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RESEARCH ARTICLE

CHARACTERIZATION AND PROCESS OPTIMIZATION OF ULTRASOUND EXTRACTED POLYSACCHARIDES FROM THE "OPUNTIA FICUS INDICA" CLADODES

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ABSTRACT

Opuntia Ficus Indica (OFI) cladodes are rich in pectin rich polysaccharides can be used nutraceutical, medical and pharmaceutical purposes as a cheap source of raw material. In this study characterization and Response Surface Methodology (RSM) of ultrasonic assisted extraction (UAE) of water-soluble crude polysaccharides (CPS) of OFI cladodes were studied using Box-Behnken Design (BBD). Effect of power intensity, set temperature and processing time investigated, Optimal conditions for yield maximization is found as 345.5 W, 304.5 K and 28.5 min. respectively with the predicted yield as 18.58% dry base polysaccharide extract and this value was validated by experiments at optimal condition having the value as 18.48±0.35%. General composition of the extracts at the optimal condition was 7.5±2.22% moisture content, 14.4±0.87 ash, 0.18±0.05 protein, 82.12±7.2% total sugar content (SC) in glucose, 72.75±6.8% SC in galacturonic acid in dry basis. Degree of esterification was found 40.57±3.11% proving that extracted polysaccharides was low-methoxy (LM) pectin-based material which can be used a good alternative and cheap source for industrial LM pectin. Additionally, this study depicts the importance of the temperature control and temperature rise of UAE systems since depending on power, time and temperature combination, temperature inside the reactor can be increased 7-11.8%.

Keywords: *Opuntia Ficus Indica, Cladode Polysaccharides, Response Surface Methodology, Ultrasound Assisted Extraction*

1. INTRODUCTION

Opuntia Ficus Indica (OFI) is a South American cactus species that comes from the *Cactacea* (cactus) family. Under the *Platyopuntia* species of the *Opuntia* species, it stands out with its altered and flattened branches called cladode which has thorns (1-2 cm) and glochids (0.15-0.2 cm) on the surface [1] OFI; Mexico, Iran, Tunisia, Eritrea, Ethiopia, Argentina, Peru, Bolivia, Brazil, America, Spain, Italy, Israel, Morocco, is grown in semi-arid countries such as South Africa and Turkey. OFI is known as Chumbera in Spain, Higo De Las Indias in India, Fico D in India in Italy, and Barbarie in France. Due to its high degree of ecological adaptation, this plant can be found in almost all climatic conditions [2].

OFI fruit, seed and cladodes are used in commercial areas as nutraceutical and functional products in many countries such as Mexico, Chile and Spain. The fruit part of OFI is consumed fresh or pre-processed; The seed part is used in the cosmetics industry in Morocco and Tunisia in products such as fruit juice, fruit juice concentrate, coloring food, powder products, soft drinks, jam and marmalade [2]. OFI cladodes are characterized by flat bodies with oval or elongated shape and exhibit photosynthetic properties. It can be used for various products such as high dietary fiber powders, juices and nectars, canned cladode products and brine. Young cladodes are consumed in Mexico and Chile as fresh vegetables or added to foods such as sauces, salads and soups. In addition, it is used to protect fresh fruits [3], encapsulating agent for bioactive components, gallic acid and betalains [4–6] and edible film formation [7, 8]. The main components of cladodes are carbohydrate polymers containing a mixture of pectin and mucilage. Looking at the chemical composition of the cladodes; It contains 91% moisture, 4.5% carbohydrates, 1.5% protein, 0.2% fat, 1.3% ash. In addition, it is reported that cladodes contain 11mg \ 100g vitamin C 30 µg \ 100g carotenoid with its rich dietary fiber content up to 43% [9–11] Since ancient times, cactus plants have been used to heal diseases and wounds. Today, it is seen that cactus fruits and the body part called cladodes are still used as therapeutic agents. Scientific data on the medicinal properties of cactus are constantly expanding [4, 12].

OFI has become the focal point of many researches nowadays as it has been involved in the treatment of various diseases such as diabetes, high cholesterol, high blood pressure, stomach diseases, and rheumatic pain in traditional folk medicine and in many countries. OFI contains bioactive components that strengthen the immune and nervous system, reduce oxidative stress-related diseases, sweep free radicals, reduce lipid peroxidation, and increase glutathione level [2, 13, 14].

OFI cladodes are composed of gel part and fiber part where high molecular weight polysaccharides (mucilage) are found. The mucilage in the cladodes; It has been reported to consist of arabinose, galactose, xylose and rhamnose and a small amount of galacturonic acid as neutral sugars. Therefore, cladodes are reported in the literature as rich in polysaccharides as pectin source [15, 16]. Pectin is a common additive in the food industry, which contains different amounts of methyl esters and dissolves in different degrees of neutralization, forming a gel-like structure with sugar and acid under favourable conditions. Apple pulp, orange peel, sunflower tray and sugar beet are the most common raw materials for pectin production. It is stated that the products obtained in the techniques applied in polysaccharide extraction from OFI cladodes are mostly low methoxy (LM) pectin. LM pectin form a gel in a wider pH range (pH: 2-6) with multivalent cations (such as Ca + 2) at sugar-free or low sugar concentrations [12, 17–19].

Extraction phenomenon is the way of obtaining polysaccharide from plant tissue by obeying mass transfer rules. Numerous methods can be applied to extract biological materials such as plant base polysaccharides. Among these methods, besides maceration with hot water or solvent extraction, supercritical fluid extraction, subcritical water extraction, enzymatic extraction, microwave-assisted or ultrasound-assisted extraction or combinations thereof can be said [19–25]. Although this process requires long times, high amount of chemical and energy in conventional techniques, emerging technologies such as ultrasound which eases this process while improving solvent penetration, enhances suspension quality and overcomes mass transfer limiting interface [26–28]. Ultrasound assisted extraction (UAE) is a “Green Chemistry” technique due to requiring minimum amount of chemical, energy and time at lower temperatures while ensuring comparable amount of yield compared to conventional techniques such as hot water or solvent extraction [23, 29].

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Sonication systems are divided into two application groups associated with applied power and sound frequency called high power low frequency and low power high frequency ultrasound. Industrial applications such as extraction require intensive energy to enhance heat and mass transfer. Applied low frequency high power (around 20kHz) sonicator is used in forms such as bath or probe systems. Probe systems satisfies intensive energy with high efficiency so that these systems are more useful than bath system especially for processes like extraction which requires strong mixing and cell wall disruption. In addition to mass transfer enhancement, in literature it was observed that sonication systems improve heat transfer regardless of changing conditions like viscosity and ensures stable transfer rates in whole reactor unlike conventional heating system which provides limited and diminishing heat transfer rates [30]. Ultrasound-assisted applications play a role in increasing extraction rate and efficiency [25, 31]. Extractable components appear in the tissue by being damaged, and ultrasound applications have been reported to increase the extraction kinetics and the quality of the extract [20]. Studies in the literature show that ultrasound has a positive effect on polysaccharide extract yield [23]. In the literature for the extraction of pectin from OFI cladodes; microwave assisted and ultrasound assisted extraction methods, alkali applications, methods using different solvents to precipitate mucilage such as isopropyl alcohol, ethanol and acetone have been reported in various yields ranging %7-25 (dry base) [10, 15, 17, 18, 32].

System modeling is a useful tool for not only predicting rate and yield but also for understanding the major ultrasound factors and their effects on the extraction process. In this study, correlation among convective heat transfer coefficients and mass transfer rate, which limits concentration in solvent and particle velocity were inspected for selected sonication parameters such as time, temperature and amplitude using experimental observations which were obtained by Response Surface Methodology (RSM).

In this study, polysaccharide of OFI cladodes were extracted by UAE method investigating the effect of applied power, temperature and processing time on the yield of extract. Properties of polysaccharide in optimal condition was investigated by chemical properties of extracts in terms of degree of esterification and total sugar composition.

2. MATERIAL AND METHOD

2.1. Materials

OFI cladodes were freshly harvested obtained in Jun, 2019 from trial lands located in Mersin/Erdemli/Turkey which was set for European Union Project for OFI studies. Average dimensions of the cladodes were 323±7 mm toll, 152±4 mm wide having 10±3 thickness (2:3 of it was skin) with 135±15 g. Average pH of mucilage par of samples were 5.71±0.07. Samples were cleaned in warm water after the thorns and epidermis were removed before treatments. The cleaned parts were cut into small pieces (~ 1cm) and weighed and dried in the oven at 60°C for 24 hours. The dried Cladodes were grounded in a high speed disintegrator (Knife Mill GRINDOMIX GM 200, Retsch GmbH, Haan, Germany) to obtain homogenate bulk which was fine powder (Particle size: 360±45 µm). All samples were kept at 4^{o}C for further treatment. All chemicals used for analyses were purchased from Sigma-Aldrich (St. Louis, MO, USA) in analytical grade.

2.2. Ultrasound Assisted Extraction Procedure

Samples were treated into distilled water satisfying 1:15 solid liquid ratio and pH is adjusted to 2.8 with hydrochloric acid as advised in previous studies [11] which was modification of the method

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developed by Bagherian et al. [49]. Sonication process was applied using probe (14 mm diameter, 90 mm height) type sonicator (Hielscher UP400S, 24 kHz, Germany) at sonic power up to 400 W. 250 ml volumetric flask was used at the same liquid height of 40±5 mm and merged depth of probe was 35±5 mm. Flask was covered by water jacket for temperature control and additional magnetic stirring was applied in reactor to provide homogeneity. Applied sonication energy was recorded through using wattmeter (VOLTCRAFT Energy Check 3000, Germany) and temperature on the wall (Two) and inside the reactor (Tm) was noted to monitor temperature increase inside the reactor by prolonged extraction (Fig 1).

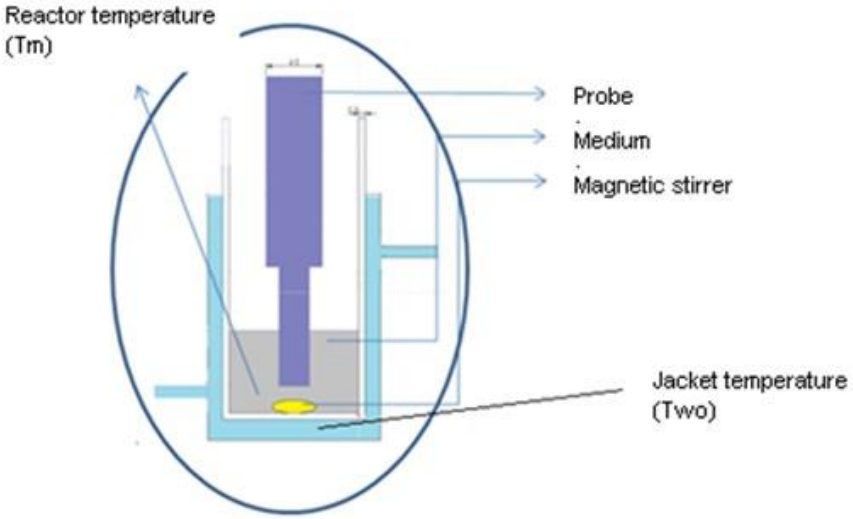

Figure 1. Demonstration of sonication system.

2.3. Calculation of Crude Polysaccharide (CPS) Yield

After sonication, water-sample mixture was centrifuged at 4000 rpm for 15 min. then filtered in order to obtain water soluble part. Settled part was not considered for further calculation. Sevag method was applied to permeate for protein removal [33]. After removal of the Sevag reagent, 200 ml of anhydrous ethanol was added and the mixture was kept 24 h at 4^{o}C to precipitate high molecular weight carbohydrates such as polysaccharides while dissolving mono and oligosaccharides into ethanol. Crude polysaccharide (CPS) was collected after centrifugation at 5000 rpm for 15 min and vacuum drying the final precipitate at 40±5^{o}C. CPS yield was determined by given equation (1) [20].

$$\text{CPS yield} = \frac{\text{weight of crude polysaccharide}}{\text{pretrated sample weight}} x\ 100 \quad (1)$$

2.4. Calculations about Ultrasonic Processors

Applied power was controlled by setting amplitude ratio (%) of equipment. Power level was recorded and it was validated by calorimetric method while measuring the temperature change of water inside the insulated flask by using equation (2) [34]. It was observed that applied sonication power "P" (W) was %80-90 of input power of the equipment that could be read on wattmeter. Further calculations were based on this outcome.

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$$P = C_P m(\frac{dT}{dt})_{t=0} \quad (2)$$

In equation (2), heat transfer capacity "Cp" and mass of water "m" are constants and their values are 4180 J/kgK and 100 g respectively. (dT/dt)t = 0 is the initial slope of temperature inside water "T" versus time "t" plot. Calculated power levels are comparable to equipment manual and it was correlated with heat transfer calculations. For further calculations, it was thought that determining the exact temperature inside the reactor was vital and effective temperature "T_{eff}" was determined by integrating Tm by processing time for each processing condition at given process time. The purpose was to see the temperature increase and average temperature during reaction.

2.5. Experimental Design, Validation and Data Analysis

In this study a three level three factor Box Behnken Design (BBD) consisting 17 experimental runs with 5 replicates at centre point in Response Surface Methodology (RSM) was developed to optimize UAE conditions. The design variables were ultrasonic power (X1), set temperature (X2), and time of the extraction process (X3) with all other parameters kept constant. Considering the preliminary trials and data in the literature, minimum and maximum values of independent variables were selected as 288-303 K, 10-30 minutes and 150-350 W by adjusting amplitude. Trials were randomized against the risk of unexpected effects occurring in the responses. Independent variables and their coded levels and actual values are shown in Table 1. All experiments were performed in triplicate, but mean values were used for modeling. The data were analysed using analysis of variance (ANOVA) by Design Expert software (Trial version 7.0.0; Stat-Ease, Inc. USA). The confidence level for statistical significance was set at a probability value of 0.05. Best models representing the system within BBD models were used for obtain maximum yield. Obtained results were also compared to the conventional method.

Table 1. Coded actual values used in Box-Behnken Design.

			Coded levels		
Codded	**Variable**	**Unit**	**-1**	**0**	**1**
X1	Power	W	150	250	350
X2	Temperature	K	288	318	303
X3	Time	min.	10	20	30

Linear, squared and interaction terms were investigated for best fitted model by checking lack of fit, R^2, adjusted R^2 and prediction error sum of squares (PRESS). Important independent variables were evaluated by analysis of variance (ANOVA) for each response. Equation validations were controlled by model ANOVA statistics. Response surface plots of selected modes were generated by statistical calculations of regression coefficients. Determined optimal values of the test variables were given as un-coded units after determining coded units [25]. Optimum condition was validated by comparing results of five experiments with predicted result obtained by generated model. For this purpose, t-test was applied.

2.6. Characterization of extracts

2.6.1. Proximate analysis.

Moisture, ash, protein and total carbohydrate content of CPS were analysed. Moisture content was determined by AOAC 930.15, ash content was determined by AOAC 942.05 [35]. Protein

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concentration was calculated from the nitrogen content (% Nitrogen x 6.25) to be obtained by element analysis (Elemental Analyzer, Perkin-Elmer Model 240) [36]. Total sugar content (SC) was evaluated by the phenol-sulphuric acid colorimetric method [33]. Briefly, 1.0 ml polysaccharide sample solution was mixed with 1.0 ml 5% phenol and 5 ml 98% H2SO4. The absorbance at 490 nm was recorded after standing for 15 min. The sugar content (SC) (%) was then calculated by equation (4) [37, 38]. Where; "C", sugar concentration (mg/mL) calculated using calibration curve, "N", dilution factor, "V_L" is the amount of solution (mL) [39].

$$\text{SC (\%dry weight)} = \frac{\text{C x N x } V_L}{\text{dried sample weight (CPS)}}\ x\ 100 \quad (4)$$

2.6.2. Esterification degree analysis.

Esterification value was one of the most important properties of pectic extracts to identify industrial application area [40]. In this study degree of esterification was determined by using modified method developed by Açıkgöz et al [41] and Bayar et al [17]. Briefly 0.1 g dried pectin dissolved in 1 mL ethanol and then added to 20 mL deionized water by stirring for 2 h at 40^{o}C. Solution was titrated with 0.1 M NaOH for neutralization and this volume was noted as "V1". Then 10 mL 0.1 M HCl was added to sample to disappear pink color. The solution was titrated again with 0.1 M NaOH and volume was noted as "V2". Degree of esterification was calculated by the equation (5) [17, 41].

$$\text{DE (\%)} = \frac{\text{V2}}{\text{V1+V2}}\ x\ 100 \quad (5)$$

3. RESULTS

Polysaccharides of OFI cladodes extracted in water after adjusting pH 2.8 by HCl as advised by previous researchers to maximize yield of pectic polysaccharide [11]. Leflish et al found the best condition by adjusting pH 2.26 [10] with mucilage polysaccharide content while, optimal pH was determined as 1.5 after mucilage removal [17]. In this study UAE was applied to mucilage of OFI cladodes and the effect of extraction conditions were evaluated on pH 2.8 adjusted water- OFI powder suspension.

3.1. UAE extraction and RSM optimization

RSM is helpful method to evaluate statistical models such a complex process as extraction phenomenon. Due to the adequacy tests, it was observed that quadratic model was the best model representing the system among other models while showing lowest and insignificant lack of fit, biggest Fisher's value or lowest p-value, highest R^2, adj-R^2 and pred-R^2 and the lowest predicted residual sum of squares "PRESS" value. ANOVA results (Table 2) of the selected quadratic model showed that correlation value was low enough to be reliable for representing the response, pred-R^2 and R^2 values were close to each other showing the good relation between experiments and predictions.

Table 2. BBD ANOVA table for CPS.

Source	Sum of Squares	F Value	p-value Prob> F
Model	79,386	84,849	< 0.0001
X1-Power	33,171	319,077	< 0.0001

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X2-Temperature	14,933	143,646	< 0.0001
X3-Time	17,405	167,424	< 0.0001
X1X2	4,818	46,346	0.0003
X1X3	1,664	16,007	0.0052
X2X3	0,240	2,310	0.1724
X1^2	0,040	0,383	0.5556
X2^2	0,386	3,712	0.0954
X3^2	6,582	63,310	< 0.0001
Residual	0,728		
Lack of Fit	0,519	3,321	0.1383
Pure Error	0,208		
Cor Total	80,114		
C.V. %	2,153		
PRESS	8,633		
R-Squared	0,991		
Adj R-Squared	0,979		
Pred R-Squared	0,892		
Adeq Precision	31,173		

Linear effects of parameters were predominant factors on responses while interaction effects and quadratic effect of time were still important ($p<0.05$). Finally, diagnostic analysis (Fig 2) carried on controlling fitness quality of the model because CPS model was important for further modeling steps in order to generate variable during mass transfer calculations. Diagnostic plots (Fig 2) showed that residuals were in normal distribution (Fig 2a), predicted values versus predictions given at Table 3 were laying on 45^{o} trend to each other (Fig 2b), and residuals were staying in reliable limits (Fig 2c-d). Developed BBD quadratic model with codded values are given in equation (6).

$$CPS = 15.66 + 2.04X_1 + 1.37X_2 + 1.48X_3 - 1.10X_1X_2 + 0.65X_1X_3 - 1.25X_3^2 \quad (6)$$

Table 3. Predicted and observed values of CPS.

Run number	**X1**	**X2**	**X3**	**Predicted values**	**Observed values**
1	-1	0	1	13.20	13.33±0.41
2	0	-1	1	14.52	14.75±0.86
3	0	0	0	15.66	15.67±0.33
4	0	1	-1	14.30	13.95±0.83
5	0	1	1	17.26	16.63±0.65
6	-1	1	0	16.09	15.92±0.56

7	1	0	1	18.50	18.41±0.49
8	0	0	0	15.66	15.30±0.25
9	1	1	0	17.97	18.08±0.62
10	0	-1	-1	11.56	11.09±0.76
11	1	-1	0	17.43	17.18±0.43
12	-1	0	-1	11.54	11.89±0.17
13	0	0	0	15.66	15.80±0.30
14	-1	-1	0	11.15	10.63±0.71
15	0	0	0	15.66	15.90±0.35
16	0	0	0	15.66	15.62±0.20
17	1	0	-1	14.32	14.39±0.42

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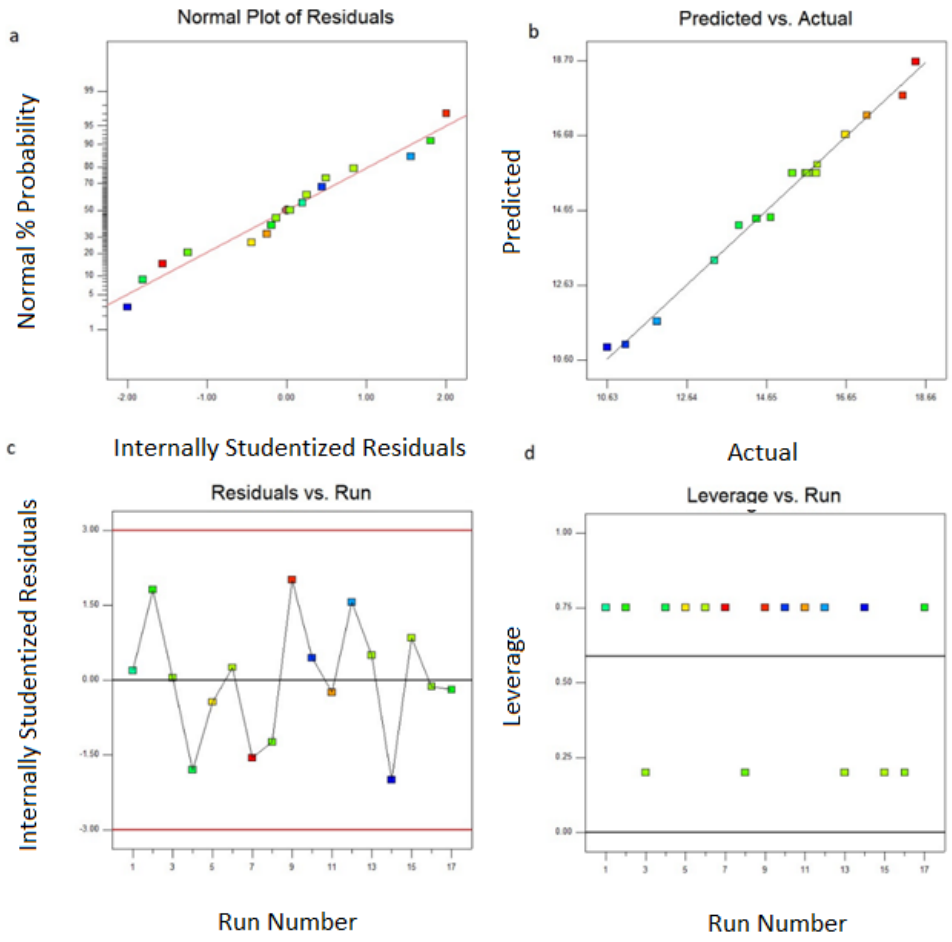

Figure 1. CPS model diagnostic plots.

Polysaccharide yield is highly dependent on extraction condition. Conventionally water soluble pectin rich CPS was extracted from plant tissue by using severe conditions under high temperature (80-90^{o}C) and long processing time (30 min-120 min) [11, 42–44]. Previous researches found the yield of polysaccharide on fresh weight was 1.2% after 80°C for 30 min extraction [44]. In this study the yield in fresh weight was 2.4%±0.46 due to conditions. On the other hand considering moisture content of cladodes 91%±5 and comparing results in dry basis average yield was calculated 14.97%±0.32 and it was still higher than conventional methods which was 12.01% [44], 10.24% [11]. Besides conventional method microwave assisted extraction with several conditions found beneficial to increase the yield up to 25% [32] which is higher than our findings. On the other hand, enzymatic extraction is also promising green method to extract cladode polysaccharides providing 17.91% in previous studies [19] which is lower than UAE yield at 350W, 318K and 30 min extraction providing 18.50% yield (Table 3), however, these variation could be expressed by differences between plant

material (maturity, species, harvesting), pre-treatment and separation technique of CPS and majorly extraction conditions. As given on response plots (Fig 3) positive effect of set temperature on the yield was important. That could be explained by easing cavitation, decrease in surface tension and solvent viscosity, improved solute solubility and enhancing solvent penetration into the solid matrix by means of increasing temperature. As a consequence, mass transfer rate increased and partial-hydrolyzation occurred which breaks weak bonds which are holding polysaccharide intact into the plant cell matrix [45–47]. Although temperature was important, the most affective factor was sonication power which causes cavitation phenomena disturbing cell wall material to ensure leaching of extractable materials [48]. Stronger sonication power caused sharpening in cavitation effect which was responsible for micro streaming and micro jets [29].

For all experiments Tm and Two were recorded and it was observed that set temperature was not reliable although jacket was applied to keep temperature constant during UAE treatment. However, all parameters increase the ambient temperature regardless of how low the set temperature was. As recorded during experiments at least 5 K increment was observed and the maximum temperature increase was recorded as 11.8% after 30 min treatment at 288 K, 350 W treatment. Especially temperature increase was more rapid for low temperature practices. This outcome was important to make further modeling calculation in other temperature sensitive transfer variables such as heat and mass transfer coefficient. On the other hand, in literature, it was investigated that sonication ensures uniform and perfect heat distribution comparing to conventional techniques [30]. Prolonged treatment had positive effect on the yield of extract and in previous studies it was found beneficial up to 25 min but decrease was observed due to structural changes and decomposition of pectic polysaccharides with longer extraction periods [52].

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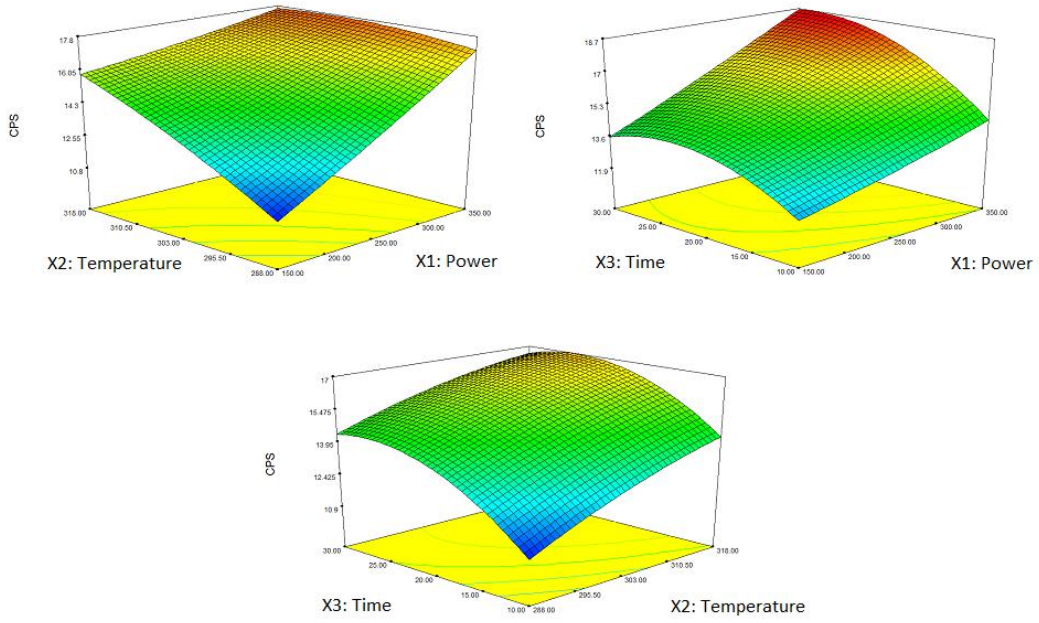

Figure 2. Response plots of CPS yield under ultrasonic conditions.

Optimal conditions were determined to maximize the yield of CPS under given extraction parameters and it was found as 345.5 W, 304.5 K and 28.5 min extraction and predicted yield was 18.58%. Average yield of five experiments run under optimal condition was 18.48±0.35% which was not different from predicted yield statistically ($p>0.05$).

3.2. Characterization of extracted CPS

Extracted CPS were highly fresh having 92±5% moisture content. Therefore, extracts were dried before further characterization. Chemical composition of dried CPS obtained under optimal conditions were 7.5±2.22% moisture content, 14.4±0.87 ash, 0.18±0.05 protein, 82.12±7.2% SC in glucose, 72.75±6.8% SC in galacturonic acid in dry basis. Chemical composition of CPS served similar results as previous researches declared [44]. Protein content was low compared to previous studies which were 0.32% [11, 17, 19], it can be explained by Sevag procedure application in current work to remove protein. Variation on galacturonic acid content was strongly related to extraction condition which had important role on pectin recovery. Ultrasonic extraction and microwave extraction techniques applied previously resulted in 68.8% and 60.66% galacturonic acid and 80.02% and 85.31% total sugar content [19, 17].

Degree of esterification is important property for CPS to identify industrial application. It is known that pectin serves diversified DE (%) due to nature of plant material. It is stated that the products obtained in the techniques applied in polysaccharide extraction from OFI cladodes are mostly LM (low methoxy) pectin [17, 42]. Measured degree of esterification of CPS found as 40.57±3.11% and

this is compatible to previous studies and can be perceived as LM pectin. On the other hand this value is higher than conventionally extracted polysaccharides which was calculated as 30.67% [53].

4. DISCUSSION

In this study, time, temperature and ultrasonic power were selected as optimisation variables. However, it was observed that extract yield was highly affected by various factors as studied plant matrix, application temperature, time, solvent, solid to liquid ratio, pH and process variables as power etc… [51,52]. For ultrasound assisted and microwave extraction procedures of pectic polysaccharides, applied conditions by previous researches were varying as 15-75^{o}C, 10-60 min., pH 1.5-10, 1:5-1:40 (g/ml), 300-900 W microwave power or 50-400 W ultrasonic power while optimisation was applied by RSM design considering three factors of extraction parameters [50-51]. pH was advised to adjust to 2.8 in Bayar et al. [19], since water soluble polysaccharides as pectin in plant material was extracted in acidic conditions as described by previous researchers as well [17,50,51]. It was found that pectin yield was positively affected by increasing pH from 1.5 to 5.5 while considering other parameters as constant [17]. On the other hand, highest pectin yield of plant extracts found between pH 2-3 [52]. Therefore, pH was set to 2.8 in this study to evaluate selected sonication parameters which was also important level to keep protein content low in the extract by keeping away the isoelectric pH of plant protein as discussed in previous studies [11,17]. In water extraction of OFI cladode pectin, solid to liquid ratio was selected as 1:15 g/ml [11,19], while mostly studied ratio was between 1:10-1:30 [50,52]. 1:15 g/ml was found good enough to evaluate the effect of sonication parameters successfully depending on preliminary studies because, dilute concentration requires more chemical usage while high concentration makes extraction difficult to run due to complicating phase separation. For the pectin extraction, solid to liquid ratio was found important factor to ease dissolution of extractable material and formation of cavitation by lowering viscosity and resistant forces for dilute solutions up to 1:30 g/ml [52].

In the present study, UAE was successfully applied to obtain polysaccharide from OFI cladodes by using BBD. Polynomial model was developed to model ultrasonic conditions which were sonic power, set temperature and processing time and optimal conditions in terms of yield maximization were 345.5 W, 304.5 K and 28.5 min extraction respectively. Achieved yield was predicted as 18.58% which was validated by experiments having the average yield 18.48±0.35%. General composition of the extracts at the optimal condition was 7.5±2.22% moisture content, 14.4±0.87 ash, 0.18±0.05 protein, 82.12±7.2% SC in glucose, 72.75±6.8% SC in galacturonic acid in dry basis. In literature Bayar et al studied UAE extraction of OFI cladodes at constant 330 W ultrasonic power and optimal conditions were found as 343 K, 70 min. extraction at pH 1.5 and 1:30 g/ml having yield of 19.06% having chemical composition as 92% dry matter, 16.88% mineral, 0.32% protein 80.02% total sugar, 68.87% uronic acids [17] which were found slightly different from current study due to extraction condition and the nature of the raw material. In this study effect of sonication power was evaluated differing from previous one by studying sonication phenomenon detailly by keeping parameters as pH and solid to liquid ratio constant and the effect of varied sonication power were investigated in terms of yield and temperature increase in the reaction vessel with sonication at various power-temperature combinations. Consequently, it was found that higher sonication power increased the pectin yield but also elevated power increased reactor temperature as well which should be considered for further studies while deciding set temperature and duration.

OFI parts as fruit and cladode have several application areas for medical and food industry with their unique composition. On the other hand, OFI is wild plant and freely available on arid and semi-arid locations without any treatment and practices. Therefore, it is a cheap raw material. Although fruit part is valuable and consumed worldwide, cladodes being high in pectin can be valorised with high added value except for disposing as animal feed. OFI cladodes are mostly LM (low methoxy pectin) pectin. However, research on the use of this product in the field of food needs more research. Degree of esterification was found 40.57±3.11% proving that extracted polysaccharides was low-methoxy (LM) pectin-based material which can be used a good alternative and cheap source for industrial LM pectin.

Another outcome of the study was showing the temperature change while UAE procedure. Although jacketed system was applied to validate constant temperature on the reactor wall, prolonged extraction increased temperature inside the reactor dramatically and the average temperature in reactor media during extraction risen up to 11% for 30 min extraction depending on set temperature and sonication power. This could have additional effect on efficiency and should be considered for UAE designs for better control and prediction.

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REFERENCES

[1] Scalisi A, Morandi B, Inglese P, Lo Bianco R., (2016), Cladode growth dynamics in Opuntia ficus-indica under drought, Environ Exp Bot, 122, 158–167.

[2] Salehi E, Emam-Djomeh Z, Askari G, Fathi M., (2019), Opuntia ficus indica fruit gum: Extraction, characterization, antioxidant activity and functional properties, Carbohydr Polym, 206, 565–572.

[3] Del-Valle V, Hernández-Muñoz P, Guarda A, Galotto MJ., (2005), Development of a cactus-mucilage edible coating (Opuntia ficus indica) and its application to extend strawberry (Fragaria ananassa) shelf-life, Food Chem, 91, 751–756.

[4] Taguchi M, Harinder Makkar F, Mounir Louhaichi F, Duffy R, Moretti D., (2017), CROP ECOLOGY, CULTIVATION AND USES OF CACTUS PEAR Editorial support Book design and layout, 26–30.

[5] Sáenz C, Sepúlveda E., (2001), Cactus-Pear Juices, J Prof Assoc Cactus Dev, 4, 3–10.

[6] Garbelotti ML, Marsiglia DAP, Torres E a. F., (2003), Determination and validation of dietary fiber in food by the enzymatic gravimetric method, Food Chem, 83, 469–473.

[7] Gheribi R, Puchot L, Verge P, Jaoued-Grayaa N, Mezni M, Habibi Y, Khwaldia K., (2018),

Development of plasticized edible films from Opuntia ficus-indica mucilage: A comparative study of various polyol plasticizers, Carbohydr Polym, 190, 204–211.

[8] Allegra A, Sortino G, Inglese P, Settanni L, Todaro A, Gallotta A., (2017), The effectiveness of Opuntia ficus-indica mucilage edible coating on post-harvest maintenance of 'Dottato' fig (Ficus carica L.) fruit, Food Packag Shelf Life, 12, 135–141.

[9] Chaouch MA, Hafsa J, Rihouey C, Le Cerf D, Majdoub H., (2015), Depolymerization of polysaccharides from Opuntia ficus indica: Antioxidant and antiglycated activities, Int J Biol Macromol, 79, 779–786.

[10] Lefsih K, Giacomazza D, Dahmoune F, Mangione MR, Bulone D, San Biagio PL, Passantino R, Costa MA, Guarrasi V, Madani K., (2017), Pectin from Opuntia ficus indica: Optimization of microwave-assisted extraction and preliminary characterization, Food Chem, 221, 91–99.

[11] Bayar N, Kriaa M, Kammoun R., (2016), Extraction and characterization of three polysaccharides extracted from Opuntia ficus indica cladodes, Int J Biol Macromol, 92, 441–450.

[12] Allegra A, Inglese P, Sortino G, Settanni L, Todaro A, Liguori G., (2016), The influence of Opuntia ficus-indica mucilage edible coating on the quality of "Hayward" kiwifruit slices, Postharvest Biol Technol, 120, 45–51.

[13] Khatabi O, Hanine H, Elothmani D, Hasib A., (2016), Extraction and determination of polyphenols and betalain pigments in the Moroccan Prickly pear fruits (Opuntia ficus indica), Arab J Chem, 9, S278–S281.

[14] Lansky EP, Paavilainen HM, Pawlus AD, Newman RA., (2008), Ficus spp. (fig): ethnobotany and potential as anticancer and anti-inflammatory agents., J Ethnopharmacol, 119, 195–213.

[15] Medina-Torres L, Brito-De La Fuente E, Torrestiana-Sanchez B, Katthain R., (2000), Rheological properties of the mucilage gum (Opuntia ficus indica), Food Hydrocoll, 14, 417–424.

[16] Majdoub H, Roudesli S, Deratani A., (2001), Polysaccharides from prickly pear peel and nopals of Opuntia ficus-indica: extraction, characterization and polyelectrolyte behaviour, Polym Int, 50, 552–560.

[17] Bayar N, Bouallegue T, Achour M, Kriaa M, Bougatef A, Kammoun R., (2017), Ultrasonic extraction of pectin from Opuntia ficus indica cladodes after mucilage removal: Optimization of experimental conditions and evaluation of chemical and functional properties., Food Chem, 235, 275–282.

[18] Lefsih K, Delattre C, Pierre G, Michaud P, Aminabhavi TM, Dahmoune F, Madani K., (2016), Extraction, characterization and gelling behavior enhancement of pectins from the cladodes of Opuntia ficus indica, Int J Biol Macromol, 82, 645–652.

[19] Bayar N, Friji M, Kammoun R., (2018), Optimization of enzymatic extraction of pectin from Opuntia ficus indica cladodes after mucilage removal, Food Chem, 241, 127–134.

[20] Yilmaz T, Tavman Ş., (2016), Ultrasound assisted extraction of polysaccharides from hazelnut skin, Food Sci Technol Int. doi: 10.1177/1082013215572415

[21] Chemat F, Zill-e-Huma, Khan MK., (2011), Applications of ultrasound in food technology: Processing, preservation and extraction., Ultrason Sonochem, 18, 813–35.

[22] Chen W, Wang W-P, Zhang H-S, Huang Q., (2012), Optimization of ultrasonic-assisted extraction of water-soluble polysaccharides from Boletus edulis mycelia using response surface methodology, Carbohydr Polym, 87, 614–619.

[23] Hromádková Z, Ebringerová A, Valachovic P., (2002), Ultrasound-assisted extraction of water-soluble polysaccharides from the roots of valerian (Valeriana officinalis L.)., Ultrason Sonochem, 9, 37–44.

[24] Hromádková Z, Ebringerová a, Valachovic P., (1999), Comparison of classical and ultrasound-assisted extraction of polysaccharides from Salvia officinalis L., Ultrason Sonochem, 5, 163–8.

[25] Yılmaz T, Tavman S., (2017), Modeling and Optimization of Ultrasound Assisted Extraction Parameters using Response Surface Methodology for Water Soluble Polysaccharide Extraction from Hazelnut Skin, J Food Process Preserv. doi: 10.1111/jfpp.12835

[26] Benito-Román Ó, Alonso E, Cocero MJ., (2013), Ultrasound-assisted extraction of β-glucans from barley, LWT - Food Sci Technol, 50, 57–63.

[27] García A, Alriols MG, Llano-Ponte R, Labidi J., (2011), Ultrasound-assisted fractionation of the lignocellulosic material., Bioresour Technol, 102, 6326–30.

[28] Xu Y, Zhang L, Bailina Y, Ge Z, Ding T, Ye X, Liu D., (2014), Effects of ultrasound and/or heating on the extraction of pectin from grapefruit peel, J Food Eng, 126, 72–81.

[29] Sivakumar V, Anna JL, Vijayeeswarri J, Swaminathan G., (2009), Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather., Ultrason Sonochem, 16, 782–9.

[30] Feng H, Barbosa-Canovas G V., Weiss J., (2010), Ultrasound Technologies for Food and Bioprocessing. Springer, New York

[31] Patist A, Bates D., (2008), Ultrasonic innovations in the food industry: From the laboratory to commercial production, Innov Food Sci Emerg Technol, 9, 147–154.

[32] Felkai-Haddache L, Dahmoune F, Remini H, Lefsih K, Mouni L, Madani K., (2016), Microwave optimization of mucilage extraction from Opuntia ficus indica Cladodes, Int J Biol Macromol, 84, 24–30.

[33] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F., (1956), Colorimetric Method for Determination of Sugars and Related Substances, Anal Chem, 28, 350–356.

[34] Cheung Y-C, Wu J-Y., (2013), Kinetic models and process parameters for ultrasound-assisted extraction of water-soluble components and polysaccharides from a medicinal fungus, Biochem Eng J, 79, 214–220.

[35] AOAC., (2007), Official Methods of Analysis. 18th edn. AOAC International, Gaithersburg

[36] Hromadkova Z, Ebringerova A., (2003), Ultrasonic extraction of plant materials — investigation of hemicellulose release from buckwheat hulls, Ultrason - Sonochemistry, 10, 127–133.

[37] Albalasmeh A a., Berhe AA, Ghezzehei T a., (2013), A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry, Carbohydr Polym, 97, 253–261.

[38] Huang S, Ning Z., (2010), Extraction of polysaccharide from Ganoderma lucidum and its immune enhancement activity., Int J Biol Macromol, 47, 336–41.

[39] Zhang Y, Kong L, Yin C, Jiang D, Jiang J, He J, Xiao W., (2013), Extraction optimization by response surface methodology, purification and principal antioxidant metabolites of red pigments extracted from bayberry (Myrica rubra) pomace, LWT - Food Sci Technol, 51, 343–347.

[40] Thakur BR, Singh RK, Handa AK., (1997), Chemistry and Uses of Pectin - A Review, Crit Rev Food Sci Nutr, 37, 47–73.

[41] Açıkgöz Ç, Poyraz Z., (2006), EXTRACTION AND CHARACTERIZATION OF PECTIN OBTAINED FROM QUINCE(cydonia vulgaris pers.), Dumlupınar Üniversitesi Fen Bilim Enstitüsü Derg, 27–34.

[42] Goycoolea FM, Cárdenas A., (2003), Pectins from Opuntia spp.: A short review, J Prof Assoc Cactus Dev, 5, 17–29.

[43] Di Lorenzo F, Silipo A, Molinaro A, Parrilli M, Schiraldi C, D'Agostino A, Izzo E, Rizza L, Bonina A, Bonina F, Lanzetta R., (2017), The polysaccharide and low molecular weight components of Opuntia ficus indica cladodes: Structure and skin repairing properties, Carbohydr Polym, 157, 128–136.

[44] Dick M, Dal Magro L, Rodrigues RC, Rios A de O, Flôres SH., (2019), Valorization of Opuntia monacantha (Willd.) Haw. cladodes to obtain a mucilage with hydrocolloid features: Physicochemical and functional performance, Int J Biol Macromol, 123, 900–909.

[45] Prakash Maran J, Manikandan S, Vigna Nivetha C, Dinesh R., (2013), Ultrasound assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face centered response surface design, Arab J Chem. doi: 10.1016/j.arabjc.2013.02.007

[46] Prakash Maran J, Manikandan S, Thirugnanasambandham K, Vigna Nivetha C, Dinesh R., (2013), Box-Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide., Carbohydr Polym, 92, 604–11.

[47] Prakash Maran J, Manikandan S, Mekala V., (2013), Modeling and optimization of betalain extraction from Opuntia ficus-indica using Box–Behnken design with desirability function, Ind Crops Prod, 49, 304–311.

[48] Toma M, Vinatoru M, Paniwnyk L, Mason TJ., (2001), Investigation of the effects of ultrasound on vegetal tissues during solvent extraction, Ultrason Sonochem, 8, 137–142.

[49] Bagherian HZokaee Ashtiani., F., Fouladitajar A., and Mohtashamy M., (2011), Comparisons between conventional, microwave- and ultrasound-assisted methods for extraction of pectin from grapefruit, Chem. Eng. Process. Process Intensif., 50, 1237–1243.

[50] Sundarraj A. A., Thottiam Vasudevan R., and Sriramulu G., (2018), Optimized extraction and characterization of pectin from jackfruit (Artocarpus integer) wastes using response surface methodology, Int. J. Biol. Macromol., 106, 698–703.

[51] Sundarraj A. A. and Ranganathan T. V., (2018), Comprehensive review on ultrasound and microwave extraction of pectin from agro-industrial wastes, Drug Invent. Today, 10, 2773–2782.

[52] Maran J. P. and Priya B., (2015), Ultrasound-assisted extraction of pectin from sisal waste, Carbohydr. Polym., 115, 732–738, 2015.

[53] Forni E, Penci M, Polesello A., (1994), A preliminary characterization of some pectins from quince fruit (Cydonia oblonga Mill.) and prickly pear (Opuntia ficus indica) peel, Carbohydr Polym, 23, 231–234.