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The production of vinegar cider from spent coffee grounds

Mayura Srikanlayanukul[1] and Panwad Sillapawattana[2,*]

[1]*Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand*
[2]*Program in Environmental Technology, Faculty of Science, Maejo University, Chiang Mai, 50290, Thailand*
** Corresponding author: Panwad Sillapawattana*
E-mail: panwad.sillapawattana@rwth-aachen.de Tel: 053-873870
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Abstract

This study suggests the vinegar production method, which utilizes spent coffee grounds (SCGs) as feedstock. The SCGs were pretreated by biotic degradation to obtain sufficient amounts of glucose. To do so, spore suspension (10^7 spores/mL) from *Aspergillus oryzae* and *Rhizopus oligosporus* were prepared and 10% (v/v) of each suspension was separately introduced to the sterilized SCG mixture (1 g of SCG: 100 mL of water). It was found that *A. oryzae* could produce higher amounts of glucose (54.68 g/L ± 0.08) than *R. oligosporus* (27.28 g/L ± 0.27). *Saccharomyces cerevisiae* TISTR 5020 was then inoculated to a liquid medium containing glucose derived from the pre-treatment step under anaerobic condition at 30° C. The alcoholic fermentation was performed and gave a yield of 2.6% ethanol (v/v). After that, the vinegar was produced by the enzymatic reaction from *Acetobacter aceti* TISTR 102 at 30°C. The highest amount of acetic acid reached 1.9% within 6 days. The vinegar from the SCGs was analyzed for antioxidant activity via DPPH and ABTS methods in comparison to 4 different kinds of vinegar in the markets including white wine, red wine, apple cider and jasmine rice vinegar. The SCG vinegar was shown to have higher antioxidant activity than some types of vinegar. This is the first study that utilized SCGs as feedstock for vinegar production. With this method, SCGs were not only valorized to vinegar, but also became a novel product for health-concern persons.

Keywords: Spent coffee grounds (SCGs), Vinegar, Ethanol production, Fermentation

1. Introduction

It is undeniable that coffee is a favorite worldwide beverage. In Thailand, 90,588 tons were demanded in 2019 (Office of Agricultural Economics). The average coffee consumption rate per Thai individual is 300 cup/person annually (Department of Agriculture). The same amount of the consumed coffee obviously becomes the same amount of spent coffee grounds (SCGs), which are residues from the brewing processes (Cruz *et al.*, 2012). SCGs are mostly deposited as fertilizer or in some cases, with a small technology investment, converted to biodiesel after coffee oil extraction process via a chemical reaction called transesterification (Kondamudi *et al.*, 2008). However, before pushing for SCGs to be decomposed, one might also choose to exploit them as feedstock for biological processes. Interestingly, SCGs still possess a wide variety of organic substances such as fatty acids, lignin, cellulose, hemicellulose and polysaccharides, which can be utilized as substrates for value-added products (Campos-Vega *et al.*, 2015). Moreover, high amounts of health-benefit phenolic bioactive compounds are also found in SCGs. These include antioxidants, which are detected by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and found to be at 16-38 mg (vitamin E)/g dry weight (Shang *et al.*, 2017).

To extend the use of SCGs, the pretreatment step is required in order to obtain the desired substances, which serve as input for the production process. Wongsiridetchai *et al.* (2018) studied the pretreatment process of SCGs via alkaline hydrolysis at 50°C for 6 h. They reported that the amount of reducing sugar was account for 520 µg/mL.

Vinegar is produced by the 2-step biological processes using a wide variety of fruits as substrates. Firstly, fruit juice is fermented by yeast under anaerobic condition to yield alcohol as intermediate. Secondly, alcohol is converted to acetic acid under aerobic environment by a group of bacteria called Acetobacter. The end-product, vinegar, tastes sour and gives a fruity aroma. Apart from fruits, other distinct aroma substrates can alternatively be chosen as feedstock.

For instance, Isham *et al*. (2019) produced vinegar from glucose by exploiting *Pleurotus pulmonarius* and *Volvariella volvacea* in combination with *Saccharomyces cerevisiae*. They found that the use of two fungi and one yeast species in a ratio of 1:1:1 for 72 h could produce ethanol and citric acid at 13.45% and 2.41%, respectively.

De Leonardis *et al.* (2018) utilized olive mill wastewater with an addition of 0.2 g/L of sucrose. It was reported that the product contained 5.6% of acetic acid and 3,600 mg/L of phenolic compound.

Li *et al.* (2014) studied the feasibility of using *Hericium erinaceus* as the substrate for vinegar fermentation. The different concentration of *H. erinaceus* powder was prepared and glucose was added to reach a Brix degree value of 25. Later, 1 g/L of *S. cerevisiae* was inoculated to the media and the fermentation process was done at 27°C for 7 days. The alcohol content of 16% was detected and in turn became a substrate for the acetic acid production. After the alcohol concentration had been adjusted properly, *Acetobacter aceti* was introduced to the media. The production was done at 30°C for 9 days on shaker at 250 rpm. This study was reported to produce 4.09% of citric acid (Li *et al.*, 2014).

The present study aimed to manage the SCG waste by utilizing SCGs as substrate for acetic acid production via 2-step fermentation processes. The SCG vinegar was also tested for the antioxidant activity via DPPH and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays in comparison to four other types of vinegar, including white wine, red wine, apple cider and jasmine rice vinegar.

2. Materials and Methods

2.1 SCG component analysis

SCGs were collected from the coffee shop within the Faculty of Science, Maejo University. They were dried in a hot air oven at 105°C for 24 h. The dried SCGs were analyzed for the chemical composition according to Association of Official Agricultural Chemists (AOAC, 1998).

2.2 Biological pretreatment of the SCGs

Aspergillus oryzae and Rhizopus oligosporus were separately cultured on Potato Dextrose Ager for 7 days or until reaching the period of spore formation. The spore suspension was prepared to obtain the concentration of 107 spores/mL. Consequently, 10% (v/v) of spore suspension was inoculated to the sterilized mixture of SCGs and water (1 g SCGs : 100 mL water). The samples were incubated at ambient temperature with shaking for 5 days. The amount of reducing sugar was analyzed every day via 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959).

2.3 Ethanol production

The starter was prepared by transferring one loop of S. cerevisiae TISTR 5020 culture into a 250-mL Erlenmeyer flask containing 150 mL of yeast extract peptone dextrose medium. The culture was shaken at 150 rpm for 12-16 h at 30°C to reach the logarithmic (log) growth phase.

The biological pretreated SCG suspension was adjusted to obtain pH 4.6. After that, 5% (v/v) of the yeast culture from the previous step was transferred into the liquid medium. The fermentation process was done in triplicate under anaerobic condition at 30°C for 7 days. The samples were collected every 24 h. The sugar content was measured using DNS method and the cell growth was directly measured by staining the cells with 2% methylene blue and the viable cells were counted using a haemacytometer. Alcohol content was detected by an ebulliometer (Dujardin-Salleron, France)

2.4 Acetic acid fermentation by Acetobacter aceti TISTR 102

One loop of A. Aceti TISTR 102 culture was transferred into an Erlenmeyer flask containing 100 mL of ethanol-yeast extract medium. The culture was shaken at 150 rpm at 30°C for 48 h to reach the exponential growth phase.

The medium containing ethanol was firstly treated with 0.02 mg/mL of potassium metabisulfite (Merck, Thailand) for 24 h at room temperature for the purpose of sterilization. Later, 5% (v/v) of the A. aceti TISTR 102 starter was then inoculated to the medium containing ethanol derived from the previous fermentation process. The culture was shaken at 150 rpm at 30°C for 7 days. The samples were collected at every 24 h and measured for pH. The acetic acid content was determined via titration with 0.1 N of NaOH solution.

2.5 Determination of antioxidant activity

An antioxidant activity was determined via DPPH and ABTS assays according to Xia *et al.* (2017). Briefly, DPPH method was done by adding 20 µL of vinegar sample to 180 µL of DPPH solution and incubated in the dark at ambient temperature for 30 minutes. The absorbance was measured at 517 nm. Trolox was used as a reference. ABTS assay was based on the ability of the substances to scavenge the ABTS radical cation. To conduct the test, 10 µL of the vinegar sample was mixed with 170 µL of ABTS solution and incubated in the dark for 6 min. The absorbance was read at 414 nm using Trolox as reference compound (Xia *et al.*, 2017). Vinegar produced from SCGs (from this study) was analyzed for antioxidant activity in comparison to white wine, red wine, apple cider and jasmine rice vinegar.

3. Results and Discussion

3.1 Analysis of SCG components

The chemical components of dried SCGs were analyzed according to the AOAC standard and are illustrated in Table 1. The SCGs are an abundant source of hemicellulose (32.96%) and cellulose (17.11%), which account for about a half of the dried sample. These two sugar polymers can be digested into small sugar molecules by chemical hydrolysis and biotic degradation. The saccharide products can potentially be converted to vinegar.

Table 1 Chemical composition of SCGs reported in percentage

Compositions	Percent (%)
Dry matter	94.66
Moisture	53.4
Ash	1.42
Protein	12.39
Fiber	19.71
Fat	16.03
Cellulose	17.11
Hemicellulose	32.96
Lignin	0.34

3.2 Biological pretreatment of SCGs

The mixture of SCGs and water at a ratio of 1g : 100 mL was degraded by *A. oryzae* in comparison to *R. oligosporus* at 30°C for 5 days. The results (Table 2) demonstrate that at day 3, the highest amount of glucose was produced via degradation by both strains. Among them, *A. oryzae* could generate 54.68 g/L ± 0.08 of glucose, whereas *Rhizopus* sp. could produce 27.28 g/L ± 0.27 of glucose.

Table 2 The amount of glucose produced by filamentous fungi analyzed by DNS method

Pretreatment period (day)	Glucose content (g/L) from different test conditions		
	A. oryzae	*R. oligosporus*	Control (without test organism)
1	26.81 ± 0.54	9.37 ± 0.35	***0.***266 ± 0.08
2	36.11 ± 1.30	15.59 ± 0.42	N/A
3	54.68 ± 1.57	27.28 ± 0.27	N/A
4	46.52 ± 0.46	26.86 ± 1.06	N/A
5	13.03 ± 1.33	21.35 ± 1.34	N/A

Thus, *A. oryzae* was shown to be a promising organism for the purpose of the SCG pretreatment process. This is due to the ability of *A. oryzae* to produce cellulase, which was similar to results shown by the study of Passos *et al.* (2018). Namely, cellulase production by *A. oryzae* IOC 3999/1998, *Penicillium* sp. and *Trichoderma reesei* using soybean flour and bagasse as substrates was investigated. The results revealed that *A. oryzae* IOC 3999/1998 could produce the highest amount of cellulase at 3,641 unit/g (Passos *et al.*, 2018). In the same direction, Moreira *et al.* (2015) studied the digestion of paper pulp by *Aspergillus terreus* at 120 rpm, 50°C for 3 h. The glucose content was measured to be 60 g/L.

Biological pretreatment of SCGs was chosen in this study, because this process is safe for the consumer product, albeit it is complicated to keep all steps uncontaminated from unwanted organisms, unlike the chemical reaction process. However, in case of acid hydrolysis pretreatment, chemical residues such as 3-MCPD (3-monochloropropane-1,2-diol) and 1,3-DCP (1,3-dichloropropane-2-ol) are formed by the reaction of protein contained in substrate and HCl during the production process. These residues are defined to be carcinogens in case of routine consumption. Therefore, European Union has issued to regulate the amount of 3-MCPD contaminated in seasoning not more than 0.02 mg/kg, whereas World Health Organization has specified the amount of 1,3-DCP not to exceed 0.005 mg/kg in food (Genualdi *et al.*, 2017; Lee and Khor, 2015).

3.3 Ethanol production from SCGs by *S. cerevisiae* TISTR 5020

Alcoholic fermentation was performed in the presence of SCGs in the liquid medium. To do so, the liquid medium derived from the three-day period of SCGs pre-treated by *A. oryzae* was chosen as substrate because of the highest amount of glucose obtained (54.68 ± 1.57 g/L = approx. 5.5 %). The medium was autoclaved and 5% (v/v) of *S. cerevisiae* TISTR 5020 was inoculated into the medium. The fermentation step lasted for 7 days under anaerobic condition at 30°C. The test sample was collected every 24 h. The ethanol content, the growth rate of yeasts and the pH value during the fermentation period are exhibited in fig 1.

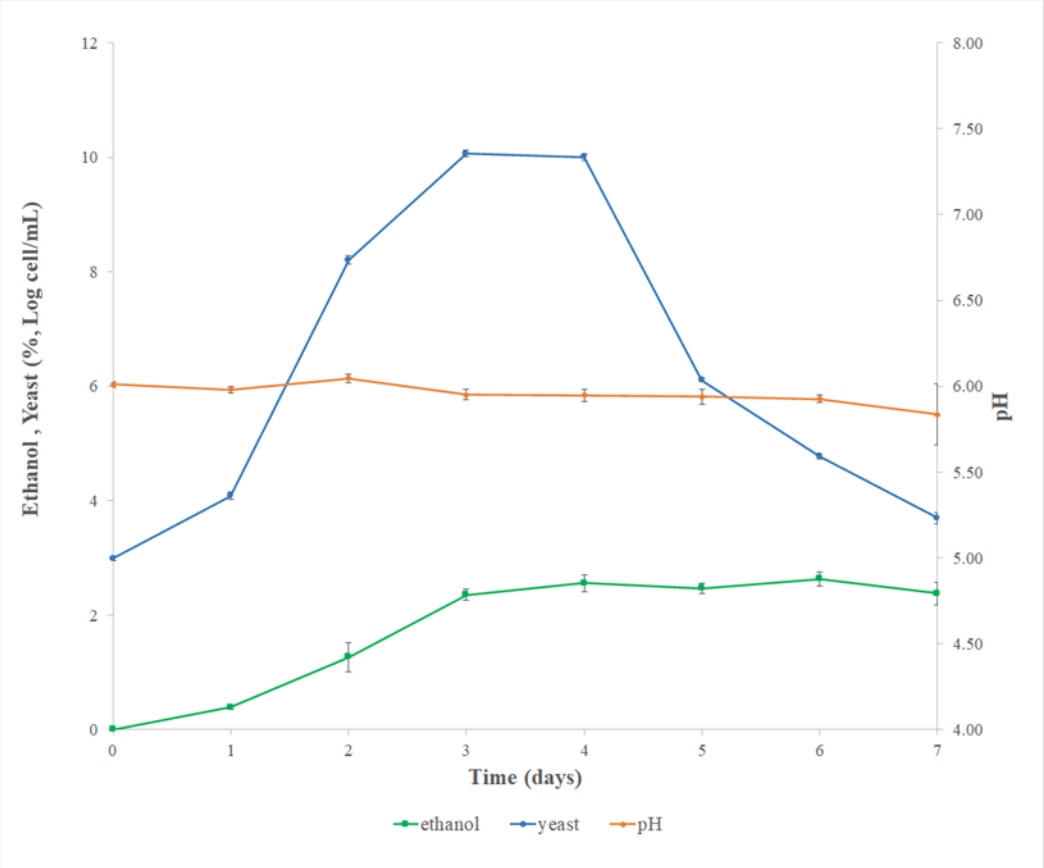

Fig 1 Ethanol content, pH value and yeast cells during the period of alcoholic fermentation utilizing SCGs as substrate.

The ethanol produced was constantly increasing and reached the highest amount of 2.6% (v/v) on day 4 and slightly reduced to 2.5% (v/v) at day 5 and from day 5-7, the percentage of alcohol was rather constant as illustrated by the green line. The growth curve of yeast cells was reported by blue line. The log phase (the period in which yeast cells was rapidly increasing) was found to be from day 0-3 and the stationary phase (the number of yeast cell stayed constant) was shown to be from day 3-4, whereas the decline phase (the amount of yeast cell was decreasing) started after day 4 until the end of the fermentation process. The pH values of the sample are shown by the red line and were found to be constant at day 6 throughout the fermentation period.

The alcohol content and the amount of yeast cells were obviously related. From fig 1, the increasing number of cells lead to the elevated amount of ethanol produced. The ethanol fermentation is a biochemical process accelerated by the action of enzymes in converting the substrate, which in this case was glucose, under anaerobic condition via the Embden-Meyerhof-Panas pathway into alcohol as product.

3.4 The production of SCG vinegar by *A. aceti* TISTR 102

The SCG vinegar production was performed by adding 5% (v/v) of *A. aceti* TISTR 102 starter to the medium containing 2.6% of alcohol from the previous step. Acetic acid fermentation was lasted for 7 days at 30°C at 150 rpm. The results are exhibited in Table 4.

Table 4 pH and acetic acid with various ethanol concentrations

Fermentation period (day)	pH value	Acid content (%)
1	5.91	0.74± 0.09
2	5.71	0.94± 0.07
3	5.78	1.03± 0.04
4	5.75	1.28± 0.10
5	5.44	1.60± 0.01
6	5.02	1.90± 0.06
7	5.07	1.90 ± 0.04

From the results, the acid content constantly increased as fermentation time passed by. The maximum percentage of acid was found at day 6 (1.9%) and remained constant at day 7. According to the notification of the Ministry of Public Health (No. 204) B. E. 2543, fermented vinegar shall contain acetic acid not less than 4 g per 100 mL at 27°C. Thus, SCG vinegar produced from this study does not meet the requirement. In order to improve the quality of SCG vinegar, higher percentage of acetic acid should be obtained. This can be accomplished either by distillation or addition of carbon source to gain sufficient amount of ethanol. However, too high concentrations of ethanol may cause toxic effects to the cells, which lead to the inhibition of cell growth, and finally reduce an amount of acetic acid produced (Sokollek and Hammes, 1997). As seen from the alcoholic fermentation, about a half of glucose was converted to ethanol and about two third of ethanol could form acetic acid. Therefore, approximately 10% glucose should be obtained in order to produce 4% of acetic acid.

3.5 Determination of antioxidant activity

An antioxidant activity tested by DPPH and ABTS assays was conducted followed Xia *et al*. (2017) The results were obtained by the calculation of DPPH and ABTS radical scavenging activity as illustrated in Table 5 and 6.

Table 5 DPPH radical scavenging activity of vinegar samples

Samples	IC_{50} (ppm)
Vitamin C	2.78 ± 0.01
Vitamin E	20.57 ± 0.03
SCG vinegar (from this research)	35.28 ± 0.07
White wine vinegar	107.04 ± 0.06
Red wine vinegar	8.03 ± 0.03
Apple cider vinegar	11.74 ± 0.02
Jasmine rice vinegar	169.93 ± 0.04

Table 6 ABTS radical scavenging activity of vinegar samples

Samples	TEAC (μM)
SCG vinegar (from this research)	0.6 ± 0.02
White wine vinegar	1.89 ± 0.03
Red wine vinegar	0.67 ± 0.01
Apple cider vinegar	1.98 ± 0.04
Jasmine rice vinegar	0.7 ± 0.06

From an antioxidant activity test via DPPH assay, the values were reported in the term of IC_{50}, which refers to the concentration level of the sample that scavenges the free radical so that 50% of the concentration is reduced. When comparing five vinegar samples, it was found that red wine vinegar possessed the highest antioxidant activity. The IC_{50} of red wine vinegar was 8.03 ppm, followed by apple cider (11.74 ppm), SCG (35.28 ppm), white wine (107.04 ppm) and jasmine rice (169.93 ppm) vinegar, respectively. Vitamin C and E were used as positive controls.

The determination of antioxidant activity via ABTS assay was performed in comparison to the Trolox standard solution and the values were expressed as TEAC (Trolox Equivalent Antioxidant Capacity). Due to the lowest TEAC value (0.6 μM), the SCG vinegar appeared to have the highest antioxidant activity. The red wine and jasmine rice vinegar appeared to have moderately the same antioxidant values (0.67 and 0.7 μM, respectively), whereas apple cider appeared to have less antioxidant activity. Both DPPH and ABTS assays revealed that SCG vinegar had more antioxidant activity than other vinegar, except red wine vinegar.

4. Conclusion

This study suggested the method to manage the SCG-waste by valorizing them to a novel product. The SCGs were found to be a suitable feedstock for vinegar production, since they contain a high content of sugar deriving substances, including hemicellulose and cellulose, which can be biotically degraded to smaller molecules of saccharide by *A. oryzae*. The amount of glucose obtained was adequate for vinegar fermentation. The use of 2.6% (v/v) of ethanol could generate 1.9% (v/v) of acetic acid. Moreover, SCG vinegar is an alternative choice for the health concerned consumers due to its health benefit as an antioxidant source.

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