Supplementary Information (SI) for Food & Function.
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Development of Pickering water-in-oil emulsions using a dual stabilization of candelilla wax and acylated EGCG derivatives to enhance the survival of probiotics (*Lactobacillus plantarum)* powder

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Supplementary Method

Cytotoxicity assay

The cytotoxicity of acylated EGCG derivatives was determined using mouse embryonic fibroblasts (NIH-3T3) by Cell Counting Kit-8 (CCK-8). Cell suspensions (100 μL/well) were inoculated in 96-well plates and placed in an incubator for 24 h of pre-culture (37°C, 5% CO_2). Acylated EGCG derivatives were dissolved in DMSO and diluted with a culture medium. 10 μL of samples with different concentrations (0, 10, 20, 40, and 80 μg/mL) were added to the culture plate and incubated for 24 h (37°C, 5% CO_2). 10 μL of CCK-8 solution was added to each well, and the plates were incubated in an incubator for 4 h (37°C, 5% CO_2). Optical density (OD) was measured using a microplate reader (H1, Boten Instrument Co., Ltd., Winooski, VT, USA) at 450 nm. Relative cell viability was obtained using the following equation: relative cell viability(%) = [OD(experimental) − OD(blank)]/[OD(control) − OD(blank)] × 100.

Supplementary Results

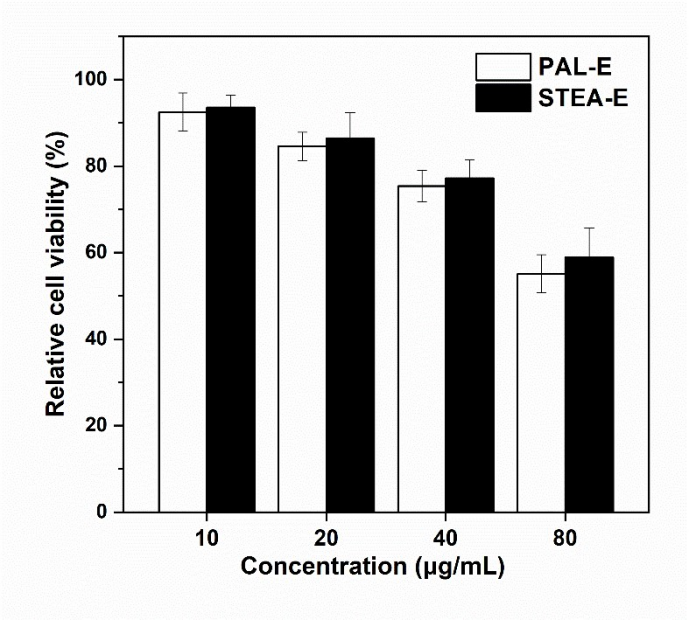

Figure S1. Cytotoxicity of acylated EGCG derivatives.

PAL-E, epigallocatechin gallate palmitate, STEA-E, epigallocatechin gallate stearate.

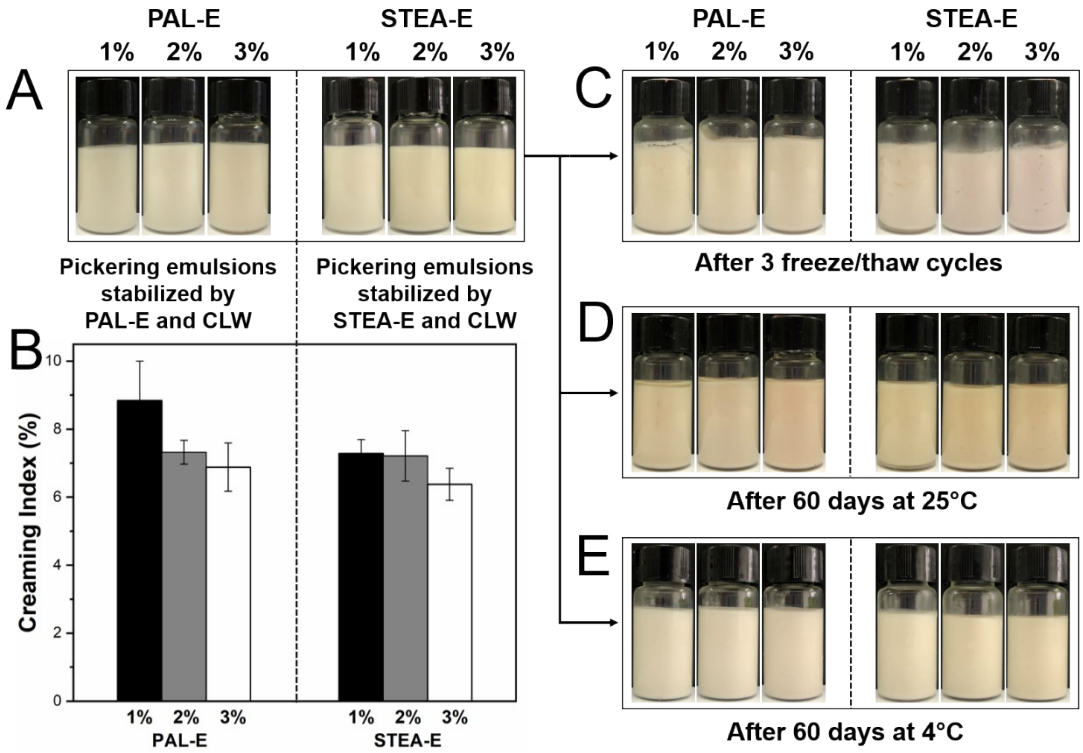

Figure S2. Physical stability of water-in-oil Pickering emulsions co-stabilized by 1-3% m/v acylated epigallocatechin gallate derivatives and 2% m/v candelilla wax at 80% oil phase volume fraction. (A) Appearance of freshly prepared emulsions; (B) Creaming index of emulsions after centrifugation; (C) Appearance of emulsions after three freeze-thaw cycles; (D) Appearance of emulsions after 60 days of storage at 25°C; (E) Appearance of emulsions after 60 days of storage at 4°C.

PAL-E, epigallocatechin gallate palmitate, STEA-E, epigallocatechin gallate stearate, CLW, candelilla wax.

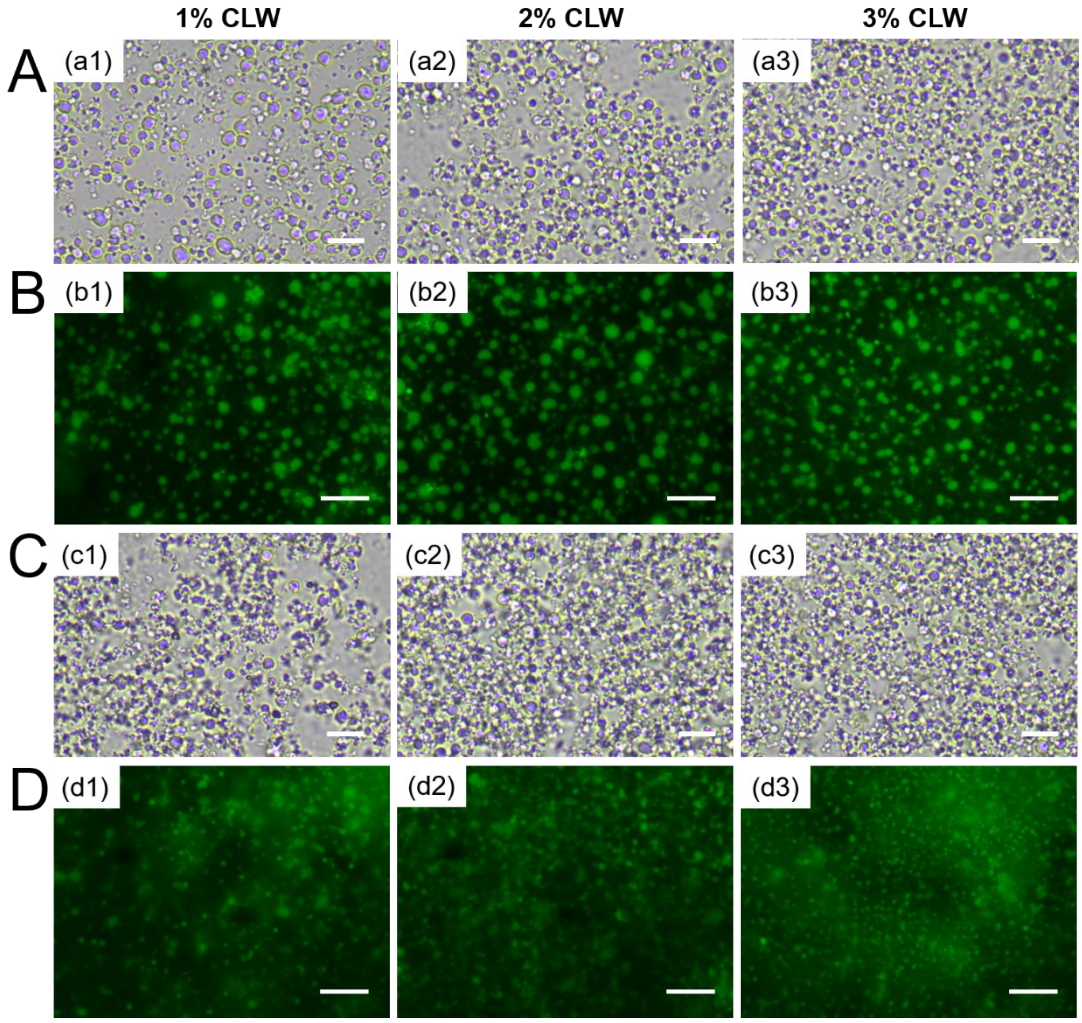

Figure S3. Microstructure of water-in-oil Pickering emulsions co-stabilized by 2% m/v acylated epigallocatechin gallate derivatives and 1-3% m/v candelilla wax at 80% oil phase volume fraction (scale bar: 10 μm). (A) Optical microscopy of epigallocatechin gallate palmitate and candelilla wax co-stabilized emulsions: a1, 1% m/v candelilla wax, a2, 2% m/v candelilla wax, and a3, 3% m/v candelilla wax; (B) Fluorescence microscopy of epigallocatechin gallate palmitate and candelilla wax co-stabilized emulsions: c1, 1% m/v candelilla wax, c2, 2% m/v candelilla wax, and c3, 3% m/v candelilla wax; (C) Optical microscopy of epigallocatechin gallate stearate and candelilla wax co-stabilized emulsions: d1, 1% m/v candelilla wax, d2, 2% m/v candelilla wax, and d3, 3% m/v candelilla wax; (D) Fluorescence microscopy of

epigallocatechin gallate stearate and candelilla wax co-stabilized emulsions: f1, 1% m/v candelilla wax, f2, 2% m/v candelilla wax, and f3, 3% m/v candelilla wax.

CLW, candelilla wax.

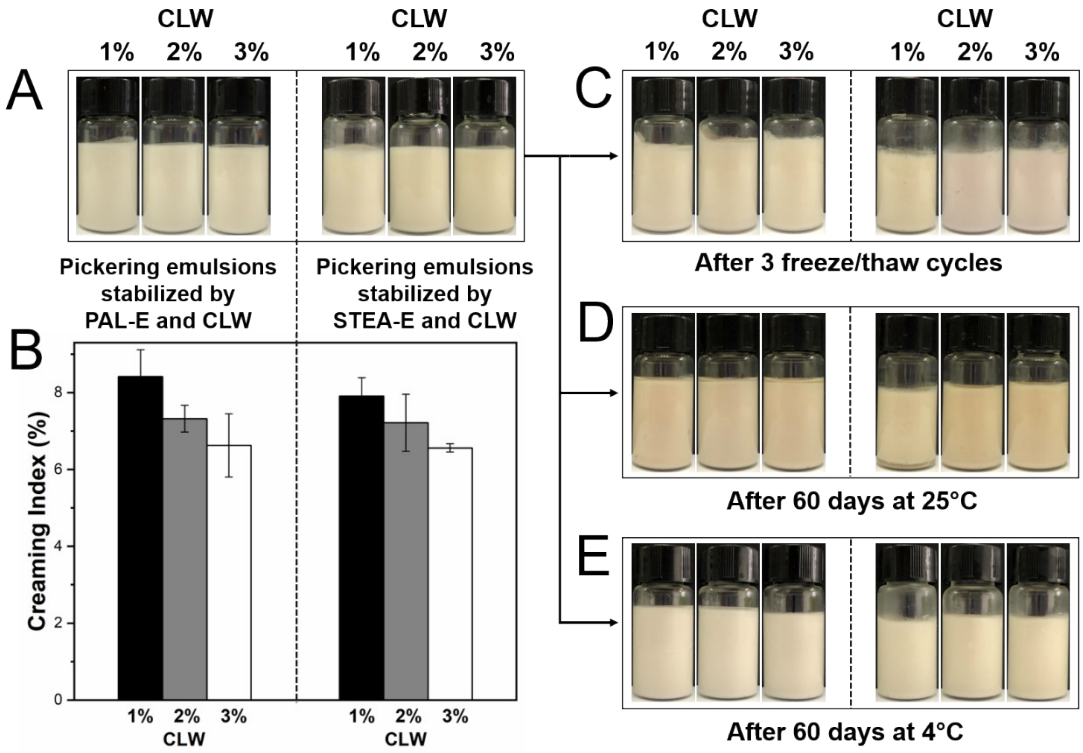

Figure S4. Physical stability of water-in-oil Pickering emulsions co-stabilized by 2% m/v acylated epigallocatechin gallate derivatives and 1-3% m/v candelilla wax at 80% oil phase volume fraction. (A) Appearance of freshly prepared emulsions; (B) Creaming index of emulsions after centrifugation; (C) Appearance of emulsions after three freeze-thaw cycles; (D) Appearance of emulsions after 60 days of storage at 25°C; (E) Appearance of emulsions after 60 days of storage at 4°C.

PAL-E, epigallocatechin gallate palmitate, STEA-E, epigallocatechin gallate stearate, CLW, candelilla wax.