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INVESTIGATING THE COMBINED ANTIYEAST EFFICACY OF PLASMA-ACTIVATED WATER AND NATURAL PHENOLICS ON PLANKTONIC DEBARYOMYCES HANSENII

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Plasma-activated water (PAW) has gained attention as a potent antimicrobial agent, demonstrating its capacity to regulate the growth of microorganisms. The production of PAW entails exposing water to cold atmospheric plasma (CAP) which introduces short and long-lived reactive oxygen and nitrogen species (RONS) such as H_2O_2, O_3, NO_2^-, NO_3^- and OH radicals. The reactive species in PAW interfere with the structural integrity and functional capabilities of microbial cells. Phenolic compounds are secondary metabolites in plants known for their health-promoting and antimicrobial properties. The combination of PAW with natural compounds as an antiyeast strategy could potentially yield additive or synergistic effects. Such an approach may broaden the antimicrobial spectrum and delay the development of microbial resistance.

This study involved the use of 1 kHz transient spark (TS) discharge PAW combined with cinnamic acid, vanillin, gallic acid and *p*-coumaric acid separately to create phenolics concentration of 2 or 1 mg/mL, and incubated with 10^6 CFU/mL of yeast *Debaryomyces hansenii* SZMC 8045Mo for 24 hours at 30 °C. In addition, 10^6 CFU/mL of *D. hansenii* in sterile tap water was directly treated with TS for 10 min and incubated with each of the four natural compounds under similar conditions. The efficacy of the PAW-phenolics was evaluated through agar plated colony counts. The results of this study suggest that the integration of PAW with natural phenolics constitutes an effective approach for combating yeast.

1. Introduction

Non-Thermal Plasma (NTP) technology serves as a green alternative that may be instrumental in the progress of agricultural production, biomedical innovations, food industry, water purification, air decontamination, and soil remediation, along with a wide array of other uses [1, 2, 3, 4, 5].

The control of fungi in agricultural, industrial and medical environments is fraught with challenges, stemming from their resilience and the multifaceted nature of these contexts [6]. Yeasts are exceptionally adaptable, able to endure extreme environmental conditions, such as low pH and high concentrations of sugar or salt, as well as surviving in cold storage [7]. Emerging technologies, including NTP and the incorporation of bioactive compounds such as phenolics, have shown substantial effectiveness in mitigating pathogenic and non-pathogenic microbes [8]. These contemporary approaches provide advantageous alternatives to traditional chemical interventions.

The utilization of plasma-activated water (PAW) in conjunction with bioactive compounds such as natural phenolics offers a promising avenue for microbial management [9]. PAW contains short-lived reactive species such as nitric oxide (NO), superoxide (O_2^-), ozone (O_3), hydroxyl radical (OH), peroxynitrate ($OONO_2^-$) and peroxynitrite ($ONOO^-$), and long-lived species such as nitrates (NO_3^-), nitrites (NO_2^-) and hydrogen peroxide (H_2O_2) [10]. These reactive species can disrupt the cellular integrity of yeasts, resulting in cell death [11]. The antifungal action of natural bioactive compounds, including phenolics, is primarily due to their ability to compromise cellular and membrane structures, denature proteins, and disrupt fungal metabolic processes [12, 13]. By integrating plasma with phenolics, it is possible to target yeasts through multiple pathways, thereby improving efficacy and reducing the potential for antifungal resistance. Furthermore, this combined approach can lead to a reduction in the concentrations of phenolics needed, which may help to minimize adverse effects on non-target organisms.

2. Methods

The transient spark (TS) discharge was generated through a power electrode connected to a high voltage DC power supply through a 10 MΩ resistor. A metal ring as a grounded electrode was submerged in the tap water/yeast-containing water. The treatment time for the transient spark was 10 min/10 mL. The schematic diagram of the TS plasma setup is shown in Figure 1.

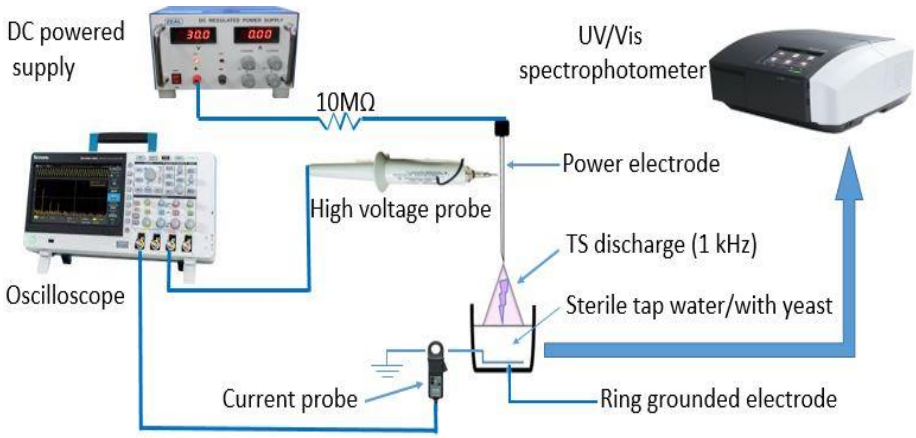

Fig. 1. The experimental setup of the transient spark discharge generating system

Two approaches to antiyeast treatment were investigated: (1) the direct application of TS discharge to 10 mL of *D. hansenii* suspension at a concentration of 10^6 CFU/mL, followed by incubation with either 2 or 1 mg of phenolic compounds; and (2) an indirect method in which transient spark PAW was used to dissolve 2 or 1 mg of phenolics, which were then incubated with 10^6 CFU/mL of yeast. The effectiveness of the plasma-phenolics treatment was evaluated by counting the number of yeast colonies on agar plates after a 24-hour incubation at 30°C.

3. Results and discussion

In the context of directly treating yeasts with TS discharge followed by the incubation with natural phenolics, it was observed that plasma-cinnamic acid displayed the highest level of antiyeast activity, followed by plasma-vanillin, plasma-gallic acid, and plasma-*p*-coumaric acid, respectively (Figure 2). Plasma-cinnamic acid showed a complete growth inhibition at both 2 and 1 mg/mL phenolic concentration (Figure 2). Plasma-vanillin resulted in growth inhibition values of 3.16 and 2.21 log at 2 and 1 mg/mL, respectively (Figure 2). Additionally, plasma-gallic acid and plasma-*p*-coumaric acid produced growth inhibition of 1.46 and 1.35 log at a concentration of 2 mg/mL, respectively (Figure 2).

In an indirect treatment scenario where PAW was used to dissolve phenolic substances at concentrations of 2 or 1 mg, followed by incubation with 10^6 CFU/mL of yeast, PAW-cinnamic acid completely inhibited growth at a concentration of 2 mg/mL and achieved a growth inhibition of 3.2 log at 1 mg/mL (Figure 3). PAW-vanillin demonstrated growth inhibition of 2.1 log and 1.6 log at 2 mg/mL and 1 mg/mL, respectively (Figure 4). Additionally, PAW-gallic acid produced growth inhibition of 1.2 log at 2 mg/mL and 1.1 log at 1 mg/mL, while PAW-*p*-coumaric acid resulted in growth inhibition of 1.4 log at 2 mg/mL and 1.0 log at 1 mg/mL (results not shown).

The combination of plasma and phenolics generally exhibited varying growth inhibitory effects on *D. hansenii* SZMC 8045Mo, with direct plasma treatment showing a higher antiyeast activity than the indirect treatment. Cinnamic acid, gallic acid, and *p*-coumaric acid, identified as hydroxybenzoic acids, exhibited varying levels of antiyeast activity when combined with PAW. These variations may be explained by the distinct chemical properties of each compound [12]. The dynamics between RONS

and phenolic functional groups, together with the antiyeast efficacy of the individual compounds, could have been pivotal in determining the final results. The presence of RONS in PAW may have augmented the effectiveness of phenolics by altering their chemical structure, enhancing solubility, and promoting effective penetration into yeast cells. In cases where antiyeast activity was notably high, such as with plasma-cinnamic acid, the combined oxidative stress from RONS and phenolics may have effectively compromised the yeast's defense mechanisms.

The plasma-phenolics antiyeast strategy holds promise in applications such as agriculture and food safety, where fungal contamination is a significant challenge [14]. Research continues to explore the optimization of PAW treatment and phenolic compound concentrations for maximizing their antiyeast efficacy [15,16,17].

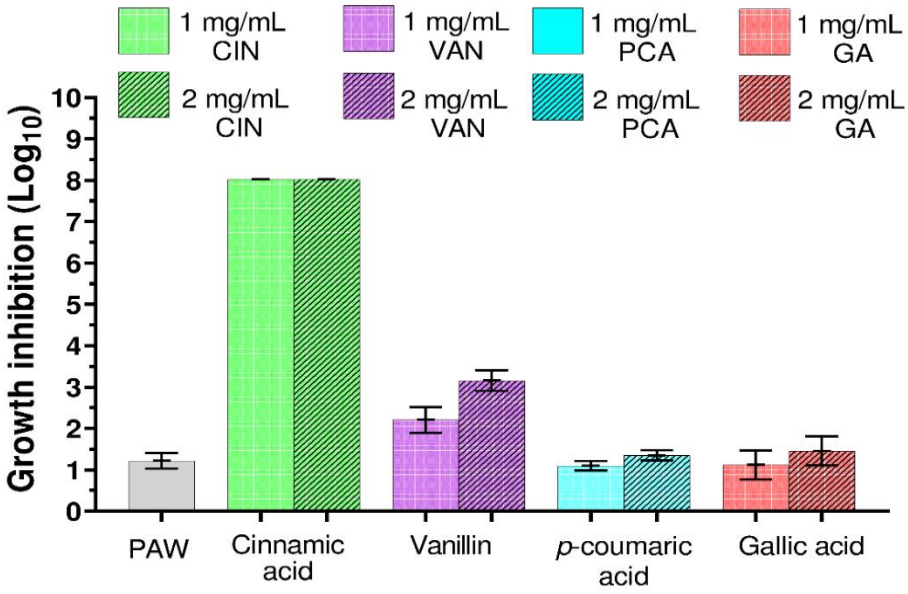

Fig. 2. Growth inhibition of direct transient spark treatment on 10 mL of 10^6 CFU/mL *D. hansenii* SZMC 8045Mo, followed by a 24-hour incubation in phenolics.

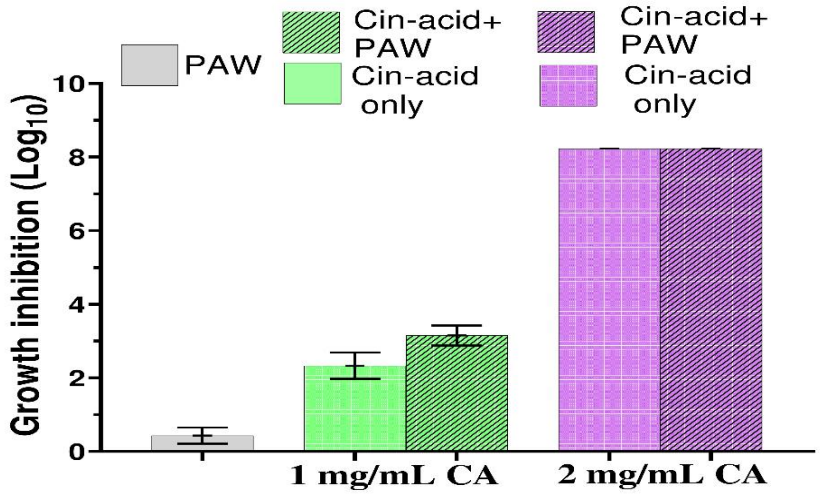

Fig. 3. Growth inhibition of transient spark PAW combined with cinnamic acid (CA) on *Debaryomyces hansenii* SZMC 8045Mo.

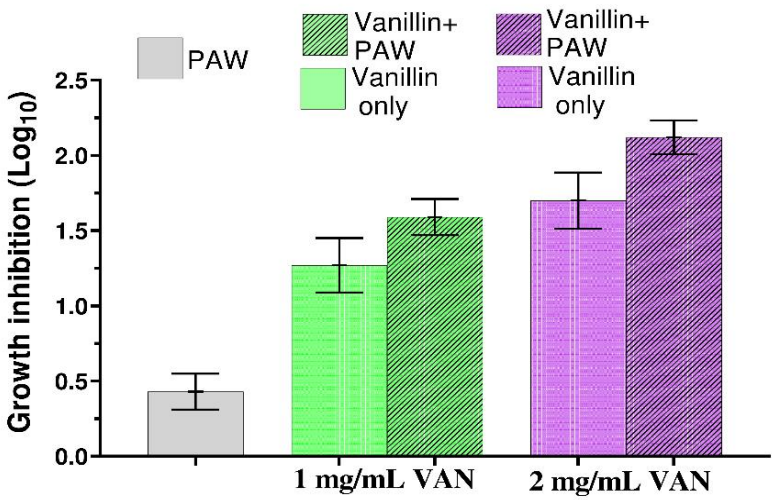

Fig. 4. Growth inhibition of transient spark PAW combined with Vanillin (VAN) on *Debaryomyces hansenii* SZMC 8045Mo.

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5. References

[1] Giardina A, Lofrano G, Libralato G, Siciliano A, Marotta E, and Paradisi C 2024 *Front. Environ. Chem.* **5** 1416702.
[2] Xu Y and Bassi A 2024 *Biotechnol. Prog.* e3511.
[3] Maccaferri C, Gherardi M, and Laurita R 2024 *Front. Phys.* **12** 1399720.
[4] Kooshki S, Pareek P, Mentheour R, Janda M, and Machala Z 2023 *Environ. Technol. Innov.* **32** 103287.
[5] Hatzisymeon M, Tataraki D, Tsakiroglou C, Rassias G, and Aggelopoulos C 2021 *Sci. Total Environ.* **786** 147420.
[6] Davies C R, Wohlgemuth F, Young T, Violet J, Dickinson M, Sanders J, Vallieres C, and Avery S V 2021 *Fungal Biol. Rev.* **36** 15-26.
[7] Segal K C, Romero A L Alcaraz L D, López O G, Torres R N, Sandoval G, and González J 2022 *Microorganisms* **10** 794.
[8] Matan N, Puangjinda K, Phothisuwan S, and Nisoa M 2015 *Food Control* **50** 291-296.
[9] Gustavo J, El Kadri H, Wantock T, Harle T, Seggiani M, Danti S, Di Stefano R, and Velliou E 2020 *Molecules* **26** 1890.
[10] Pareek P, Kooshki S, Tóth P, and Janda M 2024 *Chem. Eng. J.* **493** 152583.
[11] Polčic P and Machala Z 2021 *Inter. J. Mol. Sci.* **22** 2247.
[12] Kimani B G, Kerekes E B, Szebenyi C, Krisch J, Vágvölgyi C, Papp T, and Takó M 2021 *Foods*, **10** 1652.
[13] Kimani B G, Takó M, Veres C, Krisch J, Papp T, Kerekes E B, and Vágvölgyi C 2022 *Foods* **12** 1338.
[14] Liu X, Li Y, Wang S, Huangfu L, Zhang M and Xiang Q 2021 *LWT* **146** 111447.
[15] Kimani BG, Ramin M, Zdenko M 2024 *ICPM. IWPCT* **10** 127.
[16] Ramin M, Kimani BG, Zdenko M 2024 *ICPM. IWPCT* **10** 94.
[17] Oleksandr G, Kimani BG, Zdenko M 2024 *ICPM. IWPCT* **10** 97.