Optimization of extraction process of inulin from Indian millets (jowar, bajra and ragi)—characterization and cost analysis

Debolina Banerjee¹ · Ranjana Chowdhury¹ · Pinaki Bhattacharya²

Revised: 13 September 2017 / Accepted: 27 September 2017 / Published online: 10 October 2017
© Association of Food Scientists & Technologists (India) 2017

Abstract Prebiotic biomolecule, namely, inulin was extracted from Indian millets, namely, jowar (*Sorghum vulgare*), bajra (*Pennisetum glaucum*) and ragi (*Eleusine coracana*). Through qualitative assessment using Fourier Transform Infrared spectroscopy, the presence of functional groups of inulin in the above mentioned Indian millets were verified. The values of degree of polymerization of inulin derived from jowar, bajra and ragi were determined to be 27, 39 and 23 respectively. A comparative analysis of growth of *Lactobacillus casei* was carried out in presence of both lactose and inulin extracted from three millets and the commercial one. It was observed that the bajra inulin and lactose combination exhibited the best bacterial growth. The prebiotic effectiveness of different varieties of inulin was calculated to be in the following order: bajra > jowar > ragi > commercial inulin. Therefore the results on bajra inulin were highlighted in this article. Inulin yield from bajra was optimized as a function of temperature, HCl concentration and heating period. The maximum inulin yield (0.4727 g/g bajra) was obtained at temperature 70 °C, HCl concentration of 0.8 M and heating period of 60 min. The prebiotic activity score of bajra inulin (= 3.2) was measured to be much higher than commercial inulin (= 1.0). Growth dynamics

of *Lactobacillus casei* on lactose, bajra inulin and mixture of lactose and bajra inulin were found to be of Monod type, Haldane type and Multi-substrate-summative type respectively. The techno-economic analysis based on the production cost of inulin from raw bajra seeds suggested that it was much cheaper than commercial inulin.

Keywords Extraction of inulin · Indian millets · Bajra inulin · Response surface methodology (RSM) · Growth dynamics of *Lactobacillus casei* · Cost analysis

Introduction

Jowar (*Sorghum vulgare*), bajra (*Pennisetum glaucum*) and ragi (*Eleusine coracana*) are the multibeneficial grains which are consumed by the large population of India due to their high availability and low cost. As reported in the literature, jowar contains essential nutrients like iron, calcium, potassium, phosphorous, B-vitamins and phytochemicals and has shown potential usefulness in reducing obesity. Bajra is high in protein as compared to other cereals and contains all essential amino acids and is rich in folate, potassium, magnesium, copper, zinc, vitamin E, B-complex, calcium and iron. Bajra helps maintaining cardiovascular health and reduces acidity problems, controls cholesterol, prevents diabetes and minimizes the risk of cancer. On the other hand, ragi is a powerhouse of health benefiting nutrients that help in reducing weight and also works as a treatment for multiple diseases like brittle bones, osteoporosis, anaemia and diabetes. It is a natural relaxant that helps in relieving stress and anxiety (Michaelraj and Shanmugam 2013). Since these cereals are constitutionally similar to wheat (Koehler and Wieser 2013), they are expected to contain prebiotic molecules

Electronic supplementary material The online version of this article (doi:10.1007/s13197-017-2901-4) contains supplementary material, which is available to authorized users.

✉ Ranjana Chowdhury
ranjana.juchem@gmail.com

¹ Chemical Engineering Department, Jadavpur University, Kolkata 700032, India

² Chemical Engineering Department, Heritage Institute of Technology, Kolkata 700107, India

like inulin besides arabinoxylan found in the latter (Samanta Koruri et al. 2016). Although these coarse cereals had long been considered as sources of dietary fibre, no efforts had so far been made to extract prebiotic inulin from them. A significant work on extraction, determination of degree of polymerization and prebiotic effect evaluation of inulin from Jerusalem artichoke had been reported (Li et al. 2015). Some pioneering research studies have been reported on prebiotics derived from natural sources, as well as on the determination of degree of polymerization of different oligosaccharides, the effect of inulin on some physicochemical properties of frozen yogurt etc (Gordon Syngai et al. 2016; Rezaei et al. 2014; da Silva et al. 2014). A recent literature also reports on the detailed study on extraction, partial characterization and the potential application of crude polysaccharides from defatted coconut residue as a prebiotic source (Mohd Nor et al. 2017). The work presented in this article, reported the extraction and optimization of inulin production from different Indian millets. This would also strengthen the initiative of Government of India to enhance the production of millets (Jain Passi and Jain 2014) and popularize these coarse cereals in the global market.

The present article would focus on the extraction of inulin from Indian millets and on the optimization of operating conditions through Response Surface Methodology (RSM) technique. The extracted inulin would be characterized both qualitatively and quantitatively. The effect of inulin samples, extracted from different millets, on the growth of probiotic organism, namely, *Lactobacillus casei* (*L. casei*) would be studied and the prebiotic activity scores (Huebner et al. 2007) with reference to *L. casei* and *Escherichia coli* (*E. coli*) would be determined. The best inulin source among the millet samples would be selected. Since lactose is the predominant carbohydrate source for *L. casei* and one of the expected applications of the prebiotic inulin derived from millets would be in dairy products, the growth dynamics of *L. casei* in presence of both lactose and inulin will be determined. A techno-economic analysis would be made on the production of the best inulin extract under optimum condition using the cost of commercial inulin, extracted from chicory roots, as the reference for comparison.

Materials and methods

Chemicals

Standard chicory derived inulin purchased from Himedia, India was used. TLC silica gel G60 Aluminium sheets 20 × 20 cm (Merck HX816976, Germany), n-butanol

(Merck, India), oxalic acid (Merck, India), calcium hydroxide (Merck, India), benzene (Ranbaxy, India), acetic acid (Merck, India), methanol (Merck, India), orcinol (Merck, India), ethanol (Merck, India), sulphuric acid (Merck, India) and HCL (Merck, India) were used.

Natural inulin sources

Indian millets, namely, jowar, bajra and ragi, collected from markets have been used for extraction of inulin.

Microorganism

Lactobacillus casei (2651 1951 RPK) and *E. coli* (2065ATCC 8739) were purchased from National Collection of Industrial Microorganisms (NCIM), Pune.

Analytical instruments

FTIR Spectroscope 8400 (Shimadzu), UV visible Spectrophotometer (Varian) were used.

Analytical methods

FTIR (Fourier transform infrared spectroscopy)

In order to verify the presence of inulin, the three selected food materials was analysed using FTIR. For all the test cases comparison with the pure food grade inulin was carried out.

TLC (thin layer chromatography)

Quantitative analysis of extracted inulins was done with respect to the commercial inulin using TLC followed by spectrophotometric analysis. The protocol of the TLC analysis, as described by Samanta Koruri et al. (2014), was used.

Determination of degree of polymerization

The degree of polymerization, or DP, normally represents the number of repeating units in the polymer chain (Fried 2000). Degree of polymerization may be of two types, namely, number average DP and weight average X_w ones. For step-growth polymerization, DP and X_w are correlated to the monomer conversion, p by $DP = \frac{1}{1-p}$ and $X_w = \frac{1+p}{1-p}$. The unique properties of natural macromolecules were attributed to their high molecular weights (MacGregor and Greenwood 1980). The degree of polymerization, defined by the number of structural units specifies the size of the natural polymers. The molecular weight of that

macromolecule is simply the sum of the macromolecule is simply the sum of the molecular weights of the structural units.

The fructans like inulin are polymers of D-fructofuranose units (MacGregor and Greenwood 1980). To determine the degree of polymerization, the extracted inulin samples were first hydrolyzed and then followed by DNS (3-5 Di-nitro salicylic acid) method to determine the glucose concentration in the hydrolysate. On the other hand, inulin content of the extract was determined using the TLC method. The DP was calculated using the following procedure:

According to Simonovska (2000), the degree of polymerization, n , may be correlated with the contents of glucose and fructose in inulin as shown below:

$$n = \frac{F_i}{G_i} + 1 \quad (1)$$

If the general formula of Inulin be GF_{n-1} .

Where G = glucose, F = fructose, F_i = Content of fructose originating from inulin sample, G_i = Content of glucose originating from inulin sample.

Again, according to Curcio et al. (2014)

$$i = 0.9 * F_i + G_i \quad (2)$$

Therefore, by dividing both sides of Eq. 2 by G_i ,

$$\frac{i}{G_i} = 0.9 * \frac{F_i}{G_i} + 1 \quad (3)$$

Therefore, by using Eqs. 1 and 3

$$i = 0.1 * G_i + 0.9 * n * G_i \quad (4)$$

Thus, by determining the content of inulin, i and that of glucose, obtained through hydrolysis, the value of DP may be calculated from Eq. 4.

Comparative analysis of bacterial growth

The performance of inulin extracted from different sources, namely, jowar, bajra and ragi was compared with that of commercial inulin with respect to their influence on growth of *L. casei*.

Response surface methodology

Design-Expert 8.1 (Stat-Ease, Inc., Minneapolis, USA) software was used for the optimization of the operating parameters for the extraction of inulin.

Experimental design and optimization

The face centered central composite design (FCCD) was used to design the experimental runs to optimize the yield

of inulin with respect to temperature, HCl concentration and heating period in terms of ± 1 levels created by entering the factors. The basic protocol used for extraction of inulin from garlic, wheat, oat and daliah (Samanta Koruri et al. 2014) was followed. To ascertain reproducibility of the data, each experimental run was conducted in triplicate. Face-centered CCD was used for the optimization study of extraction process of inulin where extraction temperature was varied from 24 to 70 °C, HCl concentration was varied from 0.5 to 2 M and heating period was varied from 20 to 180 min. In a typical experimental run, all the operating variables were pre-set at predetermined design values. The experiments were conducted randomly to avoid systematic biasness.

Growth dynamics of *Lactobacillus casei*

Experiments were conducted in batch mode using inulin (0.164–0.63 g/L) and lactose (10–40 g/L) individually as carbohydrate sources for *L. casei*. Initial pH and temperature for all experiments were maintained at optimum values, i.e., 7 and 37 °C respectively. For each experiment, incubation was done for 28 h and samples were collected at 2 h interval. All experiments were performed in triplicate.

Theoretical analysis

Statistical modelling and optimization using RSM

Design-Expert 8.1 (Stat-Ease, Inc., Minneapolis, USA) software was used to build the experimental design, the surface graphs and to analyze the results. A design layout was created using CCD table with 20 experimental runs. The parameters (temperature, HCl concentration and heating period) were optimized for obtaining maximum inulin yield. The generalized equation might be written as follows:

$$Y_k = b_{k0} + \sum_{i=1} b_{ki}X_i + \sum_{i=1} b_{kii}X_i^2 + \sum_{i=1} \sum_{j=1} b_{kij}X_iX_j \quad (5)$$

where Y_k is response variable, b_{k0} is a constant intercept; b_{ki} , b_{kii} , and b_{kij} are the linear, quadratic and interaction regression coefficients, respectively. X_i and X_j represent the coded values of the process variables (factors).

Prebiotic activity score

The effectiveness of a specific prebiotic molecule on probiotics was quantitatively described by Huebner et al. (2007) by defining prebiotic activity score. The higher the prebiotic activity score the more effective is the prebiotic. Quantitatively Huebner et al. (2007) proposed an equation

to calculate the prebiotic activity score as mentioned below:

Prebiotic activity

$$\text{score} = \left(\frac{\log \frac{N_{24}}{N_0} |_{\text{prebiotic}}}{\log \frac{N_{24}}{N_0} |_{\text{glucose}}} \right)_{\text{probiotic}} - \left(\frac{\log \frac{N_{24}}{N_0} |_{\text{prebiotic}}}{\log \frac{N_{24}}{N_0} |_{\text{glucose}}} \right)_{E.coli} \quad (6)$$

where N_{24} = Number of cells of 24 h growth culture of *L. casei* and *E. coli* in de-Mann Rogosa, Sharp (MRS) and Modified de-Mann Rogosa Sharp (MMRS) media containing 20 g/L glucose and 20 g/L inulin respectively. N_0 = Initial number of cells of either *L. casei* or *E. coli*.

Values of N_{24} of *L. casei* and *E. coli* using inulin and glucose as carbohydrate sources were used to calculate the activity scores according to Eq. 6.

Cost analysis

Cost analysis of a small process plant for the production of pearl millet inulin under optimum condition was performed. The laboratory protocol reported by the present researchers was followed (Samanta Koruri et al. 2014). The process flow diagram shown in Fig. 1 was used. The small plant under analysis was constituted of the units detailed in Table 1.

The schedule of plant operation is shown in Table 2.

Referring to the extraction scheme of inulin shown in Fig. 1, each stirred vessel placed on a heating mantle handles 100 g of prebiotic, pearl millet suspended in slurry of 500 ml in 1 h. Since, 10 heating mantles are in

operation, total quantity of bajra which could be processed annually (M_{PA}) by this small plant is calculated as follows:

$$M_{PA}(\text{kg}) = \frac{10 * 100 * n_W * n_{dy}}{1000} \quad (7)$$

If the fractional extraction and the purity of inulin obtained under optimum condition be denoted by Q_{opt} and P_{opt} the annual production capacity of pure inulin by the small process plant Q_{IA} was given by following correlation

$$Q_{IA}(\text{kg}) = M_{PA} * Q_{opt} * P_{opt} \quad (8)$$

From the extraction scheme of inulin from bajra it was also clear that approximately 1 L of water was needed to process 100 g of millet inulin. Therefore the total annual consumption of water (W_Y) for the process of inulin extraction was as follows:

$$W_Y(\text{L}) = M_{PA} * \frac{0.75 * 1000}{100} \quad (9)$$

The quantity of HCl needed for 100 g of pearl millet under processing was approximately 1 mL. Therefore total annual consumption of HCl (H_Y) is calculated as follows:

$$H_Y(\text{L}) = M_{PA} * \frac{0.001 * 1000}{100} \quad (10)$$

The recurring cost for the plant has been calculated using the data provided in Table 3.

Total annual energy consumption (E_Y) of the plant was calculated considering the plant schedule and the power rating (PR) of each unit, i.e., mixer-grinder (PR_{MG}), heating mantle (PR_{HM}), Rotary vacuum evaporator (PR_{RVE}), Freeze dryer (PR_{FD}), magnetic stirred vessel (PR_{MGST}), refrigerator (PR_{RF}).

Fig. 1 Flow diagram of extraction process of inulin

Table 1 Units of small plant

Unit	Quantity	Capacity	Power rating (PR) (in W)	Cost (1\$ = Rs 67.93)		Write-off period (years)
				(INR)	(\$)	
Mixer-grinder	5	1.5 L	750	10,000	147.20	2
Heating mantles	10	1.0 L	100	3000	44.16	1
Magnetically stirred vessel	20	2.0 L	500	10,000	147.20	1
Refrigerator	2	190 L	400	20,000	294.40	3
Rotary vacuum evaporator	1	5 L	60	1,000,000	147,198.45	3
Freeze dryer	1	2.3 kg over 24 h	350	100,000	14,719.845	3

Table 2 Schedule of plant operation

Working hours for the plant (n_w)	8 h
Working days per year (n_{dY})	280

Table 3 Recurring cost for the plant

Grid electricity tariff (Calcutta electricity supply corporation) (E_C)	INR 6.77/kWh
Annual maintenance cost (MC_A) of freeze dryer and rotary vacuum evaporator	INR 50,000
Manpower (2 operators) (MP_A)	INR 900,000
Cost of water (INR/L)	W_C (INR 6/L)
Cost of HCl (INR/L)	H_C (INR 1000/L)
Cost of pearl millet (INR/kg)	P_C (INR 8/kg)
Cost of other Chemicals and filter cloth (INR)	Co (INR 100,000)

Therefore,

$$E_Y (\text{kWh}) = \frac{n_w * n_{dY} * (PR_{MG} + PR_{HM} + PR_{RVE} + PR_{FD} + PR_{MGST} + PR_{RF})}{1000} \quad (11)$$

Total energy cost (E_T) is calculated as follows:

$$E_T = E_Y * E_C \quad (12)$$

Total material cost (M_Y) was contributed from pearl, millet, water and HCl and cost of other chemicals, filters etc (C_{Ot}). Therefore,

$$M_Y = MP_A * P_C + W_Y * W_C + H_Y * H_C + C_{Ot} \quad (13)$$

The total annual recurring cost (R_{CY}) was the summation of energy cost (E_Y), Material cost (M_Y), manpower cost (MP_Y) and the annual maintenance cost (MC_Y)

$$R_{CY} = E_Y + MP_Y + MC_Y + M_Y \quad (14)$$

Total annual fixed cost (FC_Y) was incurred by the investment cost for the equipment, namely, mixer-grinder

(FC_{MG}), heating mantle (FC_{HM}), Rotary vacuum evaporator (FC_{RVE}), Freeze dryer (FC_{FD}), magnetic stirred vessel (FC_{MGST}), refrigerator (FC_{RF}), distributed annually over the write-off period.

Therefore,

$$FC_Y = FC_{MG} + FC_{HM} + FC_{RVE} + FC_{FD} + FC_{MGST} + FC_{RF} \quad (15)$$

$$FC_Y = \frac{Co_{MG}}{n_{wMG}} + \frac{Co_{HM}}{n_{wHM}} + \frac{Co_{RVE}}{n_{wRVE}} + \frac{Co_L}{n_{wFD}} + \frac{Co_{MGST}}{n_{wMGST}} + \frac{Co_{RF}}{n_{wRF}} \quad (16)$$

where Co = cost of equipment * number of equipment, n_{wr} = write-off period

Total annual cost (CT) was a summation of recurring (R_{CY}) and fixed cost (FC_Y)

Therefore,

$$CT = R_{CY} + FC_Y \quad (17)$$

$$E_{XT} = CT / Q_{IA} \quad (18)$$

The extraction cost (E_{XT}) per unit kg of pure inulin might be calculated using the following correlation.

Results and discussion

FTIR

The inulin samples extracted from different natural sources, e.g. jowar, bajra and ragi were analyzed using FTIR and the presence of inulin was confirmed by the comparison of FTIR spectra with that of commercial inulin, as evident from Table 4 (FTIR chromatograms of millets are provided in Online Resource, Figs. S1, S2 and S3).

TLC

The Thin Layer Chromatograms (TLC) of commercial inulin and the samples derived from three Indian millets

Table 4 Wavelength numbers of prebiotic samples

FTIR wavelength numbers for inulin				Range of functional groups
Commercial	Bajra	Jowar	Ragi	
1365	1383	1380	1408	1365 and 1390 cm^{-1} is often due to a t-Bu-group
1450	1457	1458	1547	A strong peak around 1450 cm^{-1} indicates the presence of methylene groups (CH_2) [CH_2 , CH_3 (bend)]
1633.71	1650	1651	1653	(1620–1680) $\text{C}=\text{C}$ Alkenes
2104.34	2149	2140	2134	(2100–2260) $\text{C}\equiv\text{C}$ stretch in alkynes, $\text{C}\equiv\text{N}$ stretch in Nitrile, OH bond, C–O stretch
2389.80	2928	2925	2928	2931 (C–H aliphatic)
3290	3432	3433	3416	(3200–3500) O–H bond

indicate the presence of inulin in each of the three candidate samples taken for study. From TLC experiment R_f (retention factor) values of extracted inulin was calculated and compared with standard food grade inulin. R_f values of commercial inulin, jowar inulin, bajra inulin and ragi inulin were 0.8, 0.8, 0.8 and 0.75 respectively.

The spectrophotometric analysis of the solution prepared using TLC spots was carried out using TLC data to find out the content of inulin (weight % dry basis) in each sample. The fraction of inulins in jowar, bajra and ragi extracts were found out to be 0.3022, 0.4727, and 0.3215 respectively.

Degree of polymerization (DP)

It was evident from existing literature, that commercial inulin (chicory) and garlic inulin have the DP ranging from 2 to 61 and 16 to 21 respectively (Evans et al. 2015 and Zhang et al. 2013). The DP of the three millets was calculated from Eq. 4. The degree of polymerization of jowar inulin, bajra inulin and ragi inulin achieved as 27, 39 and 23 respectively.

Comparative analysis of bacterial growth in presence of commercial and millet inulins

The purpose of this study was to carry out a comparative analysis of the effectiveness of economically extracted inulin from different Indian millets, with that of commercial inulin. The comparison was made by plotting bacterial (*L. casei*) cell concentration against time (Fig. 2) using different varieties of inulin as the co-substrate with lactose. From the plots it was clear that although for all sources of inulin the growth follows exponential pattern, the concentration of biomass at any instant varies with the source of inulin. Up to 20 h the biomass concentration at any instant was as follows: bajra inulin > jowar inulin > ragi inulin > commercial inulin (chicory). The reason behind this unique and interesting finding might be the variation of degree of polymerization (DP) of inulin obtained from

Fig. 2 Growth patterns of *L. casei* with 20 g/L naturally extracted inulins and 20 g/L lactose in comparison with 20 g/L commercial inulin

different sources (Li et al. 2015). As reported by Ricca et al. (2009), inulin is hydrolyzed to glucose and fructose by fructo-enzymes (Ricca et al. 2009). As per the present investigation, the bajra inulin had the highest DP (= 39) compared to that obtained from the jowar (= 27) and ragi (= 23) inulin and hence there was maximum availability of assailable monomer on enzymatic hydrolysis. This might be the justification for the observation of to highest biomass of *L. casei* on bajra inulin among all varieties. Hence, in the subsequent sections of this article, results on optimization of bajra inulin yield using RSM, determination of prebiotic activity score of bajra inulin and growth dynamics of *L. casei* in presence of bajra inulin was highlighted.

Dependence of bajra inulin yield on temperature, concentration of HCl and heating period

The model equation predicted using the values of response variables corresponding to the preset experimental conditions using the CCD table was used for optimization of bajra inulin yield with respect to temperature, HCl concentration and heating period in terms of ± 1 levels created by entering the factors.

Table 5 Experimental design matrix

Serial no.	Temp (°C)	HCl conc. (M)	Heating period (min)	Inulin yield (%)
1	65.00	1.25	80.00	70.2
2	47.00	1.25	100.00	45
3	24.00	2.00	20.00	16
4	70.00	0.80	60.00	90
5	47.00	0.01	100.00	27
6	47.00	1.25	100.00	45
7	24.00	0.50	180.00	28.8
8	70.00	0.50	180.00	45
9	47.00	1.25	234.54	32
10	24.00	2.00	20.00	16
11	24.00	2.00	180.00	28.8
12	47.00	1.25	100.00	45
13	70.00	0.80	60.00	90
14	47.00	2.51	100.00	29
15	47.00	1.25	100.00	45
16	8.32	1.25	100.00	16
17	47.00	1.25	100.00	45
18	70.00	2.00	20.00	18
19	47.00	1.25	34.54	18
20	47.00	1.25	100.00	45

The values of inulin yield obtained at different values of temperature, HCl concentration and heating period were shown in Table 5.

In accordance with the statistical analysis model fit summary, a quadratic model was selected as the best fitted with lower standard deviation (12.14) and PRESS value (63,057.75), predicted residuals sum of squares (−5.7179), adjusted R^2 (0.7017), regression coefficient (0.9108), predicted R^2 regression coefficient (0.8613) and adequate precision (7.756) in comparison to 2FI, i.e., two-factor interaction model. The data obtained from ANOVA (“analysis of variance”) for quadratic model showed insignificant lack of fit (sum of squares = 7913.07 > 0.05), Fischer test value (F value) of 5.97, R^2 value of 0.8430 and low C.V., “coefficient of variance” value (30.55). The model equation showed the dependence of inulin yield simultaneously on temperature, HCl concentration and heating period as follows:

$$\begin{aligned} \text{inulin yield} = & 47.96 + 15.59 * A - 5.79 * B + 0.93 * \\ & C - 18.31 * A * B \\ & - 13.68 * A * C + 0.57 * B * C - 1.00 * \\ & A^2 - 7.80 * B^2 - 8.98 * C^2 \end{aligned} \quad (19)$$

where A, B and C are the temperature, concentration of HCl and heating period respectively. The model was significant as the p value (lack of fit) is < 0.0050 (Additional data are given in Online Resource, Tables S1, S2 and S3).

Optimization study

In order to understand the effects of temperature, HCl concentration and heating period on inulin yield, three dimensional plots and contour plots have been shown in Fig. 3. It was observed that the maximum inulin yield of 0.4 (Q_{opt}) was obtained at 70 °C temperature, 0.8 M of HCl concentration and 60 min of heating period with desirability of unity.

Prebiotic activity score

Prebiotic activity score is a quantitative measure of the effectiveness of any prebiotic material, like inulin. The values of prebiotic activity score against *E. coli* were determined to be 1.0 and 3.2 respectively for commercial inulin and that extracted from bajra under optimum condition. It immediately revealed that bajra inulin had much better score indicating that this variety of the inulin molecule was superior to chicory based inulin from the perspective of prebiotic activity on *L. casei*. This result was also in agreement with the largest biomass concentration at any batch time using bajra inulin.

Growth dynamics of *L. casei* in presence of bajra inulin

A double reciprocal plot (provided in Fig. S4 of Online Resource) was made by graphing the inverse of specific

Fig. 3 a–f 3-D surface and contour plots depicting the variation of inulin yield with A: Temperature (°C), B: HCl concentration (M) and C: Heating period (min)

growth rate against the inverse of initial lactose concentration. The linearity of the plot indicated that Monod model for uninhibited growth kinetics (Shuler and Kargi 2002) was valid. Thus Eq. 20 was valid.

$$\mu_L = \frac{\mu_{\max_L} C_L}{K_{s_L} + C_L} \quad (20)$$

The values of $\mu_{\max_L}$ and K_{s_L} , i.e. the maximum specific growth rate and half saturation constant respectively for growth of *L. casei* solely on lactose, were determined from the double reciprocal plot.

In order to understand the effect of bajra inulin as sole carbon source on the growth rate of *L. casei*, experimental values of initial specific cell growth rate as a function of inulin concentration in total absence of lactose were plotted (provided in Fig. S5 of Online Resource). The nature of the plot clearly indicated that the increase in the concentration of inulin enhances the formation rate of cells up to 0.3225 g/L beyond which the growth was inhibited. Thus, the widely accepted Haldane model, applicable for substrate inhibited condition was applied to predict the cell dynamics. The Haldane equation is as follows (Shuler and Kargi 2002):

$$\mu_I = \frac{\mu_{\max_I} C_I}{K_{s_I} + C_I + \frac{C_I^2}{K_I}} \quad (21)$$

From the plot of initial values of μ against substrate concentration C_I , as provided in Fig. S5 of Online Resource, it was clearly observed that μ increased up to the concentration of inulin = 0.3225 g/L, beyond which it decreased. The values of $\mu_{\max_I}$ and K_{s_I} were determined using the slope and the intercept of the double reciprocal plot of $1/\mu$ versus $1/C_I$ within $C_I = 0.3225$ g/L and by using non-linear regression analysis ($R^2 = 0.995$) (provided in Fig. S6 of Online Resource). The value of K_I was determined using the following correlation (Shuler and Kargi 2002):

$$C_{S_{\max}} = (K_S K_I)^{0.5} \quad (22)$$

where $C_{S_{\max}}$ = Substrate concentration at which the specific growth rate is maximum, K_s indicates half substrate (inulin) saturation constant and K_I indicates Inhibition constant.

Therefore, for inulin based growth, $C_{I_{\max}} = 0.3225$ g/L.

The calculated values of kinetic parameters namely $\mu_{\max_L}$, K_{s_L} , $\mu_{\max_I}$, K_{s_I} and K_I are 0.373 h^{-1} , 0.05 g/L , 0.05 h^{-1} , 0.2 and 0.52 g/L respectively.

In order to verify the validity of the Haldane equation, the plots of the predicted (Eq. 21) and experimental values of specific growth rates against bajra inulin concentration were compared in the Online Resource (Fig. S7). It was evident from the figure that the experimental and the predicted data are well in agreement and thus the Haldane

equation was valid to express the dependence of cell dynamics of *L. casei* solely on bajra inulin.

The experimental time trajectories of biomass concentration at different initial lactose concentration (10, 20 and 30 g/L) were also plotted in the Online Resource (Fig. S8) using the concentration of bajra inulin as a parameter. The figure revealed that at each lactose concentration, the biomass concentration obtained at any time is greater if inulin was used in addition to lactose. Therefore, summative type of growth kinetic model applicable for multi-substrate growth was attempted to predict the cell growth dynamics (Blanch and Clark 1996). In composite equation form the cell growth dynamics in presence of both lactose and bajra inulin might therefore be written as

$$\mu = \frac{\mu_{\max_L} C_L}{K_{s_L} + C_L} + \frac{\mu_{\max_I} C_I}{K_{s_I} + C_I + \frac{C_I^2}{K_I}} \quad (23)$$

The constants in Eq. 23 were same as those determined for Eqs. 20 and 21 and hence it was devoid of any adjustable parameters.

Yield coefficients

The yield coefficients of *L. casei* on lactose ($Y_{X/L}$) and inulin ($Y_{X/I}$) were defined as follows (Shuler and Kargi 2002):

$$Y_{X/L} = \frac{\frac{dC_X}{dt}}{\frac{dC_L}{dt}} \quad (24)$$

$$Y_{X/I} = \frac{\frac{dC_X}{dt}}{\frac{dC_I}{dt}} \quad (25)$$

The values of concentrations of biomass and substrates at $t = 0$ and $t = 24$ h were used to calculate the yield coefficients. Therefore the equations were reduced to the following form

$$Y_{X/L} = \frac{(C_{X(t=24h)} - C_{X0})}{(C_{L0} - C_{L(t=24h)})} \quad (26)$$

$$Y_{X/I} = \frac{(C_{X(t=24h)} - C_{X0})}{(C_{I0} - C_{I(t=24h)})} \quad (27)$$

Simulation and comparison of specific growth rate and concentration of probiotics

The specific growth rate was simulated using Eq. 23 and were plotted as a function of both inulin and lactose concentrations in Fig. 4b. Using the Eq. 23, the values of μ were simulated as a function of initial concentrations of lactose and inulin using MATLAB 7 and 3D graph was generated in Fig. 4b. Similar 3D plot was generated using

the corresponding experimental values of μ in Fig. 4a. The comparison of the 3D plots in Fig. 4a, b immediately revealed that the experimental and simulated data agreed appreciably indicating the validity of the proposed Eq. 23. The mass balance equations for biomass, lactose and inulin were written as follows:

$$\frac{dC_x}{dt} = \frac{\mu_{\max_1} C_L}{K_{s_1} + C_L} + \frac{\mu_{\max_2} C_I}{K_{s_2} + C_I + \frac{C_I^2}{K_I}} \quad (28)$$

$$\frac{dC_L}{dt} = -\frac{1}{Y_{x/L}} \frac{\mu_{\max_1} C_L}{K_{s_1} + C_L} \quad (29)$$

$$\frac{dC_I}{dt} = \frac{1}{Y_{x/I}} \frac{\mu_{\max_2} C_I}{K_{s_2} + C_I + \frac{C_I^2}{K_I}} \quad (30)$$

The initial conditions are as follows:

At $t = 0$

$$\begin{bmatrix} C_x = C_{x0} \\ C_L = C_{L0} \\ C_I = C_{I0} \end{bmatrix} \quad (31)$$

Values of Yield coefficients namely $Y_{x/L}$, $Y_{x/I}$ are 0.2, 0.4 and initial concentrations of substrates and biomass namely C_{L0} , C_{I0} , C_{x0} are 10, 0.164 and 0.003 g/L respectively.

The values of concentrations of biomass, lactose and inulin were simulated by solving Eqs. 28, 29 and 30 with the initial conditions using ODE 45 of MATLAB7. The layout shown in Fig. 5 was followed for the simulation model and for its validation strategy. The simulated values of biomass were plotted in Fig. 6a–c and the experimental values were superimposed. The agreement ($R^2 > 0.95$) between the simulated and the experimental values again validated the summative model for the growth of *L. casei* simultaneously on lactose and inulin.

Therefore, it might be inferred that although classical Monod and Haldane models are valid for the growth on single carbohydrate sources, summative model explained the situation in presence of two carbohydrates. This signified the simultaneous utilization of both carbohydrates by the probiotic microorganism under study and hence no catabolite repression was present, as encountered in case of diauxic growth in presence of multiple carbohydrates including glucose (Blanch and Clark 1996; Shuler and Kargi 2002). The summative growth of probiotic *Pedio-coccus acidilactici* in presence of prebiotic inulin and a hexose sugar had been reported by Samanta Koruri et al. (2016). This might be due to the affinity of the probiotics towards the prebiotic inulin due to the presence of inulinase in these microorganisms (Oliveira et al. 2013). In absence of reported data on bajra inulin, these results could not be compared with the findings of previous works.

Cost analysis results

The commercialization of any product depends on its production cost to attain a competitive position with respect to similar products in the market. On the annual basis the following results were obtained using the correlations derived in the theoretical section. The values for P_{opt} and Q_{opt} of Eq. 8—the purity of inulin and fractional extraction obtained under optimum condition, as discussed under TLC and optimization study, were 0.473 and 0.4 respectively. Cost analysis of extraction of inulin from bajra in small scale is provided in Table 6.

The cost of 25 g commercial inulin was INR 2000. According to the lab scale experimental data the same quantity of bajra inulin might be produced by the investment of INR 127.35. Although, it was understandable that more purification of the pearl millet inulin was required before its full commercialization, it was expected that pearl

Fig. 4 3D plot of specific growth rate (μ) as a function of lactose and bajra inulin concentrations **a** experimental data, **b** simulated data

Fig. 5 Layout for simulation model and strategy for validation

Fig. 6 a–c Simulated values of biomass concentration of *L. casei* with respect to time

Table 6 Cost analysis of extraction of inulin from bajra in small scale

Parameter	Significance	Value
M _{PA}	Quantity of bajra processed	2240 kg
Q _{IA}	Quantity of pure inulin extracted	423.808 kg
W _Y	Volume of water used, mass of bajra processed	16,800 L
H _Y	Volume of HCl used, mass of bajra processed	22.4 L
E _Y	Energy consumption	4838.4 kWh
E _T	Total energy cost	INR 32,756
M _Y	Total material cost (bajra + water + HCl + other chemicals + filter cloths etc.)	INR 241,120
R _{CY}	Total annual recurring cost	INR 1,223,876
FC _Y	Total annual fixed cost	INR 935,000
C _T	Total annual cost	INR 2,158,876
Ex _T	Extraction cost of inulin	INR 5094.091/kg

bajra inulin would also be a close and cost effective competitor of commercial inulin presently available in Indian market. The improvement with respect to cost of bajra inulin could be obtained due to the operation at the optimum condition determined under the present investigation. Perhaps the higher cost of commercial inulin derived from chicory was due to higher price of chicory with respect to bajra in Indian market and due to the extraction at non-optimal condition. However, the information about the condition of extraction of commercial inulin from chicory was not available.

Conclusion

The optimum conditions for the extraction of inulin from Indian millets, namely jowar, bajra and ragi were determined after the qualitative and quantitative assessment of raw materials with respect to inulin content. The degree of polymerization of inulin derived from different millets were 27, 39 and 23 respectively for jowar inulin, bajra inulin and ragi inulin which were comparable to that of chicory inulin (2–60 DP). Bajra inulin showed the best prebiotic activity score and overall growth for *L. casei*. *L. casei* followed summative type growth patterns in presence of bajra inulin and lactose. For bajra, the yield of extracted inulin was correlated to temperature, HCl concentration and heating period by a quadratic model equation. The maximum yield was obtained at temperature, HCl concentration and heating period of 70 °C, 0.8 M and 60 min respectively. From the techno economic analysis done under the present investigation it was calculated that 25 g bajra inulin will cost INR 127.35 against INR 2000

for commercial inulin. It was expected that the milk products containing *L. casei* may be fortified by bajra inulin and synbiotic preparation may be designed by using *L. casei* and bajra inulin.

Acknowledgements The first author (Debolina Banerjee) acknowledges the financial support offered by Council of Scientific and Industrial Research (CSIR), India by providing Senior Research Fellowship (File Number: 9/96(0725)2K12-EMRI).

References

- Blanch HW, Clark DS (1996) Biochemical Engineering. Marcel Dekker, New York, pp 192–195
- Curcio S, Ricca E, Calabrò V, Iorio G (2014) Effect of the degree of polymerization of inulin on the rate of hydrolysis using immobilized inulinase. Food Technol Biotechnol 52(3):317–324
- da Silva IM, Rabelo MC, Rodrigues S (2014) Cashew juice containing prebiotic oligosaccharides. Food Sci Technol 51(9):2078–2084
- Evans M, Joseph A, Ratcliffe I, Peter A (2015) Determination of the degree of polymerisation of fructans from ryegrass and chicory using MALDI-TOF mass spectrometry and gel permeation chromatography coupled to multiangle laser light scattering. Food Hydrocol 55:1–8
- Fried JR (2000) Polymer science and technology. Prentice Hall of India, New Delhi, p 25
- Gordon Syngai G, Gopi R, Bharali R, Dey S, Alagu Lakshmanan GM, Ahmed G (2016) Probiotics—the versatile functional food ingredients. J Food Sci Technol 53(2):921–933
- Huebner J, Wehling RL, Hutkins RW (2007) Functional activity of commercial prebiotics. Int Dairy J 17:770–775
- Jain Passi S, Jain A (2014) MILLETS: the nutrient rich counterparts of wheat and rice. Press Information Bureau, Government of India. <http://pib.nic.in/newsite/mbErel.asp>
- Koehler P, Wieser H (2013) Chemistry of cereal grains. German Res Center Food Chem 87:11–45
- Li W, Zhang J, Yu C, Li Q, Dong F, Wang G, Gu G, Guo Z (2015) Extraction, degree of polymerization determination and prebiotic effect evaluation of inulin from Jerusalem artichoke. Carbohydr Pol 121:315–319
- MacGregor EA, Greenwood CT (1980) Polymers in nature. Wiley, Chichester, pp 6–289
- Michaelraj PSJ, Shanmugam A (2013) A study on millets based cultivation and consumption in India. Int J Market Financ Serv Manag Res 2(4):49–58
- Mohd Nor NAN, Abbasiliasi S, Marikkar MN, Ariff A, Amid M, Lamasudin DU, Manap MYA, Mustafa S (2017) Defatted coconut residue crude polysaccharides as potential prebiotics: study of their effects on proliferation and acidifying activity of probiotics in vitro. J Food Sci Technol 54(1):164–173
- Oliveira RPS, Casazza AA, Aliakbarian B, Perego P, Converti A, Oliveira MN (2013) Influence of fructooligosaccharides on the fermentation profile and viable counts in a symbiotic low fat milk. Braz J Microbiol 44(2):431–434
- Rezaei R, Khomairi M, Aalami M, Kashaninejad M (2014) Effect of inulin on the physicochemical properties, flow behavior and probiotic survival of frozen yogurt. J Food Sci Technol 51(10):2809–2814
- Ricca E, Calabrò V, Curcio S, Iorio G (2009) Fructose production by chicory inulin enzyme hydrolysis: kinetic study and reaction mechanism. Process Biochem 44:466–471

- Samanta Koruri S, Banerjee D, Chowdhury R, Bhattacharya P (2014) Studies on prebiotic food additive (inulin) in Indian dietary fibre sources garlic (*Allium sativum*), wheat (*Triticum* spp.), oat (*Avena sativa*) and dalia (BULGUR). Int J Pharm Pharm Sci 6(9):278–282
- Samanta Koruri S, Pandey A, Chowdhury R (2016) Prebiotic influence of *Plantago ovata* on free and microencapsulated *L. casei*—growth kinetics, antimicrobial activity and microcapsules stability. Int. J Pharm Pharm Sci 8(8):89–97
- Shuler ML, Kargi F (2002) Bioprocess engineering basic concepts. Prentice Hall of India, New Delhi, pp 57–199
- Simonovska B (2000) Determination of inulin in foods. J AOAC Int 83(3):675–678
- Zhang N, Huang X, Zeng Y, Wu X, Peng X (2013) Study on prebiotic effectiveness of neutral garlic fructan in vitro. Food Sci Hum Wellness 2:119–123