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Fermentative production of L(+)-lactic acid using hydrolyzed acorn starch, persimmon juice and wheat bran hydrolysate as nutrients

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ABSTRACT

The use of hydrolyzed acorn starch as a novel carbon source for L(+)-lactic acid production was proposed. The effects of carbon–nitrogen ratio and growth factor on the fermentations were studied by single factor experiments. A lower carbon–nitrogen ratio could enhance L(+)-lactic acid production, and the expensive yeast extract could be replaced by the cheap persimmon juice providing growth factor for L(+)-lactic acid production when wheat bran hydrolysate was used as the nitrogen source. The dosages of wheat bran hydrolysate and persimmon juice in the medium were statistically optimized by response surface methodology (RSM). The yield of L(+)-lactic acid reached 45.78 g/100 g dry acorn with a final concentration of 57.61 ± 1.37 g/l and a productivity of 1.60 ± 0.12 g/l h when the batch fermentation was carried out in a 5 l bioreactor under the optimal conditions of wheat bran hydrolysate 24.55 g/l and persimmon juice 12.30 g/l. Comparative batch fermentations using different raw materials such as acorn, cassava, corn and glucose showed that both the yield and the productivity of L(+)-lactic acid production were the highest when the hydrolyzed acorn starch was used as the carbon source. Therefore, the acorn could be used as a new substitute of grain raw material in L(+)-lactic acid production.

1. Introduction

Lactic acid (LA) is an organic acid named 2-hydroxypropionic acid or 2-hydroxypropanoic acid. There are two optical isomers of lactic acid, L(+)-lactic acid and D(−)-lactic acid. They are considered to be the most important carboxylic acid because of their wide applications in many fields such as food, pharmacy, textile, leather, and chemical industries (Litchfield, 1996; John et al., 2007a). In recent years, the demand for L(+)-lactic acid has been increasing considerably, owing to its new application as a monomer in the preparation of polylactic acid (PLA), a type of environment-friendly alternative to petrochemicals plastics (Datta and Henry, 2006; Wee et al., 2006).

Microbial fermentation is the main method of L(+)-lactic acid production in industry (Patnaik et al., 2002; John et al., 2009). Since the chemical formula of L(+)-lactic acid is $C_3H_6O_3$, carbon source is the main raw material in L(+)-lactic acid production. The selection of a specific raw material depends mainly on its availability, composition, and price. Thus, some cheap raw materials such as molasses, starchy and cellulosic materials have been used as the carbon sources in L(+)-lactic acid production (Hofvendahl and Hahn-Haerdal, 2000). The starchy materials consisted of glucose are easily hydrolyzed into fermentable sugars. Therefore, the starchy materials like corn, rice, unpolished rice, wheat, rye, barley and so on, are the preferred carbon sources used in L(+)-lactic acid production at present (Lu et al., 2009). These materials belonging to grain are usually processed into food and feedstuff to meet the needs of human and livestock. They are also used as production materials in fermentation industry because they are rich in starch. However, in some countries, grain is not advocated to be used as the raw material in the production of some chemicals such as industrial L(+)-lactic acid because of its high price and short supply. Non-grain raw material such as cassava used in fermentative production greatly attracted researchers (Roble et al., 2003; John et al., 2007b; Timbuntam et al., 2008).

There are many non-grain raw materials that are rich in starch and other natural nutrients. For instance, acorn obtained from oak tree is one of such materials. Acorn has been utilized as a source of starch, oil and protein over 6000 years in the Northern Hemisphere (Masaka and Sato, 2002; Abrahamson and Layne, 2003). Today, acorn, particularly its starch, is still consumed as a supplementary food of grain in some areas such as Korea, China and Japan (Nieto et al., 2002; Canellas et al., 2007). Meanwhile, another bioactive fruit, persimmon, has interested researchers in recent years. As a good source of sugars, vitamin, organic acids, antioxidants, carotenoids and polyphenols, persimmon is now a popular fruit from temperate to tropical regions where it is consumed as a health food (George and Redpath, 2008; Veberic et al., 2010). Due to their high contents of starch, amino acid and vitamin, acorn and persimmon

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have great potential as fermentation substrates. Unfortunately, there are no reports that the two types of fruit have been used in fermentation.

The objective of this work is to propose a new utilization of the hydrolyzed acorn starch and persimmon juice processed from the two types of non-grain raw materials as the carbon source and growth factor in L(+)-lactic acid production. This work is a part of a broader investigation about the new utilization of non-grain raw materials in fermentation field.

2. Methods

2.1. Samples of acorn, persimmon, wheat bran, cassava and corn

The samples of acorn (*Quercus variabilis* BL) and persimmon (*Diospyros kaki* Thunb. Lt) were obtained from Luotian county of Hubei province. The wheat bran and corn were obtained from Qichun county of Hubei province. The cassava tuber was purchased from Guangxi province of China where the cassava is very rich.

2.2. Saccharifications of acorn, cassava and corn starch, hydrolysis of wheat bran and preparation of persimmon juice

The saccharification of acorn starch was achieved by double enzymes method. Dry acorn powder was mixed with water by a ratio of 1:2 (w/v). After pH was adjusted to 6.3, the α-amylase (5000 U/g, Beijing Shuangxuan Microbe Medium Products Factory, China) was added with a dosage of 10 U/g dry acorn. The mixture was heated to 65 °C and maintained about 40 min with agitation in a 5 l bioreactor (Biostat B., Braun). After the liquefaction of acorn starch was finished, the mixture of dextrin was cooled to 58–62 °C, the pH was adjusted to 5.2, and then the glucoamylase (100,000 U/g, Shanghai Senhao Fine Chemicals Co., Ltd., China) was added with a dosage of 100 U/g dry acorn. Here, the dosages of the two enzymes were based on the starch content of the used materials. This process was maintained about 40 h with agitation. When the saccharification was finished, the mixture was cooled to room temperature and centrifuged at 7000 rpm for 5 min. The supernatant was adjusted to the desired sugar concentration and used as the main carbon source for L(+)-lactic acid production. The saccharifications of the starch of the cassava and corn were the same as that of the acorn starch. The conversion rates of the starch into sugar of the acorn, cassava and corn were 90.62%, 90.37% and 91.15%, respectively.

The hydrolysis of wheat bran was achieved by acid-hydrolysis method (Gao et al., 2007). Acid-hydrolysis is an effective method to obtain the nutrients such as amino acids, peptides and sugars from high protein material. The dry wheat bran powder was mixed with 1 mol/l of H_2SO_4 by a ratio of 1:3 (w/v). Then the slurry was heated to 121 °C and maintained for 20 min. After a sufficient hydrolysis, the mixture was cooled to room temperature, neutralized by $CaCO_3$ powder, and centrifuged at 7000 rpm for 5 min. The supernatant was collected and used as the nitrogen source for L(+)-lactic acid production.

The preparation of persimmon juice was a simple process of solid–liquid separation. After the core of the fresh persimmon was removed, the flesh was grinded into slurry. Then the slurry was mixed with water by a ratio of 1:1 (w/v) and heated to 95–100 °C, maintaining for 5 min. After being cooled to room temperature, the mixture was centrifuged at 8000 rpm for 10 min. The precipitation mainly consisted of dietary fiber can be processed into health food (Veberic et al., 2010). The supernatant was collected as persimmon juice to provide growth factor for L(+)-lactic acid production.

2.3. Strain, media and culture conditions

Lactobacillus rhamnosus HG 09, a L(+)-lactic acid producer, was used as inoculated strain. The inoculum was activated at 36 °C for 10–12 h in MRS medium (Gao et al., 2007). The media in the study of carbon–nitrogen ratio contained 80 g/l of acorn reducing sugar, 2.50 g/l of yeast extract and some wheat bran hydrolysate to make the carbon–nitrogen ratios at 40.12, 60.05 and 81.26, respectively. Here, the total carbon refers to the carbon from the acorn, wheat bran and yeast extract. The total nitrogen refers to the nitrogen from the acorn, wheat bran and yeast extract. The media in the study of replacement of yeast extract by the persimmon juice contained 80 g/l of acorn reducing sugar and 25 g/l of wheat bran hydrolysate. The media for the medium optimizations by RSM were listed in Table 1. Other media were shown further. The sugars from the acorn, cassava, corn and glucose in the medium were 80 g/l in all fermentation experiments. The concentration of the wheat bran hydrolysate (g/l) represents the dosage of the dry wheat bran used in the acid-hydrolysis process, and the concentration of the persimmon juice (g/l) represents the dosage of the fresh persimmon used in the preparation of the persimmon juice. All the media were sterilized at 121 °C for 15 min. Flask experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml medium at 42 °C with a shaking speed of 150 rpm. The ampliative fermentation was carried out in a 5 l bioreactor (Biostat B., Braun) with a working volume of 3.5 l. The culture temperature and agitation speed were maintained at 42 °C and 150 rpm, respectively. The pH of shaking flask and bioreactor were both automatically controlled by 5% (w/v) sterilized $CaCO_3$ which was added into the media before the beginning of the fermentations. The produced lactic acid reacted with $CaCO_3$ instantly and the pH of the fermentation broth was maintained at neutral level. The inoculation amount of all the fermentations was 8% (v/v).

2.4. Statistical optimization of medium by RSM

Response surface methodology (RSM) is an integration of statistical and mathematical techniques. It is a useful method for the modeling and analysis of engineering problems in many fields (Box and Wilson, 1951). In this method, the independent variables are assumed to be continuous and controllable by experiments with negligible errors. Meanwhile, a suitable approximation for the true functional relationship between independent variables

Table 1
Central composite design (CCD) for the medium optimization with experimental and predicted values for L(+)-lactic acid production. The initial acorn sugar used in the medium was 80 g/l in all fermentation experiments.

Fermentation experiment	Variable (X_i, medium component)		Response (Y_i, L(+)-lactic acid)	
	X_1 Wheat bran hydrolysate (g/l)	X_2 Persimmon juice (g/l)	Experimental (g/l)	Predicted (g/l)
1	12.93	10	47.06 ± 1.14	48.87
2	20	2.93	47.05 ± 1.07	47.31
3	15	5	44.23 ± 1.04	43.75
4	25	15	57.04 ± 1.13	56.17
5	15	15	55.14 ± 1.15	54.33
6	25	5	54.22 ± 1.12	53.93
7	27.07	10	56.02 ± 1.21	55.55
8	20	10	54.22 ± 1.22	54.55
9	20	10	54.37 ± 1.18	54.55
10	20	10	54.37 ± 1.22	54.55
11	20	10	55.42 ± 1.03	54.55
12	20	10	54.36 ± 1.23	54.55
13	20	17.07	55.12 ± 1.18	56.19