

Effects of Long-Term Storage on Exosome-Like Nanoparticle Characteristics in Fingerroot Juice

Intiporn Mingsakul¹, Wittaya Panvongsa², Ladawan Khowawisetsut^{3,4}, Narinee Srimark⁴, Sarinya Kokmat¹, Arthit Chairoungdua^{1,*}

¹Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand.,

²Department of Tropical Nutrition & Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.,

³Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.,

⁴Center of Excellence for Microparticle and Exosome in Diseases, Research Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

**Corresponding Author Email: arthit.chi@mahidol.ac.th*

Abstract

Plant exosome-like nanoparticles (PELNs), the nanovesicles secreted by plants, hold significant potential as cosmetic and therapeutic agents. The exosome-like nanoparticles isolated from fingerroot, a Southeast Asian plant, have been reported to exhibit selective cytotoxicity against colorectal cancer cells, highlighting their potential as a therapeutic material for cancer. To reduce fluctuations caused by variations in growth environment or harvest season, fingerroot juice is typically extracted and stored at -20 °C for long periods. However, the characteristics of fingerroot exosome-like nanoparticles (FELNs) after being stored have not been studied. In this study, freshly stored and 1-year stored juice underwent sucrose density gradient ultracentrifugation for FELN isolation. During the enrichment step using a sucrose cushion, the fresh-juice isolation band was thicker and exhibited less smear compared to that after 1 year of storage. Following the purification step, both groups exhibited comparable thickness and a similar distribution pattern. After isolation, FELNs were analyzed for morphology and size distribution using transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA), respectively. The FELNs from both storage conditions showed similar intact and membrane-bound vesicle morphology. The NTA results showed a normal distribution, with mean sizes of 125.51 nm for fresh juice and 118.3 nm for juice stored for one year. Furthermore, the particle size distribution values at D10, D50, and D90 for both fresh juice and juice stored for one year were comparable. Additionally, particle concentrations remained consistent, indicating that long-term storage had minimal impact on FELN characteristics and ensuring reliable handling for future applications.

Keywords: Plant-derived nanovesicles, Fingerroot, Isolation methods, Characterization