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Subcritical water hydrolysis of sugar beet pulp towards production of monosaccharide fraction

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ABSTRACT

Sugar beet pulp represents one of the most abundant by-product in food industry in terms of underexploited opportunities and amounts produced. In order to produce added-value product from this lignocellulosic material, subcritical water hydrolysis was applied in this study. Response surface methodology was used to evaluate the effects of hydrolysis parameters and optimize conditions for various responses. Hydrolysis temperature, hydrolysis time and HCl concentration were investigated as independent variables. The results obtained indicated highly promising application of corresponding hydrolysis technique towards valorization of proposed raw material. Relatively high yield of fermentable sugars with the addition of acid agent and the higher hydrolysis rates of lignocellulosic material compared to the previously published studies were obtained. Hydrolysis efficiency was higher than 50% and total monosaccharide yield reached up to 5.669 mg/mL which is significantly higher than previously reported results.

1. Introduction

As the oil markets entered a new era of insecurity in the market access, fluctuation in oil prices and environmental concerns are rising. Therefore, sugar factories are forced to consider finding new ways of energy supply in order to maintain sustainable development required to compete the growing sugar market. The sufficient knowledge regarding processes needed to produce valuable products from the polysaccharide-rich material, such as sugar beet pulp (SBP), is suggesting more comprehensive valorization of the proposed raw material (Sasaki et al., 2003; Mudhoo et al., 2011; Prado et al., 2014).

Sugar beet pulp, fibrous by-product left after the sucrose extraction from sugar beet cossettes, is normally pressed, dried, and mostly used as a cattle feed (Rombouts and Thibault, 1986, Asadi, 2006). However, this lignocellulosic material is rich in polysaccharides, particularly in cellulose, arabinan, galactan and pectin (Kelly, 1983). Therefore, as an agroindustrial residue, it represents especially favourable raw material for biomass fractionation, which can be performed in order to produce high-value products (Mussatto, 2016).

One of the possible solutions for valorisation of this kind of agroindustrial residue, could be the production of fermentation substrate adequate for generation of various combustible products, such are liquid alkanes, bioethanol, etc. According to Serrano-Ruiz and Dumesic such substrate can be obtained by using lignocellulosic material, such as SBP (Serrano-Ruiz and Dumesic, 2011). According to Prado et al., production of fermentation substrate, in the form of fermentable sugars, can be performed through hydrolysis of cellulose to glucose or hemicellulose to pentoses and hexoses (Prado et al., 2014).

Various hydrolytic technologies and different biological and non-biological treatment methods have been developed both for solubilisation or fractionation of lignocellulosic material (Gírio et al., 2010). Most of the proposed technologies were based on application of acid catalyst (Saeman, 1945; Malester et al., 1992) or enzyme (Gusakov et al., 2007) in order to conduct saccharification of cellulose and other lignocellulosic material to corresponding monomers. Recently, numerous researchers have suggested introduction of high pressure techniques, such as supercritical and subcritical techniques, in the area of agri-food by-products valorization (Toor et al., 2011; Prado et al., 2014; Pavlić et al., 2016). Their application in biomass and food waste

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streams processing has been particularly interesting due to recovery-extraction of valuable phytochemicals such as phenolics (phenolic acids, flavonoids), saccharides (simple sugars, starch, hemicellulose and cellulose) and bioactive compounds (alkaloids, essential oils, etc.) (Saldana and Valdivieso-Ramírez, 2015). Subcritical water (SW), as pressurized fluid, has gained special attention due to environmental aspect, since it is non-toxic to human health, safe to work with and has negligible environmental effect (Herrero et al., 2013). A considerable number of review articles have identified subcritical water as an effective solvent, catalyst and reactant for hydrolytic conversions and extractions (Brunner, 2014; Gbashi et al., 2017; Knez et al., 2017). Subcritical water attracted great attentions especially due to significant change of water properties, especially dielectric constant, caused by altered temperature. Furthermore, subcritical water, also has appropriate physical properties (lower viscosity but higher diffusivity) which favors the diffusion into the solid matrix and the release of compounds from solid to liquid phase (Teo et al., 2010). Therefore, application of subcritical water has significantly increased recently and this techniques has been applied for hydrolysis/extraction of various compounds such as polyphenols (Pavlić et al., 2016; Mayanga-Torres et al., 2017), sugars (Mayanga-Torres et al., 2017; Yedro et al., 2017), pectin (Wang et al., 2014), , proteins (Powell et al., 2016) and others.

In this study subcritical water has been applied for hydrolysis of sugar beet pulp. The main scope of research was to investigate the operating conditions (process parameters effects) of sugar beet pulp hydrolysis using subcritical water with the addition of acid agent. The investigation was conducted using designed experiments and the results were analysed by application of response surface methodology (RSM).

2. Material and methods

2.1. Raw material characterization

SBP used in this study was obtained in the technological process of sugar production from the sugar factory "Šajkaška" (Žabalj, Serbia). Exhausted sugar beet cossettes with initial moisture content of 88–90% were pressed using industrial scale biconical press (Babbini, Italy) removing the certain amount of "sweet water" (containing 4–5% of sucrose). After removal of "sweet water", obtained pressed SBP contained 31.5% d.m. Corresponding SBP was used in the further experiments. The physicochemical properties of SBP regarding protein, insoluble and soluble fiber content were determined by AOAC Official Methods (AOAC, 2005; AOAC, 1991; AOAC, 1990). All experiments were performed in triplicates and results were expressed as mean ± standard deviation.

2.2. Subcritical water hydrolysis (SWH)

SWH was performed in batch-type high-pressure extractor (Parr Instrument Company, USA) with internal volume 450 mL and maximum operating pressure of 200 bar and temperature 350 °C, connected with temperature controller (4838, Parr Instrument Company, USA). Auto tuning for each temperature was performed by temperature controller prior extraction. Detailed procedure was previously described elsewhere (Zeković et al., 2014). For each experimental run, 10.0 g of SBP was mixed with 200 mL of solvent and all extractions were performed at regulated isobaric conditions (50 bar). Stirring was employed by magnetic stirrer (1000 rpm) in order to increase mass and heat transfer, and prevent local overheat on the inner walls of reactor. Temperature (150, 200 and 250 °C), hydrolysis time (25, 35 and 45 min) and HCl concentration in solvent (0.5, 1 and 1.5%) were independent variables. Total time of the process could be separated to heating, stationary (hydrolysis) and cooling phase. Moment when temperature reaches stationary phase (150 ± 2 °C; 200 ± 2 °C; 250 ± 2 °C) was chosen as start of hydrolysis and from that point, until cooling, hydrolysis time was measured. Therefore, time in heating and cooling phase was excluded from the total hydrolysis time (Alaejos et al., 2008). Hydrolysis time was measured during stationary phase since it was the only constant period for each experimental run, therefore, only this period could be appropriately used as independent variable in applied experimental design. Hence, heating phase lasted for 16, 23 and 32 min for 150, 200 and 250 °C extractions, respectively, while cooling phase in ice bath was approximately 10 min for all experimental runs. After hydrolysis, liquid and solid phase were immediately filtered through filter paper under vacuum (V-700, Büchi, Switzerland). Liquid phase was collected into glass flasks and stored at 4 °C until the analysis. Total hydrolysis yield (Y) was determined by vacuum evaporation and further drying of certain volume (10 mL) of crude liquid extract. Results were expressed as percentage of total extractable solids per 100 g of SBP.

2.3. Determination of pH

The pH of the obtained SBP hydrolysates was determined using a digital pH meter (WTW, Germany).

2.4. Determination of total reducing sugar content

Total reducing sugars (open-chain form sugars with an aldehyde group or a free hemiacetal group) were determined by Luff-Schoorl method. This redox titration is based on iodometric determination of the unreduced Cu^{2+} ions (oxidizing agent) remaining after the reaction with reducing sugars (Egan et al., 1981).

2.5. Determination of saccharides and hydroxymethylfurfural (HMF)

Samples were adjusted to pH∼ 4 by adding 10 M NaOH, diluted 10 times with methanol and filtered through 0.45 μm pore size regenerated cellulose syringe filters (Agilent Technologies) before injection into the HPLC system. HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD) and an evaporative light-scattering detector – ELSD (Agilent G4218A LT-ELSD) on an Agilent, ZORBAX Carbohydrate, 5 μm, 4.6 × 250 mm column, at a flow-rate of 1.400 mL min^{-1}. Solvent gradient was performed by varying the proportion of solvent A (water) to solvent B (acetonitrile) as follows: initial 85% B; 0–14 min, 85–80% B; 14–30 min, 80-60% B. The total running time and post-running time were 30 and 5 min, respectively. The column temperature was 30 °C. HMF (≥98.0%), D-(+)-galactose (≥99.0%), D-(−)-fructose (≥99.5%), sucrose (≥99.5%), D-(+)-glucose monohydrate (≥99.5%) and L-(+)-arabinose(≥99.0%) analytical standards were obtained from Sigma-Aldrich. Stock solutions were prepared by dissolving 10 mg of standards in 10 mL water. Working standards were prepared by appropriate dilution of these solutions with deionized water to obtain concentration range of 0.001-0.300 mg/mL for HMF and 0.01-3.00 mg/mL for other investigated analytes. The injected volume of samples and standards was 50 μL and it was done automatically using autosampler. HMF was detected using DAD at 285/10 nm with reference wavelength set at 500/100 nm and the spectra were acquired in the range 210–400 nm. Arabinose, fructose and glucose were detected using ELSD detector set up as follows: nitrogen carrier gas pressure 3.5 bar, temperature 40 °C, gain 2.

2.6. Experimental design

Response surface methodology (RSM) was applied to evaluate the effects of hydrolysis parameters and optimize conditions for various responses. Box-Behnken experimental design (BBD) with three numeric factors on three levels was used. Design consisted of fifteen randomized runs with three replicates at the central point. Temperature (X_1; 150, 200 and 250 °C), hydrolysis time (X_2; 25, 35 and 45 min) and HCl concentration (X_3; 0.5, 1 and 1.5%) were investigated as independent variables. Independent variables were coded in range from −1 to 1, so the units of variables were irrelevant. The response variables were fitted to the quadratic polynomial model (Eq. (1)) which is generally able to describe relationship between the responses and the independent variables (Bezerra et al., 2008):

$$Y = \beta_0 + \sum_{3}^{i=1}\beta_i X_i + \sum_{3}^{i=1}\beta_{ii} X_i^2 + \sum \sum_{3}^{i<j=1} \beta_{ij} X_i X_j \quad (1)$$

where: Y represents the response variable, X_i and X_j are the independent variables affecting the response, and β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively. The experimental design and multiple linear regression analysis were performed using Design-Expert v.10 (License No.: 1046-3991-1553-5125, Stat-Ease, Minneapolis, Minnesota, USA). The results were statistically tested by analysis of variance (ANOVA) with the significance levels of 5%. The adequacy of the models was evaluated by the coefficient of multiple determination (R^2) and *p*-values for the model and lack of fit testing. Optimal hydrolysis conditions were determined considering total hydrolysis yield, total monosaccharides content and total reducing sugars content as responses (Ferreira et al., 2007).

3. Results and discussion

The chemical composition of raw material used in hydrolysis process is presented in Table 1. As already mentioned in previous section, dry matter content (DMC) of SBP sample was 31.5%, indicating highly successful partial reduction (60–70%) of the wet pulp water content using low-cost mechanical dewatering (Šereš et al., 2017). Pressed pulp sucrose content (1.02 wt% wet basis) represents the result of a proper management in the pulp treatment stage (Van der Poel, 1998). Total fiber content in SBP corresponds to the cellulose, hemicellulose and pectin content in sugar beet root. Having regard to the solubility of each fiber, value of 61.59% relates to the cellulose and hemicellulose as water insoluble fraction and 24.22% relates to the pectin content as water soluble fiber (Asadi, 2006; Van der Poel, 1998).

For the purpose and aim of these experiments and due to the possibility to hydrolyse to valuable monomers (monosaccharides), cellulose and hemicellulose along with sucrose content will be further discussed.

Hydrolysis products of sucrose, cellulose and hemicellulose, as well as total hydrolysis yield, are presented in Table 2. Total hydrolysis yield (*Y*) values ranged from 7.18% up to 15.84% suggesting significant impacts of selected hydrolysis parameters regarding total yield of the hydrolysis process. RSM was used to estimate the corresponding impacts and the results obtained are presented in Table 3 and Fig. 1. Parameters presented in Table 3 (sum of squares, df and mean square) were used for calculation of F-values and p-values, which were used as predictors of fitting experimental data with applied models. (Montgomery, 2001). The RSM proposed model had significantly high value of coefficient of determination ($R^2 = 0.99$ for the response *Y*), which suggested good fit between experimental data and predicted values. According to statistically significant p-value ($p < 0.05$) for the model, it was possible to conclude that applied model provides proper representation of experimental data. Lack of fit testing confirmed adequacy of fitting experimental data to a second-order polynomial model since p-value for lack of fit was insignificant ($p > 0.05$).

Table 1
Raw material (pressed sugar beet pulp) characterisation (wt%, dry matter basis).

Sucrose	3.25 ± 0.18
Protein	9.09 ± 0.24
Total fiber content	85.81 ± 1.79
Soluble fiber content	24.22 ± 1.03
Insoluble fiber content	61.59 ± 1.33
Cellulose	31.11 ± 0.94
Fat	1.00 ± 0.07

Temperature showed the strongest impact on total hydrolysis yield. Both linear and quadratic effect of hydrolysis temperature had the most prominent influence on total hydrolysis yield values. Pronounced negative effect of the temperature increase is clearly illustrated in Fig. 1. This negative effect could be explained as a result of excessive monosaccharide degradation at higher temperatures, above 150 °C, which lead to the formation of final degradation products, organic acids, furfural and 5-HMF, among others (Sasaki et al., 1998; Prado et al., 2014). Furthermore, the complete hydrolysis of sugar beet hemicellulose (consisting of arabinan and galactan (Šušić et al., 1994)) at 150 °C during 25–45 min is confirmed by relatively high values of arabinose and galactose content obtained in the corresponding experimental runs. Previous researchers reported complete hydrolysis of hemicellulose at 190–230 °C during 2–15 min or in 30–60 min at 140 °C (McMillan, 1994; Allen et al., 1996). Therefore, further temperature increase, during the suggested time of hydrolysis process, accelerates degradation of hemicellulose hydrolysis products and stimulates the successful cellulose saccharification. However, since the monosaccharide degradation rate at proposed temperatures is higher than cellulose hydrolysis rate, decrease in total process yield occurred. This was confirmed in other experiments where no arabinose content was detected. Detected galactose in experimental runs conducted at higher temperature and short/medium process time (runs 4, 5 and 6) represents a result of its generally higher thermal stability compared to arabinose (Khajavi et al., 2005; Usuki et al., 2008).

The addition of HCl showed significant impact on total yield values in both quadratic effect and interaction with hydrolysis temperature (Table 3). Moderate positive effect of increased HCl content is more pronounced in experimental runs conducted at higher hydrolysis temperatures. Moreover, it can be noticed that lowest yield values are obtained in the experiments where temperature is set at the maximum and HCl concentration at minimum level. Numerous researchers concluded that acid addition represents an important factor in increasing hydrolysis rate of various lignocellulosic materials (Sun and Cheng, 2002; Taherzadeh and Karimi, 2007). The HCl molecules serve as promoters for cellulose hydrolysis process significantly contributing to the total hydrolysis yield values. Therefore, the balance of inevitable monosaccharide degradation at higher temperatures and cellulose hydrolysis process is slightly moved towards cellulose saccharification resulting in higher yield values. This effect cannot be noticed in the experimental runs conducted at lower temperatures due to the significantly lower degradation rates of monosaccharides and slower cellulose hydrolysis. Furthermore, strong effect of HCl concentration – hydrolysis time interaction is illustrated in Fig. 1 and confirmed by low p value ($p = 0.0504$). As expected, higher values regarding total hydrolysis yield are obtained during the longer hydrolysis periods with the rising HCl concentration During the 45 min of hydrolysis process, total hydrolysis yield is increased (almost exponentially) with the rise of HCl content. Determination of regression coefficients in Eq. (1) using method of least squares provided model equation for determination of

Table 2
Total yield, reducing sugars and monosaccharides obtained by subcritical water hydrolysis.

Run	Temperature [°C]		Time [min]		HCl conc. [%]		Y [%]	Content [mg/mL]					
	coded	natural	coded	natural	coded	natural		Glucose	Fructose	Arabinose	Galactose	ΣMonosaccharides	TRS
1	0	200	−1	25	1	1.5	10.88	2.156	nd	nd	nd	2.156	5.252
2	−1	150	0	35	−1	0.5	15.84	1.773	nd	2.529	0.404	4.706	7.215
3	−1	150	−1	25	0	1	14.70	2.548	nd	2.654	0.467	5.669	6.803
4	0	200	0	35	0	1	10.76	1.003	0.173	nd	0.238	1.414	5.311
5	0	200	0	35	0	1	10.24	1.657	nd	nd	0.25	1.907	4.185
6	1	250	−1	25	0	1	7.90	0.946	0.21	nd	0.234	1.390	3.617
7	1	250	1	45	0	1	7.58	1.723	nd	nd	nd	1.723	3.769
8	0	200	0	35	0	1	9.60	1.737	nd	nd	nd	1.737	4.464
9	1	250	0	35	−1	0.5	7.18	0.539	nd	nd	nd	0.539	3.979
10	1	250	0	35	1	1.5	8.84	2.488	nd	nd	nd	2.488	4.040
11	0	200	1	45	−1	0.5	9.96	1.01	nd	nd	nd	1.010	5.020
12	0	200	−1	25	−1	0.5	11.24	1.046	nd	nd	nd	1.046	5.402
13	−1	150	0	35	1	1.5	14.88	2.259	nd	1.787	0.367	4.413	6.720
14	0	200	1	45	1	1.5	11.70	1.851	nd	nd	nd	1.851	5.495
15	−1	150	1	45	0	1	14.56	2.067	nd	1.933	0.294	4.294	6.868
16		200	35		0		7.88	0.292	nd	0.25	nd	0.542	3.626

TRS – total reducing sugars.
nd – not detected.
Y – Total hydrolysis yield.

Table 3
Analysis of variance (ANOVA) of the investigated responses (T − temperature; t − time).

Response	Source	Sum of squares	df	Mean square	F − value	p − value
Total hydrolysis yield	Model	108.76	9	12.08	72.03	< 0.0001
	T	101.39	1	101.39	604.37	< 0.0001
	t	0.11	1	0.11	0.63	0.4631
	HCl conc.	0.54	1	0.54	3.22	0.1325
	T x t	$8.100 \cdot 10^{-3}$	1	$8.100 \cdot 10^{-3}$	0.048	0.8348
	T x HCl conc.	1.72	1	1.72	10.23	0.0240
	t x HCl conc.	1.10	1	1.10	6.57	0.0504
	T^2	2.75	1	2.75	16.37	0.0099
	t^2	0.055	1	0.055	0.33	0.5904
	$(HCl\ conc.)^2$	1.43	1	1.43	8.53	0.0330
	Residual	0.84	5	0.17		
	Lack of Fit	0.16	3	0.055	0.16	0.9139
	Pure Error	0.68	2	0.34		
	Cor Total	109.60	14			
ΣMonosaccharides	Model	34.10	9	3.79	70.27	< 0.0001
	T	20.94	1	20.94	388.37	< 0.0001
	t	0.24	1	0.24	4.43	0.0891
	HCl conc.	1.63	1	1.63	30.17	0.0027
	T x t	0.73	1	0.73	13.53	0.0143
	T x HCl conc.	1.26	1	1.26	23.31	0.0048
	t x HCl conc.	0.018	1	0.018	0.34	0.5875
	T^2	8.89	1	8.89	164.95	< 0.0001
	t^2	$3.577 \cdot 10^{-3}$	1	$3.577 \cdot 10^{-3}$	0.066	0.8070
	$(HCl\ conc.)^2$	0.15	1	0.15	2.78	0.1565
	Residual	0.27	5	0.054		
	Lack of Fit	0.14	3	0.048	0.77	0.6090
	Pure Error	0.13	2	0.063		
	Cor Total	34.37	14			
Total reducing sugars	Model	20.07	9	2.23	14.07	0.0048
	T	18.61	1	18.61	117.39	0.0001
	t	$7.605 \cdot 10^{-4}$	1	$7.605 \cdot 10^{-4}$	$4.80 \cdot 10^{-3}$	0.9475
	HCl conc.	$1.485 \cdot 10^{-3}$	1	$1.485 \cdot 10^{-3}$	$9.37 \cdot 10^{-3}$	0.9267
	T x t	$1.892 \cdot 10^{-3}$	1	$1.892 \cdot 10^{-3}$	0.012	0.9172
	T x HCl conc.	0.077	1	0.077	0.49	0.5162
	t x HCl conc.	0.098	1	0.098	0.62	0.4681
	T^2	0.60	1	0.60	3.79	0.1090
	t^2	0.16	1	0.16	1.00	0.3629
	$(HCl\ conc.)^2$	0.69	1	0.69	4.34	0.0917
	Residual	0.79	5	0.16		
	Lack of Fit	0.10	3	0.035	0.10	0.9519
	Pure Error	0.69	2	0.34		
	Cor Total	20.86	14			

df – degrees of freedom.

total hydrolysis yield within investigated experimental domain (Table 4).

Total monosaccharide content (MC) obtained in the process of SWH is presented in Table 2. Values regarding obtained monosaccharides varied in the wide range, from 0.539 mg/mL up to 5.669 mg/mL. The goodness of the estimated model used for data evaluation is suggested by coefficient of determination value of $R^2 = 0.99$. According to significant p-value for the model and insignificant p-value for the lack of fit, it could be confirmed that applied model represented adequate fit with experimental data (Table 3). Similar to the results of the total hydrolysis yield analysis, process temperature showed foremost influence on obtained monosaccharide content. Both quadratic and linear effect of the hydrolysis temperature had extremely low p–values ($p < 0.0001$) confirming the most significant impact of this variable on corresponding response. Furthermore, temperature also exhibited statistically significant effects on corresponding response in interaction with other input factors (Table 3). The exponential rise of the total monosaccharide content with the temperature decrease suggested intensive degradation of obtained monosaccharides at higher hydrolysis temperatures (Fig. 2). Pentoses content was particularly influenced by temperature degradation. SBP used in presented experiments is characterized by high content of hemicellulose and cellulose (30.48% and 31.11%, respectively), therefore, significant amount of pentoses originating from hemicellulose could be expected. However, even at the lowest hydrolysis temperatures applied, only arabinose content was detected (Table 2). As previously reported by Usuki et al. (2008) arabinose showed the most significant resistance to the degradation among the pentoses tested, while other pentoses rapidly degraded at the applied experimental conditions (200–240 °C). Concerning hexoses detected, glucose and galactose, higher resistance to degradation compared to pentoses was expected (Khajavi et al., 2005). Glucose, due to more stable cyclic form compared to other aldohexoses, (spending less time in its reactive open-chain form) withstands higher temperatures more successfully than galactose which was not detected in experiments at higher temperatures and longer residence time. Furthermore, the total amount of glucose monomers in the raw material is far greater than galactose monomers indicating significantly higher ultimate yield of glucose even at the degradation kinetics similar to galactose. Moreover, response surfaces (Fig. 2), obtained by RSM analysis, clearly illustrate the increase of total monosaccharide content by decreasing the hydrolysis time and temperature. The main product of both pentoses and hexoses degradation is HMF. The HMF content in the obtained hydrolysates is presented in Fig. 3a. It could be noticed that HMF content generally decreased with the rising HCl concentration suggesting further acid catalyzed degradation of HMF to furfural, organic acids or other final degradation products. Despite the formation of organic acids, HCl addition mostly determined the final pH of the produced hydrolysates as illustrated in Fig. 3b. Furthermore, HCl addition showed highly significant effect regarding total monosaccharide content (Table 2). In the ex-

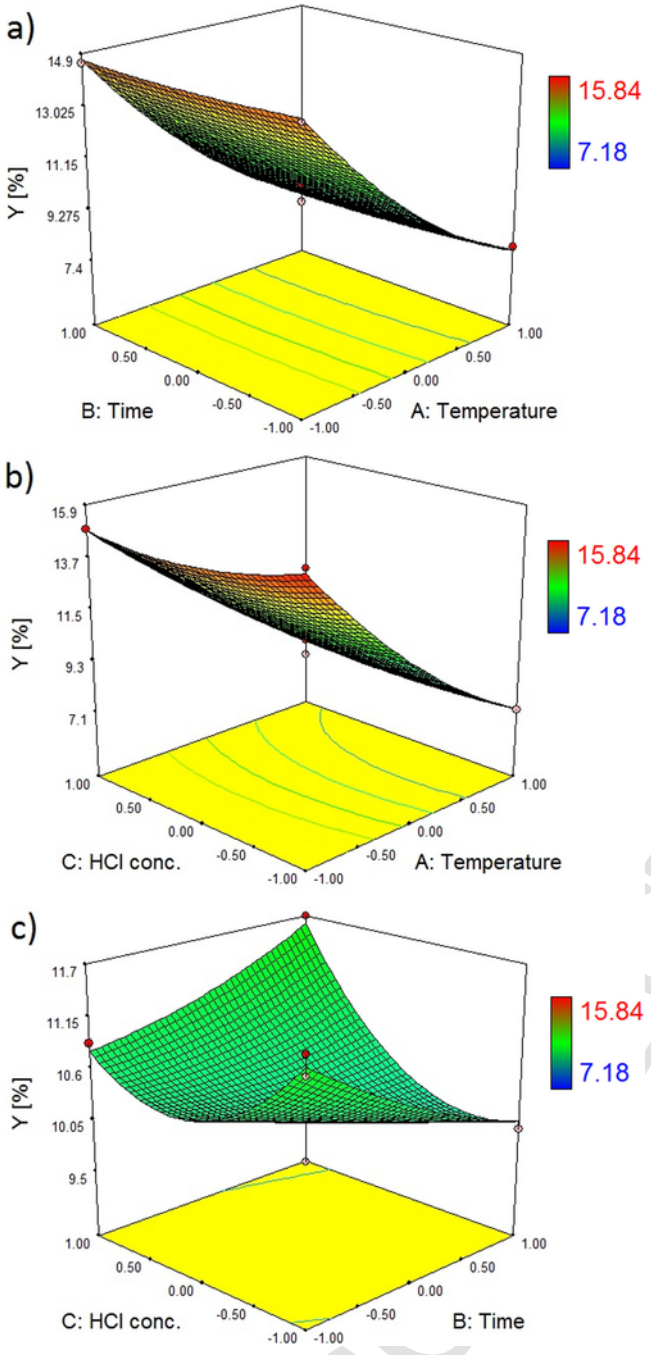

Fig. 1. Obtained response surface plots presenting combined influence of corresponding factors regarding total hydrolysis yield.

periments conducted in shorter residence time and higher temperatures, HCl addition had greatly positive effect on the total monosaccharide content, increasing the corresponding response up to 200%. Previous researchers also reported similar conclusions in terms of increased pulping efficiency in experiments where high temperature, short residence time and medium level of catalyst were used (López et al., 2008). The corresponding increase occurred due to the increased hydrolysis rate of both cellulose and hemicellulose which is catalyzed by H_3O^+ ions, resulting in higher glucose content as concluded in previous section. Predictive second-order polynomial model equation for SWH of total monosaccharide content was presented in Table 4.

Since all monosaccharides obtained in the hydrolysis process represent the monomers of sugar beet insoluble fibers, cellulose and hemicellulose (except small amount obtained by sucrose hydrolysis), corresponding monosaccharide yield regarding insoluble fiber content was calculated and presented in Fig. 4. The starting content of insoluble fiber (9.7 mg/mL) in all samples to be hydrolyzed was calculated using Eq. (2):

$$m_{ins} = \frac{m_s . m_{dm} \cdot w_{ins}}{V_{solvent} \cdot 10000} \quad (2)$$

where m_{ins} represents mass of insoluble fiber per mL of solvent (mg/mL), m_s refers to the sample mass (mg), m_{dm} refers to sugar beet pulp dry matter content (%), w_{ins} represents sugar beet pulp insoluble fiber content (%) and $V_{solvent}$ refers to the total solvent volume (mL).

The most successful insoluble fiber hydrolysis to monomers, 58.44%, is achieved in experimental run 3 where lowest hydrolysis temperature and residence time were applied, followed by medium HCl addition. High value obtained, over 50%, suggested that hydrolysis products originated from both hemicellulose and cellulose hydrolysis. Moreover, it can be noticed that all experimental runs at 150 °C had hydrolysis efficiency over 44% indicating highly favorable operating conditions regarding SWH of sugar beet pulp. In contrast, the lowest value of 5.56% is obtained in the experiment where the highest hydrolysis temperature and the lowest HCl addition were used.

The most successful production of reducing sugars was obtained in the experiments conducted at 150 °C with insoluble fiber to reducing sugars conversion ranging from 69 to 74%. Further temperature increase had evident negative effect on total monosaccharide yield and hence decreasing the total reducing sugars content. However, temperature increase did not have significant effect on the remaining reducing sugars content (oligosaccharides-histogram red), suggesting that further hydrolysis occurred within obtained oligosaccharides (production of shorter oligosaccharide chains) (Fig. 4).

Total reducing sugar content of the obtained hydrolysates ranged from 3.626 to 7.215 mg/mL (Table 2). The proposed model obtained by RSM analysis had high value of coefficient of determination ($R^2 = 0.96$) indicating good fitting properties of the experimental results which was confirmed by p-values for the model and lack of fit (Table 3). Temperature effect was the only input factor that showed statistically significant impact on the obtained results. The response surfaces slopes presented in Fig. 5 confirm strong impact of temperature factor regarding reducing sugar yield. However, with the confidence level of $p = 0.10$, quadratic effect of HCl addition also reported significant influence. The time factor had no significant influence since the proposed raw material (hemicellulose and cellulose) is relatively easily transformed from inert chains to oligo- or polysaccharides containing free reducing end in the first 25 min of hydrolysis process. The separation of already one monomer opens the reducing end and allows detection of corresponding molecules. Moreover, in further prolonga-

Table 4
Second-order polynomial equations for investigated response variables.

Response	Second-order polynomial model equation
Total reducing sugars	$TRS = 4.65 - 1.52X_1 + 0.01X_2 - 0.01X_3 + 0.02X_1X_2 + 0314X_1X_3 + 0.16X_2X_3 + 0.40X_1^2 + 0.21X_2^2 - 0.43X_3^2$
Total monosaccharides	$TM = 1.69 - 1.64X_1 - 0.15X_2 + 0.45X_3 + 0.38X_1X_2 + 0.56X_1X_3 - 0.07X_2X_3 + 1.58X_1^2 + 0.06X_2^2 - 0.23X_3^2$
Total hydrolysis yield	$Y = 10.20 - 3.56X_1 - 0.12X_2 + 0.26X_3 - 0.05X_1X_2 + 0.66X_1X_3 + 0.53X_2X_3 + 0.86X_1^2 + 0.12X_2^2 + 0.62X_3^2$

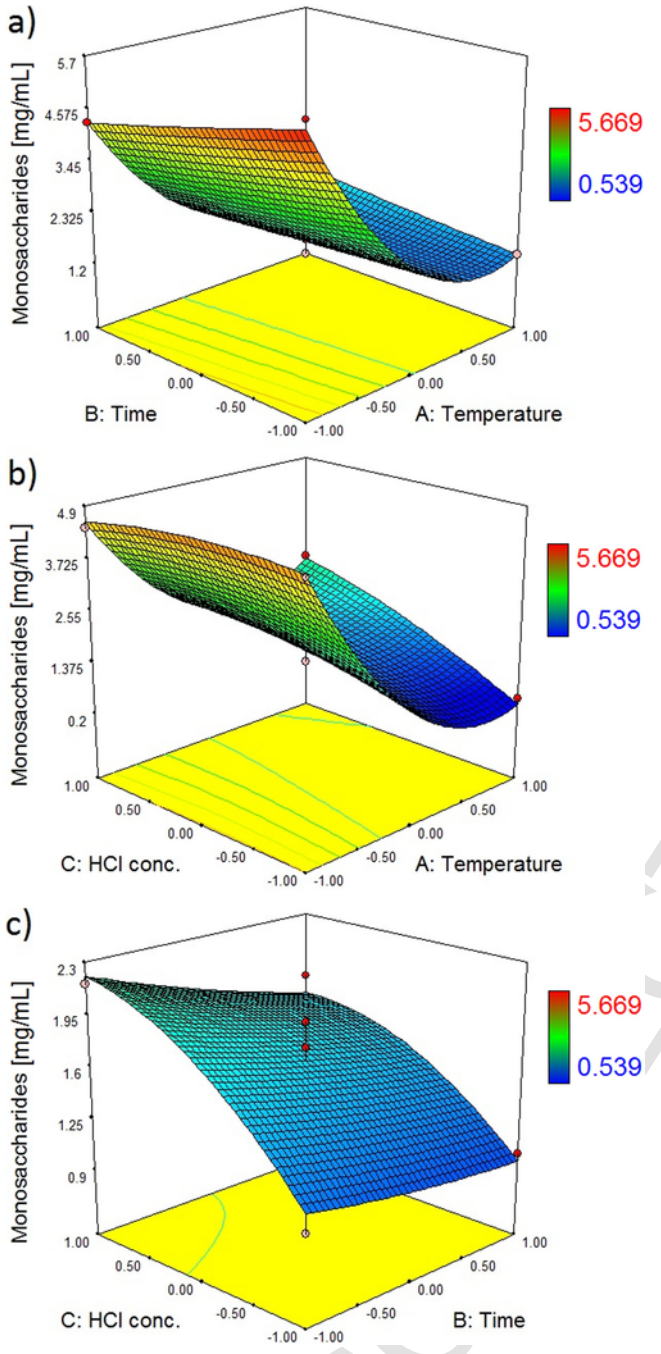

Fig. 2. Influence of corresponding input factors on obtained total monosaccharide content.

tion of hydrolysis process from 25 to 45 min, at constant temperature and HCl conc, a balance between monosaccharides degradation and newly formed reducing sugars is established. The exception is the lowest temperature where the prolongations lead to the increase of reducing sugar content (Fig. 4). Total reducing sugar content refers to both monosaccharides and oligosaccharides obtained in the process of SWH. The difference between total monosaccharides and reducing sugar content represents the amount of cellobiose, cellotriose and other oligosaccharides with reducing properties. The corresponding carbohydrates represent mostly intermediate products of incomplete cellulose hydrolysis and cannot be valorized in terms of fermentation or methanogenesis, but represent an important indicator of hydrolysis progress and efficiency. The corresponding hydrolysis efficiency regarding total reducing sugars content is reported in Fig. 4. The extremely high values regarding obtained reducing sugars percentage originating from insoluble fiber content pinpoint the opportunities of producing value-added products from SBP through SWH. Predictive second-order polynomial model equation for determination of total reducing sugar content was presented in Table 4.

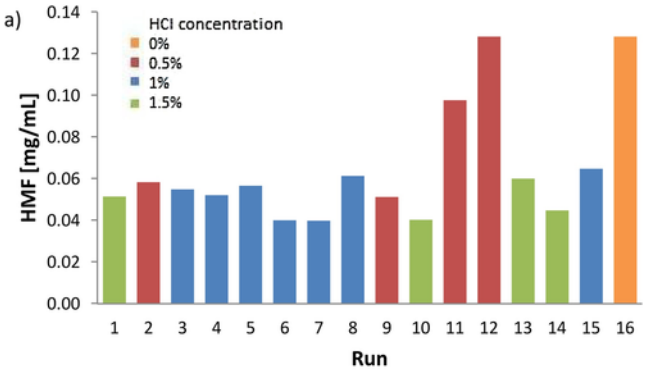

Fig. 3. HMF content and pH of the obtained hydrolysis products.

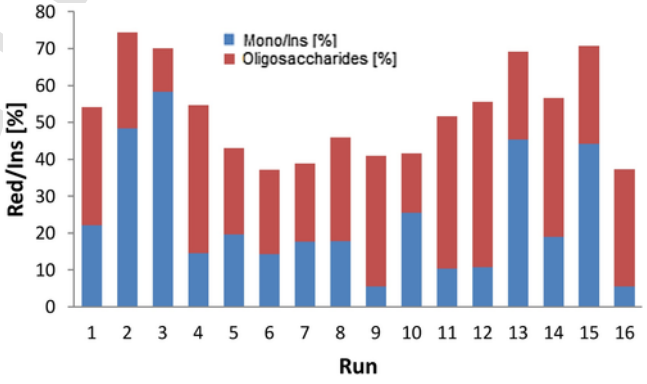

Fig. 4. Total monosaccharide (Mono) and reducing sugars (Red) yield from total insoluble fibers (Ins). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Optimized extraction conditions for maximized Y, TRS and total monosaccharides were temperature of 150 °C, extraction time of 25 min and HCl concentration of 0.5%. Observed desirability function for this optimized system was 0.878, while predicted values of Y, TRS and total monosaccharides were 16.36%, 7.54 mg/mL and 5.30 mg/mL, respectively.

4. Conclusion

Total content of cellulose and hemicellulose in SBP was 61.59% (dry matter basis) indicating high saccharification potential of corre-

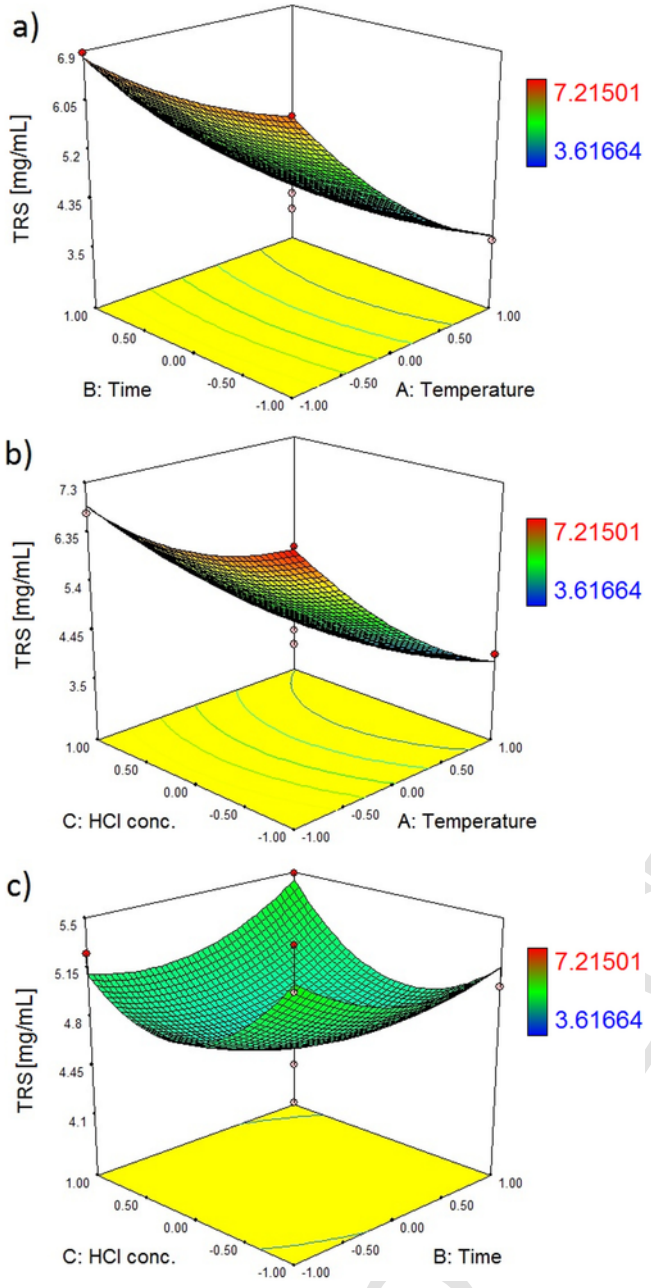

Fig. 5. Influence of corresponding input factors on obtained total reducing sugar content.

sponding raw material. Total hydrolysis yield values ranged from 7.18% up to 15.84% with the highest yield obtained at lowest hydrolysis temperatures applied. HCl addition had greatly positive effect on hydrolysis yield values and total monosaccharide content, increasing the total monosaccharide content up to 200%. High hydrolysis efficiency values obtained, over 44%, suggested highly favorable operating conditions regarding SWH of sugar beet pulp. Rapid monosaccharide degradation at highest temperatures applied lead to the lowest production of corresponding lignocellulosic monomers.

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References

Association of Official Analytical Chemists (AOAC), 1990. Protein and Ash Contents in Food and Food Products, Final Action, AOAC Official Method 985.29.
Association of Official Analytical Chemists (AOAC), 1991. Total, Soluble and Insoluble Dietary Fiber in Foods. AOAC Official Method 991.43.
AOAC International, 2005. Official Methods of Analysis of AOAC International. AOAC International, Gaithersburg.
Alaejos, J., López, F., Pérez, A., Rodríguez, A., Jiménez, L., 2008. Influence of the holm oak soda pulping conditions on the properties of the resulting paper sheets. Bioresour. Technol. 99 (14), 6320–6324.
Allen, S.G., Kam, L.C., Zemann, A.J., Antal, M.J., 1996. Fractionation of sugar cane with hot, compressed, liquid water. Ind. Eng. Chem. Res. 35, 2709–2715.
Asadi, M., 2006. Beet-sugar Handbook. John Wiley & Sons.
Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta 76, 965–977.
Brunner, G., 2014. In: In: Erdogan Kiran (Ed.), Hydrothermal and Supercritical Water Properties. Supercritical Fluid Science and Technology Series Vol. 5, Elsevier.
Egan, H., Kirk, R., Sawyer, R., 1981. The Luff Schoorl method. Sugars and preserves. Pearson's Chem. Anal. Foods 8, 152–153.
Ferreira, S.C., Bruns, R.E., Ferreira, H.S., Matos, G.D., David, J.M., Brandao, G.C., da Silva, E.G.P., Portugal, L.A., dos Reis, P.S., Souza, A.C., Dos Santos, W.N.L., 2007. Box-Behnken design: an alternative for the optimization of analytical methods. Anal. Chim. Acta 597, 179–186.
Gírio, F.M., Fonseca, C., Carvalheiro, F., Duarte, L.C., Marques, S., Bogel-Łukasik, R., 2010. Hemicelluloses for fuel ethanol: a review. Bioresour. Technol. 101, 4775–4800.
Gbashi, S., Adebo, O.A., Piater, L., Madala, N.E., Njobeh, P.B., 2017. Subcritical water extraction of biological materials. Sep. Purif. Rev. 46, 21–34.
Gusakov, A.V., Salanovich, T.N., Antonov, A.I., Ustinov, B.B., Okunev, O.N., Burlingame, R., Emalfarb, M., Baez, M., Sinitsyn, A.P., 2007. Design of highly efficient cellulase mixtures for enzymatic hydrolysis of cellulose. Biotechnol. Bioeng. 97, 1028–1038.
Herrero, M., Castro-Puyana, M., Mendiola, J.A., Ibañez, E., 2013. Compressed fluids for the extraction of bioactive compounds. TrAC—Trend. Anal. Chem. 43, 67–83.
Kelly, P., 1983. Sugar beet pulp—a review. Anim. Feed Sci. Technol. 8, 1–18.
Khajavi, S.H., Kimura, Y., Oomori, T., Matsuno, R., Adachi, S., 2005. Degradation kinetics of monosaccharides in subcritical water. J. Food Eng. 68, 309–313.
Knez, , Hrnčič, M.K., Čolnik, M., Škerget, M., . Chemicals and value added compounds from biomass using sub-and supercritical water. J. Supercrit. Fluid
López, F., Alaejos, J., Rodríguez, A., Jiménez, L., 2008. Pulping of holm oak wood. Influence of the operating conditions. Bioresour. Technol. 99, 819–823.
Malester, I.A., Green, M., Shelef, G., 1992. Kinetics of dilute acid hydrolysis of cellulose originating from municipal solid wastes. Ind. Eng. Chem. Res. 31, 1998–2000.
Mayanga-Torres, P.C., Lachos-Perez, D., Rezende, C.A., Prado, J.M., Ma, Z., Tompsett, G.T., Timko, M.T., Forster-Carneiro, T., 2017. Valorization of coffee industry residues by subcritical water hydrolysis: recovery of sugars and phenolic compounds. J. Supercrit. Fluid 120, 75–85.
McMillan, J.D., 1994. Pretreatment of lignocellulosic biomass. In: Himmel, M.E., Baker, J.O., Overend, R.P. (Eds.), Enzymatic Conversion of Biomass for Fuels Production. American Chemical Society, Washington, DC, pp. 292–324.
Montgomery, D.C., 2001. Design and Analysis of Experiments, 5th ed. John Wiley & Sons Inc., New York.
Mudhoo, A., Forster-Carneiro, T., Sánchez, A., 2011. Biohydrogen production and bioprocess enhancement: a review. Crit. Rev. Biotechnol. 31, 250–263.
Mussatto, S.I., 2016. Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery. Elsevier.
Pavlić, B., Vidović, S., Vladić, J., Radosavljević, R., Cindrić, M., Zeković, Z., 2016. Subcritical water extraction of sage (Salvia officinalis L.) by-products—process optimization by response surface methodology. J. Supercrit. Fluid 116, 36–45.
Powell, T., Bowra, S., Cooper, H.J., 2016. Subcritical water processing of proteins: an alternative to enzymatic digestion?. Anal. Chem. 88, 6425–6432.
Prado, J.M., Follegatti-Romero, L.A., Forster-Carneiro, T., Rostagno, M.A., MaugeriFilho, F., Meireles, M.A.A., 2014. Hydrolysis of sugarcane bagasse in subcritical water. J. Supercrit. Fluid 86, 15–22.
Rombouts, F.M., Thibault, J.F., 1986. Feruloylatedpectic substances from sugar-beet pulp. Carbohyd. Res. 154, 177–187.
Šušić, S., Petrov, S., Kukić, G., Sinobad, V., Perunović, P., Koronsovac, B. et al. 1994. Osnovi tehnologije šećera. Yugoslavian sugar industry (Jugosećer, Belgrade).
Saeman, J.F., 1945. Kinetics of wood saccharification-hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Ind. Eng. Chem. 37, 43–52, 3.
Saldana, M.D., Valdivieso-Ramírez, C.S., 2015. Pressurized fluid systems: phytochemical production from biomass. J. Supercrit. Fluid 96, 228–244.
Sasaki, M., Kabyemela, B., Malaluan, R., Hirose, S., Takeda, N., Adschiri, T., Arai, K., 1998. Cellulose hydrolysis in subcritical and supercritical water. J. Supercrit. Fluid 13, 261–268.
Sasaki, M., Adschiri, T., Arai, K., 2003. Fractionation of sugarcane bagasse by hydrothermal treatment. Bioresour. Technol. 86, 301–304.
Serrano-Ruiz, J.C., Dumesic, J.A., 2011. Catalytic routes for the conversion of biomass into liquid hydrocarbon transportation fuels. Energ. Environ. Sci. 4, 83–99.
Sun, Y., Cheng, J., 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol. 83, 1–11.
Taherzadeh, M.J., Karimi, K., 2007. Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bioresources 2, 472–499.
Teo, C.C., Tan, S.N., Yong, J.W.H., Hew, C.S., Ong, E.S., 2010. Pressurized hot water extraction (PHWE). J. Chromatogr. A 1217, 2484–2494.

Toor, S.S., Rosendahl, L., Rudolf, A., 2011. Hydrothermal liquefaction of biomass: a review of subcritical water technologies. Energy 36, 2328–2342.
Wang, X., Chen, Q., Lü, X., 2014. Pectin extracted from apple pomace and citrus peel by subcritical water. Food Hydrocolloids 38, 129–137.
Yedro, F.M., Grénman, H., Rissanen, J.V., Salmi, T., García-Serna, J., Cocero, M.J., 2017. Chemical composition and extraction kinetics of Holm oak (Quercus ilex) hemicelluloses using subcritical water. J. Supercrit. Fluid 129, 56–62.
Zeković, Z., Vidović, S., Vladić, J., Radosavljević, R., Cvejin, A., Elgndi, M.A., Pavlić, B., 2014. Optimization of subcritical water extraction of antioxidants from Coriandrum sativum seeds by response surface methodology. J. Supercrit. Fluid 95, 560–566.