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Research Article

The use of commercial wine yeast *Saccharomyces cerevisiae* EC1118 for cassava ethanol production at high solids loading

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Abstract Cassava is one of the most commonly imported raw materials for ethanol fermentation for the manufacture of food-grade distilled spirits in Korea. In cassava-producing countries, such as the Lao PDR, cassava can be considered low-price biomass for the production of bioethanol. In this study, the commercial wine yeast *Saccharomyces cerevisiae* EC1118 was tested for ethanol fermentation using cassava powder at a high solids loading (30%, w/v). α-Amylase and glucoamylase were used for the hydrolysis of cassava starch into glucose. To identify a suitable fermentation process for cassava, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) were compared. From the complete enzyme hydrolysis of cassava, 254.1 g/L of glucose was obtained. SSF showed higher ethanol titer during the first 12 h of fermentation, but SHF showed a higher ethanol titer after 24 h of fermentation. Finally, there was no significant difference between SHF and SSF in the final ethanol titer after 48 h fermentation (133.6 and 130.6 g/L, respectively). In summary, both SHF and SSF are applicable for ethanol production with high solid cassava using wine yeast EC1118 under the test conditions.

Keywords bioethanol, cassava, yeast, simultaneous saccharification & fermentation

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1. Introduction

The use of fossil fuels as a major energy source leads to the accumulation of atmospheric greenhouse gases (GHGs) and ultimately global climate change (Liu et al., 2021; Sonthalia and Kumar, 2021). As an alternative to gasoline, bioethanol, which is produced by the fermentation of plant sources, such as corn or sugarcane, can contribute to carbon reduction (Azhar et al., 2017; Liu et al., 2021). Cassava is a low-cost, high-starch crop grown in tropical and subtropical regions (Li et al., 2017; Sivamani and Baskar, 2018). Cassava is currently used for starch

production and alcohol fermentation (Latif and Müller 2014; Li et al., 2017). With the increasing demand for bioethanol, cassava can be used as an alternative source in the production of bioethanol near growing areas, thus making it cost-competitive.

Saccharomyces cerevisiae strains are commonly used in the food industry and laboratories because they are easy to culture and are relatively straightforward in a genetic sense (Zheng et al., 2019). In addition, *S. cerevisiae* strains have been widely used for bioethanol production because of their high yield (Lee et al., 2017). *S. cerevisiae* can tolerate various stresses under industrial fermentation conditions, such as low pH, which prevents bacterial contamination (Zheng et al., 2019).

For bioethanol production from starch crops, such as corn and cassava, two processes are required: 1) hydrolysis of starch into glucose by amylase and glucoamylase and 2) fermentation of glucose to ethanol by *S. cerevisiae*. The two processes can be performed sequentially, which is called separate hydrolysis and fermentation (SHF) (Dahnum et al., 2015). In SHF, the hydrolysis and fermentation processes are performed in different reactors (Lee et al., 2010). Alternatively, these two processes can be performed in one reactor by adding enzymes and yeast cells together; the combined process is called simultaneous saccharification and fermentation (SSF) (Cantarella et al., 2004; Lee et al., 2010; Zhu et al., 2012). Depending on the type of enzyme, yeast cells, raw materials, and fermentation conditions, the fermentation profiles of SHF and SSF can vary greatly (Zhu et al., 2012).

In this study, the commercial wine yeast *S. cerevisiae* EC1118 was tested for its ability to ferment high-solids cassava (30% w/v). In addition, the SHF and SSF of high-solid cassava were compared to design a suitable fermentation process.

2. Materials and methods

2.1. Yeast strain and culture conditions

S. cerevisiae EC1118, a commercial wine yeast strain, was used in this study (Jung et al., 2021; Kong et al., 2018). Yeast cells were precultured in YP medium (10 g/L yeast extract and 20 g/L Bacto™ peptone) containing 20 g/L glucose for 24 h at 30℃ under aerobic conditions (250 rpm). Precultured yeast cells were collected by centrifugation at 3,314 ×*g* for 5 min and washed twice with sterile distilled water. The initial cell concentration during fermentation was adjusted to 1.0 of an optical density at 600 nm (OD_{600}).

2.2. Sample preparation and liquefaction

Peeled and frozen cassava was produced in 2020 and purchased online (World Food, Vietnam). The samples were dried at 60℃ for 24 h (10% moisture content), ground, and stored at -80℃ until use. For fermentation, distilled water was added to 15 g of cassava powder in a 250-mL flask to make a total volume of 50 mL (30% w/v). The solution was sterilized at 121℃ for 15 min. For liquefaction, 5 μL of α-amylase (Liquoflow pHlex DS, Novozymes, Bagsvaerd, Denmark) was added to the cassava solution, and the flask was incubated at 80℃ for 1 h with continuous agitation at 100 rpm (Fig. 1).

2.3. Separate hydrolysis and fermentation (SHF)

For SHF, 5 μL glucoamylase (Saczyme GO 2X, Novozymes, Bagsvaerd, Denmark) was added to the liquefied cassava solution, and the flask was incubated at 30℃ and 150 rpm for 48 h until the glucose concentration increased. Concentrated nitrogen in a volume of 4 mL (100 g/L yeast extract and 200 g/L peptone) was added to 50 mL of saccharified cassava solution. Yeast cells were

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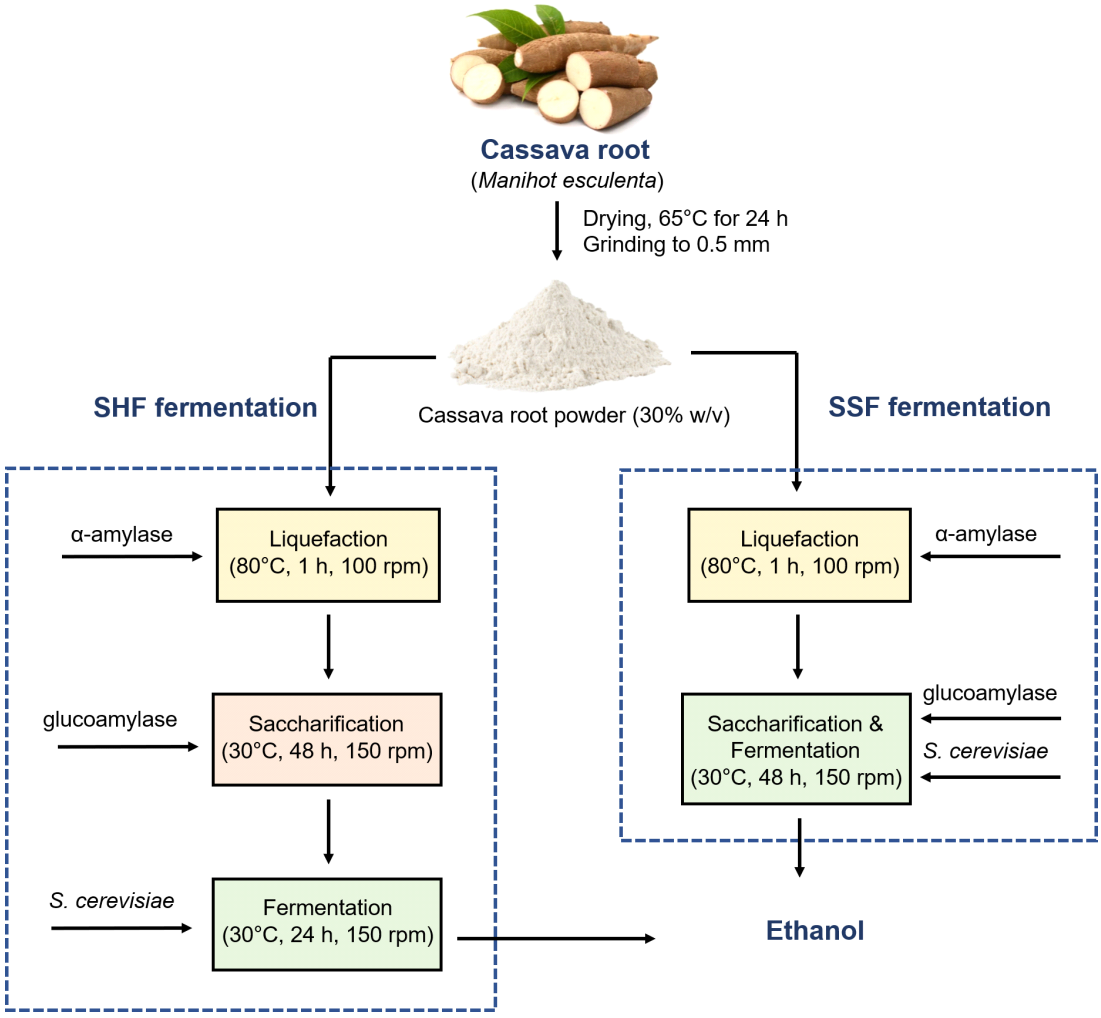

Fig. 1. Schematic diagram of cassava ethanol production by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) in this study.

inoculated at an initial cell density of OD_{600}=1.0. Fermentation was performed at 30℃ and 150 rpm for 24 h until the ethanol concentration increased.

2.4. Simultaneous saccharification and fermentation (SSF)

For SSF, 5 mL of glucoamylase, 5 mL of the nitrogen source, and 1.0 OD_{600}, of the yeast cells were added to the liquefied cassava solution. Fermentation was performed at 30℃ and 150 rpm for 48 h until the ethanol concentration increased. All fermentations were performed in triplicate. For comparison of fermentation parameters between SHF and SSF, Student's t-test was performed at $p<0.05$.

2.5. HPLC analysis

The glucose, fructose, glycerol, acetate, and ethanol concentrations of the hydrolysis and fermentation samples were analyzed through high-performance liquid chromatography (HPLC 1260 series, Agilent Technologies, Santa Clara, CA, USA) equipped with a Rezex-ROA Organic Acid H+ column (8%, 150 mm×4.6 mm; Phenomenex Inc.,

Torrance, CA, USA). The analytes were eluted with 0.005 N H_2SO_4 at 0.6 mL/min and 50℃, as previously described (Kim et al., 2019).

3. Results and discussion

3.1. Glucan content of cassava

To measure the total glucan content of cassava, α-amylase and glucoamylase were sequentially added according to the manufacturer's recommendations, as described in the Materials and Methods. During glucoamylase treatment, glucose concentration was monitored every 12 h until it increased (Fig. 2). After α-amylase treatment with 30% (w/v) cassava powder solution (0 h), the concentrations of glucose and fructose were 33.0 and 5.6 g/L, respectively. When glucoamylase was added, glucose concentration significantly increased until 48 h, and the increase in fructose concentration was marginal. Lastly, from the saccharification of 30% (w/v) cassava powder solution, 254.1 g/L glucose and 14.7 g/L fructose were produced (Table 1). Using a conversion

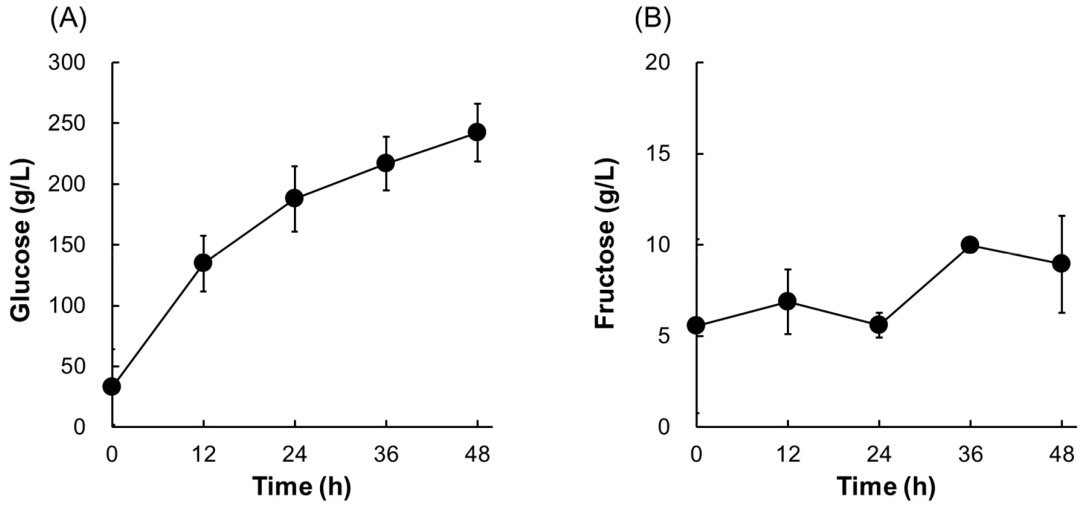

Fig. 2. Enzyme hydrolysis of cassava powder solution (30%, w/v). Glucose (A) and fructose (B) concentrations were monitored for 48 h until the level of glucose increased. Hydrolysis was performed by adding glucoamylase to the liquefied cassava solution, and the flask was incubated at 30℃ and 150 rpm.

Table 1. Fermentation parameters from SHF and SSF of high solids loading of cassava by wine yeast *S. cerevisiae* EC118

Fermentation mode	Incubation time (h)	Glucose (g/L)	Fructose (g/L)	Ethanol (g/L)	Ethanol yield (g/g)	Ethanol productivity (g/L-h)	Glycerol (g/L)	Acetate (g/L)
Hydrolysis	48	254.1±12.2	14.7±2.5	-	-	-	2.7±0.7	3.7±1.1
SHF[1)]	12	136.6±4.4	11.6±0.5	60.2±1.1	0.47±0.04	5.01±0.06	8.1±0.4	2.7±0.3
	24	3.4±0.4	3.6±0.2	130.6±1.5	0.49±0.02	5.17±0.06	9.5±0.2	1.9±0.2
SSF[2)]	12	38.2±7.0	2.5±0.4	84.4±15.5	0.32±0.02	7.04±0.53	5.0±0.5	2.0±0.2
	24	5.4±0.1	2.9±0.4	115.5±2.4	0.44±0.06	4.81±0.65	5.6±0.9	2.8±0.1
	48	5.1±0.9	2.6±0.5	133.6±3.6	0.51±0.01	2.78±0.07	6.1±0.4	2.6±0.5

[1)]SHF, separate hydrolysis and fermentation.
[2)]SSF, simultaneous saccharification and fermentation.

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factor (1.1 g glucose/g glucan), it was determined that the 30% (w/v) cassava powder solution contained 231 g/L glucan, which accounts for 85.6% glucan (g/g dry matter) of the cassava powder.

α-Amylase and glucoamylase are commonly used for the liquefaction and saccharification of starch (Yu et al., 1995). Sequential treatment of α-amylase and glucoamylase at their optimal temperatures (80-100℃ and 30-50℃, respectively) efficiently yields glucose (Jadhav and Singhal, 2013). Fresh cassava contains 32-35% starch (80-90%, g/g dry matter) (Bantadjan et al., 2020), which is consistent with the results of this study.

3.2. Separate hydrolysis and fermentation (SHF) of cassava

For SHF, a saccharified solution of 30% (w/v) cassava powder was inoculated with *S. cerevisiae* EC1118, a commercial wine yeast strain. Fermentation was conducted at 30℃ and the concentrations of ethanol and other metabolites were monitored every 12 h (Fig. 3). The glucose concentration

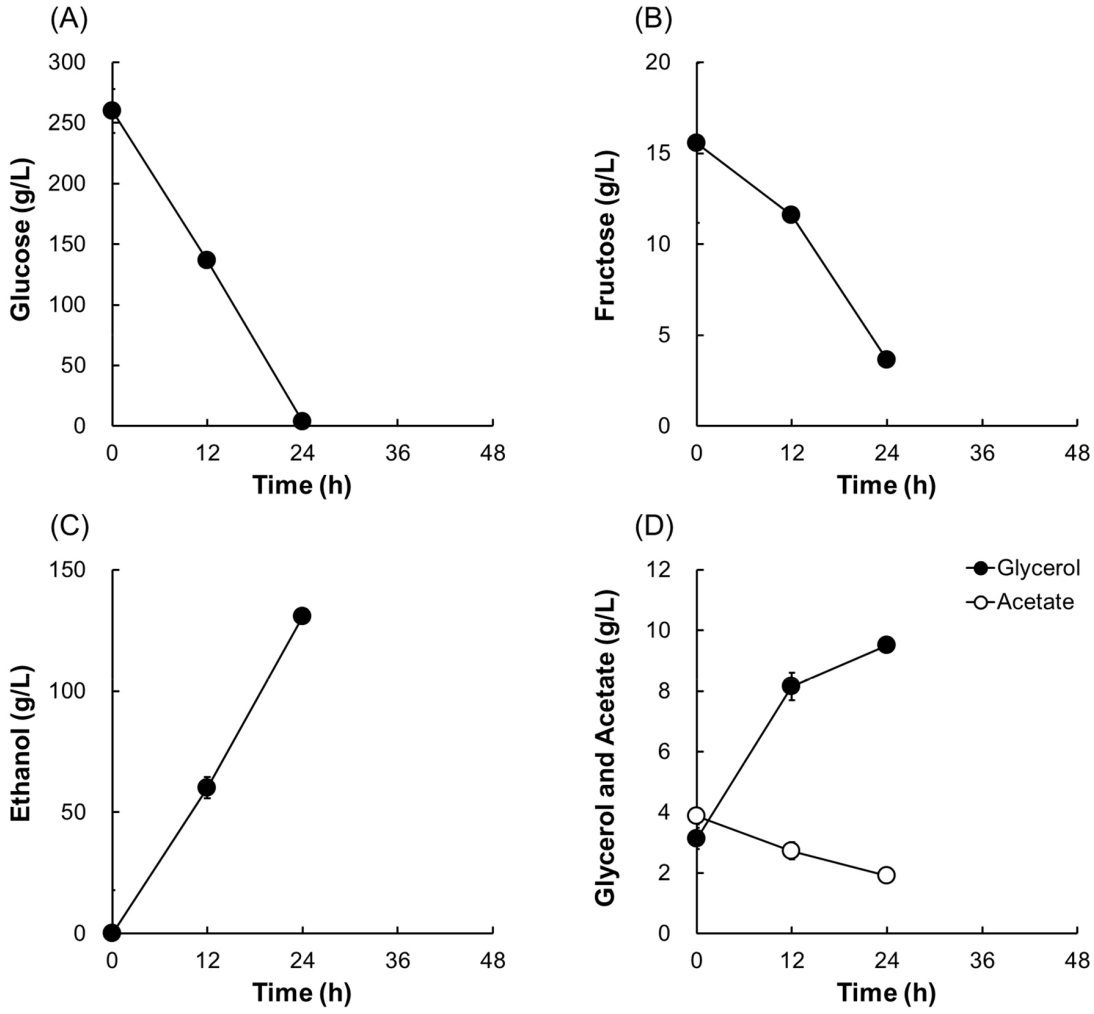

Fig. 3. Separate hydrolysis and fermentation (SHF) of high solids loading of cassava by wine yeast *S. cerevisiae* EC1118. Hydrolysis was performed by adding glucoamylase to the liquefied cassava solution, and the flask was incubated at 30℃ and 150 rpm for 48 h. Saccharified cassava solution (30%, w/v) was fermented at 30℃ and 150 rpm, and glucose (A), fructose (B), ethanol (C), glycerol and acetate (D) concentrations were monitored for 24 h until the level of ethanol increased.

decreased rapidly as the ethanol concentration increased linearly during 24 h of fermentation. At 24 h, glucose was depleted (256.5 g/L glucose consumed), and 130.6 g/L of the maximum ethanol concentration was achieved. Some fructose (11.9 g/L) was also consumed, and 9.5 g/L of glycerol was produced as a byproduct. Acetate concentration showed a slight reduction from 3.9 to 1.9 g/L. The ethanol yield was calculated as 0.49 (g ethanol/g sugar consumed), which was close to the theoretical maximum value (0.51 g/g). These results suggest that wine yeast EC1118 is suitable for cassava ethanol production at high solids loading (30%, w/v), resulting in 〉130 g/L ethanol at a near theoretical yield.

3.3. Simultaneous saccharification and fermentation (SSF) of cassava

For SSF, the liquefied solution of 30% (w/v) cassava powder was mixed with glucoamylase and yeast cells, as described in Materials and Methods. During SSF at 30℃, the glucose concentration was maintained below 50 g/L and the ethanol concentration increased rapidly for 12 h (Fig. 4).

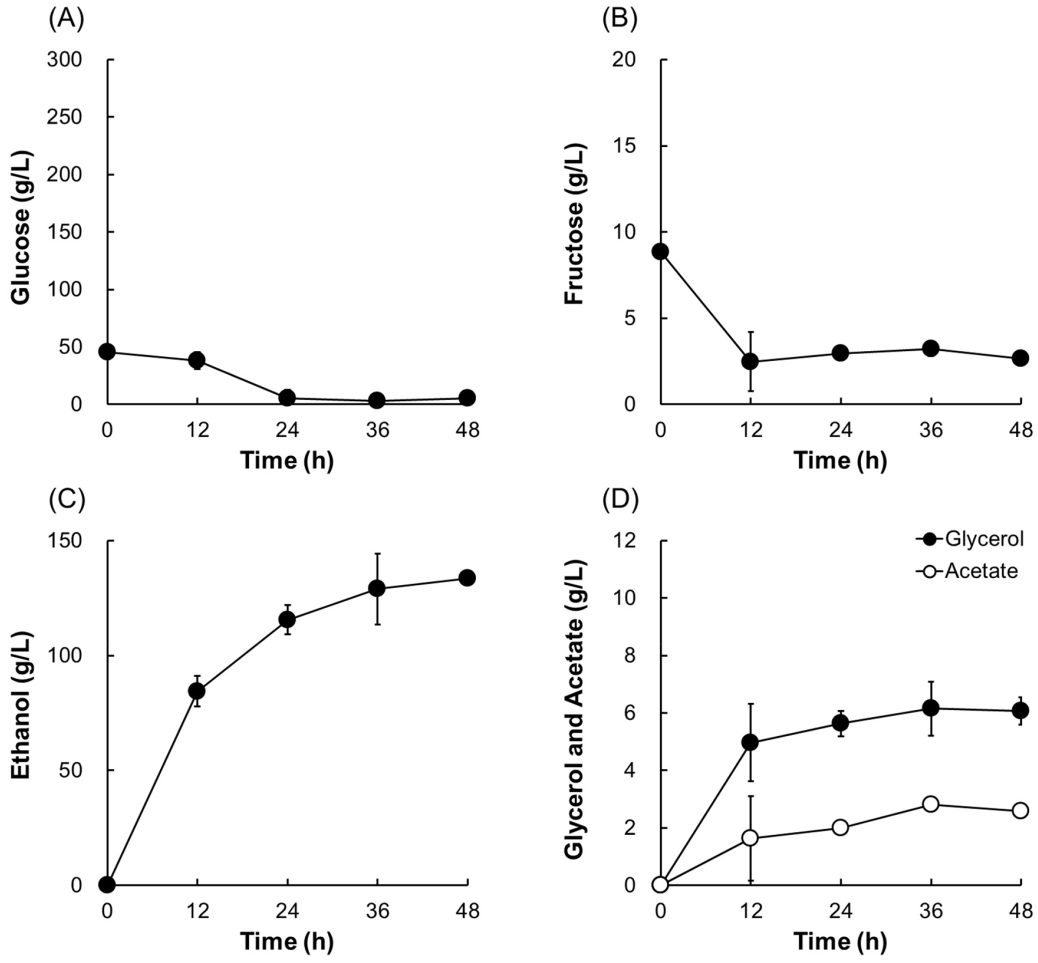

Fig. 4. Simultaneous saccharification and fermentation (SSF) of high solids loading of cassava by wine yeast *S. cerevisiae* EC1118. Liquefied cassava solution (30%, w/v) was incubated with glucoamylase, the nitrogen source, and OD_{600} 1.0 of yeast cells at 30℃ and 150 rpm, and glucose (A), fructose (B), ethanol (C), glycerol and acetate (D) concentrations were monitored for 48 h until the level of ethanol increased.

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Ethanol production continued for 48 h of fermentation, and the maximum ethanol concentration was 133.6 g/L. The final glycerol and acetate concentrations were 6.1 and 2.6 g/L, respectively. The ethanol yield was calculated as 0.51 (g ethanol/g sugar consumed). With respect to the maximum ethanol titer and yield, SSF had slightly higher values than SHF, although the difference was not statistically significant (Table 2). These results suggest that both SHF and SSF of high-solids cassava by wine yeast EC1118 can be used for efficient ethanol production.

The initial glucose concentration is one of the various factors affecting ethanol productivity. High concentrations of glucose can cause osmotic stress in yeast cells, especially when the initial cell density is low. In addition, osmotic stress can be one of the factors inhibiting the fermentation of high solids loading. Because hydrolyzed glucose is instantly consumed by yeast cells during SSF, the glucose concentration during fermentation can be kept low to a level that does not inhibit fermentation (Dahnum et al., 2015; Siriwong et al., 2019; Sovorawet and Kongkiattikajorn, 2012). In the present study, SSF showed significantly higher ethanol productivity (7.04 g/L-h) than SHF (5.01 g/L-h) during the first 12 h of fermentation. After 48 h of fermentation with sufficient cell growth, the ethanol productivity was not statistically significant (Table 1). The increased accumulation of glycerol in SHF might suggest that SHF was subjected to higher osmotic stress than SSF.

4. Conclusions

In the present study, it was confirmed that the commercial wine yeast *S. cerevisiae* EC1118 can produce 〉130 g/L ethanol from 30% (w/v) solid loading of cassava powder at a near theoretical yield. Both SHF and SSF have been successfully applied to cassava ethanol production. Although there was no statistically significant difference in

Table 2. Comparison of fermentation profiles with previous studies

Fermentation mode	Cassava samples	Solid loading (w/v)	α-Amylase	Liquefaction conditions	Glucoa-mylase	Saccharifi-cation conditions	Fermentation conditions	Fermen-tation time	Ethanol (g/L)	Ethanol yield (g/g)	Reference
SHF[1)]	Starch	20%	0.9 mg/g	85℃, 3 h	1.5 mg/g	85℃, 90 min	10 L, 30℃, 70 rpm	72 h	43.5	0.44	(Wangpor et al., 2017)
	Root	35%	150,000 U/mL	93℃, 2 g, 2 h	580 U/g	61.5℃, 2 g, 48 h, pH 4.2	30 ℃, 3 g	48 h	104.7	0.4	(Sakdaronnarong et al., 2020)
	Root	30%	0.33 mg/g	80℃, 100 rpm, 1 h	0.01%	30℃, 150 rpm, 48 h	30℃, 150 rpm	24 h	130.64	0.50	This study
SSF[2)]	Pulp	10%	10.25 U/mL	50℃, 2 h, pH 6.9	43.43 U/mL	50℃, 2 h	30 ℃,100 rpm	74 h	27.4	0.29	(Siriwong et al., 2019)
	Chip	25%	0.7 g/kg	100℃, 90 min	0.5 mg/kg		Continuous, 33 ℃, 0.05 vvm	24 h	78.8	0.43	(Choi et al., 2010)
	Starch	20%	0.3%, v/w	40℃, 72 h	0.1%, v/w	60℃, 1 h	5 L, 200 rpm	72 h	81.86	0.43	(Krajang et al., 2021)
	Root	30%	0.33 mg/g	80℃, 100 rpm, 1 h	0.01%		30℃, 150 rpm	48 h	133.6	0.51	This study

[1)]SHF, separate hydrolysis and fermentation.
[2)]SSF, simultaneous saccharification and fermentation.

ethanol titer and yield between SHF and SSF, SSF showed higher initial ethanol productivity and lower glycerol accumulation, suggesting less osmotic stress during the fermentation of high solids loading. The maximum ethanol concentration in this study was 133.6 g/L, which was the highest titer reported for cassava fermentation.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Kim IJ, Kim SR. Formal analysis: Phachanseesoulath N, Kim S. Methodology: Phachanseesoulath N, Kim S. Validation: Shin J. Writing - original draft: Shin J, Park J, Kim R, Geum S, Jeong D. Writing - review & editing: Kim IJ, Kim SR.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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