Original Article

Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating on the shelf life of peanut

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ABSTRACT

In this paper, we aimed to investigate and compare the effect of the normal type (0.1% and 0.3%) of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating and its nanocapsulated type (0.1% and 0.3%) on the shelf life of peanut. For doing this, moisture content, weight change, peroxide value, thiobarbituric acid value, total yeast and mold count, and the percentage of aspergillus mold development in peanut were studied under different treatments. According to the obtained results, the peanut coated with whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil (0.3% concentration) had the lowest moisture content (6.5%), weight change (10.3%), peroxide value (1.31 mEq O_2/kg oil), thiobarbituric acid value (0.62 mg Malone aldehyde/kg oil), total yeast and mold count (2.27 log CFU/g), and aspergillus mold development percentage (65.4%). On the other hand, this treatment acquired the highest sensory analysis score (texture: 3.6, color: 3.7, flavor: 3.35, total acceptability: 3.6). Briefly, the quality of samples coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil was better than the control sample.

Keywords: Shiraz thyme, Peanut, WPC, Shelf life, Nanoliposome.

Introduction

Peanut is a valuable herbal oilseed, which after soybean and rapeseed oilseeds comes as the third 1-year cultivated crop. As far as the quality of peanut is concerned, moisture absorption and oxidation cause plenty of problems (e.g. weight change), and consequently, the quality of peanut is influenced by these adverse reactions [1]. Over the past few decades, undesirable chemical preservatives and consumer's increasing information have caused a growing interest in the use of natural preservatives (e.g. edible films and herbal essential oils) [2]. Edible coatings and films prevent the transfer of moisture, oxygen, and soluble substances to food by creating a thin layer around the food [3]. The amino acids of whey protein form intramolecular and intermolecular bonds (van der Waals, hydrogen, hydrophobic, and electrostatic bonds) and a network structure in aqueous solutions. These coatings are suitable seals against the penetration of steam, oxygen, fat, and aroma [4]. Also, herbal extracts and essential oils own antibacterial features, and they are used as preservatives in food because they have a controller role against the growth of pathogenic bacteria and spoilage agents. Biologically, most of the essential oils are unstable and insoluble in water and defectively are attached to target places. Therefore, encapsulating them using liposomes decreases the reaction of these compounds with environmental factors (oxygen, water, light), reduces evaporation, enhances their transmission, and their uniform distribution in the final product [5]. According to the obtained results by researchers, the bioavailability of encapsulated compounds in nanoliposomes (with nanometric dimensions) is more than ordinary nanoliposomes [6]. In recent decades, extracts and essential oils have been used to increasing the shelf life of food, reducing fungal growth time and preventing the production of mycotoxins [7]. Also, owing to the hydrophobic property of the

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essential oils, they penetrate to the phospholipids of bacterial membrane and mitochondria, so that they damage the structure of bacteria by leaking the ions and cell contents and eventually, cause bacteria destruction. Shiraz thyme (*Zataria multiflora*) belongs to the Lamiaceae family [8]. This plant geographically grows in Iran, Afghanistan, and Pakistan and is generally used to make foods favorable [9]. In traditional medicine, Shiraz thyme (*Zataria multiflora*) was used as a disinfectant and anti-inflammatory. The same as the other essential oils, Shiraz thyme (*Zataria multiflora*) has some beneficial properties. For instance, owing to its phenolic compounds, it has antimicrobial effects. Thus, it can be said that the higher the phenolic content of the essential oil, the greater its antimicrobial properties [10]. Carvacrol, Thymol, and Eugenol are the main phenolic compounds of Shiraz thyme (*Zataria multiflora*) essential oil [11]. According to the conducted researches, usage of Shiraz thyme (*Zataria multiflora*) essential oil, because of its antioxidant and antimicrobial properties, is influential in food preservation [12]. The objective of this study was to investigate the effect of whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil coating on the shelf life of peanut.

Materials and Methods

Firstly, raw Valencia peanuts were purchased from a peanut packaging plant in Astaneh Ashrafiyeh in autumn. The whey protein concentrate (80% Arla Foods, Denmark) was used to prepare the coating solution. The whey protein solution with a concentration of 10% (W/V) was obtained when we dissolved 10 g of whey protein concentrate in 100 mL distilled water. In order to dissolve the proteins, a magnetic stirrer was used for 15 minutes. Afterward, the obtained mixtures were placed in a hot water bath (Ben Marie) at 90°C for 30 minutes for denaturation of the proteins. Then, they were cooled to room temperature, and glycerol with a ratio of 1 to 3 was added to whey protein concentrate and homogenized [13]. The method developed by Durling et al. was used to prepare the Shiraz thyme (*Zataria multiflora*) essential oil from its dried leaves [14]. The liposomes used to encapsulate thyme were prepared by the method offered by Bozorgnejad et al., (2012) [15]. Then, the prepared liposomes with 0.13% linoleic acid (pH=4.5) (pH was adjusted using hydrochloric acid (0.1 N)) were placed in a shaker apparatus for 30 minutes at 40° C. Next, thyme with a ratio of 1 to 4 was added to liposome and surfactant mixture, and the obtained blend was shaken for 2 hours at room temperature. The resulting mixture was dried in a spray-drying apparatus at 5.26 ml/min with 100% aeration, so that the inlet and outlet temperatures were 105 and 68°C, respectively [16]. In the next stage, various concentrations of Shiraz thyme (*Zataria multiflora*) essential oil (the nanocapsulated and normal types of essential oil with concentrations of 0.1% and 0.3%, respectively) were added to the whey protein concentrate solution. For preparing samples, the sheath and second skin of peanut were separated, and intact kernels were selected and weighed. Peanut enrobing was done by immersing them in the prepared solution. Peanut samples were put into a mesh container and then, the container was dipped in a solution of whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil (0.1%). The peanuts were dipped in the coating solution for 2 separate 30-second periods. Accordingly, different treatments (whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil with 0.3% concentration, whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil with 0.1% and 0.3% concentrations) were coated, and the control sample was prepared by immersing in distilled water. Then, the samples were pulled out of the solution and using an oven, they were dried for 12-14 h at 30 °C to obtain the appropriate moisture (3.8–4.1%) [17]. Finally, 50 g of peanuts were packed in polyethylene bags and stored for 4 months at room temperature. During the storage period, 3 packs of each treatment were randomly picked up and physical, chemical, and microbial analyses were performed for all of them.

Physicochemical analyses

• Moisture:

Moisture was measured according to the AOAC method and was calculated using the equation A.1 [18]:

Eq. (A.1):

$$\text{Moisture percentage}=\frac{W_1-W_2}{W_0}\times 100$$

Where W_1 is the weight of the sample, W_2 is the weight of the sample and weighing boat before the oven, and W_0 is the weight of the sample and weighing boat after the oven.

• Weight change:

In order to determine the weight change, fresh peanut samples were weighed at the beginning and end of the storage period, and weight changes were calculated using the equation A.2 [19]:

Eq. (A.2):

$$\text{Weight change percentage}=\frac{W_2-W_1}{W_1}\times 100$$

Where W_1 is the initial weight of the sample and W_2 is the secondary weight of the sample.

• Sensory analysis:

Peanut samples were evaluated by 20 trained assessors (10 females and 10 males) using the 5-point Hedonic Scale (1=very low, 2= low, 3=medium, 4=high, 5=very high). Factors evaluated by assessors included hardness (the initial force required to fracture the grain), the degree of color, flavor desirability, and overall acceptance. Sensory analysis was carried out in triplicates on days 0, 30, 60, 90, and 120 of the storage period [20].

• Oil extraction:

Peanut kernel oil was extracted by the cold method. Firstly, 30 g peanut kernel from each sample were crushed using a mortar, and 1.5 times of the sample volume n-hexane solvent was added. Secondly, the sample was slightly stirred for initial aeration and then, covered with a foil and placed in a dark place for 12 hours. During the mentioned time, the peanut-hexane mixture was stirred using a magnetic stirrer. Then, peanut particles were separated by a vacuum filtration system and a Whitman No. 1 filter. The sample was placed under the hood to separate the existent solvent from the oil. The extracted oil was kept in amber glasses at -27 °C until the peroxide and thiobarbituric acid value analyses were carried out [21].

- **Determination of peroxide value:**

Peroxide value refers to the peroxide concentration and is a measure of oxidation or rancidity of oils. The peroxide value indicates the number of mEq of peroxide per 1000 g of sample. Peroxide value was calculated during the storage period according to the reference method [22]. In this method, we weighed 5 g of the oil sample in a 250-mL Erlenmeyer flask and added 30 ml of the acetic acid and chloroform mixture (3 to 2 ratio), and completely dissolved them. Then, 0.5 ml of saturated potassium iodide solution was added to the flask, and the obtained solution was placed in a dark place for 1 minute. After that, 30 mL of distilled water was added, and then, it was titrated with sodium thiosulphate solution (0.01 N) until the yellow color appeared. Following that, 0.5 mL of starch reagent was added. Titration was continued until the blue color disappeared. In the control test, all of the mentioned stages were performed without the presence of the sample. Peroxide value was calculated in mEq O_2/kg oil using equation A.3:

Eq. (A.3): $$PV=\frac{(S-B)\times N\times 1000}{W}$$

Where PV is peroxide value in mEq O_2/kg oil, S is the volume of sodium thiosulfate used for oil sample titration in mL, B is the volume of sodium thiosulphate used for control sample titration in mL, N is sodium thiosulphate normality in M/L and W is oil sample weight in g [22].

- **Thiobarbituric acid measurement:**

The amount of thiobarbituric acid extracted from peanut oil was measured according to the AOCS method [23].

Microbiological Analysis:

- **Total yeast and mold count:**

In this step of the project, 10 g of peanuts were crushed next to a flame and under a laminar flow hood using a sterilized mortar. The obtained peanut powder was sterilized with 90 ml of sodium chloride solution (0.85%) and then, was transferred to a stomacher bag and homogenized. After homogenization, 10^{-2} and 10^{-3} dilutions were prepared from 10^{-1} dilution. Afterward, 0.1 mL of each dilution was added to the DG18 Agar medium using an adjustable pipette and was spread to the culture medium using a cell spreader. After a few minutes, the plates were placed in an incubator and were incubated at 20 to 25°C. Eventually, the number of molds and yeasts were counted after 5 days [24].

- **Aspergillus mold development percentage:**

In order to calculate the Aspergillus mold development percentage, we disinfected 10 peanuts from each treatment in Vitex solution (3%), and then, they were rinsed with distilled water and placed in separate plates. Next, the plates were incubated at 25°C. The number of infected peanuts in all of the plates was counted after 5 days, and the development percentage of the mentioned mold in the treatments was specified in comparison to the control sample [25].

- **Statistical design:**

The present study was carried out in a completely randomized design format with the factorial arrangement in triplicate. The variance analysis was performed using the SPSS software. Also, the Duncan test was employed to perform mean comparisons at a 95% confidence coefficient. Graphs were drawn using Excel software.

Results and discussion:

Physicochemical analyses

- **Moisture:**

Moisture is one of the factors which can affect the texture and grain quality and can increase the shelf life of the nuts. Moisture content can be one of the crucial reasons for deterioration reactions in grains. Microbial and chemical spoilage and production of mycotoxins are causes of high moisture in peanuts. The dryness of peanuts and firm texture in peanuts can be the result of low moisture. Moisture damages the sheath, kernel and in some cases can increase the growth and production of Aflatoxin by Aspergillus Flavus. Moisture requirement at the ideal growth temperature (30-35°C) is at its minimal level. Relative moisture of 80% or more facilitates mold growth and cause an increase in nut's weight. Optimum moisture of peanut for preventing mold growth is less than 10% [26]. The analysis of variance (ANOVA) proved that the effect of time and concentration of essential oil on moisture content was significant ($p < 0.05$). According to figure 1, whey protein film-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil with 0.3% concentration had lower moisture content (6.5%) than other treatments and control samples. The control sample demonstrated the highest moisture content (9.55%), which is consistent with the results of Javanmard and Ramezan (2009) and Maghsoudlou et al., (2012) [19, 27]. The conducted research by Chen (1995) illustrates that whey protein concentrate films

can reduce the water vapor transmission rate by 75.6% at 55% relative moisture and 23° C [28].

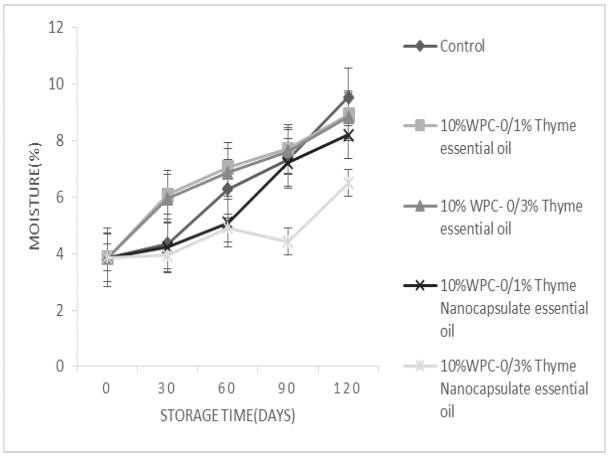

Figure 1. Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating (The normal type and nanocapsulated type) on the moisture content of peanut during the storage period.

• Weight change:

The weight change in peanut can be stated as the change in moisture content in contrast to the initial moisture content. In figure 2, the effect of whey protein-Shiraz thyme (*Zataria multiflora*) essential oil coating on peanuts weight loss prevention has been clearly illustrated. Analysis of variance (ANOVA) showed that the effect of storage time and concentration of essential oil on the percentage of weight change was significant ($p<0.05$). In uncoated samples contained more absorbed moisture, higher weight change was observed (20.4%). The outcomes showed that the weight change derived from moisture absorption in the treatment coated by whey protein film-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil (0.3% concentration) causes the weight loss reduction in peanut by 10.3%, which is consistent with the results of previous investigations [29].

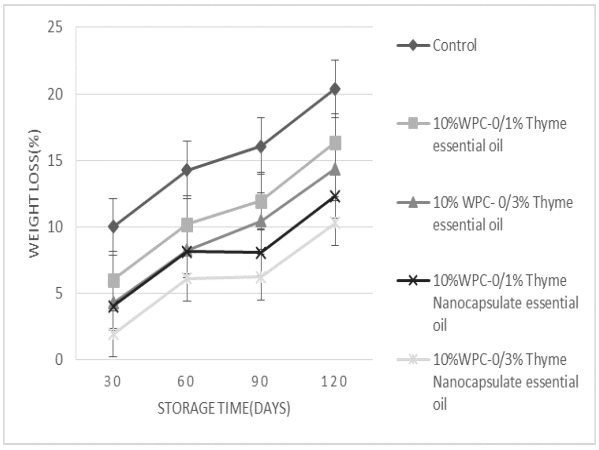

Figure 2. Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating (The normal type and nanocapsulated type) on the weight loss percentage of peanut during the storage period.

• Sensory analysis:

The results achieved from variance analysis indicated that the effect of time and essential oil concentration on sensory properties of peanut (taste, color, texture, and general acceptance) was significant ($p<0.05$). The mean of results obtained from the sensory analysis showed that peanut coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil with 0.3% concentration and control sample acquired the highest overall acceptance score (3.6) and the lowest overall acceptance score (1.8), respectively (Table 1). The Whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil coating caused maintaining the moisture in peanut and prevented texture hardening. Also, the coating preserved the color and taste of the samples and prevented the peanuts from oxidation. The results of this study are consistent with the results of Maghsoudlou et al., (2012) and Omidbeygi et al., (2007) [19, 30].

• Determination of peroxide value:

There are lots of factors such as oxidation of lipids, which can adversely affect the aroma, color, nutritional value, and production of toxic compounds during food processing, storage, and distribution. Figure 3 exhibits the results of peanut peroxide value. As can be seen in the chart, during the storage period, the peroxide value of all treatments had an upward trend. Nevertheless, the coated samples always had a lower peroxide value than the control sample (4/22 mEq O_2/kg oil), which is consistent with the results of Haq et al., (2013) and Javanmard and Ramezan (2009) [27, 31]. The lowest peroxide value was observed in the samples coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil (0.3% concentration) (1/31 mEq O_2/kg oil). Thymol and Carvacrol are the major compounds of Shiraz thyme (*Zataria multiflora*) essential oil which offer good antioxidant activity [30].

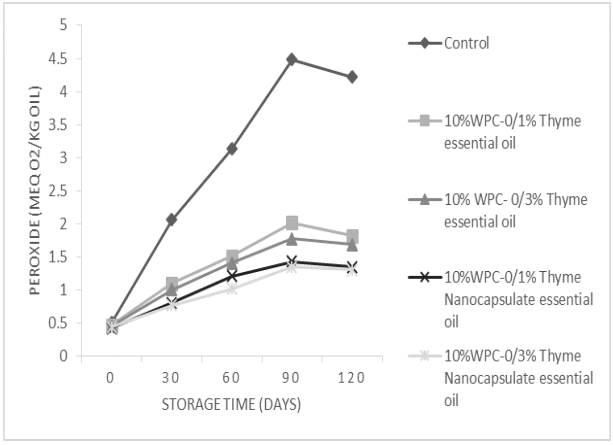

Figure 3. Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating (The normal type and

nanocapsulated type) on the peroxide value of peanut during the storage period.

- **Thiobarbituric acid measurement:**

The results of the analysis of variance showed that the effect of time and essential oil concentration on thiobarbituric acid value was significant ($p < 0.05$). The mean of the results obtained from thiobarbituric acid measurement showed that peanuts coated with the whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil (0.3% concentration) had the lowest thiobarbituric acid (0.62 mg Malone aldehyde/kg oil) and the control sample had the highest amount of thiobarbituric acid (0.93 mg Malone aldehyde/kg oil) (Figure 4). Decomposition of primary oxidation products (hydroperoxide) into secondary products (aldehydes and ketones) results in increased malondialdehyde and increased thiobarbituric acid value during the storage period [32]. The presence of phenol, carvacrol, and hydroxyl groups in Shiraz thyme (*Zataria multiflora*) essential oil correlates with its antioxidant effect. Accordingly, they are able to inhibit the activity of free radicals [33]. Also, the main reason for low thiobarbituric acid value is the impermeability of whey protein concentrate coating against oxygen [34]. Min and Krochta (2007) investigated thiobarbituric acid value as an indicator of secondary products in roasted and coated peanuts with whey protein [35]. Eventually, their results showed that thiobarbituric acid value did not show any significant difference between the coated and uncoated samples ($p>0.05$) after 45 and 60 days of storage at 35 and 50 °C, but after the mentioned period, there was a significant difference between the control and coated treatments containing ascorbic acid ($P < 0.05$). [35] The results of this study are consistent with the findings of Shon et al., (2012) and Wu et al., (2000) [34, 36]. Wu et al., (2000) also reported that protein coatings have a greater effect than polysaccharide coatings on the prevention of lipid oxidation.

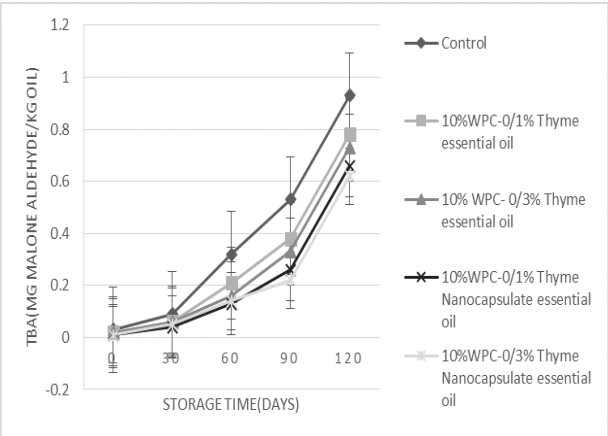

Figure 4. Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating (The normal type and nanocapsulated type) on the thiobarbituric acid value of peanut during the storage period.

Microbiological Analysis:

- **Total yeast and mold count:**

The results of the total yeast and mold count in peanuts are provided in figure 5. Over the storage period, mold and yeast growth had an upward trend in all treatments and control samples. There is a wide range of factors, which can affect the mold and yeast growth, such as a relative increase in peanut moisture content during storage, presence of oxygen in the packing air, and appropriate temperature [37]. The coating prevents mold and yeast growth by reducing oxygen and moisture permeability [38]. According to the results, there was always a significant difference in the total count of mold and yeast between the control sample and the coated treatments ($p < 0.05$), over the entire storage period. As shown in figure 5, the treatment coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil with 0.3% concentration had the lowest quantity of total mold and yeast count (2.27 log CFU/g). The highest quantity of this factor (3.21 log CFU/g) was observed in the control sample. The results of this study are consistent with the results of Javanmard and Ramezan (2009) and Omidbeygi et al. (2007) [27, 30].

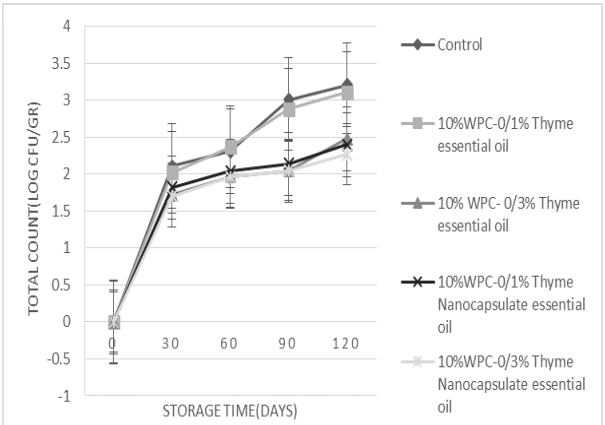

Figure 5. Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating (The normal type and nanocapsulated type) on total yeast and mold count of peanut during Storage period.

- **The percentage of Aspergillus mold development:**

According to figure 6, the percentage of Aspergillus mold development in all of the samples had an upward trend during the storage period. Variance analysis (ANOVA) showed that the effect of time and essential oil concentration on the percentage of Aspergillus mold development was significant ($p < 0.05$). The treatment coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil (0.3% concentration) had the lowest Aspergillus mold development (65.4%). Also, the control sample showed the highest percentage of Aspergillus mold development (78.36%).

Javanmard and Ramezan (2009) and Ziani et al., (2010) achieved similar results [27, 38]. Javanmard and Ramezan (2009), investigated the effect of different concentrations of whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil coating on inhibition of Aspergillus Flavus' growth on pistachio kernels, and their study showed that this coating inhibited Aspergillus Flavus' growth and as the concentration of thyme essential oil increased, its effect increased, too [27].

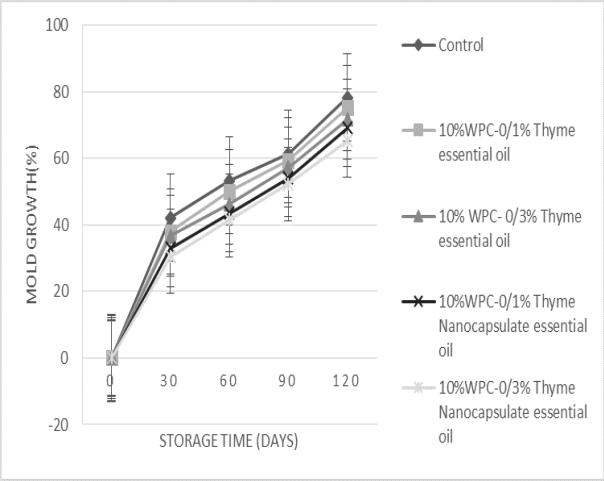

Figure 6. Effect of whey protein concentrate-Shiraz thyme (*Zataria multiflora*) essential oil coating (The normal type and nanocapsulated type) on the Aspergillus mold development percentage of peanut during the storage period.

Conclusion

The outcomes of this study proved that the whey protein concentrate coating can be employed as a good barrier against the penetration of moisture and gases and also, can improve the quality and increase the shelf life of peanuts. The treatment coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil (0.3% concentration) had the lowest moisture content, weight change, peroxide value, thiobarbituric acid, total mold and yeast count, and percentage of Aspergillus mold development. The sensory properties of these samples were scored higher than other treatments by assessors. All in all, the samples coated with whey protein concentrate-nanocapsulated Shiraz thyme (*Zataria multiflora*) essential oil had better quality than the control sample. Therefore, it can be claimed that when a natural compound is used as a coating, the problems caused by oxidative reactions and growth of Aspergillus species, which lead the product to quality reduction, reduce considerably, and owing to the beneficial features of this method, an advantageous food product can be produced and the peanut contamination problem during transportation and distribution can be solved.

Declaration of conflicting interests:

The authors declare that there is no conflict of interest.

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Table 1- Effect of whey protein concentrate-Shiraz thyme (Zataria multiflora) essential oil coating (The normal type and nanocapsulated type) on the Sensory analysis of peanut during the storage period. In every column and row, small letters illustrate that there is a significant difference (p<0/05), and capital letters imply that there are no significant differences (p>0/05).

Treatments	Storage time (day)				
	0	30	60	90	120
		Texture			
Control	4.65a	3.70g	2.90i	2.30kl	1.80m
10%WPC-0/1% Thyme essential oil	4.65a	4.15e	3.40h	2.72j	2.20l
10% WPC- 0/3% Thyme essential oil	4.65a	4.35d	3.35h	2.90i	2.40k
10%WPC-0/1% Thyme Nanocapsulate essential oil	4.65a	4.45bc	3.45h	2.90i	2.40k
10%WPC-0/3% Thyme Nanocapsulate essential oil	4.65a	4.55b	4.05ef	4.00g	3.60g
		Color			
Control	4.75a	3.80gh	2.90l	2.72m	2.20n
10%WPC-0/1% Thyme essential oil	4.75a	4.30d	3.50j	2.90l	2.40n
10% WPC- 0/3% Thyme essential oil	4.75a	4.40c	3.90f	3.00l	2.50mn
10%WPC-0/1% Thyme Nanocapsulate essential oil	4.75a	4.50bc	3.95ef	3.20k	2.70m
10%WPC-0/3% Thyme Nanocapsulate essential oil	4.75a	4.60b	4.00e	3.20k	3.70i
		Flavor			
Control	4.80a	3.65f	2.80j	2.15l	1.65n
10%WPC-0/1% Thyme essential oil	4.80a	4.00e	3.35i	2.35kl	1.85mn
10% WPC- 0/3% Thyme essential oil	4.80a	4.25cd	3.35i	2.50k	2.00l
10%WPC-0/1% Thyme Nanocapsulate essential oil	4.80a	4.40bc	3.55h	2.72k	2.20kl
10%WPC-0/3% Thyme Nanocapsulate essential oil	4.80a	4.40bc	3.60gh	3.61gh	3.35i
		Overall acceptability			
Control	4.65a	3.45ef	2.90g	2.31hi	1.80j
10%WPC-0/1% Thyme essential oil	4.65a	3.95d	3.35f	2.56h	2.15i
10% WPC- 0/3% Thyme essential oil	4.65a	4.10c	3.60ef	2.97g	2.45hi
10%WPC-0/1% Thyme Nanocapsulate essential oil	4.65a	4.35c	3.80e	2.99g	2.40hi
10%WPC-0/3% Thyme Nanocapsulate essential oil	4.65a	4.45bc	4.00d	3.90d	3.60ef