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# Biological production of citric acid in submerged culture of *Aspergillus niger* using cassava pulp wastes

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### Abstract

Utilization of cassava pulp wastes for citric acid production was investigated using *Aspergillus niger* in a submerged culture. A series of experiments were designed on various fermentation parameters to establish the optimal conditions for citric acid production from cassava pulp. This study revealed that production parameters such as cassava pulp concentration, initial pH, incubation temperature, agitation, and nitrogen source and fermentation period had effect on the amount of citric acid produced from cassava pulp. Citric acid concentration increased as the concentration of cassava pulp increases up to 20% with maximum citric acid concentration of  $14.9 \pm 0.413$  g/l after 120 hours of fermentation. pH 5.5 was the optimum with maximum citric acid concentration of  $16.8 \pm 0.23$  g/l after 120 hours of fermentation. Incubation temperature at 30° C was the optimum, with citric acid concentration of  $19.15 \pm 0.43$  g/l. Increased in agitation speed from 100 to 225 rpm gave the maximum citric acid concentration of  $25.2 \pm 0.32$  g/l after 120 hours of fermentation. Soybean meal supplementation was the best maximum citric acid concentration of  $28.2 \pm 0.51$  g/l. Evaluating the effect of different concentration of soybean meal shows that 0.3 % supplementation was the optimum with maximum concentration of  $31.2 \pm 0.35$ g/l from cassava pulp after 120 hours of fermentation. The result suggested that citric acid can be accumulated using cassava pulp by *Aspergillus niger* in submerged culture during fermentation. Cassava pulp if well harnessed can be used for large scale citric acid production.

## 1. Introduction

Citric acid which is also known as 2-hydroxy-propane-1, 2, 3-tricarboxylic acid ( $C_6H_8O_7 \cdot H_2O$ ) is a weak organic acid, with a pH of 0.2, which occurs naturally in all citrus fruits (Makut and Ekeleme, 2018). It is a solid in its pure form at room temperature and has a melting point of 153°C (Almoussa et al., 2018). Naturally, citric acid is produced by metabolic pathways that take place in a living cell via tricarboxylic acid cycle (Nwoba et al., 2012). Citric acid is commonly used in food and beverages, detergents, pharmaceuticals, cosmetics, toiletries and other industries (Majumder et al., 2010). The beverages and food industries account for about 75 % of the world's citric consumption, mainly as an ingredient in carbonated drinks and an acidulant (Show et al., 2015). Industrially, metal finishing and cleaning accounts for the largest use of citric acid, followed by lubricants, chelating agents, animal feeds and plasticizer (Bauweleers, et al., 2014).

The consumption of citric acid is expected to multiply owing to its demand and numerous applications (Gruben et al., 2014; Weyda et al., 2014; Van der staat et al., 2014; Omojasola et al., 2014). Based on market trend, it is apparent that there will be a surge in the global citric acid production by looking for

alternatives that are more economical, more environmental friendly and have higher production yield (Show et al., 2015). The citric acid cost is high, mainly due to the high cost of the substrates and this has necessitated the search for cheap and easily available substrate for citric acid production (Ezea et al., 2015). Many developing countries within the tropics have a lot of underutilized cassava pulp that can be converted to citric acid. Cassava pulp is a waste generated within the tropics from cassava during fufu process of fermentation. Currently disposal of this waste product poses considerable economic and environmental problems as they are underutilized. Development of process for value addition and processing of cassava pulp is a sure way of reducing the cost of citric acid production.

The objective of our study was to investigate citric acid production from cassava pulp that is abundant in developing countries within the tropics and also develop some optimization strategies to enhance citric acid production.

## 2. Material and methods

### 2.1 Microorganism and culture maintenance

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*Aspergillus niger* strain was obtained from Department of Microbiology University of Nigeria, Nsukka. The cultures were maintained on potato dextrose agar (PDA) slants at 4°C and sub-cultured at intervals.

## 2.2 Inoculums preparation

The spores of *Aspergillus niger* was harvested from potato dextrose Agar slant using a sterile solution of 0.01% Tween 80. The inoculation wire loop was used to dislodge the spores and to ensure proper mixing of the culture with the Tween 80. A 10 ml of  $5 \times 10^7$  spores/ml was counted using haemocytometer.

## 2.3 Substrate and pretreatments

Cassava pulp residue was obtained from Fufu processing site at Nsukka in Enugu State of Nigeria. The waste was sundried, ground and sieved into flour using Muslim cloth. The flour was thermally pretreated to gelatinize the starch by suspending different concentration into 100ml of distilled water. The sample was sterilized with an autoclave at 121°C for 15 minutes.

## 2.4 Submerged fermentation

Submerged fermentation was carried out using a 250ml foam-plugged Erlenmeyer flask. Different concentrations of cassava flour 5 to 25 % were weighed using DENVER Instrument, Model: MXX- 123 USA and suspended in 100ml nutrient medium containing  $\text{NH}_4\text{NO}_3$ , 2 g/l;  $\text{KH}_2\text{PO}_4$ , 0.2 g/l;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g/l;  $\text{Fe}(\text{SO}_4)_2 \cdot 7\text{H}_2\text{O}$ , 0.01 g/l and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g/l before pretreatment. The sample was inoculated with 10ml of *Aspergillus niger* spores and incubation at 30°C for 144 hours.

## 2.5 Effect of initial pH on citric acid production

The effect of initial pH on citric acid production was carried out by adjusting the pH to 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 using 0.1M HCl and 0.1M NaOH before Pretreatment.

## 2.6 Effect of incubation temperature on citric acid production

The effect of incubation temperature on citric acid production by *Aspergillus niger* was carried out by incubating under the following temperature 20°C, 25°C, 30°C, 35°C and 40°C for 144 hours.

## 2.7 Effect of agitation on citric acid production from cassava pulp

The effect of agitation condition on citric acid production was carried out by incubating the flasks in the rotary incubator shaker (model: VWR International by B. Bran Scientific & Instrument Company England) at 100, 125, 200, 225, 300 and 325 rotations per minutes (rpm) for 144 hours.

## 2.8 Evaluation of the effects of local nitrogen sources on citric acid production

The effects of extracts of the following local nitrogen sources on citric acid production were evaluated; Fiofio (*Cajanus cajan*), Ukwa (African bread fruit) *Treculia africana* and soybean (*Glycine max*). These were achieved by cracking and removing the hard coat followed by boiling 100 g of each in 1000 ml of distilled water in a pressure cooker for 40 minutes. After boiling the remaining broth was sieved and diluted to 1000 ml with distilled water. Thereafter, 0.2 ml of the each extract was added in 100ml containing the cassava pulp medium and autoclaved at 121°C for 15 min. After cooling the *Aspergillus niger* was inoculated and incubated at 30°C for 144 hours. Different

concentrations of soybean extract were investigated. The concentrations were; 0.1, 0.2, 0.3, 0.4 and 0.5 % of soybean extract.

## 2.9 Analytical techniques

Citric acid was estimated using pyridine acetic anhydride method as reported by Marrier and Boulet (1958). A 1 ml of diluted culture filtrate along with 1.30 ml of pyridine was added in the test tube and swirled briskly. Then 5.70 ml of acetic anhydride was added in the test tube. The test tube was placed in a water bath at 32°C for 30 min. The absorbance was measured on a Spectrophotometer 722S B. Bran Scientific and Instrument Company, England at 420 nm against the blank and the citric acids of the samples were estimated with reference standard. The pH of the sample was determined using digital pH meter (DENVER Instrument, Model: UB- 10058245 ultraBASIC USA).

## 3. Results

### 3.1 Effect of different concentrations of cassava pulp

Figure 1 shows the effect of different concentrations of cassava pulp on citric acid production by *Aspergillus niger*. Citric acid concentration increased as the concentration of cassava pulp increased up to 20 % with maximum concentration of  $14.9 \pm 0.413$  g/l citric acid after 120 hours of fermentation.

### 3.2 Effect of pH on citric acid production from cassava pulp

Figure 2 shows the effect of initial pH on citric acid production from cassava pulp using *Aspergillus niger*. pH 5.5 was the optimum with maximum citric acid concentration of  $16.8 \pm 0.23$  g/l after 120 hours of fermentation. There was a reduction in citric acid concentration from pH 6.5 to pH 8.5.

### 3.3 Effect of temperature on citric acid production

Incubation temperature at 30 ° C was the optimum with maximum citric acid concentration of  $23.7 \pm 0.42$  g/l after 120 hours of fermentation (fig 3). The citric acid concentration increased from 20°C with the optimum at 30°C. There was a reduction in citric acid concentration from 35°C to 40°C throughout the fermentation time.

### 3.4 Effect of agitation speed on citric acid production

Citric acid concentration increased as the agitation speed increased from 100 rpm to 225 rpm with maximum concentration of  $25.2 \pm 0.32$  g/l citric acid after 120 hours of fermentation (fig 4). There was a decreased in citric acid concentration between 300 rpm and 325 rpm agitation speed.

### 3.5 Effect of local nitrogen sources on citric acid production

Evaluation of different local nitrogen sources; *fio fio*, soybean, ukwa with the control shows that soybean was the best for citric acid yield with maximum citric acid concentration of  $28.2 \pm 0.51$  g/l after 120 hours of incubation (fig 5). This was followed by *fio fio*, though there was no significant difference between the *fio fio* enriched medium with the control. Ukwa did not show any enhancement when compared with the control. Different soybean concentrations as the best nitrogen were evaluated. As the concentration of soybean extract increased up to 0.3 %, the citric acid concentration of  $31.2 \pm 0.35$  g/l after 120 hours of fermentation (fig 6).

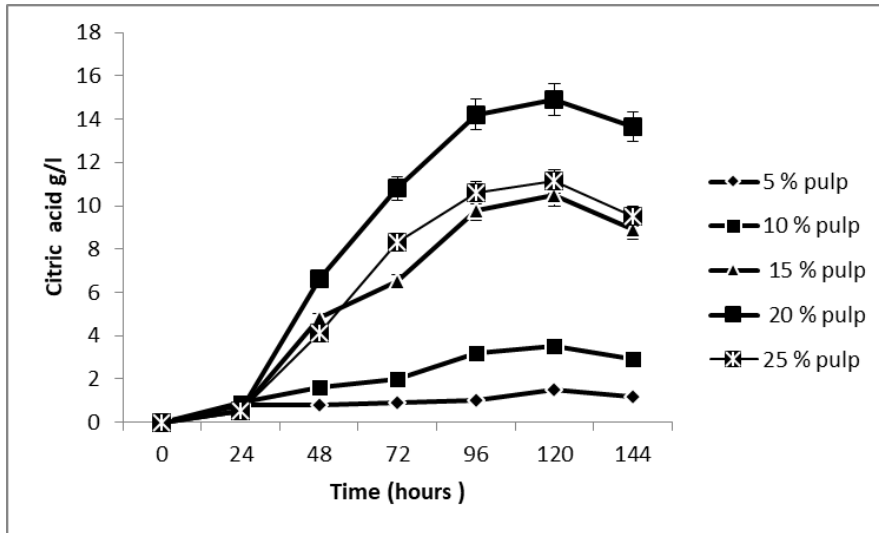


Figure 1 Effect of different concentrations of cassava pulp on citric acid production by *Aspergillus niger* in submerged culture

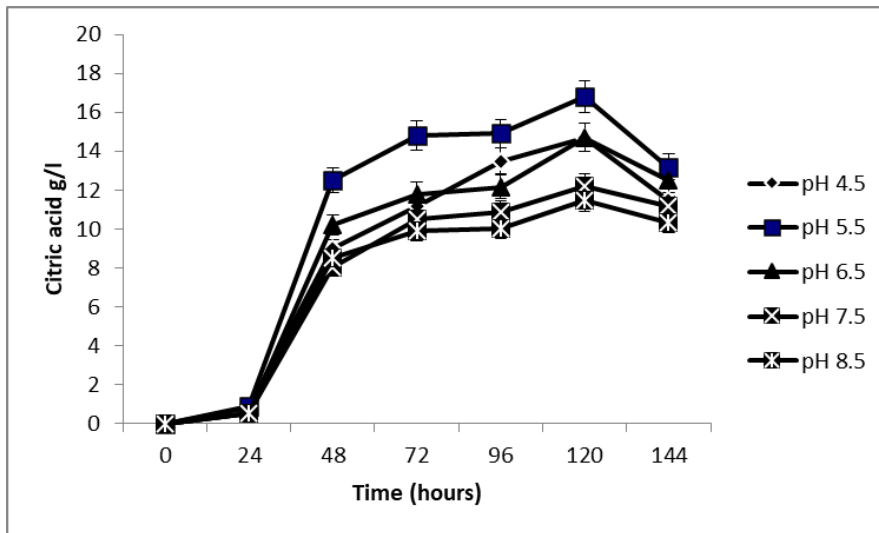


Figure 2 Effect of pH on citric acid production from cassava pulp by *Aspergillus niger* using submerged culture

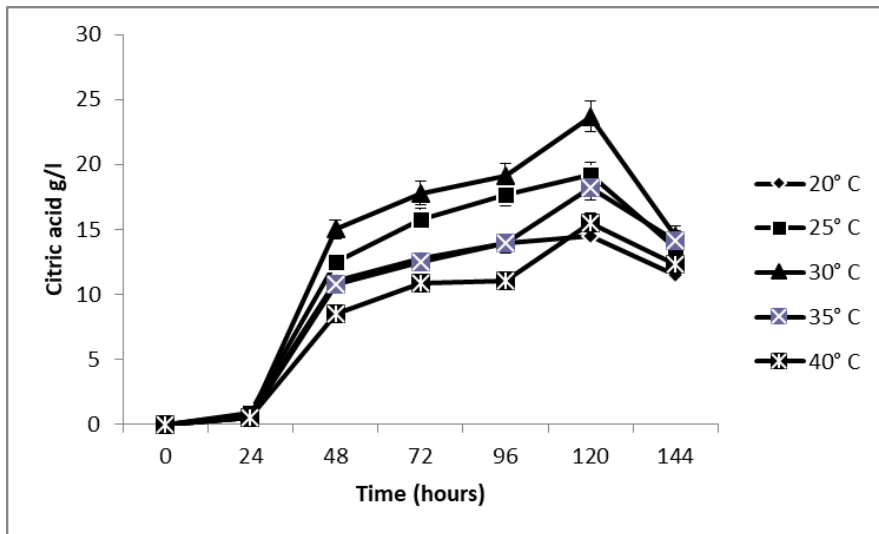


Figure 3 Effect of different temperature on citric acid production from cassava pulp by *Aspergillus niger* using submerged culture

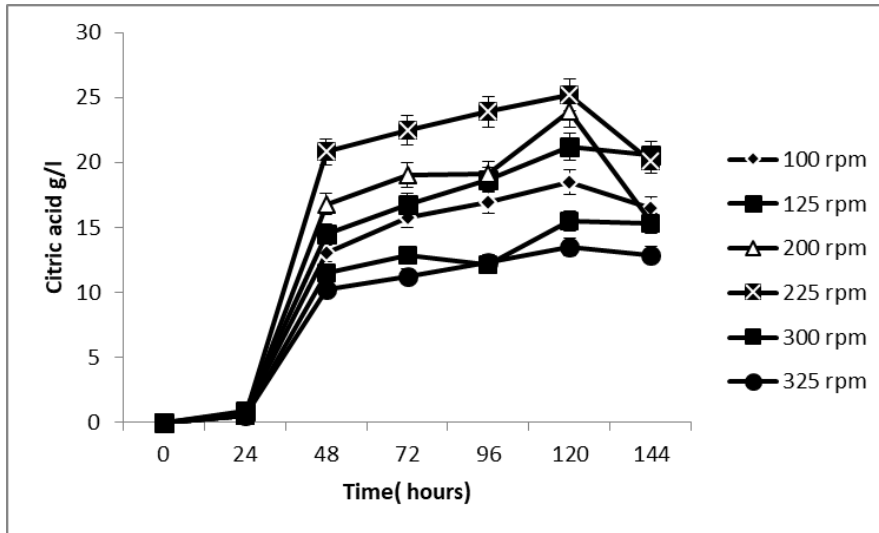


Figure 4 Effect of agitation on citric acid production from cassava pulp by *Aspergillus niger* using submerged culture

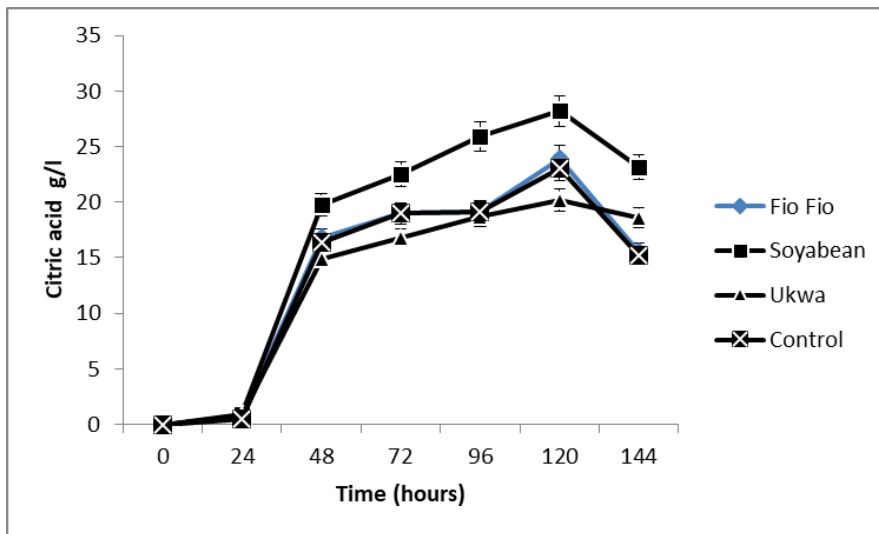


Figure 5 Effect of local nitrogen source on citric acid production from cassava pulp by *Aspergillus niger* using submerged culture

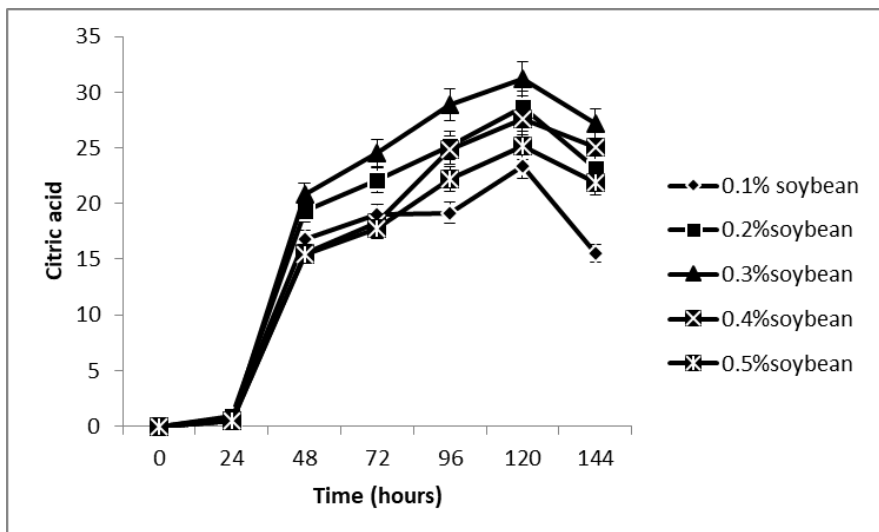


Figure 6 Effect of different temperature on citric acid production from cassava pulp by *Aspergillus niger* using submerged culture

#### 4. Discussion

The production of citric acid by *Aspergillus niger* cultured on 20% cassava pulp showed that the highest yield of 31.2 g/l after 120 hours of fermentation is in consistent with **Okareh et al. (2016)** who reported 30.54 g/l and 28.93 g/l citric acid by

*Aspergillus niger* and indigenous microflora during production of citric acid from solid state fermentation of sugar cane waste. This is in accordance with **Morgunova et al. (2018)** who reported the same range of biomass concentration during citric acid production from different renewable raw materials. **Auta et al. (2014)** reported similar concentration of *parkia biolobosa* fruit

pulp as substrate during citric acid production by *Aspergillus niger*. This suggested that *Aspergillus niger* can utilize different substrates for citric acid production.

#### 4.1 Effect of pH

pH 5.5 was the optimum with maximum citric acid concentration of 16.8 g/l after 120 hours of fermentation. There was a reduction in citric acid concentration from pH 6.5 to pH 8.5. This is in agreement with **Lodhi et al. (2001)** who made similar observation during production of citric acid from waste bread by *Aspergillus niger*. **Chetan et al. (2018)** reported the same pH range during comparison of citric acid production from *Aspergillus niger* in solid and suspension state fermentation. Similar pH was reported by **Auta et al. (2014)** during citric acid production by *Aspergillus niger* from *parkia bigobosa* fruit pulp. This shows that pH requirement depends on the strain and culture condition. The initial pH of a medium must be optimized and defined to suit the microorganism, substrate and production technique (**Show et al., 2015; Torrado et al., 2011**). A high pH often results in the deactivation of the enzyme necessary for citric acid production. During fermentation, the pH of the medium is important because a low pH reduces the risk of contamination (**Ayeni et al., 2019**).

#### 4.2 Effect of temperature

Incubation temperature at 30°C was the optimum with maximum citric acid concentration of 23.7 g/l after 120 hours of fermentation. The citric acid concentration increased from 20°C with the optimum at 30°C. There was a reduction in citric acid concentration from 35°C to 40°C throughout the fermentation time. This is in agreement with **Satheeshkumar et al. (2019)** who reported 30°C as the optimum temperature for citric acid production during utilization of fruit waste for the production of citric acid by *Aspergillus niger*. Similar result was reported by **Chetan et al. (2018)** during comparative study on citric acid production by *Aspergillus niger* in solid and suspension state fermentation. **Lodhi et al. (2001)** reported 37°C during production of citric acid from waste bread by *Aspergillus niger*. This result suggested that different temperature can be an optimum depending on the temperature of an organism.

#### 4.3 Effect of agitation

Citric acid concentration increased as the agitation speed increased from 100 rpm to 225 rpm with maximum concentration of 25.2 g/l citric acid after 120 hours of fermentation (fig 4). There was a decreased in citric acid concentration between 300 rpm and 325 rpm agitation speed. This result agreed with (**Anand et al., 2008**) who reported that citric acid production increased from 170 to 230 rpm in a stirrer fermenter using *Aspergillus niger*. (**Mohamed et al., 1995**) reported that increasing agitation rate up to 225 rpm increased production of citric acid but reduced the size of pellets formation by *Aspergillus niger*. Increase in agitation speed to some extent helps to circulate oxygen in the fermentation medium. Economical, it is better to gradually increase the aeration rate by monitoring and regulating the agitation speed. Extreme agitation can incur some losses. It can destroy the organism's ability to synthesis and accumulate citric acid.

#### 4.4 Effect of different nitrogen sources on citric acid production

Soybean was the best for citric acid yield with maximum citric acid concentration of 28.2 g/l after 120 hours of incubation. This was followed by *fiio foo*, though there was no significant difference between the *fiio fio* enriched medium with the control.

*Ukwa* did not show any enhancement when compared with the control. As the concentration of soybean extract increased up to 0.3 %, the citric acid concentration equally increased after 120 hours of fermentation. Though, there is little or no information on the of local nitrogen extract such as *fiio fio*, soybean and *ukwa*. The type of nitrogen source affects the synthesis of citric acid as well as fungal growth (**Show et al., 2015**). **Makut and Ekeleme, 2018** reported an improved citric acid production using soybean cake by *Aspergillus niger* and *Trichoderma viride*. Similar observation has been noted by **Kudzai et al. (2016)** in a medium supplemented with  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  as nitrogen sources. This implies that using local nitrogen extract as the source of nitrogen may be cost effective for citric acid production. The concentration of nitrogen has been found to have a strong effect on the production of citric acid. Nitrogen is not only part of cell's protein, but also necessary for cellular metabolism (**Show et al., 2015**).

## 5. Conclusion

In conclusion, this study revealed that cassava pulp waste has the potential to be used as substrate in the production of citric acid by *Aspergillus niger*. In addition, soybean extract has a great potential in citric acid production from cassava pulp when combined with other culture parameters such as pH, temperature and agitation.

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## Declaration of interest

There is no conflict of interest in this work.

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