Optimization of Ethanol Production from Garcinia Cambogia Residues and the Effects of its Medicinal Component on Production Yield

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Summary: Garcinia cambogia, a Chinese herbal medicine, was popular due to its effect for weight loss. The main medical component inside was determined to be hydroxycitric acid (HCA). To realize the resource technology of garcinia cambogia residue, Optimum ethanol production from residues was investigated, and the effects of remaining HCA on the ethanol yield were investigated. A Plackett-Burman experimental design was used to screen the significance of several influencing factors, and cellulase, yeast extract, and KH_2PO_4 were observed to exert important effects. The optimum ethanol fermentation conditions were determined through an orthogonal design to include a cellulase concentration of 100 U/g, a yeast extract concentration of 15 g/L, and a KH_2PO_4 concentration of 1.0 g/L. The ethanol concentration obtained under optimal conditions was 4.0 g/L. The remained HCA in the residues showed minimal influences on ethanol fermentation and could even increase ethanol yield at low concentrations.

Key words: Garcinia cambogia residue, Plackett-Burman design, Ethanol fermentation, Hydroxycitric acid.

Introduction

Considering that traditional fossil fuel resources have continued to dwindle but demands for fuel continue to increase, the discovery and development of alternative energy sources is an important endeavor [1]. Berndes et al. estimated that, in 2050, the potential global bio-energy supply will range from less than 100 EJ/year to over 400 EJ/year [2, 3]. Biofuel may present in the solid, liquid, and gas states and the major biofuels include bio-ethanol, biodiesel, biogas, bio-methanol, bio-syngas ($CO+H_2$), bio-oil, bio-char, and bio-hydrogen. Bio-ethanol is an alternative gasoline; 60% of this biofuel is produced from sugarcane and 40% is obtained from other crops. About 60 billion liters of bio-fuel was reportedly produced worldwide in 2007 [4]. Bio-ethanol can sink about 90% CO_2 and 60%–80% SO_2 when blended with 95% gasoline [5, 6]; the fuel has been obtained from various types of biomass, which are naturally available on earth.

Biomass, which contributes about 14% of the world's energy, is the fourth largest source of energy after petroleum, coal, and natural gas [7]. Therefore, bioconversion of lignocellulosic wastes into bio-ethanol has attracted great interest as a means of producing renewable energy and reducing environmental problems. Unfortunately, bioconversion efficiency is often limited by the low biodegradability of lignocellulosic wastes, which are mainly composed of cellulose, hemicellulose, and lignin. A number of biomass resources are available in the form of industrial, municipal solid, forestry, and agricultural wastes [8, 9]. One such biomass resource comes from the traditional Chinese herbal extraction industry, which produces about 1.5 million tons of solid waste annually after extraction of active medical components from natural plants. This solid waste, called "herbal extraction residue," decays easily and is potentially harmful to the environment. However, because of their abundance in cellulose, hemicellulose, and lignin, solid wastes can be employed as a renewable energy source. Reusing and recycling this valuable biomass resource is a very urgent and significant but difficult aim [10].

Herbal extraction residues contain certain active ingredients and other elements, such as crude fiber, starch, crude fat, crude protein, and amino acids. Several means for utilizing these residues are available, including re-extraction of other effective ingredients, re-use as fodder or fodder additive, production of organic fertilizer, treatment of wastewater, cultivation of edible fungi, and fermentation for protein and vinegar production [11].

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Garcinia plants are evergreen trees or shrubs that are widely distributed in tropical Asia, Africa, and Polynesia. The weight loss effect of garcinia cambogia has recently drawn wide consumer interest [12], and the emergence of a large amount of garcinia cambogia extraction process residue has been observed. Proper pretreatment of garcinia cambogia residues, a type of cellulosic waste, is thus of great importance for its utilization. Ethanol fermentation may achieve excellent resource recovery, but high ethanol yields require optimization of the culture medium. The Plackett-Burman (P-B) design method is a nearly saturated two-level experimental approach developed in the late 20th century. The method requires only a minimal number of tests to estimate the factors influencing main effects, but it is accurate and efficient for screening important factors; thus, this method has been widely used in optimization of microorganism culture conditions [13]. The P-B design method is adopted in the present study to determine the proper culture conditions to achieve high ethanol yields from garcinia cambogia residues. Because the extraction residue may also contain other medicinal materials, the effect of the substrate on ethanol yield is also investigated in this study.

Experimental

Raw materials

Garcinia cambogia residues were obtained from a traditional Chinese herbal extraction factory in Sanyuan County, Shanxi Province, China. The composition of the residues is shown in Table-1. The cellulose content of the residues was 28%, thus this resource may be a potential substrate for ethanol fermentation. After crushing, 10.0 g of the residue was mixed with 1% H_2SO_4 solution 100 ml and sterilized at 121 °C for 2 h, after which the treated residue was used for ethanol fermentation. Lignocellulose was analyzed by an SLQ-6 fiber tester (Shanghai King James Instruments Co. Ltd.), and C, N, and H contents were determined by an EA3000 elemental analyzer (Leeman, China).

Table-1: Composition of garcinia cambogia residue.

Parameter	Content %
Cellulose	27.47
Hemicellulose	8.39
Lignin	15.48
C	44.277
N	1.020
H	6.098
TS	93.18
VS	97.56

Strains and enzyme preparation

Angel yeast was provided by Angel Yeast Co. Ltd. and used for ethanol fermentation. Enzymes included cellulase (15000 U/g; Fine Chemical Research Institute, Tianjin City, China), saccharifying enzyme (100000 U/g; Beijing Aoboxing Biotechnology, LLC), phytase (5000 U/g), and pectinase (10000 U/g; Shandong Sukahan Bio-technology Co., Ltd.).

Ethanol fermentation

Ten grams of pretreated garcinia cambogia residue was added to a 250 mL glass Erlenmeyer flask. The final fermentation broth had a volume of 150 mL. The pH of the residue was adjusted to 5.0 using 1 mol/L NaOH solution. In the P-B experiment, the corresponding condition was referred to as the experimental design for enzymes and nutrients in Table-2. The fermentation yeast was added at a concentration of 0.03 g (yeast)/g (residue) to the broth. The fermentation experiment was carried out on a rotary shaker for 3.5 d at 37 °C.

Experimental design

Plackett-Burman experiment

In the test, Plackett-Burman design was selected as the method to optimize the culture media for ethanol production from garcinia cambogia residue. Various kinds of enzymes were added in order to examine the effect of their effects on ethanol production, and nitrogen sources and inorganic salts were used to test their influence on ethanol fermentation. Table-2 showed the factors in the design of P-B experiment, N = 16 was selected, wherein each factor had 2 levels, corresponding parameters were showed in the table, totally 15 factors were used in the experiment and 2 was chosen as blank factor which named N and O. There were four kinds of enzymes in the experiment, named glucoamylase, cellulase, phytase and pectinase. Since garcinia cambogia residue contained cellulose component, thus cellulase should be important, and ethanol fermentation usually needed glucoamylase, thus these two enzymes were added. Furthermore, phytase can hydrolyze phytic acid and could eliminate the inhibitory effect by phytic acid [14], pectinase hydrolysis of pectin can effectively promote the cell wall lysis to release nutrients, so pectinase was also selected. Nitrogen sources included three kinds, two organic nitrogen sources, yeast extract and peptone, one inorganic nitrogen source $(NH_4)_2SO_4$. Inorganic salts were selected as 6 kinds of commonly used inorganic salt, which were $MgSO_4$, KH_2PO_4, $CaCl_2$, $MnSO_4$, $FeSO_4$ and $ZnSO_4$, respectively.

Table-2: Factors and levels in Plackett-Burman design.

No.	Factor	Factor levels	
		Low level (-1)	High level (+1)
A	Cellulase / U·g^{-1}	0	100
B	Glucoamylase / U·g^{-1}	0	100
C	phytase / U·g^{-1}	0	100
D	pectinase /U·g^{-1}	0	100
E	yeast extract / g·L^{-1}	0	15
F	peptone / g·L^{-1}	0	15
G	$(NH_4)_2SO_4$/ g·L^{-1}	0	2.0
H	$MgSO_4$/ g·L^{-1}	0	1.0
I	KH_2PO_4/ g·L^{-1}	0	1.5
J	$CaCl_2$/ g·L^{-1}	0	0.3
K	$MnSO_4$/mg·L^{-1}	0	1.5
L	$FeSO_4·7H_2O$/ mg·L^{-1}	0	5.0
M	$ZnSO_4$/ mg·L^{-1}	0	1.4
N	blank		
O	blank		

Orthogonal experiment

According to the experimental P-B results, factors determined to be of significance were selected as factors in the orthogonal experimental design, as described in Table-3. Ethanol yield was used as an indicator to confirm optimum conditions.

Table-3: Orthogonal experimental design.

Factor	Cellulase/ U·g^{-1}	Yeast extract/ g·L^{-1}	KH_2PO_4/ g·L^{-1}	Blank
	A	B	C	D
level 1	50	10	1.0	1
level 2	100	15	1.5	2
level 3	150	20	2.0	3

Effect of hydroxycitric acid (HCA) on ethanol fermentation

Hydroxycitric acid (HCA) has been determined to be the main medicinal substrate for garcinia cambogia [15]. Although the extraction process was carried out for medicinal production, HCA traces are likely to remain in the residue. Thus, evaluating the effect of HCA on ethanol production is important. To avoid interfering effects from other ingredients, the fermentation culture medium included glucose (100 g/L), yeast extract (15 g/L), and KH_2PO_4 (1.0 g/L) and was autoclaved at 121 °C for 20 min. Several garcinia cambogia extraction solutions (HCA concentration, 60%; Xi'an tianrui Bio-Tech Co., Ltd) were used in six experiments. The corresponding extraction liquid concentrations were 0 (control), 2.5, 5.0, 10.0, 15.0, and 20.0 g/L. The fermentation experiment was performed as described in Section 2.3, and sampling was conducted at predetermined times to measure ethanol and sugar concentrations.

Analytical method

Sampling at predetermined times was conducted, and 5 mL of the fermentation broth was obtained each time. After centrifugation at 10000 r/min for 5 min, the supernatant was subjected to determinations of reducing sugar and ethanol contents. The DNS method was used to determine sugar contents [16], and ethanol concentrations were analyzed by gas chromatography (Shimadzu GC-2010) with a flame ionization detector. A scanning electron microscope (SEM, JSM-6501A, JEOL; Japan) was used to detect the microscopic structure of the residue samples. All experiments were repeated three times, and average results are reported.

Results and Discussion

Screening of important factors by the Plackett-Burman method

Table-4 showed the ethanol concentrations determined in the P-B design, the corresponding results were analyzed by Statistica 6.0. Table-5 described the result of the P-B design. Corresponding influential factors were screened. The p value was selected to determine influencing factors, and a 97% confidence interval ($p < 0.03$) was regarded as significant. From the P (a) in Table-5, cellulase may be observed to have the lowest p value of 0.010. To increase the significance of the model, the least-significant factor (the factor with the highest p value), zinc sulfate, was removed. The new model was then analyzed and a new factor sequence was obtained, as shown in P (b). Among the investigated variables, cellulase ($p = 0.001$), yeast extract ($p = 0.029$), and KH_2PO_4 ($p = 0.027$) were regarded as significant factors ($p < 0.03$). Other factors studied were statistically insignificant, although this result does not mean they have no effect on ethanol fermentation. Table-1 showed that garcinia cambogia residues were rich in cellulose. Thus, cellulase content was a significant factor because it could utilize the substrate. Table-1 further showed that the nitrogen content of garcinia cambogia residues is very low. Addition of a nitrogen source would increase the C/N ratio, which was beneficial for microorganism growth. Among the three nitrogen sources studied, yeast extract was determined to be the most significant. Besides cellulase and nitrogen, phosphate exerted a remarkable effect on ethanol yield, possibly because KH_2PO_4 presented some buffering capacity and changes in the concentration of phosphate could strongly promote the growth of yeast cells [17].

Table-4: Design and result for P-B design with 16 factors.

Number	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Ethanol Concentration /g·L^{-1}
1	-1	-1	-1	-1	1	1	1	1	1	1	-1	-1	-1	-1	1	1.7
2	1	-1	-1	-1	-1	-1	-1	1	1	1	1	1	1	-1	-1	5.2
3	-1	1	-1	-1	-1	1	1	-1	-1	1	1	1	-1	1	-1	2.5
4	1	1	-1	-1	1	-1	-1	-1	-1	1	-1	-1	1	1	1	5.6
5	-1	-1	1	-1	1	-1	1	-1	1	-1	1	-1	1	1	-1	3.8
6	1	-1	1	-1	-1	1	-1	-1	1	-1	-1	1	-1	1	1	6.3
7	-1	1	1	-1	-1	-1	1	1	-1	-1	-1	1	1	-1	1	2.0
8	1	1	1	-1	1	1	-1	1	-1	-1	1	-1	-1	-1	-1	5.0
9	-1	-1	-1	1	1	1	-1	1	-1	-1	-1	1	1	1	-1	2.4
10	1	-1	-1	1	-1	-1	1	1	-1	-1	1	-1	-1	1	1	2.7
11	-1	1	-1	1	-1	1	-1	-1	1	-1	1	-1	1	-1	1	2.4
12	1	1	-1	1	1	-1	1	-1	1	-1	-1	1	-1	-1	-1	6.0
13	-1	-1	1	1	1	-1	-1	-1	-1	1	1	1	-1	-1	1	3.1
14	1	-1	1	1	-1	1	1	-1	-1	1	-1	-1	1	-1	-1	3.2
15	-1	1	1	1	-1	-1	-1	1	1	1	-1	-1	-1	1	-1	2.3
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5.7

Table-5: Results analysis for Placett-Burman design.

Number	Factor	p (a)	p (b)
A	Cellulase / U·g^{-1}	0.010	0.001
B	Glucoamylase / U·g^{-1}	0.263	0.164
C	Phytase / U·g^{-1}	0.286	0.185
D	Pectinase /U·g^{-1}	0.166	0.085
E	Yeast extract / g·L^{-1}	0.080	0.029
F	Peptone / g·L^{-1}	0.534	0.441
G	$(NH_4)_2SO_4$/ g·L^{-1}	0.145	0.069
H	$MgSO_4$/ g·L^{-1}	0.099	0.040
I	KH_2PO_4/ g·L^{-1}	0.076	0.027
J	$CaCl_2$/ g·L^{-1}	0.585	0.498
K	$MnSO_4$/mg·L^{-1}	0.698	0.632
L	$FeSO_4·7H_2O$/ mg·L^{-1}	0.084	0.031
M	$ZnSO_4$/ mg·L^{-1}	0.761	
N	Blank		
O	Blank		

Optimization of fermentation parameters by the orthogonal experimental design

Based on the experimental P-B results, cellulase, yeast extract, and KH_2PO_4 were determined to be significant factors influencing ethanol yield. Acquiring the optimum levels of these factors was important to achieve maximum ethanol concentrations. An L_9 (3^4) orthogonal experiment was used in the present study, and results were shown in Table-6. Analyses of ranges and variances revealed that the degree of influence of the three factors on ethanol concentration showed the order yeast extract > cellulase = KH_2PO_4. This showed that the garcinia cambogia residue might be in need of nitrogen source, adjusting the yeast extract content properly can effectively promote ethanol fermentation. Table-6 also showed that the ethanol concentration increased with increasing yeast extract content. However, when the yeast extract was applied at a concentration of 20 g/L, the ethanol decreased, likely because the yeast extract functioned as a nitrogen source for microorganism growth. Thus, excessively high nitrogen concentrations would result in an overabundance of microorganisms and competition for nutrients. As the growth phase of yeast also requires oxygen, an overabundance of microorganisms in the culture medium could cause oxygen shortage, which was detrimental to their growth. Yeast extract decomposition caused nutrient accumulation in the product and a corresponding change in pH in the culture medium, which, in turn, could result in reductions in microbial growth rate and decreases in ethanol yield. Addition of cellulase helped release sugars within the garcinia cambogia residue and increased ethanol production. However, excessive addition of cellulase could not continuously increase ethanol concentrations because of the limited cellulose content in the residues, also this would increase the cost for enzymes. The optimal conditions of the orthogonal experiment were A2B2C1 and A2B2C3; maximum ethanol concentrations could be obtained under both conditions. From economical point of view, we should adopt the method with less agent utilization, the best condition was determined to be A2B2C1. Under this condition, the cellulase dosage was 100 U/g, the yeast extract concentration was 15 g/L, and the KH_2PO_4 concentration was 1.0 g/L. Verification experiments showed that the optimum ethanol concentration obtained from garcinia cambogia

residue fermentation was 4.0 g/L under optimum conditions. This result demonstrated that the orthogonal test design exhibited a high confidence level.

Since garcinia cambogia residues may be regarded as a type of lignocellulosic waste, the equation for cellulose ethanol fermentation could be utilized for the process with the residue for ethanol, corresponding equation was showed in 3–1 and 3–2. According to these equations, the theoretical ethanol concentration obtained from cellulose could be 56.1%, which meant 10 g of garcinia cambogia residue could yield 1.45 g of ethanol.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6 \quad (3–1)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (3–2)$$

For comparison, the following parameters were defined:

Theoretical ethanol quantity = mass of garcinia cambogia residue × TS × cellulose composition × 56.61%

Actual ethanol recovery rate (%) = actual ethanol quantity/theoretical quantity × 100%

Ethanol yield (%) = ethanol quantity in fermentation broth/dry mass of the substrate × 100%

Based on the result we obtained, the ethanol recovery rate was calculated as 41.4%, and the ethanol yield was 6.45%. Liu utilized corn straw as a substrate, adopted a mixture of microorganisms and achieved an optimum ethanol yield of 12.03%. This yield was markedly higher than that in the present study, likely because we only inoculated yeast that could utilize glucose. By contrast, Liu's work included utilization of pentose. The cellulose contents in corn straw were also higher than that in garcinia cambogia residue. This discrepancy reveals that pentose must be employed in future studies [18].

Cristina and Silvia Bolado investigated the effect of different pretreatment methods on wheat straw ethanol fermentation, they found hydrolysates of washed alkaline peroxide pretreated biomass provided the highest sugar concentrations, which were 31.82 g/L glucose, and 13.75 g/L xylose, with ethanol concentrations reaching 17.37 g/L [19].

Analysis of Scanning Electron Microscope Photos

Figs. 1A, 1B, and 1C respectively show SEM micrographs of untreated, treated (soaking in 1% of H_2SO_4 at 121 °C for 2 h), and fermented garcinia cambogia residues. Residues in Fig. 1A demonstrated a smooth surface and dense structure, which caused great difficulties during enzymatic hydrolysis of cellulose [20]. The residue in Fig. 1B showed a relatively rough and uneven surface with obvious holes; this result demonstrates that dilute acid pretreatment could damage the structure of the residue and increased the accessibility of the cellulose to the enzyme [21]. Fig. 1C showed residue had a loose and porous structure; this result indicated that the cellulose and other organic matter in the residue were fully utilized.

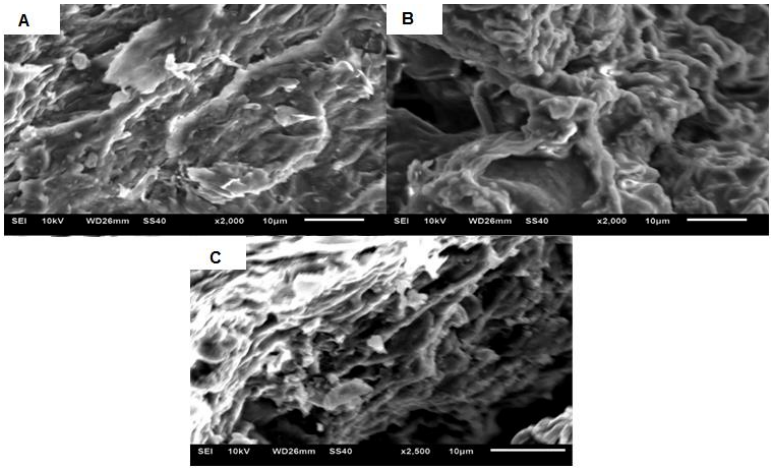
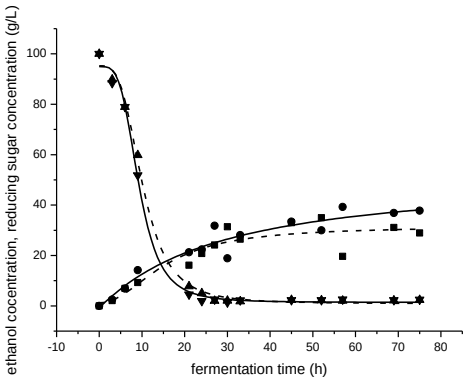


Fig. 1: Scanning electron microscope photos of garcinia cambogia residue.

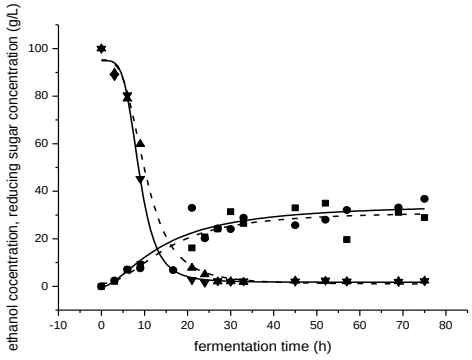
(A) untreated garcinia cambogia residues, (B) treated residue (soaking in 1% of H_2SO_4 at 121 °C for 2 h), (C) garcinia cambogia residues after fermentation 3.4 Effect of hydroxycitric acid (HCA) on ethanol fermentation.

Table-6: L_9 (3^4) orthogonal experimental results and analysis.

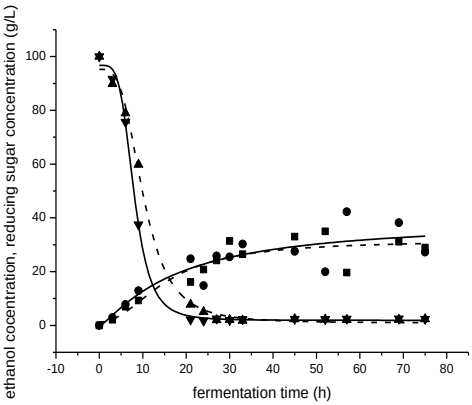
Factor	A. Cellulase / U·g^{-1}	B. yeast extract / g·L^{-1}	C. KH_2PO_4 / g·L^{-1}	D. Blank	Ethanol Content / g·L^{-1}
1	50	10	1.0	1	2.8
2	50	15	1.5	2	2.9
3	50	20	2.0	3	2.7
4	100	10	1.5	3	3.3
5	100	15	2.0	1	3.8
6	100	20	1.0	2	2.8
7	150	10	2.0	2	3.3
8	150	15	1.0	3	4.2
9	150	20	1.5	1	2.1
Average 1	2.800	3.133	3.267	2.900	
Average 2	3.300	3.633	2.767	3.000	
Average 3	3.200	2.533	3.267	3.400	
Range	0.500	1.100	0.500	0.500	
Optimum level	2	2	1，3		



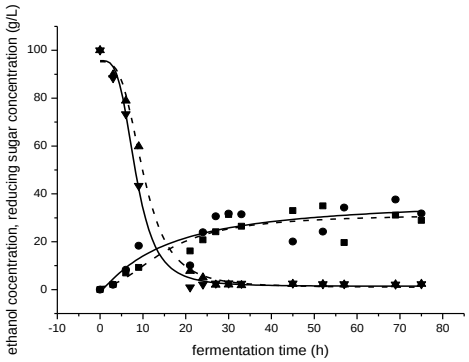
A: 1.5 g/L HCA



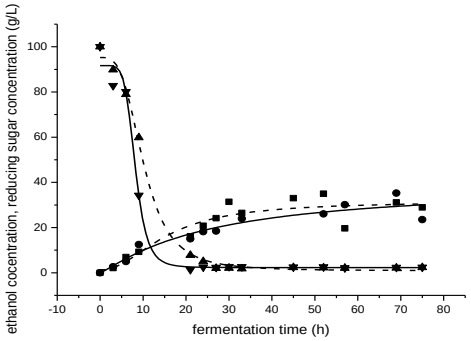
B: 3.0 g/L HCA



C: 6.0 g/L HCA



D: g/L HCA



E: 12.0 g/L HCA

Fig. 2: Effect of different HCA concentration on ethanol fermentation.

●Ethanol concentration with HCA added, ■ ethanol concentration for control, ▼ reducing sugar with HCA added, ▲ reducing sugar for control, — simulations of ethanol production with different HCA added, - - - simulations of ethanol production for the control group

The active component in garcinia cambogia was known as HCA, and its composition in dry garcinia cambogia had been determined to be 16% [12]. Analysis of the effects of different HCA concentration on ethanol production from glucose was carried out as described in Section 2.4.3. Since the HCA concentration in the extraction liquid of garcinia cambogia was 60%, the corresponding HCA concentrations in 2.4.3 for the six fermentation runs were 0 g/L, 1.5 g/L, 3.0 g/L, 6.0 g/L, 9.0 g/L, and 12.0 g/L, correspondingly. After fermentation, the ethanol and reducing sugar contents were measured. Cao et al. demonstrated that utilization of a logistic equation could simulate the ethanol fermentation process and reveal changes in ethanol and sugar concentration[22]. Thus, such an equation could be regarded as a good tool for ethanol fermentation analysis. In this study, the software Origin 8.1 was adopted for simulations of ethanol production with different HCA concentrations. The corresponding

sugar and ethanol concentrations obtained were shown in Fig. 2.

Results showed that addition of HCA could enhance the sugar consumption rate. A previous research indicated that HCA reduces blood and hepatic lipid concentrations as well as serum glucose, insulin, c-peptide, and leptin levels [23, 15]. While this showed HCA might be helpful for the sugar utilization. The ethanol yields obtained from addition of 1.5–9 g/L HCA were higher than that obtained without HCA. However, when the HCA concentration was 12 g/L, ethanol yield and fermentation velocity decreased. This result illustrated that excessively high HCA concentrations caused decreases in ethanol. Considering these results and the fact that garcinia cambogia residues likely feature HCA concentrations lower than 12 g/L, utilization of this residue was not expected to exert HCA effects on ethanol fermentation. Thus, garcinia cambogia residues may be regarded as a suitable substrate for ethanol fermentation.

Conclusion

Garcinia cambogia residue was used in the present study to produce ethanol. A P-B design was used to screen significant influencing factors, and cellulase, yeast extract, and KH_2PO_4 were determined to be important parameters affecting ethanol production. Optimum conditions determined through an orthogonal experimental design included a cellulase concentration of 100 U/g, a yeast extract concentration of 15 g/L, and a KH_2PO_4 concentration of 1.0 g/L. The ethanol concentration obtained under optimum concentrations was 4.0 g/L, the ethanol yield was 0.06 g/g, and the actual ethanol recovery yield was 41.4%. Addition of HCA enhanced the glucose consumption rate, and low concentrations of HCA could improve ethanol yield.

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References

1. X. Y. Cheng and C. Z. Liu, Enhanced biogas production from herbal-extraction process residues by microwave-assisted alkaline pretreatment, *J. Chem. Technol. Biotechnol.*, **85**, 127 (2010).
2. G. Berndes, M. Hoogwijk and R. Van den Broek, The contribution of biomass in the future global energy supply: a review of 17 studies, *Biomass Bioenerg.*, **25**, 1 (2003).
3. A. Popp, H. Lotze-campen, M. Leimbach, B. Knopf, T. Beringer and N. Bauer, On sustainability of bioenergy production: integrating co-emissions from agricultural intensification, *Biomass Bioenerg.*, **35**, 4770 (2010).
4. A. Demirbas, Bioethanol from cellulosic materials: a renewable motor fuel from biomass, *Energ. Sources.*, **27**, 327 (2005).
5. R. C. Saxena, D. K. Adhikari and H. B. Goyal, Biomass-based energy fuel through biochemical routes: a review, *Renew Sust. Energy Rev.*, **13**, 167 (2009).
6. Y. L. Lu and N. S. Mosier, *Current technologies for fuel ethanol production from lignocellulosic plant biomass*, *Genetic Improvement of Bioenergy Crops*, Springer, P.161 (2008).
7. J. Sheehan and M. Himmel, Enzymes, energy, and the environment: astrategic perspective on the U.S. Department of Energy's research and development activities for bioethanol, *Prog. Biotechnol.*, **15**, 817 (1991).
8. I. Demiral and S. Sensoz, The effect of different catalysts on the pyrolysis of industrial wastes (olive and hazelnut bagasse), *Bioresour. Technol.*, **99**, 8002 (2008).
9. J. L. Zheng, Pyrolysis oil from fast pyrolysis of maize stalk, *J. Anal. Appl. Pyrolysis.*, **83**, 205 (2008).
10. P. Wang, S. H. Zhan, H. B. Yu, X. F. Xue and N. Hong, The effects of temperature and catalysts on the pyrolysis of industrial wastes (herb residue), *Bioresour. Technol.*, **101**, 3236 (2010).
11. B. Zhu and X. X. Tian, On the Comprehensive Utilization of Herbal Medicine Dregs, *Journal of Zunyi Normal College*, **15**, 78 (2013).
12. K. Z. Wang, Diet food rookie- Garcinia cambogia, *Plants*, 05, 9 (in Chinese) (1995).
13. C. Teera, O. T. Sompong and P. Poonsuk, Statistical Optimization of medium components affecting simultaneous fermentative hydrogen and ethanol production from crude glycerol by thermotolerant Klebsiella sp. TR17, *International Journal of Hydrogen Energy*, **39**, 751 (2014).
14. J. S. Liu, M. L. Jin, D. L. Gong and F. Y. Guo, Influence on the fermentation with different raw corn flour by applying phytase, *Chemical*

Engineer., **70** (2014).
15. R. B. Semwal, D. K. Semwal, I. Vermaak and A. Viljoen, A comprehensive scientific overview of Garcina cambogia, *Fitoterapia.,* **101**, 134 (2015).
16. G. L. Miller, Use of DNS reagent for determination of reducing sugars, *Analytical Chemistry,* **31**, 426 (1959).
17. C. M. Pan, Y. T. Fan, Y. Xing, H.W. Hou and M. L. Zhang, Statistical optimization of process parameters on biohydrogen production from glucose by Clostridium sp. Fanp2, *Bioresour. Technol.,* **99**, 3146 (2008).
18. K. S. Liu and J. C. Han, Changes in mineral concentrations and phosphorus profile during dry-grind processing of corn into ethanol, *Bioresour. Technol.,* **102**, 3110 (2011).
19. C. Toquero and S. Bolado, Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing, *Bioresour. Technol.,* **157**, 68 (2014)
20. L. T. Fan, Y. H. Lee and D. H. Beardmore, Mechanism of the enzymatic hydrolysis of cellulose: Effect of major structural feratures of cellulose on enzymatic hydrolysis, *Biotechnol. and Bioeng.,* **22**, 177 (1980).
21. J. Z. Zhang, X. X. Ma and J. L. Yu, The effects of four different pretreatments on enzymatic hydrolysis of sweet sorghum bagasse, *Bioresour. Technol*., **102**, 4585 (2011).
22. Y. L. Cao, S. H. Wu, W. Q. Wu and C. J. Huang, Study on Ethanol Batch Fermentation with Different Initial Glucose Concentration, *Liquor －Making Science & Technology,* 09, 20 (in Chinese, English abstract) (2014).
23. Y. J. Kim, K. Y. Kim, M. S. Kim, J. H. Lee, K. P. Lee and T. Park, A mixture of the aqueous extract of Garcinia cambogia, soy peptide and L-carnitine reduces the accumulation of visceral fatmass in rats rendered obese by a high fat diet, *Genes. Nutr.,* **2**, 353 (2008).