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EFFECT OF GLUCOSE SUPPLEMENTATION ON CITRIC ACID PRODUCTION BY ASPERGILLUS NIGER FROM SOME AGRO-INDUSTRIAL WASTES USING SOLID-STATE FERMENTATION

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ABSTRACT

The effect of different glucose concentration on citric acid production from wheat bran, rice bran and orange pulp by Aspergillus niger under condition of solid-state fermentation was studied. The results obtained show that supplementing the agricultural wastes with 5% glucose produced the highest amount of citric acid. The values obtained are 36.49g/kg, 35.58g/kg and 46.53g/kg from wheat bran, rice bran and orange pulp respectively. The values obtained from 3% glucose supplementation of the wastes are 37.27g/kg, 33.54g/kg and 41.93g/kg from wheat bran, rice bran and orange pulp respectively, the least yields of citric acid were from the agricultural wastes without glucose, yields of 32.66g/kg, 28.82g/kg and 33.62g/kg were obtained from wheat bran, rice bran and orange pulp respectively.

Key Words: Citric acid, glucose supplementation, Aspergillus niger, wheat bran, rice bran, orange pulp, solid-state fermentation.

INTRODUCTION

Citric acid (2-hydroxy-propane-1, 2, 3-tricarboxylic acid) derives its name from the Latin word citrus. It was first isolated from lemon juice in 1784 by Carl Scheele, a Swedish Chemist (Wikipedia, 2006). It has also been synthesized from glycerol and dichloroacetate and from several other synthetic routes using different starting materials, but chemical methods have proved uncompetitive with fermentation mainly because the starting materials are worth more than the final product (Papagianni, 2007). Solid state fermentation also known as the 'Koji' process was first developed in Japan where abundant raw materials such as fruit wastes and rice bran are readily available. It is the simplest method for citric acid production and it has been an alternative method for using agro-industrial residues (Vandenberghe, *et al.*, 2000; Soccol *et al.*, 2005; Soccol *et al.*, 2006). Citric acid accumulation is strongly influenced by the type and concentration of carbon source. The presence of carbohydrates which are rapidly taken up by microorganisms has been found essential for a good production of citric acid. Among the easily metabolized carbohydrates, sucrose is the most favourable carbohydrate source followed by glucose, fructose, and galactose. Routinely, 14% to 18% sucrose is used to achieve high yields (Legisa and Gradisnik-Grapulin 1995; Soccol *et al.*, 2006).

MATERIALS AND METHODS

ORGANISM AND CULTURE MAINTENANCE

A cellulase producing *Aspergillus niger (A.niger)* isolate obtained from the Microbiology department, Ahmadu Bello University, Zaria, Kaduna state, was used for this work.

PREPARATION AND PROXIMATE ANALYSIS OF THE AGRICULTURAL WASTES.

The wheat bran used in this work was kindly supplied by Ideal Flour Mills, Kaduna. The rice bran was obtained from a small scale Rice Milling Industry in Pilgani, Langtang North Local Government Area of Plateau state. The orange pulp was obtained by expressing the juice between the palms of the hands. The pulp was removed, washed, sun-dried and ground using pestle and mortar. All the wastes were sieved to obtain fine particles. Proximate analysis of the wastes was carried-out at the Biochemistry Laboratory, Industrial Chemicals Research Unit of the National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna state. The parameters determined were percentage carbohydrate, protein, lipids, crude fibre, ash and moisture.

INOCULUM PREPARATION

The spores of the isolates were harvested from slant bottles of 4-6 days old cultures by washing with sterile distilled water containing 0.8% Tween 80(Poolyoxyethylene- sorbitanmonooleate) and enumerated using a haemocytometer (Lasure *et al.*, 2003).

FERMENTATION TECHNIQUE

All fermentations were carried-out in 500ml flasks. Twenty grams of each of the wastes were placed in three sets of three flasks. The first sets of three flasks were without sugar supplementation while the second and third sets were supplemented with 3% and 5% glucose respectively. The initial moisture was adjusted to 70% of the wastes with the nutritive solution. The nutritive solution contained 3.1g of NH_4NO_3/litre, 0.15g of KH_2PO_4/litre; 2.2g of $MgSO_4.7H_2O$/litre dissolved in 35ml of distilled water. Three percent (v/v) methanol was also added. The pH was adjusted to 5.5 using 1M HCl (Usani *et al.*, 1998 and Soccol *et al.*, 2005). Potassium ferrocyanide and refined vegetable oil were not added in this case. The inoculum used was 10^7 spores/g of waste (ten millilitre of the spores suspension). The length of fermentation was 120 hrs.

ANALYTICAL METHOD

Citric acid was extracted by adding 200ml of distilled water to each flask and heated to 60^0C. The content of each flask was stirred with a glass rod and filtered through Whatman filter paper. The filtrate from each flask was then used for the determination of citric acid and reducing sugars (Usani *et al.*, 1998 and Soccol *et al.*, 2005). Citric acid was determined daily titrimetricaly as described by the British Pharmacopea, 1973 & Al-Delaimy and El-Holi, 2003. Dry mycelia weight was determined as described by Al – Delaimy and El-Holi, (2003) and Haq *et al*, (2003). Residual sugar was determined from the filtrate obtained above using the 3, 5-Dinitrosalycilic acid (DNS) method (Miller, 1959).

RESULTS

PROXIMATE ANALYSIS

Table 1 depicts the proximate composition of the wastes. This analysis show that orange pulp have the largest quantity (68.50%) of the portions (carbohydrate and crude fibre) that can be fermented to citric acid while wheat bran contain 62.99% and rice bran contain 62.65%.

Table 1: Proximate analysis of wheat bran, rice bran and orange pulp

Wastes	% moisture content	% ash content	% fat content	% crude protein	% crude fibre	% carbohydrate
Wheat bran	9.87	18.30	3.80	5.38	27.72	34.93
Rice bran	14.44	4.83	1.30	16.44	9.33	53.66
Orange pulp	21.79	3.76	0.70	5.25	9.47	59.03

CITRIC ACID YIELDS

Table 2 show citric acid yields, residual sugar concentration and mycelia weight of *A. niger* from the three agric. wastes used.

Figure 1 depicts comparison of citric acid yield by *A. niger* isolate without glucose supplementation, with three percent glucose supplementation and with five percent glucose supplementation during solid-state fermentation of wheat bran, rice bran and orange pulp.

WHEAT BRAN

The highest citric acid yield (37.27g/kg) obtained was from the fermentation of wheat bran supplemented with 3% glucose, followed by a citric acid yield of 36.49g/kg from 5% percent glucose supplemented wheat bran and the least citric acid yield was 32.66g/kg from wheat bran without glucose supplementation. Residual sugar concentration of 13.44g/kg, 13.80g/kg and 14.80g/kg were obtained from fermentation of wheat bran without glucose, wheat bran with 3% glucose and wheat bran with 5% glucose respectively (Table 2). Mycelia weight of 24.76kg, 25.66g/kg and 26.22g/kg were obtained from fermentation of wheat bran without glucose, wheat bran with three percent glucose and wheat bran with five percent glucose respectively (Table 2).

RICE BRAN

The highest amount (35.58g/kg) of citric acid obtained was from rice bran supplemented with five percent glucose, followed by a yield of 33.54g/kg from three percent glucose supplemented rice bran and the least citric acid yield (28.82g/kg) was from rice bran without glucose supplementation. Residual sugar concentration after the fermentation of rice bran without glucose, with three percent glucose and with five percent glucose was 9.20g/kg, 14.10g/kg and 13.44g/kg respectively (Table 2). Mycelia weight obtained from the fermentation of rice bran without glucose, with three percent glucose and with five percent glucose was 23.94g/kg, 25.02g/kg and 26.00g/kg respectively (Table 2).

ORANGE PULP

The highest citric acid yield of 46.53g/kg was from orange pulp supplemented with five percent glucose, followed by a yield of 41.93g/kg from orange pulp supplemented with three percent glucose and the least yield (33.62g/kg) was from orange pulp without glucose. Residual sugar concentration obtained were 15.20g/kg, 15.90g/kg and 15.90g/kg from orange pulp without glucose, with three percent glucose and with five percent glucose respectively (Table 2). Mycelia weight obtained from the fermentation of orange pulp without glucose, with three percent glucose and with five percent glucose was 24.50g/kg, 25.88g/kg and 26.74g/kg respectively (Table 1).

DISCUSSION

Sugar supplementation was found to enhance citric acid yield from all the agricultural wastes studied. The highest citric acid yields were obtained from the wastes supplemented with 5% glucose and the lowest yields from the wastes without sugar supplementation. The only exception being 3% glucose supplemented wheat bran which gave higher yield than 5% glucose supplemented whaet bran (Figure 1). Kiel *et al.*, (1981) reported that no citric acid was produced by *A. niger* from solid-state fermentation of cotton waste without sugar supplementation while 347g/kg of citric acid was produced when supplemented with 14% sucrose. The highest citric acid yields obtained from wheat bran (37.27g/kg) and rice bran (35.58g/kg) were much lower than those obtained by Soccol *et al.,* (2006), who reported a yield of 85g/kg from wheat bran and 127g/kg from rice bran. However, the yield obtained from orange pulp (46.53g/kg) was higher than that reported by Soccol *et al.,* (2006) which was 46.00g/kg. The yield of citric acid obtained from solid – state fermentation of other agro-industrial wastes were also much higher than the yields obtained in this study. A yield of 347g/kg and 269g/kg were obtained from cassava bagasse (Soccol *et al.*, 2006 and Vandeberghe *et al.*, 2004 respectively). Hang and Woodams, (1998) reported a yield of 250g/kg from corncobs while Hang (1998) reported a yield of 883g/kg from grape pomace. Khare *et al.*, (1998) reported a lower yield of 29g/kg from carrot waste compared to the agricultural wastes studied.

Table 2: Citric acid yields, residual sugar concentration and mycelia weight of *A. niger* from the three agric. wastes with and without sugar supplementation

Agric. Wastes	Citric acid yield (g/kg)	Residual sugar (g/kg)	Mycelia weight (g/kg)
Wheat bran without glucose	32.66	13.40	24.76
Wheat bran with 3% glucose	37.27	13.80	26.22
Wheat bran with 5% glucose	36.49	14.50	26.22
Rice bran without glucose	28.82	9.20	23.94
Rice bran with 3% glucose	33.54	14.10	25.02
Rice bran with 5% glucose	35.58	13.44	26.00
Orange pulp without glucose	33.62	15.20	24.50
Orange pulp with 3% glucose	41.93	15.90	25.88
Orange pulp with 5% glucose	46.53	15.90	26.74

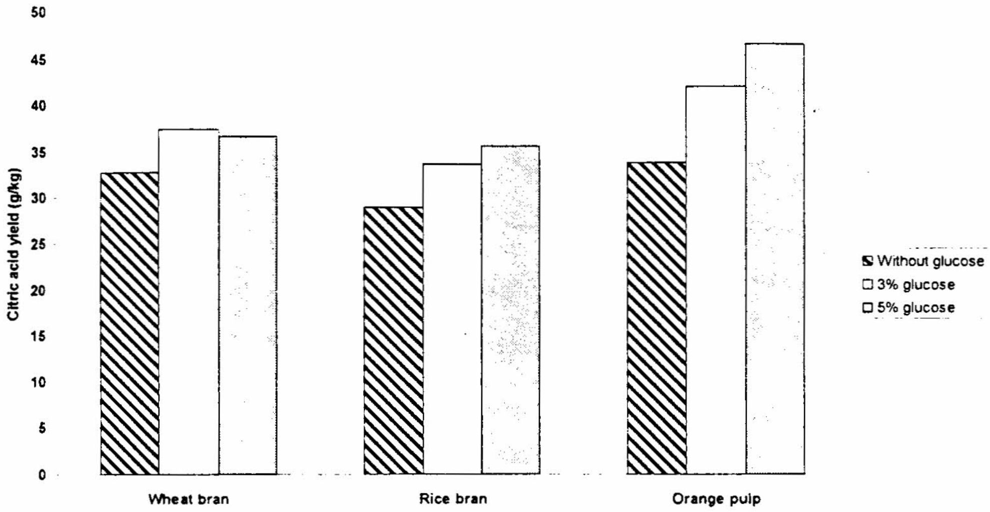

Figure 1: Comparison of citric yield by *A. niger* with 3% and 5% glucose and without glucose during solid-state fermentation of wheat bran, rice bran and orange pulp

CONCLUSION

Sugar supplementation was found to improve citric acid production by *Aspergillus niger* from whaet bran, rice bran and orange pulp under condition of solid-state fermentation. Increasing the level of sugar supplementation coupled with strain improvement studies may further increase the yield of citric acid from these wastes.

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