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Title　：Development of chitosan-based nanocomposite enriched with essential oil as antifungal coating for fresh fruit application
(生鮮果実の防カビコーティング剤としての精油添加キトサン系ナノコンポジットの開発)

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Thesis Summary

Edible packaging (coatings and films) has been used to improve the shelf life of fresh fruit products. The preparation process of edible packaging depends on the properties of the ingredients and the application end use. The objective of this study were to 1) develop antifungal nanocomposite coating formulated from chitosan (Chi), Indonesia origin essential oil, and nanoparticles; 2) evaluate the functional properties of the coating films. These objectives were achieved by optimizing the raw material dosage selection and blending sequence.

In the first work, the properties of an antifungal coating film made from Chi combined with zinc oxide nanoparticles (ZNPs) and Indonesian sandalwood essential oil (SEO) were investigated. Incorporation of 0.5% SEO or 0.025% ZNPs with 0.5% SEO into 0.8% Chi coating solution showed outstanding inhibitory effects on mycelium growth and spore germination of *Penicillium italicum*. The antifungal mechanism was explained by measuring the loss of spore membrane integrity. The antifungal effect was also confirmed *in vivo* by testing on tangerine fruit. The biocompatibility of these coatings was analyzed using Fourier transform infrared spectroscopy, scanning electron microscopy, atomic force microscopy, and fluorescence microscopy. Significant increases in pH, appearance viscosity, and transparency and a decrease in light transmittance were found with 0.8% Chi plus 0.025% ZNPs and 0.5% SEO compared with the control. Chi films incorporating SEO and ZNPs can be used as an edible film and coating to reduce chemical use.

Further work, a novel formulation of composite coating comprising 0.8% Chi incorporating 0.025% CuO nanoparticles (CuO) and 0.5% Indonesian cedarwood essential oil (CEO) was fabricated by casting method. FTIR, CLSM, and SEM analyses were employed to characterize the biocompatibility of each formulation. In addition, the physico-chemically properties of the composite coatings were characterized. The color (L^*), light transmission, zeta potential, and roughness of Chi were significantly ($P < 0.05$) altered negatively by the presence of CuO or CEO; the color (a^*, b^*, and ΔE), apparent viscosity, and transparency also changed positively as a consequence of CuO and CEO incorporation. The antifungal features of the pure Chi coating against *P. italicum* and *P. digitatum* were improved synergistically by CuO and CEO, confirmed by *in vitro* and *in vivo* assays. The composite coatings obtained in this work had potential applications for active primary food

packaging, particularly for fresh postharvest commodities.

Finally, a novel composite edible coating film consisting of 0.8% Chi and 0.5% SEO was developed. Cellulose nanofibers (CNFs) were used as a stabilizer agent of oil-in-water Pickering emulsion. We found four typical groups of CNF level-dependent emulsion stabilization, including (1) unstable emulsion in the absence of CNFs; (2) unstable emulsion (0.006–0.21% CNFs); (3) stable emulsion (0.24–0.31% CNFs); and (4) regular emulsion with the addition of surfactant. Confocal laser scanning microscopy was used to characterize the droplet diameters and morphology. Antifungal tests against *Botrytis cinerea* and *P. digitatum*, between emulsion coating stabilized with CNFs (Chi/SEOpick) and Chi or Chi/SEO was tested. The effective concentration of CNFs (0.24%) improved the performance of Chi coating and maintain Chi/SEO antifungal activity synergistically confirmed with a series of assays (*in vitro*, *in vivo*, and membrane integrity changes). The incorporation of CNFs contributed to improve the functional properties of CS and SEO-loaded Chi including light transmission at UV and visible light wavelengths and tensile strength. AFM and SEM were employed to characterize the biocompatibility of each coating film formulation. Emulsion-CNF stabilized coating had potential applications for active coating for fresh fruit commodities.