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Research Article

The Effect of Free and Encapsulated Casein Hydrolyzates on the Oxidation Rate and Structural Properties of Mayonnaise

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Casein is known as a source of bioactive peptides. In this study, the effects of mayonnaise enrichment with casein hydrolyzates on the oxidation process during 6 months were investigated. For this purpose, mayonnaise (≈60% oil) was treated with casein hydrolyzates and encapsulated casein hydrolyzates at concentrations of 100 and 200 ppm and compared with synthetic antioxidants (TBHQ). The results showed that the addition of casein hydrolyzates decreased the oxidation (peroxide, thiobarbituric acid, anisidine, and Totox indices) with a positive dose-response from 100 to 200 ppm. At similar concentrations, samples containing encapsulated casein hydrolyzates performed better than nonencapsulated casein hydrolyzates in retarding the oxidation and were able to compete with TBHQ. The results of scanning electron microscopy (SEM) also showed an improvement in the structure of samples containing encapsulated casein hydrolyzates. Therefore, the use of encapsulated casein hydrolyzates as natural antioxidants is recommended in functional foods and food emulsions such as mayonnaise.

1. Introduction

Mayonnaise is one of the most popular sauces in the world, which is produced by the emulsification of oil, eggs, and vinegar. Oxidation reactions in mayonnaise are influenced by many factors such as chemical composition, physicochemical properties of the aqueous and oily phases, the interfacial region, and the type of surfactants [1]. The mechanism of lipid oxidation in food emulsions such as mayonnaise is complex and is not fully understood. In food emulsions, oxidation reactions occur in the interfacial membrane. Therefore, the properties of this membrane have an important effect on the rate of oxidation. The oxidation rate in oil-in-water oil emulsions such as mayonnaise occurs faster than pure oils [2]. Oxidation in mayonnaise, which occurs due to the high oil content and unsaturated fatty acids, reduces its quality during storage. This phenomenon causes the development of off-flavor, undesirable odor, and discoloration of mayonnaise. Therefore, preventing oxidation has an important effect on increasing the shelf life of mayonnaise [1]. Today, due to the importance of various diseases such as cardiovascular diseases, most recent researches have focused on the application of functional compounds from natural resources. Bioactive ingredients include vitamins, fatty acids, carotenoids, sterols, polyphenols, amino acids, peptides, proteins, and probiotics. Among the bioactive compounds, hydrolyzed proteins have been considered food-drug substances and functional additives in various food formulations [3, 4]. These compounds are rich in low molecular weight peptides (bioactive peptides) that have high digestibility, low toxicity, and allergenicity. The other biological properties include antioxidant and antimicrobial activities [5, 6]. Milk casein is widely recognized as a source of nutritious and bioactive peptides [7, 8]. The bioactive peptides derived from casein have antioxidant activity and antihypertensive and antimicrobial activities [9]. Casein hydrolyzates are more effective inhibitors of lipid oxidation than casein but have disadvantages such as bitter taste and excessive moisture absorption (leading to physical instability during storage, chemical reactions, microbial spoilage, and functional characteristic decline) which have significantly affected their application in food formulations [10]. One of the efficient methods to reduce these disadvantages is the encapsulation of bioactive compounds and protein hydrolyzates. This process is also a good way to deliver bioactive peptides. In recent years, various aspects of encapsulation of bioactive peptides derived from protein hydrolyzates have been investigated [11]. Various researchers have reported the effect of the encapsulation process on the physical properties and bitterness of casein [12], whey protein [13], chicken [14], flaxseed [15, 16], fish [17, 18], soy protein [19], and algae [20] hydrolyzates. However, so far, no research has been carried out on the effect of this process on improving the antioxidant activity of casein bioactive peptides in mayonnaise. Therefore, the study aimed to evaluate the effect of casein hydrolyzates on the oxidative stability and structural properties of mayonnaise during storage.

2. Materials and Methods

2.1. Materials. Casein and pancreatin were purchased from Sigma-Aldrich (USA). Other chemicals with analytical grades were provided by Merck Company (Germany).

2.2. Casein Hydrolysis. For the enzymatic hydrolysis, casein (5% w/v) was dissolved in 0.2 M phosphate buffer (pH: 8; 50°C) and completely hydrated at room temperature for 30 min. Then, pancreatin (2.5% w/w) was added to the solution. The reaction temperature was 40°C, and the reaction was carried out at 200 rpm for 30–60 minutes. To inactivate the enzyme activity, the reaction medium was placed in a water bath (90°C) for 15 minutes. The solution was cooled to ambient temperature and then centrifuged (Part azma-rfs-refri. Iran) at 5000 rpm for 10 minutes. The supernatant was separated, lyophilized, and stored at −20°C [12].

2.3. Encapsulation of Casein Hydrolyzates. The encapsulation of hydrolyzates was carried out in the form of liposomes through the lipid film hydration method, using chloroform and lecithin as the capsule wall material. Lecithin (10 mL) was dissolved in chloroform, and after its complete dispersion, the organic solvent was removed in a rotary evaporator at 50°C until the formation of lipid film dried using a rotary evaporator to form a thin lipid film. The chloroform was removed for 18 h in a vacuum desiccator at room temperature. The lipid film was hydrated in 20 mL of phosphate buffer pH 7.4, containing lyophilized protein hydrolyzate (2 ml), incubated (Behdad. Iran) at 60°C for 5 min, and then placed at room temperature for 10 min. Sonication of dispersion was carried out for 1 min using an ultrasonic bath (Bandelin Sonopuls, Germany) and then kept in cold water for 5 min. A control capsule was produced under the same conditions without a hydrolyzate sample [21].

2.4. Mayonnaise Preparation. Mayonnaise was prepared by mixing the oily phase including antioxidant-free soybean oil (60%), water, vinegar, eggs, salt, sugar, citric acid, mustard, and xanthan. Casein hydrolyzates and encapsulated casein hydrolyzate (100 and 200 ppm) were added to the aqueous phase of mayonnaise. A sample was prepared with synthetic antioxidant (TBHQ) at 100 ppm. A sample without any antioxidant was considered as a control. The samples were packed in glass containers and stored at 4°C for 6 months [22].

2.5. Evaluation of Oxidation Process. The oil of mayonnaise was extracted by cold method [22]. The oxidation progress was determined by measuring peroxide, thiobarbituric acid, anisidine, and Totox values according to the procedures previously characterized [22].

2.6. Scanning Electron Microscopy (SEM). The microstructure of samples was investigated using scanning electron microscopy (SEM; LEO 440I, England) at four magnifications of 100, 250, 500, and 1000. Comparison of the average diameter and pore area of images with the same scales and magnifications is determined by Microstructure Mesument.exe software.

2.7. Statistical Analysis. All experiments were performed in a completely randomized design with three replications. Analysis of variance (ANOVA) was performed by Duncan's test using SPSS software (version 20) to confirm the differences between the data ($p < 0.05$).

3. Results and Discussion

3.1. Peroxide Value (PV). The peroxide value of mayonnaise samples enriched with casein hydrolyzates is shown in Figure 1. As can be seen, at the beginning of the experiment (month 1), no significant difference was observed between the peroxide values in different groups ($p > 0.05$). During storage, the peroxide value of the control sample was significantly higher than other groups ($p < 0.05$). The peroxide value was reduced by the addition of casein hydrolyzates. At similar concentrations, samples containing encapsulated casein hydrolyzate performed better in reducing the peroxide value. The difference between the antioxidant activity of M-200, M-100, and H-200 was not significant ($p > 0.05$). Also, with increasing the concentration of casein hydrolyzate to 200 ppm, the peroxide value of mayonnaise decreased significantly ($p < 0.05$). At the end of the storage, the highest peroxide value was related to the control sample (5.83 meq/kg oil), and the lowest peroxide value was related to the sample containing TBHQ followed by mayonnaise enriched with 200 then 100 ppm encapsulated casein hydrolyzate, respectively.

Peroxide value determines the hydroperoxides or primary oxidation products. As observed, in the initial stages of oxidation, the formation of peroxides was slow and then their formation increased rapidly, and at this stage, the determination of the peroxide value is a good sign of the oxidation of mayonnaise. In the control sample, the peroxide value increased until the fifth month of the storage and then

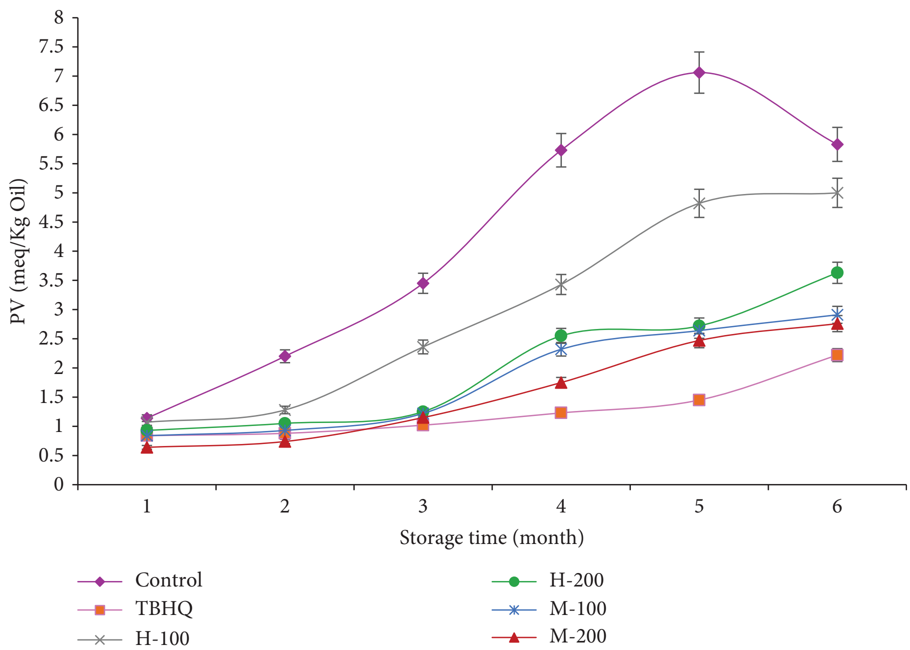

Figure 1: Peroxide value in different samples of mayonnaise during 6 months. H-100 and H-200: mayonnaise containing 100 and 200 ppm casein hydrolyzate, respectively. M-100 and M-200: mayonnaise containing 100 and 200 ppm encapsulated casein hydrolyzate, respectively.

decreased, which is due to the formation of the secondary oxidation products [22]. The higher antioxidant activity of encapsulated samples compared to normal samples should be due to the reduction of the oxygen level by capsules. The increase in peroxide value over time is due to the mayonnaise oxidation. According to the national standard of Iran (ISIRI), the standard limit of peroxide value for edible oils is 5 meq/kg oil, and in the control sample, it was higher than the standard limit at month 4 [23]. On the other hand, the control sample was acceptable until the third month of storage. Mayonnaise contains polyunsaturated fatty acids, and as the degree of unsaturation increases, the oxidation increases. Mayonnaise is an oil-in-water emulsion, its oily phase is in contact with the water, and the aqueous phase carries high amounts of oxygen which increases oxidation during storage [1]. In treatments enriched with synthetic (TBHQ) and natural (casein hydrolyzates) antioxidants, the peroxide value did not exceed the standard limit during storage. The antioxidant activity of casein hydrolyzates has been proven in many previous studies [24, 25]. Caseins contain polar fragments containing phosphorylated serine residues and serine-serine-serine-glutamicacid-glutamic acid sequences that act as effective chelators by forming complexes with calcium, iron, and zinc [26]. The antioxidant capacity of hydrolyzed proteins has been attributed to inhibiting free radicals, acting as metal chelators, oxygen scavengers, or hydrogen donors, and forming a layer around oil droplets [27]. The short-chain bioactive peptides, an increase of amino acids and active groups after enzymatic hydrolysis, increase the antioxidant properties of the hydrolyzed proteins. For example, amino acids such as tyrosine and cysteine in milk and whey proteins inhibit free radicals [8]. Histidine-containing peptides act as metal ion chelators, oxygen scavengers, and inhibitors of hydroxyl radicals. Aromatic amino acids such as tryptophan also have antioxidant activity in food systems [5]. Suetsuna et al. [28] identified bioactive peptides derived from casein that have antioxidant activity. The amino acid sequence of tyrosine-phenylalanine-tyrosine-proline-glutamic acid-leucine showed antioxidant activity. Also, the glutamic acid-leucine sequence is important for antioxidant activity [8].

3.2. Thiobarbituric Acid (TBA). The thiobarbituric acid (TBA) in mayonnaise samples is shown in Figure 2. As can be seen at the beginning of the experiment (month 1), no significant difference was observed between the TBA in different groups ($p > 0.05$). During storage, TBA in the control sample was significantly higher than other treatments ($p < 0.05$). The TBA was reduced by the addition of casein hydrolyzates. At similar concentrations, samples containing encapsulated casein hydrolyzate performed better in reducing the TBA. Also, by increasing the concentration of casein hydrolyzate to 200 ppm, the TBA of samples decreased significantly ($p < 0.05$). At the end of the storage, the highest TBA was related to the control sample (7.11 mg MDA/kg oil) and the lowest TBA was related to the sample containing TBHQ, and mayonnaise enriched with 200 and 100 ppm encapsulated casein hydrolyzate.

In other words, during the storage, casein hydrolyzates similar to that of TBHQ reduced TBA or secondary oxidation products in mayonnaise. Similar to the peroxide value, TBA changes pattern depended on the concentration of casein hydrolyzates. Bioactive peptides exhibit antioxidant properties by metal ions chelating (Fe^{2+}/Cu^{2+}) and lipid peroxidation inhibition [5]. Various studies have confirmed

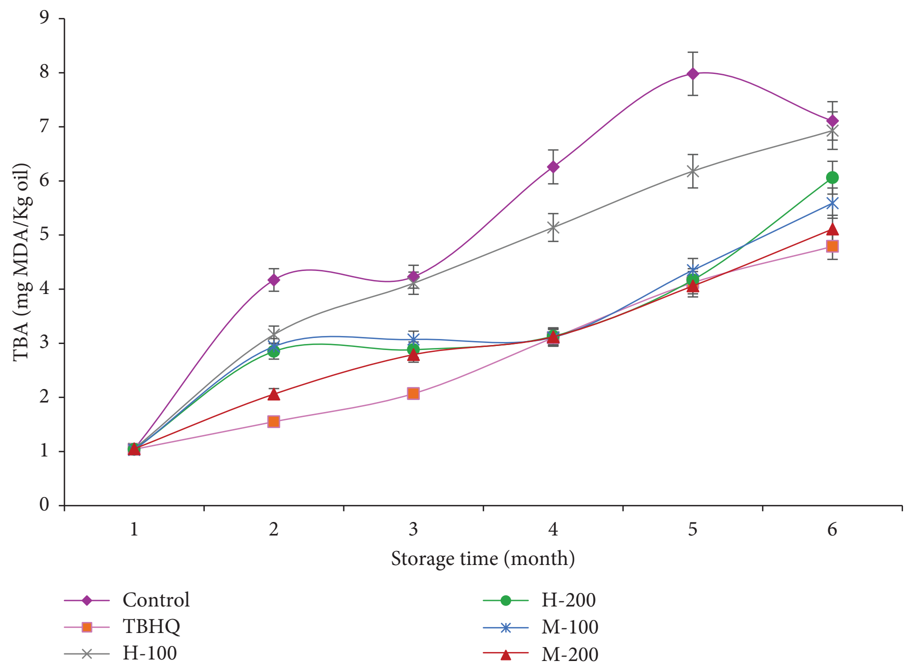

Figure 2: Tiobarbitoric acid in different samples of mayonnaise during 6 months. H-100 and H-200: mayonnaise containing 100 and 200 ppm casein hydrolyzate, respectively. M-100 and M-200: mayonnaise containing 100 and 200 ppm encapsulated casein hydrolyzate, respectively.

the antioxidant activity of milk proteins especially after enzymatic hydrolysis [29, 30]. The antioxidant activity of casein hydrolyzates has been previously reported in ground meat [31]. The researchers confirmed that bioactive peptides derived from casein prevent the spoilage in meat products and extend the shelf life of the product [32].

3.3. Anisidine Value (AV). The anisidine value of mayonnaise samples enriched with casein hydrolyzates is shown in Figure 3. As can be seen, at the beginning of the experiment (month 1), no significant difference was observed between the anisidine values in different groups ($p < 0.05$). The anisidine value of the control sample was significantly higher than other treatments ($p < 0.05$) and was reduced by the addition of casein hydrolyzates. At similar concentrations, samples containing encapsulated casein hydrolyzate acted significantly better than nonencapsulated ones in reducing the anisidine value ($p < 0.05$). Also, with increasing the concentration of casein hydrolyzate to 200 ppm, the anisidine value of mayonnaise decreased significantly ($p < 0.05$). At the end of the storage, the highest anisidine value belonged to the control sample (10.55 mmol/kg oil). The lowest anisidine value was observed in the sample containing TBHQ, and then, mayonnaise enriched with 200 and 100 ppm encapsulated casein hydrolyzate, respectively.

These results are consistent with the peroxide value because the formation and deformation of hydroperoxides occurred at this stage which led to the formation of secondary compounds [22]. According to the national standard of Iran (ISIRI), the standard limit of anisidine value in edible oils is 10 mmol/kg, and in the control sample, it was higher than the standard limit at month 6 [23]. Thus, the control sample was acceptable until the fifth month. However, enriched treatments showed a shelf life of up to 6 months because bioactive peptides have antioxidant properties and prevent lipid oxidation. For example, bioactive peptides derived from camel milk hydrolyzates delayed oxidation in minced fish [29]. The hydrolyzates derived from porcine blood effectively inhibited lipid oxidation in pork meat emulsions [33]. Gelatin-derived bioactive peptides by preventing the oxidation of lipids and proteins in shrimp increased their shelf life [34]. Similar results have been reported about fish protein hydrolyzates [35].

3.4. Totox Index. Totox (total oxidation) value of mayonnaise samples enriched with casein hydrolyzates is shown in Figure 4. As can be seen, Totox value of the control sample was significantly higher than other treatments ($p < 0.05$). With the addition of casein hydrolyzates, Totox value was decreased. At similar concentrations, casein hydrolyzates performed better when encapsulated. Also, by increasing the concentration of encapsulated casein hydrolyzate to 200 ppm, Totox value decreased significantly ($p < 0.05$). At the end of the storage, the highest Totox value was related to the control sample (22.21), and the lowest Totox value was related to the sample containing TBHQ, and mayonnaise enriched with 200 and 100 ppm encapsulated casein hydrolyzate.

Casein hydrolyzates at high concentrations also acted as antioxidants and delayed the oxidation in mayonnaise. Recent researches suggest that hydrolyzed proteins can bind to metals and prevent oxidative reactions in oil-in-water

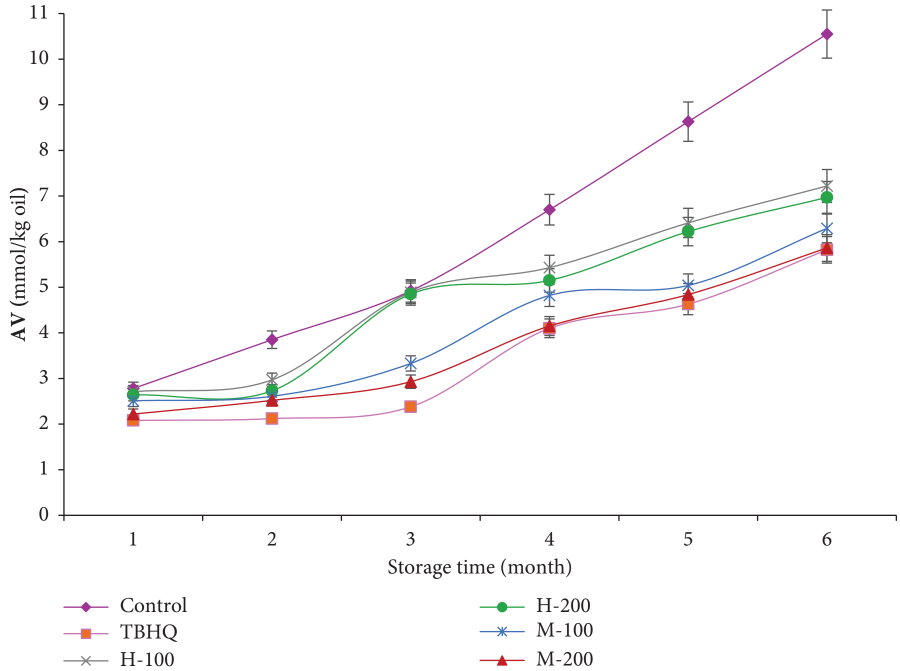

Figure 3: Anisidine value in different samples of mayonnaise during 6 months. H-100 and H-200: mayonnaise containing 100 and 200 ppm casein hydrolyzate, respectively. M-100 and M-200: mayonnaise containing 100 and 200 ppm encapsulated casein hydrolyzate, respectively.

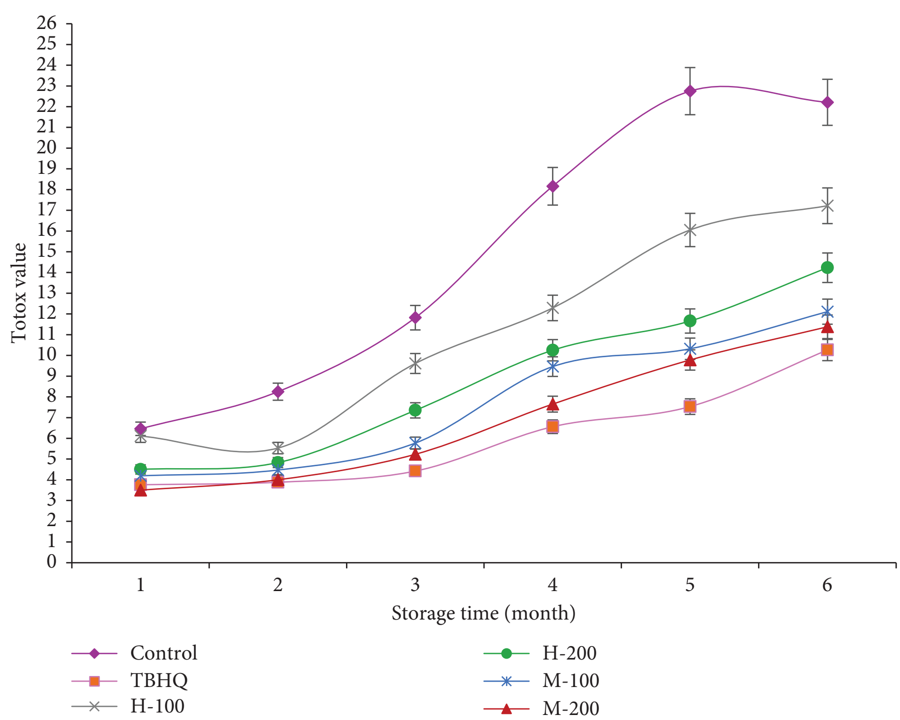

Figure 4: Totox value in different samples of mayonnaise during 6 months. H-100 and H-200: mayonnaise containing 100 and 200 ppm casein hydrolyzate, respectively. M-100 and M-200: mayonnaise containing 100 and 200 ppm encapsulated casein hydrolyzate, respectively.

emulsions, which are used as natural antioxidants and chelators in food emulsions. The mechanism of antioxidant peptides can be attributed to the formation of membranes and films around emulsion droplets and the inhibition of oil access to oxidative agents [36]. Therefore, bioactive peptides derived from casein hydrolysis are natural and functional sources of antioxidants than the primary protein, and their activity is not affected by denaturation. This is due to the higher concentration of antioxidant amino acids such as histidine, lysine, proline, and tyrosine in hydrolyzed casein

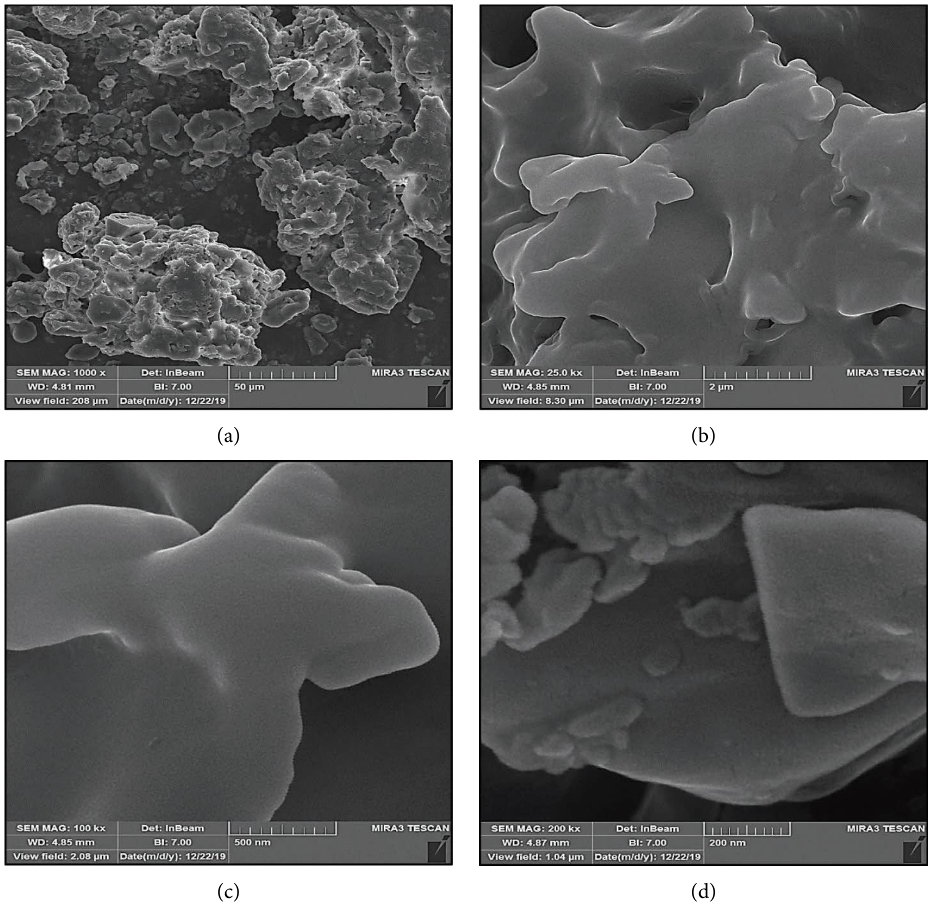

Figure 5: Scanning electron microscopy (SEM) results for mayonnaise samples: (a) control; (b) TBHQ; (c) H100; (d) M100 in 200000x magnifications.

than in pure casein [37]. The antioxidant peptides derived from whey protein [38], soybean [39], rice bran [40], egg protein [41, 42] and seafood such as fish [43, 44], shrimp [45], and algae [46] have been confirmed previously.

3.5. SEM Analysis. As can be seen in Figure 5(a), the ingredients were spread evenly in the control sample. One of the compounds observed was spherical fat cells. Fat cells are about 0.1–22 μm. TBHQ caused a significant microstructure change in the mayonnaise samples (Figure 5(b)). Unlike the control sample, less uniformity was observed in the samples containing casein hydrolyzate. Also, the aggregates were also shown in these samples. The sample containing casein hydrolyzate had denser assemblages than the control sample. In addition, there were more single casein micelles in this sample. The casein hydrolyzate in the sample resulted in higher aggregates (Figure 5(c)). As well shown, the microstructure of this sample had smoother surfaces than the control sample. Also, the individual micelles in this sample were much less than those in the two samples mentioned, which is probably due to the greater interactions between the compounds. The encapsulated casein hydrolyzate increased the dispersion of these aggregates (Figure 5(d)). Compared to the sample containing casein hydrolyzate, the density was reduced and a small number of single casein micelles were observed again. The samples in this sample had much smoother surfaces than the other samples.

4. Conclusion

The results of this study showed the antioxidant efficacy of casein hydrolyzates and their encapsulation on mayonnaise through oxidation indicators such as peroxide, thiobarbituric acid, anisidine, and Totox values. Also, casein hydrolyzates encapsulation showed a better effect on maintaining antioxidant activity during the storage of mayonnaise. Therefore, considering the results of this study, the encapsulation of casein hydrolyzates can be considered a suitable and efficient method to maintain antioxidant activity in food emulsions such as mayonnaise.

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.

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