ABSTRACT

The effect of dried potato peels after acid hydrolysis (potato peel hydrolysate- PPH) supplementation on the mycelial growth and citric acid production of *Aspergillus niger* CA16 was examined in medium containing sucrose and Prescott salt. In shake flask studies, addition of PPH resulted into 6-fold increment in biomass and 5 fold increment in citric acid production at 13th day of fermentation. Supplementing PPH, therefore, can not only reduce fermentation media cost by replacing Prescott salt but also can be used along with Prescott salt for the enhanced production of citric acid from sucrose.

INTRODUCTION

Citric acid is the principal organic acid found in citrus fruits. It is a commercially valuable microbial metabolite which is produced mainly by submerged fermentation of starch- or sucrose-based media using the filamentous fungus *Aspergillus niger*. Global production of citric acid has reached 1.7 million tons per year. Global production is expected to increase at annual growth rate of 5% due to expanding utilization of citric acid in biomedicine, biopolymer production and various other applications. The market value of citric acid is expected to exceed $2 billion in 2009[1]. Since it is a commodity chemical, it is necessary to use inexpensive and readily available raw materials for industrial production processes. Starchy materials could be suitable substrates for citric acid production because they are cheap and renewable. Very recently, citric acid fermentation by gamma ray induced mutant strains of 14/20 and 79/20 of *A. niger* using two starchy substrates like pumpkin and cane molasses were investigated in this laboratory[2].

Potato (*Solanum tuberosum* of the Solanaceae family) is the world’s most widely grown tuber crop and the fourth largest food crop in terms of fresh produce after rice, wheat, and maize (corn). Nutritionally, potatoes are best known for their carbohydrate content with starch being the predominant form of carbohydrate. Potato peels are waste by-products of the potato-processing industry. They are a good source of vitamin C, vitamin B6, copper, potassium, manganese, and dietary fiber. They also contain a variety of phytonutrients which are a natural source of antioxidants that help to prevent cellular deterioration of the body. The phytonutrients found in potato skins as well as the flesh include polyphenols, carotenoids, flavonoids, and caffeic acid[3].
Shukla and Kar studied potato peel as a solid-state substrate for thermostable –amylase production by thermophilic Bacillus isolates. Mukherjee et al. used potato peel and I. cylindrica grass mixed in a ratio of 1:1 (w/w) for the maximum production of alkaline protease. Arotupin reported that potato peel supported the highest polygalacturonase production followed by ripe banana peel, then orange bagasse, ripe plantain peel, unripe plantain peel, soluble starch, unripe banana, and cassava peel. Mabrouk and El Ahwany studied the production of -mannanase by Bacillus amylolequifaciens 10A1 cultured on potato peels. They reported that potato peels at 14 g/l as carbon source and ammonium nitrate as a nitrogen source produced maximum enzyme activity (61.5 U/mg protein). Recently the antioxidant activity of potato peel extract has been studied in food systems. Singh et al. reported potato peel extract to have the potential to offer protection against acute liver injury in rats because of its antioxidant property. Singh and Rajini investigated the ability of potato peel extract to protect erythrocytes against oxidative damage, in vitro. However, there is no report on the supplementary effect of potato peel on the production of citric acid.

Potato peel in dried form can be hydrolyzed using HCl to get more accessible sugars. Potato hydrolysate thus obtained can enhance the growth and citric acid production by A. niger which when used with sucrose as supplement. With this hypothesis, experiments were carried out to produce potato peel hydrolysate and to examine the effect of supplementing this hydrolysate for the enhanced production of citric acid from sucrose by Aspergillus niger CA 16.

**MATERIALS AND METHODS**

**Preparation of potato peel powder was undertaken as follows**

The resulting powder was subjected to acid hydrolysis at different ratios. Care was taken to apply less volume of hydrochloric acid as possible in order to minimize the dilution of sugar present in the potato peel powder. The amount of dried matter (potato peel powder) therefore must be increased to the maximum. However, it was found that if the ratio of powder to acid is between 1:1 to 1:10– the powder did not swell properly. When the ratio was increased to 1:15-1:20 (e.g., 1 g of powder in 20 ml of 0.05N HCl) the powder can be dissolved but could not be filtered. Finally, a clear filtrate was obtained using 1:30 ration as shown in Figure 2:

- 10 g of potato powder was mixed with 300 mL of 0.05 N HCl
- The suspension was sterilized at 121°C, 15 lbs pressure for 15 minutes
- Filtered with thin cloth and the clear brown hydrolysate was kept at 4°C until further use
- Sugar concentration in the hydrolysate was estimated using anthrone method

![Flow chart for the preparation of potato peel hydrolysate (PPH)](image)
Aspergillus niger CA16 was maintained in Potato dextrose agar (PDA) slants. Sucrose concentration of 14%, pH 5.0 and incubation temperature of 30°C were used throughout the fermentation. The following parameters were selected to find out which one was better for citric acid fermentation: Sugar with Prescott salt \((\text{NH}_4\text{NO}_3 \ 2.23\text{ g/L; K}_2\text{HPO}_4 \ 1.00\text{ g/L and MgSO}_4 \ 7\text{H}_2\text{O} \ 0.23\text{ g/L})\); sugar without Prescott salt; sugar with PPH with Prescott salt and sugar with PPH without Prescott salt. Fermentation was carried out in 100 ml conical flasks containing 20 ml medium. Titration was done from the 7th day of incubation. Total titrable acid values were determined by freshly prepared 0.1 N NaOH. Citric acid and residual sugar were estimated between 3-13 days of culture broth by the Marrier and Boulet method and Anthrone sulphuric acid method, respectively.

RESULTS AND DISCUSSION

The sugar concentration in the potato peel powder was found to be in the range of 11-14% (w/w). In the present study, potato peel hydrolysate constituted 50% v/v of the medium. It was found that the presence of Prescott salt is necessary for mycelial development and the biomass increased two folds in the presence of Prescott salt. However, addition of potato peel hydrolysate without Prescott salt resulted into 8-fold increment in biomass compared to the culture with only sucrose in the medium. Finally, when both Prescott salt and PPH were added to the medium, the biomass increased to 6 folds compared to the culture with sucrose and Prescott salt. Therefore, it can said that supplementing PPH can be used to replace Prescott salt and thereby can reduce the fermentation media cost but also can be used along with Prescott salt to further enhance the growth of A. niger CA16.

However, instead of biomass, more attention must be paid to the formation of citric acid when attempting to improve citric acid production using conventional substrate e.g., sucrose. In this study, it was found that, the addition of Prescott salt is necessary for citric acid production and without Prescott salt citric acid production was hampered. When Prescott salt was replaced with PPH, the citric acid production was doubled at 7th day and remained constant even at 13th day of fermentation. In contrast, the addition of both Prescott salt and PPH resulted into 2.5 fold increment in citric acid production at 7th day. The citric acid production was gradually increased and 5 fold increment of citric acid production was observed at 13th day of fermentation.

CONCLUSION

The result signifies the use of potato peel hydrolysate on the enhanced production of citric acid by A. niger CA16. Optimization of the concentration of supplementation is now ongoing to further improve and implement the process in large scale.

REFERENCES