Optimization of alkali-catalyzed organosolv treatment of spent coffee grounds for obtaining polysaccharides

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ABSTRACT

The coffee industry is growing rapidly and generating increasing amounts of spent coffee grounds annually. Spent coffee grounds contain high levels of polysaccharide, which needs in-depth research to obtain and transform into value-added products. This study was carried out to optimize the alkali-catalyzed organosolv treatment of spent coffee grounds to enrich the polysaccharide content. A three-factor central composite design of the response surface model was used to optimize the treatment variables including reflux time, NaOH, and acetone concentration to yield the highest polysaccharide level. As a result, the maximum polysaccharide content was 73.13% obtained at a reflux time of 4.5 h, 62% acetone with 0.91% NaOH. The polysaccharide-rich material from spent coffee ground was composed of 39.37% mannan, 10.40% glucan, and 9.33% galactan. Partial removal of lignin and protein was observed during the treatment. Enzymatic hydrolysis of the spent coffee polysaccharides released the highest reducing sugars of 5583 mg/L using an enzyme cocktail containing 4% of cellulase and 1% of mannanase after 96 h. The enzymatic hydrolysate contained 3190 mg/L mannose and 1790 mg/L glucose, showing a feasible transformation of spent coffee polysaccharides.

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1. Introduction

Spent coffee grounds (SCGs) have no commercial value and are usually discarded as solid waste or sent to compost facilities. In recent years, the increasing need for waste reduction and environmental protection has stimulated the search for possible methods of valorizing this waste. SCGs contain large amounts of organic compounds including lipid, cellulose, hemicellulose, and lignin. Currently, SCGs have been investigated for the production of biodiesel, activated carbon, and a source of sugars (Preethu et al., 2007; Mussatto et al., 2011; Caetano et al., 2012){Mussatto, 2011 #1; Caetano, 2012 #20}.

Being a porous and carbon-rich material, SCGs have been studied to become an inexpensive heavy metal adsorbent to replace traditional materials (Fiol et al., 2008).

Polysaccharides account for about 50% of total SCGs (Trinh et al., 2022). Arabinogalactan, galactomannan and cellulose are major polysaccharides in the SCGs, in which galactomannan is the most abundant component. Galactomannan has been noted as an excellent stiffener and stabilizer of emulsions, which shows potential in the food, pharmaceutical, and cosmetic industries (Ballesteros et al., 2015). Some findings have shown the antimicrobial and antioxidant capacities of polysaccharides extracted from SCGs (Ballesteros et al., 2015) and the immunostimulatory properties provided by coffee galactomannans and arabinogalactans (Simões et al., 2009). The prebiotic potential of spent coffee mannan has been also proved since it improves the health of human intestinal microflora (Asano et al., 2003; Gniechwitz et al., 2007). Besides being applied in the food and biomedical sector, spent coffee polysaccharides have been performed with potential uses in the manufacture of biodegradable materials.

The application of spent coffee polysaccharides is limited due to the presence of other components such as lignin, phenolic compounds, protein, and lipids attached to its structure. In our previous study, SCGs were treated using alkali and solvent to remove these components and make changes in SCG's structure. As a result of this, we obtained polysaccharide-rich materials that were effectively hydrolyzed into soluble sugars (Trinh et al., 2022). Combining alkali and solvent significantly improved the delignification of SCGs because both agents can cleave the ester linkages between polysaccharides and lignin. From these important findings, the present study was conducted to optimize the alkali-catalyzed organosolv treatment of SCGs to achieve the highest polysaccharide level. Polysaccharides derived from SCGs can be applied in various sectors such as foods, medicals, and biomaterials. The conversion of SCGs into value-added products not only provides benefits for the coffee industry, improves income for farmers, but also reduces environmental impacts.

2. Materials and Methods

2.1. Materials

The SCGs were obtained from branched coffee shops in Ho Chi Minh City from October 2022 to December 2022. The collected samples were mixed and dried at 50°C until their moisture content reached below 10% before storing at room temperature.

Cellulase enzymes (700 EGU/g) and Mannanase enzymes (500 U/g) were provided by Novozyme and LeafCleanTech, respectively. 3,5-Dinitrosalicylic acid (DNS) was purchased from Duksan (Korea). Citric acid monohydrate and sodium sulfite were purchased from Merck. Sodium citrate trihydrate, Rochelle salt, sodium hydroxide, phenol, and n-hexane were supplied by Xilong (China).

2.2. Methods

2.2.1. Defatting

The SCGs were defatted using the process described previously with some modifications (Trinh et al., 2022). Briefly, 100 g of SCGs were immersed in n-hexan overnight with a ratio of material to solvent of 1:5 (w/v). The lipid extraction was conducted by sonication for 30 min twice. The defatted SCGs were separated by filtration and dried in an oven at 60°C. Its moisture content was measured before further experiments.

2.2.2. Alkali-catalyzed organosolv treatment of spent coffee grounds

Polysaccharide-rich materials were obtained by removing lipid, lignin, protein, and some phenolic compounds in the SCGs matrix. Defatted SCGs were treated by refluxing (0.5 - 6.5 h) using aqueous acetone (10 - 90%) with a ratio of material to solvent of 1:10. Alkali (NaOH) functioned as a catalyst with a concentration range of 0.1 - 1.3% (w/v). After processing, the solid was washed intensively with distilled water to remove residual alkali and other degradation products until the pH value reached 7.0. The solid was dried at 70°C and stored at 4°C before determining the polysaccharide content.

2.2.3. Experimental design

Optimization of polysaccharide content in SCGs was conducted using a central composite design (CCD) of response surface methodology (RSM). Three independent variables were reflux time (X_1, 0.5 - 6.5 h), NaOH concentration (X_2, 0.1 - 1.3%), and acetone concentration (X3, 10 - 90%). There were 21 experimental runs including eight factorial points, six axial points, and seven center points. The significance level of the response surface model and the equation terms were studied by analysis of variance (ANOVA) with 95% confidence on Minitab 16 software. The quality of fit of the regression model was evaluated through the coefficient of determination (R^2), adjusted correlation coefficient (adj-R^2), and predictive correlation coefficient (pred-R^2. Independent variables and their levels used in the response surface design are presented in Table 1.

Table 1. Independent variables and their levels used in the response surface design

Independent variables	Unit	Coded	Coded factor level				
			- α	-1	0	+1	+ α
Reflux time	h	X_1	0.5	2	3.5	5	6.5
NaOH concentration	%	X_2	0.1	0.4	0.7	1	1.3
Acetone concentration	%	X_3	10	30	50	70	90

2.2.4. Determination of polysaccharide content

Polysaccharide content in the treated SCGs was determined by measuring the total reducing sugars released during the complete hydrolysis process using sulfuric acid reported previously. Polysaccharide-rich materials were hydrolyzed with 72% aqueous sulfuric acid for 60 min at 30°C, then distilled water was added to the mixture to dilute the acid to a 4% concentration, which was autoclaved for 60 min at 121°C. The acid hydrolysate was made up to a volume of 100 mL. Total reducing sugar content was quantified using 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959). Briefly, 1 mL of sample or glucose standard was mixed with 1 mL of DNS reagent. The mixture was kept in a boiling water bath for 5 min, then cooled down by immersing in a cold water bath before adding 3 mL of water. The absorbance of the mixture was measured using a spectrophotometer at 515 nm. Glucose was used as the standard with the range of 50 - 300 mg/L (Trinh et al., 2022).

2.2.5. Chemical compositions of spent coffee grounds

The moisture content of SCGs was measured by gravimetric analysis after drying in an oven at 105°C until obtaining constant weight mass. The nitrogen content of SCGs was estimated by the Kjeldahl method according to TCVN 10791:2015 (VS, 2015) and the protein content was measured using an appropriate Nitrogen Factor of 6.25. Ash is the inorganic residue remaining after dry oxidation at 550°C. The ash content of SCGs was quantified by gravimetric analysis according to TCVN 8124:2009/ISO 2171:2007 (VS, 2009). Acid-insoluble lignin or Klason lignin is the residual fraction after removing the ash by the complete acid hydrolysis of SCGs (Sluiter et al., 2008). Acid insoluble lignin was estimated by gravimetric analysis of the dry solid residue on the crucibles subtracted from the ash.

2.2.6. Enzymatic hydrolysis of SCGs

Polysaccharides obtained from SCGs can be used as a source of sugars through enzymatic hydrolysis. The treated SCGs were hydrolyzed using a combination of commercial polysaccharide-degrading enzymes including cellulase (Cellulast, 0 - 5%, enzyme to substrate) and mannanase (0 - 5%) at different ratios. The control experiment was conducted without adding an enzyme. Enzymatic hydrolysis experiments were performed in 0.05 M citrate buffer at pH 4.8 with a ratio of material to buffer of 1:25 (w/v). The mixture was incubated at 50°C in a shaker at 120 rpm. The hydrolysate was collected every 24 h and then filtered through a nylon membrane of 0.22 µm before determining the total reducing sugars and monosaccharides (Trinh et al., 2022).

2.2.7. Analysis of monosaccharides

Monosaccharides including mannose, glucose, and galactose in the acid and enzymatic hydrolysate were determined by high-performance liquid chromatography (HPLC Agilent 1200 Infinity II) (Sluiter et al., 2008). The Rezex RPM-Monosaccharide Pb+2 (8%) column (100 x 7.8 mm) was stabilized at 40°C. The refractive index (RI) detector was set up at 80°C. The mobile phase was deionized water with a flow rate of 0.2 mL/min. Monosaccharide standards were prepared in the range of 100 - 1000 mg/L (Trinh et al., 2022).

3. Results and discussion

3.1. Optimization of treatment conditions of SCGs for obtaining polysaccharides

The experimental matrix, actual values, and predicted values are shown in Table 2. In the optimized model, the significance level $P < 0.05$ was used to test the effect of independent variables and their interaction with the response variable. The effect of reflux time (X_1), NaOH concentration (X_2), and acetone concentration (X_3) on polysaccharide content (Y) was expressed by a regression equation as follows:

$$\text{Polysaccharides (\%)} = 44.94 + 1.19\,X_1 + 62.70\,X_2 - 0.058\,X_3 - 1.731\,X_1^2 - 63.66\,X_2^2 - 0.010327\,X_3^2 \times 3.82\,X_1 \times X_2 + 0.1782\,X_1 \text{ x } X_3 + 0.5809\,X2 \times X3$$

Table 2. Response surface central composite design for polysaccharides

Run	Treatment conditions			Polysaccharide content	
	Time (h)	NaOH concentration (%)	Acetone concentration (%)	Actual value (%)	Predicted value (%)
1	2.0	0.4	30	63.73	64.96
2	5.0	0.4	30	53.75	52.80
3	2.0	1.0	30	66.06	64.15
4	5.0	1.0	30	58.19	58.86
5	2.0	0.4	70	46.07	44.88
6	5.0	0.4	70	52.73	54.10
7	2.0	1.0	70	57.59	58.01
8	5.0	1.0	70	75.84	74.10
9	0.5	0.7	50	54.72	55.19
10	6.5	0.7	50	59.06	59.12
11	3.5	0.1	50	40.70	40.22
12	3.5	1.3	50	58.41	59.42
13	3.5	0.7	10	58.41	58.63
14	3.5	0.7	90	53.49	53.79
15	3.5	0.7	50	72.81	72.74
16	3.5	0.7	50	71.55	72.74
17	3.5	0.7	50	72.50	72.74
18	3.5	0.7	50	73.98	72.74
19	3.5	0.7	50	72.54	72.74
20	3.5	0.7	50	72.54	72.74
21	3.5	0.7	50	72.54	72.74

Table 3 shows an analysis of variance (ANOVA) for the response surface quadratic model. The regression model was statistically significant ($P < 0.05$) and the reflux time, NaOH concentration, and acetone concentration significantly affected the polysaccharide content. The coefficient of determination $R^2 = 0.9909$ demonstrated experimental data were fitted well to the predicted model. The value of the predicted determination coefficient of 0.9437 (pred-R^2) was high indicating the reliability of predicted values. In addition, the predicted-R^2 was in reasonable agreement with the adjusted-R^2 (the difference < 0.2). Furthermore, the p-value of lack of fit was not statistically significant ($P > 0.05$) demonstrating the regression model was compatible with the actual experiments.

Table 3. ANOVA for response surface quadratic model

Factor	Df	Adj Sum of Squares	Adj Mean Square	F	*P*
X_1	1	15.47	15.467	8.76	0.0143
X_2	1	368.35	368.353	208.51	0.0000
X_3	1	23.42	23.421	13.26	0.0045
X_1*X_1	1	381.49	381.491	215.95	0.0000
X_2*X_2	1	825.31	825.305	467.17	0.0000
X_3*X_3	1	429.02	429.024	242.85	0.0000
X_1*X_2	1	23.64	23.635	13.38	0.0044
X_1*X_3	1	228.72	228.724	129.47	0.0000
X_2*X_3	1	97.18	97.181	55.01	0.0000
Residual					
Lack of fit	5	14.62	2.29	4,79	0,0553
Model Summary					
S		R-sq	R-sq (adj)	R-sq (pred)	
1.32913		99.09 %	98.28 %	94.37 %	

The effect of reflux time, NaOH concentration, and acetone concentration on polysaccharide content is illustrated in Figure 1. As seen in the RSM plot, polysaccharide content increased as reflux time, NaOH and acetone concentration increased. The highest polysaccharide content was achieved for a 4.5 h reaction in 62% aqueous acetone in the presence of 0.91% NaOH. However, polysaccharide content gradually decreased by prolonging residence time and increasing acetone and NaOH concentration. Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin, which are linked together to form a complex and rigid structure. The treatment of lignocellulose with alkali generally breaks linkages between lignin - polysacc harides matrix, thus lignin was partially removed during the processing, leading to increased polysaccharide content in the treated solids (Chen et al., 2008; Garlock et al., 2012). In addition, organic solvents have a prominent effect on disrupting hydrogen and ether bonds, thereby a large amount of lignin was eliminated during organosolv treatment (Yin et al., 2021). However, hemicellulose was easily degraded under harsh treatment conditions such as long reaction time and high alkali concentrations (Harun & Geok, 2016), resulting in a decrease in the polysaccharide content in the treated sample as clearly shown on the RSM plot.

The maximized polysaccharide content was predicted as 74.41% using the optimized model. The treatment of SCGs was conducted for 4.5 h refluxing in 62% aqueous acetone and 0.91% NaOH, thereby polysaccharide content in the treated materials was found to be 73.13%. The t-test at a 5% significance level showed no significant difference between the actual value and the predicted value. Therefore, the optimized conditions in this experiment were suitable for the treatment of SCGs to obtain the highest polysaccharide content. Polysaccharide content in the treated SCG significantly increased by 1.6 times compared to that in the defatted sample (45.24%). SCG polysaccharides can be used to produce biodegradable films, coatings, adsorbents, biopolymers, or additives in food and feed industry (Simões et al., 2009).

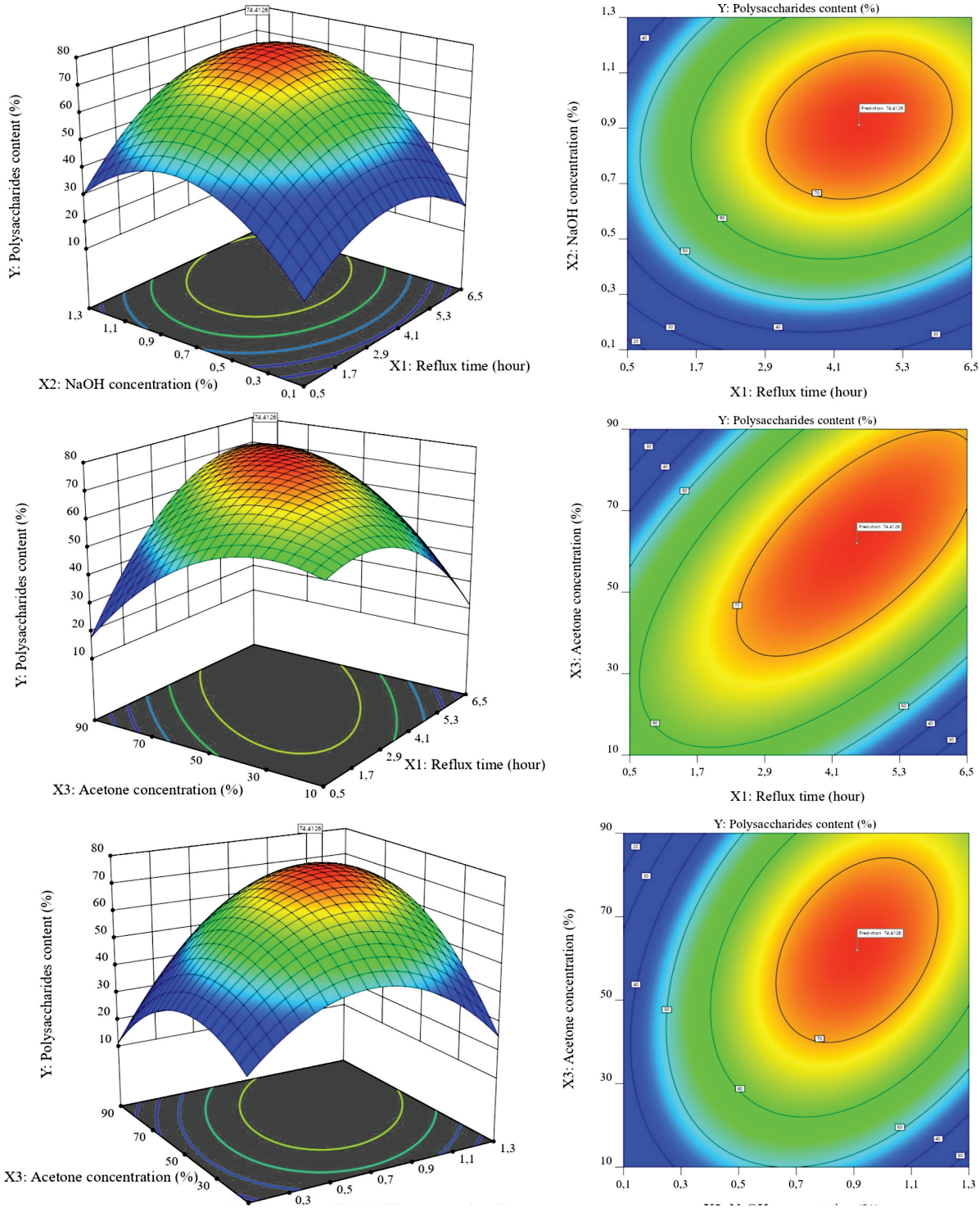

Figure 1. Response surface and contour plots showing the effect of the factors on polysaccharide content.

3.2. Chemical composition of polysaccharide-rich materials

The SCG polysaccharides were obtained by treating SCG with alkali and acetone at optimized conditions. Polysaccharides including glucan, galactan, and mannan in the untreated (defatted sample) and treated solids were analyzed by measuring glucose, galactose, and mannose in the acid hydrolysate of SCGs using HPLC. As shown in Figure 2, polysaccharide content in the treated solids significantly improved as compared to untreated SCGs. Mannan was the most abundant polysaccharide identified in the treated material, accounting for 39.37% of the solids and 1.5-fold higher than that in the untreated sample. Similarly, glucan and galactan were enriched in the obtained solid with values of 10.40% and 9.33%, respectively. The enhancement of mannan, glucan, and galactan content in the treated samples was attributed to the effective removal of some components during the alkali-catalyzed organosolv treatment such as lignin and protein as shown in Figure 3. The findings were in agreement with our earlier studies (Trinh et al., 2022) The mannan level obtained by the integrated alkali-catalyzed organosolv method in this study was higher than in the SCG treated with individual ethanol (Nguyen et al., 2017) or alkaline (Trinh et al., 2022). Polysaccharide-rich materials derived from SCGs can be used as a potential source of prebiotics, sugars, and adsorbents, which would be applied in medical, food and feed industry, or biomaterial sectors (Simões et al., 2009).

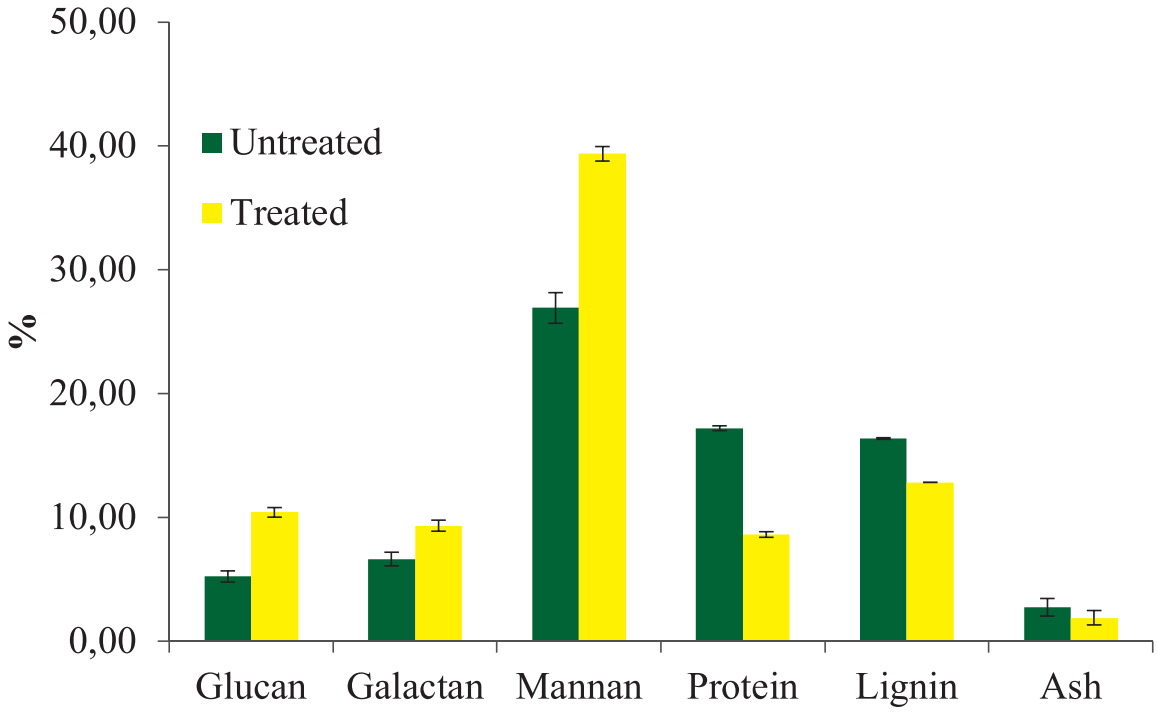

Figure 2. Chemical components of polysaccharides derived from spent coffee grounds.

3.3. Enzymatic hydrolysis of SCG polysaccharides

The SCGs-derived polysaccharides were rich in mannan, glucan, and galatan, which make them to be promising to produce soluble sugars. Enzymatic hydrolysis was an effective method to achieve high sugar yields from various lignocellulosic biomass (Trinh et al., 2018). Generally, enzymatic hydrolysis of biomass containing cellulose and hemicellulose requires a mixture of cellulase and hemicellulase to increase hydrolysis efficiency (Tang et al., 2020). Cellulast is a commercial cellulase that has been widely used in the treatment of lignocellulosic materials because it can break β-1.4-glycosidic bonds in the structure of both cellulose and hemicellulose (Roy et al., 2020). Mannanase enzymes belonging to the hemicellulase group can catalyze the hydrolysis of the β-1.4-mannosidic bonds in the main chain of mannans that are found as the major polysaccharide in SCGs (Liao et al., 2014). To improve the release of sugar during the hydrolysis of SCG polysaccharides, our study performed a combination of cellulase and mannanase enzymes with different proportions at 5% of enzyme loading (enzyme to the substrate).

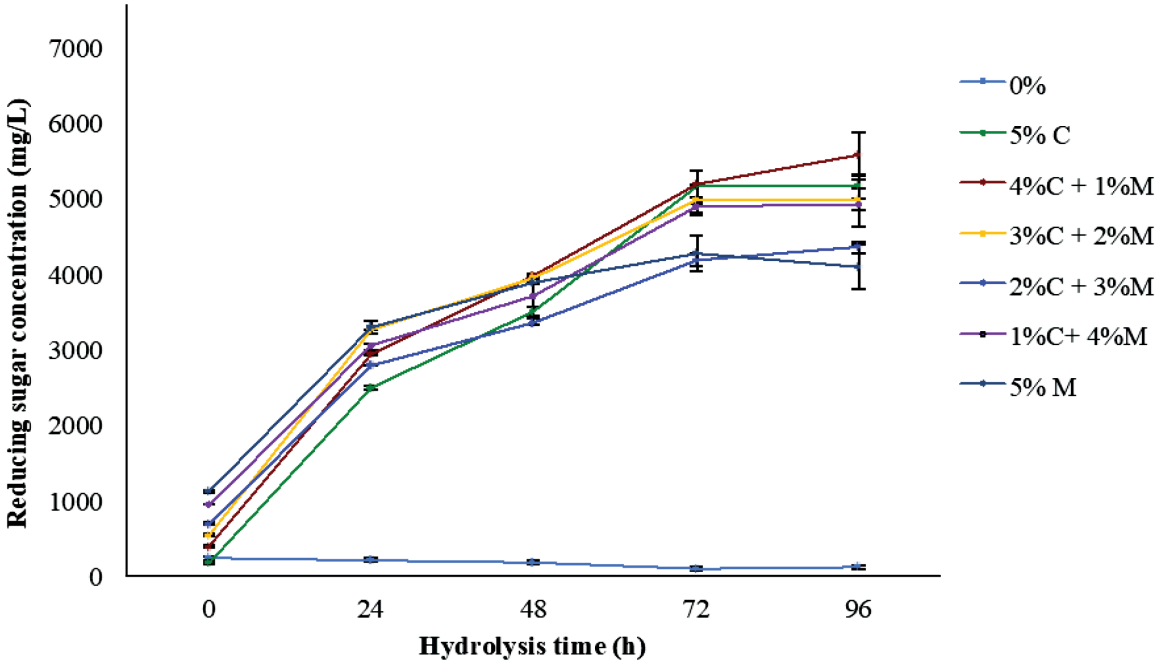

Figure 3. Enzymatic hydrolysis of spent coffee ground polysaccharides. C: Cellulase; M: mannanase.

Figure 3 shows the release of total reducing sugars during enzymatic hydrolysis of SCG polysaccharides. Reducing sugar concentrations reached 5141 mg/L and 4074 mg/L when using individual cellulase and mananase after 96 h, respectively. The findings indicated cellulase is more effective than mananase to hydrolyze SCG polysaccharides. The treatment using 4% of cellulase and 1% of mannanase (enzyme to the substrate) showed the highest reducing sugar yield of 5583 mg/L and it was statistically different from the other treatments ($P < 0.05$). The use of an enzyme combination is regarded as a promising strategy to enhance the hydrolysis efficiency of SCGs because of its synergistic effects. Additional mannanase has been reported to give better sugar yield than using only cellulase or xylanase for the hydrolysis of SCGs (Jooste et al., 2013). However, increasing mannanase loading from 2 - 4% didn't have a positive effect on sugar yields as compared to using cellulase alone.

The data revealed the major role of cellulase with a suitable amount of mannanase required to enhance the hydrolysis efficiency of SCGs. Enzymatic hydrolysate obtained at the optimal treatment after 96 h contained 3190 mg/L mannose and 1790 mg/L glucose. Enzymatic conversion yield from polysaccharides to soluble sugars was estimated about 431.4 mg/g which were higher than our previously reported results using Viscozyme (Trinh et al., 2022). Additionally, alkali-catalyzed organosolv treatment made changes in SCG's structures and compositions, facilitating the enzyme accessibility to its substrate. However, galactose was not found in the hydrolysate (Figure 4).

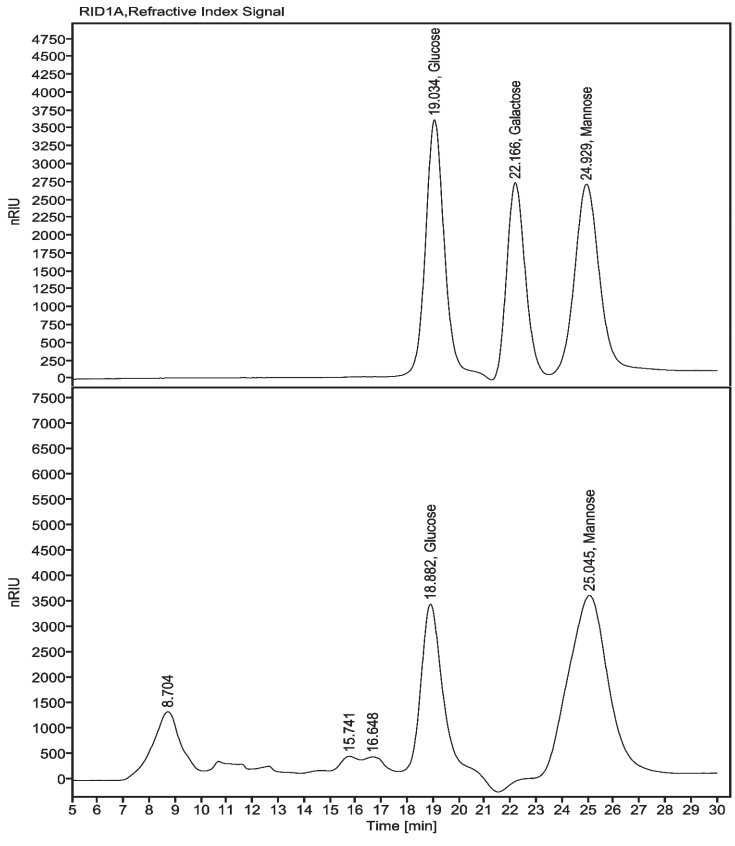

Figure 4. Chromatogram of sugar standards (above) and spent coffee hydrolysate (below).

5. Conclusions

In this study, the highest polysaccharide content reached 73.13% at optimized conditions including a reflux time of 4.5 h, 62% acetone with 0.91% NaOH. The polysaccharides from SCGs were composed of 39.37% mannan, 10.40% glucan, and 9.33% galactan. Lignin and protein were partially removed during the processing. The use of the enzyme mixture containing 4% of cellulase and 1% of mannanase gave the highest sugar production of 5583 mg/L through 96 h of hydrolysis. Enzymatic hydrolysis of the SCG polysaccharides released 3190 mg/L mannose and 1790 mg/L glucose.

Conflict of interest

The authors have no conflicts of interest to declare.

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