AMERICAN JOURNAL OF BIOCHEMISTRY AND BIOTECHNOLOGY

RESEARCH ARTICLE

Influence of Zein-Fucoidan Nanoparticles Loaded with Curcumin on the Maillard Reaction Between Casein and Lactose

Shiyang Li[1], Yufan Liu[1], Zhengpeng Wei[2], Falan Li[1], Jinmei Wang[2], Shaoxuan Yu[1]*, Yuanda Song[1,3]*

[1]College of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo 255049, Shandong, China
[2]Rongcheng Taixiang Food Products Co., Ltd, Rongcheng 264309, Shandong, China
[3]School of Basic Medicine, Qilu Medical University, Zibo 255300, Shandong, China

*Corresponding Author: yusx89sdut@126.com, ysong@sdut.edu.cn

Abstract: The influence of curcumin-encapsulated zein-fucoidan nanoparticles (CZFNPs) on the formation of Maillard reaction products (MRP) at the early, intermediate, and advanced stages in a casein-lactose (CN-Le) reaction system was systematically studied. As controls, the effects of zein-fucoidan nanoparticles (ZFNPs) and free curcumin (Cur) were also evaluated. The results showed that CZFNPs effectively inhibited the formation of fructosamine, glyoxal (GO), as well as 3-deoxyglucosone (3-DG), markedly decreased the hydrolysis of CN, and consequently decreased the generation of fluorescent products and total Nε-(carboxymethyl) lysine (CML). Moreover, the inhibitory effects increased with the concentration of CZFNPs and heating time. In contrast, ZFNPs and Cur exhibited much weaker inhibitory effects on the formation of MRP, particularly after 48 h of heating. Furthermore, CZFNPs reduced the 5-hydroxymethylfurfural (5-HMF) content compared with Cur. Therefore, this work provides additional insights into the application of composite nanoparticles loaded with polyphenols in food.

Keywords: Zein-Fucoidan Nanoparticles, Curcumin, Maillard Reaction, Casein, Lactose

Received: 03-09-2025 | **Revised:** 07-01-2026 | **Accepted:** 17-02-2026 | **DOI:** 10.3844/ajbbsp.2026.22.02.029

Introduction

Owing to their nontoxic, biocompatible, and biodegradable properties, composite nanoparticles fabricated from food-derived proteins and polysaccharides have attracted considerable attention as delivery systems for drugs and nutrients in both medical and food applications [1]. For example, negatively charged fucoidan can naturally adsorb onto the surface of self-assembled zein nanoparticles with positive charges via charge-driven interactions coupled with intermolecular hydrogen bonds, thereby forming composite nanoparticles of zein and fucoidan (ZFNPs) [2, 3]. Dispersions of ZFNPs were shown to remain stable without precipitation under various conditions, such as pH 2-8, salt concentration below 25 mM, heating at 80°C for 60 min, and storage at 4°C for 28 days [2, 4, 5]. Moreover, the incorporation of the lipophilic bioactive compounds, including eugenol, resveratrol, and curcumin (Cur), into ZFNPs effectively slowed their release during simulated gastrointestinal digestion and improved in vitro bioaccessibility [2, 4, 5]. Therefore, ZFNPs show great potential for nutraceutical applications.

The Maillard Reaction (MR) occurs widely during food processing and storage [6]. As a type of nonenzymatic browning, this reaction initiates with the chemical interaction of reducing sugars with amino groups in protein chains or free amino acids. Through a cascade of subsequent reactions, it yields a complex set of compounds, commonly classified as Maillard reaction products (MRs) [6, 7]. Although MR promotes the formation of characteristic aromas and pigments, it simultaneously alters the nutrient composition of food, as essential amino acids may be degraded and undesirable compounds may develop [8]. Numerous studies have reported the influences of food constituents (e.g., carbohydrates, proteins, and lipids), manufacturing parameters (e.g., thermal conditions, duration, ultrasound, and microwave heating), and food additives (e.g., antioxidants, polyphenols, and proteases) on the MR [9-11]. For example, Na et al. [12] found that the addition of dihydromyricetin (DMY), procyanidin (PC), and epigallocatechin gallate (EGCG) could effectively inhibit the MR in low-lactose milk (LLM). We recently observed interactions between casein (CN) and both ZFNPs and Cur-loaded ZFNPs (CZFNPs) via hydrogen bonds, which altered their structures and inhibited the dissociation and hydrolysis of CN micelles during heating [3]. However, their impacts on the MR between CN and sugars, as well as on the generation of MRP, remain poorly characterized.

The present study investigated how CZFNPs affect the MR between CN and lactose (Le) and the formation of MRP. Firstly, fluorescence changes in CN-Le systems with and without CZFNPs were monitored using a fluorescence spectrophotometer. The effect of ZFNPs alongside unencapsulated Cur was also measured as controls. Then, levels of Nε-(carboxymethyl) lysine (CML), α-dicarbonyl compounds, 5-gydroxymethylfurfural (5-HMF), as well as fructosamine were quantified using reversed-phase HPLC and UV-visible spectrophotometer, respectively. Changes in the molecular mass of CN were evaluated via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The findings may provide additional insights into the application of polyphenol-loaded food-grade composite nanoparticles.

Materials and Methods

Materials

Both CN of bovine origin and zein protein were sourced through Sigma Chemical Co. (St. Louis, MO, USA). Cur (purity ≥ 97%), nitrotetrazolium blue chloride (NBT, 98% purity), glyoxal (GO, 40% aqueous solution), and 5-HMF (purity > 99%) were supplied by Shanghai-based Aladdin Biochemical Technology Co., Ltd. (China). Fucoidan with 98% purity and of brown seaweed origin was sourced via Shanghai-based Macklin Biochemical Co., Ltd. O-Phthalaldehyde (OPA, 98% purity), o-phenylenediamine (OPDA, purity ≥ 98.5%), 2-mercaptoethanol, oxalic acid, tetrapotassium hexacyanoferrate trihydrate, and zinc acetate were supplied by Shanghai-based Sinopharm Chemical Reagent Co., Ltd. 3-Deoxyglucosone (3-DG, purity ≥ 98%) was supplied by Beijing-based Bailingwei Chemical Reagent Co., Ltd. CML (purity ≥ 97%) was supplied by Shanghai-based Yuanye Biotechnology.

Preparation of CZFNPs and ZFNPs

CZFNPs and ZFNPs were produced based on the method reported by our group [3].

Cumulative Release Rate of Cur

In vitro cumulative release of Cur from CZFNPs at different times was measured using a dialysis bag diffusion technique [13]. Briefly, 1 mL of 32 μg/mL CZFNPs solution was placed in a pretreated dialysis tubing (3500 Da cutoff). Then the tubing was immersed in 100 mL of ultrapure water with 1 wt% Tween-80 and incubated at 60℃ in the dark under stirring. Tween-80 improved the dispersion and structural integrity of Cur. At predetermined time points, 1 mL of dialysate was removed and replaced with an equivalent quantity of ultrapure water. Dialysate samples were analyzed for absorbance at 425 nm (Abs425) with a Thermo Scientific™ Multiskan™ FC microplate reader (Thermo Fisher Scientific Inc., Massachusetts, USA). Cur content in solution was calculated using a calibration curve. The cumulative amount and percentages of released Cur at different times were calculated.

Preparation and Storage of Samples

CN and Le were separately dispersed in 0.2 mol/L phosphate buffer solution (PBS, pH 6.7) to obtain stock solutions at concentrations of 96 and 200 mg/mL [14]. Meanwhile, Cur powder was dissolved in absolute ethyl alcohol to obtain a 1 mg/mL stock solution. Then, equal volumes (1 mL each) of CN and Le solutions were combined, and the total volume was tuned to 4 mL using PBS. To prepare the CN-Le systems containing CZFNPs, ZFNPs, and Cur, namely CZFNPs-CN-Le, ZFNPs-CN-

Le, and Cur-CN-Le, different volumes of CZFNPs, ZFNPs, and Cur solutions were added to CN-Le mixtures, and their final volumes were also tuned to 4 mL with PBS. A series of concentrations expressed as the concentration of Cur (4, 8, and 16 μg/mL) was obtained. The pure CN solution was used as the blank control. All the solutions were thoroughly mixed and incubated at 60℃ for 12, 24, and 48 h to observe the early stages of MR and study the reaction mechanism. After incubation, the reactions were quenched by rapid cooling on ice, while the samples were preserved at -80℃ until subsequent analysis.

Determination of Fluorescence

The sample solutions were diluted 5 times, and the fluorescence of AGEs was measured with an RF-5301PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 352 nm excitation as well as 444 nm emission [14]. The percentage inhibition of fluorescent AGE formation by CZFNPs, ZFNPs, and Cur was determined as follows:

$$\text{Inhibition rates } (\%) = \frac{F_c - F_s}{F_c - F_b} \times 100$$

Where Fc, Fs, and Fb are fluorescent intensities at 444 nm of the CN-Le system, the CN-Le systems containing CZFNPs, ZFNPs, or Cur (CZFNPs-CN-Le, ZFNPs-CN-Le, or Cur-CN-Le), and the blank control (CN), respectively.

Determination of CML

Sample Reduction and Hydrolysis

Samples were pretreated following the method prescribed in prior studies, with minor modifications [15, 16]. Briefly, equal volumes (2 mL each) of the sample solution, borate buffer (0.2 mol/L, pH 9.5), and freshly prepared sodium hydroxide solution (2 mol/L in 0.1 mol/L NaOH) were fully mixed. The solution was maintained at 4℃, protected from light, for a 12-hour reduction period. After the reduction, 1 mL HCl (6 mol/L) was incorporated, and hydrolysis proceeded at 110℃ for 24 hours under nitrogen. Subsequently, HCl was removed via nitrogen purging, and the remaining hydrolysate was dissolved in 2 mL of 0.2 mol/L borate buffer (pH 9.5). Finally, the resulting mixture was passed through a 0.22 μm polyether sulfone (PES) syringe filter.

Sample Derivatization and Analysis

For derivatization, an OPA solution was prepared by combining 50 mg OPA with methanol (5 mL), adding 250 μL 2-mercaptoethanol, and bringing the total volume to 50 mL using a 0.2 mol/L sodium-based buffering system at pH 9.5 [15]. The hydrolysate (1 mL) was blended with an equal volume of OPA reagent and reacted for exactly 180 s.

Total CML was quantified using reversed-phase HPLC (RP-HPLC) analysis [16]. An Agilent 1260 Infinity II LC system with a fluorescence detector and a Waters Xbridge C18 column (4.6×250 mm, 5 μm) was used for the experiment. During the analysis, the derivatives (10 μL) were introduced into the HPLC system. Column temperature was set at 32℃. Solvent A, a mixture of acetonitrile and 15 mmol/L sodium phosphate buffer (pH 7.2) at a 17:83 ratio (v/v), and solvent B, composed of acetonitrile, served as the mobile phase, with a flow rate set to 1 mL/min. The elution program for solvent B increased from 0% to 30% over 0−10 min, then from 30% to 60% during 10−20 min, maintained at 60% for 3 min, and finally decreased from 60% to 0% between 23 and 28 min. Fluorescence detection of OPA-derivative occurred at excitation 340 nanometers and emission 455 nanometers. Total CML levels were quantified using a calibrated series ranging from approximately 0 to 100 μg/mL.

Determination of Fructosamine

Fructosamine levels were quantified using NBT reduction [14]. A 0.5 mmol/L NBT solution was formulated by adding the appropriate quantity of NBT reagent to a 0.1 mol/L sodium carbonate buffer (pH 10.4). Sample solution (100 μL) was added to 400 μL NBT solution, mixed completely, and incubated at 37℃ for 30 min. Later, the Abs530 of the mixture was recorded with a UV-2600 spectrophotometer (Shimadzu, Japan). Inhibition rates for CZFNPs, ZFNPs, and Cur regarding fructosamine formation were calculated using the following equation:

$$\text{Inhibition rates } (\%) = \frac{A_c - A_s}{A_c - A_b} \times 100$$

Where Ac, As, and Ab are the Abs530 values of the CN-Le system, the CN-Le systems containing CZFNPs, ZFNPs, or Cur, and the CN solution, respectively.

Measurement of α-Dicarbonyl Compounds

GO and 3-DG levels were quantified following established procedures with slight adjustments [17]. Firstly, the derivatization of GO and 3-DG was conducted by combining 1 mL sample solution with 1 mL 0.05 mol/L OPDA aqueous solution and reacting at 25℃ for 2 h away from light. A 0.22 μm nylon membrane was employed to clarify the derivatized sample. Agilent 1260 Infinity II LC equipped with Waters Xbridge C18 (4.6 mm × 250 mm, 5 μm) enabled simultaneous separation and detection of target compounds at 315 nm via diode array detection. The injection volume was set to 10 μL; the column temperature remained at 40℃, and the flow rate was 1 mL/min. Eluent A comprised 0.1% formic acid in water, while eluent B consisted of methanol. Phase B gradient: 30% to 60% (0−10 min), 60% to 30% (10−12 min), 30% hold (12−15 min). The concentrations of derivatives of GO and 3-DG were determined by external standards (0, 0.1, 0.5, 1, 2.5, 5 μg/mL).

Determination of the Molecular Weight of CN

Following the procedure reported previously [3], SDS-PAGE enabled evaluation of CN molecular weight distribution before and after the MR in the presence or absence of CZFNPs, ZFNPs, and Cur.

Measurement of 5-HMF

Quantification of 5-HMF followed the procedure reported by Aktağ and Gökmen [18]. Briefly, 0.5 mL oxalic acid solution (0.15 mol/L) was combined with 1 mL solution, followed by heating under boiling water for 30 min. For clarification, Carrez I and II solutions (250 μL) were introduced into the mixture after cooling to room temperature. Carrez I solutions contained 15 g tetrapotassium hexacyanoferrate trihydrate per 100 mL water, whereas Carrez II solutions contained 30 g zinc acetate in 100 mL water. Centrifugation (12,000 rpm, 5 min) was performed, and the resulting supernatant was collected. After dilution to 2 mL with ultrapure water, the supernatant was filtered using a 0.22 μm PES membrane, and 5-HMF was quantified via an Agilent 1260 Infinity II LC system with diode array detection. Separation utilized a Waters Xbridge C18 column (5 μm, 4.6 mm × 250 mm) with the column compartment kept at 25℃, and detection was carried out at 284 nm. Chromatographic separation employed acetonitrile as phase A and ultrapure water as phase B. The gradient applied to phase B proceeded as follow: 4.5% → 12.5% (0−4 min), 12.5% → 18.5% (4−7 min), 18.5% → 24.5% (7−10 min), 24.5% → 30% (10−12 min), 30% → 100% (12−15 min), 100% hold 5 min, 100% → 4.5% (20−25 min). A flow velocity of 1 mL/min was applied, with an injection amount of 10 μL. 5-HMF concentration in the samples was quantified based on a calibration curve spanning 0.1-20 μg/mL.

Statistical Analysis

All samples underwent triplicate analysis, with results reported as mean ± standard deviation. Differences were considered significant at $p < 0.05$, and data were evaluated using one-way ANOVA followed by Duncan's multiple range test in SPSS v22 (IBM, USA).

Results and Discussion

Natural polyphenols exhibit stronger antioxidant activities with greater MR inhibition compared with synthetic compounds, while maintaining lower toxicity. In recent years, they have become a common focus of research in living organisms and in food systems [19, 20].

Cumulative Release of Cur

Cumulative Cur release from CZFNPs was obtained using a dynamic dialysis method under thermal treatment at 60℃ for 48 hours. As exhibited in Fig. S1, the cumulative release of Cur increased rapidly and then slowly with the increase of heating time. When CZFNPs were heated at 60℃ for 24 and 48 h, the release rate of Cur was 80% and 95.6%, respectively. Therefore, Cur could be successfully and continually released into the solution from CZFNPs.

Impacts of CZFNPs on the Generation of Fluorescence

Fluorescence development during MR is generally used as an indicator of the reaction rate and MRP formation [14, 21, 22]. Fluorescence variations of CN-Le mixtures with or without varying levels of CZFNPs, ZFNPs, and Cur are shown in Fig. 1. As a blank control, the fluorescence of the CN solution increased only slightly with heating time (Fig. 1A). This likely results from heat-induced denaturation, molecular breakdown, and oxidative modification of CN [23]. In contrast, the fluorescence of the CN-Le system increased markedly with heating time, particularly at 24 and 48 h, indicating the enhanced formation of fluorescent products due to the presence of Le. This result aligns with earlier findings [24].

DOI: 10.3844/ajbbsp.2026.22.02.029

Fig. 1B illustrates how varying CZFNP concentrations influence fluorescence in CN-Le systems subjected to 60℃ for 24 and 48 h. After 24 h of heating, the inhibition rates of 4, 8, and 16 μg/mL CZFNPs were -17.5%, 9.2%, and 45.8%, respectively. After 48 h of heating, the inhibition rates increased to 5.2%, 16.8%, and 57.9%, respectively. At 24 h, 4 μg/mL CZFNPs enhanced fluorescence, although the inhibitory effect of CZFNPs became more pronounced as concentration and heating duration increased ($p < 0.05$). The different effects at various concentrations might be related to the following reasons. Firstly, CZFNPs could induce changes in CN conformation, such as a decrease in α-helix and an increase in random coils [3], which improve the flexibility of CN, thereby exposing internal groups, promoting their reaction with Le, and ultimately promoting the formation of fluorescence. However, with increasing CZFNPs concentration, the interactions between CZFNPs and CN were enhanced, producing larger, more robust aggregates [3] and increased system viscosity [25], thereby inhibiting the MR between amino acid residues and Le and product formation. Moreover, CZFNPs gradually released Cur during heating, effectively avoiding the thermal degradation of Cur, while Cur could inhibit fluorescence formation by quenching free radicals and capturing dicarbonyl compounds [14].

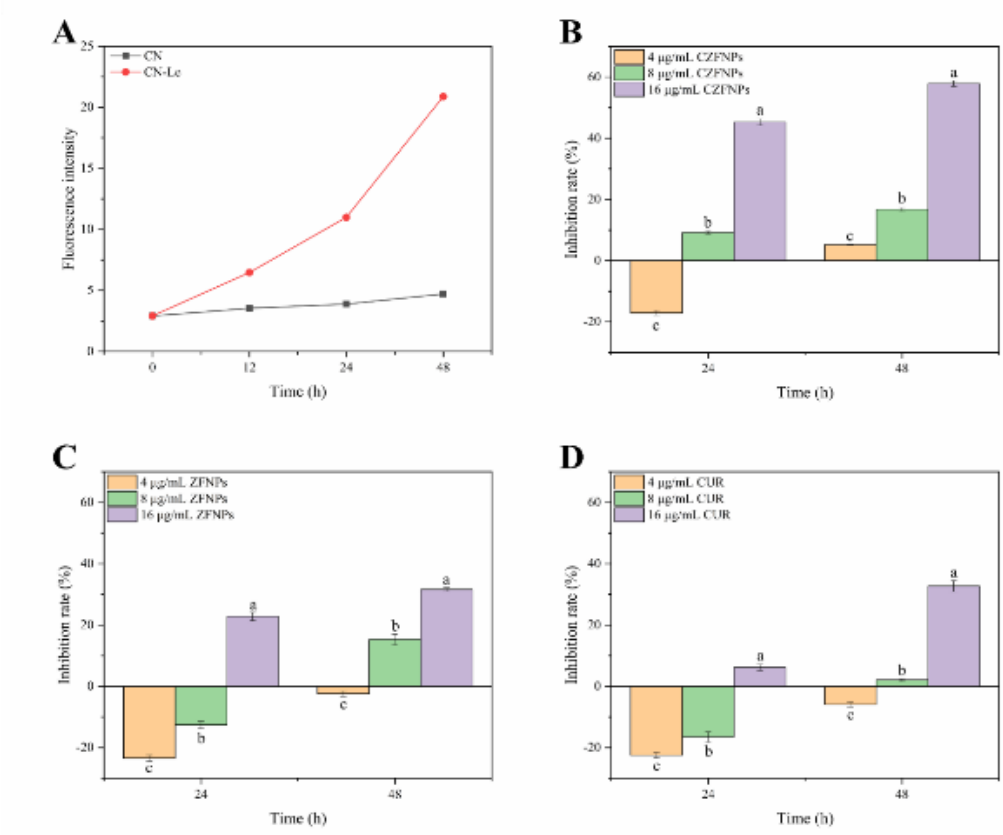

Fig. 1: Effect of prolonged heating on the formation of fluorescent AGEs in CN and CN-Le systems (A). Inhibition rates of CZFNPs (B), ZFNPs (C), and Cur (D) at different concentrations on the generation of fluorescent AGEs in the CN-Le system, which was heated at 60℃ for 24 or 48 h. Differences between groups at the same heating interval are considered statistically significant ($p < 0.05$) when indicated by different lowercase letters

Fig. 1C and D present the effects of ZFNPs and free Cur on fluorescence formation, showing trends comparable to those of CZFNPs. At low concentrations (4 and 8 μg/mL), ZFNPs could also induce structural changes and exposure of internal groups of CN, thereby promoting the formation of fluorescent products. At a ZFNP concentration of 16 μg/mL, fluorescence generation was significantly inhibited, with inhibition rates of 22.8% at 24 h and 31.6% at 48 h. As discussed above, this might be due to the increase in CN stability and solution viscosity. Similarly, Cur showed an inhibitory effect on the generation of fluorescence when its concentration increased to 16 μg/mL, and the inhibition rates were 6.1% and 32.7% at 24 and 48 h, respectively. At low concentrations, Cur mainly existed in the free form in solution, was unstable under heating conditions, reacted directly with amino groups in CN, and thereby promoted the MR [26]. While Cur at high concentrations could form composite colloidal particles with CN through self-assembly [27], which might increase the thermal stability of Cur, and then

contribute to playing an inhibitory role and reducing the fluorescence formation. Remarkably, CZFNPs showed a much stronger effect on the inhibition of fluorescence in the CN-Le system compared to Cur at the same concentrations.

Based on the above results, 16 μg/mL of CZFNPs and the corresponding concentrations of ZFNPs and Cur were selected to carry out the follow-up experiments.

Impacts of CZFNPs on the Generation of CML

CML, a non-fluorescent advanced-stage MRP, was used as a representative marker [28]. After 24 h of heating, CML levels in the CN-Le system were significantly higher than those in the CN control and other treatments (Fig. 2A). Thus, adding 16 μg/mL CZFNPs, ZFNPs, or Cur suppressed CML formation. However, no significant differences were observed among the treated groups, and CML levels in all treatments remained above those of the CN control. When the heating time was extended to 48 h (Fig. 2B), the amount of CML in the CN and CN-Le groups increased by 15.3 and 27.8 μg, respectively. This was in accordance with a previous result, which showed that more intense thermal treatment can promote the cyclization, dehydration, dehydrogenation, and rearrangement of active substances, such as Amadori products and furfural compounds, and accelerate their reactions with amino compounds, thereby increasing the accumulation of harmful products, including CML [29]. On the contrary, the CML content in the treated groups with CZFNPs, ZFNPs, and Cur decreased by 10.1, 1.9, and 2.0 μg, respectively, and was lower than that in the CN group. However, the CML content in the treated groups with ZFNPs or Cur was obviously higher than that in the CZFNP-treated group. This trend agreed with fluorescence results, indicating that CZFNPs exhibited a stronger inhibitory effect on MR with the increase of heating time.

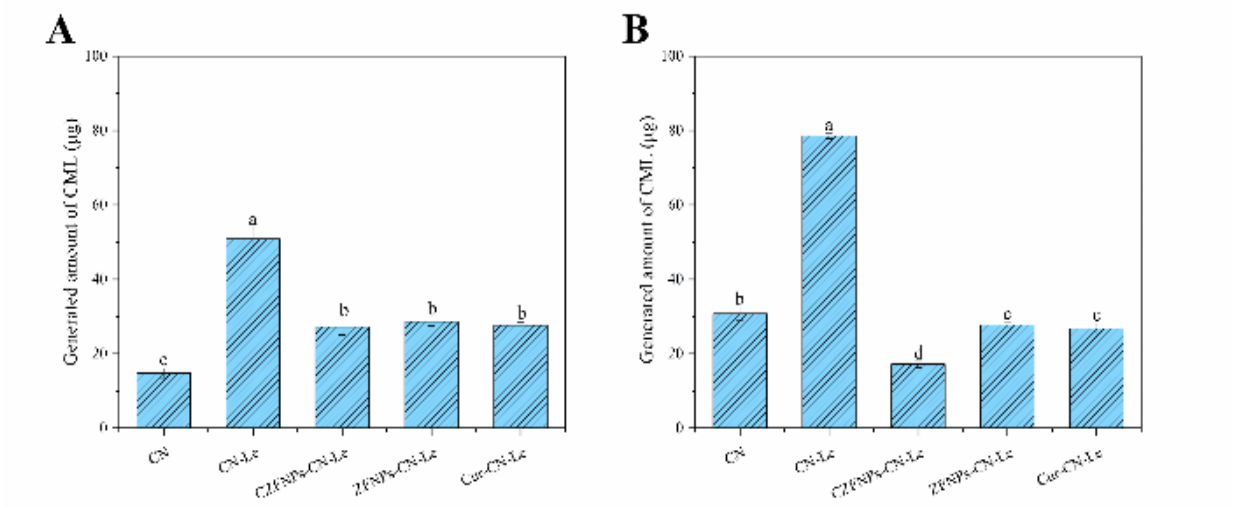

Fig. 2: Effects of CZFNPs, ZFNPs, and Cur on CML levels in CN-Le systems heated at 60℃ for 24 (A) and 48 h (B). Significant differences ($p < 0.05$) among various groups are denoted by distinct lowercase letters

Influences of CZFNPs on the Production of Other MRP

To investigate the basis of CZFNP-mediated inhibition of fluorescence and CML formation, its influences on the generation of other MRP and on the hydrolysis of CN were studied as well. For example, fructosamine is considered a key intermediate in the early phase of the MR. α-Dicarbonyl compounds (e.g., GO and 3-DG) form during the MR's middle phase via the oxidation or breakdown of reducing sugars, Schiff base, and Amadori rearrangement products and subsequently contribute to advanced glycation end products (AGE) formation in the final MR phase [30].

Influences of CZFNPs on the Production of Fructosamine

After 24 h of thermal treatment, CZFNPs, ZFNPs, and Cur inhibited fructosamine formation by 9.2%, 7.4%, and 2.7%, respectively (Fig. 3). CZFNPs and ZFNPs exhibited significantly stronger inhibitory effects compared with Cur. Our previous work [3] has shown that when CZFNPs and ZFNPs were blended with CN, they might attach to the protein surface as a result of their surface characteristics. Furthermore, Fourier Transform Infrared Spectroscopy (FTIR) indicated hydrogen bonding between CZFNPs/ZFNPs and CN. The excellent inhibition of CZFNPs and ZFNPs may be partially due to the capacity to bind amino groups of CN, thereby blocking the formation of fructosamine.

DOI: 10.3844/ajbbsp.2026.22.02.029

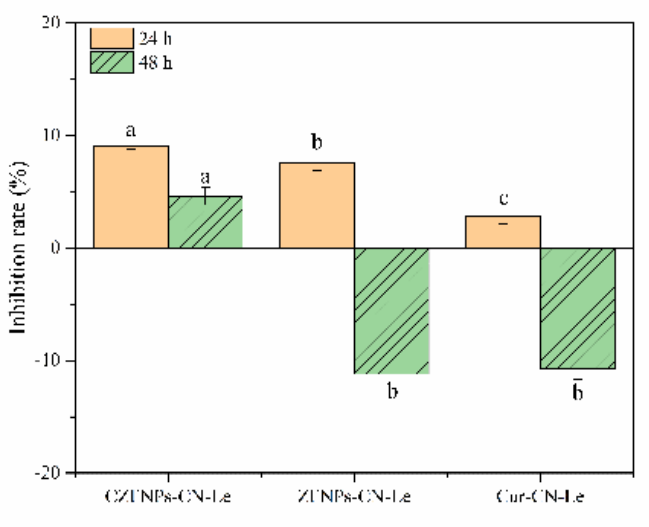

Fig. 3: Impact of CZFNPs, ZFNPs, and Cur on fructosamine formation in CN-Le systems heated at 60℃ for 24 and 48 h. Significant differences ($p < 0.05$) within each heating interval are denoted by distinct lowercase letters across the experimental groups

After 48 h of thermal treatment, the inhibitory effect of CZFNPs was significantly reduced, but still with an inhibition rate of 4.8%. In contrast, ZFNPs and Cur promoted the formation of fructosamine. With prolonged heating, interactions of Le carbonyl groups with CN amino groups may significantly increase, and thermal decomposition of Cur, fucoidan, and zein may occur, leading to the promotion of fructosamine generation [14]. It was previously reported that Cur, along with glucose, could promote MR and acrylamide formation in crust-like food systems with extended baking time [26]. Nevertheless, interactions among zein, fucoidan, and Cur during CZFNP formation, such as electrostatic and non-covalent forces, may enhance CZFNP thermal robustness [2].

Impacts of CZFNPs on the Formation of α-Dicarbonyl Compounds

No matter how long the heating time was, the amount of GO in the CN group was very small (Fig. 4A and B). With the addition of Le, the amount of GO in the CN-Le system sharply increased and was about 200 times higher than that in the CN group. However, CZFNPs, ZFNPs, and Cur significantly reduced the amount of GO in CN-Le systems, and the effects of CZFNPs and ZFNPs were obviously better than that of Cur, especially when the heating time was 48 h. Fig. 4C shows that 3-DG levels in the CN-Le system exceeded those in CN and the other treatments after 24 h of thermal treatment. Therefore, CZFNPs, ZFNPs, and Cur significantly inhibited the formation of 3-DG in CN-Le systems, and they were about equally effective. In comparison with the thermal treatment of 24 h, the amount of 3-DG in CN and CN-Le groups separately increased by 15.7 and 27.4 μg when the heating time increased to 48 h (Fig. 4D). On the contrary, the amount of 3-DG in CZFNPs-CN-Le, ZFNPs-CN-Le and Cur-CN-Le groups decreased from 29.5, 30.1, 29.8 to 23.0, 29.6 and 29.3 μg, falling below the levels observed in the CN treatment. Therefore, CZFNPs, ZFNPs, and Cur significantly inhibited the formation of 3-DG in CN-Le systems as well. The inhibitory effects can be attributed to the antioxidant activities of the nanoparticle components, including Cur, fucoidan, and zein [31, 32]. Polyphenols have been reported to trap α-dicarbonyl compounds via electrophilic substitution on the benzene ring [33]. The trend of these data was in accordance with that of CML.

Changes in the Molecular Weight of CN With and Without CZFNPs

The effects of CZFNPs, ZFNPs, and Cur on CN molecular weight were analyzed in CN-Le systems subjected to 48 h of heating at 60℃. Fig. 5 shows that natural CN (Lane 1) exhibited primary protein bands at approximately 25-35 kDa [34]. Before thermal treatment, the CN-Le, CZFNPs-CN-Le, ZFNPs-CN-Le, and Cur-CN-Le groups (Lanes 3, 5, 7, and 9) showed protein banding patterns consistent with the natural CN. Thus, Le, CZFNPs, ZFNPs, and Cur did not notably alter CN molecular weight, and their association occurred via non-covalent interactions, as demonstrated in our previous work [3].

However, after 48 h of thermal treatment, the strength of protein bands in CN (Lane 2) and CN-Le systems (Lane 4) significantly decreased, and their positions moved to where the molecular weights were lower than 25 kDa, indicating the hydrolysis of CN [35]. It can be speculated that the produced polymers with lower molecular weights, peptides, or amino acids more easily participate in MR as substrates, resulting in more MRP formation.

DOI: 10.3844/ajbbsp.2026.22.02.029

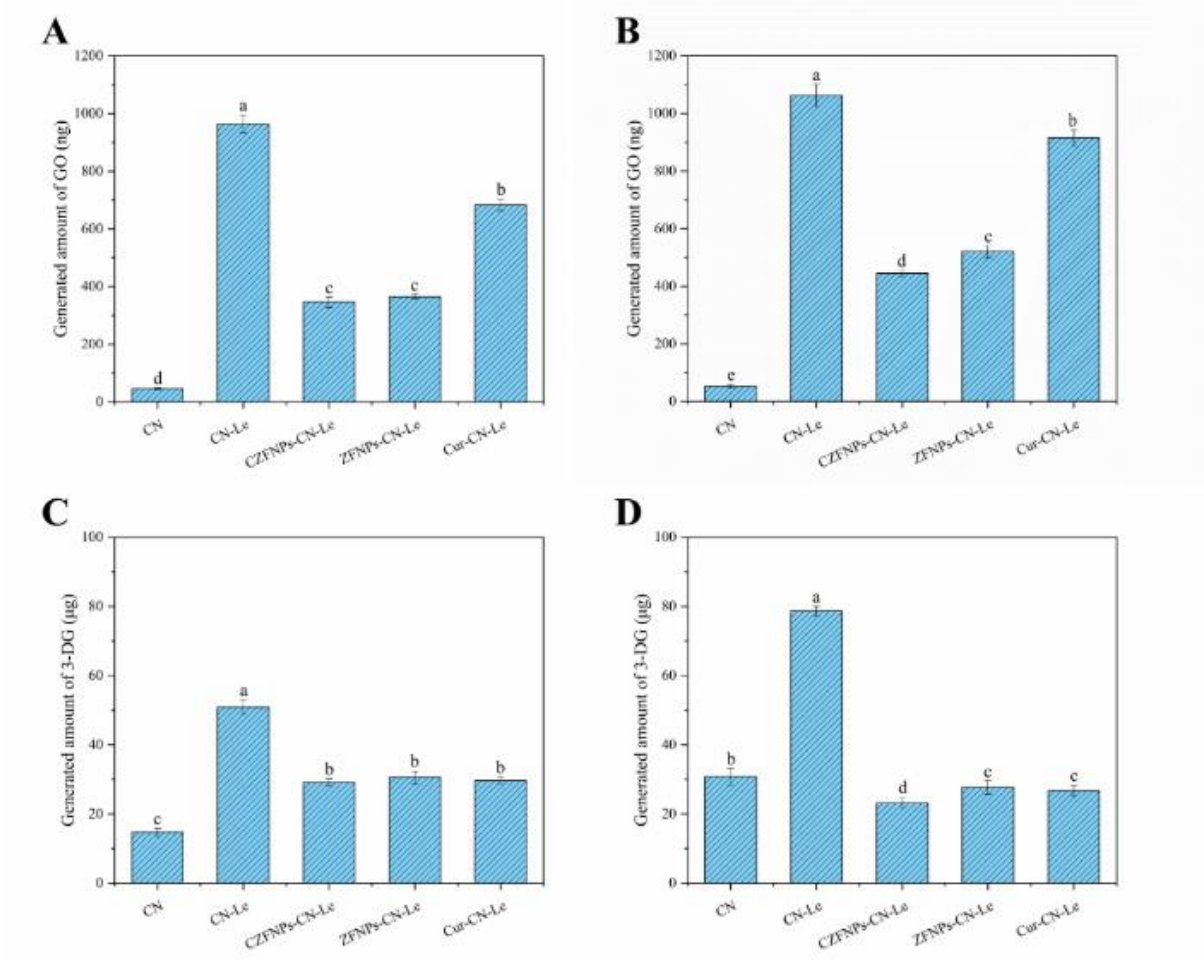

Fig. 4: Effects of CZFNPs, ZFNPs, and Cur on GO (A and B) and 3-DG (C and D) formation in CN-Le systems heated at 60℃ for 24 (A and C) and 48 h (B and D). Distinct lowercase letters denote statistically significant differences ($p < 0.05$) among treatments

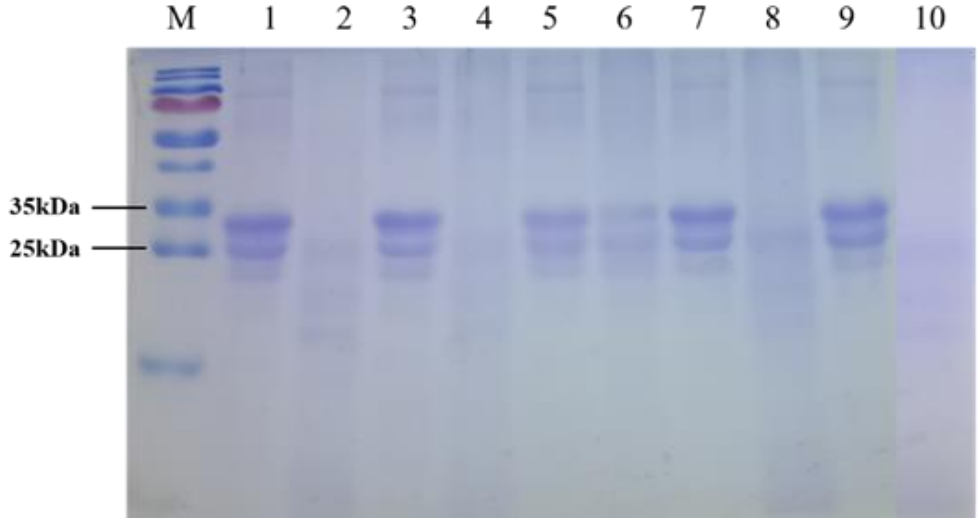

Fig. 5: SDS-PAGE profiles of the samples from different groups after heating. Lane M shows protein markers ranging from 25 to 160 kDa. Lanes 1, 3, 5, 7, and 9 were CN, CN-Le, CZFNPs-CN-Le, ZFNPs-CN-Le, and Cur-CN-Le systems before thermal treatment, respectively. Lanes 2, 4, 6, 8, and 10 were CN, CN-Le, CZFNPs-CN-Le, ZFNPs-CN-Le, and Cur-CN-Le systems that were heated at 60℃ for 48 h, respectively

When CZFNPs were added into CN-Le systems (Lane 6), the protein bands appeared at the same positions as those groups without heating (Lane 5, for example), and the strength of protein bands was significantly enhanced compared with CN-Le (Lane 4), which suggested that CZFNPs prevented the hydrolysis of CN, namely protecting its structural stability during heating. The positions of protein bands in ZFNPs-CN-Le (Lane 8) slightly moved to where the molecular weights were lower, but their strength was also stronger than that in CN-Le (Lane 4). Thus, the effect of ZFNPs was similar to but weaker than that of CZFNPs. Moreover, protein band intensity in the heated Cur-CN-Le group closely matched that of CN, indicating that Cur had little effect on CN.

Impacts of CZFNPs on the Formation of 5-HMF

HMF serves as an indicator of product quality and a potential adulterant across various food industries, including coffee, fruit juices, milk, sauces, honey, and cereals [36]. Therefore, the impact of CZFNPs on the generation of 5-HMF was also detected. There are two main ways in which 5-HMF is formed in cow milk: the enolization of the Amadori product of the MR and the degradation and isomerization of lactose [37]. As shown in Fig. 6, no matter how long the heating time was, the trend of the amount of 5-HMF generated in different groups was similar. Specifically, 5-HMF levels in the CN-Le system were significantly elevated relative to CN ($p < 0.05$), but remained lower than those in the systems containing Cur, CZFNPs, and ZFNPs. Therefore, 16 μg/mL of Cur, CZFNPs, and ZFNPs promoted the generation of 5-HMF in the CN-Le system, which was heated at 60℃ for 24 and 48 h. However, compared with Cur, CZFNPs decreased 5-HMF levels. This was consistent with the result of fructosamine, in which CZFNPs also showed a better inhibitory effect because of the higher thermal stability. On the contrary, it was reported that EGCG, DMY, and PC at concentrations from 0.1 to 0.5 mg/mL effectively inhibited the 5-HMF content of LLM [12]. Similarly, [38] found that the addition of tea polyphenols could inhibit the 5-HMF content of brown fermented milk Similarly, Li et al. [38]. The differences might be due to the different polyphenols, reaction systems, and thermal conditions, which can obviously influence the MR.

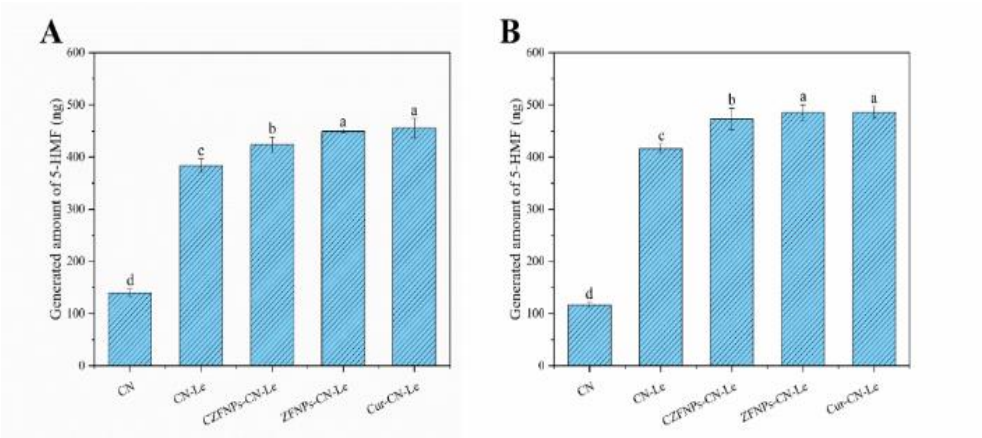

Fig. 6: Effects of CZFNPs, ZFNPs, and Cur on the amount of 5-HMF in CN-Le systems, which were heated at 60℃ for 24 (A) and 48 h (B). Distinct lowercase letters denote significant variations ($p < 0.05$)

Conclusion

CZFNPs significantly influenced the MR between CN and Le, inhibited the generation of fluorescence and CML, and the inhibitory effect rose with the increase of concentration and heating time. Two pathways may explain the excellent inhibitory effects of CZFNPs. Firstly, CZFNPs could block the formation of fructosamine and the hydrolysis of CN via binding to the free amino groups of CN through non-covalent interactions. Moreover, CZFNPs could reduce the content of α-dicarbonyl compounds (e.g., GO and 3-DG) in the CN-Le system as they could sustainably release Cur during heating. In addition, CZFNPs reduced the amount of 5-HMF in comparison with Cur, which might be attributed to the higher thermal stability. Therefore, before the application of composite nanoparticles, especially those loaded with polyphenol compounds, in food, it may be necessary to identify their influences on MR and MRP formation.

It should be noted that all conclusions are drawn from a simplified model system under a specific thermal condition, and the effects of CZFNPs need verification in real, complex food matrices and at higher processing temperatures. Furthermore, the potential impact of MRP inhibition on the desirable food attributes, such as flavor and color, should be considered.

Acknowledgment

We are grateful to the editing team for their dedicated work in the publication process. Additionally, we acknowledge the publishers for supplying the resources and platforms that have made the dissemination of our work possible.

Funding Information

This work is supported by Shandong Provincial Youth Innovation Team Development Plan of Colleges and Universities (Grant No. 2022KJ226) and Natural Science Foundation of Shandong Province (Grant No. ZR2019BC104).

Author's Contributions

Shiyang Li: Methodology, investigation, data collection, data analysis, writing original draft.
Yufan Liu: Investigation, data collection, formal analysis.
Zhengpeng Wei: Formal analysis, advising, fund supporting, validation.
Falan Li: Fund supporting.
Jinmei Wang: Validation.
Shaoxuan Yu: Methodology, conceptualization, writing original draft, writing review and editing, fund acquisition, Supervision.
Yuanda Song: Advising, writing review and editing, supervision.

Ethics

This article is an original and unpublished piece. The corresponding authors hereby confirm that all co-authors have read and approved the manuscript, and that the research involves no ethical issues.

References

1. Shah, B. M., Palakurthi, S. S., Khare, T., Khare, S., & Palakurthi, S. (2020). Natural proteins and polysaccharides in the development of micro/nano delivery systems for the treatment of inflammatory bowel disease. International Journal of Biological Macromolecules, 165, 722-737. https://doi.org/10.1016/j.ijbiomac.2020.09.214
2. Zhang, H., Jiang, L., Tong, M., Lu, Y., Ouyang, X.-K., & Ling, J. (2021). Encapsulation of curcumin using fucoidan stabilized zein nanoparticles: Preparation, characterization, and in vitro release performance. Journal of Molecular Liquids, 329, 115586. https://doi.org/10.1016/j.molliq.2021.115586
3. Xu, S., Yu, S., Zhang, Z., Pang, G., Zhang, Y., Wang, X., Xiao, H., & Song, Y. (2023). Influences and potential mechanisms of zein-fucoidan nanoparticles loaded with and without curcumin on casein before and after thermal treatment. Food Hydrocolloids, 145, 109108. https://doi.org/10.1016/j.foodhyd.2023.109108
4. Liu, Q., Qin, Y., Jiang, B., Chen, J., & Zhang, T. (2022). Development of self-assembled zein-fucoidan complex nanoparticles as a delivery system for resveratrol. Colloids and Surfaces B: Biointerfaces, 216, 112529. https://doi.org/10.1016/j.colsurfb.2022.112529
5. Lei, Y., & Lee, Y. (2024). Stabilization of zein nanoparticles with tween-80 and fucoidan for encapsulation of eugenol via a nozzle simulation chip. Food Research International, 188, 114514. https://doi.org/10.1016/j.foodres.2024.114514
6. Ge Pan, G., & Melton, L. D. (2007). Nonenzymatic Browning of Lactose and Caseinate during Dry Heating at Different Relative Humidities. In Journal of Agricultural and Food Chemistry (Vol. 55, Issue 24, pp. 10036-10042). https://doi.org/10.1021/jf072257n
7. Yan, H., Yu, Z., & Liu, L. (2022). Lactose crystallization and Maillard reaction in simulated milk powder based on the change in water activity. Journal of Food Science, 87(11), 4956-4966. https://doi.org/10.1111/1750-3841.16335
8. Jaeger, H., Janositz, A., & Knorr, D. (2010). The Maillard reaction and its control during food processing. The potential of emerging technologies. Pathologie Biologie, 58(3), 207-213. https://doi.org/10.1016/j.patbio.2009.09.016
9. Li, H., Ping, Y., Niranjan, K., Wu, Q., Chen, Z., Zhang, L., Zhao, B., & Liu, K. (2024). Structure, antioxidant properties and AGEs (advanced glycation end products) formation of modified wheat gluten protein after enzymatic hydrolysis and Maillard reaction. Journal of Food Composition and Analysis, 136, 106795. https://doi.org/10.1016/j.jfca.2024.106795
10. Shi, B., Guo, X., Liu, H., Jiang, K., Liu, L., Yan, N., Farag, M. A., & Liu, L. (2024). Dissecting Maillard reaction production in fried foods: Formation mechanisms, sensory characteristic attribution, control strategy, and gut homeostasis regulation. Food Chemistry, 438, 137994. https://doi.org/10.1016/j.foodchem.2023.137994
11. Anilkumar, K., Tangirala, S., Hema, V., Sunil, C. K., & Rawson, A. (2025). Effect of ultrasound assisted novel and conventional frying on Maillard reaction products and quality of jackfruit chips. Journal of Food Measurement and Characterization, 19(6), 4337-4353. https://doi.org/10.1007/s11694-025-03256-z
12. Na, Z., Liu, S., Bi, H., He, X., & Liu, T. (2024). Inhibitory effects of polyphenols on the Maillard reaction in low-lactose milk and the underlying mechanism. Journal of Dairy Science, 107(12), 10512-10526. https://doi.org/10.3168/jds.2024-25306

13. Yu, S., Wang, S., Xie, Z., Yu, S., Li, L., Xiao, H., & Song, Y. (2021). Hyaluronic acid coating on the surface of curcumin-loaded ZIF-8 nanoparticles for improved breast cancer therapy: An in vitro and in vivo study. Colloids and Surfaces B: Biointerfaces, 203, 111759. https://doi.org/10.1016/j.colsurfb.2021.111759
14. Liu, Y., Yu, S., Liu, L., Yue, X., Zhang, W., Yang, Q., Wang, L., Wang, Y., Zhang, D., & Wang, J. (2017). Inhibition of the double-edged effect of curcumin on Maillard reaction in a milk model system by a nanocapsule strategy. LWT, 84, 643-649. https://doi.org/10.1016/j.lwt.2017.06.037
15. Drusch, S., Faist, V., & Erbersdobler, H. F. (1999). Determination of NE-carboxymethyllysine in milk products by a modi®ed reversed-phase HPLC method. In Food Chemistry (Vol. 65, Issue 4, pp. 547-553). https://doi.org/10.1016/s0308-8146(98)00244-1
16. Abdi, F., golchinfar, Z., Tabibiazar, M., Taghvimi, A., & Ghorbani, M. (2021). Effect of tannic and gallic acid on glycation of egg white protein and formation N-(Carboxyl methyl) lysine. Food Bioscience, 43, 101245. https://doi.org/10.1016/j.fbio.2021.101245
17. Yu, H., Zhong, Q., Guo, Y., Xie, Y., Cheng, Y., & Yao, W. (2020). Potential of resveratrol in mitigating advanced glycation end-products formed in baked milk and baked yogurt. Food Research International, 133, 109191. https://doi.org/10.1016/j.foodres.2020.109191
18. Aktağ, I. G., & Gökmen, V. (2021). Investigations on the formation of α-dicarbonyl compounds and 5-hydroxymethylfurfural in fruit products during storage: New insights into the role of Maillard reaction. Food Chemistry, 363, 130280. https://doi.org/10.1016/j.foodchem.2021.130280
19. Gugliucci, A., Bastos, D. H. M., Schulze, J., & Souza, M. F. F. (2009). Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins. In Fitoterapia (Vol. 80, Issue 6, pp. 339-344). https://doi.org/10.1016/j.fitote.2009.04.007
20. Khan, M. W. A., Otaibi, A. A., Alhumaid, A. F. M., Alsukaibi, A. K. D., Alshamari, A. K., Alshammari, E. M., Al-Zahrani, S. A., Almudyani, A. Y. M., & Sherwani, S. (2021). Garlic Extract: Inhibition of Biochemical and Biophysical Changes in Glycated HSA. Applied Sciences, 11(22), 11028. https://doi.org/10.3390/app112211028
21. Hu, T.-Y., Liu, C.-L., Chyau, C.-C., & Hu, M.-L. (2012). Trapping of Methylglyoxal by Curcumin in Cell-Free Systems and in Human Umbilical Vein Endothelial Cells. In Journal of Agricultural and Food Chemistry (Vol. 60, Issue 33, pp. 8190-8196). https://doi.org/10.1021/jf302188a
22. Xue, C., Deng, P., Quan, W., Li, Y., He, Z., Qin, F., Wang, Z., Chen, J., & Zeng, M. (2022). Ginger and curcumin can inhibit heterocyclic amines and advanced glycation end products in roast beef patties by quenching free radicals as revealed by electron paramagnetic resonance. Food Control, 138, 109038. https://doi.org/10.1016/j.foodcont.2022.109038
23. Liu, H., Grosvenor, A. J., Li, X., Wang, X., Ma, Y., Clerens, S., Dyer, J. M., & Day, L. (2019). Changes in Milk Protein Interactions and Associated Molecular Modification Resulting from Thermal Treatments and Storage. Journal of Food Science, 84(7), 1737-1745. https://doi.org/10.1111/1750-3841.14663
24. Pinto, M. S., Léonil, J., Henry, G., Cauty, C., Carvalho, A. F., & Bouhallab, S. (2014). Heating and glycation of β-lactoglobulin and β-casein: Aggregation and in vitro digestion. Food Research International, 55, 70-76. https://doi.org/10.1016/j.foodres.2013.10.030
25. Yu, S., Pang, G., Li, S., Lv, S., Wei, Z., Wang, J., Xiao, H., & Zhu, L. (2025). Impacts of zein-fucoidan nanoparticles with and without curcumin on gel properties of golden threadfin bream (Nemipterus virgatus) surimi. Food Chemistry, 468, 142415. https://doi.org/10.1016/j.foodchem.2024.142415
26. Hamzalıoğlu, A., Mogol, B. A., Lumaga, R. B., Fogliano, V., & Gökmen, V. (2013). Role of curcumin in the conversion of asparagine into acrylamide during heating. In Amino Acids (Vol. 44, Issue 6, pp. 1419-1426). https://doi.org/10.1007/s00726-011-1179-5
27. Rahimi Yazdi, S., & Corredig, M. (2012). Heating of milk alters the binding of curcumin to casein micelles. A fluorescence spectroscopy study. Food Chemistry, 132(3), 1143-1149. https://doi.org/10.1016/j.foodchem.2011.11.019
28. Rahbar, Samuel, & Figarola, James L. (2002). Inhibitors and Breakers of Advanced Glycation Endproducts (AGEs): A Review. Current Medicinal Chemistry-Immunology, Endocrine & Metabolic Agents, 2(2), 135-161. https://doi.org/10.2174/1568013023358889
29. Sahu, A., Kasoju, N., & Bora, U. (2008). Fluorescence Study of the Curcumin−Casein Micelle Complexation and Its Application as a Drug Nanocarrier to Cancer Cells. Biomacromolecules, 9(10), 2905-2912. https://doi.org/10.1021/bm800683f
30. Şen, D., & Gökmen, V. (2022). Kinetic modeling of Maillard and caramelization reactions in sucrose-rich and low moisture foods applied for roasted nuts and seeds. Food Chemistry, 395, 133583. https://doi.org/10.1016/j.foodchem.2022.133583
31. Chen, Y., Wang, Y., He, L., Wang, L., Zhao, J., Yang, Z., Li, Q., & Shi, R. (2024). Zein/fucoidan-coated phytol nanoliposome: preparation, characterization, physicochemical stability, in vitro release, and antioxidant activity. In Journal of the Science of Food and Agriculture (Vol. 104, Issue 12, pp. 7536-7549). https://doi.org/10.1002/jsfa.13575
32. Sinurat, E., Marraskuranto, E., Sihono, Artanti, N., Zakaria, Z. A., & Randy, A. (2025). Ultrasound extraction of fucoidan and its antioxidant activities from tropical brown seaweeds. International Journal of Biological Macromolecules, 311, 143592. https://doi.org/10.1016/j.ijbiomac.2025.143592
33. Ahmadi, F., Shokoohinia, Y., Javaheri, Sh., & Azizian, H. (2017). Proposed binding mechanism of galbanic acid extracted from Ferula assa-foetida to DNA. Journal of Photochemistry and Photobiology B: Biology, 166, 63-73. https://doi.org/10.1016/j.jphotobiol.2016.11.011
34. Ng, S. W., Lu, P., Rulikowska, A., Boehm, D., O'Neill, G., & Bourke, P. (2021). The effect of atmospheric cold plasma treatment on the antigenic properties of bovine milk casein and whey proteins. Food Chemistry, 342, 128283. https://doi.org/10.1016/j.foodchem.2020.128283
35. Azdad, O., Mejrhit, N., El Kabbaoui, M., Chda, A., Ouahidi, I., Tazi, A., Bencheikh, R., & Aarab, L. (2018). Effect of heating and enzymatic hydrolysis on casein cow milk sensitivity in Moroccan population. Food and Agricultural Immunology, 29(1), 424-433. https://doi.org/10.1080/09540105.2017.1391179
36. Martins, F. C. O. L., Alcantara, G. M. R. N., Silva, A. F. S., Melchert, W. R., & Rocha, F. R. P. (2022). The role of 5-hydroxymethylfurfural in food and recent advances in analytical methods. Food Chemistry, 395, 133539. https://doi.org/10.1016/j.foodchem.2022.133539

37. Wu, J.-W., Hsieh, C.-L., Wang, H.-Y., & Chen, H.-Y. (2009). Inhibitory effects of guava (Psidium guajava L.) leaf extracts and its active compounds on the glycation process of protein. Food Chemistry, 113(1), 78-84. https://doi.org/10.1016/j.foodchem.2008.07.025
38. Li, H., Zhang, Y., Jiang, Y., Li, J. X., Li, C., Zhao, Y., Li, C., Jie, R. Q. D., Zulewska, J., Li, H., & Yu, J. (2023). Application of tea polyphenols as additives in brown fermented milk: Potential analysis of mitigating Maillard reaction products. Journal of Dairy Science, 106(10), 6731-6740. https://doi.org/10.3168/jds.2022-22973