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Lactic Acid Fermentation from Coffee Ground Waste Hydrolysate by *Lactobacillus rhamnosus*

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Abstract: Lactic acid is an important organic acid that is widely used in the food, pharmaceutical, and cosmetic industries. Lactic acid was produced from coffee ground waste which contains fermentable sugars and is increasingly generated from our daily dietary life. Among 114 strains of *Lactobacillus* species, *Lactobacillus rhamnosus* ATCC 10863 was selected for the production of lactic acid from coffee ground waste. Through alkali pretreatment and saccharification, cellulose and hemicellulose in coffee ground waste were converted into fermentable sugars. Pretreatment experiments were conducted at various alkali solution, concentrations, and times. Alkali pretreatment with 35 g/L of KOH at 121℃ for 60 min, the highest concentration of fermentable sugars was produced. The optimum concentration of Viscozyme L was 2% when saccharification was proceeded at 55℃ for 7 days. The productivity of lactic acid fermentation was the highest (0.59 g/L/h) at 100 g of coffee ground waste (1x concentration), whereas the lactic acid concentration was the highest at 600 g of coffee ground waste (6x concentration). As the concentration of coffee ground increased, the lactic acid concentration was also increased, however, the amount was not proportional to the coffee ground waste used. In this study, it was found that coffee ground waste could be used as a culture medium for *Lactobacillus rhamnosus* ATCC 10863 through pretreatment and saccharification for the production of lactic acid.

Keywords: Coffee ground waste; *Lactobacillus rhamnosus*; lactic acid; fermentable sugars; saccharification; alkali pretreatment

1 Introduction

Lactic acid is an organic acid which is responsible for the taste of soured milk and is widely used as an acidifier and food preservative. In cosmetic industry, lactic acid derivatives such as lactic acid esters are widely used as intermediates [1,2] for moisturizing, antibacterial, skin regeneration, and oral hygiene products due to their hygroscopicity and emulsifying properties [3]. In addition, it is also used as an adjunct to the synthesis of dermatologic drugs and osteoporosis in the pharmaceutical industry [4]. Global demand of lactic acid is expected to increase to 1,960,100 tons by 2020 [5] due to the ongoing utilization of lactic acid in many industries. Regarding the industrial use of lactic acid, 85% of production is mostly used in food-related industries [6]. Studies are being conducted specifically for the efficient production of L-lactic acid, which is mainly used in the food industry and biomedical fields. Microbial fermentation can reduce the cost of lactic acid production by using relatively low-price raw materials such as cellulose, hemicellulose, and starch [7]. Lactic acid bacteria that produce only the L-form can be used to reduce or even eliminate the cost of separating the L-form isomer from a mixture of L-form and D-form lactic acid [8]. Although lactic acid is occasionally produced by *Rhyzopus* [4,9] and *Enterococcus* [1], its main avenue of production is *Lactobacillus* strains, which are gram-positive and facultative anaerobic microorganisms.

Coffee is one of the most popular beverages in the world. The International Coffee Organization (ICO) estimated that 8.5 billion kilograms of coffee were produced in 2014, most of which were consumed in the EU, United States, Brazil, and Japan [10]. Coffee ground waste is an insoluble residue that remains after coffee beans are dehydrated, milled, and brewed. Previously, instant coffee producers have opted to drop large quantities of coffee ground waste into landfills, which can alter the ecosystem around the landfill. In addition, due to the anti-nutritional activity of tannin contained in coffee ground waste, addition of coffee ground waste to animal feeds is also limited [11,12]. In some countries, coffee ground waste has been used as a fuel for industrial boilers due to its high heat of combustion (5,000 kcal/kg) [13,14]. In Oi et al.'s experiment [15] coffee grounds were used for the production of methane by a mixture of microorganisms consisting of methane bacteria obtained from sludge. Mussatto et al. produced ethanol from coffee silver skin (epidermis) and coffee ground waste pretreated by using H_2SO_4 to increase conversion of fermentable sugars [16,17]. Caetano et al. [18] extracted oil from coffee ground waste and evaluated the quality of biodiesel after transesterification.

Coffee ground waste is rich in fermentable sugars for fermentation. However, it requires pretreatment to resolve the covalent bonds between lignin and hemicellulose. The decomposition of lignocellulose results in the hydrolysis of hemicellulose and cellulose, making it an industrially important monosaccharide. In this study, we optimized the conditions for pretreatment and saccharification of coffee ground waste to increase conversion into fermentable sugars and further into lactic acid.

2 Materials and Methods

2.1 Microorganisms

A total of 114 *Lactobacillus* strains were used for the utilization of various carbon sources and lactic acid production. They were 17 types of ATCC strains and 97 types of KLB strains [19].

2.2 Analysis of Coffee Ground Waste Composition

The compositions of carbohydrates in coffee ground waste were measured using the standard National Renewable Energy Laboratory (NREL) method [20]. Dehydrated coffee ground waste was mixed with 72% (w/v) H_2SO_4 and treated for 48 h at 30°C. Then, the concentration of H_2SO_4 was adjusted to 4% after heat treatment in an autoclave (Vision Scientific Co., Ltd., Korea) at 121°C for 2 h. The mixture was then separated into a solid and liquid phase. Concentration of carbohydrates (liquid phase) was analyzed by HPLC LC-10AD (Shimadzu, Japan) with RI detector RID-10A (Shimadzu, Japan). The column used to determine the carbohydrate composition of coffee ground waste was Aminex HPX-87P (Bio-Rad, USA). The column oven (CTO-10A, Shimadzu, Japan) was maintained at 60°C. The mobile phases for coffee ground waste compositions analysis consisted of distilled deionized water (ddH$_2$O) at a 0.5 mL/min flow rate. The solid phase was dried until a constant weight at 105°C in a drying oven (Vision Scientific Co., Ltd., Korea). The ash and lignin contents were determined by weight measurements of initial and final weights after igniting of dried solid phase in a muffle furnace (Daihan Scientific Co., Ltd., Korea) at 550°C for 4 h.

2.3 Pretreatment of Coffee Ground Waste

In order to use coffee ground waste as a medium, pretreatment and saccharification conditions must be optimized for efficient conversion into fermentable sugars. Coffee ground waste was dehydrated by a freeze drier (Ilshin Lab. Co., Korea) for 2 days, after which alkali pretreatment was performed. The alkali solutions used for hydrolysis were KOH, NaOH, and Ca (OH)$_2$. About 100 g of dehydrated coffee ground waste was added to each alkali solution (1 L) at different concentrations. The concentrations of alkali solution used were 2.5 g/L, 5 g/L, 20 g/L, 35 g/L, and 50 g/L, and the treatment times were 15 min, 30 min, 45 min, 60 min, 75 min, and 90 min. The treatment temperature was performed at 100°C. After alkali treatment, the samples were washed with water until effluent water became colorless. The washed sample

was dried at 55°C in a drying oven (Vision Scientific Co., Ltd., Korea). The dried sample was then mixed with 100 mL of ddH$_2$O and adjusted to pH 5.8 for enzyme reaction.

2.4 Saccharification of Pretreated Coffee Ground Waste

Viscozyme L (Novozyme, Denmark) was used for the saccharification of coffee ground waste. Viscozyme L mainly contains cellulase, xylanase, and β-galactosidase. To determine the concentrations of Viscozyme L for efficient saccharification, the concentration of Viscozyme L was varied among 1%, 2%, 4%, and 6%. Pretreated coffee ground waste and Viscozyme L were mixed and reacted in a shaking incubator (Vision Scientific Co., Ltd., Korea) at 55°C for 3 days.

2.5 Cultivation

Different culture media were used for preculture and main culture of *L. rhamnosus* ATCC 10863. De Man, Rogosa and Sharpe (MRS) medium (glucose 20 g/L, protease peptone 10 g/L, beef extract 10 g/L, ammonium citrate 2 g/L, sodium acetate 5 g/L, K$_2$HPO$_4$ 2 g/L, tween 80 1 g/L, MgSO$_4$·7H$_2$O 0.1 g/L, and MnSO$_4$·4H$_2$O 0.05 g/L) was used for preculture, and the coffee medium prepared by pretreatment and saccharification of coffee ground waste was used for main culture. The pH of MRS medium and coffee medium were adjusted to pH 6.5 before fermentation. The effect of the amount of coffee ground waste on the conversion of sugars into lactic acid was investigated by using coffee medium prepared from three different amounts of coffee: 100 g of coffee ground waste (1x), 300 g of coffee ground waste (3x), and 600 g of coffee ground waste (6x). *L. rhamnosus* ATCC 10863 precultured in MRS medium was transferred to a 3 L jar fermenter (KBT, Korea) containing 1 L of coffee medium. Bacterial cells were cultured with an agitation rate of 30 rpm under anaerobic conditions at 37°C and maintained at pH 6.5 with 1 M NaOH.

2.6 Analysis of Fermentable Sugars and Lactic Acid

The concentration of fermentable sugars in coffee medium prepared from coffee ground waste and the concentrations of D-lactic, L-lactic acid, and total lactic acid after fermentation were analyzed by HPLC. To determine the uptake of fermentable sugars in coffee medium by *L. rhamnosus* ATCC 10863, the residual concentration of fermentable sugars was measured. The samples for sugar and lactic acid analysis were prepared by using a 0.2 μm filter (Sartorius, Germany). The instrument used for analysis was HPLC LC-10AD (Shimadzu, Japan) equipped with RI detector RID-10A and UV/Vis detector SPD-10A (Shimadzu, Japan). The UV wavelength was 254 nm. Fermentable sugar and lactic acid concentrations were analyzed by using Aminex HPX-87P and HPX-87H (Bio-Rad, USA) column, respectively. Column temperature was maintained at 60°C. The mobile phase was ddH$_2$O for sugar analysis and 0.014 M sulfuric acid solution for lactic acid analysis. The flow rates were 0.5 mL/min for ddH$_2$O and 0.6 mL/min for sulfuric acid. Chiralpak Ma$^+$ (Daicel, Japan) column was used for analysis of D-Lactic acid and L-lactic acid. Column temperature was maintained at room temperature. The mobile phase was 0.2 mM anhydrous copper (II) sulfate solution, and the flow rate was 0.5 mL/min.

3 Results and Discussion

3.1 Analysis of Coffee Ground Waste

The contents of fermentable sugars from coffee ground waste were analyzed by the NREL method. Coffee ground waste consisted of 43% hemicellulose, 37% lignin, 7% cellulose, and 13% other substances such as ash and proteins (Tab. 1). The maximum content of total sugars obtained from 100 g of coffee ground waste was 12.21 g of glucose, 3.19 g of xylose, 9.33 g of arabinose, 6.67 g of fructose, and 5.22 g of mannose. The composition of coffee reported by Mussatto et al. [16] was 30.4% galactose, 19.0% glucose, and 3.8% arabinose. The compositional difference can be attributed to the type of coffee bean [21] since the composition of ingredients can vary depending on the types of coffee beans used in the blending. The

coffee used in the Mussatto et al. [21] study was a mixture of arabica and robusta, and the coffee used in this study was arabica.

Table 1: Composition of coffee ground waste

Components	Composition (%)
Hemicellulose	43
Lignin	37
Unknown	11
Cellulose	7
Ash	2

3.2 Conversion of Coffee Ground Waste into Fermentable Sugars

The pretreatment process was optimized to increase the yield of fermentable sugars in coffee ground waste after saccharification. During acid pretreatment, the high temperature increased the rate of hemicellulose degradation, thus causing the toxic compounds such as furfural and 5-hydroxymethy-furfural (HMF) [22]. Although alkali treatment requires additional saccharification, it does not produce toxic substances. Therefore, alkali pretreatment was chosen it improve the enzymatic degradation of lignin and conversion of carbohydrate polymers into soluble fragments. After alkali pretreatment, enzymatic saccharification was performed by 2% Viscozyme L.
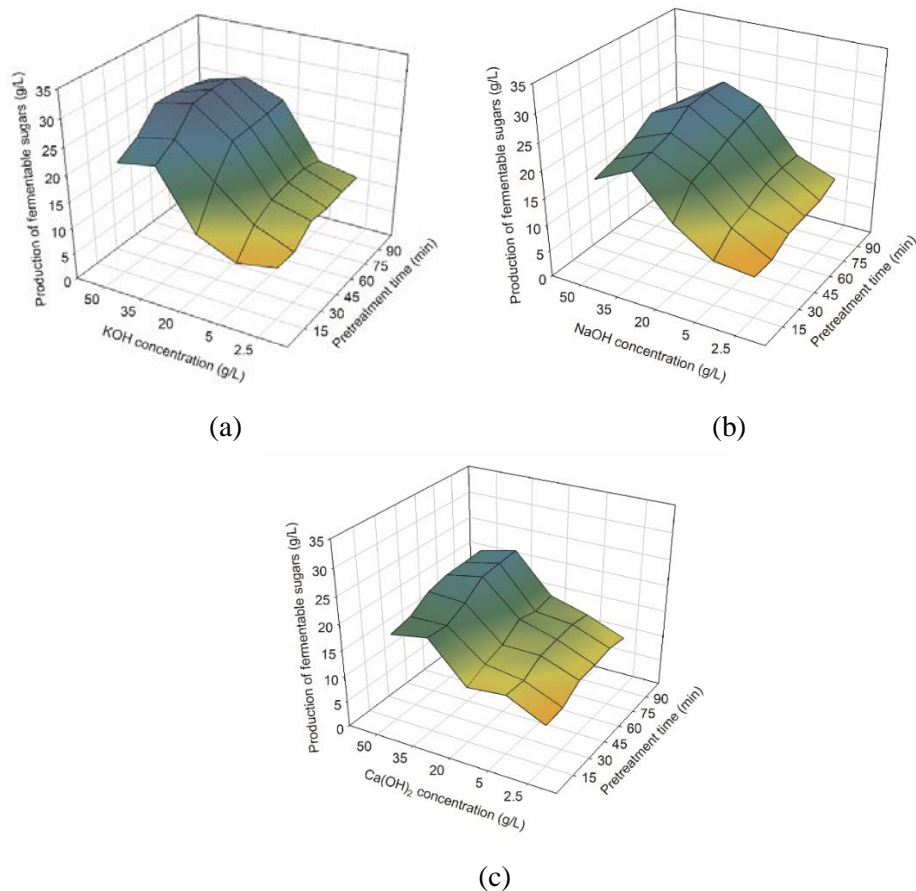

(a)   (b)

(c)

Figure 1: Effects of concentration and pretreatment time of various alkali solutions on production of fermentable sugars. (a): KOH, (b): NaOH, and (c): Ca (OH)$_2$

To select alkali solution with high pretreatment efficiency, various concentrations of KOH, NaOH, and Ca (OH)$_2$ were repared (Fig. 1). At 35 g/L of KOH, the highest yield of fermentable sugars was obtained (29.40 g/L), which was 87.45% total sugars in coffee ground waste. Fermentable sugars included glucose, xylose, arabinose, and fructose. The production of fermentable sugars was increased by 1.27-fold compared to that at 2.5 g/L of KOH. The amount of fermentable sugars produced increased up to at 35 g/L of KOH concentrations. However, a slightly low level of fermentable sugar production was observed at 50 g/L of KOH. The maximum content of fermentable sugars was obtained after 60 min. Pretreatment times of 15, 30, and 45 min seemed to be insufficient for conversion of hemicellulose and cellulose into fermentable sugars. However, there was a noticeable loss of fermentable sugars due to degradation of pretreated coffee ground waste after 75 min. Some pretreated coffee ground waste particles are so small that they passed through the mesh screen during the washing process.

For NaOH pretreatment, the highest fermentable sugar concentration was obtained at 35 g/L (25.83 g/L) after 75 min of pretreatment. A fermentable sugar concentration of 87.86% was obtained at 35 g/L of KOH after 60 min of pretreatment. At 35 g/L of NaOH, the production of fermentable sugars was 2.43-fold higher than that at 2.5 g/L of NaOH and 1.17-fold higher than that at 50 g/L of NaOH. The highest fermentable sugar concentration was reached after 75 min. In the case of Ca (OH)$_2$, the concentration of fermentable sugars was highest (23.11 g/L) at 35 g/L of Ca (OH)$_2$ after 90 min of pretreatment. A fermentable sugar content of 78.58% was obtained after KOH pretreatment.

Based on the results of the pretreatment using the three different alkali solutions, maximum production of fermentable sugars was obtained at 35 g/L of alkali pretreatment, and the degradation of lignin and hemicellulose with Ca (OH)$_2$ occurred more slowly than with other alkali solutions. The highest concentration of fermentable sugars was obtained at 35 g/L of KOH after 60 min of pretreatment.

The enzyme used for saccharification was Viscozyme L, which contains not only hemicellulase and cellulase but also xylanase and β-glucanase. After the enzyme reaction, arabinose, mannose, and fructose were produced as well as glucose and xylose. The highest amount of fermentable sugars was produced by 2% Viscozyme L (Fig. 2). When the concentration was higher than 2%, more fermentable sugars were produced, although the amount was not proportional to the enzyme used. The maximum yield (0.29 g of fermentable sugar/g of coffee ground waste) was obtained upon pretreatment with 35 g/L of KOH for 60 min and saccharification by 2% Viscozyme L. Total fermentable sugar content of 80.28% was estimated by the NREL method. Kwon et al. [23], Choi et al. [24], and Ravindran et al. [25] has been used coffee ground waste for the production of fermentable sugars. Our yield was slightly lower than those of other reports, due to different pretreatment methods, saccharification conditions, and raw materials.
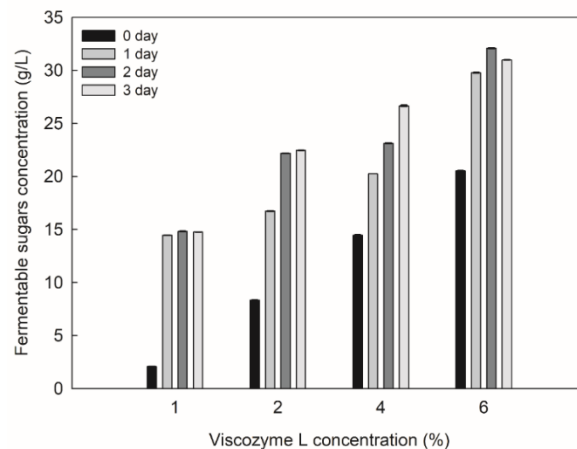

Figure 2: Effects of Viscozyme L concentration on the production of fermentable sugars

3.3 Production of Lactic Acid from Coffee Medium

Lactobacillus rhamnosus ATCC 10863 was selected from 114 *Lactobacillus* strains for the production of lactic acid from coffee medium due to the advantage in high production of lactic acid and utilization of various carbon sources (data not shown). *L. rhamnosus* ATCC 10863 was cultured in coffee medium prepared by pretreatment of coffee ground waste for 60 min at 121°C, followed by saccharification with 2% Viscozyme L at 55°C for maximum 7 days in accordance with the various concentration of coffee ground waste (1x, 3x, and 6x concentration). The concentration of fermentable sugars in 1x coffee medium was 27.54 g/L. *L. rhamnosus* ATCC 10863 consumed all fermentable sugars in coffee medium within 66 h (Fig. 3). The maximum lactic acid concentration was 24.95 g/L at 42 h and was maintained until the end of fermentation. Productivity of 1x coffee medium (0.59 g/L/h) was much higher than those of 3x and 6x coffee media. Cells grown on 3x coffee medium produced 42.44 g/L of lactic acid, and the L/D lactic acid ratio was similar to that of 1x coffee medium. The initial concentration of fermentable sugars in the medium was 56.08 g/L, and the time required for complete consumption of fermentable sugars was 90 h. When *L. rhamnosus* ATCC 10863 was cultured in 6x coffee medium, 106.89 g/L of fermentable sugars were consumed after 468 h. The maximum lactic acid concentration (107.28 g/L) was higher than those of other coffee medium concentrations. However, lactic acid productivity (0.23 g/L/h) was the lowest when compared to other two coffee medium concentrations due to the long fermentation time.
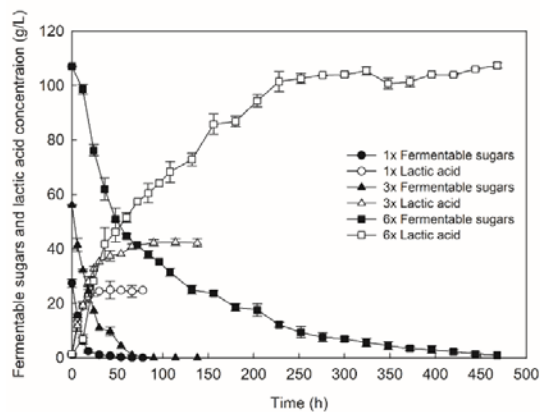

Figure 3: Production of lactic acid of *Lactobacillus rhamnosus* ATCC 10863 by using coffee medium

The results for the concentration of fermentable sugars in coffee medium showed that the consumption rate of fermentable sugars was increased when using 3x coffee medium. However, the consumption rate of fermentable sugars in 6x coffee medium was lower than those of 1x and 3x coffee media during the first 12 h. In 6x coffee medium, the reduction in the consumption of fermentable sugars is probably due to the substrate inhibition of the medium caused by the high sugar concentration (106.89 g/L). In conclusion, the lactic acid concentration was the highest in 6x coffee medium, whereas the lactic acid productivity was highest in 1x coffee medium. The highest ratio of L-lactic acid was 96.8% (L-form/total lactic acid) obtained at 6x concentration.

4 Conclusion

In this study, lactic acid was produced from coffee ground waste after pretreatment and saccharification. *L. rhamnosus* ATCC 10863 was cultured in coffee medium for lactic acid production. When pretreatment was performed with 35 g/L of KOH at 121°C for 60 min and 2% Viscozyme L was used in the saccharification process, maximum production of fermentable sugars was obtained (0.29 g of fermentable sugar/g of coffee ground waste). The highest lactic acid productivity (0.59 g/L/h) was obtained from 1x coffee medium. The lactic acid concentration (107.28 g/L) was highest when *L. rhamnosus* ATCC 10863 was grown on 6x coffee medium. As a result, more than 90% fermentable sugars in coffee ground waste

was converted into lactic acid. The production of lactic acid using coffee ground waste as a biomass can be reduced environmental pollution caused by waste, and it can be a substitute for petrochemical-based products, which will be contributed to the elimination of future energy problems.

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