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COPRODUCTION OF PROTEASE, AMYLASE AND LIPASE FROM FRUIT AND VEGETABLE WASTE USING *ASPERGILLUS NIGER* FCC-ASQ-06

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ABSTRACT

In this study, multi-enzyme (amylase, protease and lipase) production from *Aspergillus niger* FCC-ASQ-06 was evaluated. Different fruit (banana peel, orange peel, lemon) and vegetable (pea peel) waste were tested as carbon sources. Maximal amylase and lipase production were 4.43 U/g DM and 6.44 U/g DM when banana peel was used as carbon source whereas highest protease production of 3.66 U/ g DM was noted when lemon peel was used as carbon source. Production rate further increased with fermentation time and maximum enzyme activities were noted after 144 h fermentation time when banana peel was used as carbon source. In the next step, effect of nitrogen sources on different enzyme production were checked. Maximal protease, amylase and lipase production were 12.4 U/ g DM, 13.4 U/g DM and 15.6 U/g DM, respectively when peptone and yeast extract were used as nitrogen source. Maximal enzyme production was noted from pH 6-8. Fermentation medium cost is always technical barrier in industrial scale processes for bioethanol, organic acids and enzyme production (lipase, amylase and protease). These enzymes have applications in various industries including food industry, saccharification of starchy materials, detergent and textile industry. This is why, there is need to find cost effective medium for multienzyme production. Utilization of fruit and vegetable waste in biotechnology industry will certainly reduce commodity production cost and solve pollution problems.

1. INTRODUCTION

The most significant industrial enzymes are proteases due to their wide range of applications in food, cosmetics, detergent, pharmaceutical, leather and synthetic bio-technology. Protease market value has reached to more than 65% of total industrial enzyme market [1-4].

Lipases are employed in food additive, detergent, oleo chemical, bio-diesel, biodegradable polymers and textile industries [5]. Amylases find their applications in food, saccharification of starchy materials, pharmaceuticals, detergent and textile industries [6-7]. These enzymes could be produced from micro- and macro-organisms [8]. Microbial enzymes are preferred over animal and plants source enzymes due to their stability, higher production rate, biochemical versatility, and availability of huge number of microbial strains. Enzymes are produced under solid-state and submerged fermentation. Solid

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state fermentation is advantageous over submerged fermentation due to no substrate processing needed. Plant based biomass could directly be employed in solid state fermentation this will save energy and cost [9]. Many processes have been developed for the utilization of agro-industrial residues as raw material to produce bulk chemicals bio-ethanol, organic acids and enzymes. Utilization of plant based biomass as substrate will save fermentation medium cost and reduce pollution that could occur due to accumulation of solid waste [10].

Therefore, in present study multi-enzyme production was optimized using fruit and vegetable waste to reduce the fermentation operation and capital cost. Fermentation medium cost is always technical barrier in industrial scale processes for bio-ethanol, organic acids and enzyme production (lipase, amylase and protease). These enzymes have applications in various industries mentioned above. This is why, there is need to find cost effective medium for multi-enzyme production. Utilization of fruit and vegetable waste in biotechnology industry will certainly reduce commodity production cost and solve pollution problems.

2. MATERIALS AND METHODS

Raw material

The waste material lemon peel, banana peel, orange peel and pea peel were collected from household waste from Hyderabad, Sindh, Pakistan.

Solid state fermentation

The fermentation medium consists of 10 g/L of banana peel, orange peel, lemon peel or pea peel in separate flasks, peptone 3 g/L, potassium dihydrogen phosphate (KH_2PO_4) 1.2 g/L, $MgSO_4$ 1.2 g/L and $(NH_4)_2SO_4$ 0.6 g/L. The initial pH was adjusted to 6.0 with 0.1N NaOH or 0.1N HCL.

Effect of time

Fermentation medium (50 mL) containing banana peel, lemon peel, orange peel or pea peel (10 gL^{-1}) powder as a carbon source was taken in separate flasks and KH_2PO_4 (0.6 gL^{-1}), peptone (5 gL^{-1}), $MgSO_4$ (0.6 gL^{-1}), $(NH_4)_2SO_4$ (0.3 gL^{-1}) in 250 mL conical flask.

The cotton plugged flask was autoclaved for 20 mint at 121 °C. The sterilized medium was cooled at room temperature and each flask was inoculated from 2 mL of *Aspergillus niger* suspension. Fermentation was allowed at 30 °C for 72 hours. In the next step, time of incubation was evaluated using different peels powder in separate flasks in 50 mL fermentation medium containing peel powder as carbon source and KH_2PO_4 1.2 g/L, peptone 5 g/L, $MgSO_4$ 1.2 g/L, $(NH_4)_2 SO_4$ 0.6 g/L. Fermentation was allowed at 30 °C for 10 days, samples were collected after 24 h interval.

Effect of nitrogen source

Effect of different organic and inorganic nitrogen compounds on multi-enzyme production was evaluated using different peel powder. In 50 mL fermentation medium containing 10 g/L of peel powder as carbon source and KH_2PO_4 1.2 g/L, different nitrogen compounds 5 g/L, $MgSO_4$ 1.2 g/L, $(NH_4)_2 SO_4$ 0.6 g/L. Fermentation was allowed at 30 °C for 6 days, samples was collected after 144 h.

pH Effect

Effect of initial pH on multi-enzyme production was evaluated using banana peel, orange peel, lemon peel and pea peel as carbon source in separate flasks. In 50 mL fermentation medium containing 10 g/L peel powder as carbon source and KH_2PO_4 1.2 g/L, yeast extract 5 g/L, $MgSO_4$ 1.2 g/L, $(NH_4)_2 SO_4$ 0.6 g/L. Fermentation was allowed at 30 °C for 6 days, samples were collected after 144 h.

Enzyme activities

Amylase activity was calculated by adopting the reducing sugars procedure described by elsewhere [11]. Activity of lipase was also calculated according to modified method reported elsewhere [12]. Protease activity in the culture supernatant was assayed according to method adapted from Penner et al., 1967 [13].

3. RESULTS AND DISCUSSION

Cost of fermentation medium is one of the financial issues in industrial scale processes for production of commodity products like, bio-ethanol, organic acids, microbial enzyme production and many more. Therefore, researchers in various laboratories are trying to develop cost effective fermentation processes by using low cost substrate (agro-industrial waste). This is why, in present study, solid state fermentation process was evaluated and developed for multi-enzyme production from fruit (banana peel, orange peel and lemon peel) and vegetable (pea peel) Waste. This strategy has provided practical approach towards production of multi-enzyme from same fermentation medium using low cost material that will reduce enzyme production cost and eliminate the possible pollution problem that could occur due to discharge of food waste.

Figure 1 shows the results of solid substrate fermentation using fruit and vegetable waste as carbon source. Four different waste materials lemon peel, banana peel, pea peel and orange peel were evaluated as energy source for amylase, protease and lipases production from *Aspergillus niger*. Amylase activities were 4.43, 3.56, 3.07 and 4.09 U/g DM with banana, lemon, orange and pea peel, respectively. Protease activities were 2.045, 3.66, 3.34 and 2.50 U/g DM with banana, lemon, orange and pea peel, respectively. Lipases activities were 6.44, 5.15, 0.22 and 4.09 U/g DM with banana, lemon, orange and pea peel, respectively. Production rate of each enzyme varied with carbon source and maximal production of amylase and lipase were noted when banana peel was used as carbon source whereas protease activity was highest in lemon peel medium. Literature suggests that enzyme production varies with carbon source due to nature of microorganisms and micronutrients present in each carbon source also vary. These micronutrients have certain effect on gene regulation for production of enzymes and microbial growth. Several studies have reported optimization of enzyme production in solid state fermentation as well as in submerged fermentation condition. Each microorganism behave differently in each fermentation condition due to composition of each medium also vary. Imrana et al., 2017 [14] have produced protease from *Bacillus licheniformis* BCC-02-50 using molasses as carbon source and yeast extract as nitrogen source after 36 h in submerged fermentation condition. Qureshi et al., 2016 have obtained maximal amylase and protease concentration when wheat straw was supplemented in solid state fermentation.

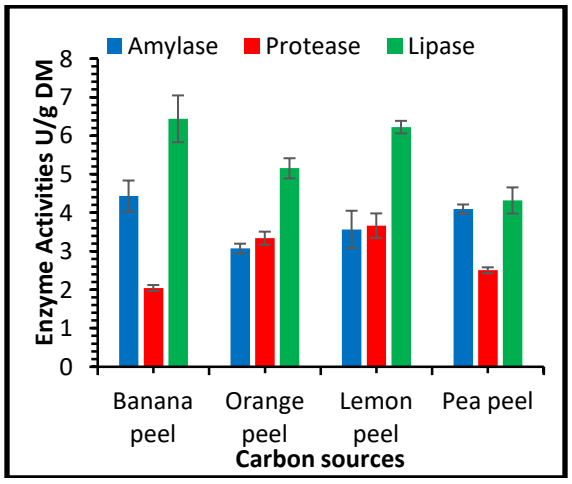

Figure 1. Impact of various plant materials on enzyme's production isolated from different carbon sources.

Figure 2.1. showed that enzyme production using banana peel as carbon source enhanced with the passage of time and highest concentration of lipase was obtained at 120 h after that lipase concentration gradually decreased, whereas, highest concentration of amylase was obtained at 144 h after that activity decreased. Maximal protease concentration was obtained at 144 h. Effect of orange peel as carbon source on enzyme concentration was determined and results are depicted in figure 2.2. Enzymes concentration increased with fermentation time maximal lipase concentration was achieved after 120 h, highest protease and amylase concentrations were seen after 144 h. Figure 2.3 shows lipase, protease and amylase production using lemon peel as carbon source. In this case maximal enzyme activities were noted after 144 h for all tested enzymes. The time dependent production of enzymes using pea peel as carbon source is shown in figure 2.4. Similar results were observed for lipase, amylase and protease production after 144 h. Results clearly suggests that highest enzyme production was related to microbial growth. Growth dependent lipase production was observed by [15]. In our previous study [16], solid state fermentation of wheat straw from Bacillus BBXS-2 produced maximum amylase and protease concentration after 120 h.

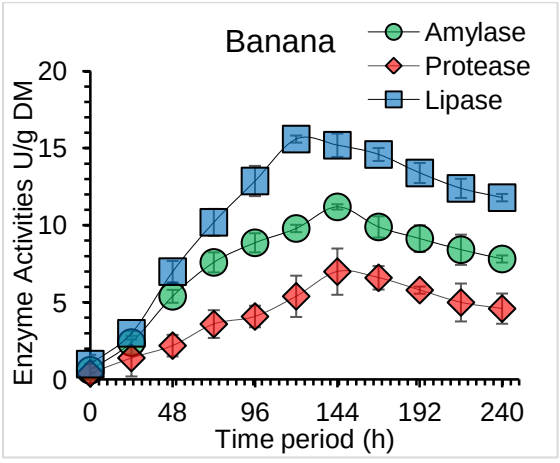

Figure 2-1. Time dependent production of enzymes using banana peel as carbon source

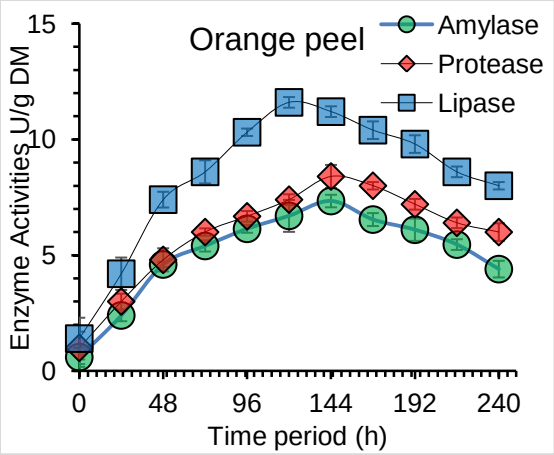

Figure 2-2. Time dependent production of enzymes using orange peel as carbon source

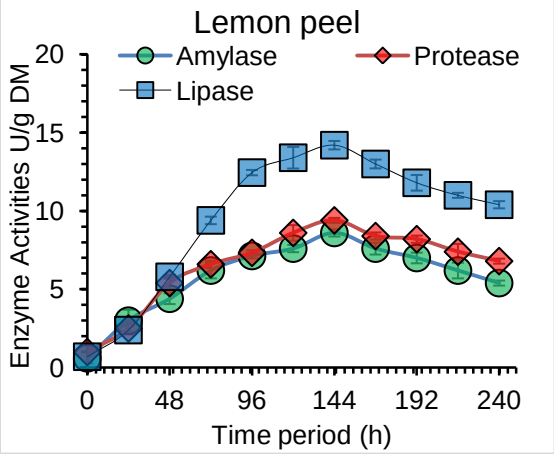

Figure 2-3. Time dependent production of enzymes using lemon peel as carbon source

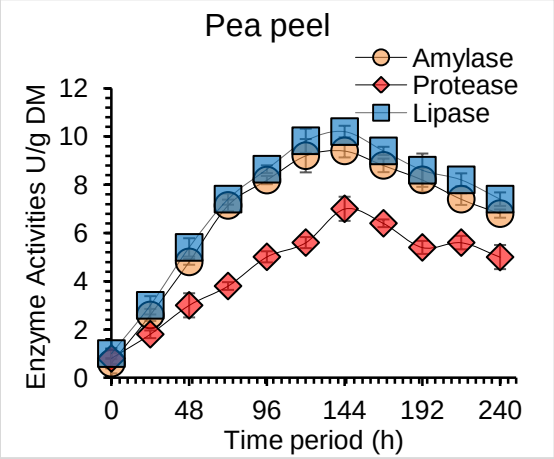

Figure 2-4. Time dependent production of enzymes using pea peel as carbon source

Effect of different nitrogen sources (peptone, yeast extract, tryptone, sodium nitrate, ammonium chloride and urea) on the production of different enzymes (amylase, protease and lipase) from *Aspergillus niger* was evaluated (Figure 3). Different fruit (banana-, lemon- and orange-peel) and vegetable (pea peel) wastes were used as carbon sources. Figure 3.1 shows that the maximum lipase production of 15.4 U/g DM was noted when nitrogen source was peptone. The highest amylase activity of 13. 4 U/g DM was obtained from yeast extract medium and maximal protease production (10 U/g DM) was observed from tryptone medium.

Figure 3.2 show that lipase production was 13.6 U/ g DM when tryptone was supplemented in the fermentation medium. Whereas, maximal protease concentration of 12.4 U/g DM and amylase production of 10.4 U/g DM were obtained when nitrogen source was yeast extract. Figure 3.3 shows the impact of organic and inorganic sources of nitrogen using lemon peel as carbon source on different enzyme production. All enzyme activities were highest in case of organic nitrogen sources, highest lipase production was obtained from peptone, highest amylase production was obtained from tryptone whereas protease concentration reached 11.4 U/g DM in fermentation medium supplemented with yeast extract. The impact of variuos nitrogen sources on lipase, amylase and protease production from *Aspergillus niger* is shown in figure 3.4, pea peel was used as a carbon source. Highest amylase and protease concentration were obtained from yeast extract and lipase activity from tryptone fermentation medium. Peptone was found best nitrogen source for amylase production from Bacillus sp BCC 021-50 under submerged fermentation conditions [17]. Imrana et al., 2017 have reported amylase production from *Bacillus clausii* MCC 233-50 under submerged fermentation conditions and urea was found best nitrogen source for amylase production [18].

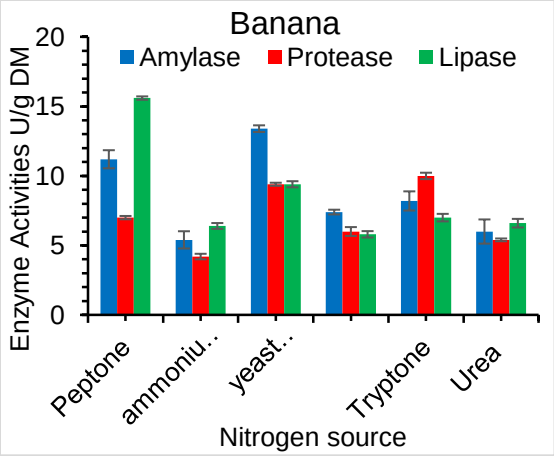

Figure 3-1. Impact of nitrogen sources on enzymes using banana peel as carbon source

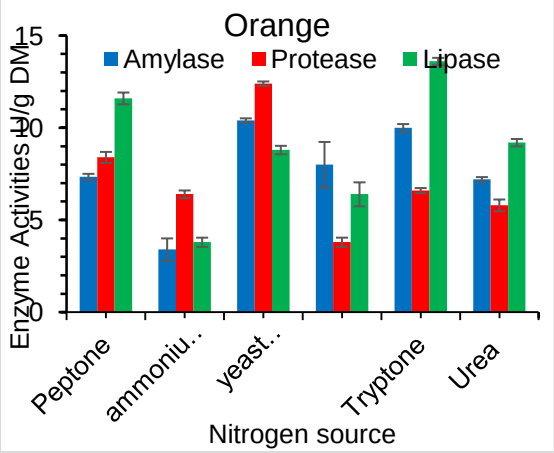

Figure 3-2. Impact of nitrogen sources on of enzymes using orange peel as carbon source

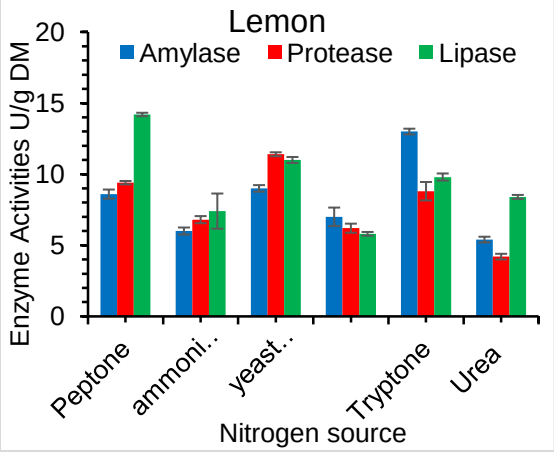

Figure 3-3. Impact of nitrogen sources on enzymes using lemon peel as carbon source.

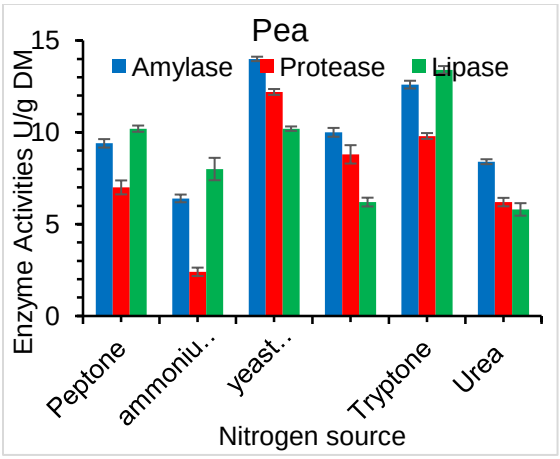

Figure 3-4. Impact of nitrogen sources on enzymes using pea peel as carbon Source

Figure 4.1-4.4 shows the impact of pH on enzyme production under different fermentation conditions using different carbon sources. In different conditions, highest enzyme concentrations (protease, lipase and amylase) were noted from pH 6-8. Microorganisms produce different enzyme at different fermentation conditions probably due to different genes are involved in synthesis of each enzyme. Enzymes are protein in nature and synthesized using microbial metabolic machinery. Beg et al., 2003 [19] have reported proteases production under alkaline conditions. [16] have reported amylase and protease coproduction under open fermentation conditions using wheat straw as substrate, maximum enzyme production was noted at pH 8.5. [20] have reported amylase production from Bacillus sp. BCC 01-50 under submerged fermentation condition and maximum concentration was obtained at pH 8.0 when molasses mineral medium was used.

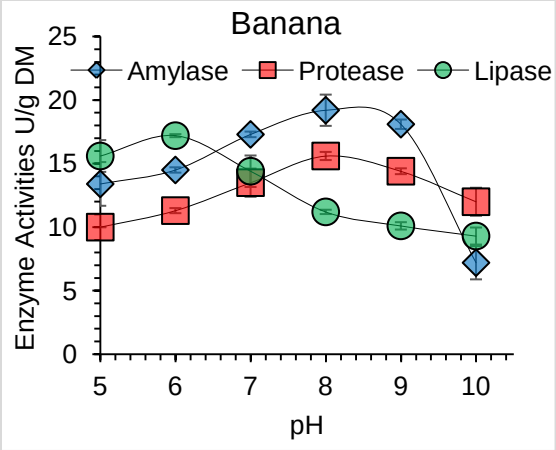

Figure 4-1. Impact of pH on enzymes production from banana peel

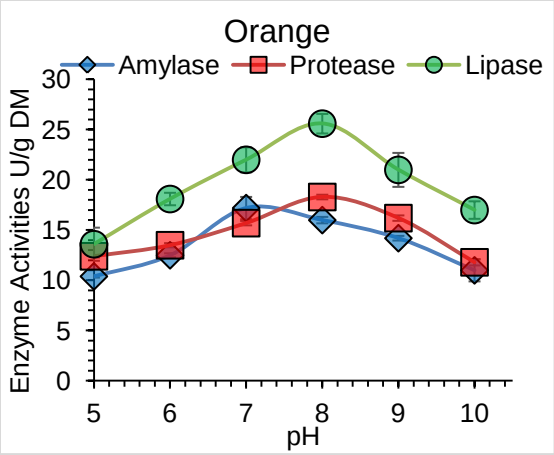

Figure 4-2. Impact of pH on enzymes production from orange peel

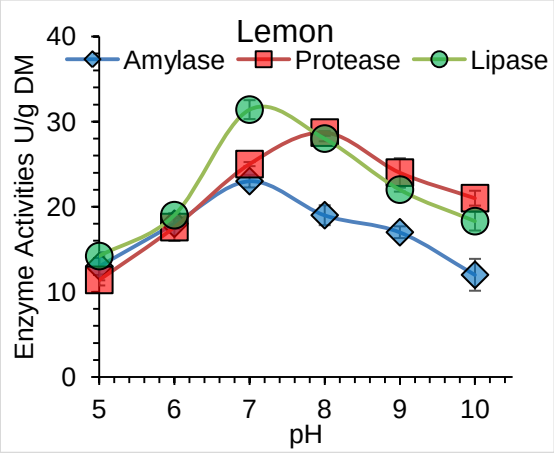

Figure 4-3. Impact of pH on enzymes production from lemon peel

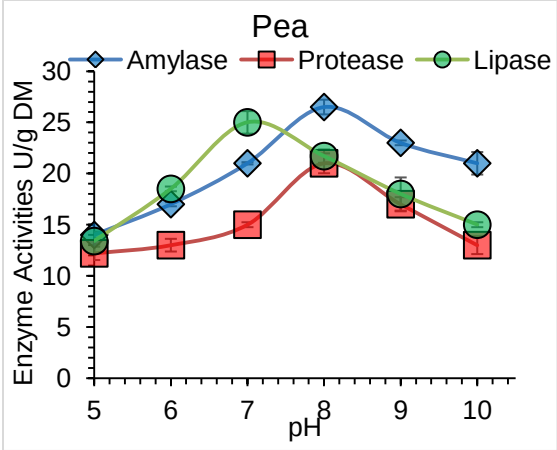

Figure 4-4. Impact of pH on enzymes production from pea peel

4. CONCLUSION

In present study, multi-enzyme (amylase, protease and lipase) production from *Aspergillus niger* was evaluated. Different fruit (banana peel, orange peel, lemon peel) and vegetable (pea peel) waste were tested as carbon sources. Maximal amylase and lipase production were 4.43 U/g DM and 6.44 U/g DM when banana peel was used as carbon source whereas highest protease production of 3.66 U/ g DM was noted when lemon peel was used as carbon source. Production rate further increased with fermentation time and maximum enzyme activities were noted after 144 h fermentation time when banana peel was used as carbon source. In the next step, effect of nitrogen sources on different enzyme production were checked. Maximal protease, amylase and lipase production were 12.4 U/ g DM, 13.4 U/g DM and 15.6 U/g DM, respectively when peptone and yeast extract were used as nitrogen source. Maximal enzyme production were noted from pH 6-8. Fermentation medium cost is always technical barrier in industrial scale processes for bioethanol, organic acids and enzyme production (lipase, amylase and protease). These enzymes have applications in various industries including food industry, saccharification of starchy materials, detergent and textile industry. This is why, there is need to find cost effective medium for multienzyme production. Utilization of fruit and vegetable waste in biotechnology industry will certainly reduce commodity production cost and solve pollution problems.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding publication of this article.

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