Inulin, FTIR, TLC, HPLC.

INTRODUCTION

Studies on prebiotics and probiotics are recently attracting the attention of scientists and technologists due to immense potential of these two closely related bio-systems. Prebiotics are basically any non-digestible food ingredients that can benefit the human body by stimulating the growth and activity of one or more species of probiotics in the large intestine and produce short chain fatty acids (SCFAs) resulting in a decrease of the colon pH [1]. Prebiotics may be added to food preparations containing probiotics to enhance the stability of food matrix and viability of probiotic cultures [2].

Prebiotics are mostly oligosaccharides in chemical nature. Full-spectrum prebiotics, e. g., oligofructose-enriched inulin (OEI) provide the full range of molecular link lengths from 2-64 links per molecule and nourish bacteria throughout the colon. Inulin has been defined as a polydisperse carbohydrate material consisting mainly, if not exclusively, of β -(2-1) fructosyl-fructose links [3]. A few reports are available, showing the positive effect of inulin on the growth of probiotic organism [4, 5, 2]. Huebner, 2007 reported that inulin enhanced the growth of *L. Casei* [6]. In addition to the inclusion of inulin in yogurt, it is also used as a substitute of fat in low fat emulsions like salad dressings etc [7].

For the fortification of food products with prebiotic, like inulin, the possibility of extraction of the latter from all available natural food grade resources should be investigated. Inulin producing plant species are found in several monocotyledonous and dicotyledonous families, including Liliaceae, Amaryllidaceae, Gramineae, and Compositae [8]. Usually, food stuffs, rich in dietary fibre, serve as prebiotic sources.

In spite of several resources of inulin, only one inulin containing plant species namely Chicory (*Chichorium intybus*) has been used to produce inulin commercially so far, possibly because of technical snags for exploring other resources. It is now established that inulin is naturally available in several fruits and vegetables. Among them garlic, onion, wheat, oat, dalia, banana etc. are some of the common

Indian vegetables and fruits which have been reported to be rich in inulin by earlier researchers [9, 10]. It may be noted that no systematic and programmed investigation is available on qualitative and quantitative assessment of inulin in these Indian vegetable stuffs. Although a few scattered information have been reported in the literature review articles [11, 12] on the content of inulin in several Indian vegetables but these are only on piece meal basis and are not suitable for commercial exploitation.

Considering the importance of isolation and purification of inulin from its native source, in the present investigation an attempt has been made to isolate and characterise inulin quantitatively and qualitatively from four candidate materials namely- garlic, oat, wheat, and dalia. Qualitative identification of inulin has been done using Fourier transformed infrared (FTIR) while quantitative determination has been carried out using thin layer chromatography (TLC) followed by spectrophotometry and high performance liquid chromatography (HPLC).

A pure food grade sample of inulin has been used as standard for the purpose of comparison. This investigation presents a programmed study on isolation and characterisation of inulin from four abundantly available food materials.

MATERIALS AND METHODS

Materials

Garlic (*Allium sativum*), wheat (*Triticum spp*) oat (*Avena sativa*) and dalia (**Bulgur**) were purchased from local market. Moisture contents of all prebiotic sources are provided in Table 1. Food grade inulin purchased from Himedia, India, was used. TLC Silica gel G 60 Aluminium sheets 20 x 20 cm (Merck HX 816976, Germany), benzene (Ranbaxy, India), acetic acid (Merck, India), methanol (Merck, India), resorcinol (Merck, India), ethanol (Merck, India), sulphuric acid (Merck, India), HCL (Merck, India), HPLC water (Merck, Germany), Acetonitrile (Merck, Germany), Column: C-18 Phenomenex (250 x 4.6 mm x 5 μ) were used.

Analytical Instruments

Shimadzu FTIR Spectroscope 8400, Varian UV-visible spectro photometer Cary 50 Bio, Shimadzu Corporation Reverse Phase with RI detector HPLC (Model no: CBM-Ro A) were used.

Determination of Inulin in Natural Prebiotics

Inulin in different natural prebiotics was qualitatively and quantitatively determined using thin layer chromatographs (TLC).

TLC (Thin Layer Chromatography)

The standard Inulin solutions of different concentrations (5g/L, 10g/L, and 20g/L) were hydrolysed using 5% oxalic acid and heated in boiling water bath for 30 minutes [13]. 1 mL of each inulin solution was hydrolysed with 4 ml oxalic acid. Filtrate obtained from suspensions of aqueous test solutions of raw natural prebiotics at concentration of 20g/L each were hydrolysed using the same protocol. Approximately 10 µl of each hydrolysed sample was applied to spot on the aluminium sheet (TLC Silica gel G 60 Aluminium sheets) by a micropipette (make: Biohit proline 0.5 to 10 µl) and was developed for ~ 3 h in the n-butanol: acetic acid: methanol solvent (4:2:2) [14] mixture in a closed chamber. The mobile phase (solvent) was at the bottom of the tank. The mobile phase gradually moved upward carrying the spotted samples. Once the solvent front moved ~ 2/3 of the sheet, the sheet was removed; the solvent front was marked immediately and dried with a portable dryer. The sheet was sprayed with spraying reagent which had been prepared by mixing 0.2% orcinol in sulphuric acid and was incubated in hot air oven for 15 minutes at 70°C. Dark brown spots appeared for different samples. R_f values (Relative Fronts of the solute and the solvent) were calculated using the following equation.

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Distance moved by solvent}} \dots \dots \dots (1)$$

Distance moved by solvent

From the TLC of each sample spot obtained at the same position as inulin, was scraped out and collected in a sample vial containing 10 mL distilled water each and OD values of supernatants were determined with the spectrophotometer at 540 nm, to quantify the concentration of inulin. Standard curve (not shown) generated using OD values of TLC spots corresponding to pure inulin solutions of different concentrations have been used to determine inulin concentration in the natural prebiotic extracts.

Extraction of Inulin

The prebiotic native sources, namely, garlic, wheat, oat, and dalia were washed properly with distilled water. In case of garlic, peeling of outer skin was done manually and the core portion was taken for the preparation of paste. 100 g garlic cloves were cut into small pieces and with addition of 150 mL distilled water was blended with Mixer-Grinder. The protocol for chicory reported by Mavumengwana was followed with some modification to extract inulin from garlic, wheat, dalia and oat. The flow sheet showing different operations in the protocol is shown in figure 1.

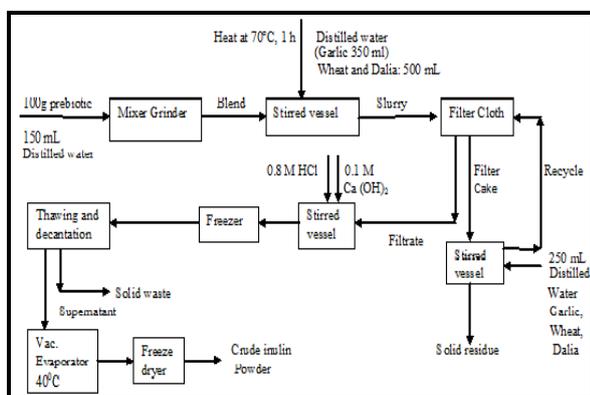


Fig. 1: Process of inulin extraction from natural prebiotic sources

According to the protocol, suspensions of the aqueous pastes of different samples were prepared. The volume of distilled water used for the preparation of suspension was 750 mL for all samples except wheat, for which 900 ml water was required. The suspension was heated up to 70°C and was subsequently screened. Heating up to 70°C had been recommended by previous workers [15, 16], to deactivate the inulinase, which might have otherwise led to the conversion of inulin to fructose. The solid cake from the filter cloth was resuspended several times in distilled water and was recycled to the filtration unit. Finally the filtrate was treated with calcium hydroxide to raise the pH up to 8.0. The pH was readjusted to 7.0 by using 0.8 M HCl. After the adjustment of pH, filtrate was frozen at -22°C for 3h and was thawed. The supernatant was evaporated under vacuum to evaporate water and to obtain crude inulin powder. The crude inulin powder obtained from garlic, wheat, dalia and oat were analysed quantitatively and qualitatively using HPLC. Quantity of inulin in the extracts of prebiotic samples was also determined using DNS method. Chemical bonds present in native inulin powder were assayed using FTIR spectroscopy.

Characterization of Extracted Inulin Powder

HPLC (High Performance Liquid Chromatography)

The extracted inulin powder derived from each natural prebiotic was analysed using HPLC to determine the concentration of inulin. Solutions (1g/ml) of standard inulin and extracted inulin powder of each natural prebiotic sample were prepared in distilled water. Similarly, solutions of pure samples were also made maintaining concentration of 0.2g/mL. The sample was subsequently freeze dried to obtain the solution of 1g/mL. 20 µl of each sample solution was injected through the column using water: acetonitrile (80:20) solvent system with a flow rate of 1 mL/min at column temperature of 25.8°C.

DNS (3-5 Di-nitro salicylic Acid method)

1g of natural inulin powder of each prebiotic sample was taken in 5 ml distilled water and 5 ml solution of 4:1 acetonitrile: water was added. This solution was allowed to precipitate. The solvent was decanted and after repeated washing with distilled water the final suspension of 4 mL was divided into two parts. One part was taken directly for fructose determination using DNS (3, 5- Di-nitro salicylic Acid) method and another part was hydrolysed with 5% oxalic acid following the same protocol used in case of TLC analysis was further analysed using DNS [17]. Since extracted inulin powder contents inherently associated sugar molecules, DNS analysis of extracted inulin powder will provide the concentration of sugars associated with inulin powder. On the other hand after hydrolysis of inulin when the sample is subjected to DNS analysis, concentration of sugar obtained from the breakdown of inulin structure will be achieved. The difference of the sugar content between the hydrolysed product and the parent inulin powder will be the fructose content in the inulin. The concentration of pure inulin in the extract inulin powder is obtained by the simple molar ratio relationship given by Mavumengwana.

$$[I] = 162 \times [F]/180 \dots \dots \dots (2)$$

Where [I] = concentration of inulin, g/L and [F] = concentration of fructose obtained from inulin, g/L.

FTIR (Fourier Transformed Infrared Spectroscopy) Analysis

In order to verify the presence of inulin in the four selected food materials, under study, FTIR analysis has been carried out to arrive at a conclusive decision. For all the test cases comparison has been done with that of the pure food grade inulin.

RESULTS AND DISCUSSIONS

TLC Analysis

Results obtained from thin layer chromatogram (Figure not shown) indicate the presence of inulin in each of the four candidate samples taken for study. From TLC experiment R_f values of inulin have been calculated. These values have been compared with the R_f value obtained for standard food grade inulin. Results are shown in Table 1.

Table 1: R_f values of prebiotic food samples and inulin

Samples	R _f Values
Inulin	0.8
Garlic	0.8
Wheat	0.9
Oat	0.92
Daliah	0.9

A quantitative approach has been made using TLC data to find out the content of inulin (weight % dry basis) in each sample. The results are shown in Table 2.

Table 2: Concentrations of inulin in different prebiotic

Natural prebiotics	Quantity of Inulin Extract (powders)(%)(w/w)
Garlic	16.6
Wheat	13.07
Oat	8.94
Dalia	14.95

Indian food materials

It is evident from the Table that garlic contains the maximum amount of inulin whereas oat contains the least amount of inulin. Such results are, however, expected. Garlic being a fibrous vegetable and containing high fructose should possess a high concentration of inulin. On the other hand, oat, a digestible cereal containing the least amount of fructose in it out of the four samples investigated, possesses the least quantity of inulin.

The OD values obtained by spectrophotometric analysis of aqueous solutions of coloured spots on TLC plates have been used for the calculation of the percentage of inulin present in the above-mentioned natural prebiotic materials. The concentration of inulin in each natural prebiotic source, calculated on a dry weight basis, has been provided in Table 2. It is evident that garlic contains the maximum concentration (16.6%) of inulin. The concentration of inulin in garlic is also determined to be 15.69% using HPLC analysis.

Estimation of Inulin in extract powder by DNS method

In order to find out the quantity of inulin content (weight % dry basis) in inulin extract powder obtained from different natural prebiotic samples, the DNS method has been applied. The results obtained from these experiments are shown in Table 3.

Table 3: Percentage of inulin content in the inulin extracts powder

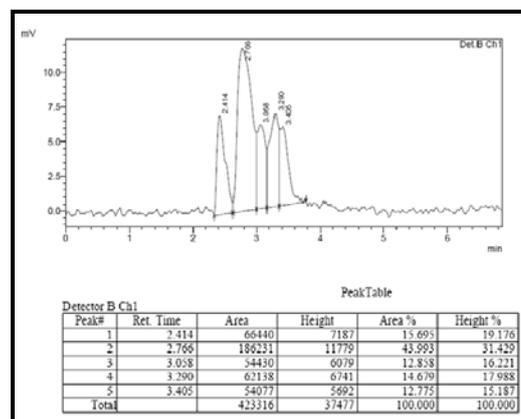
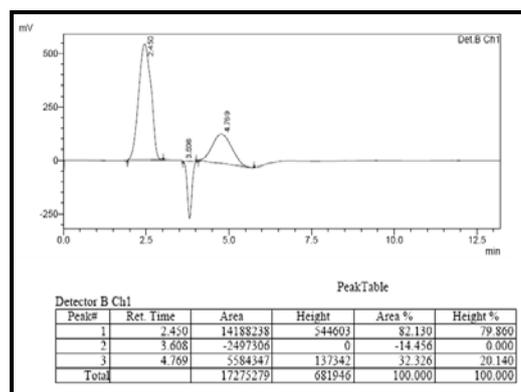
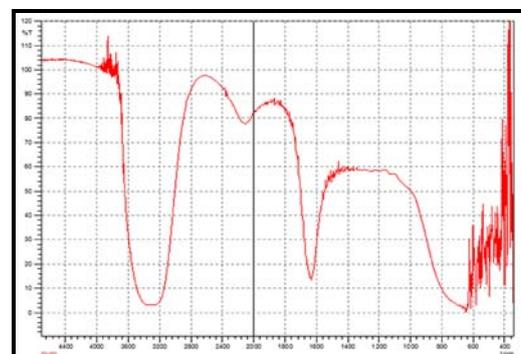
Natural prebiotic sample	Fructose content, g/L	Inulin content, g/L (from Equation 2)	Percentage inulin in extract powder
Garlic	276.3	248.67	99.46
Wheat	216.5	194.85	77.94
Oat	148.08	133.28	53.31
Dalia	247.64	222.88	89.15

From the table, it also appears that the purity of inulin obtained from garlic is the maximum compared with the other prebiotic samples. Since extracted inulin is a mixture of pure inulin and associated sugars, it may appear that the quantity of extracted inulin is greater than the quantity of pure inulin present in the native source. The difference in quantity of extracted inulin and inulin actually present is minimum in the case of garlic because of the very low content of other carbohydrates present in it. However, for other samples studied in the present investigation, an appreciable difference is noticed in this regard.

HPLC

In order to ascertain the presence of inulin in garlic, a qualitative experiment has been performed using HPLC technique. The chromatogram attached (Figure 2 and 3) for raw garlic sample and

the standard inulin powder (obtained from the market) clearly indicates the peak corresponding to the retention time (RT) 2.45 minutes for standard inulin and RT 2.414 minutes for the garlic sample. Evidently, this close resemblance of RT is a qualitative indication of the presence of inulin. The chromatogram data when processed shows a quantitative content of inulin of 15.7% by weight dry basis in the garlic sample, while for standard inulin powder, this value is 82.13%. Similar observations were made for other prebiotic samples, viz., wheat, oat, and dalia, used for the present investigation. It may be noted that the result obtained from HPLC is comparable to that from TLC.

**Fig. 2: HPLC chromatogram of garlic****Fig. 3: HPLC chromatogram of inulin****Fig. 4: FTIR chromatogram of inulin**

Qualitative analysis of prebiotic samples for their inulin content using FTIR

Although TLC, HPLC, and DNS analyses of the prebiotic samples used in the present investigation have confirmed the presence of inulin in

them, in order to take a conclusive decision FTIR analysis of the samples has been carried out. The FTIR chromatogram of the standard food grade inulin sample (used for comparison purpose) and four other samples are shown in figures 4 and 5 respectively.

By comparing above two figures the distributions of chemical bonds in the standard sample and the sample under study have been evaluated and the results are shown in Table 4.

On closure examination it reveals that in each of the candidate food materials inulin is present. A careful qualitative comparison also shows that chemical composition of garlic extract is almost similar to the standard inulin throughout the wavelength scanned (Table 4 and figure 5). This indicates that the garlic is enriched with inulin and is a potential source of the same. On the other hand chromatograms of wheat, oat and dalia have shown the similarity to the inulin less than that of garlic. A close comparison of chromatograms (figures 4 and 5 and table 4) evidently indicates that the centroid of the main maximum of pure inulin sample and the hydrolysed garlic sample fall on the same wavelength between 3240.41 to 3348.42 cm^{-1} . Therefore it may be stated that garlic is highly enriched in inulin. The centroid of the wheat lies higher than that in the spectrum of inulin about 40 cm^{-1} , whereas the centroid of

oat and dalia shift to about 31 cm^{-1} higher than that of inulin in each individual case (figures 4 and 5 and table 4). This may be due to the influence of different interfering ions present in these food stuffs [23]. The mass fractions of inulin in wheat, oat and dalia are also appreciable.

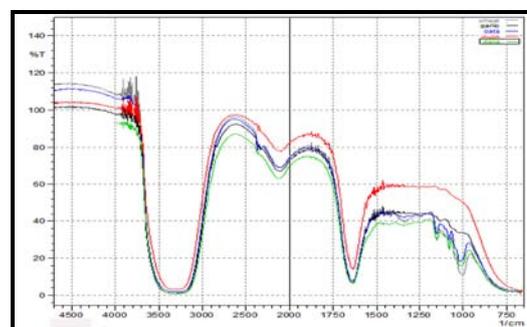


Fig. 5: FTIR chromatogram comparison between inulin, garlic, wheat, oat and dalia

Table 4: Wavelength numbers of prebiotic samples

FTIR wavelength numbers					Range of functional groups
Inulin	Garlic	Wheat	Oat	Dalia	
1633.71	1633.71	1633.71	1635.64	1633.71	(1620-1680) -C=C- Alkenes [18]
1965.46	1965.46	1963.53	1905.67	1869.02	(1638.84) C=O [19] (1908-1960) O-H stretch first overtone, C=O stretch second overtone, O-H stretch/O-H bend combination
1988.61	1988.61	1988.61	1940.39	1909.53	(1980) Asym N-H stretch/N-H in plane bend; C-N stretch combination [20]
2015.61	2015.61	2015.61	----	1944.25	
2038.76	2036.83	2038.76	----	1967.39	
2061.90	2061.90	2061.90	----	1990.54	
2086.98	2088.91	2086.98	----	2115.91	(2100-2260) -C≡C- stretch in alkynes, -C≡N- stretch in Nitrile, OH bond, C-O stretch [21]
2104.34	2100.48	2100.48	2104.34	----	
2351.23	2353.16	2358.94	2358.94	2358.94	
2389.80	----	2328.08	2330.01	----	
3240.41	3240.41	3278.99	3271.27	3271.27	(3200-3500) O-H [22]
3290.56	3290.56	---	---	---	

CONCLUSION

The present investigation on isolation of prebiotic from different natural sources reveals that the Indian food samples, namely garlic, wheat, oat and dalia contain the prebiotic inulin. The concentration of inulin in each food component has been determined using the combination of TLC supported by spectrophotometric methods and HPLC. The extraction of inulin was also performed for all prebiotic food samples under study. The inulin in extract was analysed qualitatively and quantitatively using HPLC and DNS methods. The purity of inulin extract is 99.46 % in case of garlic and this is the maximum value obtained from the samples studied in the present investigation. The powder was characterised regarding the distribution of chemical bonds using FTIR and was satisfactorily compared with standard inulin. The flow chart used for the purpose of extraction of inulin is a commercially viable process. The evidence of high concentration of inulin in these food stuffs also supports the daily intake of them for the maintenance of good health.

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