# การใช้เทคโนโลยีพลาสมาเสมือนร่วมกับโซเดียมคาร์บอเนตเพื่อควบคุมโรคขั้วหวีเน่าของกล้วยหอมทอง
# Emulated Plasma Technology with Sodium Carbonate Treatment to Control Crown Rot Disease in 'Hom Thong' Bananas

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## Abstract

The postharvest treatment with emulated plasma technology combined with food preservatives to control crown rot disease caused by *Lasiodiplodia theobromae* on 'Hom Thong' bananas was investigated. The experiments were separated into three parts. First, the antifungal effect of food preservatives in vitro test, sodium carbonate (SC), and potassium sorbate (PS) at 0 (control), 0.5, 1.0, 1.5, and 2.0% (w/v) amended in PDA was observed. The mycelium growth was significantly inhibited by 1.0, 1.5, and 2.0% SC (w/v). Second, the effect of emulated plasma-activated water (EPAW) at different exposure times, 0 (control), 20, 40, and 60 min, on the severity of crown rot on bananas was examined. After 6 days of storage at ambient temperature, the disease severity of all EPAW treatments was less than that of untreated bananas. Third, treatment of EPAW at 20 min was selected to combine with 1% SC to obtain the emulated plasma-sodium carbonate solution (EPAS-1% SC). The result showed that EPAS-1% SC treatment could reduce the crown rot disease severity (2.67 scores) in comparison to the untreated one (3.83 scores), and it displayed similar results to those using fungicide one (2.3 scores) during ambient temperature storage for 6 days. Therefore, the result implies that EPAS-1% SC treatment can potentially control crown rot disease in 'Hom Thong' bananas.

**Keywords:** banana, crown rot, emulated plasma technology, food preservatives

## บทคัดย่อ

การศึกษาเทคโนโลยีพลาสมาเสมือนร่วมกับสารกันอาหารเน่าเสียเพื่อควบคุมโรคขั้วหวีเน่าของกล้วยหอมทองที่มีสาเหตุจากเชื้อรา *Lasiodiplodia theobromae* แบ่งออกเป็น 3 ส่วน ส่วนที่ 1 ศึกษาผลของสารกันอาหารเน่าเสียต่อการเจริญของเชื้อราในอาหาร PDA ที่ผสมโซเดียมคาร์บอเนต (SC) และโพแทสเซียมซอร์เบต (PS) ความเข้มข้น 0 (ชุดควบคุม) 0.5, 1.0, 1.5 และ 2.0% (W/V) พบว่า SC ความเข้มข้นตั้งแต่ 1.0 % (W/V) สามารถยับยั้งเส้นใยเชื้อราได้สมบูรณ์ ส่วนที่ 2 การศึกษาผลของการใช้น้ำพลาสมาเสมือน (EPAW) ที่ระยะเวลา 0 (ชุดควบคุม) 20 40 และ 60 นาที ต่อความรุนแรงของการเกิดโรคขั้วหวีเน่าของกล้วยหอมทอง พบว่า หลังการเก็บรักษาที่อุณหภูมิห้องนาน 6 วัน การแช่กล้วยใน EPAW ทุกระยะเวลาที่ทดสอบสามารถลดความรุนแรงของโรคได้เมื่อเปรียบเทียบกับชุดควบคุม ดังนั้น EPAW ที่เวลา 20 นาที จึงถูกเลือกมาใช้ร่วมกับ 1.0% SC เพื่อผลิตเป็นสารละลายพลาสมาเสมือนโซเดียมคาร์บอเนต (EPAS - 1% SC) การทดสอบส่วนที่ 3 ผลการทดลองพบว่า EPAS - 1% SC สามารถลดความรุนแรงของโรค (2.67 คะแนน) ได้เมื่อเปรียบเทียบกับชุดควบคุม (3.83 คะแนน) และให้ผลดีใกล้เคียงกับการใช้สารกำจัดเชื้อรา (2.3 คะแนน) การทดลองนี้แสดงให้เห็นว่าการใช้ EPAS - 1% SC มีศักยภาพในการควบคุมโรคขั้วหวีเน่าของกล้วยหอมทอง

**คำสำคัญ:** กล้วย เทคโนโลยีพลาสมาเสมือน สารกันอาหารเน่าเสีย โรคขั้วหวีเน่า

## Introduction

'Hom thong' Banana (*Musa sapientum*) is one of the foremost broadly grown and traded natural products in the world due to its nutritional esteem (Voora *et al*., 2022), which has the potential to export. But

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the limitation of bananas is directly related to the postharvest disease: crown rot caused by various fungi, especially *L. theobromae* (Jitareerat and Uthairatanakij, 2012; Reyes *et al*., 1998). Fungicides are still the most common practice in controlling crown rot disease, but it is not safe. Alternative safe treatments should be considered, such as food preservatives, which can be enhanced their antifungal effect in cooperating with physical treatments (Ditschun and Winter, 2000). In previous studies about food preservatives, potassium sorbate (PS) inhibited pathogen growth and controlled the fruit rot in dragon fruit (Jitareerat *et al*., 2018). Sodium carbonate (SC) also reduced crown rot disease in bananas (Zoeir *et al*., 2017). Innovative physical treatment like plasma-activated water (PAW) or plasma-activated solution (PAS) has the potential to inactivate microbes due to its containing an ionized medium with reactive oxygen species (ROS), charged ions, and UV rays to damage microorganism cells (Misra *et al*., 2011). Ahmad *et al*. (2022) demonstrated that the use of emulated plasma-activated water (EPAW) or emulated plasma-activated solution-$H_2O_2$ (EPAS-$H_2O_2$) could kill *C. gloeosporioides*, a causal agent of anthracnose in chili seeds. As above information, this study was to investigate the EPAW combined with SC on the control of crown rot in bananas.

## Materials and Methods

The experiments were divided into three parts. The pathogen utilized during this serial experiment was *L. theobromae*, a major pathogen of crown rot disease. The first part, the antifungal effect of food preservatives on mycelium growth was determined. A mycelial dish (0.5 cm ∅) was placed at the centre of the PDA plate contained with potassium sorbate (PS) or sodium carbonate (SC) at 0 (control), 0.5, 1.0, 1.5, and 2.0% (w/v), each treatment had 4 replications. The diameter of mycelium was measured every day until the control reached the edge of the Petri plates. The result is presented as the inhibition percentage. Second, the effect of EPAW to control crown rot was investigated as described by Ahmad *et al*. (2022). Bananas were treated with EPAW at different exposure times, 0 (control), 20, 40, and 60 min. All samples were kept at room temperature, and the disease severity was assessed in terms of an index of a scale of 0-7 scores (Alvindia, 2013). Third, EPAW at 20 min was chosen to be combined with 1% SC to obtain the emulated plasma-activated solution (EPAS-1% SC) compared with that 150 ppm prochloraz, EPAW at 20 min. and non-treated (control) group. Each treatment consists of 6 replications (banana crowns). During the treatment, micro nanobubbles served to circulate water, ensuring that all samples were appropriately contacted with EPAW or EPAS. Bananas were packed in the cartoon box at room temperature for 6 days, and the disease severity of crown rot was assessed. The experiment was laid out on a Completely Randomized Design (CRD). All data were subjected to ANOVA conducted using the Statistical Analysis System (SAS, Ver.9, Inc., USA).

## Results and discussion

### Effect of food preservatives on mycelial growth of *L. theobromae*

SC treatments at 1.0, 1.5, and 2.0% completely inhibited *L. theobromae* growth (100% inhibition) (Figure 1A). Whereas PS, at the highest concentration (2.0%), could inhibit the growth by 55.63% (Figure 1B). SC was found to be the most effective food preservative in inhibiting mycelium growth compared to PS. SC, as salts, offered inhibition on the pathogen. As reported previously by Fallik *et al*. (1996), growth inhibition is likely due to a direct impact on the pathogen, causing decreased fungal cell turgor pressure, collapsed hyphae and spores, and inability to sporulate and release extracellular enzymes. The other inhibitory effect of SC is due to its high pH. SC media was found to have high pH compared to PS media, pH was 10.03 and 6.75 (data not shown). According to Youssef *et al*. (2014), the pH of salts directly inhibits fungus growth as pH significantly impacts antifungal activity.

**Effect of emulated plasma-activated water (EPAW) on crown rot disease**

EPAW treatment showed less disease severity than control (Figure 2). The lower disease severity on EPAW treatment might be due to the inactivation of the pathogen. According to Critzer *et al*. (2007) and Misra *et al*. (2011), reactive species produced by plasma conditions are frequently associated with direct oxidative effects on the outer surface of microbial cells. Among EPAW at different exposure times, there are no significant differences, so the minimum exposure time of EPAW at 20 min. was chosen to be the next treatment as it was the good condition to combine with the selected food preservative, which is sodium carbonate at 1%.

**Study the combined effect of emulated plasma solution of 1% sodium carbonate (EPAS - 1% SC).**

The EPAS-1% SC could control crown rot on bananas. As shown in Figure 3B, where the disease severity of EPAS-1% SC treated bananas was lower than that of EPAW and control by having significantly lower scores, moreover, the result also demonstrated the similarity as fungicide treatment. Bananas treated with EPAS gained lower disease severity due to EPAS effect on pathogens. According to Ahmad *et al*. (2022), EPAS treatment destroyed fungi by destroying their cell walls, which may have been driven by the generation of ROS and the contribution of that gas' physical properties in the emulated plasma condition. Besides, through their interactions with biological material like DNA, protein and lipids, the plasma agents contribute to the lethal effect. However, the ability of plasma to kill pathogenic organisms while excluding the host or activate various pathways in different organisms makes it quite selective (Misra *et al*., 2011). Additionally, the emulated plasma condition was generated under 1% SC solution. SC controls postharvest disease through direct pathogen effects and host defence mechanisms (Talibi *et al*., 2011; D'aquino *et al*., 2020).

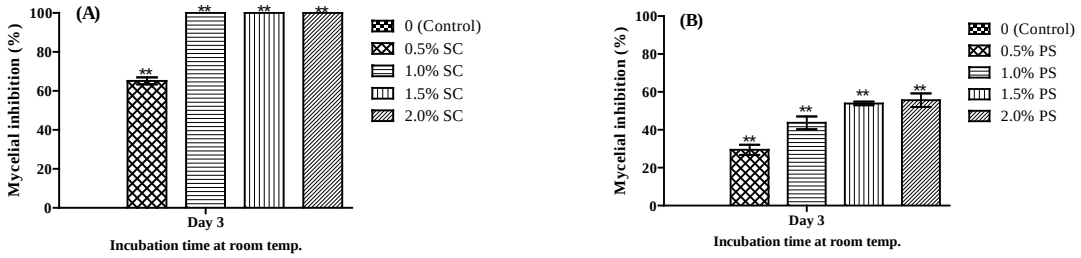



**Figure 1.** Effect of sodium carbonate (A), potassium sorbate (B) at 0 (control), 0.5, 1.0, 1.5 and 2.0% (w/v) on mycelium growth inhibition of *L. theobromae* after 3 days incubation at room temperature.

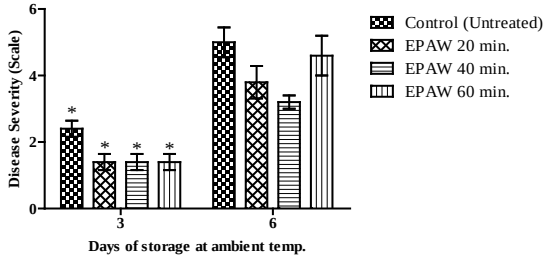
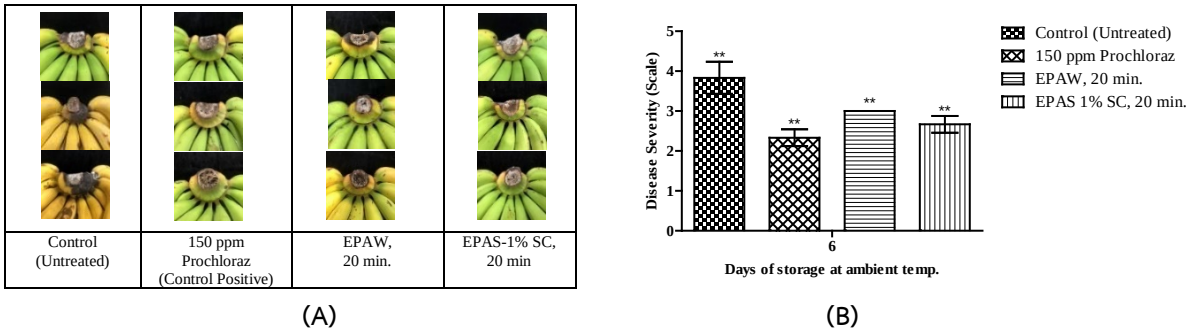



**Figure 2.** Severity of crown rot disease in banana cv. Hom Thong after treating with emulated plasma activated water (EPAW) at different exposure times, 0 (control), 20, 40, and 60 min during storage at ambient temperature for 6 days.




(A) (B)

**Figure 3.** Illustration of crown rot symptom (A) and severity of crown rot disease (B) in banana cv. Hom Thong after treatment with emulated plasma-activated water (EPAW) combined with 1% sodium carbonate (EPAS-1% SC) 20 min, compared with EPAW, prochloraz, and the untreated control fruit during storage at ambient temperature for 6 days.

## Conclusion

EPAS-1% SC treatment could reduce crown rot disease, similar to fungicide treatment after 6 days of storage. Therefore EPAS-1% SC is potentially controlling crown rot disease in 'Hom Thong' bananas.

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