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Short Communication

Coconut Cake: A Novel Substrate for Citric Acid Production under Solid State Fermentation

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Use of sunflower seed cake and coconut cake for citric acid production under solid state fermentation with two strains of *Aspergillus niger* MTCC 281 and KLCN2 were investigated. The coconut cake was found to be a better substrate than sunflower seed cake and yielded maximum amount of citric acid with the strain KLCN2 after 96 h of fermentation. The sugar consumption efficiency was 76. 4 per cent.

Key words: Coconut cake, sunflower seed cake, citric acid, *Aspergillus niger*, solid state fermentation.

Citric acid, first discovered by Sheele in 1784 as a constituent of citrus fruits, is one of the major important organic acids produced by modern biotechnological means (1). It has multitude usage in food, beverages, pharmaceuticals, chemical flavour enhancement, preservative, plasticizer and synergistic sequestering agent, cosmetic industries and other miscellaneous industries (2-5). Citric acid is conventionally produced by surface or submerged fungal fermentation of sucrose or molasses (3,4,6). As non-conventional substrates, various agricultural wastes are also being used for citric acid production.

The sunflower seeds and coconuts are mainly used for extraction of oil. The huge quantity of oil cake generated still contains considerable amount of fermentable sugars and other nutrients that can serve as valuable raw material for a number of fermentative processes. Presently, this useful byproduct is used either as cattle feed or disposed without treatment which causes environmental pollution. Hence, attempts were made in this study to use the sunflower seed cake and coconut cake as substrates for citric acid production through solid state fermentation by strains of *Aspergillus niger*.

The samples collected from local oil mills were ground separately in a waring blender and analyzed for moisture, total carbohydrates, fats and pH as per the methods described by Ranganna (7). Trace metal elements (Cu^{2+}, Fe^{2+}, and Mn^{2+}) were estimated by an atomic absorption spectrophotometer as per Arnold et al (8).

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Aspergillus niger MTCC 281 (procured from IMTECH, Chandigarh, India) and KLCN2 (isolated from spoiled orange) were used for the preparation of spore suspensions. One mL spore suspension of each strain containing $1x10^7$ cfu mL^{-1} was inoculated into the flasks containing 40 g of each autoclaved substrate (adjusted to initial moisture content of 55%), and incubated at 30°C for 7 d. Fermented mouldy substrates were aseptically withdrawn at every 24 h intervals and extracted with distilled water (9) and analyzed for citric acid (10) and residual sugars (11).

Maximum yield of citric acid on both the substrates was obtained after 72 h in case of *A. niger* MTCC 281 and 96 h in case of *A. niger* KLCN2 (Fig 1). The yield of citric acid decreased sharply as fermentation period increased irrespective of the substrates and strains.

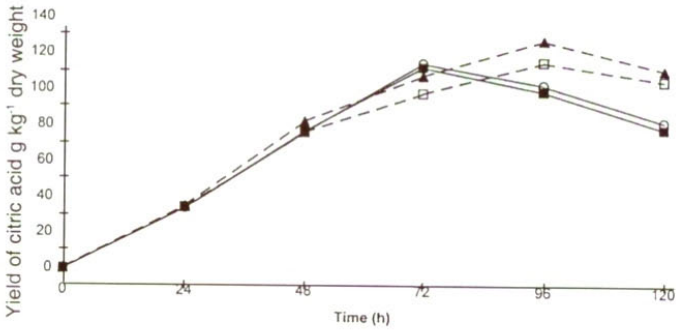

Fig. 1. Yield of citric acid by strains of *Aspergillus niger* on coconut and sunflower seed cake. (o) *A. niger* MTCC281 on coconut cake, (■) *A. niger* MTCC281 on sunflower seed cake, (▲) *A. niger* KLCN2 on coconut cake and (□) *A. niger* KLCN2 on sunflower seed cake.

Citric acid yield by *A. niger* MTCC 281 was 1.3 times more with coconut cake as substrate (108.9 g kg^{-1} with 57.8% sugar consumption) than with sunflower seed cake (84.5 g kg^{-1} with 74.6% sugar consumption) after 72 h. Similarly, the yield of citric acid by *A. niger* KLCN2 was 1.1 times more (128.6 g kg^{-1} with 76.4% sugar consumption) after 96 h on coconut cake than on sunflower seed cake. This proved coconut cake to be a better substrate than sunflower seed cake for production of citric acid under solid state fermentation. This was because of the organically rich nature of the coconut cake with higher reducing sugars and fat content (Table 1).

Table 1. Biochemical composition of sunflower seed cake and coconut cake.

Composition	Sunflower seed cake	Coconut cake
pH	5.0	5.8
Moisture	3.7	10.8
Total carbohydrates	28.9	45.3
Reducing sugars	16.7	20.0
Fat	7.4	10.0
Fe^{2+}	223.8	130.1
Mn^{2+}	13.2	24.2
Cu^{2+}	14.1	17.2

All values in per cent except pH. Trace elements μg $(100g)^{-1}$

Generally citric acid yield is considerably enhanced when the substrates are amended with oils and fats (12-15). The unsaturated fatty acids act as alternative hydrogen acceptors during fermentation, allowing the mould to metabolize the substrate actively for longer period leading to improved yield of citric acid (12). The higher amount of fatty acids (Table 1) in the coconut cake might have lead to higher citric acid yields than the sunflower seed cake. The coconut cake also contains lower amount of Fe^{2+} and higher amount of Cu^{2+} and Mn^{2+} than the sunflower seed cake. Higher levels of Fe^{2+} in the substrates are known to reduce citric acid production, enhancing aconitase activity (3,16). It is also observed that Mn^{2+} at low concentration favours citric acid production (17) and at higher levels inhibits the process. On the other hand, Cu^{2+} promotes citric acid yield. The adverse effect, if any, due to high Fe^{2+} and Mn^{2+} appears to be effectively balanced by the Cu^{2+} content in the coconut cake, as opined by Mattey (18). Further, the solid state fermentation process itself has the inherent capacity to overcome whatever adverse effects metal ions may have on citric acid production and facilitated by the undefined complex organic composition of the substrates (19).

Initial pH level of the substrate is also one of the crucial factors for successful citric acid fermentation. The initial pH of the substrate in turn depends upon the carbon sources used during fermentation. When glucose and sucrose or other relatively pure substrates are used for fermentation, the substrate pH needs to be adjusted to 2-3. When a crude substrate is used high initial pH is required (19), as the non-ionic nutrients may prevent germination of the spores (20). As such, the coconut cake having high initial pH serves as a better substrate for citric acid production than the sunflower seed cake.

On the whole, the coconut cake emerged as a better substrate than the sunflower seed cake due to its high organic content coupled with the high levels of Cu^{2+} and low pH.

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