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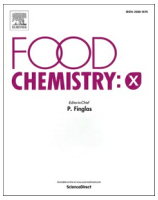

Multifaceted roles of foam-mat freeze-dried catechins nanoencapsulation to enhance catechins stability and bioaccessibility, and quality of green tea catechins-fortified milk

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ABSTRACT

Catechins, widely used as functional ingredients and health supplements, face usage limitations due to their poor stability and bioaccessibility. In this study, encapsulation techniques including nanoemulsion and foam-mat freeze-drying were utilized to enhance the stability and bioaccessibility of catechins. Catechins nanoemulsion (CaNE) was ultrasonically fabricated and foam-mat freeze-dried CaNE (FD-CaNE) was prepared by mixing with a blend of maltodextrin and gum arabic as wall material and foaming with hydroxypropyl methylcellulose before freeze drying. Unencapsulated catechins (UN-Ca), CaNE, and FD-CaNE were fortified in pasteurized milk to improve its functional properties. FD-CaNE was shown to be the best at preserving total flavonoid content (TFC) and catechins' antioxidant activity (AA), retarding lipid oxidation, and inhibiting bacterial growth. In vitro gastrointestinal digestion test, digested CaNE and FD-CaNE showed better bioaccessibility of catechins by having higher percentage of TFC recovery and AA than the digested UN-Ca. This study has proved that FD-CaNE can be used as bioactive food ingredients to enhance the stability and bioaccessibility of catechins in food matrices and digestive system, and to improve quality and shelf life of catechins-fortified milk.

1. Introductions

Milk and dairy products play a crucial role in health-promoting benefits due to their high nutritional value based on the presence of necessary critical macro- and micro-nutrients (Cimmino et al., 2023). Although milk possesses numerous bioactive components, such as casein and whey proteins that are important for physiological and biochemical functions, several studies have shown that milk has a limited amount of phenolic compounds, which has the potential to prevent the oxidative stress-related diseases in humans, such as Alzheimer's disease, cancer, diabetes, obesity, and vascular disease. In response to the increased customer demand for healthier foods, food companies have tended to manufacture functional dairy products that incorporate additional bioactive ingredients, particularly phenolic compounds (Di Maio, Pittia, Mazzarino, Maraschin, & Kuhnen, 2019; Rezagholizade-shirvan et al., 2024).

Catechins, polyphenol compounds present in many plants and an important component of tea leaves, are an extremely interesting bioactive substance that can be used as a food fortification ingredient in a wide range of food products to provide potential health benefits (Rashidinejad et al., 2021). Besides enhancing the anti-oxidative function, reducing the oxidation of lipids and extending the product's shelf life are other advantages of adding catechins in food (Jansson et al., 2019; Ruengdech & Siripatrawan, 2021). However, their application is limited owing to the problems related with their stability (Bhushani, Karthik, & Anandharamakrishnan, 2016). Catechins are prone to degradation or deactivation by various environmental and processing conditions such as pH, temperature, oxygen, and light, resulting in the loss of their health benefits (Wang et al., 2022). Moreover, catechins have poor oral bioavailability resulting from instability in the gastrointestinal tract and limited membrane permeability across the intestine (Peng et al., 2018; Rashidinejad et al., 2021). Encapsulation, a technique that encloses active agents inside the matrix of a carrier material has been reported to improve the stability, controlled release, and bioavailability of bioactive compounds when incorporated into foods (Huang, Song, Li, & Guan, 2025; Xu et al., 2024). One of the

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encapsulation techniques widely used to improve the antioxidant activity (AA), antimicrobial property, and bioaccessibility of bioactive compounds is nanoemulsion system due to its small particle size (<200 nm) and high encapsulation capacity (Gadkari, Shashidhar, & Balaraman, 2017; Li et al., 2023; Ruengdech & Siripatrawan, 2021). Although nanoemulsion systems are beneficial, liquid-formed nanoemulsions have limited applications because various mechanisms, such as gravitational separation, flocculation, coalescence, and Ostwald ripening, can lessen emulsion's kinetic stability during storage (Gadkari et al., 2017). Moreover, there is a risk of microbial growth when nanoemulsions are stored at room temperature, resulting in a short shelf life. To overcome such problems, techniques have been developed to convert nanoemulsion from liquid to solid form such as powder, to avoid changes in the characteristics of nanoemulsions when stored for a long period and to enhance their industrial applications (Park et al., 2019; Ruengdech & Siripatrawan, 2022). Foam-mat freeze-drying (FMFD), a combination of a foaming and freeze-drying method, has been developed as an economical alternative to conventional drying for the encapsulation of nanoemulsions of bioactive chemicals and essential oils in order to preserve their functional activity and stability (Ruengdech & Siripatrawan, 2022). Pilong, Mishra, Ruengdech, and Siripatrawan (2023) suggested that foam-mat freeze-dried clove essential oil nanoemulsion can effectively preserve stability, antimicrobial activity, and major volatiles of clove oil as compared to the liquid-formed nanoemulsion.

Although catechins nanoemulsion (CaNE) have been currently reported, to the best of our knowledge, a practical application of foam-mat freeze-dried CaNE (FD-CaNE) in foods has never been investigated. This research is the first to study the functional stability of FD-CaNE, as a food fortification component, in a model food. Therefore, this research aimed at evaluating the stability of FD-CaNE in real food application and to investigate its effects on the qualities and shelf life of pasteurized milk. Physicochemical changes including color, titratable acidity (TA), pH, AA, and lipid oxidation of the catechins-fortified milk during storage were evaluated. The bioaccessibility of catechins during in vitro digestion was also investigated. Moreover, microbiological test was performed to assess the antimicrobial activity of the encapsulated catechins.

2. Materials and methods

2.1. Materials

Green tea catechins (Polyphenon 60), Tween® 80 (Polysorbate 80), and all digestive enzymes were purchased from Sigma-Aldrich Co. Ltd. (Taufkirchen, Germany). Maltodextrin (DE 10–15) and gum Arabic were purchased from Shandong Duqing Inc. (Heze, Shandong, China). Hydroxypropyl methylcellulose (Methocel™ K4M, methoxyl 19.0–24.0 % and hydroxypropoxyl 7.0–12.0 %, food grade) was supplied by Dow Chemical Company, USA. Medium Chain Triglyceride (MCT) oil was obtained from Talent Co., Ltd. (Bangkok, Thailand).

2.2. Fabrication of CaNE

CaNE fabrication started with a coarse emulsion preparation, MCT oil (7.5 % w/w) was gradually dropped into the aqueous phase which comprised of Tween® 80 (5 % w/w), Polyphenon 60 (1 % w/w) and deionized water and was vigorously mixed at 16,000 rpm for 5 min using a homogenizer (Antrieb X10/25, Ystral GmbH Maschinenbau + Processtechnik, Germany). The coarse emulsion was then sonicated at electrical power of 400 W, frequency of 24 kHz, and amplitude of 50 % for 10 min using an ultrasonic processor (UP400S, Hielscher Ultrasonics GmbH, Teltow, Germany). The sample was placed in an ice bath (4 ± 2 °C) throughout the emulsification process to maintain cool temperature and prevent sudden heat.

2.3. Preparation of FD-CaNE

FD-CaNE was produced by FMFD following the method of Ruengdech and Siripatrawan (2022). Hydroxypropyl methylcellulose (HPMC) gel was prepared by dissolving of HPMC (2 % w/w) in hot water at (60 °C). CaNE was mixed with a blend of maltodextrin (MD) and gum Arabic (GA) (5 % w/w) using a magnetic stirrer until completely dissolved. CaNE foam was generated by slowly pouring 30 mL mixed CaNE into 90 mL HPMC gel using an electric hand mixer (Kenwood HM320, Kenwood Limited, Havant, United Kingdom) for 10 min. The CaNE foam was then poured into the stainless-steel container, and stored in a freezer (Sanyo MDF-236, Sanyo Electric Co., Ltd., Osaka, Japan) at −35 °C for 24 h. A freeze dryer (Labconco model 195, Labconco Corporation, England) operating at −50 °C, 130 × 10^{-3} mbar was used to dried the frozen foam for 48 h. After the freeze-drying process, the dried foam sample was ground using a laboratory blender at high speed (Two-Speed 3390D25, Waring® Commercial Co., McConnellsburg, PA, USA), passed through a sieve (Mesh No.50), and sealed in an aluminum foil bag. The properties of CaNE and FD-CaNE are shown in Table S1.

2.4. Milk fortification

Commercial fresh pasteurized milk with 3.3 % (w/v) fat was fortified with different forms of catechins including unencapsulated catechins powder (UN-Ca), CaNE, or FD-CaNE at a final concentration of 1 mg catechins/mL. Non catechins-fortified milk was used as a control. The catechins-fortified milk and control were re-pasteurized at 72 ± 1 °C for 10 min by immersing the sample beakers in a water bath maintained at 75 °C, then rapidly cooling down to 25 °C using chilled water. Subsequently, the pasteurized milk was aseptically filled into 150 mL transparent polyethylene terephthalate (PET) bottles and stored at 4 °C.

2.5. Milk quality assessment

2.5.1. Color

Colorimetric parameters (L^*, a^*, and b^* representing lightness, redness, and yellowness, respectively) were obtained using a colorimeter (Colorflex, Reston, VA, USA). Color difference (ΔE) was calculated according to the equation given below:

$$\Delta E = \sqrt{\left(L_t^* - L_0^*\right)^2 + \left(a_t^* - a_0^*\right)^2 + \left(b_t^* - b_0^*\right)^2} \quad (1)$$

The Whiteness Index (WI) was determined using Eq. 2, while the Browning Index (BI) was computed using Eq. 3, based on the method outlined by Palou, López-Malo, Barbosa-Cánovas, Welti-Chanes, and Swanson (1999).

$$WI = 100 - \sqrt{\left(100 - L_t^*\right)^2 + \left(a_t^{*2} + b_t^{*2}\right)} \quad (2)$$

$$BI = \frac{x - 0.31}{0.17} \times 100; x = \frac{a_t^* + 1.75L_t^*}{5.645L_t^* + a_t^* - 3.012b_t^*} \quad (3)$$

where L_0^*, a_0^*, and b_0^* were the variable as brightness, redness, and yellowness at initial time and L_t^*, a_t^*, and b_t^* represented the variable as brightness, redness, and yellowness at any time 't'.

2.5.2. TA and pH

TA value was estimated as a percentage of lactic acid by titrating milk samples with 0.1 mol/L NaOH. A pH meter (SevenCompact™, Mettler-Toledo (Thailand) Ltd., Bangkok, Thailand) was used to measure the pH value of samples.

2.5.3. Microbiological property

To determine the microbial populations in pasteurized milk, the aerobic plate count (APC) was carried out in accordance with the guidelines from the U.S. Food and Drug Administration (FDA) (2001).

Ten milliliters of milk samples were added to 90 mL of 0.1 % (w/v) sterile peptone water, mixed for 30 s, and then serially diluted. The sterile plate count agar media was poured into a Petri dish containing one milliliter of inoculum from each dilution and kept at 35 °C for 48 h.

2.5.4. Total flavonoid content (TFC) and AA

Phenolic compounds present in milk samples were extracted following the procedure described by Di Maio et al. (2019). Briefly, 15 mL methanol was added to 5 mL milk in a screw-capped test tube. After 30 s shaking, the mixture was incubated for 40 min at room temperature (25 °C) before being centrifuged (4000 ×*g*, 15 min, 25 °C), and the supernatant was collected for analyses.

Estimation of TFC in milk samples was conducted by a spectrophotometric method using $AlCl_3$ as a derivatizing agent and rutin (RE) as a standard. In a test tube, milk supernatant (0.1 mL) was mixed with 5 % (w/v) $NaNO_3$ (0.2 mL). After 5 min incubation, 10 % (w/v) $AlCl_3$ (0.2 mL) and 1 mol/L NaOH (1 mL) were added, the mixtures were incubated for 15 min at room temperature. Absorbance of the samples and standards was measured at 510 nm using a double beam spectrophotometer (UV-1900i, Shimadzu corporation, Tokyo, Japan). TFC was calculated from the calibration curve and reported as mg RE/mL.

The AA of the catechins-fortified milk was evaluated by 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH) and ferric reducing/antioxidant power (FRAP) assays.

Milk contains naturally occurring phenolic compounds, particularly flavonoids, which possess antioxidant properties (Muniandy, Shori, & Baba, 2016). To fully understand the influence of nanoencapsulation and FMFD on the stability of catechins fortified in milk, the TFC and AA of unfortified milk (control) sample were calculated using Eq. (4).

$$Retention\ (\%) = \frac{C_{St} - C_{Ct}}{C_{Si} - C_{Ci}} \times 100 \quad (4)$$

where C_{Si} and C_{St} are the amounts of chemical (TFC, DPPH, or FRAP) values of catechins-fortified milk at the initial time and any time 't', respectively. C_{Ci} and C_{Ct} are the amounts of chemical values of control milk at the initial time and any time 't', respectively.

2.5.5. Oxidative stability

To evaluate the efficacy of catechins' AA, changes in primary and secondary lipid oxidation products occurring in milk during storage were measured. The lipid hydroperoxide (primary product) content of pasteurized milk, reported as peroxide value (PV), was determined following the American Oil Chemists Society (AOCS, 1995).

Thiobarbituric acid reactive substances (TBARS) were used to evaluate the development of malondialdehyde (MDA) and other aldehydes (secondary products). The TBARS method was adapted according to Siripatrawan & Makino (2025). Milk samples (1 mL) were diluted with distilled water (1:4 v/v) and combined with 4 mL sodium dodecyl sulfate (SDS) solution and 0.1 mL ethylenediamine tetraacetic acid (EDTA) solution before being vortexed for 10 s, and then incubated in a 95 °C water bath for 60 min. After cooling down to 25–29 °C in an ice bath, the samples were mixed with pyridine and butanol solutions and centrifuged at 1600 ×*g* for 20 min (25 °C). The absorbance of the top organic layer of each sample was measured at 532 nm by a spectrophotometer. The absorbance values were converted to milligram of malonaldehyde per kilogram of sample using a predetermined standard curve.

2.6. In vitro digestion

The fortified milk and control samples were subjected to a two-phase in vitro digestion, which included gastric and small intestinal phases according to the procedure explained by Yang and McClements (2013) with modifications. Briefly, the gastric phase (GP) of digestion was initiated by preparing simulated gastric fluid (SGF). NaCl (2 g), HCl (7 mL), and pepsin (3.2 g) were added into a flask and the final volume was made up to 1 L and then the pH was adjusted to 1.2 using 1.0 mol/L HCl. An aliquot (10 mL) of milk sample was diluted with 10 mL of distilled water and then mixed with an equal volume of GF (20 mL). The mixture was adjusted to pH 2.5 using 1 mol/L NaOH and incubated at 37 °C in a covered shaking water bath (95 rpm) for 2 h. After that, the gastric digesta (30 mL) was subjected to intestinal digestion phase (IP) by adjusting pH to 7.0 using 1 mol/L NaOH. Simulated small intestinal fluid (SIF) comprising 4.8 mg/mL pancreatic lipase (2.5 mL), 5 mg/mL bile extract solution

(4 mL), and 750 mmol/L $CaCl_2$ solution (1 mL) was added to the gastric digesta and incubated for 2 h at 37 °C in a covered shaking water bath (95 rpm). After completion of gastric or intestinal phases, an aliquot of the digested sample was centrifuged at 13,000 ×*g* for 15 min at 4 °C. A mixture of 1 % (*w/v*) ascorbic acid and 0.28 % (w/v) H_3PO_4 at a ratio of 9: 1 (*v*/v) was added into the supernatant to stop the degradation of catechins after digestion and stabilize them until TFC and AA measurement (Xie, Kosińska, Xu, & Andlauer, 2013). To avoid interferences induced by the digestive enzymes and buffers used in the digesting process, identical conditions were applied to a blank (without the added sample). The bioaccessibility of catechins was presented as the percentage of recovery of TFC, DPPH, and FRAP. The recovery was calculated by comparing the quantity of TFC, DPPH, and RFAP recuperated at the end of digestion to the total amount in the starting samples.

$$Recovery(\%) = \frac{C_{DS}}{C_{IS}} \times 100 \quad (5)$$

where C_{DS} and C_{IS} are the amounts of chemical (TFC, DPPH, or FRAP) values in the digested samples and in the initial samples of each phase, respectively, after subtracting the chemical values of the digested control milk.

2.7. Statistical analysis

Data were expressed as mean ± standard deviation which was obtained from the triplicate tests. Data were subjected to analysis of variance (ANOVA), and Duncan's new multiple range test was used to compare means at a 95 % confidence level.

3. Results and discussions

3.1. Appearance and color

Catechins are highly prone to degradation and epimerization during heating process and storage, leading to their losses and color changes. Previous studies confirmed that degradation of catechins cause apparent changes in the color of tea products, which may become unacceptable to consumers during distribution and storage (Jansson et al., 2017; Wang et al., 2022). The effect of catechins on the appearance of catechins-fortified milk is shown in Fig. S1. The color of pasteurized milk altered from white to pale pink color when catechins were added, most noticeably in the UN-Ca sample. A similar result was obtained by Jansson et al. (2019) who found that lactose-reduced UHT milk turned to pink color upon the addition of green tea extract. The apparent pink color is probably attributed to the larger molecular weight oxidation products, e.g., epigallocatechin gallate (EGCG) dimer (Liu, Kang, & Yan, 2021). These colored compounds are similar to the pinkish-brown substance developed when EGCG is heated or stored for long-term (Li, Taylor, Ferruzzi, & Mauer, 2013). The results revealed that milk containing nanoemulsified catechins had lighter shade of pink than that containing free catechins, demonstrating an effectiveness of the nanoemulsion system to prevent catechins oxidation. Moreover, with respect to consumer perception of milk, using CaNE or FD-CaNE may not have adverse effects on the organoleptic quality of milk.

To better understand the color changes during storage, colorimetric

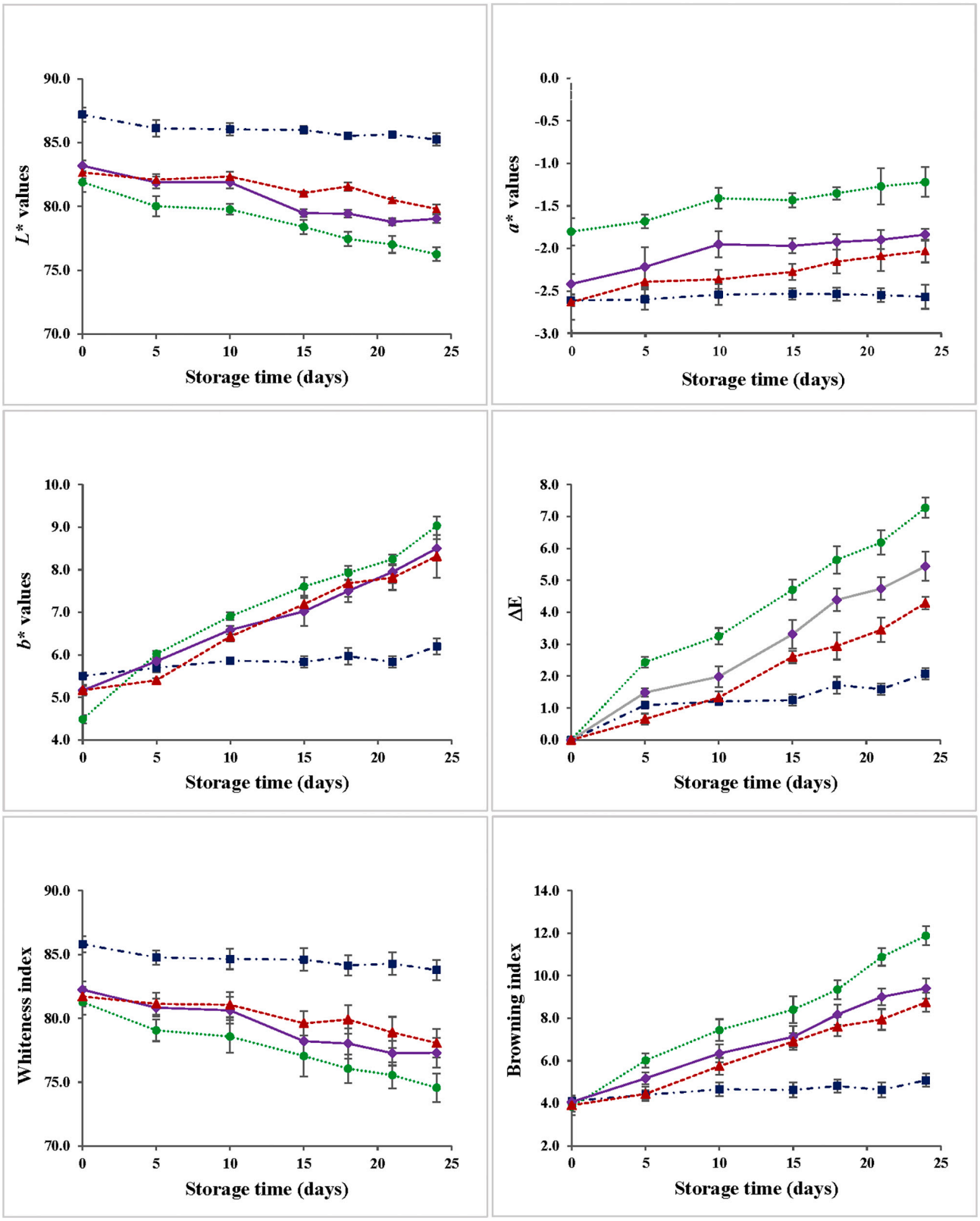

Fig. 1. color parameters (lightness, L^*; redness, a^*; yellowness, b^*; total color change, ΔE; whiteness index, WI; and browning index, BI) of unfortified and catechins-fortified milk during storage at 4 °C for 24 days.
(= 0, = 5, = 10, = 15, = 18, = 21, and = 24 days)

Table 1
Titratable acidity (TA) and pH values of unfortified and catechins-fortified milk during storage at 4 °C.*

Properties	Storage time (days)	Control	UN-Ca	CaNE	FD-CaNE
TA (%)*	0	0.155 ± 0.01^{ab}	0.152 ± 0.01^{a}	0.153 ± 0.01^{ab}	0.156 ± 0.01^{ab}
	5	0.157 ± 0.01^{ab}	0.153 ± 0.01^{ab}	0.155 ± 0.01^{ab}	0.154 ± 0.01^{ab}
	10	0.170 ± 0.01^{bcd}	0.166 ± 0.01^{abcd}	0.161 ± 0.01^{abc}	0.163 ± 0.01^{abc}
	15	0.191 ± 0.01^{efgh}	0.182 ± 0.01^{def}	0.175 ± 0.01^{cde}	0.177 ± 0.01^{cde}
	18	0.205 ± 0.01^{hi}	0.195 ± 0.02^{fgh}	0.187 ± 0.01^{efg}	0.190 ± 0.01^{efgh}
	21	0.223 ± 0.01^{jk}	0.207 ± 0.01^{hij}	0.196 ± 0.01^{fgh}	0.202 ± 0.01^{ghi}
	24	0.246 ± 0.01^{l}	0.229 ± 0.01^{k}	0.217 ± 0.01^{ijk}	0.215 ± 0.01^{ijk}
pH*	0	6.79 ± 0.02^{a}	6.76 ± 0.02^{ab}	6.74 ± 0.03^{ab}	6.74 ± 0.02^{ab}
	5	6.76 ± 0.03^{ab}	6.75 ± 0.02^{ab}	6.74 ± 0.02^{ab}	6.75 ± 0.03^{ab}
	10	6.71 ± 0.04^{bc}	6.71 ± 0.04^{bc}	6.72 ± 0.03^{bc}	6.73 ± 0.04^{bc}
	15	6.62 ± 0.03^{d}	6.66 ± 0.05^{cd}	6.67 ± 0.04^{cd}	6.66 ± 0.02^{cd}
	18	6.55 ± 0.03^{fg}	6.60 ± 0.02^{de}	6.62 ± 0.05^{de}	6.63 ± 0.03^{de}
	21	6.33 ± 0.04^{h}	6.51 ± 0.03^{g}	6.57 ± 0.03^{fg}	6.59 ± 0.04^{ef}
	24	6.08 ± 0.04^{j}	6.22 ± 0.04^{i}	6.28 ± 0.03^{hi}	6.33 ± 0.03^{h}

* Data are presented as mean (n = 3) ± standard deviation (SD). The different letters indicate a significant difference ($p < 0.05$) in the values.

parameters (in terms of L^*, a^*, b^*, ΔE, WI, and BI) of unfortified and catechins-fortified milk were measured as displayed in Fig. 1. The results revealed that L^* and WI values of milk fortified with catechins significantly ($p < 0.05$) decreased with increasing storage time, whereas a^*, b^*, ΔE, and BI markedly increased. However, there was no change in the chromatic properties of the unfortified milk (control) throughout the storage. The increases in a^* and b^* values revealed a formation of reddish and yellowish pigments as a result of catechins oxidation. The fact that catechins are easily oxidized when exposed to light or high temperature, and their byproducts (e.g., theaflavins, thearubigins, and theabrownins) cause a yellowish-brown color shift (Li et al., 2013). These results are consistent with the studies of Jansson et al. (2017) who found that a^* and b^* values of catechins-fortified milk increased during storage.

Color difference (ΔE) can be defined as the distance between two colors. It has been used to describe colors that a person can feel with an unaided eye as similar, identical, or wholly distinct. The ΔE value of all catechins-fortified milk noticeably increased throughout the storage time. UN-Ca sample had the highest ΔE value (7.27), followed by CaNE (5.44) and FD-CaNE (4.29) at the end of storage. According to the literature (Mokrzycki & Tatol, 2011), the ΔE value of 3.5–5 define that clear difference in color is noticed whereas $\Delta E > 5$ define that observer notices two different colors. Therefore, the results indicated that the color alteration of unencapsulated catechins was more drastic and perceptible than that of encapsulated catechins. When comparing the encapsulated samples with each other, FD-CaNE exhibited enhanced protection against oxidation. A foam matrix of HPMC, maltodextrin, and gum Arabic acted as a protective barrier, reducing catechin exposure to oxygen, light, and other external agents, thereby slowing down the oxidation process. As a result, catechins in FD-CaNE remained more stable, preserving their original color and antioxidant activity, leading to lower a^*, b^*, and ΔE values compared to CaNE (Ruengdech & Siripatrawan, 2022).

The WI is one of the most important quality parameters for milk products. Initially, WI of the unfortified milk (85.82) exhibited higher than those of catechins-fortified milk

(81.27–82.25). WI values of catechins-fortified milk reduced steadily during storage, however, there was no variation in unfortified milk. At the end of storage time, WI values decreased to 74.57, 77.31, and 78.07 for UN-Ca, CaNE, and FD-CaNE, respectively. WI values decreased as a result of the rise in the brown color product during storage (Jansson et al., 2017). Contrary to WI results, BI of samples significantly increased with storage duration, excepting that of the control. When compared among the catechins-fortified milk, the increasing rate of BI of both CaNE and FD-CaNE was lower than that of the UN-Ca. An increase in BI values might be attributed to both the accelerated Maillard reaction induced by catechins and the oxidation of EGCG, yielding dimers and quinones, which also produce brown pigments (Jansson et al., 2019).

According to the color results, the changes in colorimetric parameters of encapsulated catechins-fortified milk were less than those of unencapsulated one. These demonstrated that the prevention of color changes due to catechins oxidation and degradation during storage can be achieved by encapsulating catechins using a nanoemulsion system. Moreover, encapsulation of CaNE by FMFD could enhance the stability of catechins in milk.

3.2. TA and pH

Table. 1 presents the changes in TA and pH values of catechins-fortified milk throughout 24 days of storage at 4 °C. Initially, the TA values of all samples were comparable, ranging from 0.152 % to 0.156 %. The TA values of all samples increased with increasing storage time, however, the rates of TA increase in all catechins-fortified milk were less than that of the control. At the end of the storage period, the control had the highest TA value, followed by UN-Ca, CaNE, and FD-CaNE, respectively. However, the TA values of the catechin-treated samples were not significantly different. These results were supported by the fact that adding catechins (both non-encapsulated and encapsulated) to milk could lower total acidity (TA) during storage due to their antimicrobial properties, inhibiting the growth of lactic acid bacteria and other acid-producing microorganisms. Their mechanism of action includes disrupting bacterial cell membranes and interfering with enzymatic activities essential for bacterial metabolism (Najgebauer-Lejko, 2014).

On the other hand, pH value of the samples decreased throughout storage. The initial pH values of all samples ranged from 6.74 to 6.79, which were within the acceptable range (6.60–6.80) for commercial pasteurized milk (Ziyaina, Govindan, Rasco, Coffey, & Sablani, 2018). The pH value gradually decreased during the first 10 days of storage, but noticeably decreased thereafter. The pH value of the control at 21 days was 6.33, which was less than the standard limit (pH = 6.5), indicating that the sample was not acceptable (Ziyaina et al., 2018), whereas pH values of UN-Ca, CaNE, and FD-CaNE were 6.51, 6.57, and 6.59, respectively. However, the pH of all fortified milk dropped below 6.5 over 24 days of storage. The changes in pH values were directly related to lactic acid formation during storage, with a strong correlation between pH and TA values ($R^2 = 0.912$).

3.3. Microbial growth

Microbiological analysis is generally used to indicate the shelf life of pasteurized milk because microbial metabolism significantly affects food safety, food quality, and human health. According to Notification of the Ministry of Public Health of Thailand (2013), Re: Other milk products (No. 352), the APC in pasteurized milk should not exceed 5×10^4 CFU/mL (4.7 log CFU/mL) at all times from manufactured date to the expiry date on the label.

Fig. 2 illustrates APC of unfortified (control) and catechins-fortified pasteurized milk during 24 days of storage at 4 °C. An increasing trend of microbial growth in all samples was observed from the processing day up to the end of storage and it was found that the highest

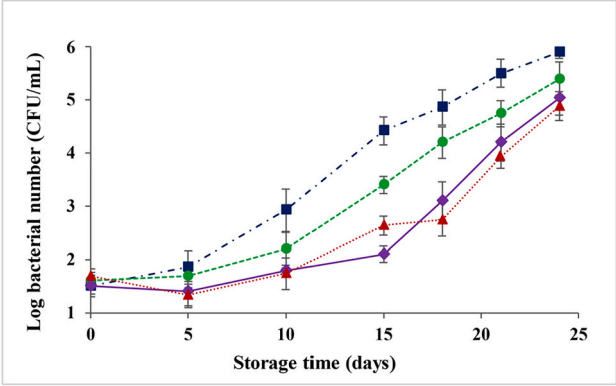

Fig. 2. Aerobic plate count (APC) of unfortified and catechins-fortified milk during storage at 4 °C for 24 days.
(= 0, = 5, = 10, = 15, = 18, = 21, and = 24 days)

microbial number was in the control sample considered at the same storage time. At the initial storage time, the APC of all samples were below 2.0 log CFU/mL. A sharp, statistically significant ($p < 0.05$) increase in APC, was noted at 15-day of storage, and subsequently, there was a significant difference ($p < 0.05$) in the microbial count between the catechins-fortified samples and the control. The APC of the control exceeded the standard limit on the 18-day of storage whereas that of UN-Ca, CaNE, and FD-CaNE were 4.20, 3.15, and 2.75 log CFU/mL, respectively, which is still considered fit for consumption. The lower microbial growth of the catechins-fortified samples was probably attributed to the antimicrobial efficacy of catechins (Siripatrawan & Noipha, 2012). Finally, the number of total microorganisms of UN-Ca and nanoencapsulated catechins simples were above 4.7 log CFU/mL on the 21- and 24-day of storage, respectively. The lower microbial growth of encapsulated catechins samples indicated that the nano-encapsulated catechins had higher antibacterial action than free-formed catechins. These behaviors could be explained by the fact that smaller droplets facilitate the penetration of bioactive compounds through the bacterial cell wall, resulting in being more efficient at destroying bacteria (Chuesiang et al., 2019; Kongboonkird, Chuesiang, Ryu, & Siripatrawan, 2024). These results were consistent with the study of Ruengdech and Siripatrawan (2021) who found that CaNE could extent the shelf life of high pressure processed coconut milk better than unencapsulated catechins.

3.4. TFC and AA stability

Fig. 3 summarizes the percentage of TFC retention and AA of catechins-fortified milk during 24 days of storage at 4 °C. The results showed that there was a marked difference in the retention of TFC in three catechins-fortified milk samples (Fig. 3a). Non catechins-fortified milk had an initial TFC of 0.18 ± 0.01 mg RE/mL, whereas catechins-fortified milk had TFC ranging from 0.67 to 0.73 mg RE/mL at the beginning of storage. The TFC of catechins-fortified milk obtained by the flavonoid test was lower than the predicted values (estimated from the additional quantity), which may be attributed to an interaction between particular catechins and milk proteins, leading to a formation of (+) catechin-β-casein. As a result, free surface hydrophobic sites that react with the TFC analytical reagents decreased, resulting in a marked decrease in free flavonoids (Yuksel, Avci, & Erdem, 2010). The TFC retention of all samples significantly ($p < 0.05$) decreased with increasing storage time. The decrease in flavonoids of the catechins-fortified milk might be attributed to catechins epimerization and oxidation (Kim et al., 2020). FD-CaNE had the highest TFC retention (55.59 %) at the end of the shelf life (day-24), followed by CaNE (47.49 %) and UN-Ca (26.29 %). The higher TFC retention of the encapsulated catechins samples indicated that encapsulating catechins in oil droplets via nanoemulsion could enhance catechins stability by preventing it from reacting with external stimuli, e.g., O_2, UV-light, and metal ions (Ruengdech & Siripatrawan, 2021). These results agree with those of Gadkari et al. (2017) who found that the emulsification system provided a protective barrier to emulsified green tea extract which preserved higher polyphenol content and AA than unemulsified green tea catechins. Interestingly, the results showed that FD-CaNE could preserve higher catechins in milk during storage than CaNE. This could be explained by the fact that nanoemulsified catechins encapsulated by the FMFD approach are entrapped not only in oil droplets but also in the matrix of wall materials (HPMC, MD, and GA), which provides a strong structural support to prevent catechins from interacting with substances in the food systems or being destroyed by the stimuli in the environment (Sarabandi, Jafari, Mahoonak, & Mohammadi, 2019).

To confirm the protection efficiency of nanoemulsion system, the retention of AA for catechins-fortified milk was investigated and presented as DPPH and FRAP values (Fig. 3b and c). The results of AA were consistent with those of TFC retention, with DPPH and FRAP levels decreasing with storage time. At the end of the storage time, the retention of DPPH (27.56 %) and FRAP (30.98 %) of UN-Ca samples was lower than that of emulsified catechins samples. This result is similar to the previous reports which suggested that the CaNE could retain AA of catechins (DPPH and FRAP) by 8–12 % more than free catechins when fortified in high-pressure processed coconut milk and stored at 4 °C for 21 days (Ruengdech & Siripatrawan, 2021). Moreover, the DPPH and FRAP recovery of FD-CaNE during storage were higher than those of CaNE. Similar results were obtained by Zokti, Sham Baharin, Mohammed, and Abas (2016) who worked on the encapsulated catechins extracts by spray drying using MD, GA, and chitosan as wall materials to enhance the antioxidant efficiency of mango drinks. The encapsulated catechins were more stable in the supplemented mango drinks than the unencapsulated catechins, resulting in better preserving AA in the mango drinks during storage. The decrease in AA of catechins-fortified milk with storage time was considered to be attributable to the decline in bioactive components of catechins. With increasing storage periods, catechins with high AA, namely EGCG, epicatechin (EC), and epicatechin gallate (ECG) are epimerized to non-epimerized catechins with lower activity; gallocatechin-3-gallate (GCG), catechin (C), and gallocatechin (GC), respectively (Kim et al., 2020).

3.5. Oxidation stability

The evolution of lipid oxidation, reported as PV and TBARS, of unfortified and catechins-fortified milk during 24 days of storage is shown in Fig. 4. The results showed that lipid oxidation of all milk samples

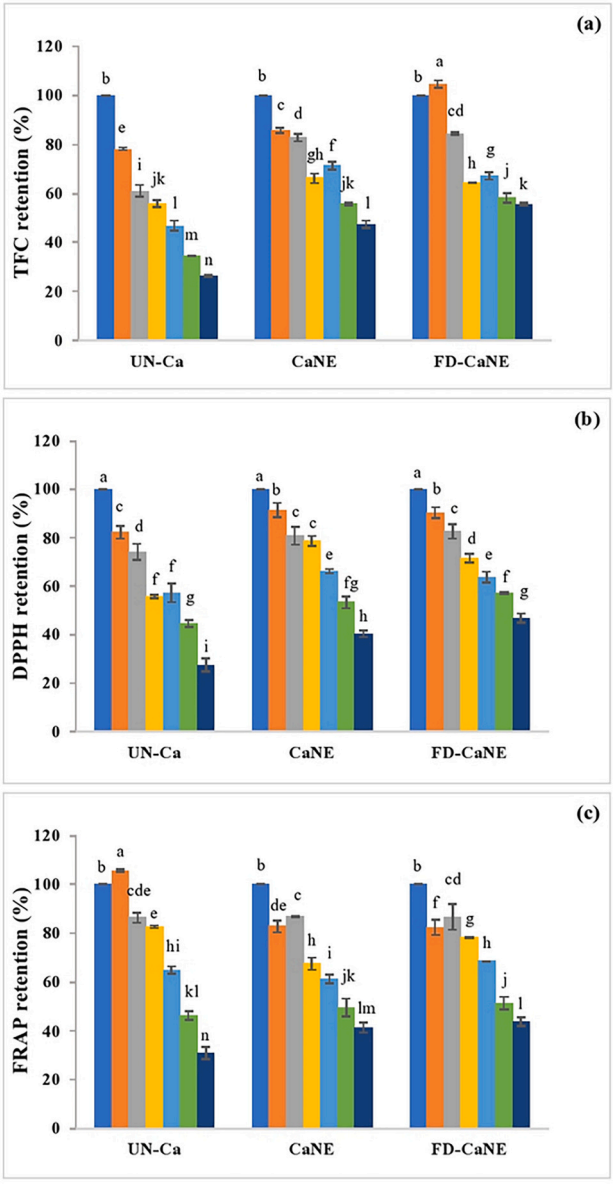

Fig. 3. Retention of TFC (a) and antioxidant activity (DPPH (b) and FRAP (c)) of catechins-fortified milk during storage at 4 °C for 24 days.

(Control UN-Ca CaNE FD-CaNE)

increased with increasing storage time. The PV of unfortified milk markedly increased from 3.6 to 31.7 meq O_2/kg during the first 18 days before slightly increasing to 32.2 meq O_2/kg at the end of storage duration (Fig. 4a). This suggests that in the latter stage of milk fat oxidation, the rate of hydroperoxide breakdown to secondary oxidation products predominated that of hydroperoxide formation (Lan et al., 2024). The TBARS of the control sample was higher than those of the others throughout the storage duration, ranging from 0.182 to 1.61 mg MDA/kg. As expected, catechins could significantly inhibit lipid oxidation in pasteurized milk during storage at 4 °C. All catechins-fortified milk had lower hydroperoxide and malondialdehyde production rates than control sample. These results agree with the study of Jung (2011) who found that green tea catechins (25 ppm) inhibited hydroperoxide and malonaldehyde formation in milk after 30 h of light exposure by 82.1 % and 75.0 % inhibition, respectively.

The oxidation inhibition efficiency of catechins in different systems was in the descending order as FD-CaNE > CaNE > UN-Ca. The TBARS values of the UN-Ca sample were equivalent to those of the encapsulated catechins-fortified milk up to day-15 of storage. After 15 days, MDA generation in the UN-Ca milk increased more rapidly than in the samples fortified with encapsulated catechins ($p < 0.05$). At the end of storage time, TBARS value of the UN-Ca (1.08 mg MDA/kg) was significantly higher than that of CaNE (0.828 mg MDA/kg) and FD-CaNE (0.787 mg MDA/kg). These results could be explained by the fact that there was considerable degradation and oxidation of unencapsulated catechins during the storage, implying a reduction in their antioxidant properties. The control oxidation results were consistent with the AA results, suggesting that nanoencapsulation can enhance the stability of bioactive components and AA of catechins in catechins-fortified milk during storage.

The results obviously suggested that FD-CaNE enhances its ability to inhibit lipid oxidation more effectively than unencapsulated catechins. These behaviors were probably explained that the HPMC foam matrix acts as a protective barrier, shielding catechins from environmental factors, which can accelerate oxidation and degradation. This encapsulation could preserve catechins' antioxidant activity as well. Additionally, nanoemulsification enhances catechins' stability and prolongs their antioxidant effects in lipid-based systems (Li et al., 2023). Another key mechanism is the improved interaction between nanoemulsified catechins and lipid radicals, as nanoemulsions facilitate catechins' localization at the oil-water interface, where lipid oxidation begins. This proximity allows catechins to more effectively neutralize lipid peroxyl radicals, slowing the oxidation chain reaction and extending their protective effects (Ganguly et al., 2024).

3.6. *In-vitro bioaccessibility of catechins*

The effect of nanoencapsulation on catechins bioaccessibility was studied in comparison to unencapsulated catechins by digesting them under the simulated gastrointestinal system. The percent recovery of TFC and AA measured by DPPH and FRAP assays of the digested catechins-fortified milk are shown in Fig. 5. The control sample was also digested, and its TFC and AA were determined. The chemical values obtained from the control were subtracted from the samples with catechins. The TFC recovery of nanoencapsulated catechins samples in both GP and IP were significantly ($p < 0.05$) higher than those of the unencapsulated catechins as shown in Fig. 5a. The total TFC recovery of unencapsulated catechins after two phases of digestion was only 29.07 %, whereas CaNE and FD-CaNE exhibited 43.34 % and 49.88 %, respectively. The results indicated that the nanoencapsulation technique significantly preserved catechins in simulated gastrointestinal conditions. This was probably due to the MCT oil engulfing and protecting parts of the catechins from degradation during digestion (Peng et al., 2018). These observations agreed with those of Bhushani et al. (2016) who found that the nanoemulsion system with 10 % (*w/v*) sunflower oil may reduce the degradation of major catechins in vitro simulated conditions with improving the percentage of recovery of nanoemulsified catechins in GP and IP by 53.18 % and 26.20 %, respectively, when compared to unencapsulated catechins. Noticeably, the TFC recovery of IP was significantly ($p < 0.05$) lower than that of GP in all samples. These results agree with the studies of Peng et al. (2018) who reported that the stability of catechins during IP was considerably ($p < 0.05$) lower than GP (based on statistical comparison of GP and IP recovery of catechins). These results are supported by the fact that the degradation of catechins is pH dependent and they are susceptible to auto-oxidation under neutral or slightly alkaline conditions. Since pH values of the GP and IP during in vitro digestion were 2.5 and 7.0, respectively, the loss of catechins was observed to be higher under intestinal conditions.

FD-CaNE showed a significant ($p < 0.05$) higher TFC recovery of intestinal digestion than CaNE. The higher bioaccessibility of catechin nanoemulsions encapsulated within a foam matrix composed of HPMC foam matrix (including HPMC, maltodextrin, and gum Arabic)

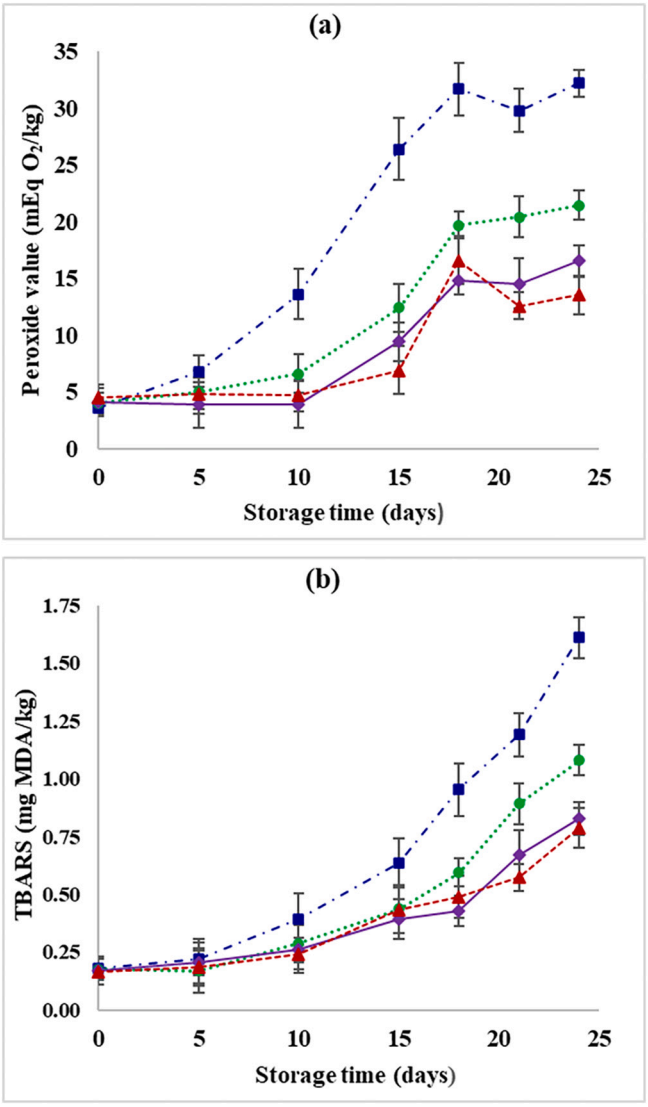

Fig. 4. Peroxide (a) and TBARS (b) values of unfortified and catechins-fortified milk during storage at 4 °C for 24 days.
(= 0, = 5, = 10, = 15, = 18, = 21, and = 24 days)

compared to unencapsulated catechins, can be attributed to several factors. However, the primary reason is likely their protective barriers to catechin degradation in the digestive system. The HPMC matrix possesses protective properties that help prevent catechin degradation caused by various factors, such as oxygen, and pH change including digestive enzymes, which can induce oxidation and structural breakdown, leading to a loss of antioxidant activity. The presence of an appropriate encapsulation system plays a crucial role in minimizing these losses, thereby preserving the functional properties of catechins. The previous studies reported that the bioavailability of the catechins increased by more than 50 % (Son, Chung, Ko, & Shim, 2016) when coated with hydroxypropyl methylcellulose phthalate (HPMCP), a phthalic acid ester of HPMC, indicating that coating with coating agent such as HPMCP could be an effective way to improve the catechins stability. Moreover, the study found that the encapsulation of phenolic compounds using various wall materials, including maltodextrin (MD), microcrystalline cellulose (MCC), hydroxypropyl methylcellulose (HPMC), and xanthan gum, at optimal concentrations, resulted in a maximum bioaccessibility of 99 %. This finding indicates that these microparticles effectively protect phenolic compounds from degradation as they pass through the intestinal tract (Fernández-Repetto, Gómez-Maqueo, García-Cayuela, Guajardo-Flores, & Cano, 2023).

The percentage of AA recovery measured with DPPH and FRAP assays of the catechins-fortified milk under simulated gastrointestinal conditions are shown in Fig. 5b and c. The AA of the UN-Ca sample was reduced by more than 40 % in GP and higher than 50 % in IP. As a result, total AA recovery decreased by almost 70 %, which could be related to the reduction of flavonoid compounds in the digested milk. The AA of encapsulated catechins were likewise reduced by gastrointestinal digestion, however to a smaller amount than free catechins. The total DPPH and FRAP recovery for CaNE and FD-CaNE were more than 40 % which were higher than those of UN-Ca, indicating that the AA can be preserved by the nanoemulsion technique. Although FD-CaNE had a slightly higher AA recovery than CaNE during digestion in GP and IP, they exhibited no differences in maintaining DPPH and FRAP values of catechins in milk after complete digestion.

4. Conclusion

Pasteurized milks fortified with catechins formed pink color and had higher antioxidant activity, less lipid oxidation, lower microbial growth, and longer shelf life than unfortified milk. By employing nanoemulsion

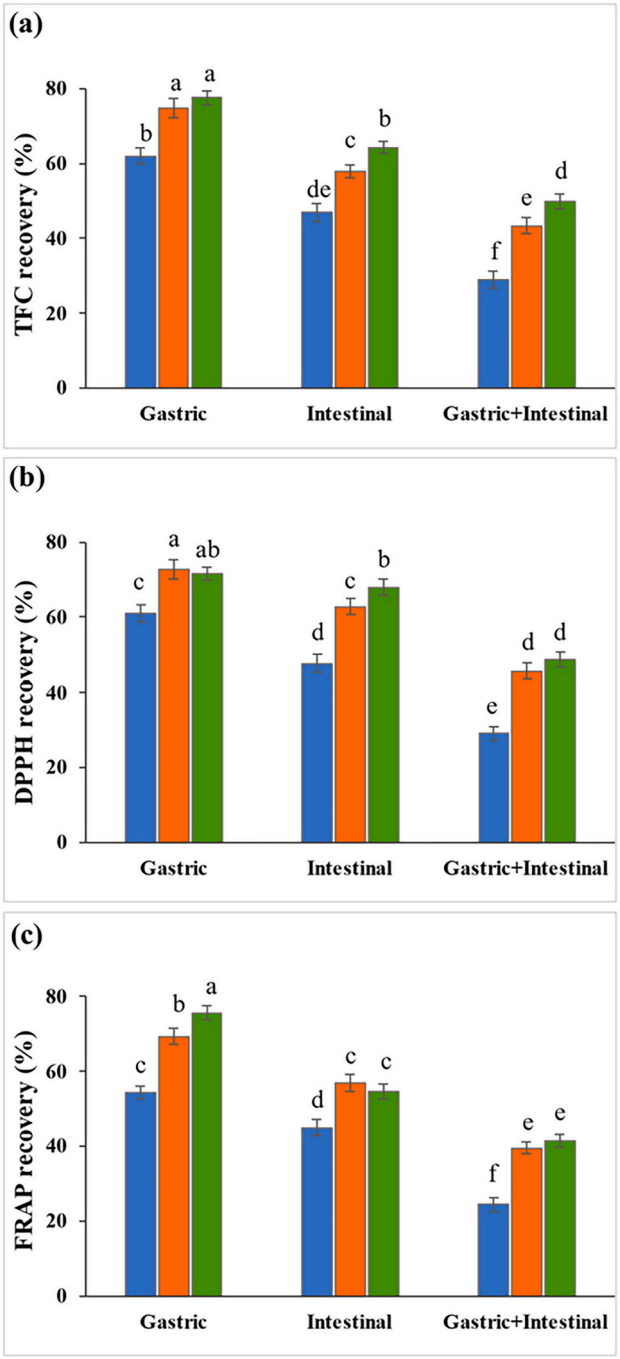

Fig. 5. Percentage of digestive recovery of TFC (a) and antioxidant activity (DPPH (b) and FRAP (c)) of catechins-fortified milk following in vitro gastrointestinal digestion.

(= UN-Ca = CaNE = FD-CaNE)

in combination with foam-mat freeze-drying techniques, the stability of catechins fortified in milk can be improved. FD-CaNE showed the lowest color change and the highest retention of TFC and AA, presumably because catechins were encircled with oil droplets and covered by polysaccharide-based wall materials, playing a role as a barrier to protect catechins from being damaged by external stimuli. Due to the higher amount of preserved active catechins during storage, the increasing rate of TA value, microbial growth, and lipid oxidation observed in the FD-CaNE sample were lower than that in other samples, resulting in a longer shelf life. Additionally, FD-CaNE showed a higher recovery of AA and flavonoid content in the gastrointestinal system, suggesting that FD-CaNE can enhance the bioaccessibility of catechins, which possibly raises their potential health benefits.

This study has demonstrated the feasibility of using FD-CaNE as food fortification in milk to increase the nutritional and antioxidant values, as well as to preserve desirable quality of milk. The product is expected to meet the consumer demand for nutritious and healthy beverages, making it desirable for commercialization. However, the results showed that milk fortified with CaNE had slightly different color from regular milk. Therefore, it is of great importance to investigate the consumer acceptance for the catechins-fortified milk to ascertain the product marketability. The sensory evaluation is, hence, recommended for our future research.

CRediT authorship contribution statement

Anchalee Ruengdech: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Dharmendra K. Mishra:** Writing – review & editing, Data curation. **Ubonrat Siripatrawan:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Ethical statement

The research presented does not involve any animal or human study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102391.

Data availability

Data will be made available on request.

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