Journal of Food Quality

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Research Article

Improvement in Entrapment Efficiency and *In Vitro* Digestion Stability of Lutein by Zein Nanocarriers with Pepsin Hydrolysis

Yan Jiao,[1] He Han,[1] Ying Chang,[1] Dajing Li,[2] and Asad Riaz[2]

[1]*College of Food and Biological Engineering, Qiqihar University, Qiqihar, China*
[2]*Institute of Farm Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing, China*

Correspondence should be addressed to Yan Jiao; jiaoyan_3000@126.com and Ying Chang; achang-01@163.com

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Zein is one of the popular bioactive carriers and play critical roles in the promotion of stability, absorption, and utilization of the nutrients and bioactive ingredients. The application of zein delivery systems for the encapsulation of bioactive ingredients has recently gained increasing interest. The aim of this work was to modify zein by pepsin and prepare the lutein-loaded zein nanoparticle (LZN) and the lutein-loaded zein hydrolysate nanoparticle (LZHN), respectively. The effects of zein hydrolysation on entrapment efficiency and *in vitro* digestion stability of lutein were also evaluated in this study. Hydrolysation of zein by the pepsin has important effects on lutein embedding. The optimal hydrolysis conditions, including the pepsin concentration (1.5%), temperature (55°C), and time (4 h), enhanced the entrapment efficiency (EE) of lutein by $93.82 \pm 2.82\%$ as compared to $85.18 \pm 3.28\%$ of the untreated zein, respectively. In contrast to LZN, LZHN had better structural characteristics, the average particle size decreases from 158.40 ± 3.22 nm to 112.2 ± 1.56 nm, and LZHN showed better dispersivity and zeta potential. The stability and release assays in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) showed that hydrolyzed zein nanocarriers by pepsin improved the digestion stability and promoted the release of lutein under gastrointestinal digestive conditions. These results suggest that hydrolyzed zein with pepsin may act as an effective carrier for lutein delivery and shows many potential advantages compared with the zein.

1. Introduction

Lutein is a nutritional and functional ingredient and widely exists in a variety of foods. Lutein belongs to the xanthophyll class of carotenoids; it has the peculiarity of having two hydroxyl groups in its structure, which determines its exclusive behavior, and exhibits various biologically significant activities in the body [1, 2]. It has many health benefits such as being antioxidant and preventing oxidative damage [3,4], inhibiting inflammation [5], prevention of atherosclerosis [6], and especially the unique protective actions against ocular disease [7]. For these reasons, lutein is a vital substance for promoting and maintaining good health. Unfortunately, lutein is an unstable bioactive substance; in particular, it is easy to decompose and be deactivated under gastrointestinal digestive conditions in the body. In addition, lutein has high hydrophobicity and poor absorption after oral administration, as well as low bioavailability [8]. As a result, the application of lutein in foods and pharmaceutical industries has been greatly restricted. In order to improve the physical and chemical properties of lutein, and to effectively promote the absorption and utilization of lutein, novel edible protein delivery systems have been developed to enhance the water solubility, *in vitro* stability and *in vivo* stability, and release of lutein. They have played an important role in protecting active ingredients from degradation and inactivation [9, 10].

Zein is one of the commonly used biological active carriers and exhibits good self-assembly and delivery properties in encapsulation of bioactive components. As an edible carrier, zein has been recognized as safe as a direct human food ingredient by the Food and Drug Administration (FDA); therefore, it has been widely used for preparation of nanodelivery systems [11, 12].

Many functional and nutritional components have been successfully encapsulated into zein and synthesize zein nanoparticles to improve their physicochemical characteristics, such as tomato oleoresin, antimicrobial lysozyme, and essential oils [13]. The microstructure, chemical stability, and bioavailability of these active ingredients were significantly improved by embedding them in the zein carrier. However, as a bioactive material carrier, natural zein has some disadvantages in encapsulation and transport of lutein. Zein contains a high proportion of hydrophobic amino acids and it has low solubility which tends to aggregate in aqueous systems and easily form larger polymer particles. Additionally, the low stability and poor controlled-release performance of zein in gastrointestinal conditions have a significant impact on the delivery of the internal active substances and these defects hinder the application of zein as appropriate carriers for bioactive components [14].

To overcome these drawbacks, the recent research focuses on constructing the modified protein nanodelivery systems. In particular, the zymolytic proteins have been revealed to have many advantages as a carrier of active substances. Compared to the proteins, the protein hydrolysates or peptides possess smaller size, better polydispersity, and higher water solubility. For example, corn protein hydrolysate could be developed as a novel nanovehicle to enhance the physicochemical stability and *in vitro* bioaccessibility of vitamin D3 [15]. Zein hydrolysates could be used as oral delivery vehicles to enhance the physicochemical stability and *in vitro* bioaccessibility of curcumin [16]. Zein hydrolysate and tannic acid complex could be employed as an emulsifier in constructing a physical stable nanoemulsion delivery system and showed a remarkable increase in physical stability, high alga oil encapsulation efficiency, and antioxidative properties [17].

However, the zymolytic zein still has some drawbacks: different zein hydrolysates have different properties and their carrier capacities are affected by the conditions of enzymatic hydrolysis and characterization of enzyme. According to the research of Wang et al., the hydrolysis of zein by pepsin could improve the dispersity and stability of zein in water [18]; if zein hydrolysates could be used as nanocarriers for lutein, the solubility and digestion stability of lutein can be improved significantly, and the functional properties and *in vivo* utilization of the lutein will be obviously enhanced.

In this work, zein hydrolysates were prepared by pepsin, the effect of zein hydrolysis on entrapment efficiency of lutein was assessed, and a lutein-loaded nanocomplex system based on zein hydrolysates was first established for improving *in vitro* digestion stability and release of lutein. In particular, their structural characterization and morphology were also investigated.

2. Materials and Methods

2.1. Materials. Lutein was obtained from Sigma Aldrich (St. Louis, MO, USA). Zein was obtained from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). The pepsin was provided by Novo Nordisk (Bagsvaerd, Denmark). Petroleum ether and ethanol were from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China). All other chemicals and reagents used were of analytical grade.

2.2. Hydrolysis of Zein. In order to investigate the effect of zein hydrolysation on the entrapment efficiency of lutein, the zein was hydrolyzed by pepsin according to the method of Jin et al. [19] with some modifications. Based on our previous researches, the hydrolysis time, pepsin dosage, and hydrolysis temperature were mainly effective factors affecting the entrapment efficiency of lutein; therefore, we optimized the three hydrolysis conditions. Briefly, 0.5 g of zein was suspended and homogenized in 25.0 mL of purified water, pepsin (0.5–3.0% w/v) was, respectively, added, and the solution was adjusted to a pH of 3.0 with hydrochloric acid. Then, the mixture was hydrolyzed, respectively, at a certain temperature (40°C–65°C) for 1–6 h. The hydrolysis reaction was finished by deactivating enzyme at 95°C for 5 min. Then, the hydrolysate was freeze-dried (LD-53, Millrock, USA) and stored at 4°C for later use.

2.3. Preparation of LZ and LZH Nanoparticles. The LZ and LZH nanoparticles were prepared by a liquid-liquid dispersion method, according to our previous method with a slight modification [20]. 2.5 mL of zein or hydrolysate (ZH) (2.0 mg/mL) was dissolved in 10 mL ethanol by ultrasonic treatment for 30 s, and 5 mL lutein solution (40 μg/mL) was added dropwise to the zein or ZH solution and stirred under magnetic stirring (1000 rpm) at 20°C for 30 min. In order to prevent the insoluble precipitation, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was injected into 22.5 mL of an aqueous phase and the mixed solution was transferred to a rotary evaporator and incubated in a 50°C water bath. Ethanol was removed under a reduced pressure during the evaporation reaction. The LZN and LZHN suspension were collected and kept at 20°C for further analysis.

2.4. Entrapment Efficiency (EE) Measurement. The encapsulation efficiency was calculated by our previously described method with a slight modification [18]. Briefly, 3.0 mL of LZH and 3 mL of petroleum ether were mixed by vortexing vigorously for 1.0 min at 20°C. The mixed sample was centrifuged at 3000 rpm for 5 min to collect the petroleum ether supernatant. The operation was repeated three times.

The concentration of lutein was determined by HPLC at 445 nm (Column: YMC-C_{30}, 250 × 4.6 mm, 5 μm particle size, Agilent Technologies, Germany) as described by Li et al. 2015 [21]. The mobile phase was methyl tert-butyl ether and methanol at a linear gradient from 0 min (5 : 95, v/v) to 30 min (30 : 70, v/v) and a flow rate of 0.9 mL/min, the column temperature was maintained at 25°C, and 20 μL of sample was injected.

Two calibration curves were, respectively, made by dissolving the lutein standard in petroleum ether or alcohol

in a range from 0.2 to 1.0 mg/L, and the EE (%) of lutein in the nanoparticles was calculated by the following formula:

$$\mathrm{EE}\,(\%) = \left(\frac{1 - W_1}{W_0}\right) \times 100\%. \quad (1)$$

W_1 and W_0 were the weight of free amount of unloaded lutein in petroleum ether and initial weight of lutein added in the LZ or LZH nanoparticles, respectively.

2.5. Particle Size, Polydispersity Index (PDI), and Zeta Potential Analyses. The particle size, PDI, and zeta potential of the LZH and LZHN nanoparticles were characterized by a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). LZN and LZHN suspension were filtered with 0.45 μm membrane before measurement. Mean particle sizes and zeta potential values were calculated as the average of triplicate measurements.

2.6. Morphology Analysis. The morphology of the LZN and LZHN was observed with a transmission electron microscopy (TEM) (Hitachi H-7650, Japan). One droplet of the LZN or LZHN was put on a copper grid of 400 mesh and dried in the air for 5 min. Then, the negative staining was performed with 2% phosphotungstic acid solution for 1 min. After air drying at 20°C, the samples were observed under the TEM [9].

2.7. Stability Evaluation of Lutein in LZ and LZH Nanoparticles during In Vitro Digestion. Simulate gastric (SGF) and simulated intestinal fluid (SIF) model were adopted to evaluate the digestion stability of lutein in LZN and LZHN by the method of Frenzel and Steffen-Heins [22] with some modifications. In order to produce the SGF, 2.0 mL of 0.8% pepsin solution (800–2000 U/mg of protein) was added to the 100 mL of distilled water, and the pH value of the solution was adjusted to 2.0 with hydrochloric acid. The SIF was prepared by dissolving 0.18 g sodium hydroxide, 0.81 g potassium dihydrogen phosphate, 0.48 g pancreatin, and 0.52 g bile salts into 100 mL purified water.

In vitro digestion stability of LZ and LZH nanoparticles were assessed by measuring the degration rate of lutein in SGF and SIF separately according to the method described by Davidov-Pardo et al. [23] with a slight modification. Briefly, 10 mL of LZ or LZH solution was added to 30 mL SGF or SIF separately. The mixed solution was incubated at 37°C with continuous shaking at 100 rpm and sampled after 2–10 h, and then 0.5 mL suspensions were diluted with 10 mL ethanol, the sample was centrifuged at 6000 rpm for 10 min, and the supernatant was filtered through a membrane filter (0.45 μm). The lutein content was assayed based on methods described above. The degradation rate of lutein was calculated by the following equation:

$$\text{degradation rate}\ (\%) = \frac{M_0 - M_t}{M_0} * 100\%. \quad (2)$$

where M_0 and M_t represent the lutein weight before and after *in vitro* digestion, respectively.

2.8. In Vitro Release of Lutein in LZN and LZHN. In vitro release of lutein from LZN and LZHN was evaluated by the method of our previous research, with some modifications [24]. 20 mL of each of LZN and LZHN solution was mixed with 20 mL SGF or SIF, and ten portions of mixed samples were prepared. The suspensions were incubated in a shaking water bath (100 rpm) at 37°C and sampled successively after 1–10 h, 0.5 mL suspensions were mixed well with 5 mL petroleum ether and then vortexed for 1.0 min, the sample was centrifuged at 6000 rpm for 10 min, and the supernatant was collected. The extracted lutein content was subsequently assayed as described above. All measurements were performed in triplicate. The cumulative release was calculated by the following equation:

$$\text{cumulative release}\,(\%) = \frac{C_n}{C} * 100\%, \quad (3)$$

where C_n and C were the lutein content of cumulative release for some time and loaded initially in the LZN and LZHN, respectively.

2.9. Statistical Analysis. All measurements were performed in triplicate, and the experimental results were statistically tested for significance ($p < 0.05$) for analysis of variance using SPSS software. All data were reported as the mean ± standard deviation (SD).

3. Results and Discussion

3.1. Influence of Zein Hydrolysis Time on Entrapment Efficiency of Lutein. The effect of zein hydrolysis at different times on the entrapment efficiency of lutein was studied. The zein was hydrolyzed with pepsin (1.5%) at 50°C for 1–6 h. Figure 1 indicates the variation in the entrapment efficiency of lutein in connection with hydrolysis time. It could be observed that the unhydrolyzed zein (0 h) showed lower entrapment efficiency of lutein. Instead, the entrapment efficiency of hydrolyzed zein by pepsin displayed a gradual increase with increase of hydrolysis time. When the zein was hydrolyzed for 4 h, the entrapment efficiency of lutein reached a maximum value. This is because when the zein was hydrolyzed, the pepsin hydrolysis caused structural destruction of zein, some polypeptides increased gradually, the polypeptides were combined with lutein, and the entrapment efficiency of lutein was increased. But the hydrolysis degree of zein reached the maximum at 4 h, the small molecular peptides and amino acids increased, and the entrapment efficiency of lutein was no longer increased and even showed a slight decrease after 4 h. Therefore, the entrapment efficiency of lutein was higher at 4 h hydrolysis of zein, where medium sized zein and polypeptides could be easily formed, which were beneficial to improve the encapsulation efficiency [25, 26].

3.2. Influence of Pepsin Dosage on Entrapment Efficiency of Lutein. The optimal pepsin dosage during the hydrolysis process is a crucial factor for encapsulation capacity of zein. In Figure 2, various pepsin dosages (0.5%, 1.0%, 1.5%, 2.0%,

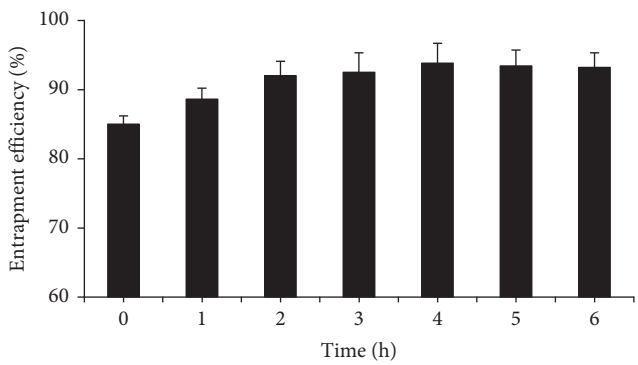

Figure 1: Effect of zein hydrolysis time on entrapment efficiency of lutein. The other hydrolysis conditions were kept constant: pepsin dosage 1.5%, hydrolysis temperature 50°C, and pH 3.0. Data were expressed as mean values ± standard deviation ($n = 3$).

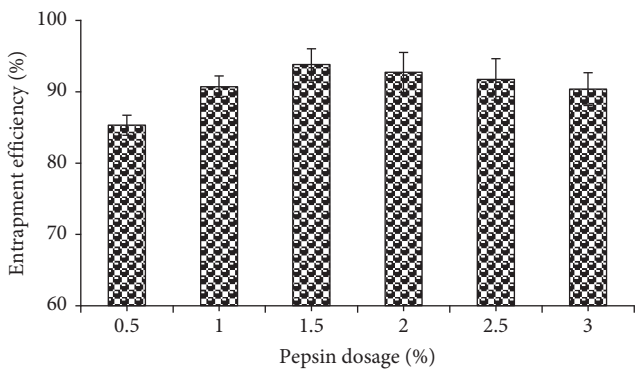

Figure 2: Effect of pepsin dosage on entrapment efficiency of lutein. The other hydrolysis conditions were kept constant: hydrolysis temperature 50°C, pH 3.0, and hydrolysis time 4 h. Data were expressed as mean values ± standard deviation ($n = 3$).

2.5%, and 3.0%) were used for hydrolysis of zein at 50°C for 4 h, the results showed that the entrapment efficiency of lutein increased with an increase in the pepsin dosage up to 1.5% in the hydrolysis of zein, and the higher entrapment efficiency of lutein (92.85 ± 2.25%) resulted from the 1.5% pepsin dosage of hydrolysis of zein. Unfortunately, there was no homologous increase seen for higher pepsin dosage (2.0–3.0%). These results indicate that the optimal pepsin dosage is key to hydrolyze zein to obtain a high EE of lutein, the suitable pepsin dosage could cause limited enzymatic hydrolysis, they could damage the structure of zein and produced polypeptides which led to higher encapsulation capacity, and the redundant pepsin was useless for encapsulation of lutein with hydrolysate [27]. Therefore, the hydrolysis of zein with 1.5% pepsin was the most appropriate method to improve the encapsulation capacity of zein.

3.3. Influence of Hydrolysis Temperature of Zein on Entrapment Efficiency of Lutein. The zein was hydrolyzed with pepsin (1.5%) at different temperatures (40°C, 45°C, 50°C, 55°C, 60°C, and 65°C) for 4 h. The result is shown in Figure 3. With the increase of the hydrolysis temperature, a significant increase in EE of lutein was found to be 93.82 ± 2.82%. The

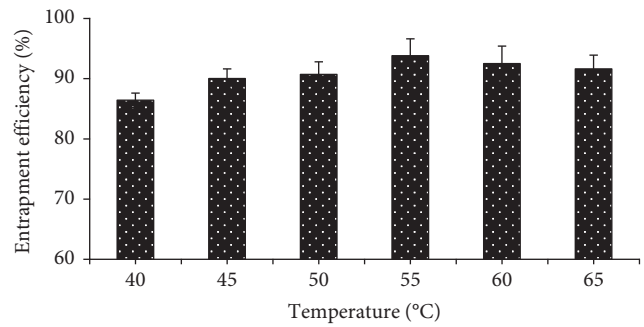

Figure 3: Effect of zein hydrolysis temperature on entrapment efficiency of lutein. The other hydrolysis conditions were kept constant: pepsin dosage 1.5%, hydrolysis time 4 h, and pH 3.0. Data were expressed as mean values ± standard deviation ($n = 3$).

EE decreases gradually when the temperature raises above 55°C. These results indicate that temperature is critical for enzymes hydrolysis; optimal temperature is known to be the chief reason for enzymes hydrolysis of zein and has an important influence on structure and property of zein. On one hand, the enzyme reaction has an optimal temperature and high temperatures could deactivate enzymes. On the other hand, the suitable temperature is a benefit for modification of zein [28]. Therefore, the optimal temperature is 55°C. The zein was hydrolyzed with 1.5% pepsin at 55°C for 4 h, the zein was appropriately modified, the maximum EE could be reached at 93.82 ± 2.82%, and once the temperature reached 55°C, there would not be any further significant microstructure damage; increase of hydrolysis temperature of zein to more than 55°C cannot lead to high EE of lutein which could be due to excessive degradation of zein and pepsin loss. Therefore, the hydrolysis temperature of zein at 55°C was the most optimum temperature.

3.4. Physicochemical Characterizations

3.4.1. Particle Size, Polydispersity Index (PDI), and Zeta Potential Analyses. The lutein-loaded zein nanoparticles (LZN) and lutein-loaded zein hydrolysate nanoparticle (LZHN) were successfully prepared by liquid-liquid dispersion method [29], and the average particle size, PDI, and zeta potential of samples were measured, respectively.

The particle size analysis revealed that the average particle size of the LZN and LZHN was obviously different (Figures 4(a) and 4(b)); the average particle size of the LZN was 158.40 ± 3.22 nm, while the zein was hydrolyzed; the macromolecular zein was broken down into small proteins and peptides; the size and hydrophobicity decreased [30], and when the lutein was loaded in zein hydrolysate to form LZHN, the average particle decreased to 112.24 ± 1.56 nm. Therefore, the size of LZHN was smaller than the size of LZN.

The PDI values of LZN and LZHN were less than 0.5, and both of them displayed good polydispersity index. The PDI of LZN was 0.147 ± 0.026, but it was lower in LZHN (0.039 ± 0.008) on account of the hydrolysis of zein, and possessed better dispersion. The reason may be that the

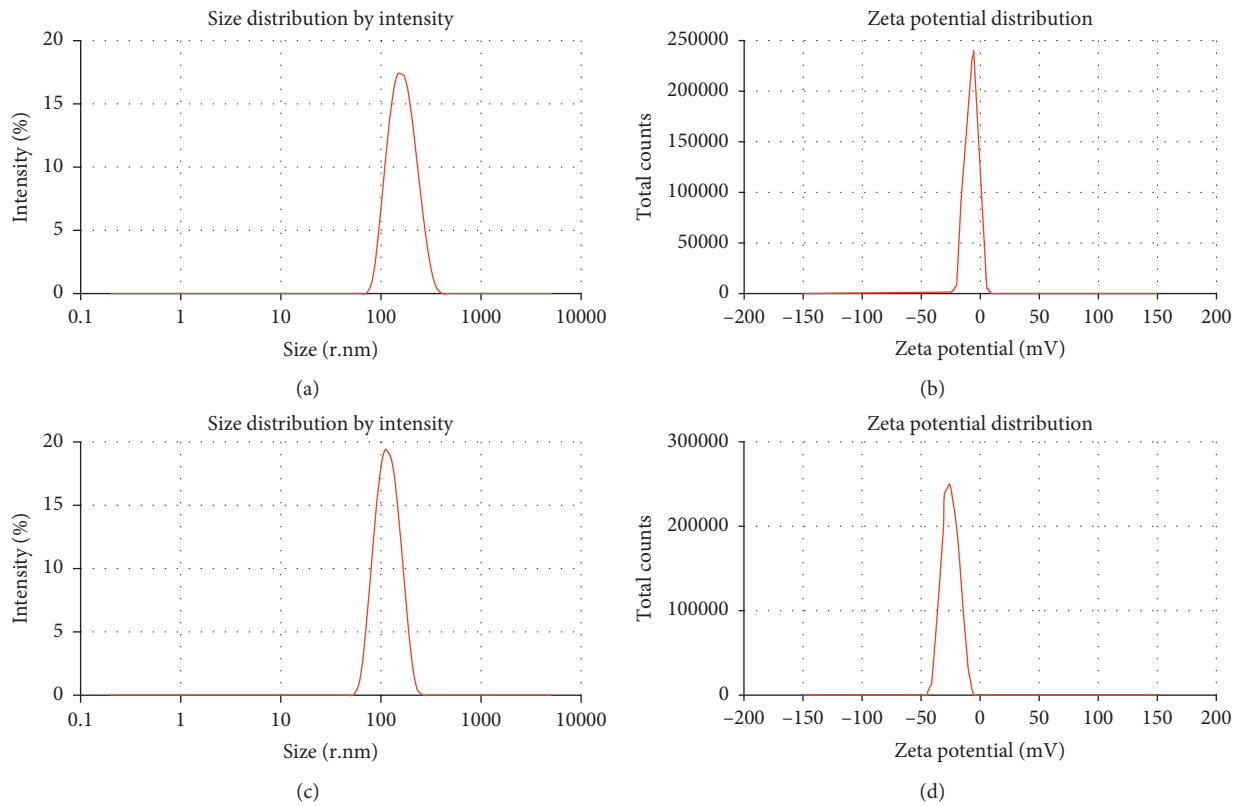

Figure 4: The average particle size (a, c) and zeta potential (b, d) of LZN (a) and LZHN (b).

water-soluble groups in zein increased due to proteolysis, and the solubility and dispersion of LZHN were therefore improved. It also means that zein hydrolysate is helpful to improve the stability and release of lutein-loaded zein nanoparticles and improve the solubility and dispersity of lutein in water.

Zeta potential diagram showed that LZN possessed a lower negative charge of -5.8 ± 0.06 mV (Figure 4(b)), and this indicated that the LZN was unstable and prone to aggregation. Compared to the LZN, LZHN possessed an average negative charge of -25.6 ± 1.06 mV (Figure 4(d)), and hydrolysis resulted in a significant change in zeta potential. The obvious changes of zeta potential revealed that there were more negative charge groups in the zein hydrolysate after enzymatic hydrolysis, and they could prevent zein protein from aggregating into large particles and improve the stability and dispersibility of zein particles. Therefore, the entrapment efficiency and dispersity of lutein were also improved.

3.4.2. Transmission Electron Microscopy (TEM). The microstructure of the lutein-loaded zein nanoparticle (LZN) and the lutein-loaded zein hydrolysate nanoparticle (LZHN) was examined by TEM. As shown in Figure 5, the lutein was embedded in the zein and hydrolysate through self-assembly. LZN (Figures 5(a) and 5(c)) and LZHN (Figures 5(b) and 5(d)) showed spherical shape nanoparticles of approximately 100–200 nm in diameter, consistent with the previous examined results of particle size, and they formed an embedded core-shell structure to protect lutein. LZN displayed bigger size than LZHN and tended to assemble and constitute larger nanoscale particles, leading to an increase in particle size [31]. Instead, LZHN showed smaller nanoparticles and seemed to be well distributed in the suspension system. The hydrolysis decreased the hydrophobic interaction of zein and exhibited a regular spherical carrier structure; zein hydrolysate did not cause lutein aggregation and exhibited a higher entrapment efficiency.

3.5. In Vitro Digestion Stability. The digestion stability of lutein was investigated by measuring the lutein degradation rates of the uncoated lutein, LZN, and LZHN in SGF and SIF. Figure 6 indicates that the uncoated lutein decomposed rapidly in SGF and SIF, and lutein degradation rates reached $42.93 \pm 1.78\%$ and $34.77 \pm 2.07\%$ after 10 h of incubation, respectively.

When the lutein was coated with LZ, the lutein was found to degrade at a slower rate than the uncoated lutein. Degradation rates of lutein in SGF (Figure 6(a)) and SIF (Figure 6(b)) were $32.32 \pm 1.36\%$ and $27.42 \pm 1.31\%$, respectively. The lutein stabilized by the LZHN complex showed a remarkable stability, and the lutein degradation rates decreased to $26.81 \pm 1.53\%$ and $25.66 \pm 0.66\%$ after 10 h of incubation, respectively.

This could be attributed to the fact that the EE of lutein in LZHN was higher than LZN, and the free lutein content decreased in the digestive juice system whereas zein and zein

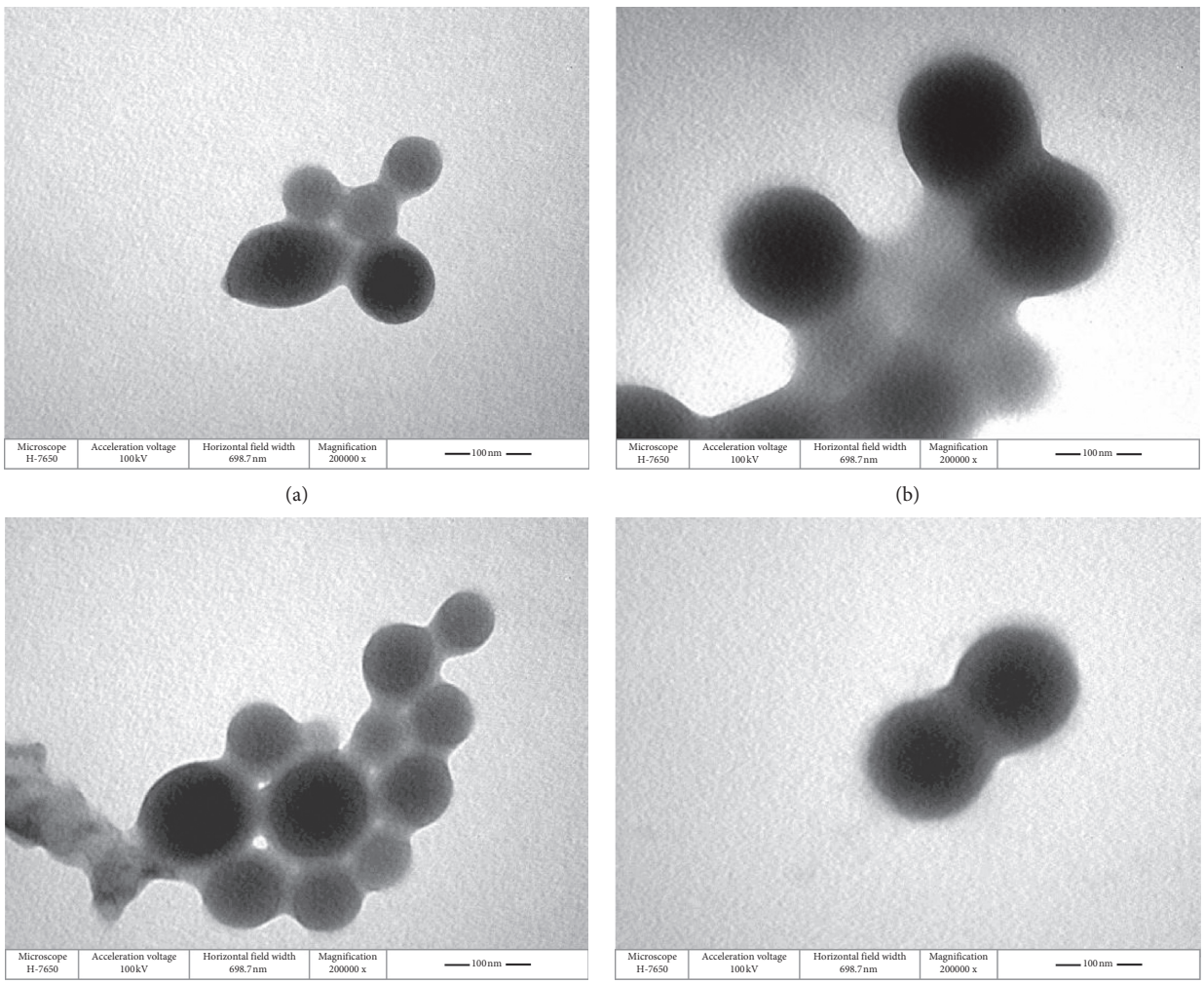

Figure 5: Transmission Electron Microscope (TEM) images of zein and lutein-loaded zein nanoparticle (LZN) (a, c), and zein hydrolysate nanoparticle and lutein-loaded zein hydrolysate nanoparticles (LZHN) (b, d).

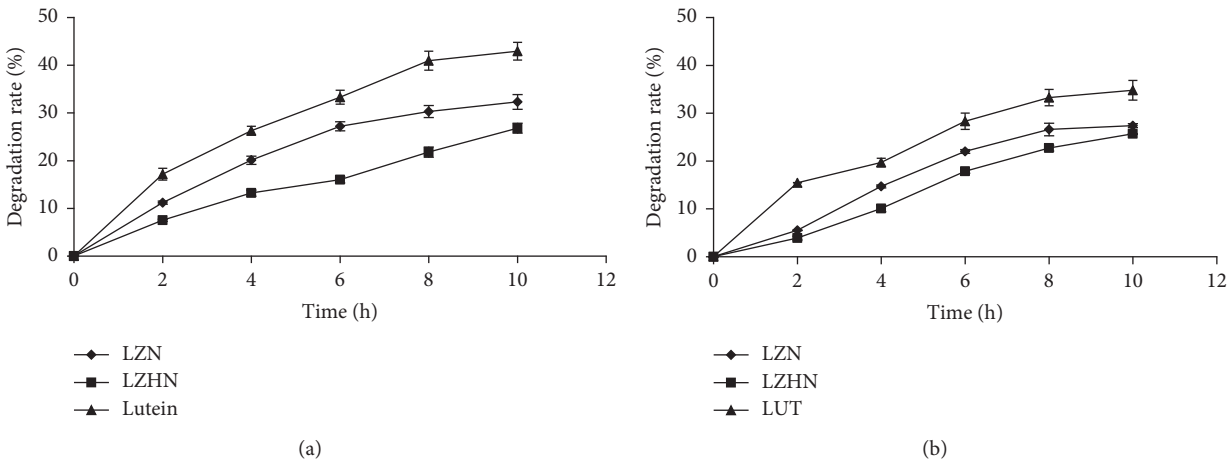

Figure 6: Lutein degradation rate of free lutein, LZN, and LZHN during digestion in SGF (a) and in SIF (b) for 10 h.

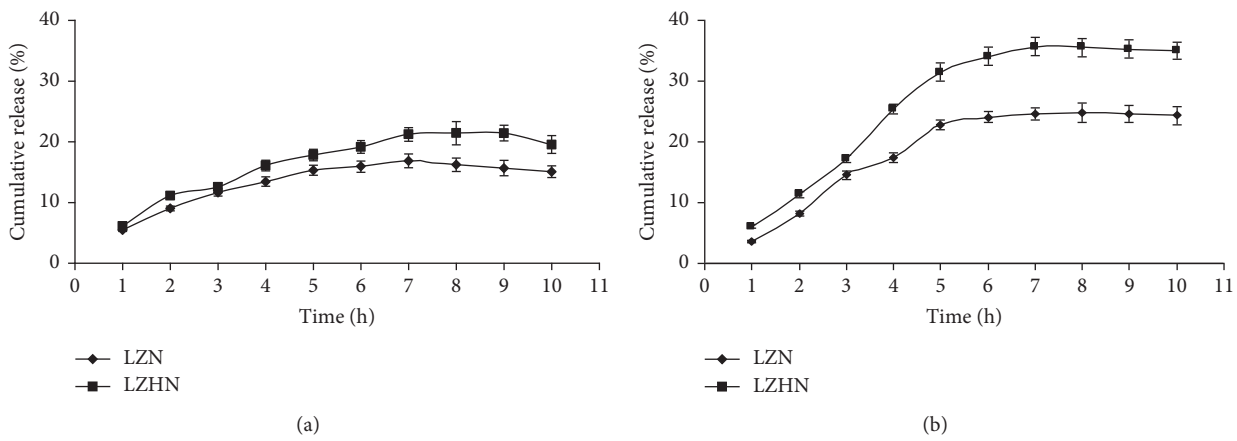

Figure 7: Lutein cumulative release of LZN and LZHN during digestion in SGF (a) and in SIF (b) for 10 h.

hydrolysate could protect embedded lutein from degradation [32]. Furthermore, the zein hydrolysate could decrease the following hydrolysis by pepsin and therefore protect loaded lutein from degradation. The results revealed that zein hydrolysates displayed better stability and they could prevent degradation of lutein in SGF and SIF. For this reason, LZN and LZHN could decrease the degradation of lutein in acidic and alkaline conditions and increased the digestion stability of lutein.

The result verified that free lutein was more susceptible to degradation than its encapsulation in nanoparticles, and the stability of lutein could be increased in digestive process by embedding lutein in LZN and LZHN. Particularly, the digestion stability of the lutein loaded in zein hydrolysate was much higher than the lutein loaded in zein.

3.6. In Vitro Release Characteristics. The lutein release from LZN and LZHN was performed for 10 h in SGF and SIF, respectively. Figure 7 showed that lutein cumulative release of LZN and LZHN was 16.86 ± 0.96% and 21.22 ± 0.84% after 7 h of incubation in SGF, respectively (Figure 7(a)). And the cumulative release decreased slightly due to the degradation of lutein. But the lutein cumulative release of LZN and LZHN increased to 24.03 ± 0.92% and 34.08 ± 1.48% after 6 h of incubation in SIF (Figure 7(b)), respectively. And then the cumulative release reached the sustained release after 6 h of incubation. The results showed that LZHN released much more encapsulated lutein in SIF than in SGF, and the lutein in LZHN was released faster than in LZN in the same time under the SGF and SIF traditions.

The results revealed that lutein cumulative release in LZHN was higher than that of zein in SGF and SIF, and the release of lutein was better in alkaline intestinal solutions than acidic gastric juice. The possible reasons are that zein hydrolysate has stronger hydrophilicity than zein, it displayed good solubility and dispersibility in SGF and SIF, and the lutein loaded in the zein hydrolysate could be easily released from nanoparticles. For this reason, the cumulative release of lutein was higher, especially in intestinal fluids. In conclusion, zein hydrolysate is a suitable carrier for lutein delivery.

4. Conclusion

In this work, lutein-loaded zein and its hydrolysate nanoparticles were successfully synthesized as a novel nanocarrier for lutein. The hydrolysis of zein was found to significantly affect the entrapment efficiency of lutein. Zein hydrolysate nanoparticle exhibited excellent physicochemical characterizations, and lutein-loaded hydrolytic zein had a smaller nanoscale size and exhibited good dispersibility for the micromolecular zein and polypeptide. Meanwhile, the digestive stability and release of lutein in zein hydrolysate nanoparticles in SGF and SIF were significantly enhanced. Nonetheless, the molecular weight of the zein hydrolysate used for embedding lutein is still uncertain, and the *in vivo* absorption and bioavailability of lutein in zein hydrolysate nanoparticle is not clear.

In conclusion, enzymatic hydrolysis of zein by pepsin was a simple and effective means for improving the coating performance and load capacity, and it seems to be a superior carrier for improving the *in vitro* digestion stability of lutein. This may be useful for potential applications for development of active ingredients carrier.

Our future studies mainly focus on the development of zein hydrolysate by different sources of enzymes, the effect of molecular weight and amino acid distribution to the encapsulation and transport capacity will be studied, and the stability and *in vivo* absorption of lutein will be further investigated. The novel and effective modified zein delivery system of lutein will be the emphasis of our research.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Practical Application. The application of pepsin hydrolysis has potential for increasing the encapsulation and transport capacity of the zein. Zein is a common and important carrier of lutein, while natural zein has poor solubility and easily

aggregates in water, which displayed low embedding rate and poor release property for lutein. Pepsin hydrolysis could significantly increase the entrapment efficiency and *in vitro* digestion stability of lutein. Pepsin hydrolysis is a simple and effective method to modify the properties of zein, which can be beneficial in food industries for the load and delivery of active substance.

Conflicts of Interest

The authors declare that this article content has no conflicts of interest.

Acknowledgments

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References

[1] D. B. Rodrigues, A. Z. Mercadante, and L. R. B. Mariutti, "Marigold carotenoids: much more than lutein esters," *Food Research International*, vol. 119, pp. 653–664, 2019.

[2] E. Giordano and L. Quadro, "Lutein, zeaxanthin and mammalian development: metabolism, functions and implications for health," *Archives of Biochemistry and Biophysics*, vol. 647, pp. 33–40, 2018.

[3] E.-S. M. Abdel-Aal and I. Rabalski, "Antioxidant properties of high-lutein grain-based functional foods in comparison with ferulic acid and lutein," *American Journal of Biomedical Sciences*, vol. 5, no. 2, pp. 109–125, 2013.

[4] A. L. B. Zeni, A. Camargo, and A. P. Dalmagro, "Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities," *Pharmacology Biochemistry and Behavior*, vol. 179, pp. 63–72, 2019.

[5] M. Di Filippo, B. D. Mathison, J. S. Park, and B. P. Chew, "Lutein and β-cryptoxanthin inhibit inflammatory mediators in human chondrosarcoma cells induced with IL-1β," *The Open Nutrition Journal*, vol. 6, no. 1, pp. 41–47, 2012.

[6] A. G. Murillo and M. L. Fernandez, "Lycopene and lutein and the prevention of atherosclerosis: is supplementation necessary," *Ecronicon Nutrition*, vol. 2, pp. 466–474, 2015.

[7] P. S. Bernstein, B. Li, P. P. Vachali et al., "Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease," *Progress in Retinal and Eye Research*, vol. 50, pp. 34–66, 2016.

[8] T. Chuacharoen and C. M. Sabliov, "Stability and controlled release of lutein loaded in zein nanoparticles with and without lecithin and pluronic F127 surfactants," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 503, pp. 11–18, 2016.

[9] S. Uzun, H. Kim, C. Leal, and G. W. Padua, "Ethanol-induced whey protein gels as carriers for lutein droplets," *Food Hydrocolloids*, vol. 61, pp. 426–432, 2016.

[10] C. Zhao, X. Shen, and M. Guo, "Stability of lutein encapsulated whey protein nano-emulsion during storage," *PLoS One*, vol. 13, no. 2, Article ID e0192511, 2018.

[11] A. O. Elzoghby, W. M. Samy, and N. A. Elgindy, "Protein-based nanocarriers as promising drug and gene delivery systems," *Journal of Controlled Release*, vol. 161, no. 1, pp. 38–49, 2012.

[12] M. R. Kasaai, "Zein and zein-based nano-materials for food and nutrition applications: a review," *Trends in Food Science & Technology*, vol. 79, pp. 184–197, 2018.

[13] F. Xue, C. Li, Y. Liu, X. Zhu, S. Pan, and L. Wang, "Encapsulation of tomato oleoresin with zein prepared from corn gluten meal," *Journal of Food Engineering*, vol. 119, no. 3, pp. 439–445, 2013.

[14] Y.-H. Wang, J.-M. Wang, X.-Q. Yang, J. Guo, and Y. Lin, "Amphiphilic zein hydrolysate as a novel nano-delivery vehicle for curcumin," *Food & Function*, vol. 6, no. 8, pp. 2636–2645, 2018.

[15] Y. Lin, Y.-H. Wang, X.-Q. Yang, J. Guo, and J.-M. Wang, "Corn protein hydrolysate as a novel nano-vehicle: enhanced physicochemical stability and in vitro bioaccessibility of vitamin D3," *LWT—Food Science and Technology*, vol. 72, pp. 510–517, 2016.

[16] R. Pan, Y. Zou, J. Wang et al., "Gamma/alpha-zein hydrolysates as oral delivery vehicles: enhanced physicochemical stability and in vitro bioaccessibility of curcumin," *International Journal of Food Science & Technology*, vol. 53, no. 7, pp. 1622–1630, 2018.

[17] Y.-H. Wang, Z.-L. Wan, X.-Q. Yang, J.-M. Wang, J. Guo, and Y. Lin, "Colloidal complexation of zein hydrolysate with tannic acid: constructing peptides-based nanoemulsions for alga oil delivery," *Food Hydrocolloids*, vol. 54, pp. 40–48, 2016.

[18] H.-J. Wang, Z.-X. Lin, X.-M. Liu, S.-Y. Sheng, and J.-Y. Wang, "Heparin-loaded zein microsphere film and hemocompatibility," *Journal of Controlled Release*, vol. 105, no. 1-2, pp. 120–131, 2005.

[19] D.-X. Jin, X.-L. Liu, X.-Q. Zheng, X.-J. Wang, and J.-F. He, "Preparation of antioxidative corn protein hydrolysates, purification and evaluation of three novel corn antioxidant peptides," *Food Chemistry*, vol. 204, pp. 427–436, 2016.

[20] Y. Jiao, X. Zheng, Y. Chang, D. Li, X. Sun, and X. Liu, "Zein-derived peptides as nanocarriers to increase the water solubility and stability of lutein," *Food & Function*, vol. 9, no. 1, pp. 117–123, 2018.

[21] D.-J. Li, J.-F. Song, and C.-Q. Liu, "Stereoisomers identification and storage stability of microencapsulated marigold lutein," *International Journal of Food Properties*, vol. 18, no. 1, pp. 178–185, 2015.

[22] M. Frenzel and A. Steffen-Heins, "Whey protein coating increases bilayer rigidity and stability of liposomes in food-like matrices," *Food Chemistry*, vol. 173, pp. 1090–1099, 2015.

[23] G. Davidov-Pardo, C. E. Gumus, and D. J. McClements, "Lutein-enriched emulsion-based delivery systems: influence of pH and temperature on physical and chemical stability," *Food Chemistry*, vol. 196, pp. 821–827, 2016.

[24] Y. Jiao, D. Li, C. Liu, Y. Chang, J. Song, and Y. Xiao, "Polypeptide—decorated nanoliposomes as novel delivery systems for lutein," *RSC Advances*, vol. 8, no. 55, pp. 31372–31381, 2018.

[25] Y. Li, H. Liu, Q. Liu, B. Kong, and X. Diao, "Effects of zein hydrolysates coupled with sage (Salvia officinalis) extract on the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions," *Food Hydrocolloids*, vol. 87, pp. 149–157, 2019.

[26] X.-Q. Zheng, J.-T. Wang, X.-L. Liu et al., "Effect of hydrolysis time on the physicochemical and functional properties of corn glutelin by Protamex hydrolysis," *Food Chemistry*, vol. 172, pp. 407–415, 2015.

[27] H.-T. Tao, Y.-X. Wang, W. Liu et al., "Optimizion of enzymatic hydrolysis of zein to prepare protein hydrolysate with increased antioxidants," *Biomedical Research*, vol. 29, no. 12, pp. 2631–2636, 2018.

[28] X. Liu, X. Zheng, Z. Song et al., "Preparation of enzymatic pretreated corn gluten meal hydrolysate and in vivo evaluation of its antioxidant activity," *Journal of Functional Foods*, vol. 18, pp. 1147–1157, 2015.

[29] T. Bilenler, I. Gokbulut, K. Sislioglu, and I. Karabulut, "Antioxidant and antimicrobial properties of thyme essential oil encapsulated in zein particles," *Flavour and Fragrance Journal*, vol. 30, no. 5, pp. 392–398, 2015.

[30] Y.-H. Wang, J.-M. Wang, J. Guo, Z.-L. Wan, and X.-Q. Yang, "Amphiphilic zein hydrolysate as a delivery vehicle: the role of xanthophylls," *LWT—Food Science and Technology*, vol. 79, pp. 463–470, 2017.

[31] Y.-H. Wang, Y. Yuan, X.-Q. Yang, J.-M. Wang, J. Guo, and Y. Lin, "Comparison of the colloidal stability, bioaccessibility and antioxidant activity of corn protein hydrolysate and sodium caseinate stabilized curcumin nanoparticles," *Journal of Food Science and Technology*, vol. 53, no. 7, pp. 2923–2932, 2016.

[32] C. J. Cheng, M. Ferruzzi, and O. G. Jones, "Fate of lutein-containing zein nanoparticles following simulated gastric and intestinal digestion," *Food Hydrocolloids*, vol. 87, pp. 229–236, 2019.

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Research Article

Postharvest Application of *Aloe vera* Gel-Based Edible Coating to Improve the Quality and Storage Stability of Fresh-Cut Papaya

Vittorio Farina,[1] Roberta Passafiume,[1] Ilenia Tinebra,[1] Dario Scuderi,[1] Filippo Saletta,[1] Giovanni Gugliuzza,[2] Alessandra Gallotta,[3] and Giuseppe Sortino[1]

[1]*Department of Agricultural, Food and Forest Sciences (SAAF), Università Degli Studi di Palermo, Palermo, Italy*
[2]*CREA Research Centre for Plant Protection and Certification, Palermo, Italy*
[3]*Department Sciences of Soil, Plants and Food (DiSSPA), Università di Bari, Bari, Italy*

Correspondence should be addressed to Roberta Passafiume; roberta.passafiume@unipa.it

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Ready-to-eat products are damaged by various factors, including exposure to O_2 and CO_2, extreme temperatures, and rapid decay, due to trauma during processing. The use of natural antimicrobial agents and antioxidants might extend the shelf-life of the fruits. The aim of this work is to investigate the effects of four different antibrowning and gelling agents added into the *Aloe vera* gel-based edible coatings and applied to fresh-cut papaya. EC1 treatment consists of *Aloe vera* gel (30% v/v), EC2 contains $CaCl_2$ (5% v/v), EC3 contains K carrageenan (0.5% v/v), and EC4 contains sodium alginate (1.5% v/v) and K carrageenan (0.5% v/v). The fruits treated with EC2 showed the best results while maintaining high values in terms of firmness (that differ from the control of 42.5%), soluble solid content (that differ from the control of 14.6%), and titratable acidity (that differ from the control of 49%). Hence, the addition of $CaCl_2$ also reduces the ripening rate and loss of color without altering the product's sensory qualities. EC3 and EC4 treatments have provided an oxygen barrier and reduced respiratory rate, increasing the firmness retention and keeping a high C^* value thanks to K carrageenan and sodium alginate.

1. Introduction

During the last decade, there has been a marked change in both consumers' lifestyles and in the climate: the former has stimulated the demand by modern consumers for ready-to-eat fruits and vegetables that have been peeled, cut, washed, dried, and packaged in plastic trays and finally are marketed in refrigerated cases [1]; the second has allowed Mediterranean areas to cultivate tropical and subtropical crops [2]. During this last decade, the cultivation of papaya (*Carica papaya* L.) has also spread in these areas. It is originally from southern Mexico and Costa Rica and it was introduced as a plantation crop cultivated in protected environments throughout all tropical and subtropical regions; in fact, in the coastal areas of Sicily, papaya cultivation could represent a valid alternative to traditional crops in cold greenhouses.

Papaya is a climacteric fruit and is known for its high nutritional value: it contains low calories and is rich in vitamins and minerals such as vitamins C and A, riboflavin, folate calcium, thiamine, iron, pantothenic acid, niacin, potassium, and fibers [3]. Papayas have a very short postharvest shelf-life (7–14 days) under greenhouse conditions [4, 5]; for this reason, several researchers have developed postharvest storage procedures that help minimize quality deterioration [6] and help prolong shelf-life while maintaining high sensory and nutritional attributes. Several conservation techniques have been studied and developed to increase the shelf-life of fresh-cut fruit, such as pickling, drying, high-pressure processing, modified atmospheres, and edible coating. Some of these techniques use additives for increased effectiveness [7]. However, the organoleptic properties of these fresh-cut fruits are influenced by these preservation techniques. For example, the drying method will cause a loss of moisture, thus changing the appearance and taste of the fruit. Therefore, the edible coating could be the most favorable method for maintaining the condition of

fresh-cut fruit. The edible coating (EC) is an odorless, colorless, and tasteless substance consisting of hydrocolloids, polysaccharides, proteins, lipids, and wax and forms an invisible barrier on the surface of the fruit that separates it from the surrounding atmosphere [8]. It also reduces water loss and controls microbial growth while preserving fruit quality and giving the product better mechanical resistance [9]. A layer of edible material is especially effective in preserving postharvest quality and reducing production costs for highly perishable fruits such as the papaya [10–12]. Although its effectiveness has been tested on different fruits, its application is not yet widespread [13], especially not on fresh-cut fruits. Tavassoli-Kafrani et al. [14] have studied the application of calcium chloride, K carrageenan, and sodium alginate as film-forming agents: calcium forms links between pectic substances within the cell wall [15] giving it the potential to increase the postharvest quality of fruits and vegetables [16, 17]. In addition, the colloidal properties of alginate, salt of alginic acid isolated from brown algae, results in the formation of resistant gels or insoluble polymers by reacting with Ca^{+} after treatment with $CaCl_2$ [18]. Instead, K-carrageenan is derived from carrageenans, which are natural hydrophilic polymers [19] constituted by a linear chain of partially sulphated galactans. These biopolymer-based films extend food shelf-life by maintaining organoleptic and sensory characteristics [20] and inhibiting oxidation processes [21]. Another interesting aspect is that the products used in edible coating are totally natural and are considered safer for consumption, as they are nontoxic and economical.

For this reason, recent studies have shown that the gel extracted from different parts of plants has an antifungal and antimicrobial effect on the whole fruit [22]. *Aloe vera* gel has been applied to grapes [23], cherries [24], strawberries [25], litchis [26], and plums [27] in film form, showing excellent results in terms of browning reduction and fungal alterations on the surfaces of treated fruits. *Aloe vera* gel has been used as an edible coating for raw produce such as nectarines [28], mangoes [29], apples [30], papayas [4], and table grapes [31, 32]. Furthermore, *Aloe vera* provides 20 of the 22 amino acids required in the human diet and 7 of the 8 essential amino acids. It is a good source of vitamins that act as antioxidants that neutralize free radicals [33, 34].

Therefore, the aim of this work was to evaluate the potential activity of *Aloe vera* gel in edible coating based on calcium chloride, K carrageenan, and sodium alginate, to assess the changes in the quality attributes of fresh-cut papaya.

2. Materials and Methods

2.1. Vegetal Material. Fifty papaya fruits were harvested at green-mature ripening stage (entire surface green, with traces of yellow on the flesh), with a regular shape and uniform size, from the "Orto di Nonno Nino" farm greenhouse in Sicily (38°08′ N, 13°10′ E).

2.2. Chemicals. Food-grade sodium alginate (Keltones LV, ISP, San Diego, CA, USA) and gellan gum (Kelcogels, CPKelco, Chicago, IL, USA) were used as carbohydrate biopolymers in the coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added as a plasticizer. $CaCl_2$ (Sigma-Aldrich Chemic, Steinheim, Germany) was employed for cross-linking (0.75% v/v) per 2 min, and *Aloe vera* gel was added as an antimicrobial agent.

2.3. Preparation of Aloe vera Gel. Homogeneous leaves were selected according to size and harvested according to visual analysis. The epidermis was separated from the gel, which was manually cut into portions of 10 ± 1 mm in thickness and the sample was maintained at 4 ± 1°C in a refrigerator. The gelatinous parenchyma was triturated (Ultra-Turrax T25, Janke and Kunkle, IKa Labortechnik, Breisgau, Germany) for 5 minutes at 24.500 rpm, to form a homogeneous substance, and filtered to remove the fibrous portion. Then, a gelling agent (Gelzan™ CM Gelrite®, Sigma-Aldrich, 1% v/v) and glycerol (3% v/v) were added to improve the viscosity and plasticity of the film [4]. An additional homogenization, at 90°C for 40 minutes, was used as a microbiological stabilization of the solution.

2.4. Coating Composition. For the preparation of the edible coating, four treatments were tested in 500 ml of distilled water:

(i) CTR: control, untreated

(ii) EC1: gellan gum (1% v/v), Aloe v. gel (30% v/v), and glycerol (3% v/v)

(iii) EC2: gellan gum (1% v/v), Aloe v. gel (30% v/v), glycerol (3% v/v), and $CaCl_2$ (5% v/v)

(iv) EC3: gellan gum (1% v/v), Aloe v. gel (30% v/v), and K carrageenan (0.5% v/v)

(v) EC4: gellan gum (1% v/v), Aloe v. gel (30% v/v), sodium alginate (1.5% v/v), and K carrageenan (0.5% v/v)

All of the concentrations were selected on the basis of other studies conducted on other kinds of fruits and on papaya in which positive and statistically significant results were obtained [35–37].

The sample was homogenized with heat treatment (90°C for 45 min), and ascorbic acid (0.5% v/v) and citric acid (1% v/v) were added to prevent the browning of the solution and to maintain a pH value below 4.

The papaya fruits were washed with cold tap water to remove surface contamination and then were sanitized by immersion in sodium hypochlorite (2% v/v) in distilled water at 5°C for 2 min. After that, each papaya fruit was hand-peeled and cut into slices of 2 cm using a ceramic knife and at room temperature (9 ± 1°C). The knife and cutting board were rinsed in cool water at 8°C.

All of the treatments were applied by spraying with an airbrush (0.8 mm of nozzle diameter) powered by N_2. Finally, 100 g of fresh-cut fruits was packaged in a passive atmosphere and stored at a low temperature (5°C and 90% RH), in 90 PET trays for 12 days. Each tray (125 mm × 115 mm and 150 cc) was thermally sealed on the top with a 50 mm thick bioriented polypropylene (BOPP) film. The BOPP permeability was 2.004 mL O_2 m^{-2} d^{-1} atm^{-1}

and 3.824 mL CO_2 m^{-2} d^{-1} atm^{-1}. All of the analysis was carried out on an interval of three days up to twelve days.

2.5. Physicochemical Characterization

2.5.1. Weight Loss. The weight loss was measured every three days with a two-decimal precision digital scale (Gibertini, Italy). The value was expressed as a relative percentage and calculated as weight loss (%) = (Wi – Wt)/Wi ∗ 100 (where Wi is the initial weight and Wt is the weight measured during storage).

2.5.2. Firmness. The firmness of the pulp (*N*) was determined by a digital penetrometer (mod. 53205, Turoni, Forlì, Italia), in two opposite sides of the fruit.

2.5.3. Total Soluble Solid Content. The total soluble solid content (°Brix) was estimated by a digital optical refractometer ATAGO (Atago Co., Ltd, Tokyo, Japan).

2.5.4. Titratable Acidity and pH. The titratable acidity (gL^{-1} MA%) and the pH value were measured with a Crison Compact pH meter titrator (Crison Instruments, SA, Barcelona, Spain).

2.5.5. Flesh Color. A Minolta colorimeter (Chroma Meter CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) was used to evaluate the color of the fresh-cut papaya. The color space is shown as follows: brightness (L^* value); the hue angle, which shows the change in color of the fruit flesh (a lower h° indicates a more intense browning); and the saturation, which represents the degree of color saturation (higher C^* values indicate a brighter color and consequently a higher market value). The instrument has been calibrated using a standard white plate.

2.5.6. O_2 and CO_2 Analysis. The respiration rate (RR) of each tray was regularly monitored using a gas analyzer (CheckMate II, PBI-Dansensor (Far East) Limited., Copenhagen, Denmark). Three independent replicates were conducted for each treatment.

2.5.7. Sensorial Analysis. The sensorial analysis was carried out by 8 trained panelists (4 males and 4 females, aged 24 to 33 years) who were recruited based on previous experience as an apple sensory panelist [38, 39]. Twenty different qualitative and quantitative descriptors were evaluated: flesh color (FC), presence of filaments (PF), firmness (F), sea smell (SS), peach smell (PS), exotic fruit smell (EFS), medicines smell (MS), cheese smell (CS), burnt oil smell (BOS), acid (A), sweet (S), bitter (B), juiciness (J), mealy (M), seafood flavor (SFF), peach flavor (PF), exotic fruit flavor (EFF), medicinal flavor (MF), cheese flavor (CF), and finally, burnt oil flavor (BF). The judges evaluated the intensity of each attribute on a discontinuous scale from 1 (absence of the descriptor) to 9 (maximum intensity of the descriptor). Water was provided for rinsing their mouths between the different papaya samples. The sensory profile of each genotype was reported in spider plots.

2.5.8. Statistical Analysis. Data were presented as mean ± standard deviation. Statistical analysis was performed using the XlStat® software version 9.0 (Addinsoft, Paris, France). Data were analyzed using one-way analysis of variance (ANOVA) and Tukey's posttest with $p < 0.05$ considered significant.

3. Results and Discussions

As shown in Figure 1, weight loss during the 12-day cold storage period was characterized by higher values for the CTR treatment from the third day of storage. In this case, there was a gradual weight loss from the first to the last day of storage. Similar values were observed for the EC1, EC3, and EC4 treatments, with the former slightly different and with a greater weight loss, while the EC2 treatment was the most effective of all of the treatments and it has created a thin layer on the fruit surface that has probably reduced the weight loss. Hence, the *Aloe vera* gel reduced evaporation from the fruit surface and retained more weight. Thanks to *Aloe vera* gel-based edible coating, Guillén et al. [27] has shown an effective reduction in weight loss in both peach and plum fruits, while Benítez et al. [40] reduce the pectin depolymerization processes in fresh-cut kiwi. An important role of this positive effect could be attributed to calcium chloride: Mahajan and Dhatt [41] have reported that calcium might have delayed senescence and reduced the respiration rate and transpiration. Nevertheless, different concentrations of calcium chloride introduced in loquat [35] and strawberries [42] showed positive values regarding weight loss. Also, Ahmed et al. [28] carried out the same study on nectarine, applying 2.5% *Aloe vera* gel and suggested that the coating material contributed to maintaining the weight of the fruit and increased its postharvest shelf-life. Therefore, our data highlight that both coating materials can help reduce fruit weight loss during storage through minimal respiration.

Concerning the firmness of the papaya slices (also shown in Figure 1), the EC2 treatment showed the best results (decreases by 35.8% compared to the CTR on the 12th day). The EC1 and EC3 show similar values (24.3% and 22.3% respectively), while the CTR showed a greater decrease in firmness (78.3%) during 12 days of cold storage. These results show the beneficial effects of the *Aloe vera* coating on the shelf-life of papaya fruits, in agreement with Batisse et al. [43] and Vidrih et al. [44] as they assumed that the softening and consistency of the fruits change during fruit storage. According to many research studies carried out on fresh fruit, postharvest $CaCl_2$-based preparations have been observed to maintain food quality and fruit firmness of loquat [45] and fresh-cut papaya [46]. White and Broadley [47] demonstrate that the preservation of firmness in calcium-treated fruits may be due to its accumulation in cell walls, which facilitates the crossing of pectic polymers that increases the strength of the walls and cell cohesion [48]. The

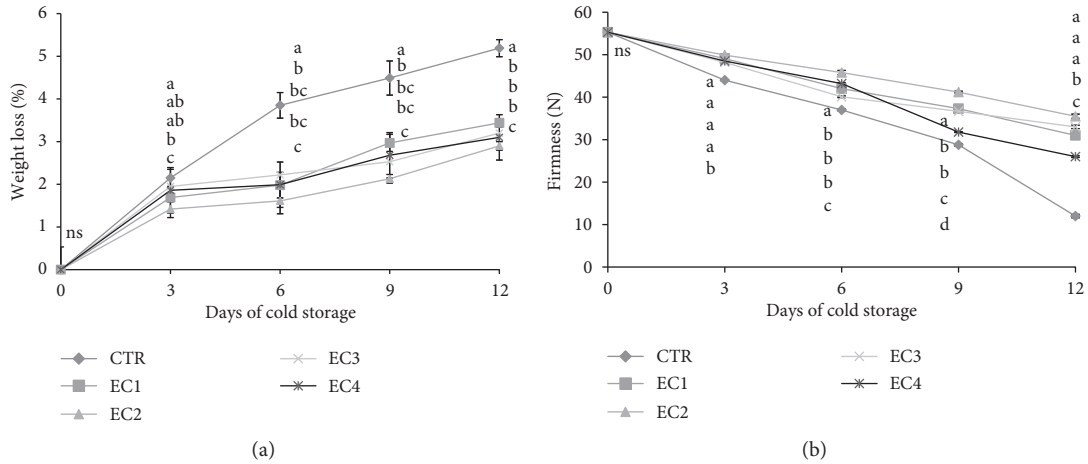


Figure 1: Trend of weight loss (%) and firmness (N) of fresh-cut papaya fruit cold stored and treated with edible coating. Values were recorded at 0, 3, 6, 9, and 12 days at 5°C and RH 90%. Significant values are indicated with equal letters in each step.

softening process in the fruit is dependent on the increase in polygalacturonase, activity of β-galactosidase, and pectinmethylesterase [49], as they are responsible for the loss of fruit quality. Narsaiah et al. [36] in their study on minimally processed papaya have demonstrated that an increase in alginate concentration increases the firmness retention. Lerdthanangkul and Krochta [50] also obtained similar results and concluded that coatings and/or films based on *Aloe vera* gel have a significant effect on the firmness of the preserved fruits.

From day 0 to day 12, the soluble solid content increased significantly between uncoated papaya fruits and coated fruits (Figure 2), starting from the same value (6.85°Brix) and reaching (in CTR) values close to 10°Brix. This increase in soluble solid content occurs due to the ripening and senescence of the fruit. In uncoated papaya fruits, the increase in soluble solid content may be due to increased degradation or biosynthesis of polysaccharides into simple sugars. It also increases moisture loss due to the accumulation of sugars in the tissues. This phenomenon probably leads to a decrease in the acid content of the fruits (TA), since acids are important substrates for respiratory metabolism. However, the coated sample showed a minimal increase in the values of the total soluble solid content, compared to the control sample. The lowest SSC at the end of the storage period was registered in fruits coated with an EC2 treatment (7.90°Brix) that provided an excellent semipermeable film modifying the internal atmosphere by reducing the ethylene production. Decreased respiration rates also slow down the metabolite use and the fruit ripening [51]. Biale [52] asserted that an increase in SSC in the flesh and the peel was observed when the fruits reach their climacteric respiration peak. In particular, the EC2 treatment showed almost gradual linearity with the lowest maximum values compared to the other treatments, on the 12th day of storage. Similar results have been reported from Li et al. [53] on the edible coating for fresh-cut kiwis and from Cortez-Vega et al. [54] on fresh-cut papaya.

The titratable acidity (TA) decreases both in the fruits subjected to coating treatments and in the untreated fruits (CTR), starting from the same value (1 g citric acid/L) (Figure 2). At 5°C and 90% RH, the fruits show a similar tendency with the TA parameter decreasing linearly over the storage period. The EC1 treatment differs from the others in that it shows a constant trend in the range of time from 0 to 6 days; from day 6 to day 12, a decreasing trend begins. The EC2, EC3, and EC4 treatments show more or less the same values during the whole storage period; the CTR is characterized by rather lower values in the storage period (0–12 days), with much lower values than the treated fruits on the 12th day. Here, the TA value did not seem to be influenced by treatment. Manganaris et al. [55] reported that calcium chloride dips did not produce any effects in TA% in fruit of peaches, but titratable acidity is directly related to the concentration of organic acids present in the fruits, which are substrates for enzymatic respiration reactions [56]. Ahmed et al. [28] and Marpudi et al. [4] reported that the TA decreases and the SSC increases in nectarines and papaya coated with *Aloe vera* gel, during storage time. This difference in the TA and SSC values is most likely due to the difference in the types of fruit and whether they were cut or whole.

During the 12 days of storage, the application of the EC helped to preserve the color attributes (Figure 3) of the analyzed fresh-cut papaya. Concerning lightness (L^*), throughout the time, the coated samples showed higher values than the controls. Regarding chroma (C), the measured parameters indicate that the saturation decreases during the storage period, so the samples tend to become darker and tending to grey (55%). A more marked decrease in saturation values is found in the papaya slices of the EC3 treatment, probably due to the presence of K carrageenan which provides an oxygen barrier and manages to reduce the respiratory rate and maturation leading to the delay of activities that cause the reduction of chroma values [57]. In addition, Martínez-Romero et al. [24] show that the color values are better in fruits treated with *Aloe vera* gel than in untreated fruits. During the storage period, a color change from warmer to colder tones was observed, which was more

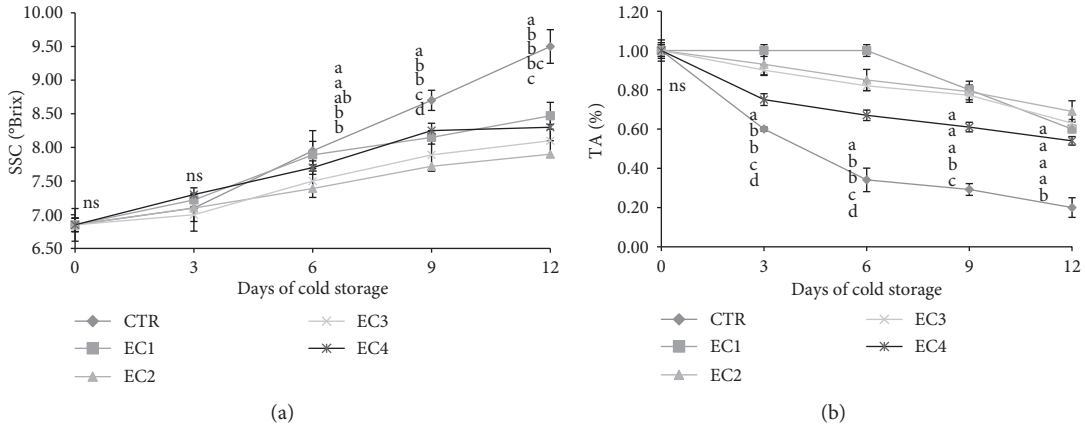


Figure 2: Trend in the soluble solid content (°Brix) and titratable acidity (%) of fresh-cut stored papaya. Values were recorded at 0, 3, 6, 9, and 12 days at 5°C and RH 90%. Significant values are indicated with equal letters in each step.

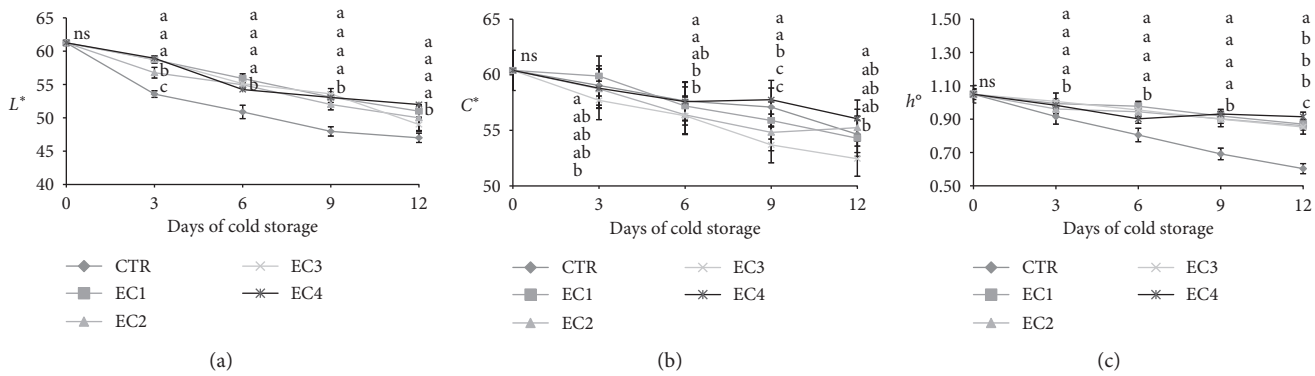


Figure 3: Brightness (L), chroma (C), and hue angle ($h°$) trends of papaya fresh-cut fruits during storage at 5°C and 90% RH. Significant values are indicated with equal letters in each step.

evident in the untreated papaya slices. In fact, as can be seen in the figure, CTR fruits suffer a greater decrease in hue angle ($h°$) values than fruits treated with EC, which instead have an almost similar and constant trend throughout the storage period. This behavior could be attributed to the presence of ascorbic acid and citric acid added in the coating formulations. These acids act as antibrowning agents. González-Aguilar et al. [58] studied color changes of fresh-cut pineapple treated with antibrowning agents for 16 days at 10°C and they reported that isoascorbic acid, ascorbic acid, or acetylcysteine significantly reduced ($p < 0.05$) the changes in the values of L^* and $h°$ compared with the control. Moline et al. [59] found that the combination of citric acid and ascorbic acid was effective in reducing the browning of fresh-cut bananas. These results, in fact, show that the treatment with EC reduces the loss of color of the fruit and preserves it longer during cold storage.

Furthermore, the O_2 and CO_2 contents (Figure 4) were determined in the trays: concerning the evolution of oxygen, during the 12 days of storage at 5°C and RH 90%, it decreased both in the treated fruits and in the CTR. As shown in the figure, the initial values of O_2 are similar for both CTR and treated fruits, while on the 12th day of storage the values decreased by 3-4% for the fruits that have benefited from the various treatments. In particular, EC2 is the most effective treatment. This behavior can once again be attributed to the presence of Ca^+ ions which are involved in the reduction of the respiration rate and metabolic processes of the fruits [60]. In the CTR, on the other hand, there is a rather rapid decrease, in fact on the 12th day, and halved values are recorded, due to the normal ripening process of the fruits. This shows that the fruit, at the end of the storage period, has a minimum respiratory activity because the oxygen has almost completely been consumed. Considering that O_2 levels are complementary to CO_2 levels, fruits have a lower respiration rate due to the high presence of CO_2.

Concerning the evolution of carbon dioxide in the fresh-cut papaya fruits, it increases both in the four treatments and in the CTR, starting from initial values of CO_2 close to 0%, the same for all. The best final CO_2 values were recorded in the fruits treated with EC2, according to Ferguson [60], Maftoonazad, and Ramaswamy [61] and Alonso and Alique [62]. Narsaiah et al. [36] confirm that the alginate associated with the edible coating forms a barrier to gas exchange. The other treatments have similar values. The CTR, on the other hand, shows a higher production of CO_2 on the 12th day.

Finally, concerning sensory analysis (Figure 5) carried out by panelists, immediately after the transformation of

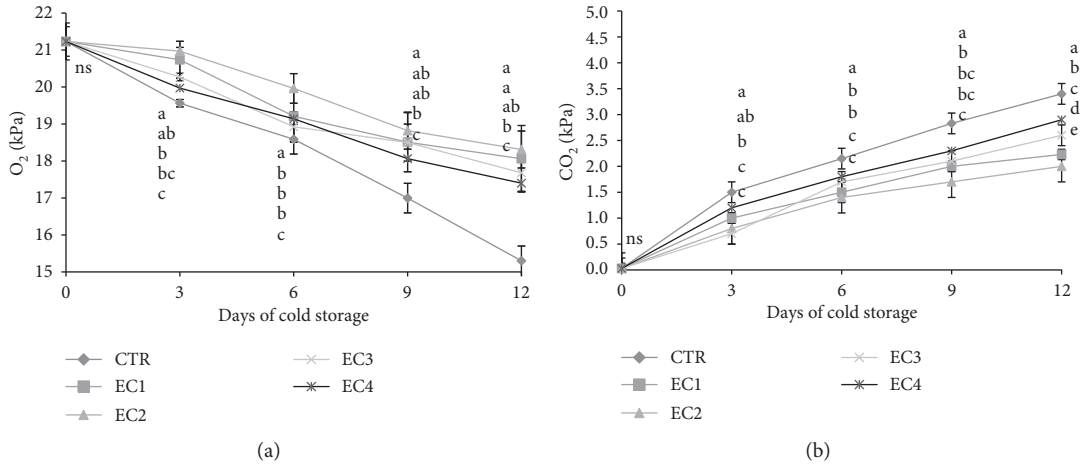


Figure 4: O_2 (kPa) and CO_2(kPa) trend of papaya fresh-cut fruits during storage at 5°C and 90% RH. Significant values are indicated with equal letters in each step.

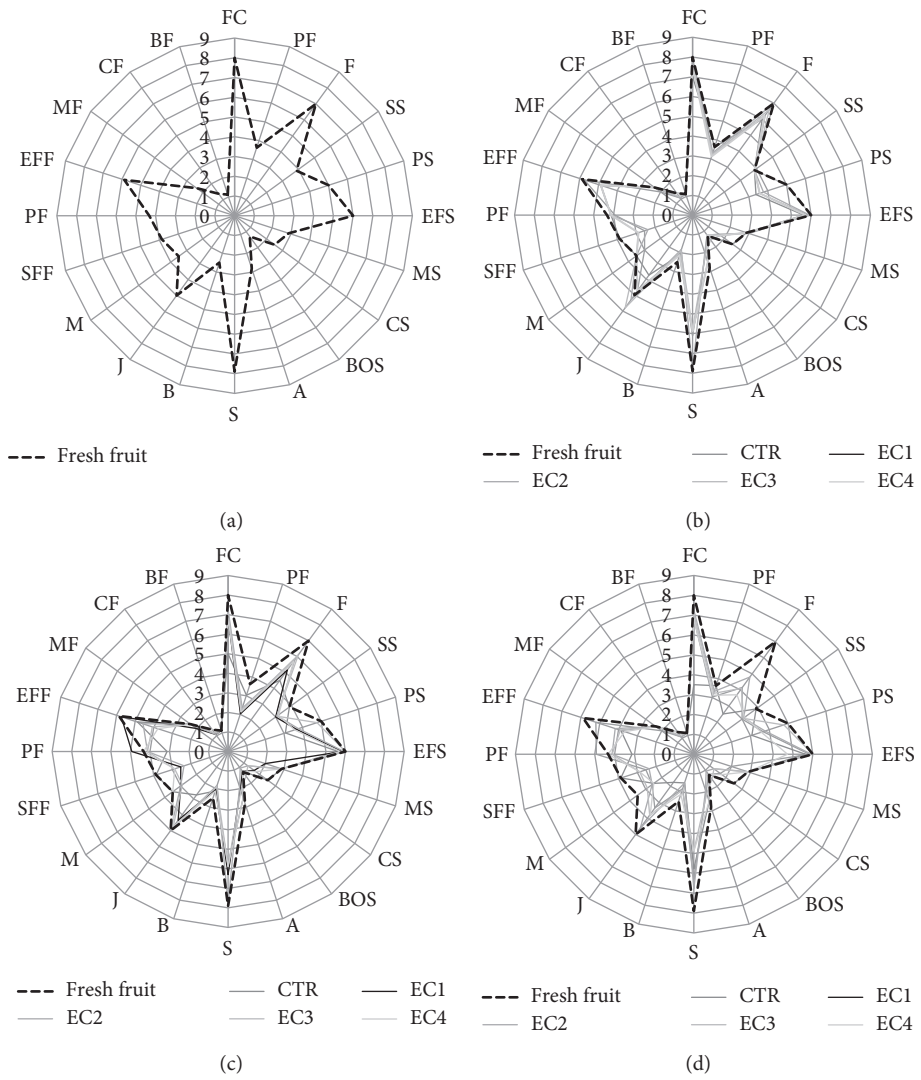
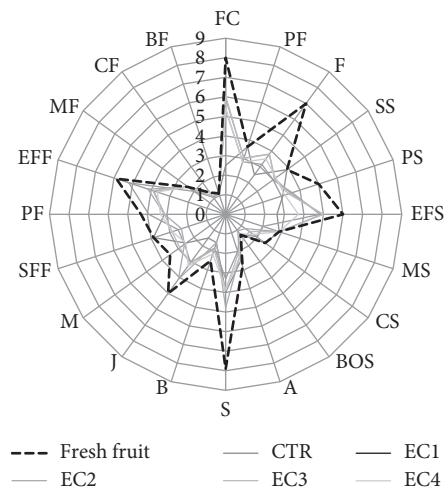


Figure 5: Continued.



(e)

Figure 5: Sensory profile of papaya fruits after (a) 0 (T0), (b) 3 (T3), (c) 6 (T6), (d) 9 (T9), and (e) 12 (T12) days of cold storage at 5°C and 90% RH. Flesh color (FC), presence of filaments (PF), firmness (F), smell of sea (SS), smell of peach (PS), smell of exotic fruit (EFS), smell of medicines (MS), smell of cheese (CS), smell of burnt oil (BOS), acid (A), sweet (S), bitter (B), juiciness (J), mealy (M), seafood flavor (SFF), peach flavor (PF), exotic fruit flavor (EFF), medicinal flavor (MF), cheese flavor (CF), and burnt oil flavor (BF).

fresh untreated fruits (CTR) into a fresh-cut (T0) product: the judges found an excellent flesh color, pleasant sweetness, good consistency when cut, medium juiciness, and smell and flavor of exotic fruits; they did not perceive the presence of filaments, bitter taste, smell and flavor of medicine, or smell of burnt oil.

On day 3, the sensory profile of the fruits treated according to the four treatments was compared with the CTR in order to analyze the differences. As shown in the figure, the EC2 treatment was the most effective in maintaining high values of sweetness and flesh consistency, good consistency when cut, good smell of exotic fruit, and high juiciness. All treatments showed similar results. The EC1 treatment gave the best results for exotic fruit flavor, whereas the EC3 treatment was the one that maintained the highest juiciness compared to the others. In the CTR, the results were similar, the difference was found in the consistency of the pulp and the juiciness decreases, and the fruits showed a slight off-flavor and cheese smell. After six days of storage, the coated fruit still had no defects, unlike the CTR, which is characterized by a slight odor and flavor of cheese and flouriness. Flesh firmness was maintained at optimal levels for all treatments, especially for the EC2 treatment, but decreased significantly in the CTR. The sweetness remains at the same value for all treatments, but the juiciness decreases, especially, once again, in the CTR. In the EC1 treatment, compared to other treatments, there is a greater flavor of peach and of exotic fruit. Even on day 9, the pulp firmness is maintained in optimal conditions, unlike CTR, where it decreases significantly, demonstrating the success of treatments based on edible coating. The EC2 treatment was the most suitable in terms of pulp firmness, juiciness, and sweetness. EC1 treatment continues to maintain an average peach and exotic fruit flavor value. The CTR shows the least encouraging results, as it has a slight medicinal odor, a cheese odor, and a low firmness when cut. On day 12, the firmness of the pulp is minimally reduced in the four treatments, continuing to maintain a good quality, unlike the CTR in which this parameter is limited, having suffered a reduction. The cut firmness decreased for all treatments, showing slightly better results in the EC3 treatment. Juiciness also decreases, maintaining the best results in EC2 treatment and the lowest in the CTR.

4. Conclusions

Our results demonstrated the ability of the natural *Aloe vera* gel-based edible coatings to maintain the quality characteristics of fresh-cut papaya. It is certain that the fresh-cut fruits have benefited from the effects of *Aloe vera* gel, but in particular, the presence of $CaCl_2$ (in EC2 treatment) has kept the highest values in terms of weight loss, firmness, SSC, TA, and respiration rate. On the other hand, K carrageenan and sodium alginate (in EC3 and EC4 treatments) have provided an oxygen barrier and reduced respiratory rate, increasing the firmness retention and keeping a high C^* value. Finally, sensorial analysis confirms these results and claims that the *Aloe vera* gel-based edible coating and the other added agents did not affect the natural taste of papaya.

Data Availability

The data used to support the findings of the study are available from the corresponding author upon request.

Additional Points

Highlights. (i) Four edible coatings based on *Aloe vera* gel were used in fresh-cut papaya. (ii) EC2 treatment (gellan gum 1% v/v, Aloe v. gel 30% v/v, glycerol 3% v/v, and $CaCl_2$ 5% v/v) evidenced the best results. (iii) The edible coatings form an excellent semipermeable barrier, delay senescence,

and reduce the respiration rate. (iv) The natural taste of the papaya was not affected by edible coatings.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] D. Casati and L. Baldi, "Il settore della IV gamma e il suo sviluppo in Lombardia: gli aspetti economici," *Bullettino dell'agricoltura. Atti della società agraria di lombardia*, vol. 148, no. 3-4, pp. 17–36, 2009.

[2] V. Farina, L. Tripodo, G. Gianguzzi et al., "Innovative techniques to reduce chilling injuries in mango (*Mangifera Indica* L.) trees under mediterranean climate," *Chemical Engineering Transactions*, vol. 58, pp. 823–828, 2017.

[3] H. N. Prajapati, R. K. Patil, and Y. M. Shukla, "Studies on biochemical changes and changes in cell wall degrading enzymes in papaya fruit inoculated with colletotrichum demetium," *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 7, pp. 1953–1961, 2017.

[4] S. L. Marpudi, L. S. S. Abbirami, R. Pushkala, and N. Srividya, "Enhancement of storage life and quality maintenance of papaya fruits using aloe vera based antimicrobial coating," *Indian Journal of Biotechnology*, vol. 10, pp. 83–89, 2011.

[5] R. M. D. A. Pimentel and J. M. M. Walder, "Gamma radiation in papaya harvested at three stages of maturation," *Scientia Agricola*, vol. 61, no. 2, pp. 146–150, 2004.

[6] M. Y. Rohani, M. Z. Zaipun, and M. Norhayati, "Effect of modified atmosphere on the storage life and quality of Eksotika papaya," *Journal of Tropical Agriculture and Food Science*, vol. 25, no. 1, pp. 103–114, 1997.

[7] N. S. Mohamed Salleh, "Development of starch and soy protein edible coating and its effect on the postharvest life of mango (*Mangifera indica* L.)," Universiti Teknologi MARA, Shah Alam, Malaysia, Doctoral dissertation, 2013.

[8] J. M. Krochta, *Protein as Raw Materials for Films and Coatings: Definitions, Current Status, and Opportunities in Protein-Based Films and Coatings*, A. Gennadios, Ed., CRC Press, Boca Raton, FL, USA, 2002.

[9] E. A. Baldwin, M. O. Nisperos-Carriedo, and R. A. Baker, "Use of edible coatings to preserve quality of lightly (and slightly) processed products," *Critical Reviews in Food Science and Nutrition*, vol. 35, no. 6, pp. 509–524, 1995.

[10] J. W. Rhim, H. M. Park, and C. S. Ha, "Bio-nanocomposites for food packaging applications," *Progress in Polymer Science*, vol. 38, no. 10-11, pp. 1629–1652, 2013.

[11] E. D. O. A. Sena, H. G. S. de Araújo Couto, A. Regina da Costa Paixão, M. P. C. Silveira, L. F. G. de Oliveira Júnior, and M. A. G. Carnelossi, "Utilização de biofilme comestível na conservação pós-colheita de pimentão verde (*Capsicum annuum* L.)," *Scientia Plena*, vol. 12, no. 8, 2016.

[12] N. B. Gol, P. B. Vyas, and T. V. Ramana Rao, "Evaluation of polysaccharide-based edible coatings for their ability to preserve the postharvest quality of Indian blackberry (*Syzygium cumini* L.)," *International Journal of Fruit Science*, vol. 15, no. 2, pp. 198–222, 2015.

[13] R. J. W. Lambert, P. N. Skandamis, P. J. Coote, and G.-J. E. Nychas, "A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol," *Journal of Applied Microbiology*, vol. 91, no. 3, pp. 453–462, 2001.

[14] E. Tavassoli-Kafrani, H. Shekarchizadeh, and M. Masoudpour-Behabadi, "Development of edible films and coatings from alginates and carrageenans," *Carbohydrate Polymers*, vol. 137, pp. 360–374, 2016.

[15] M. Demarty, C. Morvan, and M. Thellier, "Calcium and the cell wall," *Plant, Cell and Environment*, vol. 7, no. 6, pp. 441–448, 1984.

[16] E. A. Kirkby and D. J. Pilbeam, "Calcium as a plant nutrient," *Plant, Cell and Environment*, vol. 7, no. 6, pp. 397–405, 1984.

[17] F. Bangerth, "Calcium-related physiological disorders of plants," *Annual Review of Phytopathology*, vol. 17, no. 1, pp. 97–122, 1979.

[18] R. C. Keshri and M. K. Sanyal, "Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated (4±1c) storage," *Journal of Muscle Foods*, vol. 20, no. 3, pp. 275–292, 2009.

[19] F. A. Osorio, P. Molina, S. Matiacevich, J. Enrione, and O. Skurtys, "Characteristics of hydroxy propyl methyl cellulose (HPMC) based edible film developed for blueberry coatings," *Procedia Food Science*, vol. 1, pp. 287–293, 2011.

[20] R. D. Earle, U.S. Patent and Trademark Office, Washington, DC, USA, U.S. Patent No. 3, 1968.

[21] K. R. Conca and T. C. S. Yang, "Edible food barrier coatings. Activities report of the R and D Associates (USA)," *Critical Reviews in Food Science and Nutrition*, vol. 48, pp. 496–511, 1993.

[22] P. J. Zapata, D. Navarro, F. Guillén et al., "Characterisation of gels from different Aloe spp. as antifungal treatment: potential crops for industrial applications," *Industrial Crops and Products*, vol. 42, pp. 223–230, 2013.

[23] S. Castillo, D. Navarro, P. J. Zapata et al., "Antifungal efficacy of Aloe vera in vitro and its use as a preharvest treatment to maintain postharvest table grape quality," *Postharvest Biology and Technology*, vol. 57, no. 3, pp. 183–188, 2010.

[24] D. Martínez-Romero, N. Alburquerque, J. M. Valverde et al., "Postharvest sweet cherry quality and safety maintenance by Aloe vera treatment: a new edible coating," *Postharvest Biology and Technology*, vol. 39, no. 1, pp. 93–100, 2006.

[25] M. I. Pinzon, L. T. Sanchez, O. R. Garcia, R. Gutierrez, J. C. Luna, and C. C. Villa, "Increasing shelf life of strawberries (*Fragaria* ssp) by using a banana starch-chitosan-Aloe vera gel composite edible coating," *International Journal of Food Science & Technology*, vol. 55, no. 1, pp. 92–98, 2019.

[26] S. Ali, A. Sattar Khan, A. Ullah Malik, M. A. Anjum, A. Nawaz, and H. M. Shoaib Shah, "Modified atmosphere packaging delays enzymatic browning and maintains quality of harvested litchi fruit during low temperature storage," *Scientia Horticulturae*, vol. 254, pp. 14–20, 2019.

[27] F. Guillén, H. M. Díaz-Mula, P. J. Zapata et al., "Aloe arborescens and Aloe vera gels as coatings in delaying postharvest ripening in peach and plum fruit," *Postharvest Biology and Technology*, vol. 83, pp. 54–57, 2013.

[28] M. J. Ahmed, Z. Singh, and A. S. Khan, "PostharvestAloe veragel-coating modulates fruit ripening and quality of "Arctic Snow" nectarine kept in ambient and cold storage," *International Journal of Food Science & Technology*, vol. 44, no. 5, pp. 1024–1033, 2009.

[29] K. T. H. Dang, Z. Singh, and E. E. Swinny, "Edible coatings influence fruit ripening, quality, and aroma biosynthesis in

mango fruit," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 4, pp. 1361–1370, 2008.

[30] M. Ergun and F. Satici, "Use of aloe vera gel as biopreservative for "Granny Smith" and "Red Chief" apples," *The Journal of Animal and Plant Sciences*, vol. 22, no. 2, pp. 363–368, 2012.

[31] J. M. Valverde, D. Valero, D. Martínez-Romero, F. Guillén, S. Castillo, and M. Serrano, "Novel edible coating based onAloe veraGel to maintain table grape quality and safety," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 20, pp. 7807–7813, 2005.

[32] M. Serrano, J. M. Valverde, F. Guillén, S. Castillo, D. Martínez-Romero, and D. Valero, "Use ofAloe veraGel coating preserves the functional properties of table grapes," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 11, pp. 3882–3886, 2006.

[33] Z. Selamoglu, C. Dusgun, H. Akgul, and M. F. Gulhan, "In-vitro antioxidant activities of the ethanolic extracts of some contained-allantoin plants," *Iranian Journal of Pharmaceutical Research: IJPR*, vol. 16, no. Suppl, pp. 92–98, 2017.

[34] M. Erdemli, H. Akgul, B. Ege, Z. Aksungur, H. Bag, and Z. Selamoglu, "The effects of grapeseed extract and low level laser therapy administration on the liver in experimentally fractured mandible," *Journal of Turgut Ozal Medical Center*, vol. 24, no. 2, p. 1, 2017.

[35] A. Akhtar, N. A. Abbasi, and A. Z. H. A. R. Hussain, "Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage," *Pakistan Journal of Botany*, vol. 42, no. 1, pp. 181–188, 2010.

[36] K. Narsaiah, R. A. Wilson, K. Gokul et al., "Effect of bacteriocin-incorporated alginate coating on shelf-life of minimally processed papaya (*Carica papaya* L.)," *Postharvest Biology and Technology*, vol. 100, pp. 212–218, 2015.

[37] K. Vij, T. Takaya, K. Kohyama, and M. Watase, "Effects of sugars on the gel-sol transition of agarose and k-carrageenan," in *Developments in Food Engineering*, pp. 108–110, Springer, Boston, MA, USA, 1994.

[38] G. Sortino, M. Ingrassia, A. Allegra, and P. Inglese, "Sensory evaluation and suitability for fresh-cut produce of white peach [*Prunus persica* (L.) Batsch] "Settembrina di Bivona," in *Proceedings of the VIII International Peach Symposium 1084*, pp. 787–790, Matera, Italy, May 2015.

[39] V. Farina, G. Gianguzzi, and A. Mazzaglia, "Fruit quality evaluation of affirmed and local loquat (Eriobotrya japonicaLindl) cultivars using instrumental and sensory analyses," *Fruits*, vol. 71, no. 2, pp. 105–113, 2016.

[40] S. Benítez, I. Achaerandio, F. Sepulcre, and M. Pujolà, "Aloe vera based edible coatings improve the quality of minimally processed 'Hayward' kiwifruit," *Postharvest Biology and Technology*, vol. 81, pp. 29–36, 2013.

[41] B. V. C. Mahajan and A. S. Dhatt, "Studies on postharvest calcium chloride application on storage behaviour and quality of Asian pear during cold storage," *Journal of Food, Agriculture and Environment*, vol. 2, no. 3-4, pp. 157–159, 2004.

[42] J. M. García, S. Herrera, and A. Morilla, "Effects of postharvest dips in calcium chloride on strawberry," *Journal of Agricultural and Food Chemistry*, vol. 44, no. 1, pp. 30–33, 1996.

[43] C. Batisse, M. Buret, and P. J. Coulomb, "Biochemical differences in cell wall of cherry fruit between soft and crisp fruit," *Journal of Agricultural and Food Chemistry*, vol. 44, no. 2, pp. 453–457, 1996.

[44] R. Vidrih, M. Zavrtanik, and J. J. Hribar, "Effect of low O_2, high CO_2 or added acetaldehyde and ethanol on postharvest physiology of cherries," *Acta Horticulturae*, vol. 468, pp. 695–704, 1998.

[45] C. Shuiliang, Y. Zhende, L. Laiye et al., "Studies on freshness keeping technologies of loquat," *South China Fruits*, vol. 31, no. 5, pp. 28–30, 2002.

[46] T. M. M. Mahmud, A. Al Eryani-Raqeeb, S. S. Omar, A. M. Zaki, and A. E. Abdul-Rahman, "Effects of different concentrations and applications of calcium on storage life and physicochemical characteristics of papaya (*Carica Papaya* L.)," *American Journal of Agricultural and Biological Science*, vol. 3, no. 3, pp. 526–533, 2008.

[47] P. J. White and M. R. Broadley, "Calcium in plants," *Annals of Botany*, vol. 92, no. 4, pp. 487–511, 2003.

[48] G. T. Grant, E. R. Morris, D. A. Rees, P. J. C. Smith, and D. Thom, "Biological interactions between polysaccharides and divalent cations: the egg-box model," *FEBS Letters*, vol. 32, no. 1, pp. 195–198, 1973.

[49] S. Remón, M. Eugenia Venturini, P. Lopez-Buesa, and R. Oria, "Burlat cherry quality after long range transport: optimisation of packaging conditions," *Innovative Food Science & Emerging Technologies*, vol. 4, no. 4, pp. 425–434, 2003.

[50] S. Lerdthanangkul and J. M. Krochta, "Edible coating effects on postharvest quality of green bell peppers," *Journal of Food Science*, vol. 61, no. 1, pp. 176–179, 1996.

[51] Ö. Yaman and L. Bayoındırlı, "Effects of an edible coating and cold storage on shelf-life and quality of cherries," *LWT—Food Science and Technology*, vol. 35, no. 2, pp. 146–150, 2002.

[52] J. B. Biale, "Respiration of fruits," *Handbuch der Pflanzenphysiologie*, vol. 12, no. Part II, pp. 536–592, 1960.

[53] S. Li, L. Zhang, M. Liu, X. Wang, G. Zhao, and W. Zong, "Effect of poly-ε-lysine incorporated into alginate-based edible coatings on microbial and physicochemical properties of fresh-cut kiwifruit," *Postharvest Biology and Technology*, vol. 134, pp. 114–121, 2017.

[54] W. R. Cortez-Vega, S. Pizato, J. T. A. de Souza, and C. Prentice, "Using edible coatings from Whitemouth croaker (*Micropogonias furnieri*) protein isolate and organo-clay nanocomposite for improve the conservation properties of fresh-cut "Formosa" papaya," *Innovative Food Science & Emerging Technologies*, vol. 22, pp. 197–202, 2014.

[55] G. A. Manganaris, M. Vasilakakis, I. Mignani, G. Diamantidis, and K. Tzavella-Klonari, "The effect of preharvest calcium sprays on quality attributes, physicochemical aspects of cell wall components and susceptibility to brown rot of peach fruits (*Prunus persica* L. cv. Andross)," *Scientia Horticulturae*, vol. 107, no. 1, pp. 43–50, 2005.

[56] J. A. Ball, "Evaluation of two lipid-based edible coatings for their ability to preserve post-harvest quality of green bell peppers," Virginia Polytechnic Institute and State University, Blacksburg, Virginia, Doctoral dissertation, 1997.

[57] H. M. Hamzah, A. Osman, C. P. Tan, and F. Mohamad Ghazali, "Carrageenan as an alternative coating for papaya (*Carica papaya* L. cv. Eksotika)," *Postharvest Biology and Technology*, vol. 75, pp. 142–146, 2013.

[58] G. A. González-Aguilar, S. Ruiz-Cruz, R. Cruz-Valenzuela, A. Rodríguez-Félix, and C. Y. Wang, "Physiological and quality changes of fresh-cut pineapple treated with anti-browning agents," *LWT—Food Science and Technology*, vol. 37, no. 3, pp. 369–376, 2004.

[59] H. E. Moline, J. G. Buta, and I. M. Newman, "Prevention of browning of banana slices using natural products and their derivatives," *Journal of Food Quality*, vol. 22, no. 5, pp. 499–511, 1999.

[60] I. B. Ferguson, "Calcium in plant senescence and fruit ripening," *Plant, Cell & Environment*,vol. 7, no. 6, pp. 477–489, 1984.

[61] N. Maftoonazad and H. S. Ramaswamy, "Postharvest shelf-life extension of avocados using methyl cellulose-based coating," *LWT—Food Science and Technology*, vol. 38, no. 6, pp. 617–624, 2005.

[62] J. S. Alonso and R. Alique, "Influence of edible coating on shelf life and quality of "Picota" sweet cherries," *European Food Research and Technology*, vol. 218, no. 6, pp. 535–539, 2004.

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Research Article

Influence of Rosemary Extract Addition in Different Phases on the Oxidation of Lutein and WPI in WPI-Stabilized Lutein Emulsions

Duoxia Xu,[1] Zhanqun Hou,[2,3] Guorong Liu,[1] Yanping Cao,[1] Atikorn Panya,[4] Hang Xiao,[5] and Will Dixon[5]

[1]*Beijing Advanced Innovation Center for Food Nutrition and Human Health (BTBU), School of Food & Health, Beijing Engineering and Technology Research Center of Food Additives, Beijing Higher Institution Engineering Research Center of Food Additives and Ingredients, Beijing Technology & Business University, Beijing, China*
[2]*China National Research Institute of Food and Fermentation Industries Co., Ltd., Beijing 100015, China*
[3]*Beijing Key Laboratory of the Innovative Development of Functional Staple and the Nutritional Intervention for Chronic Disease, Beijing, China*
[4]*Food Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Rd., Khlong Nueng, Khlong Luang, Pathumthani 12120, Thailand*
[5]*Department of Food Science, University of Massachusetts, Amherst, MA, USA*

Correspondence should be addressed to Yanping Cao; caoyp@th.btbu.edu.cn

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The aim was to investigate rosemary extract with different addition methods affecting the physicochemical stability of WPI-coated lutein emulsions and examine the correlations between lutein degradation and WPI oxidation during storage. First, lutein emulsions containing different concentrations of rosemary extract in the oil phase were prepared. Second, lutein emulsions containing rosemary extract in the oil phase or water phase were studied along with the kinetic reaction of lutein degradation. Moreover, the impact of rosemary extract on the oxidation of WPI and their products was also determined. It was noticed that rosemary extract at 0.05 wt.% exhibited the best protection of lutein. According to the kinetics analysis of lutein degradation, the direct addition of rosemary extract in the oil phase was more suitable for retarding the degradation of lutein in emulsion than the addition in the aqueous phase due to it being partitioned at the interface. Meanwhile, it was revealed that the addition of rosemary extract in the water phase exhibited better inhibition of the WPI oxidation than addition in the oil phase. The understanding of the association and driving forces of rosemary extract in emulsion systems may be useful for the application of rosemary extract in multicomponent food systems.

1. Introduction

Lutein is a yellow pigment with two hydroxyl groups in the conjugated polyene chain presented in fruits and vegetables [1]. It has been reported that lutein has the functional properties of reducing incidences of eye diseases such as age-related macular degeneration and reducing the risk of cardiovascular diseases, atherosclerosis, and cancer diseases [2, 3]. However, the conjugated polyene chain of lutein is susceptible to degradation during processing and storage. The degradation of lutein produced a number of initial oxidation products, such as carbon-peroxyl triplet biradicals and epoxides [4]. These reactions can lead to loss of color and bioactivity of lutein in food products.

Dispersing the lipid soluble lutein into the oil phase of oil-in-water emulsions could improve its oxidative stability by emulsifying the oil phase solution with the aqueous phase containing emulsifiers [5]. Some studies have reported the stability of delivery systems enriched with lutein [6, 7]. The oxidation mechanism of lutein was comprehensively reviewed by Boon et al. [8]. Several factors could influence the degradation of lutein in oil-in-water emulsions including

size, droplet charge, interface thickness of emulsion droplets, and the addition of antioxidants.

In addition, prooxidants such as transition metals, for example, copper and iron, may lead to electron transfer between lutein and metals by forming lutein radical cation. The formed radical can undergo lutein degradation reactions, leading to lutein loss. In addition, lutein may react with the resulting and already present free radicals by adduct formation reactions or hydrogen abstraction, leading to lutein loss [9]. Therefore, the addition of antioxidant such as transition metal chelators (EDTA) or free radical scavengers (e.g., deferoxamine, α-tocopherol, and TBHQ) to emulsions was useful to increase emulsion chemical stability [10].

The use of synthetic antioxidants has become consumers' concern regarding their safety, which promotes the food industry to seek more natural alternatives [11]. Rosemary extracts contain different kinds of components such as rosmarinic acid, carnosic acid, and carnosol that provide one of the major sources of natural antioxidants, which are applied commercially at present in food products [12]. There are studies that showed rosemary extract and rosmarinic and carnosic acids were more effective when added to bulk oil than emulsified corn oil. However, it was also reported that rosmarinic acid probably acted as a prooxidant in emulsions. It was interesting to find that rosemary extracts, carnosic acid, and carnosol efficiently prevented hydroperoxide formation in bulk soybean oil, whereas they exhibited prooxidant activity in emulsions [13]. Lipid oxidation occurrence in emulsions at the surface of oil droplets instead of in the aqueous phase is expected to be the reason for this phenomenon.

Natural antioxidants are particularly difficult to evaluate in emulsions due to the complicated interfacial properties influencing the partition of the antioxidants in multiphase food systems [14]. According to the polar paradox hypothesis, lipophilic compounds are better antioxidants in oil-in-water emulsion, while hydrophilic antioxidants are more effective in the nonpolar system. However, it was reported that some antioxidative compounds did not follow the polar paradox due to the partitioning of antioxidants between the oil and water phases. It was reported that antioxidant activity is dependent on different parameters and that polarity is not the only factor to be taken into account [15, 16].

Whey protein is a useful emulsifier and can inhibit the oxidation of dispersed phase by preventing the penetration of prooxidants into the emulsified droplet [17, 18]. It was found that tryptophan, cysteine, and methionine had the most effective antioxidant activities in proteins. Tryptophan played an important role in antioxidation by allowing oxygen radicals to quench its indolic hydrogen due to serving as a hydrogen donor [19]. Amino acid residues such as tryptophan located at the interface of the oil droplets in emulsions might be preferentially impacted by oxidizing the unsaturated oil phase [20].

It has been a challenging topic for food researchers to understand antioxidant behaviors in complex food systems. Antioxidant could be dynamically distributed between oil and water phases in emulsions. The influence of the addition of rosemary extract in the different phases on the stability of the functional components in emulsion is unclear. Therefore, we evaluated the influence of addition methods (in the oil phase or water phase) of a commercial rosemary extract on the degradation of lutein in oil-in-water emulsions. The kinetic parameter reaction rate constants of process of lutein chemical stability test were presented. Moreover, the impact of rosemary extract on the oxidation of WPI and their products was also determined using spectrofluorometry. The relationship between WPI oxidation and lutein degradation was established. Moreover, the physical stability was evaluated by changes of particle size, zeta-potential, and instability index of lutein emulsions before and after storage. The study focused on developing a delivery system with proper antioxidant addition methods that improved oxidative stability for bioactive compound and protein.

2. Materials and Methods

2.1. Materials. Rosemary extract (Shenzhen Hua pure Biotechnology Co., Ltd.) was analyzed to contain 9.01% carnosol, 9.06% carnosic acid, 2.16% rosmarinic acid, 4.02% oleanolic acid, and 13.3% ursolic acid. Lutein powder (purity > 97%) was obtained from Nanjing Zelang Pharmaceutical Technology Co., Ltd. (Nanjing, China). Whey protein isolate (WPI) was obtained from Jinan SAN Chemical Co., Ltd. (Jinan, China). The manufacturer reported that it contained 97.6% protein (dry basis). Medium-chain triglyceride (MCT) oil was purchased from Lonza Inc. (Allendale, NJ, USA). Sodium azide was obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade.

2.2. Lutein Emulsion Preparation. WPI was dissolved in the 5.0 mM phosphate buffer at pH 7.0. 1.0 M NaOH was added to adjust the pH of the solution to 7.0 if necessary. The solutions were kept overnight to ensure complete dissolution and dispersion. Sodium azide (0.01 wt.%) was added in WPI solution to prevent microbial growth.

Lutein emulsion (0.5 wt.% WPI) was prepared by adding 5 wt.% MCT oil containing lutein (0.06 wt.% in the final emulsion) as the dispersed phase to 95 wt.% aqueous phase solution at room temperature. The mixture was then prehomogenized using an Ultra-Turrax high-speed blender (B25 model, Shanghai Beierte experimental equipment Co., Ltd.) at a speed of 19000 rpm for 3 min to form coarse emulsions, which were then passed through a microfluidizer processor (M-110PS model; Microfluidics International Corp., Newton, MA) three times at 50 MPa. After that, the pH was adjusted to 7.0 using 1.0 M HCl or NaOH. To prepare emulsions for chemical analysis, samples were diluted with 5 mM phosphate buffer solution to a total oil content of 1 wt.% and then transferred into screw-capped brown bottles flushed with nitrogen.

To study the effect of rosemary extract on the stability of lutein in emulsions, rosemary extract was added to oil phase (MCT) of lutein emulsion at a final concentration of 0, 0.02 wt.%, 0.05 wt.%, and 0.1 wt.%, respectively. To study the

influence of rosemary extract added in the oil phase and water phases on the oxidation of lutein and WPI in WPI-stabilized emulsions, rosemary extract was added to the oil phase and water phase at a final concentration of 0.05 wt.%, respectively.

2.3. Determination of Particle Size. Particle size of WPI-coated lutein emulsions with different concentrations and different addition methods of rosemary extract before and after storage was measured by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed detector angle of 90°. To avoid multiple scattering effects, emulsions were diluted with the same phosphate buffer solution to a final oil droplet concentration of 0.005 wt.% prior to each measurement. Results were described as cumulants mean diameter (size, nm) for droplet size.

2.4. Measurement of Zeta-Potential. The electrical charge (zeta-potential) of WPI-stabilized lutein emulsions with different concentrations and different addition methods of rosemary extract before and after storage was determined using a particle electrophoresis instrument Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK). Emulsions were diluted to a droplet concentration of 0.005 wt.% using buffer solution prior to each measurement to avoid multiple scattering effects. The instrument used the Smoluchowski approximation to obtain the zeta-potential of the droplets.

2.5. Physical Stability Analyzed by LUMiSizer. The physical stability of WPI-stabilized lutein emulsions with different concentrations and different addition methods of rosemary extract before and after storage was measured with the LUMiSizer (LUM GmbH, Berlin, Germany). It employs centrifugal sedimentation to accelerate the occurrence of instability phenomena. The integration graph characterizes the transmitted NIR light as a function of time and position over the entire sample length. The percentage of light absorbance per hour was described as the "instability index." The instrumental parameters used for the measurement were set as follows: volume, 1.8 ml of dispersion; 3000 rpm; $time_{Exp}$, 7650 s; time interval, 30 s; and temperature, 25°C [21].

2.6. Chemical Stability of Lutein Emulsion. The degradation of lutein emulsion was measured by measuring the change of lutein content in the emulsions during storage in the dark at 55°C. WPI-coated lutein emulsions with different contents and different addition methods of rosemary extract were transferred into screw-capped brown bottles flushed with nitrogen. The lutein content was measured during the storage at 55°C for 7 days. Lutein in emulsion was extracted with ethanol and n-hexane. After that, the absorbance at 440 nm was measured using a Shimadzu UVmini-1240 UV-vis spectrophotometer. The content of lutein during the storage was obtained by referring to a standard curve of lutein prepared under the same condition. The content of lutein in emulsions was calculated as relative lutein C in percent: $C_{(t)}/C_0$, where $C_{(t)}$ was the lutein concentration after storage for a period t and C_0 was the lutein concentration before storage [22].

2.7. WPI Oxidation. The oxidation of WPI in lutein emulsion was measured by measuring both the loss of tryptophan fluorescence and the emission of fluorescence by protein oxidation products in emulsions using fluorescence spectroscopy (RF-5301PC, Shimadzu Corp.). Lutein emulsion samples (e.g., without rosemary extract and with the addition of 0.05 wt.% rosemary extract in the oil phase or water phase) before and after different storage were dispensed in a quartz spectrofluorometer cell. The emission spectra of tryptophan were recorded at 362 nm with the excitation wavelength established at 283 nm. Emission spectra of WPI oxidation products were recorded at 714 nm with the excitation wavelength set at 390 nm [23].

2.8. Statistical Analysis. All emulsions were prepared in duplicate, and all measurements were performed in three times. Data were analyzed by analysis of variance (ANOVA) using the software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL).

3. Results and Discussion

The paper was organized in three different parts. First, the impact of the addition of different concentrations of rosemary extract on the physical and chemical stabilities of lutein emulsions was determined. In the second part, the stability of the lutein emulsions containing rosemary extract in the oil phase or water phase was presented. The degradation of lutein in emulsions was modeled with the first-order kinetic reaction. In the third part, the effect of rosemary extract addition method (in the oil phase or water phase) on the WPI oxidation in emulsion was assessed.

3.1. Impact of Rosemary Extract Concentration on the Physical Stability of Lutein Emulsion. The particle size, zeta-potential, and instability index of lutein emulsions were measured as a function of different concentrations of rosemary extract in the oil phase. An understanding of the effect of rosemary extract at different concentrations on the physical property can provide the basis for the chemical stability of lutein emulsions.

The mean particle size was measured before and after the lutein emulsions and 0, 0.02 wt.%, 0.05 wt.%, and 0.1 wt.% rosemary extracts were stored for 7 days at 55°C, respectively (Figure 1(a)). The droplet size of lutein emulsions without and with different concentrations of rosemary extract did not show any significant differences (216.8 ± 3.2, 220.1 ± 4.1, 220.8 ± 2.0, and 216.1 ± 3.9 nm, respectively). After storage, the mean droplet size of all the four lutein emulsions was slightly increased but did not significantly change during storage (Figure 1(a)), thus indicating that the emulsions

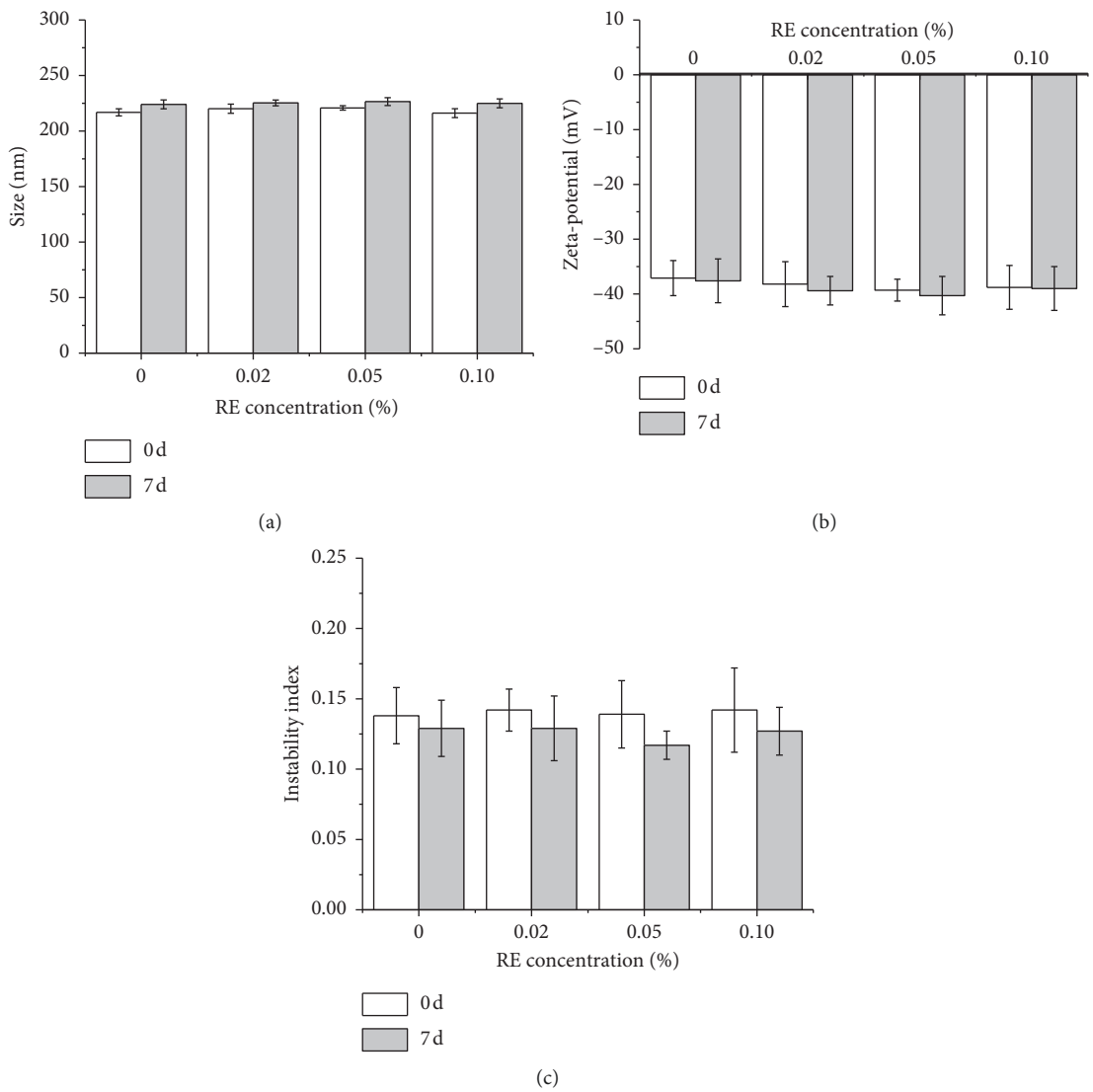

Figure 1: Effect of the different concentrations of rosemary extract (RE) (in the oil phase) on the droplet size (a), zeta-potential (b), and instability index (c) of WPI-stabilized lutein emulsion before storage (0 days) and after storage (7 days) at 55°C. Data points represent means ($n = 3$) ± standard deviations.

were stable regarding droplet coalescence and Ostwald ripening.

As can be seen in Figure 1(b), there were no significant differences in the zeta-potential of the presence of various concentrations of rosemary extract in lutein emulsions at 0 days compared with the control (the absence of rosemary extract). The droplets of lutein emulsions with 0, 0.02 wt.%, 0.05 wt.%, and 0.1 wt.% rosemary extract exhibited high negatively charged surfaces ranging from -37.1 ± 3.2 to -39.3 ± 1.9 mV ($P > 0.05$), indicating that there was enough electrostatic repulsion between the lutein droplets to prevent them from coming into close proximity.

After 7 days of storage, the zeta-potential of the lutein emulsion droplets with different concentrations of rosemary extract seems to be slightly more negative in comparison with the values at 0 days. However, there were no significant differences in the zeta-potential values in this study ($P > 0.05$). It was reported that slight decreases in a negatively charged surface of proteins could be attributed to the loss of cationic species such as amines that might react with aldehydes from lutein oxidation [20].

The effect of the addition of rosemary extract on the instability index of lutein emulsion using LUMisizer was demonstrated in Figure 1(c). It was observed that the addition of rosemary extract did not affect the instability index of lutein emulsions. However, thermal treatment during storage slightly decreased the instability indexes. It has been reported that when whey-protein-coated droplets are heated, the layer of adsorbed proteins undergoes conformational changes, leading to exposure of nonpolar and sulfhydryl groups. Thereby, the hydrophobic attraction between the droplets probably was increased [24].

Overall, the results of droplet size, zeta-potential, and instability index proved the insignificant influences of

different concentrations of rosemary extract on the physical stability of lutein emulsion. Meanwhile, before and after storage, all the four lutein emulsions (without and with different concentrations of rosemary extract) were stable to droplet aggregation.

3.2. Impact of Rosemary Extract Concentration on the Chemical Stability of Lutein Emulsion. Figure 2 shows the impact of rosemary extract concentration on the chemical degradation of lutein in oil-in-water emulsions during storage at 55°C accelerated degradation tests. As shown in Figure 2, the protective effect of rosemary extract was observed at the three concentrations (0.02 wt.%, 0.05 wt.%, and 0.1 wt.%) in comparison with the control (without rosemary extract). After storage for 7 d, the lutein emulsion without rosemary extract exhibited a lutein loss of 45.5%, while those with rosemary extract of 0.02 wt.%, 0.05 wt.%, and 0.1 wt.% exhibited lutein losses of 28.3%, 12.1%, and 24.1%, respectively. The best chemical stability of lutein emulsion was achieved for the addition of 0.05 wt.% rosemary extract followed by 0.1 wt.% and 0.02 wt.%, respectively. More rosemary extracts with concentration of 0.1 wt.% decreased their effectiveness and even caused prooxidative effect. In addition, it was suggested that phenolic antioxidants become prooxidants by regenerating peroxyl radicals [25, 26].

The effective protection of lutein by rosemary extract was due to its composition containing 9.01% carnosol, 9.06% carnosic acid, 2.16% rosmarinic acid, 4.02% oleanolic acid, and 13.3% ursolic acid. The effect of adding rosemary extract and its constituents, carnosol, carnosic acid, and rosmarinic acid, in bulk oil and emulsion has been noted by Frankel et al. [13]. It was mentioned that rosemary extract, carnosol, and carnosic acid were effective antioxidants in corn oil emulsions. Rosemary extract was described as an antioxidant that scavenges hydroxyl radicals and peroxyl radicals, preventing lipid peroxidation [26]. Therefore, rosemary extract showed the effective antioxidant properties in the lutein emulsions.

It was also reported that the charge effect of hydrophilic antioxidant ascorbic acid on pigment stability was a function not only of concentrations but also the presence of transition metal ions. According to Qian et al., ascorbic acid could act as a prooxidant attributed to its ability to convert ferric ions into more reactive ferrous ions, which promoted the formation of free radicals [27]. Therefore, it was very useful to understand the effect of rosemary extract content on the degradation of lutein emulsion. At higher concentrations, it might negatively affect lutein stability. In this study, it was shown that the addition of 0.1 wt.% extract had a less positive effect on lutein stability compared with 0.05 wt.% extract, which was probably due to high amounts of antioxidants acting counterintuitively as a prooxidant. Panya et al. proved that rosmarinic acid and α-tocopherol in the oil phase of the emulsion exhibited strong synergistic interactions in water-in-oil emulsions containing lutein [28]. It was reported that this interaction resulted in the formation of caffeic acid from rosmarinic acid, which would enhance the oxidative stability of the emulsion. A similar interaction between rosemary extract and lutein might be occurring in the lutein emulsions studied in this work.

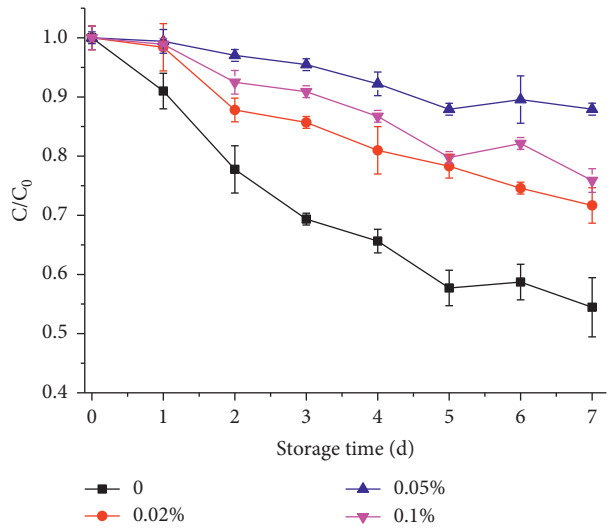

Figure 2: Effect of the different concentrations of rosemary extract (in the oil phase) on the degradation of lutein in WPI-stabilized emulsions during the storage at 55°C. Data points represent means ($n = 3$) ± standard deviations.

3.3. Influence of the Addition of Rosemary Extract in Different Phases on the Physical Stability of Lutein Emulsion. The droplet size, surface charge, and instability index of lutein emulsions were measured in the present and absence of rosemary extract in both the oil and water phases (Figures 3(a)–3(c)). The droplet size of the lutein emulsions without and with rosemary extract in oil and water phases did not change remarkably after storage at 55°C for 7 days, indicating that the three lutein emulsions were relatively physically stable. Before and after storage, zeta-potential was similar for the three lutein emulsions. The slight decrease of zeta-potential after storage could be attributed to the products of lutein oxidation interacting with the amine groups of protein or causing protein confirmation changes [20]. No obvious change of instability index was observed in lutein emulsions without and with rosemary extract in the oil or water phases. There were decreases in the instability index of the three lutein emulsions after storage. It was probably due to the heat induced WPI interaction increasing the interfacial layer thickness and resulted in droplet interactions being dominated by steric hindrance [29]. It indicated that there were no significant differences in the physical stability between the additions of rosemary extract in the oil and water phases.

3.4. Influence of the Addition of Rosemary Extract in Different Phases on the Chemical Stability of Lutein Emulsion. The course of the degradation of lutein emulsion with rosemary extract in the oil phase or water phase at 55°C was presented in Figure 4. It was found that the addition of rosemary extract in the oil phase or water phase of lutein emulsion inhibited lutein loss during storage. The protection effect of

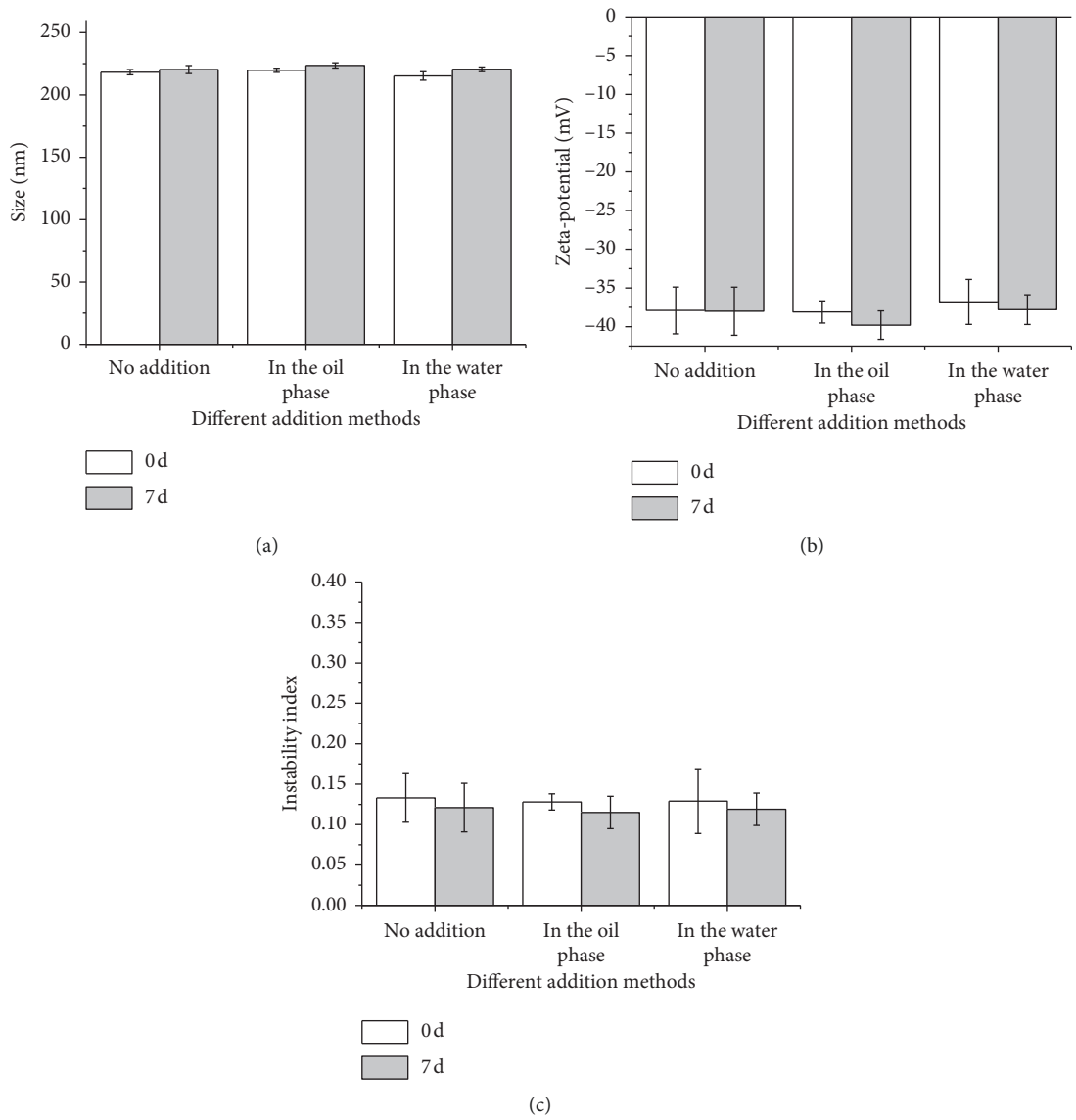

Figure 3: Effect of the rosemary extract (RE) addition in different phases (oil and water phase) on the droplet size (a), zeta-potential (b), and instability index (c) of WPI-stabilized lutein emulsion before storage (0 days) and after storage (7 days) at 55°C. Data points represent means ($n = 3$) ± standard deviations.

the addition of rosemary extract in the oil phase of lutein emulsion was much better than that in the water phase. For the emulsion in the absence of rosemary extract, lutein degraded rapidly with 45.5% loss after 7-day storage. For the lutein emulsion in the presence of rosemary extract in the oil phase and water phase, lutein degraded with 12.4% and 25.9% losses after 7-day storage, respectively. It proved that the presence of rosemary extract in the oil phase was more suitable for retarding the degradation of lutein in emulsion.

It was found that the first-order kinetic reaction was suitable for the process of lutein loss during the heat chemical stability test. The absolute values of the kinetic parameter reaction rate constant K and the goodness of fit R^2 were presented in Table 1. The lower K was, the higher chemical stability of lutein emulsion was. It showed that reaction rate constant K was dependent on the concentration of rosemary extract and the addition methods. The degradation reaction rate constant K of lutein emulsions with different concentrations and different addition phases was in the order of 0.05 wt.% oil phase ($0.81 \times 10^{-3} \cdot h^{-1}$) < 0.1 wt.% oil phase ($1.57 \times 10^{-3} \cdot h^{-1}$) < 0.02 wt.% oil phase ($2.05 \times 10^{-3} \cdot h^{-1}$) < 0.05 wt.% water phase ($2.34 \times 10^{-3} \cdot h^{-1}$) < control ($4.04 \times 10^{-3} \cdot h^{-1}$).

The first-order fitting of lutein degradation was in accordance with other researches [30]. The first-order reaction for chemical stability of lutein emulsion proved that the mechanisms of degradation of carotenoids were similar. It also indicated that the kinetic parameter of carotenoids degradation was dependent on the content and addition phase of antioxidants used. Moreover, the presence of the rosemary extract in the oil or aqueous phase of the lutein emulsions did not change the type of chemical reaction acting on the lutein.

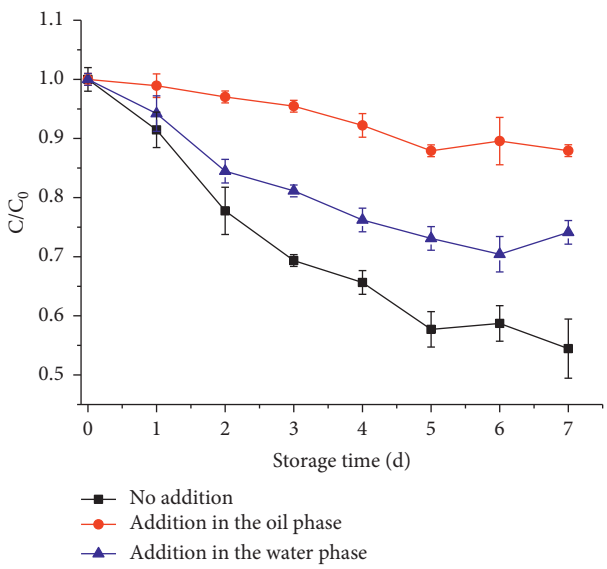

Figure 4: Effect of the rosemary extract (RE) addition in different phases (oil and water phase) on the degradation of lutein in WPI-stabilized emulsions during the storage at 55°C. Data points represent means ($n = 3$) ± standard deviations.

Table 1: First-order reaction rate constant for heat degradation of lutein emulsion with different concentrations and addition methods of rosemary extract.

Different concentration and addition methods of rosemary extract in lutein emulsion	k (h^{-1})	R^2
Control	4.04×10^{-3}	0.9304
0.02 wt.% in the oil phase	2.05×10^{-3}	0.9619
0.05 wt.% in the oil phase	0.81×10^{-3}	0.9077
0.1 wt.% in the oil phase	1.57×10^{-3}	0.9421
0.05 wt.% in the water phase	2.34×10^{-3}	0.8796

In the present study, the addition of rosemary extract in the oil phase was significantly more active than in the corresponding water phase ($P < 0.05$). The result can be explained on the basis of the interfacial properties of the antioxidants. It was assumed that, in the emulsion systems used in this study, the polar rosemary extract in the oil phase was more favorably partitioned at the interface than that in the water phase, thus becoming more protective. The trend was in accordance with the reported results exhibiting that the polar hydrophilic antioxidants ascorbic acid and Trolox were much less protective in emulsion than in bulk systems due to polar paradox [13].

3.5. Impact of Rosemary Extract in Different Phases on the Oxidation of WPI. There are some amino acids such as Cys, Trp, and Met from proteins that present antioxidative potentials with both metal-chelating and radical scavenging activities [31]. WPI oxidation in lutein emulsion occurred during the storage. Estévez et al. reported an approach for the analysis of the protein-lipid interaction and protein oxidation by the decrease of natural tryptophan fluorescence and the increase in fluorescence protein oxidation products through fluorescence spectroscopy [32]. The effects of the addition of rosemary extract in the different phases on the oxidation of WPI in lutein emulsions by decrease of tryptophan fluorescence (Figure 5(a)) and the increase in fluorescence of protein oxidation products (Figure 5(b)) were shown.

When excited at 283 nm, tryptophan residue was the only species to emit fluorescence in the range of 300–400 nm. At the beginning, the addition of rosemary extract in the oil phase or water phase in lutein emulsions did not obviously affect the initial level of tryptophan fluorescence compared with the absence of rosemary extract in emulsion. The tryptophan fluorescence decreased in the three lutein emulsions samples during storage for 7 days. During the first 2 days, the loss of tryptophan fluorescence was slight, and the differences of fluorescence intensity between the additions of rosemary extract in the different phase and the control sample were not significant ($P > 0.05$). After the storage for 3 days, the fluorescence intensity of WPI in lutein emulsions was in the order of rosemary extract in the water phase > rosemary extract in the oil phase > control (no rosemary extract). However, there were no significant differences in the fluorescence intensity of WPI between the additions of rosemary extract in the oil phase and aqueous phase in lutein emulsions. It would therefore indicate that the presence of rosemary extract in the aqueous phase and oil phase of lutein emulsions obviously improved the WPI stability. However, there were no significant differences in the protection of WPI between the additions of rosemary extract in the oil phase and aqueous phase in lutein emulsions. It was indicated that the presence of rosemary extract in the different phases of WPI-stabilized lutein emulsions did not significantly influence the oxidation of WPI. This was probably because most of the WPI molecules were adsorbed into the interface and they could interact with rosemary extract in the oil phase (transferring to the interface) and water phase (much better solubility); therefore, the effects of the addition of rosemary extract in the different phases on the WPI oxidation were similar.

The decrease of fluorescence intensity during storage of WPI-coated lutein emulsions was attributed to the oxidative degradation of tryptophan in the presence of free radical formed during lutein oxidation. Transition metal ions also acted as an initiator converting tryptophan into radicals which could react with lutein leading to its degradation [32]. The decrease of tryptophan fluorescence has been used as an indicator of oxidation of casein and BSA in oil-in-water emulsions [33]. The present result was in agreement with our previous research on the influence of WPI oxidation on the degradation of β-carotene in WPI-coated emulsions. It was found that the tryptophan fluorescence of WPI decreased in β-carotene emulsion during storage time and there was a relationship between protein oxidation and β-carotene loss [34].

The influence of addition of rosemary extract in the different phases on the WPI oxidation products was also studied (Figure 5(b)). The formation of WPI oxidation products due to the reaction of its free amino groups in lutein emulsions without and with addition of rosemary extract in the oil phase and water phase was also detected

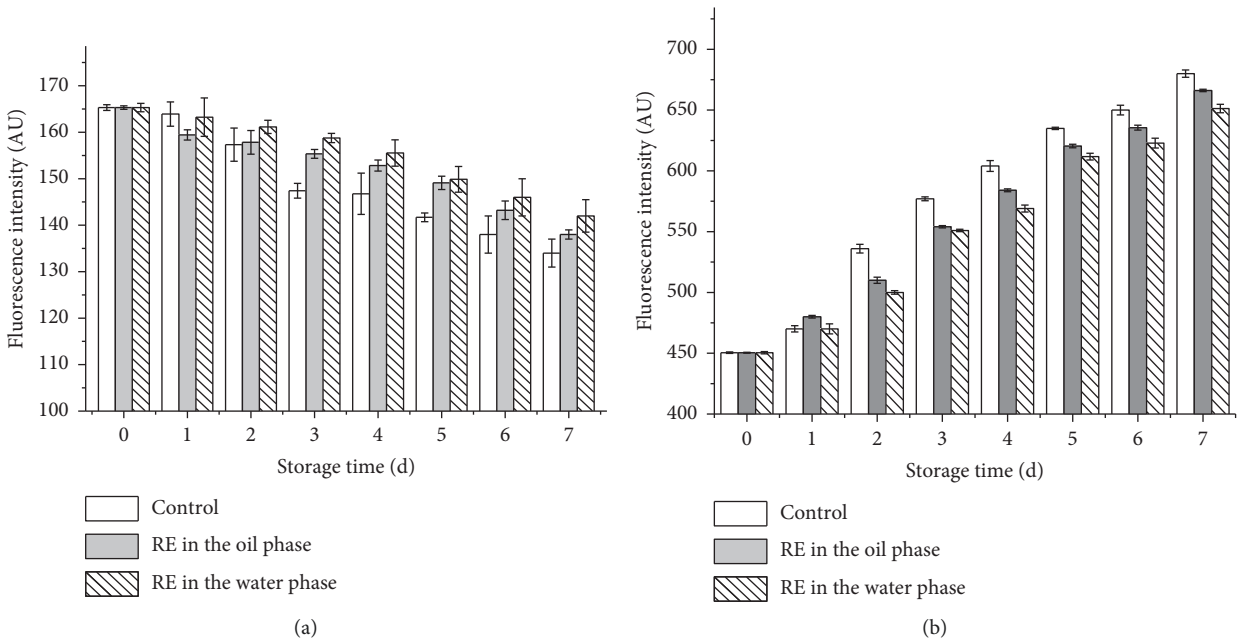

Figure 5: Decrease of tryptophan fluorescence (a) and increase of WPI oxidation products fluorescence (b) in WPI-stabilized lutein emulsions with different addition methods of rosemary extract (RE) (in the oil or water phase) during the storage at 55°C. Data points represent means ($n = 3$) ± standard deviations.

using fluorescence spectroscopy. As shown in Figure 5(b), the fluorescence intensity emitted by WPI oxidation products of the three lutein emulsions increased over storage time. Before the storage, the formation of WPI oxidation products was not influenced by the presence of rosemary extract. It was probably due to the fact that the addition of small molecular rosemary extract did not affect the structure of WPI. After storage for 2 days, the addition of rosemary extract in the oil phase and aqueous phase in lutein emulsions obviously decreased the fluorescence intensity compared with the absence of rosemary extract in emulsion. Significant differences of fluorescence intensity were observed between the additions of rosemary extract in the oil phase and aqueous phase in lutein emulsions. The presence of rosemary extract in the water phase significantly decreased the content of WPI oxidation product compared with that in the oil phase during the storage ($P < 0.05$).

It was revealed that the presence of rosemary extract in the aqueous phase of lutein emulsion would be better to inhibit the oxidation of WPI. The correlated change trend of fluorescence intensities of tryptophan and protein oxidation products was expected, since they were generated from WPI oxidation. Lutein emulsion in the absence of rosemary extract exhibited the greatest lutein degradation rate and the highest WPI oxidation product, which was a good correlation between the lutein loss and WPI oxidation. The greater protection of WPI in lutein emulsion with rosemary extract in the water phase might be a function of the better solubility of the rosemary extract mainly containing rosmarinic acid, carnosic, and so forth in the aqueous phase compared with that in the oil phase. Therefore, rosemary extract in the water phase could effectively chelate iron and scavenge free radicals for the WPI oxidation in lutein emulsion. In addition, it was found that rosemary extract in oil phase could provide active prevention against radicals generated from lutein degradation; therefore, the prevention of the radicals generated by WPI might be limited. Finally, the interaction between WPI and rosemary extract could also explain the strong protection of WPI oxidation. It was reported that antioxidant compounds could interact with amino acids [35].

4. Conclusion

Overall, the efficacy of rosemary extracts as antioxidants in WPI-stabilized lutein emulsion depends on the concentration and addition method. Rosemary extract was the most active at the certain optimal concentration of 0.05 wt.%. Exceeding it resulted in a decrease of activity and even the occurrence of a prooxidative effect. The first-order reaction kinetic parameter proved that the lutein degradation was dependent on the content and addition phase of antioxidants used. On the protection of lutein in emulsion, rosemary extracts performed better in oil phase than in water phase, whereas, on the protection of WPI, rosemary extracts performed better in water phase than in oil phase. It was assumed that, in the emulsion systems used in this study, the polar rosemary extract in the oil phase was more favorably partitioned at the interface than that in the water phase, thus becoming more protective. The greater protection of WPI in lutein emulsion with rosemary extract in the water phase might be a function of the better solubility of the rosemary extract mainly containing rosmarinic acid, carnosic acid, etc. in the water phase compared with that in the oil phase. In addition, it was found that rosemary extract in oil phase could provide active prevention against radicals generated

from lutein degradation; therefore, the prevention of the radicals generated by WPI might be limited. As a result, delivery system with rosemary extract can be developed for the emulsification of hydrophobic compounds and also in the protection of protein against oxidation during storage.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

[1] H. B. Sowbhagya, S. R. Sampathu, and N. Krishnamurthy, "Natural colorant from marigold-chemistry and technology," *Food Reviews International*, vol. 20, no. 1, pp. 33–50, 2004.

[2] A. Alves-Rodrigues and A. Shao, "The science behind lutein," *Toxicology Letters*, vol. 150, no. 1, pp. 57–83, 2004.

[3] K. Mitri, R. Shegokar, S. Gohla, C. Anselmi, and R. H. Müller, "Lute in nanocrystals as antioxidant formulation for oral and dermal delivery," *International Journal of Pharmaceutics*, vol. 420, no. 1, pp. 141–146, 2011.

[4] X. Mesnier, C. Gregory, P. Fança-Berthon, F. Boukobza, and A. Bily, "Heat and light colour stability of beverages coloured with a naturalcarotene emulsion: effect of synthetic versus natural watersoluble antioxidants," *Food Research International*, vol. 65, pp. 149–155, 2014.

[5] D. J. McClements, E. A. Decker, and J. Weiss, "Emulsion based delivery systems for lipohilic bioactive components," *Journal of Food Science*, vol. 72, no. 8, pp. R109–R124, 2007.

[6] J. Losso, A. Khachatryan, M. Ogawa, J. Godber, and F. Shih, "Random centroid optimization of phosphatidyl glycerol stabilized lutein-enriched oil-in-water emulsions at acidic pH," *Food Chemistry*, vol. 92, no. 4, pp. 737–744, 2005.

[7] C. Zhao, H. Cheng, P. Jiang, Y. Yao, and J. Han, "Preparation of lutein-loaded particles for improving solubility and stability by polyvinyl pyrrolidone (PVP) as an emulsion-stabilizer," *Food Chemistry*, vol. 156, pp. 123–128, 2014.

[8] C. S. Boon, D. J. McClements, J. Weiss, and E. A. Decker, "Factors influencing the chemical stability of carotenoids in foods," *Critical Reviews in Food Science and Nutrition*, vol. 50, no. 6, pp. 515–532, 2010.

[9] L. B. Fomuso, M. Corredig, and C. C. Akoh, "Metal-catalyzed oxidation of a structured lipid model emulsion," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 24, pp. 7114–7119, 2002.

[10] D. X. Xu, F. Yuan, Y. X. Gao, D. J. McClements, and E. A. Decker, "Influence of pH, metal chelator, free radical scavenger and interfacial characteristics on the oxidative stability of β-carotene in conjugated whey protein-pectin stabilised emulsion," *Food Chemistry*, vol. 139, no. 1–4, pp. 1098–1104, 2013.

[11] A. A. M. Botterweck, H. Verhagen, R. A. Goldbohm, J. Kleinjans, and P. A. Van Den Brandt, "Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study," *Food and Chemical Toxicology*, vol. 38, no. 7, pp. 599–605, 2000.

[12] L. S. Dias, M. E. C. Menis, and N. Jorge, "Effect of rosemary (Rosmarinus officinalis) extracts on the oxidative stability and sensory acceptability of soybean oil," *Journal of the Science of Food and Agriculture*, vol. 95, no. 10, pp. 2021–2027, 2015.

[13] E. N. Frankel, S.-W. Huang, R. Aeschbach, and E. Prior, "Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinicacid, in bulk oil and oil-in-water emulsion," *Journal of Agricultural and Food Chemistry*, vol. 44, no. 1, pp. 131–135, 1996.

[14] M. S. Brewer, "Natural antioxidants: sources, compounds, mechanisms of action, and potential applications," *Comprehensive Reviews in Food Science and Food Safety*, vol. 10, no. 4, pp. 221–247, 2011.

[15] J. J. Sun, J. H. Liu, and Z. W. Wang, "Application of tea polyphenols to edible oil as antioxidant by W/O microemulsion," *Journal of Dispersion Science and Technology*, vol. 36, no. 11, pp. 1539–1547, 2015.

[16] M. H. Gordon, F. Paiva-Martins, and M. Almeida, "Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 5, pp. 2480–2485, 2001.

[17] E. Dickinson, "Hydrocolloids as emulsifiers and emulsion stabilizers," *Food Hydrocolloids*, vol. 23, no. 6, pp. 1473–1482, 2009.

[18] K. Viljanen, A. L. Halmos, A. Sinclair, and M. Heinonen, "Effect of blackberryand raspberry juice on whey protein emulsion stability," *European Food Research and Technology*, vol. 221, no. 5, pp. 602–609, 2005.

[19] B. Hernández-Ledesma, A. Dávalos, B. Bartolomé, and L. Amigo, "Preparation of antioxidant enzymatic hydrolysates from (alpha-lactalbumin and beta-lactoglobulin. Identification of active peptides by HPLC-MS/MS," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 3, pp. 588–593, 2005.

[20] M. S. Katsuda, D. J. McClements, L. H. S. Miglioranza, and E. A. Decker, "Physical and oxidative stability of fish oil-in-water emulsions stabilized with beta-lactoglobulin and pectin," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 14, pp. 5926–5931, 2008.

[21] D. Xu, X. Wang, J. Jiang, F. Yuan, and Y. Gao, "Impact of whey protein-beet pectin conjugation on the physicochemical stability of beta-carotene emulsions," *Food Hydrocolloids*, vol. 28, no. 2, pp. 258–266, 2012.

[22] D. Xu, Z. Aihemaiti, Y. Cao, C. Teng, and X. Li, "Physicochemical stability, microrheological properties and microstructure of lutein emulsions stabilized by multilayer membranes consisting of whey protein isolate, flaxseed gum and chitosan," *Food Chemistry*, vol. 202, no. 1, pp. 156–164, 2016.

[23] M. Heinonen, D. Rein, M. T. Satué-Gracia, S.-W. Huang, J. B. German, and E. N. Frankel, "Effect of protein on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system," *Journal of Agricultural and Food Chemistry*, vol. 46, no. 3, pp. 917–922, 1998.

[24] K. Demetriades, J. N. Coupland, and D. J. McClements, "Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength," *Journal of Food Science*, vol. 62, no. 3, pp. 462–467, 1997.

[25] E. N. Frankel, S.-W. Huang, J. Kanner, and J. B. German, "Interfacial phenomena in the evaluation of antioxidants: bulk oils vs emulsions," *Journal of Agricultural and Food Chemistry*, vol. 42, no. 5, pp. 1054–1059, 1994.

[26] Y. Che Man and I. Jaswie, "Effect of rosemary and sage extracts on frying performance of refined, bleached and deodorized (RBD) palm olein during deep-fat frying," *Food Chemistry*, vol. 69, no. 3, pp. 301–307, 2000.

[27] C. Qian, E. A. Decker, H. Xiao, and D. J. McClements, "Nanoemulsion delivery systems: influence of carrier oil on β-carotene bioaccessibility," *Food Chemistry*, vol. 135, no. 3, pp. 1036–1043, 2012.

[28] A. Panya, K. Kittipongpittaya, M. Laguerre et al., "Interactions between alpha-tocopherol and rosmarinicacid and its alkyl esters in emulsions: synergistic, additive, or antagonistic effect," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 41, pp. 10320–10330, 2012.

[29] D. J. McClements, E. A. Decker, Y. Park, and J. Weiss, "Structural design principles for delivery of bioactive components in nutraceuticals and functional foods," *Critical Reviews in Food Science and Nutrition*, vol. 49, no. 6, pp. 577–606, 2009.

[30] V. Lavelli and M. C. Torresani, "Modelling the stability of lycopene-rich by-products of tomato processing," *Food Chemistry*, vol. 125, no. 2, pp. 529–535, 2011.

[31] A. Saiga, S. Tanabe, and T. Nishimura, "Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 12, pp. 3661–3667, 2003.

[32] M. Estévez, P. Kylli, E. Puolanne, R. Kivikari, and M. Heinonen, "Fluorescence spectroscopy as a novel approach for the assessment of myofibrillar protein oxidation in oil-in-water emulsions," *Meat Science*, vol. 80, no. 4, pp. 1290–1296, 2008.

[33] K. Viljanen, P. Kylli, R. Kivikari, and M. Heinonen, "Inhibition of protein and lipid oxidation in liposomes by berry phenolics," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 24, pp. 7419–7424, 2004.

[34] D. Xu, X. Wang, J. Jiang, F. Yuan, E. A. Decker, and Y. Gao, "Influence of pH, EDTA, α-tocopherol, and WPI oxidation on the degradation of β-carotene in WPI-stabilized oil-in-water emulsions," *LWT—Food Science and Technology*, vol. 54, no. 1, pp. 236–241, 2013.

[35] A. Panya, W. Temthawee, N. Phonsatta et al., "Apolarradical initiated conjugated autoxidizabletriene (ApoCAT) assay: effects of oxidant locations on antioxidant capacities and interactions," *Journal of Agricultural and Food Chemistry*, vol. 63, no. 34, pp. 7546–7555, 2015.