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Short communication

Utilization of various fruit juices as carbon source for production of bacterial cellulose by *Acetobacter xylinum* NBRC 13693

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ABSTRACT

The effective culture method to produce bacterial cellulose from fruit juices by *Acetobacter xylinum* NBRC 13693 was examined. Bacterial cellulose production from various fruit juices including orange, pineapple, apple, Japanese pear and grape were investigated, and the possibility of producing bacterial cellulose from those juices was suggested. The yields of the bacterial cellulose were increased by addition of the nitrogen source to the fruit juices. In addition, it was confirmed that the orange and Japanese pear juices were suitable medium for a bacterial cellulose production. The bacterial cellulose was produced from the various component of orange such as a peel and squeeze residue, and the bacterial cellulose of 0.65 g (dry weight) was produced from the orange of 100 g, and the solid residue from the orange was about 17.2 g.

1. Introduction

Ever since, its unique physical properties were discovered in mid-1980s (Iguchi & Yamanaka, 1997; Nishi et al., 1990; Yamanaka et al., 1989), interest has grown in bacterial cellulose which is traditionally used as *nata-de-coco*, an indigenous food in South-east Asia (Budhiono, Rosidi, Taher, & Iguchi, 1999). Bacterial cellulose produced by *Acetobacter xylinum* is pure cellulose aggregate which does not include any impurities, such as hemicellulose, pectin and lignin. It differs from plant-derived cellulose by having no intracellular cavity (Moon, Park, Chun, & Kim, 2006). Additionally, extracellularly synthesized bacterial cellulose microfibrils differ from plant cellulose with respect to their high crystallinity, high water absorption capacity and mechanical strength (Keshk & Sameshima, 2006; Klemm, Schumann, Udhardt, & Marsch, 2001). The superior physical properties of bacterial cellulose make it an interesting candidate for possible studies and uses in speaker diaphragms, tourniquet, or dietary fibers. In addition, due to its low toxicity and chemical stability, bacterial cellulose can be used in the manufacturing of artificial skin as well as paint used as a thickener for ink (Klemm et al., 2001; Shibazaki, Kuga, Onabe, & Usuda, 1993).

Mostly, fruits are sold and consumed as raw food, but most of the damaged and non-standard size ones are shelved, though some are processed to make jams, pastes and sauces. When the fruits can not be shipped because of their poor quality caused by bad weather and other natural disasters, it leads to low prices and fruits wastages. The majority of these wastes end up being discarded. However, such fruits have abundant sugars such as glucose and fructose that could be bio-converted into useful products. It has been reported that acetic acid bacterium such as *A. xylinum* can assimilate various sugars and produce bacterial cellulose (Adejoye, Adebayo-Toyo, Ogunjobi, Olaoye, & Fadahunsi, 2006; Bae & Shoda, 2004; Ishihara, Matsunaga, Hayashi, & Tisler, 2002; Verschuren, Cardona, Robert Nout, De Gooijer, & Van Den Heuvel, 2000). Although coconut juice is known to produce bacterial cellulose (Adejoye et al., 2006), no reports are available on the potential of other fruits to produce such a product.

In this work, the effective culture method to produce bacterial cellulose from various fruit juices including orange, pineapple, apple, Japanese pear and grape were investigated, and the possibility of the bacterial cellulose production from the fruit wastes was evaluated.

2. Methods

2.1. Microorganism and bacterial cellulose production from fruits juice

Acetobacter xylinum NBRC 13693 was used as the acetic acid bacteria for the production bacterial cellulose. In this study, *Citrus unchiu* Marc. (orange), *Malus domestica* Borkh (apple), *Ananas comosus* (L.) Merr. (pineapple), *Pyrus pyrifolia* var. *culta* (Japanese pear) and *Vitis* spp. (grape) fruits were purchased at a local market in Kanazawa city. These fruits were washed, crushed, squeezed and separated to the juices and the residues. The juices were filter-sterilized and stored at −15 °C for future use. The nitrogen sources in

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the Hestrin and Schramm media (Hestrin & Schramm, 1954) (2.0% peptone, 0.5% yeast extract and 0.12% citric acid) were added to the fruit juices, and the mixture was adjusted to pH 6 with disodium hydrogen phosphate buffer (medium I). The synthetic fruit medium that consisted of the nitrogen sources in HS medium and sugar reagent (glucose, fructose and sucrose) was also used for bacterial cellulose production (medium II). Sugar concentration in each experiment was decided based on the sugar content originally present in the fruit juices. The fruit juices, whose pH was adjusted to 6 was used as medium III. The bacterium was grown in the medium I–III at 30 °C.

2.2. Recovery of bacterial cellulose and analytical methods

After cultivation, the culture medium was separated into the supernatant and pellicles by centrifugation. For removal of microbial product contaminants, pellicles were successively washed with water, 2% (w/v) sodium hydroxide solution, 2 (v/v) acetic acid and water. Thereafter, the pellicles were dried in an oven at 80 °C to constant weight (Ishihara et al., 2002). The glucose, fructose and sucrose concentration in various fruit juices were analyses by HPLC (*Shimadzu* LC-9A, *Shimadzu* Co. Ltd., Kyoto, Japan) with *Ultron* PS-80P column (Shinwa Chemicals Industries Ltd., Kyoto, Japan) and RI detector. The Meicelase (*Meiji seika*, Tokyo, Japan) was used for the enzymatic saccharification of the bacterial cellulose and fruit residues (peel and squeeze residue). All reported data were the mean values and standard deviations corresponding to three triplicate samples.

3. Results and discussion

3.1. Bacterial cellulose production from fruit juices

Table 1 shows comparison of the composition of sugar in various fruit juices and pH of fruit juices. The amount of sugar in the grape juice was richest (10.3%) in these fruit juices while the amount of sugar in the Japanese pear was the least (7.3%). The detected sugars in fruit juices were glucose, fructose, sucrose, the composition of which differed according to the type fruit. Sucrose was abundantly present in the orange and pineapple juices, while the apple, Japanese pear, and grape juices recorded high values of fructose. The amount of glucose in the grape juice was the highest among these fruit juices. The pH values of these juices were quite low, apparently due to the presence of citric acid, apple acid, and tartaric acid. Therefore, it is suggested that in order for the juices to be used as growth media for bacteria, their pH should be adjusted accordingly. The bacterial cellulose production was confirmed from sucrose and fructose (data not shown). Therefore, appreciable amounts of the bacterial cellulose could be produced from fruit juices that contain a lot of sucrose and fructose.

Fig. 1 shows the amounts of bacterial cellulose production from various fruit juices in a culture of *A. xylinum* NBRC 19693 in HS medium with 2.0% peptone, 0.5% yeast extract, 0.12% citric acid (medium I) and fruit juice adjusted to pH 6 (medium II). After 14 days incubation, the production of the bacterial cellulose was confirmed from both media. However, more bacterial cellulose from *A. xylinum* NBRC 19693 cultured in medium I was produced. Moreover, it was confirmed that *A. xylinum* NBRC 19693 could not produce bacterial cellulose in the medium consisting of only sugars namely glucose, sucrose and fructose (data not shown). The nitrogen sources such as yeast extract and bactopepton was indispensable for the cell growth and bacterial cellulose production. It is therefore suggested that such nitrogen sources should be included the fruit juices media. The dry bacterial cellulose weight produced from the medium that consisted of orange juice was the maximum. Although the amount of sucrose in pineapple and orange juices is the same (Table 1), the bacterial cellulose was abundantly produced from the orange juice medium (5.9 g/l) than the pineapple medium (4.1 g/l). It is thought that this was caused by ingredients other than the sugar in the fruit juices, which have some influences on the production of the bacterial cellulose, or substrate inhibition.

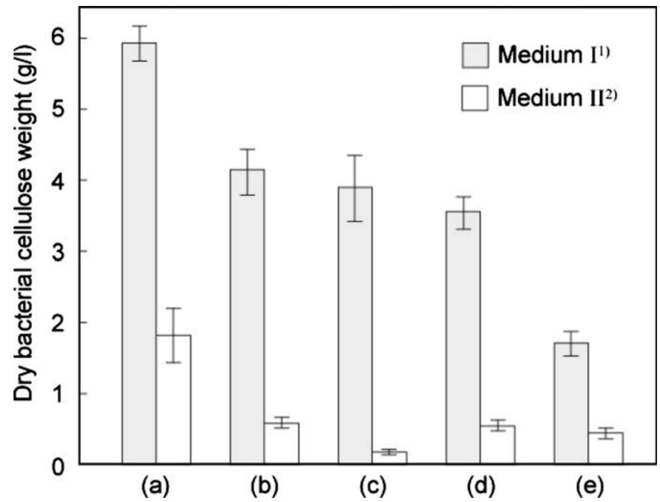

Fig. 1. Bacterial cellulose product from various fruit juices on Medium I and II. (a) Orange (b) Pineapple (c) Apple (d) Japanese pear (e) Grape. (1) Fruit juice adjusted pH 6 + nitrogen source in HS medium. (2) Fruit juice adjusted pH 6.

Table 2 shows comparison of yield of bacterial cellulose yield to sugars (glucose + fructose + sucrose) in various media. The medium I, II and III were used for bacterial cellulose production. Medium I consisted of fruit juice adjusted pH 6 and nitrogen source in HS medium. Medium II consisted of only fruit juice adjusted pH 6. Medium III consisted of sugar reagents and nitrogen source in HS medium. When the yield of the bacterial cellulose produced with medium I and medium II was compared, the yields of the bacterial cellulose with medium II were very low and the yields with medium I was higher in all the fruits. Yield of the bacterial cellulose with medium I (apple) was 19.5 times more than that with medium II (apple). The addition of the nitrogen source to the apple juice was very effective. Yields of

Table 1
Comparison of the composition of sugar in various fruit juices and pH of fruit juices.

Fruit	Comparison of sugar				pH (–)
	Glucose (%)	Fructose (%)	Sucrose (%)	Total (%)	
Orange	1.3	1.2	4.8	7.3	3.9
Pineapple	2.0	2.0	4.9	8.9	3.5
Apple	1.4	4.7	2.4	8.5	3.6
Japanese pear	1.9	4.0	0.3	6.2	4.1
Grape	4.2	5.9	0.2	10.3	4.1

Table 2
Comparison of yield of bacterial cellulose to sugars in various medium.

Fruit	Yield to sugars (%)		
	Medium I[a]	Medium II[b]	Medium III[c]
Orange	6.9 ± 0.2	2.1 ± 0.2	4.0 ± 0.2
Pineapple	3.9 ± 0.3	0.6 ± 0.1	5.9 ± 0.3
Apple	3.9 ± 0.2	0.2 ± 0.1	6.1 ± 0.2
Japanese pear	4.8 ± 0.3	0.6 ± 0.1	3.1 ± 0.3
Grape	1.4 ± 0.2	0.3 ± 0.1	8.8 ± 0.4

[a] Fruit juice adjusted pH 6 + nitrogen source in HS medium.
[b] Fruit juice adjusted pH 6.
[c] Sugar reagents + nitrogen source in HS medium.

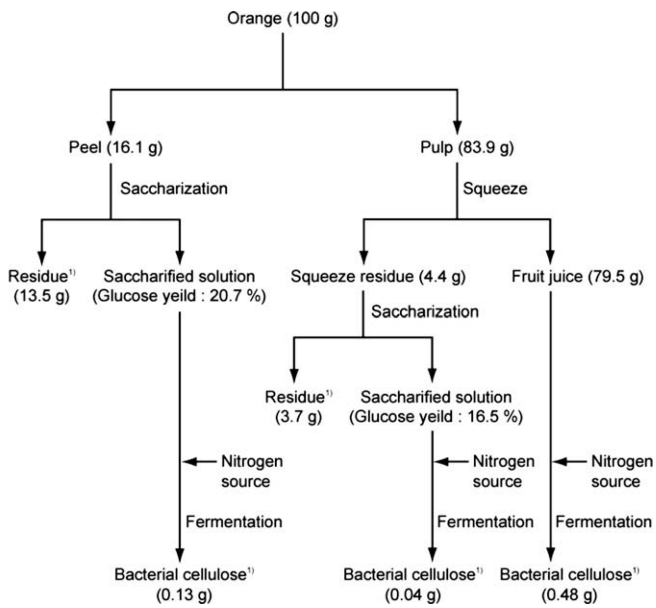

Fig. 2. Bacterial cellulose production from various orange contents. (1) Dry weight of residue and bacterial cellulose.

the bacterial cellulose produced with the medium II that used pear, pineapple, grape, and orange were 8, 6.5, 4.6 and 3.3 times more than those with medium I. Therefore, the addition of the nitrogen source to the fruit juices was effective in the bacterial cellulose production. The juice of medium I that obtained highest yield was orange juice, but the juice of medium with the lowest increase in yield by the addition of the nitrogen source was orange juice. It is thought that this is because orange juice has the nitrogen source such as protein and amino acid that enhance cell growth. Compared with the yield of the bacterial cellulose with medium III, the yields of the bacterial cellulose with medium I consisting of orange and Japanese pear increased, but the yields of the bacterial cellulose with medium I consisting of other fruits decreased. Therefore, it is thought that the orange juice and Japanese pear juice could consist of promoting substance for production of the bacterial cellulose and the pineapple juice, apple juice and grape juice could consist of inhibitory substance for production of the bacterial cellulose. From the above-mentioned observations, it is thought that the orange juice was the most suitable juice for the bacterial cellulose production.

Fig. 2 shows the flow chart of bacterial cellulose production process from various component of orange. The orange was divided by pulp and peel, and the amount of pulp and peel provided from 100 g of orange were 83.9 g and 16.1 g, respectively. The pulp was squeezed, and then divided into fruit juice and squeeze residue. The medium consisting of fruit juice adjusted pH 6 and nitrogen source in HS medium was prepared, and the bacterial cellulose of 0.48 g (dry weight) was produced from the medium. The squeeze residue and peel were saccharized by Meicelase, the glucose yield were 16.5% and 20.7%, respectively. The media consisting of the saccharized solutions adjusted pH 6 and nitrogen source in HS were prepared. And the amount of bacterial cellulose (dry weight) from the media using the squeeze residue and peel were 0.04 and 0.13 g, respectively. It was confirmed that an inhibitor for the bacterial cellulose production and the cell growth and were not included in saccharized solution. Thus, the bacterial cellulose of 0.65 g (dry weight) was produced from the orange of 100 g, and the solid residue from the orange was about 17.2 g. From the above-mentioned, the process of the effective utilization of the surplus produced and dumped orange was suggested.

4. Conclusions

The culture methods to efficiently produce bacterial cellulose from orange, pineapple, apple, Japanese pear and grape juices using *A. xylinum* NBRC 13693 were examined. The following findings were obtained:

(1) The possibility of producing bacterial cellulose from juices of fruit wastes such as orange, pineapple, apple, Japanese pear and grape was suggested.
(2) The yields of the bacterial cellulose were increased by addition of the nitrogen source to the fruit juices.
(3) It was suggested that for enhanced production of bacterial cellulose, the promoting substances could be included in the orange and Japanese pear juices.
(4) The orange juice was suitable medium for a bacterial cellulose, and the bacterial cellulose was produced from the various component of orange such as a peel and squeeze residue.

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