Two-step pretreatment for improving enzymatic hydrolysis of spent coffee grounds

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ABSTRACT

Spent coffee ground (SCG) has attracted increasing attention since it contains many useful components such as polysaccharides, protein, lipid, and bioactive compounds. The aim of this research was to enhance the enzymatic hydrolysis to release important sugars in the SCG using different pretreatment methods. Spent coffee grounds were pretreated by alkali pretreatment, organosolv pretreatment, and the combined process. The pretreated material was hydrolyzed by different commercial enzymes including Cellulast, Pectinex, Ultraflomax, and Viscozyme. Monosaccharides, total phenolic content, and antioxidant activity in the hydrolysate were measured and evaluated. The use of Viscozyme achieved the highest reducing sugar yield and showed a significant difference from other enzymes. Alkali and organosolv pretreatment were demonstrated to improve the production of sugars. The alkali pretreatment followed by organosolv treatment effectively removed lignin, resulting in only 14% lignin in the pretreated sample. The maximum reducing sugar concentration reached 6120 mg/L through two-step pretreatment and subsequent enzymatic hydrolysis, corresponding to a yield of 161 mg sugar/g substrate. The SCG hydrolysate contained 2917 mg/L mannose, 1633 mg/L glucose, and 957 mg/L galactose. Phenolic compounds were observed to be released during the enzymatic hydrolysis, giving a total phenolic content of 174.4 mg GAE/L and the SCG hydrolysate also showed an antioxidant capacity equivalent to 263.2 mg/L ascorbic acids after 120 h hydrolysis. This study demonstrated a scalable two-step pretreatment process to obtain important sugars including mannose, glucose, and galactose along with phenolic compounds for further industrial uses.

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1. Introduction

Coffee is one of the most popular beverages in the world and the second largest traded commodity after petroleum. Coffee production generates an enormous amount of solid residues namely spent coffee grounds (SCGs). About nine million tons of SCGs are released into the environment every year, which may cause serious environmental problems (Karmee, 2018). SCG has recently attracted increasing interest since it is a valuable resource of sugars, oils, antioxidants, proteins, and other high-value compounds (Peshev et al., 2018).

SCG is a lignocellulosic material and essentially consists of polysaccharide polymers and lignin. The major polysaccharides in SCG include galactomannan, arabinogalactan, and cellulose. Among the monosaccharides in SCG, mannose constitutes the largest portion (20 - 30%) of its total carbohydrate content, which make it become a promising source for mannose production (Nguyen et al., 2017). Mannose has been widely used in the food, pharmaceutical, cosmetic and

poultry industries and acts as starting material for the synthesis of drugs (Hu et al., 2016). Although carbohydrate is the most abundant fraction in SCG (up to 50%), the extraction of sugars from SCG is not simple. Like other lignocellulosic biomass, SCG structure is rigid, dense, and recalcitrant. Without any pretreatment, the bioconversion yield of polysaccharides into monosaccharides is limited.

Pretreatment methods are applied to increase the efficiency of lignocellulose hydrolysis by improving enzyme accessibility to polysaccharides. An efficient pretreatment strategy is generally simple to perform and produces high fermentable sugar yields with the minimal formation of degradation products (Ravindran et al., 2017). High lignin content restricts the efficiency of enzymatic hydrolysis of lignocellulosic biomass. Therefore, removal of lignin is a key strategy for achieving effective pretreatment and hydrolysis. Ranvindran et al. (2017) performed eight different pretreatment methods in SCG but a single process didn't give desirable results. Then, the sequential combined process using concentrated acid, and acetone pretreatment followed up with the ammonia fiber explosion pretreatment showed to achieve the maximum sugar yield. A combined process is a recent strategy since single pretreatment couldn't overcome the recalcitrance of complex biomass.

In this study, SCGs were pretreated using alkali and organosolv pretreatment. Both two methods aim to dissolve lignin by cleaving the ester linkages between polysaccharides and lignin. However, the pretreatments can cause partial degradation of hemicellulose and cellulose at severe conditions of temperatures or alkaline solution concentrations. Therefore, we performed SCG pretreatment at mild conditions and applied two-step pretreatment combining alkali and organosolv pretreatment. This approach is potential to accelerate the enzymatic hydrolysis via promoting delignification but minimizing polysaccharide degradation and expected to be feasible in large scale.

2. Material and Methods

2.1. Material

Spent coffee grounds were collected from several coffee shops in Ho Chi Minh city, Vietnam. The samples were mixed and dried at 50°C to a moisture content of below 10% before use.

Cellulast 1.5 L, Pectinex Ultra SP-L, Ultraflomax and Viscozyme (Novozyme) were supplied by Brenntag (Vietnam). Standard chemicals including glucose, galactose, mannose were purchased from Sigma Aldrich. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 3,5-Dinitrosalicylic acid (DNS), Folin-Ciocalteu, gallic acid, albumin and ascorbic acid were purchased from Merck. Other chemicals were purchased from Xilong (China).

2.2. Methods

2.2.1. Defatting

SCG was defatted using hexane (ratio of hexane:biomass is 5:1) by sonication for 30 min. The defatted biomass was dried in an oven at 60°C, and its moisture content was measured before further analysis.

2.2.2. Chemical compositions of SCG

Crude protein and ash content of SCG were quantified according to TCVN 10791:2015 and TCVN 8124:2009/ISO 2171:2007, respectively.

The qualitative analysis of the monosaccharide compositions and lignin content of SCG samples was performed according to Sluiter et al. (2008) and Trinh et al. (2018).

2.2.3. Pretreatment of SCG

Defatted SCG was pretreated using several methods. Alkali pretreatments were carried out using NaOH 1% in an autoclave at 120°C for 15 min at a ratio of biomass to the alkaline solution of 1:5. Organosolv pretreatments were conducted by mixing SCG with acetone at a ratio of biomass to solvent of 1:5 in a sonicator apparatus for 1 h. The solid residue was separated and dried for further use. The two-step pretreatment was initiated using alkali pretreatment followed by organosolv pretreatment.

2.2.4. Enzymatic hydrolysis of SCG

Pretreated SCG was hydrolyzed using several commercial polysaccharide-degrading enzymes including Cellulase, Pectinex, Ultrafomax, and Viscozyme. Enzymatic hydrolysis experiments were carried out in 50 mM citrate buffer (pH 5.0) with 4% biomass (w/v) in a shaking in-

cubator. The ratio of enzyme to SCG is 5%. The hydrolysate was collected by centrifugation for 10 min and filtered through a nylon membrane 0.22 µM before a measurement of reducing sugars and monosaccharides.

2.2.5. Determination of reducing sugars

Reducing sugar content was quantified using 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959). 1 mL of sample was mixed with 1 mL of DNS for 5 min in a boiling bath, then the mixture was kept in a cold water bath for 10 min prior to adding 3 mL of water. Glucose was used as the standard with a range of 50 - 300 mg/L.

2.2.6. Analysis of monosaccharides

Mannose, galactose and glucose were quantified by high-performance liquid chromatography (HPLC Agilent 1200 Infinity II) using a refractive index detector. The Rezex RPM- Monosaccharide Pb+2 (8%) (Phenomenex) column (100 mm × 7.8 mm) was operated at 85°C. The mobile phase is deionized water at a flow rate of 0.2 mL/min.

2.2.7. Determination of total phenolic content and antioxidant activity

The total phenolic content (TPC) in the hydrolysate was determined using the Folin-Ciocalteu colorimetric method described previously (Trinh et al., 2018). Antioxidant activity of the hydrolysate was estimated by DPPH assay according to the reported procedure (Trinh et al., 2018). Briefly, 1 mL of sample was added to 1 mL of 0.16 mM ethanolic DPPH solution. The mixture was incubated in darkness for 30 min at room temperature. Ascorbic acid was used as the standard. All results were expressed as mg ascorbic acid equivalent/L of hydrolysate (mg AAE/L).

2.3. Data analysis

All experiments were performed in triplicate. Means and standard deviations (SD) are given for three independent experiments. Statistical analysis was performed using Minitab 16. All analytical results are reported on the dry matter mass of the samples.

3. Results and Discussion

3.1. Chemical compositions of SCG

The chemical composition of SCG is highly variable depending on the type of coffee, its growing conditions and the brewing method. The largest component of SCG is polysaccharides including cellulose and hemicellulose, which make up more than 50% of the dry mass of the SCG (McNutt et al., 2018). In this study, we collected many samples from coffee shops, then mixed them up and dried before use. Mannose and galactose were identified as the main components of the hemicellulose sugars, while glucose is the major composition of cellulose (Figure 1). Total polysaccharides accounted for about 50.1% of the dry SCG. Mannose was the most abundant sugar (28.6%), followed by galactose (12.3%) and glucose (9.2%). Mannose, an important sugar, is used widely in food, medicine, cosmetic, and food-additive industries. Mannose was demonstrated to improve the immune system and give many benefits to health. However, mannose production using chemical synthesis and plant extraction cannot meet the requirements of the industry (Wu et al., 2019). Since SCG is rich in mannose, it is considered as a potential source for mannose production. Lignin is the second most abundant component in SCG, which made up 23.5% (dw). Besides, SCG also contains a significant amount of oil (9.7%), crude protein (14.5%), and small portion of phenolic compounds, ash and caffeine. Similar results have been reported in the literature (McNutt et al., 2018; Nguyen et al., 2019).

3.2. Effect of enzyme on the hydrolysis performance

Enzymatic hydrolysis of SCGs was performed using four types of commercial enzymes including Cellulast, Pectinex, Ultraflomax, and Viscozyme. Temperature is one of the most important factors affecting hydrolysis performance. The hydrolysis experiments were conducted from 35 - 55°C to optimize the working temperature for each enzyme type. Figure 2 showed that Viscozyme and Ultraflomax efficiently worked at 35 - 45°C, while Cellulast and Pectinex displayed the best performance at a wider temperature range of 35 - 50°C. Similar results were mentioned previously (Gama et al., 2015; Andlar et al., 2016). The

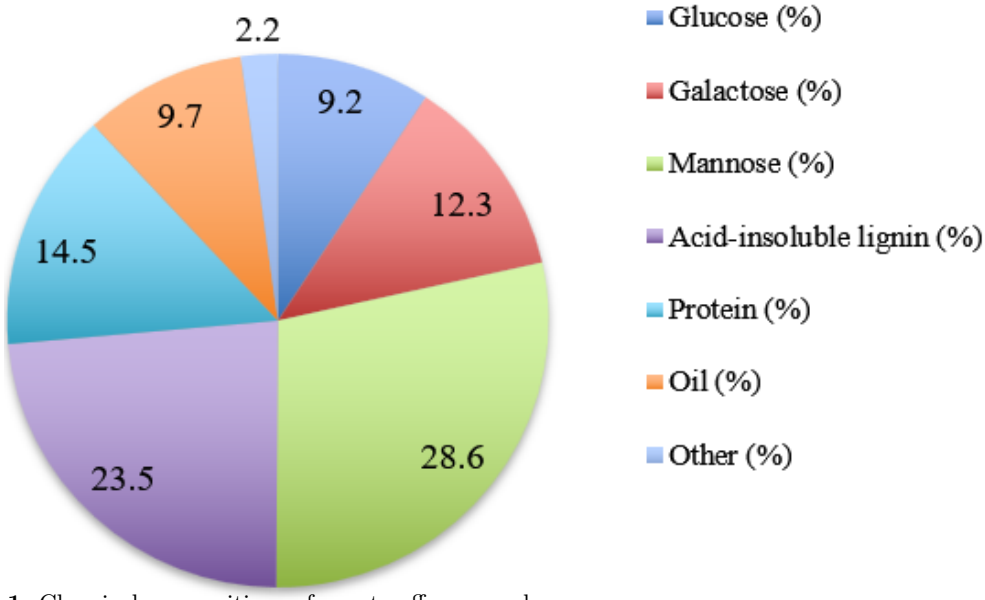

Figure 1. Chemical compositions of spent coffee ground.

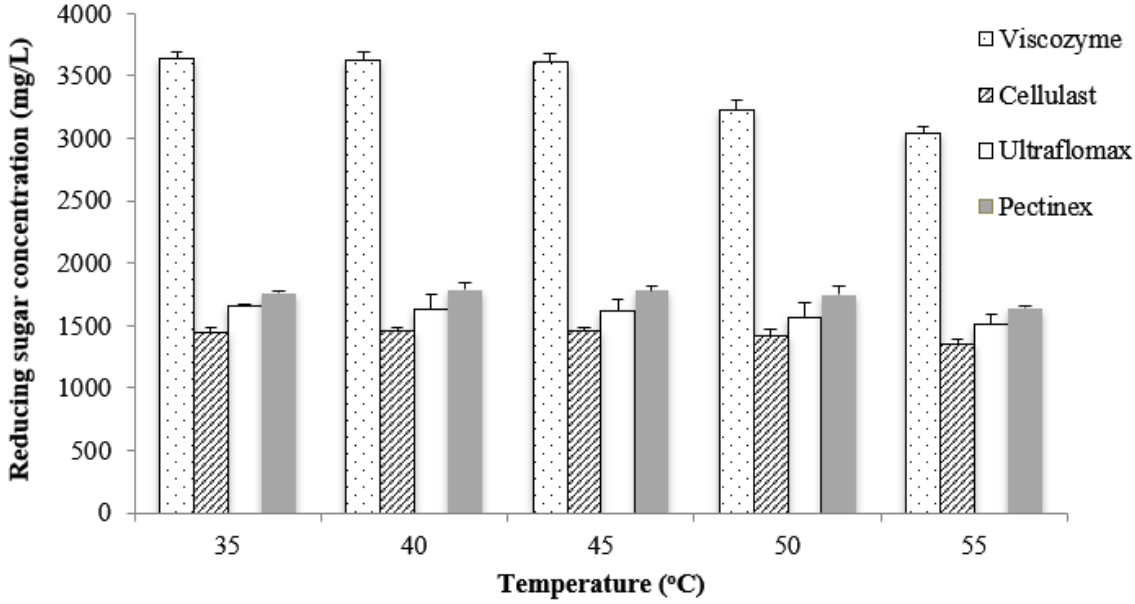

Figure 2. Effect of temperatures on the enzymatic hydrolysis of spent coffee ground.

optimized temperatures were identified within the range recommended by the enzyme manufacturer. Then, further hydrolysis experiments were carried out at 35°C for all enzyme types.

SCGs contain a high fraction of hemicellulose and cellulose, thus the enzymatic hydrolysis process required a mixture of hemicellulase and cellulase. The use of single enzyme was not effective for the hydrolysis of complex lignocellulosic biomass (Cho et al., 2020). In fact, Cellulast is only composed of cellulase leading to the lowest reducing sugar concentration as a result (Figure 3). Pectinex is composed of enzyme activities of pectinase, hemicellulase and beta-glucanase, while Ultraflomax is a cocktail of xylanase and glucanase. The use of Pectinex and Ultraflomax showed higher concentrations of reducing sugars than Cellulast. Viscozyme remarkably improved the yield of reducing sugars, giving 2.2 - 2.7 times higher than other enzymes. The highest concentration of reducing sugar was found to be 6120

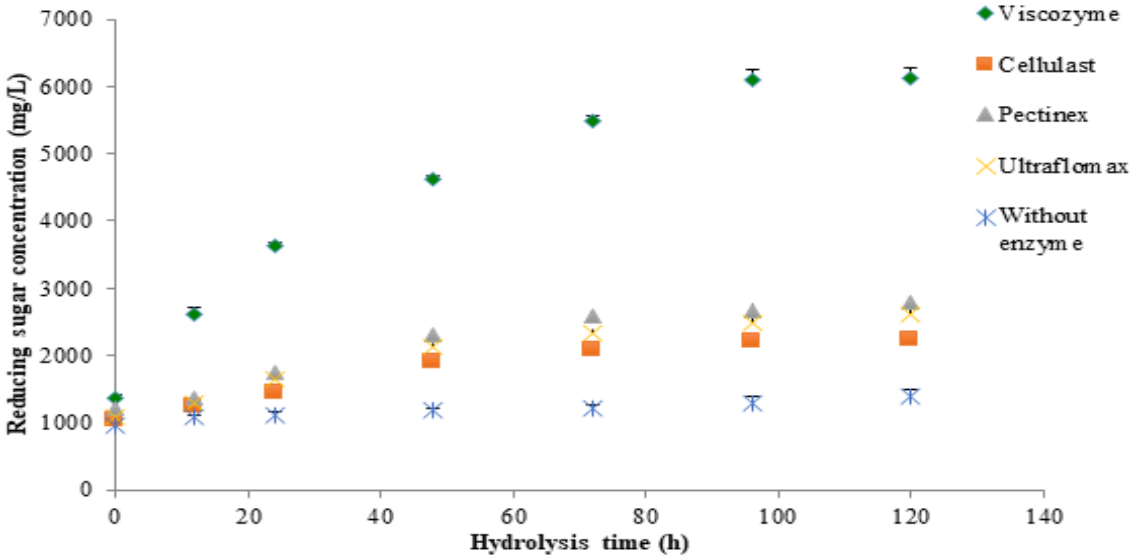

Figure 3. Effect of enzyme types on the hydrolysis of spent coffee ground.

mg/L, while only 1393 mg/L of reducing sugar was released in the experiments without using enzyme. Viscozyme is a mixture of hemicellulase, cellulase, beta-glucanase, arabinase, and xylanase which was widely used for the hydrolysis of various lignocellulose types such as SCG, sugar beet, and apple pomace (Gama et al., 2015; Andlar et al., 2016; Liu et al., 2021). Liu et al. (2021) successfully performed alcoholic fermentation based on SCG hydrolyzed with 6% Viscozyme. Another study prepared a SCG hydrolysate for effective lactic fermentation using a mixture of Viscozyme and Cellulast (Hudecova et al., 2018). Reducing sugar concentration increased rapidly within 96 h but then slowed down. Reducing sugar released at 96 h was not significantly different from that at 120 h when Viscozyme was used.

3.3. Effect of different pretreatments on the hydrolysis performance

The presence of lipid in SCGs limits the access of hydrolytic enzyme to its substrate, therefore the lipid in SCG was removed prior to further pretreatments. Pretreatment is a crucial step in the conversion of lignocellulosic biomass into soluble sugars. Pretreatment aims to decompose the complex biomass matrix, remove lignin, and increase the enzyme accessibility to polysaccharides, subsequently improving the yield of enzymatic saccharification (Trinh et al., 2018). In the study, organosolv and alkali pretreatment showed an improvement in the yield of reducing sugars, achieving 4205 mg/L and 4806 mg/L, respectively, after 120 h hydrolysis. While, enzymatic hydrolysis of untreated sample released 3565 mg/L reducing sugars at the same time (Figure 4). Generally, organosolv pretreatments occur with numerous organic or aqueous solvent mixtures at high temperature to break down the complex structure of lignocellulose and solubilize lignin. Alkali pretreatment is capable of removing lignin and a part of the hemicelluloses by destroying the linkages between lignin and other polymers, thereby facilitating enzyme access to its substrate and improving the production of fermentable sugars (Wongsiridetchai et al., 2018; Jin et al., 2019). The lignin removal and hemicellulose solubilization together facilitate exposing the accessible area of biomass for the subsequent enzymatic hydrolysis process. In this study the pretreatment with acetone was carried out using sonication method which was both effective for the dissolution of lignin and the extraction of polyphenols (Ravindran et al., 2018). The lignin content of organosolv pretreated sample was 23.5%, being lower than untreated sample (26.1%) (Table 1). While, the use of NaOH (1%) also showed a decreased lignin level, giving 21.8% in the pretreated biomass. The alkali treatment of SCG followed by organosolv pretreatment achieved the highest reducing sugar (6120 mg/L) via enzymatic hydrolysis after 120 h, which was 1.7-fold higher than the untreated sample. The two-step pretreatment significantly enhanced the production of sugars compared to the individual

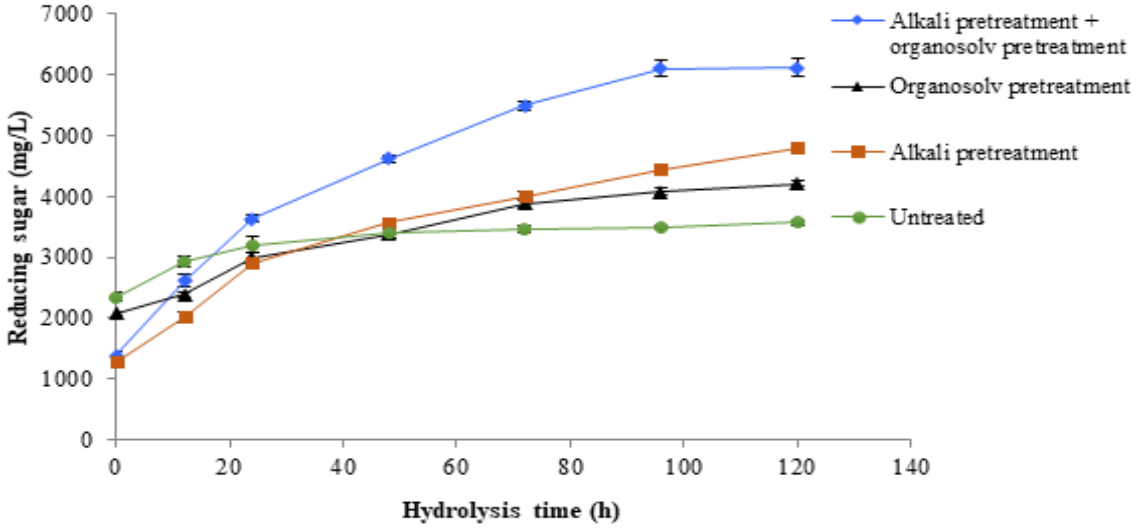

Figure 4. Effect of pretreatment methods on the hydrolysis performance.

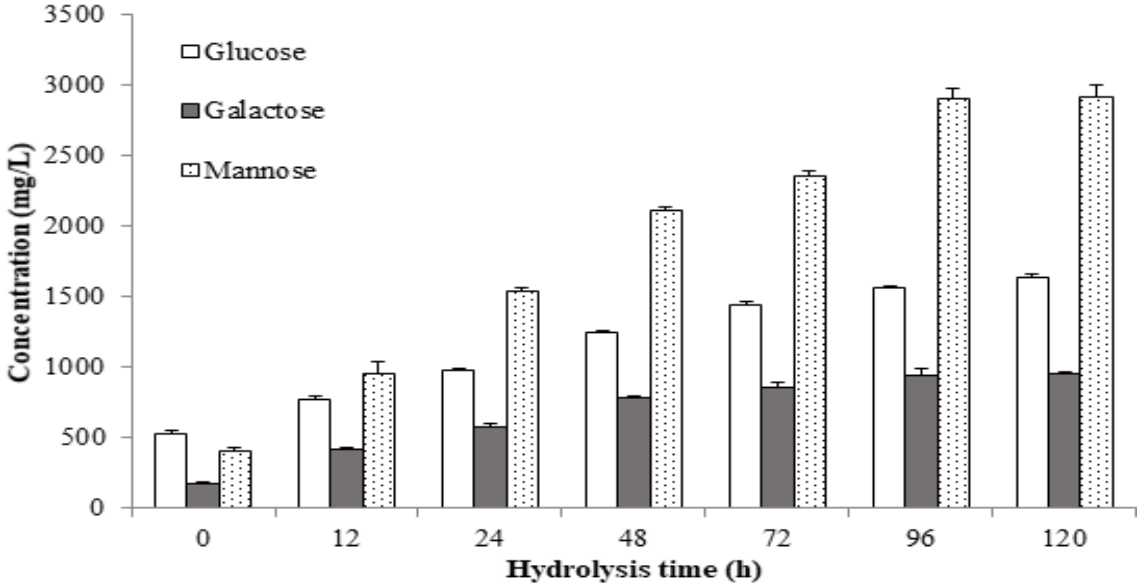

Figure 5. The production of monosaccharides during the enzymatic hydrolysis.

pretreatment process. This is attributed to the effective delignification of both processes compared to the individual step. In fact, the compositional analysis revealed that only 14% of lignin was determined in the SCG pretreated with two-step pretreatment which may explain for the remarkable improvement of biomass digestibility (Table 1). In a previous report, alkali pretreatment with NaOH (0.5 N) has been demonstrated to increase the effectiveness of enzymatic hydrolysis of SCG and produce 526 mg/L of reducing sugar (Wongsiridetchai et al., 2018). Our study successfully developed a two-step process that effectively removed lignin and subsequently enhanced the yield of enzymatic hydrolysis. The use of the combined method has been widely performed previously since single pretreatment couldn't overcome the recalcitrance of biomass (Ravindran et al., 2017; Tang et al., 2020).

3.4. Analysis of the SCG hydrolysate

Spent coffee ground was pretreated by two-step process before applying enzymatic hydrolysis with 5% Viscozyme. Figure 5 shows the release of monosaccharides during the enzymatic hydrol-

ysis within 120 h. Mannose is the most abundant sugar identified in the SCG hydrolysate with the highest concentration of 2917 mg/L after 120 h, which accounted for 47.7% of total reducing sugars. At the same time, the maximum concentration of glucose and galactose were 1633 mg/L and 957 mg/L, respectively. The corresponding yields of mannose, glucose and galactose were 76.8, 43.0 and 25.2 mg/g SCG, respectively. Currently, mannose is used as a starting material to synthesize immune-stimulatory agents, antitumor agents, vitamins, and D-mannitol (Wu et al., 2019). Mannose in the hydrolysate can be separated from other sugars using an established process mentioned in our previous study (Nguyen et al., 2019). SCG hydrolysate is rich in sugars that can be applied as a fermentation medium for the production of bioethanol (Nguyen et al., 2019; Liu et al., 2021); and organic acid (Hudeckdva et al., 2018). Besides, SCG hydrolysate also contained significant amount of polyphenols, giving 174.4 mg GAE/L of total phenolic content after 120 h hydrolysis. Choi et al. (2017) identified the presence of chlorogenic acid, gallic acid, and protocatechuic acid in the SCG, which were responsible for displaying the antioxidant activity. Numerous methods have been reported for the extraction of phenolic compounds. The use of enzymes such as cellulase, hemicellulase, pectinase is capable of breaking down the plant cell walls, thereby facilitating the release of small molecules such as phenolic compounds. Viscozyme contains enzyme activities of hemicellulase, cellulase, beta-glucanase, arabinase and xylanase, which were demonstrated to effectively hydrolyze the cell wall polysaccharides. Therefore, an increase in total phenolic content was observed during the hydrolysis process (Figure 6). Enzyme-assisted extraction is a recently used method that has shown faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to other methods (Puri et al., 2012). Moreover, the antioxidant activity of the SCG hydrolysate elevated with increasing total phenolic content during the enzymatic hydrolysis with the highest value of 263.2 mg/L ascorbic acid equivalents.

Table 1. Chemical compositions of spent coffee ground pretreated by different methods

Pretreatment method	Cellulose (%)	Galactan (%)	Mannan (%)	Acid-insoluble lignin (%)
Alkali pretreatment + organosolv pretreatment	$13.5^{a} \pm 1.2$	$14.5^{a} \pm 1.3$	$47.7^{a} \pm 1.4$	$14.0^{c} \pm 1.2$
Organosolv pretreatment	$11.4^{ab} \pm 1.1$	$13.5^{a} \pm 0.9$	$34.9^{c} \pm 1.6$	$23.5^{ab} \pm 1.4$
Alkali pretreatment	$9.4^{b} \pm 1.2$	$13.3^{a} \pm 0.6$	$39.6^{b} \pm 1.6$	$21.8^{b} \pm 1.1$
Untreated (defatted)	$10.3^{b} \pm 0.9$	$13.2^{a} \pm 0.9$	$32.8^{c} \pm 1.2$	$26.1^{a} \pm 0.8$

Different letters in a column indicate statistically significant differences ($P < 0.05$).

4. Conclusions

In this study, we demonstrated an efficient pretreatment of SCG initiated by alkali pretreatment using NaOH 1% followed by organosolv

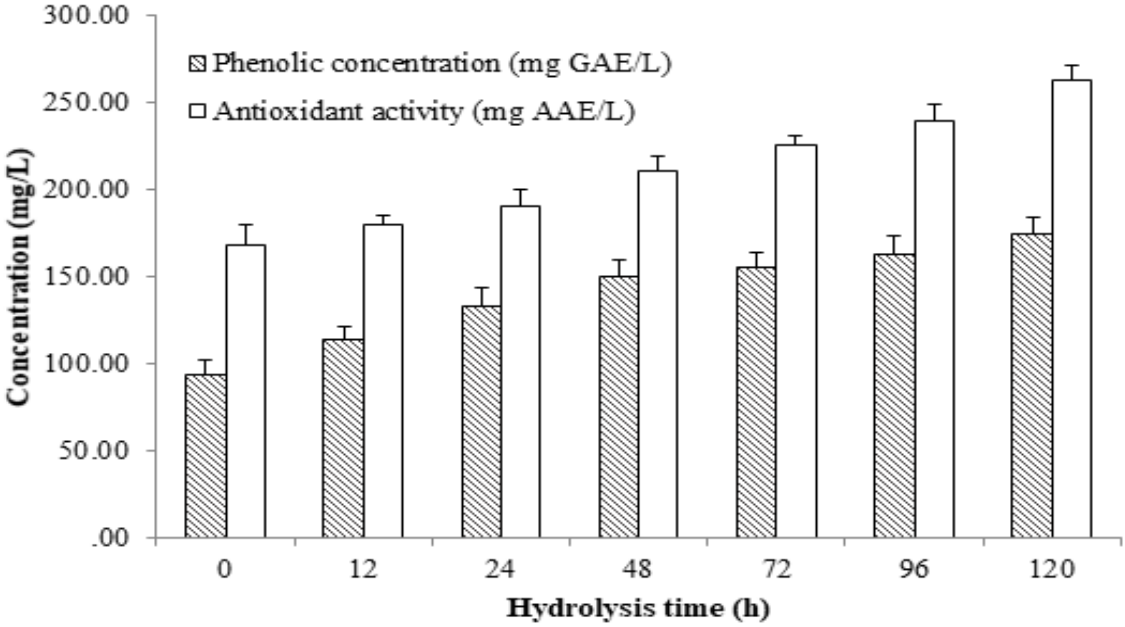

Figure 6. Total phenolic content and antioxidant activity of spent coffee ground hydrolysate.

treatment with acetone. The two-step process effectively removed lignin and improved the production of reducing sugars. The highest sugar concentration reached 6120 mg/L, corresponding to a yield of 161 mg sugar/g substrate. Mannose, the most abundant monosaccharide in the hydrolysate, accounted for 47.7% of the reducing sugars. SCG hydrolysate also contained a total phenolic content of 174.4 mg GAE/L and showed an antioxidant capacity equivalent to 263.2 mg/L of ascorbic acid.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Andlar, M., Rezic, I., Oros, D., Kracher, D., Ludwig, R., Rezic, T., & Santeka, B. (2016). Optimization of enzymatic sugar beet hydrolysis in a horizontal rotating tubular bioreactor. *Journal of Chemical Technology & Biotechnology* 92(3), 623-632. https://doi.org/10.1002/jctb.5043.

Choi, B., & Koh, E. (2017). Spent coffee as a rich source of antioxidative compounds. *Food Science and Biotechnology* 26(4), 921-927. https://doi.org/10.1007/s10068-017-0144-9.

Gama, R., Dyk, J. S. V., & Pletschke, B. I. (2015). Optimisation of enzymatic hydrolysis of apple pomace for production of biofuel and biorefinery chemicals using commercial enzymes. *3 Biotech* 5(6), 1075-1087. https://doi.org/10.1007/s13205-015-0312-7.

Hu, X., Shi, Y., Zhang, P., Miao, M., Zhang, T., & Jiang, B (2016). D-Mannose: properties, production, and applications: An overview. *Comprehensive Reviews in Food Science and Food Safety* 15(4), 773-785. https://doi.org/10.1111/1541-4337.12211.

Hudeckova, H., Neureiter, M., Obruca, S., Fruhauf, S., & Maroval, I. (2018). Biotechnological conversion of spent coffee grounds into lactic acid. *Letters in Applied Microbiology* 66(4), 306-312. https://doi.org/10.1111/lam.12849.

Jin, L. S., Salimi, M. N., & Kamal, S. Z. (2020). Optimization of pretreatment and enzymatic hydrolysis of spent coffee ground for the production of fermentable sugar. *IOP Conference Series: Materials Science and Engineering* 743, 12-30. https://doi.org/10.1088/1757-899X/743/1/012030.

Karmee, S. K. (2018). A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. *Waste Management* 72, 240-254. https://doi.org/10.1016/j.wasman.2017.10.042.

Liu, Y., Lu, Y., & Liu, S. Q. (2021). The potential of spent coffee grounds hydrolysates fermented with *Torulaspora delbrueckii* and *Pichia kluyveri* for developing

an alcoholic beverage: The yeasts growth and chemical compounds by yeast extracts. *Current Research in Food Science* 4, 489-498. https://doi.org/10.1016/j.crfs.2021.07.004.

McNutt, J., & He, Q. (2018). Spent coffee grounds: A review on current utilization. *Journal of Industrial and Engineering Chemistry* 71, 78-88. https://doi.org/10.1016/j.jiec.2018.11.054.

Nguyen, Q. A., Cho, E. J., Lee, D. S., & Bae, H. J. (2019). Development of an advanced integrative process to create valuable biosugars including manno-oligosaccharides and mannose from spent coffee grounds. *Bioresource Technology* 272, 209-216. https://doi.org/10.1016/j.biortech.2018.10.018.

Peshev, D., Mitev, D., Peevac, L., & Peev, G. (2018). Valorization of spent coffee grounds - A new approach. *Separation and Purification Technology* 192, 271-277. https://doi.org/10.1016/j.seppur.2017.10.021.

Puri, M., Sharma, D., & Barrow, C. J. (2012). Enzyme-assisted extraction of bioactives from plants. *Trends in Biotechnology* 30(1), 37-44. https://doi.org/10.1016/j.tibtech.2011.06.014.

Ravindran, R., Desmond, C., Jaiswal, S., & Jaiswal, A. K. (2018). Optimisation of organosolv pretreatment for the extraction of polyphenols from spent coffee waste and subsequent recovery of fermentable sugars. *Bioresource Technology Reports* 3, 7-14. https://doi.org/10.1016/j.biteb.2018.05.009.

Ravindran, R., Jaiswal, S., Abu-Ghannam, N., & Jaiswal, A. K. (2017) Two-step sequential pretreatment for the enhanced enzymatic hydrolysis of coffee spent waste. *Bioresource Technology* 239, 276-284. https://doi.org/10.1016/j.biortech.2017.05.049.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., & Templeton, D. (2008). *Determination of ash in biomass*. Battelle, USA: National Renewable Energy Laboratory.

Tang, S., Cao, Y., Xu, C., Wu, Y., , Li, L., Ye, P., Luo, Y., Gao, Y., Liao, Y., Yan, Q., & Cheng, X. (2020). One-step or two-step acid/alkaline pretreatments to improve enzymatic hydrolysis and sugar recovery from arundo donax L. *Energies* 13(4), 948. https://doi.org/10.3390/en13040948.

Trinh, L. T. P. , Choi Y. S., & Bae, H. J. (2018). Production of phenolic compounds and biosugars from flower resources via several extraction processes. *Industrial Crops and Products* 125, 261-268. https://doi.org/10.1016/j.indcrop.2018.09.008.

Wu, H., Zhang, W., & Mu, W., 2019. Recent studies on the biological production of D-mannose. *Applied Microbiology Biotechnology* 103, 8753-8761. https://doi.org/10.1007/s00253-019-10151-3.

Wongsiridetchai, C., Chiangkham, W., Khlaihiran, N., Sawangwan, T., Wongwathanarat, P., Charoenrat, T., & Chantorna, S. (2018). Alkaline pretreatment of spent coffee grounds for oligosaccharides production by mannanase from Bacillus sp. GA2(1). *Agriculture and Natural Resources* 52(3), 222-227. https://doi.org/10.1016/j.anres.2018.09.012.