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# Inulin from *Pachyrhizus erosus* root and its production intensification using evolutionary algorithm approach and response surface methodology

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ABSTRACT

Production of inulin from yam bean tubers by ultrasonic assisted extraction (UAE) was optimized by using response surface methodology (RSM) and genetic algorithms (GA). Yield of inulin was obtained between 11.97%–12.15% for UAE and 11.21%–11.38% for microwave assisted extraction (MAE) using both the methodologies, significantly higher than conventional method (9.9 %) using optimized conditions. Under such optimized condition, SEM image of root tissues before and extraction showed disruption and microfractures over surface. UAE provided a shade better purity of extracted inulin than other two techniques. Degree of polymerization in inulin was also recorded to be better, might be due lesser degradation during extraction. Significant prebiotic activity was recorded while evaluation using *Lactobacillus fermentum* and it was 36 % more than glucose treatment. Energy density by UAE was few fold lesser than MAE. Carbon emission was far more less in both these methods than the conventional one.

## 1. Introduction

With the sharp increase in health related problems, especially with the advent of COVID-19, enhancing immunity is one of the prescribed method to stay safe and healthy. Boosting of immunity is linked with the structure and function of microbiome. Health of gut bacteria can be enhanced by using probiotic directly or using prebiotic in order to get the beneficial effect indirectly. Apart from this, there is an increasing trend of gastrointestinal problems. In this regard, India has become one of the pioneer countries among other south-east Asians as around 10 % of its population is suffering under severe “Functional Gastrointestinal Diseases” according to a report by Boronat, Ferreira-Maia, Matijasevich, and Wang (2017). There are a plethora of synthetic drugs accessible in the market but lots of side effects are adhered with these pharmaceuticals. So now-a-days scientific communities are expressing their interests towards enriching the population of gut-friendly microbes that are already present in human system. Prebiotic compounds play a pivotal role in increasing the population of microbes in human gut. These are basically non-digestive oligosaccharides (short chain dietary carbohydrates) that show selective metabolism within system. Oligosaccharides, resistant to gastric acidity, are fermented and utilized by gut micro-biota. It stimulate the growth and/or activity of gut bacteria (Olano-Martin, Mountzouris, Gibson, & Rastall, 2001).

Mexican yam bean or Jicama (*Prachyrhizus erosus* L.), a member of fabaceae family, is an important crop in terms of its economic significance in Mexico along with various south-east Asian countries. Different polysaccharides are present in the fruit that consist of cellulose, pectic polysaccharides, xyloglucans, hetereomannans along with inulin (Ramos-De-La-Pena, Renard, Wicker, & Contreras-Esquivel, 2013). The crop is still under-utilized although it has huge commercial potential.

Inulin being a potent prebiotic substance, its extraction is very much economically important in nutraceutical as well as functional food perspective. So to explore the possibility of utilization of this under-utilized crop for the purpose of valorisation, this yam bean tuber flesh was selected for the extraction of inulin.

Generally oligosaccharides are being extracted by hot water apart from other newer techniques like ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) etc. Conventional extraction

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process uses higher temperature for extended period of time but it results in lesser yield (Liu, Fu, Chi, & Chi, 2014). UAE and MAE provide immense advantage in terms of lesser extraction time, lower energy requirement and higher efficiency. Using ultrasound mediated extraction method, inulin was earlier extracted from Burdock roots (*Arctium lappa*) (Milani, Koocheki, & Golimovahhed, 2011), Jerusalem artichoke tubers (Li et al., 2018), roots of elecampane (*Inula helenium* L.) (Petkova et al., 2015), tubers of Iranian artichoke (Abbasi & Farzanmehr, 2009). Similarly different fructo-oligosaccharides were extracted from various matrices using microwave heating. Using this technique, inulin was isolated from tubers of *Helianthus tuberosus* L. (Temkov, Petkova, Denev, & Krastanov, 2015), tubers of *Cynara scolymus* L. (Ruiz-Aceituno, García-Sarrió, Alonso-Rodriguez, Ramos, & Sanz, 2016), Burdock roots (Li et al., 2014) etc. But no study has been documented regarding extraction of inulin from yam bean tubers by ultrasound or microwave technique till date.

As per industrial purview, the optimisation of extraction conditions is quintessential in order to get maximum yield of desirable bioactives for any kind of extraction method. Using Response Surface Methodology (RSM) for the optimisation purpose is not a new approach. But limited study has been conducted regarding optimised extraction protocol for inulin from different crops. Keeping this fact in mind, the present research work was formulated with the aim to find the role of extraction techniques on the purity of inulin extracted by UAE and MAE. Prebiotic activity of the extracted inulin was also evaluated for its utilization in food. Process optimisation was done by two methods viz. Box-Behnken Design (response surface methodology) (Varghese, Bhowmik, Jaggi, Varghese, & Kaur, 2017) as well as genetic algorithm approach based on the concept of natural selection and genetics by using non-linear second order response surface model (García, García-Pedrajas, Ruiz, & Gómez-Nieto, 2018). This piece of work will be helpful for industrial application by understanding appropriate conditions for obtaining maximum prebiotic compound.

## 2. Materials and methods

### 2.1. Plant materials and reagents

Yam bean tubers were collected from a local market of Kolkata (Mechhua fruit market, N 22.57°; E 88.36°). The tubers were cut into pieces of convenient sizes (1−2 cm) and blanched in boiling water (2 L per 1 kg tubers; 100 °C) for 5 min to deactivate enzymatic activities. The blanched tuber pieces were kept overnight under oven at 50 °C for complete drying. The dried samples were then grinded using a mixer grinder into fine powder (<1 mm size). This powdered material was utilized for extraction of inulin.

Deionized water was used for extraction purpose, obtained from millipore purifier system having 18.2 MΩ cm resistance. Calcium hydroxide, phosphoric acid, 3, 5-dinitrosalicylic acid, phenol and sulphuric acid are analytical grade (Merck®India). Standard of inulin was procured from Sigma-Aldrich.

### 2.2. Instrumentation details

Ultrasonicator (VCX-750, Sonics, Sonics and Materials Inc., Newtown, USA) with 20 KHz frequency was used for the UAE purpose. It includes ultrasonic processor with a titanium probe of 13 mm diameter with amplitude (100 %) of 114 μ.Domestic microwave (CE2933, Samsung) working at power level ranged between 300−900 W and frequency of 2450 MHz.

Chromatographic analysis was done by HPLC (Waters Alliance 2695 separation module, equipped with the amino column (Waters, 250 × 4.6 mm, 5 μ), using ELSD (model 2424).

A pH meter and a UV–vis spectrophotometer (Analytik Jena AG, Germany) were used for pH and spectrophotometric analysis respectively. Centrifuge (Z326 K, Hermle AG, Germany) was used for the separation of precipitate.

### 2.3. Extraction procedure

#### 2.3.1. Ultrasound assisted extraction

Powdered material was subjected to probe ultrasound (VCX-750, Sonics, Sonics and Materials Inc., Newtown, USA) of 20 KHz frequency at 60, 80 and 100 % amplitude using three different solvent (water)-solute ratio (3.5:1, 4.5:1, 5.5:1 v/w) under extraction time of 120 s, 150 s and 180 s. Total 15 combinations were reported as output from RSM analysis based on these extraction parameters and yield of inulin was obtained for each combination. Further, inulin was obtained from the water extract of tuber powder according to Li's method [14]. Firstly $Ca(OH)_2$ was added to the extract up to pH of 11 to precipitate the protein portion. After completion of the step, $H_3(PO_4)_2$ was added to lower the pH to 8. The whole content was centrifuged at 10,000 rpm for 5 min to get the supernatant. Finally inulin powder was obtained by precipitating in excess ethanol followed by freeze drying (Labconco, USA) at -80°C.

Total sugar content and reducing sugar content were measured adapting Phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and 3, 5-dinitrosalicylic acid method (DNS) (Miller, 1959). The difference between these two values is the inulin present in the extract that was presented as percentage value as per dry weight of tuber sample.

#### 2.3.2. Microwave assisted extraction

Design of extraction method using microwave is similar to ultrasound assisted extraction where power of microwave were taken as 300 W, 600 W and 900 W along with solvent-solute ratio of 3.5:1, 4.5:1 and 5.5:1 under extraction time of 120 s, 150 s and 180 s. Fifteen different combinations were obtained by RSM analysis and the same were used for extraction of inulin. Yield of inulin was obtained for each combination following the process described earlier in Section 2.3.1.

#### 2.3.3. Conventional hot water extraction

Inulin was also extracted by heating the tuber tissues (0.5 kg) with water (1.5 L) at 90°C for 30 min along with homogenization (5000 rpm). It was repeated twice in order to complete the extraction. All the extracts were combined, filtered and processed in the way similar to other methods (discussed in Section 2.3.1) to obtain yield of inulin.

### 2.4. Optimization using response surface methodology

Box-Behnken design was used for the optimization experiment of inulin production from tuberous root of yam bean. The analysis based on BBD generally consider second order response surface model (quadratic polynomial). As model lack-of-fit was significant after using second order response surface model which ideally should remain non-significant, partial third order or cubic polynomial model can be used for fitting data based on BBD till degrees of freedom can be ensured for estimating the experimental error. Dependent variables used in the present study were coded and depicted in Table 1 and the layout of

**Table 1**
Variables (actual and coded) used for the experimental design for UAE experiment (A) and MAE experiment (B).

| Coded variable levels | | | Independent variable |
|---|---|---|---|
| 1 | 0 | −1 | |
| **A** | | | |
| 100 | 80 | 60 | Amplitude (%) |
| 180 | 150 | 120 | Time (Sec.) |
| 5.5 | 4.5 | 3.5 | Solvent/solute ratio |
| **B** | | | |
| 900 | 600 | 300 | Power (W) |
| 180 | 150 | 120 | Time (Sec.) |
| 5.5 | 4.5 | 3.5 | Solvent/solute ratio |

required experiments were done according to Table S1. Design Expert software (version 9.0.6.2) was used for the analysis of the whole experiment. Optimised data generated by the RSM was validated by real time experimental data.

Genetic algorithms are another approach to optimize the experimental condition. It is based on natural evolution and it basically imitate the Darwin's principle of "survival of the fittest". Complex optimization problems can be solved using genetic algorithms. Recently, number of studies used this technique to optimize experimental parameters (Hatami, Meireles, & Zahedi, 2010; Muthusamy, Manickam, Murugesan, Muthukumaran, & Pugazhendhi, 2019; Sodeifian, Sajadian, & Ardestani, 2016). Genetic algorithm, pioneered by Holland, is mainly used in optimization for its accuracy. Unlike other optimization techniques, it does not require initial values for the experimentation. Here, optimization was done by using exponential second order response surface polynomial based on GA approach. Optimization using non-linear model requires initial parameter values. For the present investigation, the initial parameter values for the exponential second order polynomial were obtained by fitting the exponential second order polynomial model based on the data obtained through experimentation carried out using Box-Behnken Designs used in the present study.

GA experiments were carried out in SAS® Proprietary Software 9.4 (TS1M1) by SAS Institute Inc., Cary, NC, USA. Genetic algorithm approach for optimization was carried out in R version 3.4.4.

### 2.5. SEM analysis

In order to see the effectivity of extraction procedure and disruption caused during the extraction process, surface structure of root powder of *Pachyrhizus erosus* was observed under SEM (CarlZeiss Evo-MA-10, operating at 10.0 KV/EHT) before and after the individual experiment. Three samples (UAE, MAE and conventional method) along with the initial material was used for recording of SEM image. Sample was prepared for the analysis by mounting approximately the material (0.5 mg) in powdered form on an aluminium stub having sputter-coating with palladium layer.

### 2.6. Purity profiling of inulin

For the purity estimation of inulin, free fructose present in the extracted inulin was estimated by spectrophotometrically using the method described by Saengkanuk, Nuchadomrong, Jogloy, Patanothai, and Srijaranai (2011) as well as by HPLC. For the estimation of total fructose and glucose, inulin extract as hydrolysed by 0.2 mL $L^{-1}$ HCl at 100 °C for 45 min. The hydrolysate was estimated for fructose and glucose concentration after neutralizing with NaOH solution. Inulin content in the extracted materials was calculated by following the method by Saengkanuk et al. (2011).

Chromatographic separations of hydrolysed inulin was performed by isocratic elution with 90 % A/10 % B solvent system where A and B was 80/20 acetonitrile/water with 0.2 % triethylamine and 30/70 acetonitrile/water with 0.2 % triethylamine respectively with flow rate of 1.0 mL $min^{-1}$. Gain set for ELS detector was 100 with nitrogen gas pressure of 35 psi. Inulin was hydrolysed and the fructose content was measured by HPLC.

Effect of temperature and influence of ultrasound/microwave on the degree of polymerization in inulin was also evaluated at two extreme condition of UAE and MAE, used in the RSM experiment.

### 2.7. Assessment of prebiotic effect

#### 2.7.1. Growth curve of microbe taken for prebiotic assessment

For this prebiotic effect evaluation of inulin, pure culture of *Lactobacillus fermentum* was used, which was maintained by Division of Microbiology, ICAR-IARI, New Delhi. The microbial strain was grown at 35 °C under anaerobic condition using de man Rogosa Sharp (MRS) broth. Growth of the microbe was monitored at regular time interval by measuring optical density (OD) of the medium at 622 nm.

#### 2.7.2. Prebiotic effect of inulin

Response of inulin as prebiotic was assessed by using the same culture media having similar MRS broth composition but with replacement of sugar with inulin. Growth of culture was also assessed in MRS broth with no carbohydrate source that was considered as control. Carbohydrate concentration was maintained at 2% level in all cases. The activated inoculum was incubated with 1% (v/v) and kept at 35 °C. Growth of the bacteria was observed at 12 h interval up to 72 h when growth of microbe was in stationary phase. OD values were measured as an indication of growth of bacterial culture. For further confirmation, bacterial count was also done by taking sample from each respective culture media by serial dilution method using 0.9 % NaCl solution.

### 2.8. Energy consumption

Energy density ($E_v$, J $mL^{-1}$) was calculated and compared between UAE and MAE methods. It is described as amount of energy dissipated per volume unit of extraction solvent (Chan, See, Yusoff, Ngoh, & Kow, 2017). It is measured by the following equations.

$$E_{\mathrm{v}} = P_v\ \mathrm{t} \tag{1}$$

$$P\mathrm{v} = \frac{\mathrm{m}.Cp.\ \frac{\partial T}{\partial t}}{\mathrm{V}} \tag{2}$$

Where $P_v$ is the power density (Wm$L^{-1}$); t is the extraction time (sec); m is the mass (g) of the sample; $C_p$ is the specific heat of water (4.186 J $g^{-1}$ °$C^{-1}$); $\frac{\partial T}{\partial t}$ is the heating rate (°C $s^{-1}$) during the execution of the experiment and V is the total volume (mL) of the sample.

Analysis for energy consumption is prerequisite for any technology, which has the potential to be scaled upto industry level. Total energy consumption was calculated based on the consumption of electricity by each experiment. Carbon emission was calculated by considering the fact that 1KWh produces 0.8 kg of $CO_2$.

## 3. Results and discussion

### 3.1. Comparison of extraction methods

Selection of *Pachyrhizus erosus* tubers for extraction of inulin was done with the purpose of valorization of the crop. The crop is underutilised although it is grown in different parts of the globe.

Extraction yield of inulin from *Pachyrhizus erosus* tuberous root was done by conventional hot extraction, MAE and UAE was varied across 9.9, 10.2–11.2, 10.3–11.9 % respectively. Conventional extraction was done by hot water refluxing for 30 min. Extraction efficiency did not improve upon increase in duration. Further, initial soaking for a fixed time followed by extraction or homogenization prior to extraction did not enhance extraction efficiency significantly. Better extraction of phenolic components from *Tagetes erecta* was reported by Kazibwe, Kim, Chun, & Gopal (2017), where hot water extraction, waterbath sonication and ultrafast ultrasonication were compared. Ultrasonic cleaning bath and probe system can be efficient source of extraction for better yield. Alzorqi, Sudheer, Lu, and Manickam (2017) compared hot water, Soxhlet and UAE of polysaccharides from *G. lucidum* mushroom and the result revealed that extraction yield of the polysaccharide was 63.4, 107.1 and 80.9 mg, respectively.

UAE was done by varying frequencies, time and solute to solvent ratio keeping temperature constant at 40 °C.Variations in inulin yield was recorded across all the variables (Fig. S1). Maximum extraction (12.2 %) efficiency was observed in the experiment where 100 % amplitude was used for three minutes with solute to solvent ratio of 1:4.5 and it was 19.2 % more than the conventional extraction.

Better extraction in UAE was provided by the energy delivered by the ultrasonic waves, which helped to penetrate the solvent inside the matrix, whereas, temperature governed the extraction in case of MAE. Extraction efficiency was maximum (11.2 %) in that experiment where microwave power of 900 W was exposed for 150 s with solvent to solute ratio of 5.5. it was observed that 13.5 % more extraction efficiency in MAE than conventional method. Highest pectin yield from grapefruit was recorded in MAE (27.8 %) as compared to UAE (17.9 %), done in ultrasonic bath. In MAE, 900 W for 6 min interval was used for extraction, whereas, 25 min sonication in ultrasonic bath at 70 °C (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011). Better rupture of the cells followed by better penetration of solvent inside the matrix are the reasons for better extraction and it was confirmed by the SEM data presented in future sub section. Simultaneous ultrasonic-microwave assisted extraction of inulin required much shorter time than conventional method, when it was done in burdock root (Lou, Wang, Wang, & Zhang, 2009). Extraction time for conventional extraction and ultrasonic-microwave assisted extraction method was 60 and 300 s respectively, but the yield was a shade better in conventional method (99.8 mg $g^{-1}$) than the other method (99.0 mg $g^{-1}$). Upon increase in extraction time, the later method showed degradation of inulin. UAE provided better yield from roots of globe artichoke (Castellino et al., 2020). The study reported in general 33 % increase in extraction yield by UAE than conventional hot water extraction. It was also concluded that genetic and pedo-climatic variations do contribute to the extraction yield apart from extraction method. Milani et al. (2011) reported optimum extraction condition for isolation of inulin from *Arctium lappa* keeping amplitude, temperature, time and solute to solvent ratio as variable and it was concluded that amplitude played an important role during extraction. Inulin yield from the source was 12.3 and 24.3 % when extracted by hot water and ultrasonic assisted extraction techniques.

Both UAE and MAE generates significant amount of heat during extraction in short time leads to better solubility of extractants and better extraction (Plazzotta, Ibarz, Manzocco, & Martín-Belloso, 2020; Saikia, Mahnot, & Mahanta, 2016). Yansheng et al. (2011) reported neither particle size nor solid to solvent ratio influence the extraction efficiency but variation in microwave power influenced the extraction of lactones from *Ligusticum chuanxiong*.

the significant portions of variations explained by the model is 98.45 %. It is to be noted here that, the adjusted $R^2$ will increase if only significant variables included in the model. However, for the above model, the overall lack-of-fit also remains highly significant (p-value: 0.0041) at 1 % level of significance which is not desirable as from statistical point of view, the lack-of-fit which tests the goodness of fit of the model which should remain non significant for model to be fitted well. The non significance of lack-of-fit may be due the fact that the second order model is not exactly capturing all the variations in the data and if it is so then there is still better scope for model improvement.

The above model was improved and validated in the lab for the optimization. It is to be noted that, the data under consideration were obtained based on a 15 run Box-Behken Design (BBD) with three factors which is although enough for estimating all the 10 parameters (including intercept) of a quadratic model, but the same resources are not enough to estimate all the 20 parameters (including intercept) of a cubic model. However, the existing resources can be used to estimate some more additional parameters apart from all the 10 parameters of quadratic model. Keeping this mind the analytical situation in ultrasonication data, the final model fitting was done with the above quadratic model with additional parameters as $AC^2$ and $BC^2$. Therefore, in order to improve the performance of the model and keeping the resource constraint, the following non-hierarchical cubic model with $AC^2$ and $BC^2$ has been fitted again and the results are summarized as follows:

$$y = \beta_0 + \beta_1 * A + \beta_2 * B + \beta_3 * C + \beta_{11} * A^2 + \beta_{22} * B^2 + \beta_{33} * C^2 + \beta_{12} * AB + \beta_{13} * AC + \beta_{23} * BC + \beta_{133} * AC^2 + \beta_{233} * BC^2$$

From above Table 2, based on non hierarchical cubic model, it can be observed that the overall model is highly significant at 1 % level of significance with a p-value of <0.0001. All effects are also significant at 1 % level of significance except the interaction effect of B and C which remains significant at 5 % level of significance. For the fitted model, the lack of fit test remain totally non significant at 5 % level of significance which indicates that the model is the perfect fit. As a results the model is able to explain complete variation with data with both The $R^2$ and adjusted $R^2 = 1.00$. The final fitted model is:

$$Inulin\ \ yield(\%) = 21.31 - 0.06A - 0.07\,B - 4.19C + \left(4.17 \times 10^{-5}\right)AB - 0.01AC + 0.03\,BC + \left(6.79 \times 10^{-4}\right)A^2 + \left(1.85 \times 10^{-5}\right)B^2 + 0.47\,C^2 + \left(1.13 \times 10^{-3}\right)AC^2 - \left(3.60 \times 10^{-3}\right)BC^2$$

### 3.2. Optimization of the extraction condition using RSM and GA

#### 3.2.1. Extraction parameters for UAE

The analysis based on BBD (Box-Behken Design), which generally consider second order response surface model (quadratic polynomial). For three factors (amplitude (A), time (B) and solvent to solute ratio (C) in this experiment, the second order polynomial will look like

$$y = \beta_0 + \beta_1 * A + \beta_2 * B + \beta_3 * C + \beta_{11} * A^2 + \beta_{22} * B^2 + \beta_{33} * C^2 + \beta_{12} * AB + \beta_{13} * AC + \beta_{23} * BC$$

Here, the response y = inulin content (%).

Based on second order model fitting, it has been observed that for the present experiment dataset, the overall model, A and B are highly significant at 1 % level of significance. The quadratic effect of A i.e. $A^2$ also remains significant at 1% level of significance. All other effects remain non-significant at 5 % level of significance. The $R^2 = 0.9945$ for the model indicates the model is able to explain 99.45 % variability which is quite good. The adjusted $R^2 = 0.9845$ which is also quite good, indicates

**Table 2**
Analysis of variance (ANOVA) for the BBD fitted model for optimization of inulin by UAE optimization experiment.

| Source | Sum of square | df | Mean square | F |
|---|---|---|---|---|
| Model | 4.37 | 11 | 0.40 | 17876.73** |
| Frequency (A) | 0.30 | 1 | 0.30 | 13412.46** |
| Time (B) | 5.559E-003 | 1 | 5.559E-003 | 250.17** |
| Solvent (C) | 1.106E-003 | 1 | 1.106E-003 | 49.77** |
| AB | 2.500E-003 | 1 | 2.500E-003 | 112.50** |
| AC | 1.225E-003 | 1 | 1.225E-003 | 55.12** |
| BC | 2.250E-004 | 1 | 2.250E-004 | 10.12* |
| $A^2$ | 0.27 | 1 | 0.27 | 12262.62** |
| $B^2$ | 1.026E-003 | 1 | 1.026E-003 | 46.15** |
| $C^2$ | 1.097E-003 | 1 | 1.097E-003 | 49.37** |
| $AC^2$ | 1.012E-003 | 1 | 1.012E-003 | 45.56** |
| $BC^2$ | 0.023 | 1 | 0.023 | 1040.06** |
| Residual | 6.667E-005 | 3 | 2.222E-005 | |
| Cor Total | 4.37 | 14 | | |

*, ** significance at 5 % and 1 % respectively.

**Table 3**
Comparison between optimum conditions predicted by BBD and GA models for UAE and MAE.

| Approach | Inulin (%) | |
|---|---|---|
| | Predicted | Experimental* |
| $UAE_{BBD}$ | 12.23 | 11.97 |
| $UAE_{GA}$ | 12.24 | 12.15 |
| $MAE_{BBD}$ | 11.57 | 11.21 |
| $MAE_{GA}$ | 11.53 | 11.38 |

* average of three analysis.

Optimum point is fixed as 100 % amplitude, 180 s time and solvent to solute ratio of 4.5. It can be seen that, the optimum point maximize the inulin (%) and the predicted maximum value is 12.23 % with the maximum desirability value (Fig. S2). The optimum value may lie between 12.22–12.25%. Predicted values of the experiment was validated in laboratory and presented in Table 3.

$$Inulin\ \ yield(\%) = 19.11 + \left(7.49 \times 10^{-4}\right)A_1 - 0.07B_1 - 4.25C_1 + \left(1.17 \times 10^{-5}\right)A_1B_1 - \left(8.33 \times 10^{-4}\right)A_1C_1 + 0.03\,B_1C_1 + \left(5.93 \times 10^{-7}\right)A_1^2 - \left(1.30 \times 10^{-5}\right)B_1^2 + 0.47\,C_1^2 + \left(9.17 \times 10^{-5}\right)A_1C_1^2 - \left(3.60 \times 10^{-3}\right)B_1C_1^2$$

Out of three variables, interaction between two factors is presented in the form of 3D response surface curve and their contour plots (Fig. 1A–C). 3D plots depicts the interaction between two variables keeping the third factor constant. Here, only graph with second order interaction effects are plotted. Fig. 1A and D indicates that keeping solvent to solute at ratio 4.5, maxima with highest desirability lies towards the higher percentage of ultrasonication amplitude and Time (sec). Whereas, Fig. 2B and E indicates that keeping time at 180 s, maxima with highest desirability lies towards the higher values of amplitude and intermediate values of solute to solvent ratio. Fig. 3C and F indicates that keeping ultrasonication amplitude of 100 %, maxima with high desirability lies towards the higher values of time and intermediate values of solvent to solute ratio.

### 3.2.2. Extraction parameters for MAE

Similar experiment was conducted for MAE of inulin from the same matrix. Three factors for the BBD experiments are (microwave power ($A_1$), time ($B_1$) and solvent to solute ratio ($C_1$) is a response surface design. The analysis revealed that for the given dataset, the overall model, $A_1$ and $B_1$ are significant at 1% level of significance with p-values as 0.004, <0.0001 and 0.003 respectively. All other effects remain non-significant at 5 % level of significance except the interaction $A_1B_1$ (p-

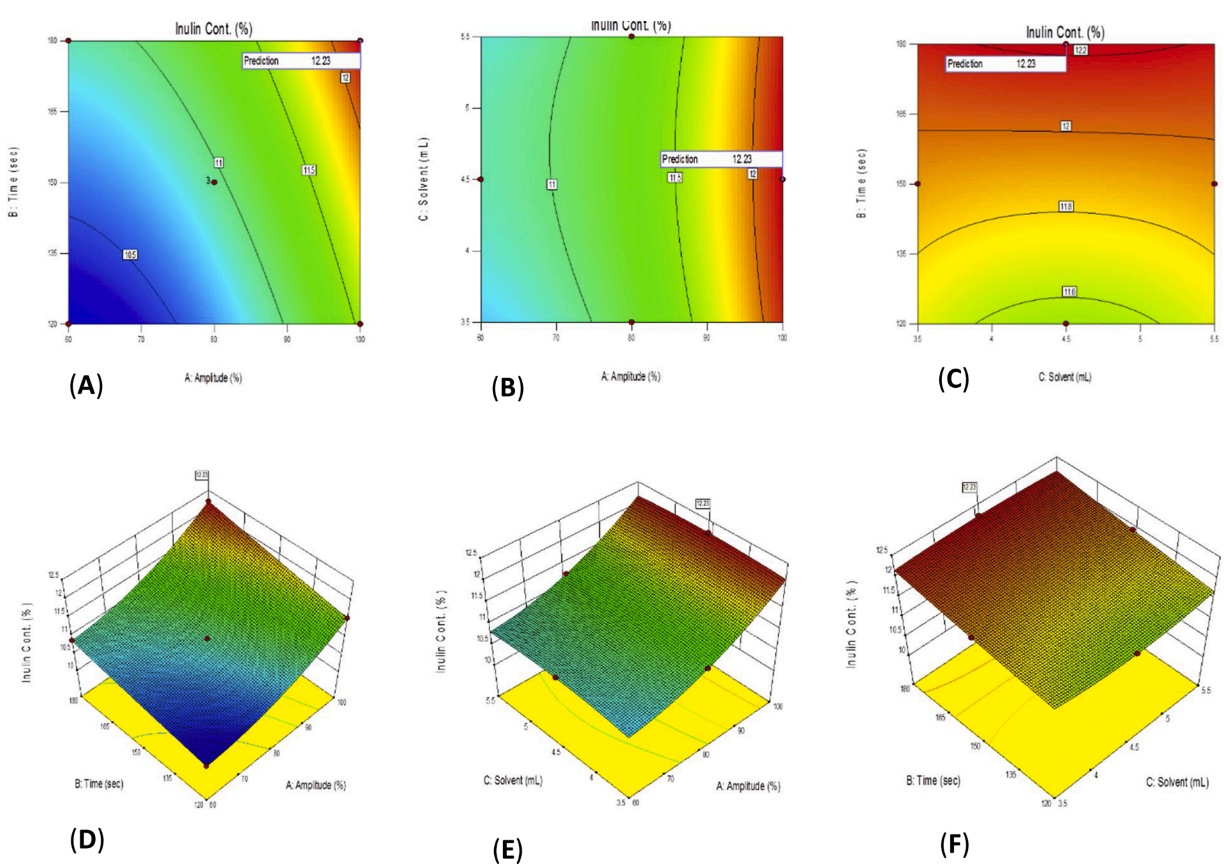


**Fig. 1.** Contour (A, B, C) and response surface plots (D, E, F) for the interaction between amplitude and time, amplitude and solvent, solvent and time in UAE optimization experiment.

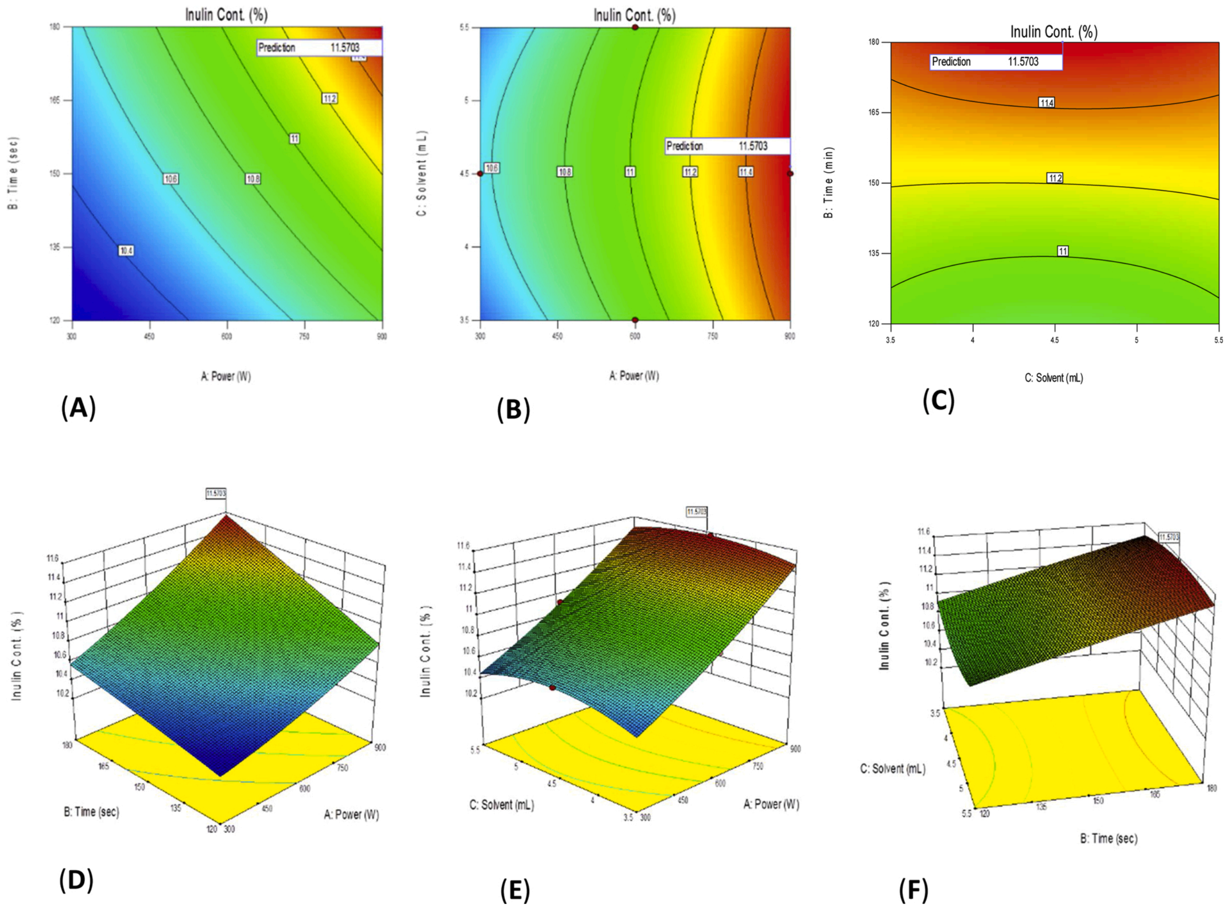


**Fig. 2.** Contour (A, B, C) and response surface plots (D, E, F) for the interaction between power and time, power and solvent, solvent and time in MAE optimization experiment.

value: 0.0310). The $R^2 = 0.9862$ for the model indicates the model is able to explain 98.62 % variability which is quite good and the adjusted $R^2 = 0.9614$. It is to be noted here that, the adjusted $R^2$ will increase if only significant variables included in the model. However, the overall lack-of-fit also remains significant (p-value: 0.0160) at 5 % level. The non-significance of lack-of-fit might be due to the fact that second order model is not exactly capturing all the variations in the data. Thus the model was further analysed to make lack of fit non-significant.

Like the analysis done in case of UAE experiment, by hit and trial method, different parametric combinations were considered and finally the model fitting was done with the above quadratic model with additional parameters as $A_1C_1^2$ and $B_1C_1^2$. So, the non-hierarchical cubic model with $A_1C_1^2$ and $B_1C_1^2$ has been fitted again and the results are summarized in Table 4. The model is highly significant at 1 % level of significance with a p-value of <0.0001. The linear effect of microwave power (p value <0.0001) and that of time (p value <0.0001) are highly significant at 1% level of significance whereas the linear effect of solvent to solute ratio remains significant at 5% level of significance. The quadratic effect of microwave power i.e. $A_1^2$ remains highly significant and the interaction effect $A_1B_1$ and $B_1C_1^2$ remains significant at 1 % level of significance whereas the effect of $A_1C_1^2$ (p value 0.0323) remains significant at 5 % level of significance. For the fitted model, the lack of fit test remain non-significant at 5 % level of significance which indicates that the model fitted really well. $R^2$ of 0.9998 and adjusted $R^2$ of 0.9992 indicates the model is now able to explain almost all the variability. The final fitted model is as follows.

Based on that, optimum point is fixed as 899.9 W of microwave power, 179.9 s time and solvent to solute ratio of 4.55. It can be seen that, the optimum point maximize the inulin % and the predicted maximum value is 11.57 % with the maximum desirability value (Fig. S3). The optimum value may lie between 11.54 to 11.60. The optimum point was validated in lab and presented in Table 3.

The two factor interaction wise contour plots and 3D plots are as follows [only graph with second order interaction effects are plotted]. Fig. 2A and D indicates that keeping time at 179.9 s, maxima with highest desirability lies towards the higher values of power and intermediate values of solvent (mL). Figs. 2B and 5 E indicates that keeping solvent at 4.5 mL, maxima with highest desirability lies towards the higher values of amplitude and time (Sec). Fig. 3C & F indicates that keeping strength at 899.9 W, maxima with high desirability lies towards the higher values of time and intermediate values of solvent to solute ratio.

### 3.3. Optimization of the extraction condition using GA

#### 3.3.1. Extraction parameters for UAE

Recently, genetic algorithm has been successfully explored for the optimization of parameters. Muthusamy et al., 2019 studied optimum extraction condition for the separation of pectin from sunflower heads by a genetic algorithm approach. Maximum experimental yield of pectin from the heads was 29.5 % as compared to 29.1 %, predicted by ANN coupled GA. Comparable prediction was recorded by RSM and ANN-GA approaches and both are found suitable for the optimization purpose. Sodeifian et al., 2016 also used both of these approaches to optimize extraction of essential oil from *Ferulago angulate* using supercritical fluid and it was concluded that ANN-GA models were found to be more

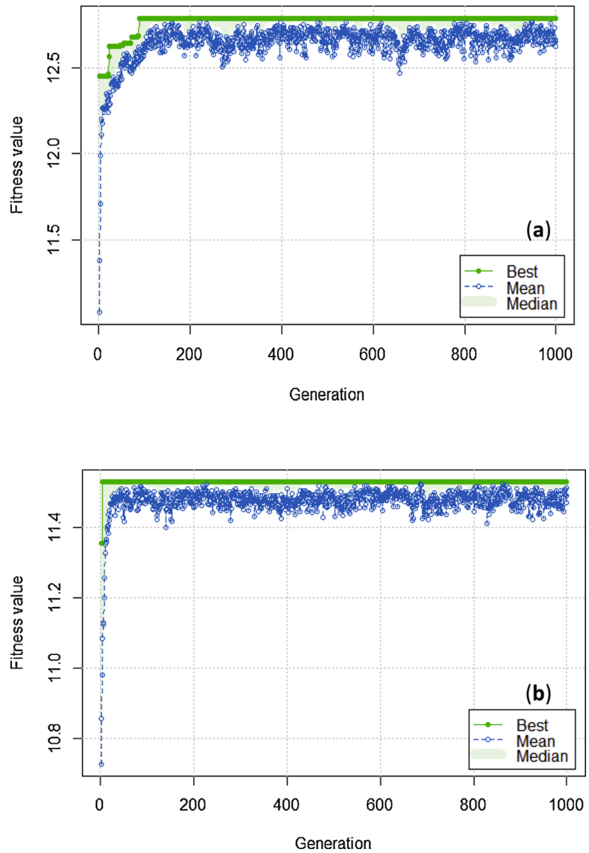


**Fig. 3.** Best fitness value vs. no. of generations in the GA experiment of UAE (a) and MAE (b) experiment.

**Table 4**
Analysis of variance (ANOVA) for the BBD fitted model for optimization of inulin by MAE optimization experiment.

| Source | Sum of square | df | Mean square | F |
|---|---|---|---|---|
| Model | 1.81 | 11 | 0.16 | 1559.52*** |
| Frequency (A) | 0.62 | 1 | 0.62 | 5912.53*** |
| Time (B) | 0.30 | 1 | 0.30 | 2865.79*** |
| Solvent (C) | 1.800E-003 | 1 | 1.800E-003 | 17.05** |
| AB | 0.044 | 1 | 0.044 | 417.79*** |
| AC | 2.500E-005 | 1 | 2.500E-005 | 0.24 |
| BC | 2.500E-005 | 1 | 2.500E-005 | 0.24 |
| $A^2$ | 0.011 | 1 | 0.011 | 99.50*** |
| $B^2$ | 5.026E-004 | 1 | 5.026E-004 | 4.76 |
| $C^2$ | 3.103E-004 | 1 | 3.103E-004 | 2.94 |
| $AC^2$ | 1.513E-003 | 1 | 1.513E-003 | 14.33** |
| $BC^2$ | 0.023 | 1 | 0.023 | 218.96*** |
| Residual | 3.167E-004 | 3 | 1.056E-004 | |
| Cor Total | 1.81 | 14 | | |

*significance at 10 %, ** significance at 5 %, *** significance at 1 %.

**Table 5**
ANOVA of the non-linear model of GA for optimization of inulin by UAE and MAE.

| Source | DF | Sum of Squares | Mean Square | F Value | p value |
|---|---|---|---|---|---|
| **UAE** | | | | | |
| Model | 10 | 1874.0 | 187.4 | 4351.16 | <.0001 |
| Error | 5 | 0.2153 | 0.0431 | | |
| Uncorrected Total | 15 | 1874.2 | | | |
| **MAE** | | | | | |
| Model | 10 | 1741.9 | 174.2 | 37754.6 | <.0001 |
| Error | 5 | 0.0231 | 0.00461 | | |
| Uncorrected Total | 15 | 1741.9 | | | |

accurate than RSM, although both the approaches showed good agreement with the experimental data. Optimised extraction yield was 0.85 and 0.86 % by the RSM and ANN-GA models respectively and it was comparable to the experimental yield (0.87 %). Hatami et al., 2010 explored genetic algorithm for the optimization of pressure and temperature for the supercritical fluid extraction of oil from clove bud using $CO_2$ as extraction solvent. Pressure and temperature was optimized for the maximum extraction of clove oil by GA approach.

For the second order response surface model fitted to the data, lack of fit remains significant at 5% level of significance. As a result a non-hierarchical third order polynomial have been fitted. Alternatively, an exponential form of second order response surface model as follows was also fitted to the data which lead to non-linear model fitting.

$$y = e^{\left(\beta_0+\beta_1*A+\beta_2*B+\beta_3*C+\beta_{11}*A^2+\beta_{22}*B^2+\beta_{33}*C^2+\beta_{12}*AB+\beta_{13}*AC+\beta_{23}*BC\right)}$$

Here, A: Amplitude, B: Time and C: Solvent to solute ratio

The non-linear model fitting was done through iterative procedure using Gauss-Newton method of non-linear least square. The convergence criteria satisfied after 5 iteration. The model remains highly significant at 1% level of significance. The estimated parameters are presented in Table 5 and Table S2.

The fitted equation is as follows:

$$Inulin\ yield(\%) = e^{\left[2.47-\left(4.10\times10^{-3}\right)A-\left(2.98\times10^{-2}\right)B+0.06C+\left(3.33\times10^{-6}\right)A^2 +\left(9.76\times10^{-5}\right)B^2 -0.006C^2+\left(1.75\times10^{-5}\right)AB-\left(5.70\times10^{-5}\right)AC +\left(2.00\times10^{-4}\right)BC\right]}$$

Since the model is highly significant, as a result the fitted model was used for genetic algorithm optimization for finding optimal solution.

Genetic algorithms, a mathematical model inspired by the famous Charles Darwin's idea of natural selection is being used for the optimization. Principle of natural selection illustrates the preservation of only the fittest individuals, over different generations. An evolutionary algorithm which improves the selection over time. Basic concept of GA is to combine the different solutions, generation after generation to extract the best information for each one. Advantage of this approach over other optimization methods is that it allows the best solution to emerge from the best of prior solutions. That way it creates new and more fitted individuals. The GA approach has been effectively used in optimization problem.

GA produces random solution in the first generations if there is no seed values (starting solutions) are provided. - Best solutions, with least or most return value based on the nature of optimization, are picked on which genetic operators is applied to produce a new solution as part of the second generation. GA produces more unique random solutions in the second generation. This process continues until the most optimal solutions is reached or the generation hard limit is reached.

GA has two basic genetic operators which are Cross Over and Mutation. Cross Over: Two parent solutions are selected and their attributes are swapped to produce modified child solutions. Mutation: A parent solution is picked and altered to produce a better solution.

The fitted non-linear second order response surface model as given above was considered as objective function. After 1000 iteration, optimization results are summarized as follows:

| | | | |
|---|---|---|---|
| **GA settings** | | | |
| Type | Real valued | | |
| Population size | 50 | | |
| Number of generations | 1000 | | |
| Elitism | 2 | | |
| Crossover probability | 0.8 | | |
| Mutation probability | 0.1 | | |
| Search domain | | | |
| | A | B | C |
| Lower | 60 | 120 | 3.5 |

(continued on next page)

(*continued*)

| | | | |
|---|---|---|---|
| Upper | 100 | 180 | 5.5 |
| **GA Results** | | | |
| Iterations | 1000 | | |
| **Fitness function value** | **12.24945** | | |

The optimum solution was obtained with 0.8 crossover probability which is quite high. The mutation probability is 0.1 which is in lower side as desirable. The final fitness value for the optimum solution is 12.25. The optimum combination comprised of ultrasonic amplitude of 100 %, time of 180 s and solvent to solute ratio of 5.59 for the inulin yield of 12.79 %.

It is to be noted here that, the fitness function value itself is the optimal value at the optimal solution point as obtained through the genetic algorithm approach. Iteration results are presented in Fig. 3a. The predicted value was validated in the laboratory and the result is presented in Table 3.

### 3.3.2. *Extraction parameters for MAE*

For optimization of MAE also, similar experiment was planned and analysed the data using genetic algorithms. The estimated parameters are presented in Table 5 and Table S3. The fitted equation in this case is as follows:

$$Inulin\ \ yield(\%) = e^{\left[2.25-\left(8.00\ \times 10^{-4}\right)A_1+\left(4.99\ \times 10^{-3}\right)B_1+0.008C_1+\left(4.79\ \times 10^{-7}\right)A_1^2 -\left(1.52\ \times 10^{-5}\right)B_1^2-\left(8.80\ \times 10^{-3}\right)C_1^2+\left(1.01\ \times 10^{-5}\right)A_1B_1-\left(9.70\ \times 10^{-6}\right)A_1C_1+\left(6.80\ \times 10^{-5}\right)B_1C_1\right]}$$

Here, A: Power, B: Time and C: Solvent to solute ratio. Since the model is highly significant, as a result the fitted model was used for genetic algorithm optimization for finding optimal solution.

| | | | |
|---|---|---|---|
| **GA settings** | | | |
| Type | Real valued | | |
| Population size | 50 | | |
| Number of generations | 1000 | | |
| Elitism | 2 | | |
| Crossover probability | 0.8 | | |
| Mutation probability | 0.1 | | |
| Search domain | | | |
| | $A_1$ | $B_1$ | $C_1$ |
| Lower | 300 | 120 | 3.5 |
| Upper | 900 | 180 | 5.5 |

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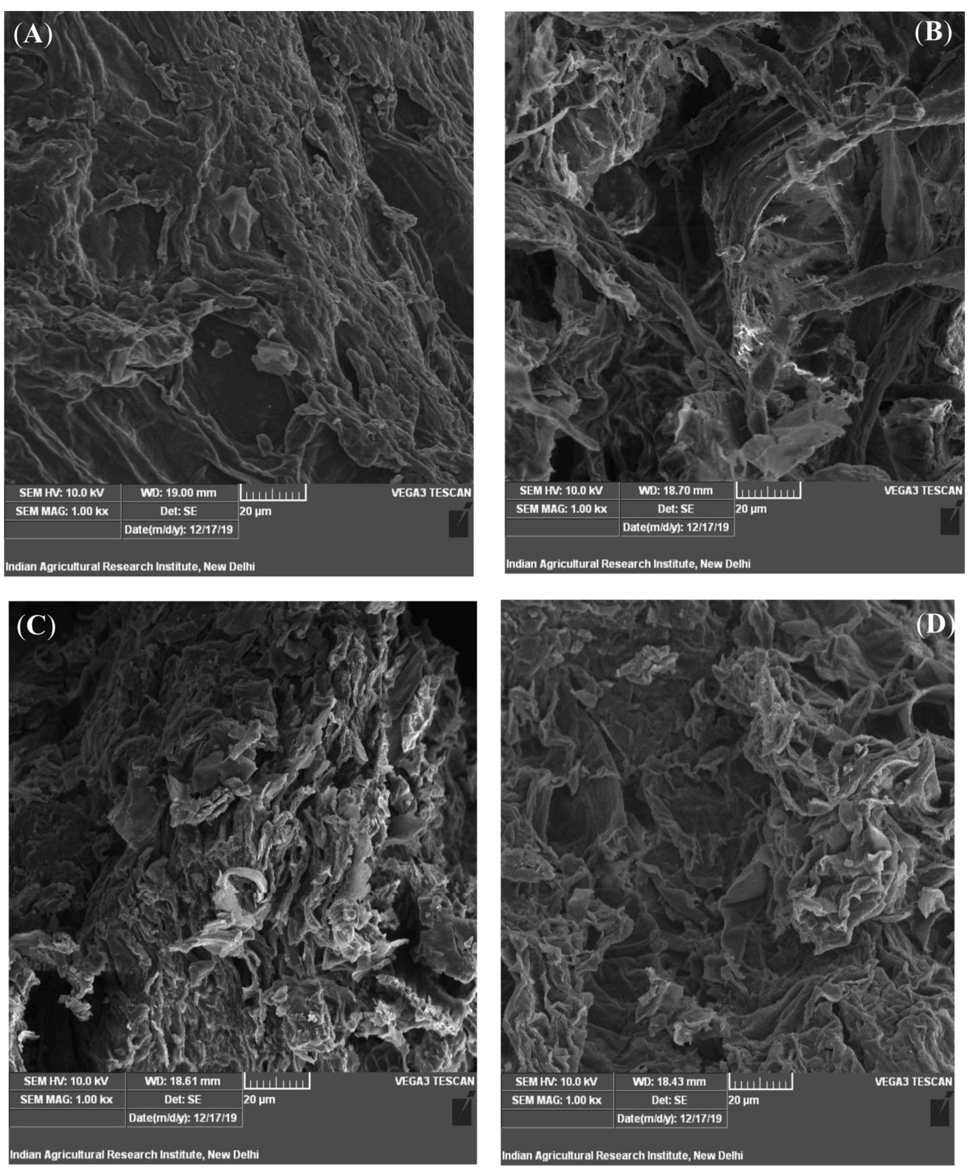


**Fig. 4.** SEM images of raw (A), UAE (B), MAE (C) and conventionally (D) extracted residual material of *Pachyrhizus erosus* tuberous root.

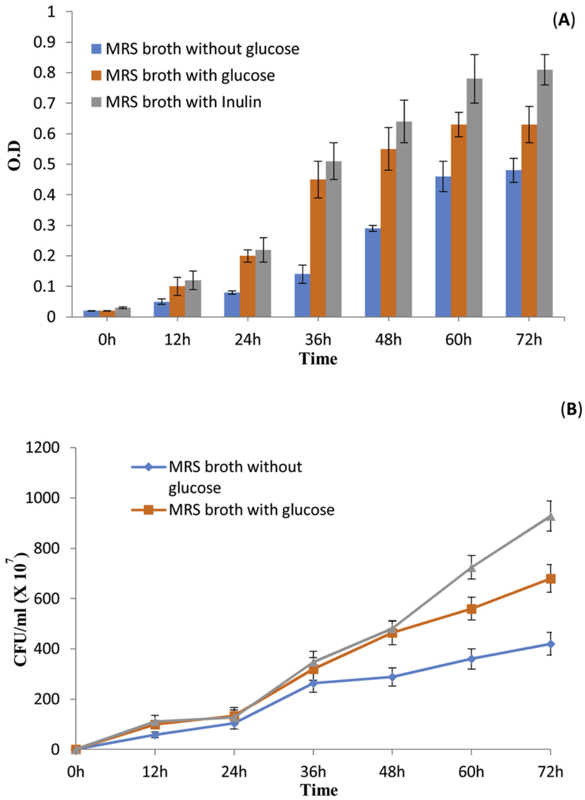


**Fig. 5.** O.D values (A) and bacterial count (B) in samples of culture media with no carbon source (without glucose), with glucose and with inulin.

(*continued*)

| | |
|---|---|
| **GA Results** | |
| Iterations | 1000 |
| **Fitness function value** | **11.53069** |

The optimum solution was obtained with 0.8 crossover probability which is quite high. The mutation probability is 0.1 which is in lower side as desirable. The final fitness value for the optimum solution is 11.53069. The optimum combination is as follows:

The optimum combination comprised of microwave power of 900 W, time of 180 s and solvent to solute ratio of 5.22 for the inulin yield of 11.53 %. Iteration results have been presented in Fig. 3b. The data came out of the analysis was required to be validated and thus the same was done in the laboratory and the result is presented in Table 3.

### 3.4. Scanning electron microscopy

Images of untreated root powder of *P. erosus* along with UAE, MAE and conventionally extracted residual materials are presented in Fig. 4A–D. Slight rupture of the outermost cells were observed in conventionally extracted residual material, when we compare with the untreated material. But significant changes in the outermost cell structure were observed in the UAE and MAE extracted residual materials. MAE and UAE technique produced severe rupture in the leaf cells while extraction of polyphenols from *Myrtus communis* leaves, which was evidenced from the SEM picture.

Interestingly, disintegration pattern in UAE and MAE are different. Dong et al., 2016 also reported similar observation while extraction of polysaccharides from *Chuanminshen violaceum*. Ultrasonic soundwaves seems to rupture more intensely than all other extraction protocols. Acoustic cavitation led to the severe damage of the outermost cells and helped in formation of bigger cracks at surface, which enhanced the maximum release of inulin from the matrix to the bulk solvent. Better disintegration facilitated better penetration of solvent inside the matrix, followed by acoustic cavitation yielded better extraction efficiency (Xia et al., 2011).

Whereas, the pattern was different in MAE residual material, where more disintegration at surface level was observed. Texture was crumbled in a significant manner (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). Microwave irradiation along with rise in temperature helped the disintegration and thus release of inulin in the adjacent solvent facilitated. Scanning electron micrograph of *P. radiata* bark upon Soxhlet, UAE and MAE of phenolics showed significant cell destruction (Aspé & Fernández, 2011).

Better yield in UAE and MAE method than conventional one was attributed to disruption of cellular structure followed by better penetration of solvent inside the matrix with acoustic cavitation/enhanced temperature. On the contrary, diffusion of solvent inside the matrix by following Fick's law of diffusion, which led to solubilization of inulin and mass transfer to the bulk solution is the major mechanism in conventional extraction method. 3.5. Purity and degree of polymerization of inulin

HPLC chromatogram of two monomers i.e. fructose and glucose present in inulin was obtained (Fig S4.) upon acidic hydrolysis with 0.1 % of HCl. Retention time of both the sugars were confirmed by running respective standards under similar condition. Being fructo-oligosaccharide, fructose is the major fraction with glucosyl moiety at terminal end (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2016). The obtained chromatogram supports the fact and confirmed. Kristo, Foo, Hill, & Corredig, 2011 estimated inulin in dairy matrix by using LC with evaporative light scattering detector with good repeatability and reproducibility. The investigation used inulinase for the hydrolysis of inulin.

Purity per cent of inulin was measured for UAE and MAE extracted inulin. In both the methods, minimum and maximum exposure condition was selected viz. for UAE, 60 % amplitude for 120 s and 100 % amplitude for 180 s; for MAE 300 MHz for 120 s and 900 MHz for 180 s for the comparison study. Data of the experiment is presented in Table 6. In general, there is no difference between maximum and minimum exposure condition of UAE and MAE. Whereas, a shade difference was observed between UAE and MAE in terms of purity of inulin. Purity was least (56.7 %) in the conventionally extracted inulin, which might be attributed to more extraction of unwanted materials due to long exposure time at boiling condition of water.

There are a few reports on effect of processing specifically heating on the degradation of inulin. Hydrolysis kinetics of fructo-oligosaccharide was studied across pH range and temperature (80–120 °C) (L'homme, Arbelot, Puigserver, & Biagini, 2003). At 90−100 °C, complete degradation of fructo-oligosaccharide oligomers was reported in 1–1.5 h. (Matusek, Merész, Le, & Örsi, 2009). In the present study, degree of polymerization was maximum in UAE extracted inulin and it was superior than the inulin extracted by MAE and conventional methods. It might be attributed to more heating in MAE and conventional methods.

**Table 6**
Purity (%) and effect of UAE/MAE on the degree of polymerization of inulin.

| Sample | Total fructose (%) | Free fructose (%) | Total glucose (%) | Inulin (% purity) | Degree of Polymerisation |
|---|---|---|---|---|---|
| $M_{300}T_{120}$ | 68.54 | 1.26 | 4.61 | 66.94 | 15.87 |
| $M_{900}T_{180}$ | 66.75 | 1.24 | 4.52 | 65.18 | 15.77 |
| $U_{60}T_{120}$ | 76.34 | 1.27 | 4.82 | 74.69 | 16.84 |
| $U_{100}T_{120}$ | 73.31 | 1.25 | 4.78 | 71.70 | 16.34 |
| Conventional method | 58.3 | 1.32 | 3.98 | 56.70 | 15.65 |

$M_{300}T_{120}$ and $M_{900}T_{180}$ represents inulin extracted by microwave with power of 300 MHz for 120 s and 900 MHz for 180 s. $U_{60}T_{120}$ and $U_{100}T_{120}$ represents ultrasound amplitude of 60 % for 120 s and 100 % for 180 s.

### 3.5. Prebiotic activity

In this study first the growth of the microbe i.e. *Lactobacillus fermentum* was observed to decide incubation period to perform prebiotic activity. It has been seen that the lag phase lasted for 18 h. Then the strain grew exponentially which suggested it was in logarithmic phase. After that stationary phase began at 60 h and they started to decline after 72 h. That's why prebiotic activity was studied up to 72 h after culture fermentation to understand the effect of inulin over microbial population.

Fig. 5 displays increase in OD value of culture medium fermented 72 h with pectin extracted from different citrus peels as sources of carbon. Higher OD value denotes better growth of microbe and efficient utilization of inulin. During logarithmic phase (up to 48 h) as microbes grew rapidly, there was minute variation between OD value of medium added with glucose and inulin as carbon source. But in the stationary phase (up to 72 h) OD value was distinctly higher in case of medium enriched with inulin compared to glucose as carbon source. This type of trend indicates effective fermentation of inulin by microbe over longer period of time.

Rubel, Pérez, Genovese, & Manrique, 2014 studied in-vitro prebiotic analysis of inulin from Helianthus tuberous L. using *Lactobacillus paracasei* and found significant prebiotic activity. As these fructo-oligosaccharides substances get fermented, different organic acids are formed that help to increase the microbiome, make these substances an effective prebiotic ingredient. Similar reports were reported by Caleffi et al., 2015, where Pfaffia inulin was found to be highly active as prebiotic when evaluated using bifidobacterial and lactobacillary populations.

The present result was further confirmed by counting bacterial colony from each respective culture media having glucose and inulin as carbon source along with control that showed similar kind of trend as of before (Fig. 5B). Higher bacterial population was observed in case of inulin than others up to 72 h, showed nearly 36.4 % increase compared to glucose as carbon source.

### 3.6. Energy density and energy consumption

Energy density was measured for optimal condition of UAE and MAE experiment in order to compare between two extraction principles. To compare the efficacy of these two extraction techniques, energy density was evaluated. Ev is the energy dissipated to the system provided by the extracting system. From the energy density value, efficient extraction system can be selected. $E_v$ delivered by optimized condition of MAE is far greater than UAE conditions. Thus, UAE proved to be energy efficient. Present result is in agreement with the results reported by Chen et al. (2018) and Plazzotta et al. (2020). These investigations used *Orthosiphon stamineus* fruit, citrus peel and peach waste as matrix for the UAE.

Analysis for energy consumption is prerequisite for any technology, which has the potential to be scaled upto industry level. Energy consumption pattern in each experiment is presented in Table 7. Total energy consumption was maximum in conventional method (180 KJ) and UAE experiments showed to be energy efficient as compared to MAE experiments. The same trend was reflected in the carbon footprint pattern also. Ultrasonication process generates ultrasound, mechanical acoustic waves and it produces acoustic cavitation during extraction. Significant amount of energy is being required to generate it and after cell disruption the energy is converted into thermal energy. Whereas, for the microwave radiation more energy is required to produce it. Similar observation was reported by Jacotet-Navarro et al. (2015). For industrial viability, requirement of energy and the emission of carbon are to important parameters needs to be evaluated. Vinatoru, Mason, & Calinescu (2017) reported in a review that 50–90 % reduction in foot print in MAE over conventional method.

**Table 7**
Comparison of energy consumption.

| Approach | Total energy consumption (KJ) | Energy density (J $mL^{-1}$) | Energy/ biomass (MJ $Kg^{-1}_{Biomass}$) | Carbon emission (kg $CO_2$ kg $extract^{-1}$) |
|---|---|---|---|---|
| $UAE_{BBD}$ | 90 | 18.61 | 90 | 20.00 |
| $UAE_{GA}$ | 90 | 18.61 | 90 | 20.00 |
| $MAE_{BBD}$ | 162 | 55.83 | 162 | 36.00 |
| $MAE_{GA}$ | 162 | 44.29 | 162 | 20.00 |
| Conventional | 180 | – | 180 | 40.00 |

## 4. Conclusion

Process intensification for the production of inulin was successfully optimized by UAE and MAE techniques by optimising the all the variables. Amplitude of ultrasonicator/power of microwave oven, time and solute to solvent ratio were optimized by GA and RSM techniques. Better extraction was achieved by UAE as compared to MAE and both these techniques were better than conventional hot extraction. Both these techniques provided comparable inulin yield. Genetic algorithm approach commensurate the optimized data, produced by RSM. Thus both or either one can be used for the optimization of inulin from the matrices. Reason behind the better extraction by UAE was confirmed by the SEM analysis of the matrices. SEM picture of the matrices after extraction revealed clear picture about the style of disruption on the cellular structure. Microfractures were observed in root tissues extracted by UAE, whereas surface modification was observed in MAE materials. When the extracted materials were compared with the initial root tissues, difference in their rupture pattern was clearly observed.

Interestingly, UAE provided a shade better purity of extracted inulin than other two techniques. Degree of polymerization in inulin was also recorded to be better. Higher temperature in MAE and conventional method might be attributed to slight degradation of inulin. Significant prebiotic activity was recorded while evaluation using *Lactobacillus fermentum* and it was 36 % more than glucose treatment. Enhancement in microbial count significantly confirmed the activity.

When both these technologies were compared for the energy efficiency, UAE provided far lesser energy density than MAE. Carbon emission was also comparatively a shade lesser in UAE experiments than other techniques. Thus UAE can be considered to be more feasible for mass production at industrial level and it should be sustainable in long run.

## CRediT authorship contribution statement

**Rohan Sarkar:** Investigation, Data curation, Formal analysis, Writing - original draft.
**Arpan Bhowmik:** Conceptualization, Investigation, Methodology, Software, Resources, Validation.
**Aditi Kundu:** Supervision, methodology, Visualization, Writing-Editing, Writing - review & editing.
**Anirban Dutta:** Supervision, Methodology, validation, Visualization, Writing - review & editing.
**Lata Nain:** Methodology, Formal analysis, Visualization, Resources.
**Goutam Chawla:** Data curation, Software, Writing - review & editing, Resources.
**Supradip Saha:** Conceptualization, Formal analysis, Funding acquisition, Writing - original draft, Writing - review & editing, Project administration, Resources.

## Declaration of Competing Interest

All the authors declare that there is no known competing financial interests or personal relationships that could have influence the investigation reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2020.117042.

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