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Research Article

Sodium Caseinate–Tannic Acid Antioxidant Complex Act as a High Internal Phase Pickering Emulsions Stabilizer

Atefeh Nourabi[1,2], Hesam Mashhadi[1,2], Mahnaz Tabibiazar[2*], Hamed Hamishehkar[3]

[1]*Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2]*Department of Food Science and Technology, Faculty of Nutrition and Food Science, Tabriz University of Medical Sciences, Tabriz, Iran.*
[3]*Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

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Abstract

Background: Emulsion-based formulations have gained attention in food and pharmaceutical products due to their unique properties. However, their use in food product formulation faces problems because of phase separation and fat oxidation, which seriously reduces the quality of the product. Therefore, finding ways to increase the physical and oxidative stability of emulsions is valuable. This work aimed to assess of physicochemical properties of sodium caseinate (SC)-tannic acid (TA) complex treated with heating and ultrasonication as well as their efficiency in physical and oxidative stability of high internal phase Pickering emulsion (HIPPEs) as antioxidant colloidal particles.

Methods: SC 1% (w/v) was mixed with a different concentration of TA (0.1, 0.3, 0.5, 0.7, 1% w/v) and the pH of solutions was adjusted to 9. The unheated, heat-treated, and ultrasonicated SC-TA complexes were analyzed to elucidate possible interaction using FTIR, fluorescence spectroscopy, and DSC. Finally, HIPPEs were prepared by mixing the prepared samples and soybean oil at a volume ratio of 25:75 v/v respectively. The microstructure of the most stable HIPPEs was assessed using SEM.

Results: Based on the FTIR results the covalent bond in heat-treated SC-TA was formed via the Maillard reaction. In addition, the reduction of free amino groups confirms Schiff base formation. All treated SC-TA samples showed a superior ability to stabilize emulsion in comparison with native SC when used as an aqueous phase of HIPPEs. The long-term physical was observed in heat-treated SC-TA HIPPEs for over two months. In PEs stabilized by SC-TA complex nanoparticles, primary and secondary oxidation product levels were significantly lower than in SC alone.

Conclusion: The fabricating antioxidant emulsions using heat-treated SC-TA are a good guarantee for the physical and oxidative stability of food formulations due to TA's intrinsic antioxidant properties and the protective role of SC-TA colloidal particles against coalescence.

Introduction

Pickering emulsions (PEs) are emulsions that are formed by the adsorption of solid particles to the oil-water interface.[1,2] So far, PEs have been fabricated with various solid colloidal particles, including silica, titania nanoparticles, cellulose, and protein-based colloidal particles.[1,3] The stability of these emulsions depended on the physicochemical properties (shape, size, wettability, and surface charge) of the adsorbed particles and the viscoelastic behavior of the interface layer.[4] Wetting of the particles with water or oil phases plays a decisive role in them. PEs can be stabilized using partial wetting of particles by oil and water, which is evaluated by measuring contact angles.[3]

Emulsions stabilized with solid colloidal particles are defined as high internal phase Pickering emulsions (HIPPEs) where the volume ratio of the dispersed phase to the continuous phase is greater than ($j \geq 0.74$).[1,3,5] The stabilizers for HIPPEs should be capable of reducing the interfacial tension between water and oil phases, forming an interfacial film, and adsorbing rapidly at the interface. In addition to being highly resistant to coalescence and flocculation, HIPPEs are widely used in pharmaceutics, cosmetics, and food industries.[1,3] Oxidative degradation of HIPPEs due to high volumes of edible oils led to off-flavor formation and nutritional quality deterioration. Based on previous reports, phenolic acids could be inhibiting lipid peroxidation due to their antioxidant activities. The antioxidant activity of phenolic acids can also be enhanced

*Corresponding Author: Mahnaz Tabibiazar, E-mail: mahnaz_tabibiazar@yahoo.com & tabibiazarm@tbzmed.ac.ir

by fabricating a dense interface layer with proteins or polysaccharides. However, there are few reports on antioxidant HIPPEs stabilized by protein-polyphenol or polyphenol-polysaccharide complex particles. Designing emulsion systems with antioxidant properties is essential because it provides a suitable template for protecting oxidation-sensitive materials.[6]

The current study used sodium caseinate (SC) as a proline-rich protein with favourable emulsification properties. SC's adsorption and stabilizing behaviour have been extensively studied as an emulsifier.[7,8] SC adsorbs quickly at hydrophobic surfaces and creates the protein layer around the oil droplet, stabilizing the oil-in-water emulsion through electrostatic and steric forces. Emulsions with a 20% internal phase can be stabilized by using native SC with a concentration of 0.2% to 2%.[3,9] Various factors including environmental changes (freeze, thaw, and pH) can affect the stability of native SC-stabilized emulsions and may induce coalescence and flocculation of emulsions.[8] To stabilize HIPPEs using SC, protein modification is inevitable. Therefore, changing the properties of SC may be an effective method to make it act as a Pickering-type stabilizer.[4] It is well known that protein-polyphenol interactions can alter protein structure and hence its functional properties.[10] Tannic acid (TA) is a water-soluble phenolic acid found in many plants as a gallic acid polymer glycoside.[7,11,12] TA is widely used as a functional food additive and exhibits excellent radical scavenging, antioxidant and antimicrobial activity and lots of biological properties such as anti-mutagenic and anti-carcinogenic effects.[7,12] TA contains active functional groups such as phenolic hydroxyl groups, hydroxyl, and carboxyl groups. The high level of phenolic hydroxyl groups (>> 25) in TA, enables it to complex with proteins, polysaccharides, alkaloids, and other substances,[7,13,14] in non-covalent interactions such as ionic pairing and hydrogen bonding.[13] TA can interact with proteins over a wide pH range via hydrophobic and hydrogen bond mediated or covalent C=N bonds under alkaline conditions.[13,15,16] Hydrogen bonding between hydroxyl groups may affect the formation of hydrophobic areas.[17] It has been reported that increased electrostatic repulsions improved the stability of emulsions formed by ovalbumin-TA.[16]

This study investigated the effect of TA interaction with SC at alkaline pH under heating and ultrasonication on the structure and function of SC and their ability to form HIPPEs. Further, the oxidative and physical stability of SC-TA stabilized emulsions were assessed.

Methods

Materials

TA (purity >99% wt) and SC (protein content of about 90%) were purchased from Sigma-Aldrich Chemical Company, USA, and stored at 4°C in the refrigerator. Soybean oil was purchased from a local supermarket. Sodium hydroxide (0.1 M NaOH) was used to adjust the pH of the samples. Deionized water was used throughout the study. All other chemicals used were of analytical grade.

Preparation of colloidal suspension

The SC solution 1% w/v in water was stirred at room temperature (25 °C) for 1h to complete hydration. Accordingly, the SC was mixed with a different concentration of TA (0.1, 0.3, 0.5, 0.7, 1% w/v), and all solutions were adjusted to pH 9.0 by using NaOH solution and stirred for 2 h. Then samples were treated in three modes: un-heated, heat-treated (80 °C for 1h), and ultrasonic-treated (Sonopuls HD 3100, Bandelin electronic, Germany) 20 kHz sonicator, for 10 minutes with 5 s on-time/5 s off-time). All samples were kept inside the ice pack to avoid temperature rise during operation. The solutions were allowed to stand overnight at 4 °C to ensure the formation of complexes before the preparation of emulsions.

Preparation of emulsions

The HIPPEs were prepared by mixing the prepared colloidal suspensions and soybean oil at a volume ratio of 25:75 v/v respectively. Ultrasonic homogenizer (Sonopuls HD 3100, Bandelin electronic, Germany) 20 kHz probe sonicator, was used for 4 minutes with 1 s on-time/1 s off-time to provide homogeneity. All emulsions were 10 mL in volume and kept inside the ice pack to prevent temperature rise during operation. The physical stability emulsion was monitored during storage.

Scanning electron microscopy (SEM)

In order to analyze the microstructure of SC and SC-TA emulsion samples, the samples were diluted 250-fold with water. The 10 µL of diluted sample was transferred in a glass coverslip until dry at room temperature. SEM photographs were taken using SU8010 (Hitachi, Co. Ltd., Japan). To coat the samples, a thin layer of gold was applied (< 20 nm) using a sputter (DST1, Nanostructured Coating Co., Iran). An excitation voltage of 25 kV was used in taking the photographs.

The particle size and zeta potential mmeasurements

the particle size and zeta potential of colloidal suspensions and emulsion samples were measured using dynamic light scattering (Zetasizer Nano-ZS90, Malvern Instruments Ltd., UK). Before analysis, the colloidal suspension and emulsion samples were diluted with water 100 and 250 fold, respectively.

Fluorescence spectroscopy

Fluorescence spectroscopy was used to investigate possible complexation between SC and TA at 25 °C. The SC solution was prepared at a constant concentration (1% w/v) and a defined amount of TA was added. The intrinsic fluorescence of SC and different treatments of SC-TA nanoparticles were measured using a spectrofluorometer (FP-750, Jasco, Japan). The excitation wavelength SC was set at 280 nm, and the emission spectrum was recorded

between 290 and 900 nm.

Contact angle measurement

For the determination of surface energy or adhesion energy, the three-phase contact angle of colloidal particles was measured using an OCA 20 AMP (Dataphysics Instruments GmbH, Germany). Tablets with dimensions of 13×2 mm were prepared from freeze-dried powder. A 5 µL of water droplet was placed on the tablet's surface and after 4 minutes of rest, the image was taken using a high-speed video camera. Contact angle measurements are repeated and averaged for each sample with three drops and three separate pellets.

Fourier transform infrared spectroscopy (FTIR)

The Fourier transform spectrophotometer (PerkinElmer, UK) measured the infrared spectra of freeze-dried colloidal suspension samples from 400 to 4000 . In order to analyze the data, Omnic software v8.0 was used (Thermo Nicolet, USA).

Differential scanning calorimetry (DSC)

The thermal behavior of freeze-dried colloidal suspension powders was examined using a Q100 model DSC (TA Instruments, DA). Aluminum pans were used to heat the hermetically sealed samples from 25 to 250 °C at a steady rate of 5 °C / min, and an empty aluminum container was used as a reference. Analyzes were performed under a nitrogen flow rate of 20 mL/min to prevent oxidation.

Measurement of free amino group content

Free amino groups were quantified as described by Habeeb with a slight modification.[18] Briefly, both 1 mL of 4% NaHCO3 (pH 8.5) and 1 mL of 0.1% 2.4.6-trinitrobenzene sulfonic acid (TNBS) were transferred to 1 mL of SC-TA heat-treated solution (5 mg/mL), followed by mixing and incubating at 40°C for 2 h in a covered water bath to avoid light. Then, 10 % (w/v) sodium dodecyl sulfate was added prior to terminating the reaction by adding 0.5 mL HCl (1.0 N). The sample was then set at room temperature for 15 min before recording the absorbance at 340 nm using a Shimadzu UV-1100 PC model spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Oxidative stability measurements

The lipid hydroperoxides measurement

To determine the LH of emulsions, measurements were taken immediately after preparation and two months after that using the AOCS Official Method Cd 8b-90 (AOCS, 2011). LH was calculated as milliequivalents of O2 per kilogram of oil based on Equations (1):

$$LH = (N \times (S-B) \times 1000)/W \quad (1)$$

where S is the volume of $Na_2S_2O_3$ used to titrate the sample, B is the volume of $Na_2S_2O_3$ used by the blank, M is the molarity of $Na_2S_2O_3$, and W is the mass of the sample (g).

Determination of malondialdehyde (MDA)

MDA was determined as a secondary oxidation product of lipid oxidation by using Yang and Xiong's method.[19] An aliquot (1 mL) of the sampled emulsion was mixed with 2 mL of thiobarbituric acid (TBA) test solution (15% trichloroacetic acid and 0.375% TBA dissolved in 0.25M HCl). In the water bath, the mixture was boiled for 15 min and cooled to ambient temperature. Afterward, the mixture was filtered through a 1.2 µm microporous membrane filter. MDA was determined by recording the filtrate at 532 nm on the same UV spectrophotometer using an external standard (1,1,3,3 tetra ethoxy propane).

Determination of released free fatty acids (FFA)

An FFA value is defined as the amount of KOH (in milligrams) needed to neutralize the free fatty acids in 1 g of oil sample. Determination of the FFA value in stable fresh emulsion and after 2 months were done according to the AOCS Official Method Cd 3d-63 (AOCS, 2009) and the following Equations (2):

$$FFA = (A-B)M \times 56.1/W \quad (2)$$

This equation takes the following variables into account: A represents the volume of standard alkali used in the titration (mL); B represents the volume of standard alkali used in the blank titration (mL); M represents the potassium hydroxide concentration (mol/L); and W represents the mass of the oil sample (g).

Antioxidant activity

The antioxidant activity of the SC-TA complex was measured using DPPH (2, 2-diphenyl-1-picrylhydrazyl), radical scavenging activity. Firstly, 100 µL of emulsion sample and 2 mL DPPH solution (0.1 mM) were mixed and incubated for 25 min at 25 °C in dark conditions. After that, by using UV spectrophotometry (Pharmacia Biotech Ltd., UK) at 517 nm, absorbances of samples and were measured. Radiation scavenging activity was calculated using the following Equations (3):

(3) DPPH activity % = (1-() × 100)

Statistical analyses

Statistical analyzes were performed using SPSS version 27.0.1 (SPSS Inc., Chicago, USA), ($p < 0.05$) and Kruskal-Wallis test. All measurements were performed in triplicate.

Result and discussion

Characterizations of SC-TA complex

The stable colloidal suspensions were prepared when the SC and TA concentrations ratio was set to 1: 0.1% w/v. Table 1 shows the particle size, PDI, zeta potential, and contact angles of SC and SC-TA complexes before (unheated) and after heating and ultrasonication treatment in pH-adjusted samples (pH 9.0). The particle size of pH-adjusted SC was greater than the native SC (without pH adjustment). In accordance with previous findings, increasing pH causes an increase in particle size because at alkaline pH, the negative charges of the protein chain increase, and as a result, the

Table 1. Characterization of prepared colloidal suspensions at pH 9.0 (SC/TA ratios is 1: 0.1% w/v).

Aqueous phase	Contact angles (θ)	Colloidal Suspension			Free amino group content (nmol/mg protein)
		Particle size (nm)	PDI	Zeta Potential (mV)	
SC (unheated)	70 ± 4	290 ± 15 [a]	0.4 ± 0.03	-23 ± 2 [a]	-
SC (heated)	71 ± 1	231 ± 21 [b]	0.3 ± 0.01	-20 ± 3 [a]	804 ± 2
SC-TA (unheated)	68 ± 2	634 ± 24 [c]	0.8 ± 0.01	-27 ± 4 [b]	-
SC-TA (heated)	75 ± 3	312 ± 18 [c]	0.3 ± 0.05	-29 ± 1 [b]	340 ± 1
SC-TA (ultrasonicated)	77 ± 1	253 ± 11 [c]	0.5 ± 0.05	-29 ± 3 [b]	-

Different letters indicate significant ($p < 0.05$) difference within the same column.

electrostatic repulsion between the chains increases.[1] The particle size of heat-treated SC was smaller than unheated samples. This may be related to heat-induced degradation.[20] The particle size of heat and ultrasonic-treated SC-TA significantly decreased, indicating that stable and compact colloidal particles were formed. The cause of this phenomenon may be related to the following: a) In heat-treated samples and alkaline pH, TA is partially hydrolyzed into glucose and gallic acid moieties due to the pKa value (pka=6),[21] b) Hydroxyl groups of TA and gallic acid are oxidized to carbonyl groups and formed o-quinone under alkaline conditions and vigorous stirring.[22] c) The covalent bond formed between amine groups of SC and carbonyl groups of gallic acid (Maillard reaction) strongly affects the SC structure.[23] Based on previous studies, TA has the ability to bind proteins via hydrogen-hydrophobic interactions or covalent C-N bonds under alkaline conditions.[14,15] The negative charge of SC-TA under different treatments increased compared to native SC (unheated SC), likely because of the formation of TA coating on the surface of SC particles.[7,11] Also, there were no significant differences in zeta potential between heated and unheated SC-TA complexes. Additionally, no significant difference was observed between heat and ultrasonic-treated samples. Similar events occur after sonicating the casein solution, which reduces particle size, but ultrasound had no effect on the zeta potential of the sonicated particles.[24] The mechanisms of interaction of heat or ultrasonic-treated SC-TA were assayed via Fluorescence spectroscopy, FTIR, and Free amino groups content.

Fluorescence measurements

Fluorescence spectroscopy is a useful method for the investigation of structural and environment polarity changes in proteins and their complexes with different chemical compounds.[7] Figure 1 shows the fluorescence spectrum of SC (unheated), unheated, heated, and ultrasonicated SC-TA complex. SC showed strong fluorescence emission with a peak at 383 nm upon excitation at 280 nm due to the presence of the Tryptophan (Trp) and Tyrosine (Tyr) residues in its structure. After adding TA, fluorescence emission intensity at 383 nm decreased, demonstrating that the interaction between TA and protein caused the fluorescence to decrease.[16] There was no significant difference in fluorescence intensity between unheated and heat-treated/ultrasonicated SC-TA

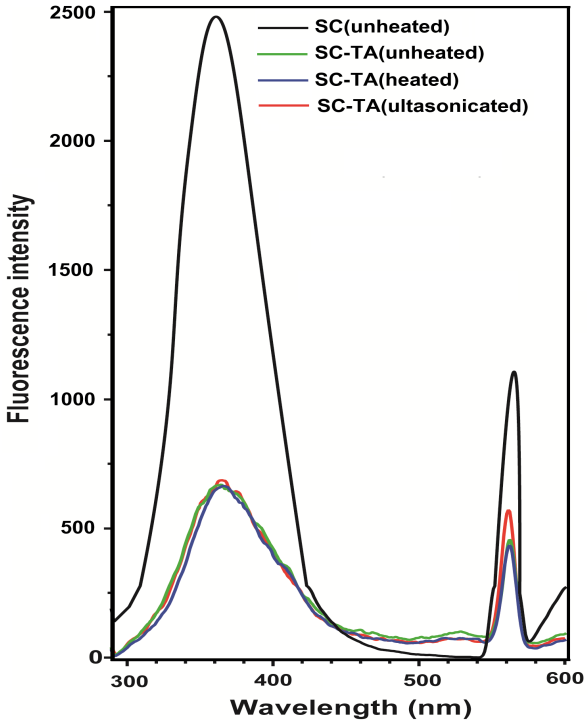

Figure 1. Fluorescence emission spectra of sodium caseinate at excitation wavelengths of 280 nm in presence of tannic acid and different treatments at pH 9.0.

Fourier transform infrared spectroscopy (FTIR)

Figure 2 shows the FTIR spectra of samples, which provide information about the structural and functional group changes of SC during various treatments with TA. In the spectra of native SC (unheated SC), the amide I at 1640 and amide II at 1540 are associated with C=O and C-N stretching of peptide bands of protein. The characteristic peaks of amide III bands at 1200–1400 are mainly associated with the stretching of the C-N bond and deformation of the N-H bond. A large peak in the range of 3500-3300 indicated intermolecular H-bonding and O-H stretching modes. According to Figure 2a, TA had two peaks around 1710 and 1613 probably due to the carbonyl stretching of the TA.[7] A very broad peak was observed in the spectrum of TA at 3363 , likely caused by many hydroxyl groups and hydrogen bonds in the molecule.[10] In SC-TA complexes, the peak intensity increased slightly more than in pure compounds (SC), indicating that SC and TA interact non-covalently. This study confirmed previous

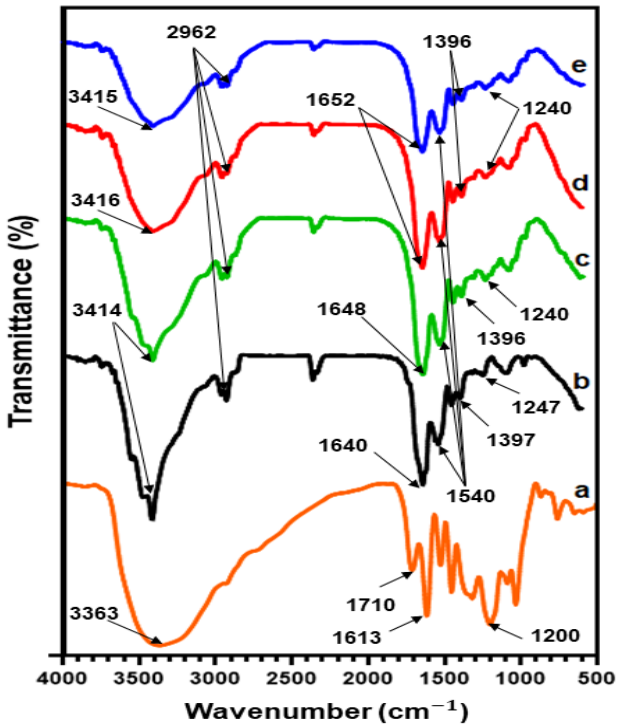

Figure 2. FTIR spectra of samples (a) tannic acid (TA), (b) sodium caseinate (SC) (unheated), (c) sodium caseinate – tannic acid SC-TA (unheated), (d) SC-TA (ultrasonicated), (e) SC-TA (heated) at pH 9.0.

findings, indicating that polyphenols were partially bonded to the protein through H-bonds.[7] Also based on another study the phenolic acid cross-linked the soy protein isolate and gelatin through hydrogen bonding.[15] However, in accordance with previous reports, the characteristic peak at 1710 of TA was hardly found in the SC-TA complex.[7] The drastic decrease in the peak intensity of amide I and amide II bands in heat-treated SC-TA showed that the Maillard reaction between SC and phenolic TA was done by applying heat in alkaline conditions. This was in line with previous studies that the intensity of amide I and amide II was reduced after the Maillard reaction.[25,26] In the heat-treated protein, Amadori compound (C=O), Schiff base (C=N), and pyrazines (C-N) are formed through of Maillard reaction which leads to several changes in the FTIR spectrum of the heat-treated SC-TA complex.

Free amino groups content

As shown in Table 1, the number of free amino groups in the heat-treated SC-TA significantly reduced compared to heat-treated SC. Reduction of free amino groups in heat-treated SC-TA showed that mainly ε-amino, imidazole, and guanidino groups of lysine, histidine, and arginine residues reacted with TA active groups, respectively. The phenolic compounds, including TA, can be oxidized to their quinone counterparts in an alkaline solution and the presence of oxygen, which can react with the free amino groups of protein, resulting in products with enhanced antioxidant activity.[27] Quinones are active components and can attack functional groups in proteins through the formation of Schiff bases and Michael addition reactions.[28]

Differential scanning calorimetry (DSC)

The DSC curves of SC (unheated), SC-TA (unheated), SC-TA (heated), and SC-TA (ultrasonicated) are shown in Figure 3. An analysis of DSC data was conducted on composite particles to evaluate their thermodynamic compatibility based on glass transition temperature (Tg) and crystalline melting temperature (Tm). SC powder alone shows an exothermic peak around 84 °C that is correlated with the Tg of SC and an endothermic peak around 223 °C associated with the Tm. Additionally, SC-TA (unheated) showed an exothermic peak around 86°C, related to a Tg, and a single endothermic peak at roughly 187 °C, related to a Tm of SC-TA (unheated). SC-TA (heated) and SC-TA (ultrasonicated) particles had exothermic peaks at temperatures of 90 °C and 92 °C, respectively, which were higher than SC and SC-TA (unheated), indicating crosslinking and complexation.[29] Previous studies have shown that emulsion thermal stability varies considerably with the types of particles and treatments used.[30] As an example, the freeze-thaw stability of the emulsion was increased by heating the soy and whey protein isolates.[31] According to a previous report, cross-linking reduces the molecular mobility of casein chains, which increases the Tg of the treated samples. These changes in thermal behavior are a good indicator of casein-phenolic acid covalent bonding.[14]

Characterizations of HIPPEs

The colloidal suspensions of SC-TA complex at the concentration ratio of 1:0.1 %wt were used for the preparation of HIPPEs at 75: 25 oil: water volumetric ratio (Figure 4A). Figure 4B and 4C shows the physical stability of the prepared emulsions after preparation and 45 days. The heated SC-TA-containing emulsion was stable for 60 days without phase separation at 6 °C. The emulsion containing sonicated SC-TA showed phase separation after 21 days, while the emulsion sample containing SC-TA was stable for 45 days (Figure 4D). The results showed that heat treatment in alkaline conditions led to the formation of nanoparticles with greater emulsification abilities. Table 2 shows the particle size and zeta potential of emulsion samples. The average particle size of TA containing emulsion either unheated or treated (heated or sonicated) significantly decreased in compared to SC and heated SC. Smaller colloidal particles reduce the interfacial energy between two immiscible liquids such as oil and water, helping to produce stable PEs.[32] Also, Heat-treated SC-TA-containing samples had more negative zeta potentials than other samples. A higher zeta potential means more stability of the colloidal suspension,[33] but in the case of an emulsion, first, these particles must be able to be absorbed in the interface to play their role. It should be noted that the energy of adsorption of particles (E) to the interface of two immiscible phases is a determining factor in the forming

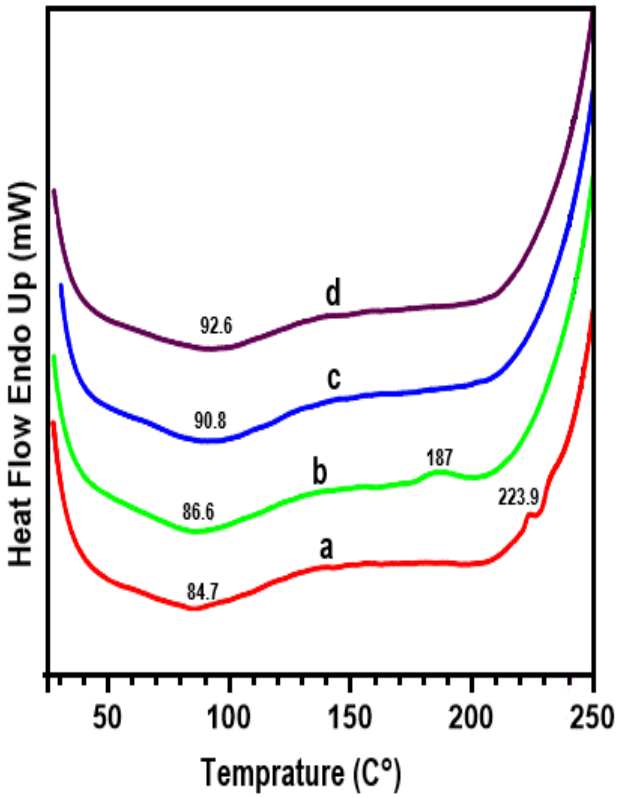

Figure 3. The DSC curves of samples (a) sodium caseinate (SC) (unheated), (b) sodium caseinate –tannic acid (SC-TA) (unheated), (c) SC-TA (heated), (d) SC-TA (ultrasonicated) at pH 9.0.

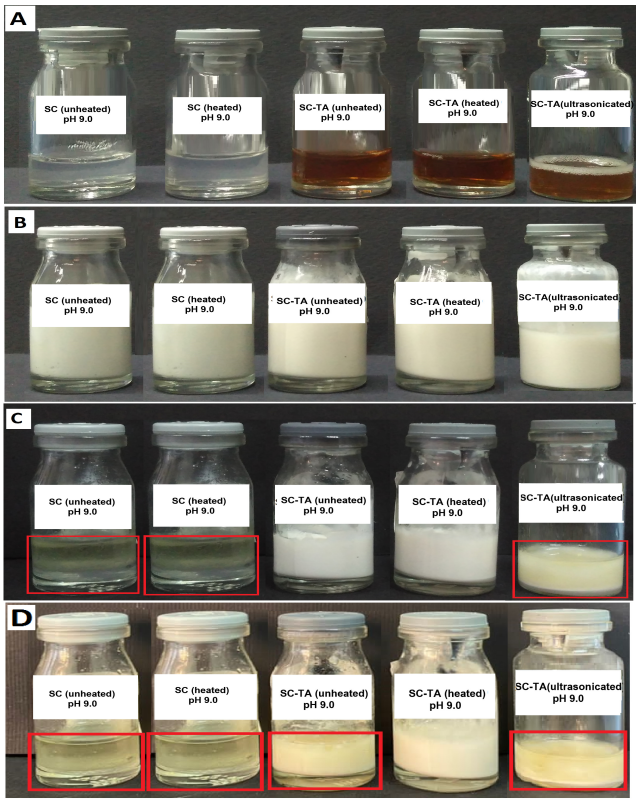

Figure 4. (A) Colloidal Suspensions, (B) high internal phase Pickering emulsion (HIPPE) on day = 0, (C) HIPPEs on day = 45, (D) HIPPEs on day = 60 at pH 9.0 (sodium caseinate – tannic acid (SC/TA) ratios is 1: 0.1 % w/v and oil in water ratio is 75: 25 respectively).

of PEs. Based on the following equation $E = \pi$, the energy required to move the particles from the interface to each of the phases is influenced by particle size, interfacial tension, and three-phase contact angles.[1,34] The contact angle of the SC and SC-TA colloidal suspensions are presented in Table 1. The contact angles of SC-TA (heated) and SC-TA (ultrasonicated) were more than heated and unheated SC. This may be due to the conformational change of SC-TA following the heat and ultrasonication treatment.

In the case of emulsions stabilized with colloidal particles, as the surface charge density increases, hydrophilicity increases, and contact angle decreases, and vice versa. Therefore, the energy required for the particles to be absorbed into the water-oil interface depends on the equilibrium between electrostatic repulsion and hydrodynamic forces (particle size) that push the particle toward the interface.[1] In the heated SC-TA with small particle size and higher surface charge, this equilibrium was established and led to the formation of stable PEs. Figure 5 shows the SEM images of the prepared emulsion samples. The SC (unheated), SC-TA (unheated), SC-TA (heated), and SC-TA (ultrasonicated) had almost smaller and spherical shapes, which confirmed the effect of adding TA and using heat and ultrasonic treatment on the particle size.

Antioxidant capacity of SC-TA complex

The antioxidant activity results showed that TA has a significant radical scavenging activity activity (Figure 6A), In line with the previous study, the powerful antioxidant

Table 2. Characterization of prepared HIPPEs at pH 9.0 (SC/TA ratios is 1: 0.1% w/v) (Time of stability. d: day).

Aqueous phase	Emulsion			
	Particle size (nm)	PDI	Zeta Potential (mV)	Physical Stability
SC (unheated)	2228 ± 15 ^{a}	0.5 ± 0.03	-42 ± 2 ^{a}	1 d
SC (heated)	2195 ± 21 ^{a}	0.4 ± 0.01	-41 ± 3 ^{a}	2 d
SC-TA (unheated)	2407 ± 24 ^{b}	1.0 ±0.01	-39 ± 4 ^{a}	45 d
SC-TA (heated)	749 ± 18 ^{c}	0.5 ±0.05	-46 ±1 ^{b}	60 d
SC-TA (ultrasonicated)	422 ± 11 ^{d}	0.2 ±0.05	-42 ± 3 ^{b}	21 d

Different letters indicate significant ($p < 0.05$) difference within the same column.

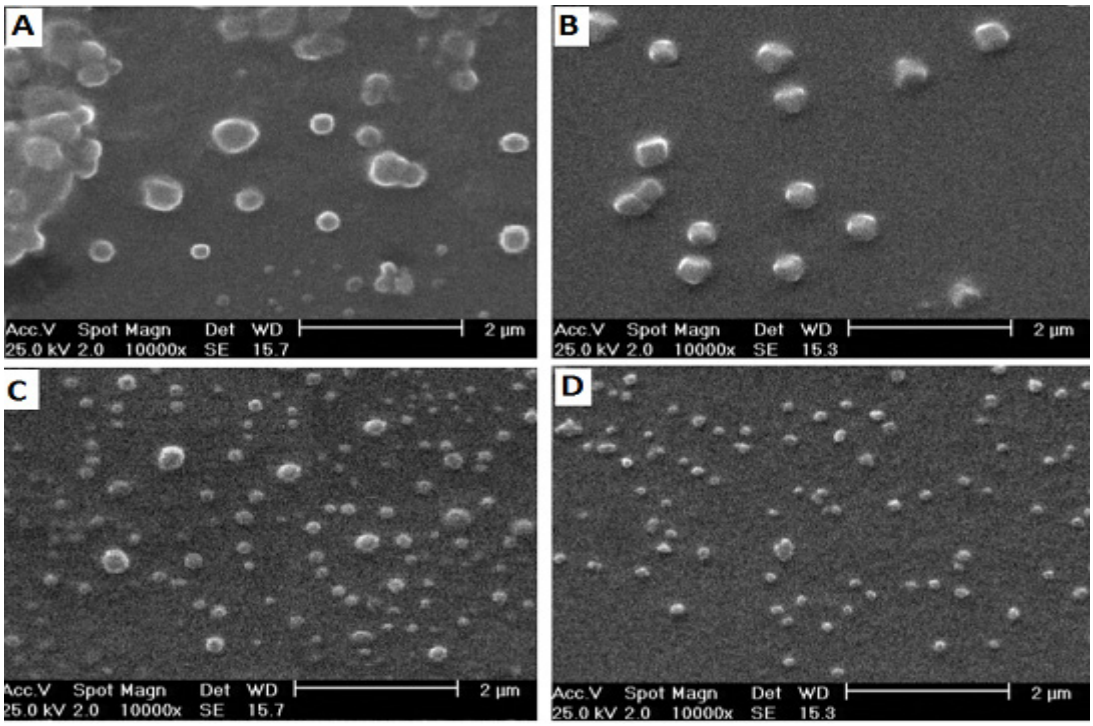

Figure 5. SEM images of emulsions samples (A) sodium caseinate SC (unheated), (B) sodium caseinate– tannic acid (SC-TA) (unheated), (C) SC-TA (heated), (D) SC-TA (ultrasonicated) at pH 9.0.

activity of TA was related to scavenging free radicals and chelating with metal ions.[11] The heated SC-TA samples exhibited more antioxidant properties than the ultrasonic and unheated samples. The results are consistent with previous reports showing that thermally processed TA possesses greater antioxidant properties than fresh TA, due to the breaking of glycosidic bonds and the release of gallic acid.[35] Therefore, in heated SC-TA samples lipid oxidation and the formation of toxic oxidation products are inhibited more strongly.[36]

Characterization of oxidative stability of HIPPEs

The oxidative stability of the prepared emulsions using treated SC-TA particles and native SC was also monitored by measuring the lipid hydroperoxide (LH), free fatty acid content (FFA), and malondialdehyde (MDA) in these samples as primary and advanced lipid oxidation marker (Figure 6B, 6C, and 6D). Lipids oxidation may restrict the shelf life of emulsion products and lead to the degradation of functional ingredients in the oil phase.[37] In PEs stabilized by SC-TA complex nanoparticles, LH, MDA, and FFA levels were significantly lower than in SC alone. The oxidative stability of PEs in oil-in-water is affected by a various factors,including composition, particle size, droplet charge, the oil-water interface structure, and the emulsifier layer's thickness at the interface. Antioxidants and pro-oxidants and pro-oxidants (intermediate metals), may also control the rate and extent of lipid oxidation.[12,38,39] In accordance with previous studies, in the stable PEs, when solid particles are placed at the water-oil interface, an obstacle is created against the pro-oxidant transfer to interfacial areas from the aqueous continuous phase.[39-41] Furthermore, LH, FFA, and MDA values in the emulsion containing heat-treated SC-TA complex were lower than others, indicating that the rate of lipid oxidation has been reduced effectively. As mentioned above, the nature of particles adsorbed to the interface plays a vital role in oxidation control.

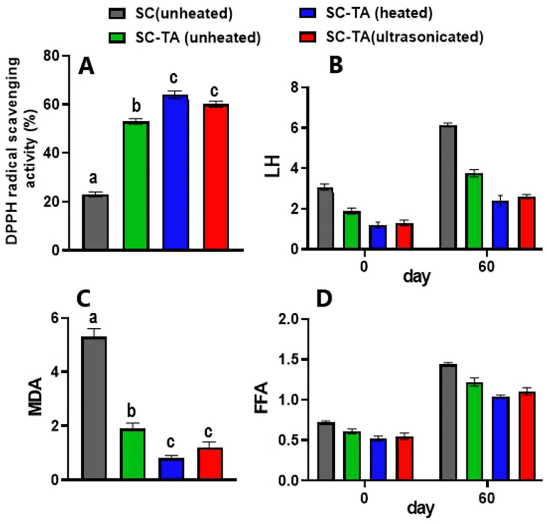

Figure 6. Antioxidant activity of sodium caseinate (SC) and sodium caseinate-tannic acid (SC-TA) complexes after different treatment (A), lipid hydroperoxide (LH) (B), malondialdehyde (MDA) (C), and free fatty acid content (FFA) (D) analysis of high internal phase pickering emulsions (HIPPEs). Different letters in each column represent significant differences ($p < 0.05$).

Based on previous reports, TA a superior antioxidant is able to enhance the emulsification attributes of zein nanoparticles and contribute to the formation of stable interfacial films. This way, it can protect oxidizable bioactive substances in the oil phase from degradation, especially some oil-soluble and unstable bioactive substances. As a result, TA in the form of nanoparticles may prove to be an effective strategy for protecting lipids from peroxidation, as well as stabilizing PEs.[11]

Conclusion

Novel edible HIPPEs stabilized by SC-TA complex particles were fabricated to enhance the oxidative stability of the emulsions. Simultaneous heat treatment under alkaline conditions was required to produce significant changes in the behavior of SC-TA particles. According to FTIR results, SC's structural conformation changed after heating in an alkaline solution due to complex formation with TA. The high physical stability of the emulsion at the mixing ratio of 75: 25 oil and water indicated the formed solid particles had been appropriately arranged at the oil and water interface. The prepared emulsion showed good physical and oxidative stability, which will benefit to food and pharmaceutical manufacturers in designing reduced-fat food formulations and encapsulation systems.

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Author Contributions

Atefeh Nourabi: Investigation, Software, Formal Analysis, Resources, Data Curation, Writing - Original Draft. Hesam Mashhadi: Writing - Review & Editing, Software, Formal Analysis. Mahnaz Tabibiazar: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Project Administration, Supervision, Writing - Review & Editing. Hamed Hamishehkar: Writing - Review & Editing.

Conflict of Interests

The authors declare no competing interests.

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