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Application of microliposome of ferula leaves extract on shelf life of beef burger during refrigerated storage

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ABSTRACT

Despite the development of methods to increase the shelf life and safety of food products, the economic loss caused by food spoilage is still considered one of the main challenges of food industry. Due to the culture of the use of natural products and functional foods, the consumer's desire for natural products with extended shelf life has increased. Phenolic compounds, like many bioactive compounds, gradually become inactive and usually cause a bitter taste in food. Microliposome is one of the effective solutions to increase the stability and reduce the unpleasant taste of bioactive compounds. In this research, the Ferula leaves was extracted with ethanol and its phenolic and flavonoid properties were determined. Then, the antioxidant properties of the extract were determined by DPPH and β-carotene/linoleic acid assay at different concentration (400, 800, 1600 and 3200 PPM). Then it was added to beef burger in the form of liposomes and the oxidative, microbial and sensory characteristics and the release rate of phenolic compounds in beef burger were investigated over two weeks' storage. The results showed that the total phenolic and flavonoid content of the Ferula leaves extract was 270.67 ± 5.8 mgGA/ g extract and 160.81±5.65 mg Quercetin/g extract, respectively. With the increase in the concentration of Ferula extract, the DPPH radical inhibition increases from 33.73 to 84.42% and β-carotene-linoleic acid from 32.56 to 74.90% at 400 to 3200 PPM. The results obtained on the shelf life of beef burger showed that the highest microbial growth and lipid oxidation were observed in the control, and the lowest one was observed in the sample containing 3200 PPM Ferula extract. Based on the oxidation test and sensory evaluation, adding microliposome of Ferula leaves extract at 1600 PPM can significantly increase the shelf life of beef burger in the refrigerator.

1. Introduction

Medicinal plants are an important part of the world's economic plants. These plants include many species and cultivars that contain biologically active compounds. The secondary metabolites produced by these plants, in addition to the pharmaceutical industry, can be used in other industries, including the food industry, in order to increase the nutritional value and enhance shelf life. Iran is located in a very suitable region in terms of geographical location. So that it is one of the biggest sources of plant diversity. Iran, with its large country and diverse climate, is one of the important centers of propagation and distribution of many plant species [1]. Therefore, the use of these valuable plants in the food industry is effective in health benefits. Ferula is one of the important wild medicinal plants in Iran. Due to indiscriminate and incorrect exploitations, many of the Ferula in Iran have been destroyed [2]. Considering that Ferula is native plant of Iran and parts of Afghanistan, it is rarely found in other parts of the world and few studies have been done on it. Ferula is monocarpic plant and after producing seeds, the plant dries up forever and dies [3]. 32 species of Ferula grow in different parts of Iran. Pharmacological studies show that this plant is a potential source of terpene derivatives, especially terpene coumarins [4]. In the phytochemical research of Ferula, various compounds such as monoterpene coumarins, terpene coumarins, phenylpropanoid derivatives and steroid compounds have been identified that have unique biological properties [5]. A variety of biological activities of Ferula extract have been reported by many researchers, which include anticancer, antioxidant, anti-inflammatory and antimicrobial effects [6-8].

Encapsulation is the technology of enclosing liquids, solids and gases in capsules to achieve a more controlled release. One of the types of lipid carriers for the microencapsulation of bioactive compounds are liposomes [9]. Liposomes are spherical or multilayered spherical vesicles composed of polar lipids, especially phospholipids, which create bilayer spherical structures in the presence of water molecules. This phospholipid can hold aqueous solutions. Liposomes have amphipathic properties and can contain a wide range of hydrophilic and lipophilic compounds. The use of liposomes for the controlled release of bioactive compounds can be effective in food quality and microbial growth controlling [10].

Animal protein plays an essential role in human nutrition, the lack of it causes disorders of growth, metabolism, reproduction, etc. due to the population increase and the need for animal protein consumption, meat production is one of the main goals of breeding in animal husbandry units [11]. Livestock sector has always been considered as one of the most important economic sectors of the society. This sector has a close relationship with the agricultural sector, the income of rural households and nutrition in urban and rural areas. Red meat is known as one of the most important food consumed by humans, which is considered as a valuable and nutritious food source due to its rich sources of protein, energy and vitamins, minerals and amino acids. In addition to the high nutritional value of red meat in Iran and the world, the industries related to the production, storage, packaging, processing and distribution of red meat play an important role in creating added value, employment and trade in the countries in the world [12]. Beef and its products, such as hamburgers, are among the food products that are prone to spoilage due to the physical and chemical conditions suitable for microorganisms, in this regard, the high amount of nutrients and high water activity. The addition of microliposome

containing Ferula extract has not been used to increase the shelf life of meat products. Therefore, in this article, an attempt is made to investigate the antioxidant effects of microliposome of Ferula extract in increasing the burger shelf life and preventing microbial and oxidative spoilage during the storage period.

2-Materials and methods

Preparation of ethanol extract of Ferula

Ferula is collected and dried in the shade and then powdered. After grinding, extraction with ethanol is done. After removing the solvent by rotary evaporator and freeze dryer, it is transferred to glass vials and kept in the freezer at -20ºC until the research is done [13].

Measurement of total phenolic and flavonoid content

The total phenolic content was measured by Folin-Ciocaltio method and the results were expressed in terms of mg gallic acid/g Ferula leaves extract. So, to the 2.5 ml of Ferula extract, 0.1 Folin-Ciocaltio reagent and 2 ml of Na_2Co_3 (7/5%) were added and the volume reached 50 ml by distilled water. After 12 to 15 h, the absorption was read at 765 nm [14]. The standard curve in terms of gallic acid was determined from the relation y = 0.008x + 0.23 with R^2= 0.98. The total flavonoid content was measured by aluminum chloride colorimetric method. In this method, 250 µL of Ferula extract (0.1%) was mixed with 75 µL of 5% $NaNO_2$. After 6 min, 150 µL of $AlCl_3$ (10%) and 500 µL of NaOH (1 M) were added to the mixture. At the end, the resulting mixture was homogenized with 2.5 ml of distilled water. The absorbance was read at 510 nm. The standard curve was in the range of 12 to 320 µg of quercetin/mL with the standard curve y=0.0048x and R^2=0.99. The flavonoid compounds were reported in mg quercetin/g extract [15].

Determination of Ferula leaves extract antioxidant activity by DPPH assay

Ferula extract (0.5 ml) in different concentrations was added to 2 ml of 6×10^{-5} M methanolic solution of DPPH free radical. After 30 min of storage in the dark under ambient temperature, the absorbance of the solution at 517 nm was read.

$$I(\%)=((A_{blank}-A_{sample})/A_{blank})\times100 \quad (1)$$

In this equation, I(%): DPPH radical scavenging activity, A_{blank}: absorbance of the control and A_{sample}: absorbance of the sample containing Ferula extract [16].

Determination of Ferula leaves extract antioxidant activity by β-carotene/ linoleic acid

assay

5 mg of β-carotene was dissolved in 10 ml of chloroform. Then, 600 µl of this solution was added to a volumetric flask containing 40 mg of linoleic acid and 400 mg of Tween 40. The dissolved chloroform was removed and 100 ml of distilled water saturated with oxygen was added to the flask. After that, the flask was vigorously stirred to form an emulsion. 2.5 ml of the prepared emulsion was added to the tubes containing 350 µL of different concentrations of the extract, and the absorption of the samples was read immediately at zero time at 470 nm. Then the lid of the tubes was closed and placed in a hot water bath at 50ºC for 120 min, and then the absorbance was read at 470 nm. The antioxidant activity of the samples was determined using the following equation:

$$I(\%)=[1-(A_{(sample\ 120)}-A_{(sample\ 0)})/(A_{(control\ 120)}-A_{(control\ 0)})]\times100 \quad (2)$$

In this equation, I(%):B-carotene/linoleic acid bleaching, A sample(120): absorbance of the sample containing Ferula extract after 120 min, A sample(0): absorbance of the sample containing Ferula extract at zero time, $A_{control\ (120)}$: The absorbance of the control sample after 120 min, $A_{control\ (0)}$: The absorbance of the control sample at zero time [17].

Preparation of microliposome containing Ferula leaves extract

In order to prepare microliposome, first, 2 g of lecithin and 2 g of Tween 80 were mixed in 38 g of distilled water and shaken for 5 h. In the next step, 4 g of Ferula leaves extract was added to the aqueous dispersion of lecithin and the whole mixture was sonicated for 600 s (1 second on and 1 second off) 40 kHz and the 40% of power. The produced microliposomes were stored in sterile bottles in dark conditions at refrigerator until use [18].

Preparation of hamburger treatments

Hamburger containing meat, soybean powder, breadcrumbs, onion, salt, vegetable oil and special spices was prepared. Ferula leaves extract was added to hamburger in microliposome at 400, 800, 1600 and 3200 PPM. A hamburger sample without extract was also used as a control. The samples were then packed in polyethylene bags and kept at 4ºC for 15 days. Oxidation, microbial and sensory analysis were performed on the samples.

Microbial analysis

Hamburger (10 g) was aseptically mixed with 45 ml of peptone water 0.9% for one min at room temperature. After that, the required dilutions were prepared and 1 ml of each dilution was placed in the PCA medium using the pour plate method. Total viable count (TVC) and Psychrotrophic bacteria count (PTC) is analyzed according to standard methods No. 5272 (Institute of Standards and Industrial Research of Iran, 2016) and standard methods No. 2629 (Institute of Standards and Industrial Industrial Research of Iran, 2012), respectively. In order to increase the accuracy of this test, 3 repetitions for each sample and 4 appropriate dilutions for each repetition were evaluated.

Determination of peroxide value (PV)

40 g of hamburger was mixed with chloroform (100 ml) and filtered with Whatman filter paper. The filtered solution (25 ml) placed in the oven at 37ºC until the chloroform evaporates completely. Then add acetic acid (37 ml) and saturated KI (1 ml) to the filtered solution (25 ml) and after one min add distilled water (30 ml) and starch (1 ml). The resulting solution was titrated with 0.01 N $Na_2S_2O_3$ until the color changed from yellow to white. The peroxide value was calculated in terms of meqO_2/kg of hamburger [19].

Determination of thiobarbituric acid (TBA)

To determine the thiobarbituric acid value, hamburger (0.2 g) was transferred to a 25 ml volumetric flask and then adjusted to volume with 1-butanol. Then, this solution (5 ml) was poured into a dry tube with a lid and TBA reagent (5 ml) was added to it. The tubes were placed in a water bath for 2 h at 95ºC and then cooled. The absorbance was read at 530 nm and TBA was determined and expressed as on mg malonaldehyde/kg hamburger [20].

Sensory evaluation

For sensory evaluation, the grilled hamburger was evaluated based on color, odor, texture and taste and overall acceptance by the panelists (15 people). Sensory evaluation was done with the 5-point hedonic method in the treatments immediately after grilling as (5: excellent, 4: very good, 3: good, 2: acceptable, 1: weak) [21].

Statistical analyses

Three experimental replicates were done. Data of four concentration levels (400, 800, 1600 and 3200 PPM) and during 15 days were expressed as the mean and standard deviation and analyzed by one-way ANOVA, followed by Duncan's multiple range test. Analyses were carried out to a significance level of $p < 0.05$, using the SAS software version 1.9 at the 5% level.

3- Results and discussion

In this research, the Ferula leaves extract was obtained with ethanol and its phenolic and flavonoid content were determined. Then, the antioxidant activity of the extract was determined by DPPH and β-carotene-linoleic acid methods at different concentration levels (400, 800, 1600 and 3200 PPM). Then it was added to the hamburger in the form of microliposomes and the oxidative, microbial and sensory properties and the release rate of phenolic compounds in the hamburger were investigated during 15 days.

The bioactive compounds of Ferula leaves extract

Phenols and flavonoids are valuable bioactive compounds in plant tissues. These compounds are only produced by plants and other oxygenic photosynthetic organisms and are necessary for the animal's food [22]. Flavonoids with the main structure of diphenylpropane with differences in the central pyran ring are widely distributed in the plant and constitute almost half of the 8000 identified phenols that can be effective in the diseases treatment [23]. The phenolic content of Ferula leaves is 270.67±5.8 mg gallic acid/g extract and its flavonoid content are 160.81±5.65 mg quercetin/ g extract (Table 1).

Table 1- Determination of the extraction yield, phenol and flavonoid content of the Ferula leaves extract

	Yield (%)	Phenolic content (mg Gallic acid/ g extract)	Flavonoid content (mg quercetin / g extract)
Ferula leaves extract	9.15	270.67±5.8	160.81±5.65

Antioxidant activity of Ferula leaves extract

The antioxidant activity of the extracts containing polyphenolic compounds is due to their ability to donate hydrogen atoms or electrons and free electrons. The antioxidant activity by DPPH method, is measured by the discoloration of purple 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. In this system, antioxidants react with DPPH free radical and make it pale or colorless by giving hydrogen or electron [24]. The color reduction is directly related to the antioxidant activity of the sample. The DPPH free radical inhibition in Ferula leaves extract is shown in Table 2. The results showed that with the increase in the extract concentration, the DPPH free

radical inhibition and antioxidant activity increase. So that, it reaches from 33.73 ± 4.05 at 400 PPM to 84.42 ± 4.2 at 3600 PPM. The significant antioxidant activity of Ferula extract is related to its high amount of phenols and flavonoids. Among phenolic compounds, flavonoids are considered stronger antioxidant compounds. Flavonoids are strong inhibitors of hydroxyl and peroxide radicals.

The β-carotene/linoleic acid method is based on the discoloration of β-carotene, the mechanism of which is the reaction of β-carotene with the free radical produced as a result of the hydroproxide formation from linoleic acid. The rate of β-carotene discoloration decreases in the presence of antioxidants. In the β-carotene/linoleic acid model, β-carotene quickly becomes colorless in the absence of antioxidants. The reason for this problem is the oxidation of β-carotene and linoleic acid and the formation of free radicals. The free radical of linoleic acid is created after the separation of the hydrogen atom by the unsaturated β-carotene molecules. Subsequently, β-carotene itself is oxidized and decomposed to some extent, and its orange color disappears, which can be evaluated by spectrometry [25]. The results of β-carotene test in different concentrations of Ferula leaves extract in Table 2 show that with increasing concentration of the extract, their inhibition also increases. In this test, 3200 PPM had the highest inhibition (90.74 ± 6.08) and statistically there is a significant difference between the lower concentrations (P≥0.05). Pabast et al. (2018) studied the effect of chitosan and savory essential oil in microliposome on increasing the shelf life of lamb meat during 20 days at refrigerator temperature. The results showed that microliposome effectively controlled microbial growth and chemical spoilage in meat, which they attributed to its antimicrobial and antioxidant activity [20].

Table 2- Antioxidant activity of Ferula leaves extract by DPPH scavenging and β-carotene/linoleic acid assay

Ferula extract	DPPH scavenging activity (%)	B-carotene/linoleic acid bleaching (%)
400 ppm	33.73±4.05^{d}	32.56±3.01^{d}
800 ppm	44.04±3.06^{c}	42.89±3.25^{c}
1600 ppm	74.43±2.02^{b}	63.62±2.07^{b}
3200 ppm	84.42±4.2^{a}	74.90±6.08^{a}

Different small letters in the same column represent significant difference ($p < .05$).

Quality evaluation of hamburger containing microliposome of Ferula extract

Oxidation

Peroxide value is one of the most widely used quality parameters of meat, which shows the total peroxides in meat fat as the primary oxidation products. The peroxide reduction after reaching its maximum during the initial stages of oxidation has been reported, which indicates the instability of peroxides and their breakdown into secondary products during the later stages of oxidation [14]. The sensitivity of meat to fat oxidation depends on various factors such as animal species, anatomical position of body muscles, storage time, packaging methods and addition of antioxidants. Plant extracts contain many antioxidant compounds that have the ability to control and inhibit oxidation. Changes in peroxide value extracted from hamburger during the storage are shown in Figure 1. The trend of changes in peroxide value in all the examined samples is increasing, and the control sample had the highest peroxide value after the storage period. Samples containing Ferula extract microliposome compared to the control sample showed significantly less oxidation, which is

probably due to the slow release of Ferula extract from microliposome, whose antioxidant activity prevents oxidation. On the last day of the storage period, the peroxide value for the samples containing 3200 PPM of Ferula extract was 2.81±0.05 meqO_2/kg, which was significantly lower than control sample 3.9±0.04 meqO_2/kg. Yekta et al. (2020) investigated the antimicrobial and antioxidant properties of hamburger with quinoa peptide nanoliposomes during 12 days of refrigeration. Among the evaluated treatments, the lowest thiobarbituric acid value, peroxide and volatile nitrogenous bases and microbial growth, staphylococcus, mold and yeast were observed for the treatment containing 5 mg/ml peptide. Therefore, nanoliposome containing quinoa peptide was effective in increasing the hamburger shelf life [26].

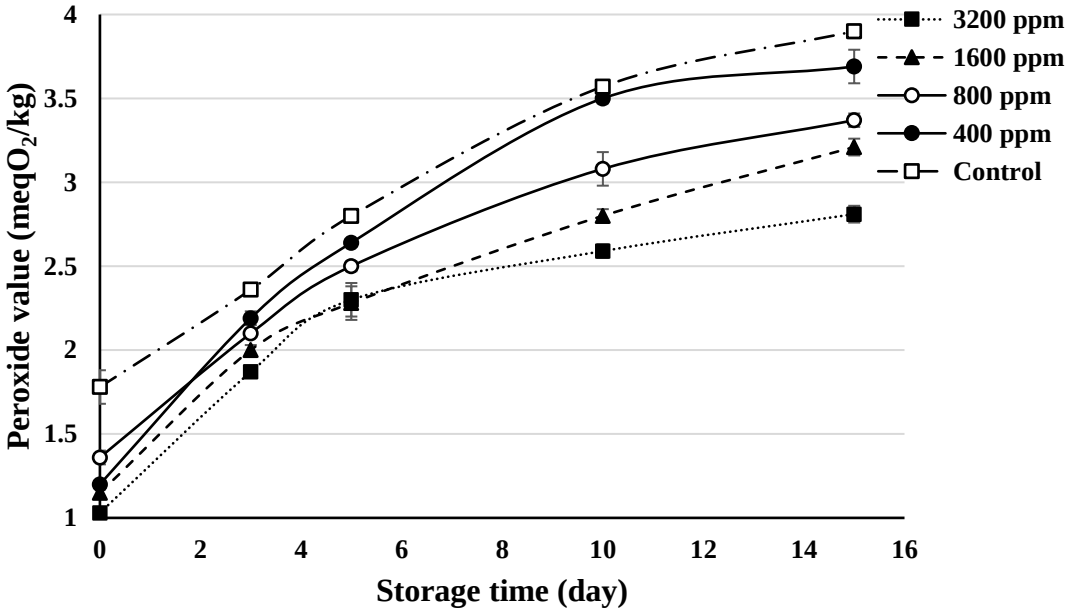

Figure 1- Changes in peroxide value of beef burger containing Ferula extract microliposome during refrigerated storage

The secondary oxidation products in meat is determined by thiobarbituric acid value. Hydroperoxides are primary products of lipid oxidation, which turn into carbonyls (aldehydes and ketones) during decomposition, which are the main secondary compounds. These compounds are more stable than hydroperoxides [27]. The results related to the changes in thiobarbituric acid value of different hamburger samples containing microliposome of Ferula extract are shown in Figure 2. It can be seen that the trend of changes in thiobarbituric acid value in all the investigated samples is increasing and as the time passed, significant changes have been shown. Also, the control had the highest thiobarbituric acid (1.0 ± 23.03 mg malondialdehyde/kg). After that, the sample containing 3200 PPM extract of Ferula extract (0.66±0.02 mg/kg), 1600 PPM (0.079±0.0 mg/kg), 800 PPM (0.83±0.04 mg/kg) 0.0 mg/kg) and 400 PPM (0.0±89.03 mg/kg). The reduction of thiobarbituric acid value compared to the control sample is related to the antioxidant effects of phenolic and flavonoid compounds of Ferula extract, which was determined in DPPH and β-carotene/linoleic acid free radical inhibition assays. Qotouri et al. (1402) studied the effect of nanoliposomes of fish oil on the technological properties and nutritional quality of hamburger with reduced fat during storage at refrigerator (4ºC). For this

purpose, the physicochemical properties of nanoliposome containing fish oil (5 and 10%) were investigated. The pH, protein, fat, ash of raw sample, water holding capacity, moisture, and cooking efficiency were calculated. The results showed that the use of nanoliposomes in hamburger has increased the water holding capacity and cooking efficiency. Also, after the cooking process, it improved the texture and color. The sensory evaluation showed that the nanoliposomes containing fish oil in the hamburger had the highest score of texture, taste, smell, color and overall acceptance [28]. Also, Islamian Amiri et al. (2021) investigated chitosan-chia seed gum enriched with microliposomes of Bay laurel essential oil to increase the shelf life of quail fillets. The results showed the microcoating of bay laurel essential oil was significantly effective on the shelf life of quail fillets compared to the control sample during 16 days of storage in the refrigerator [29].

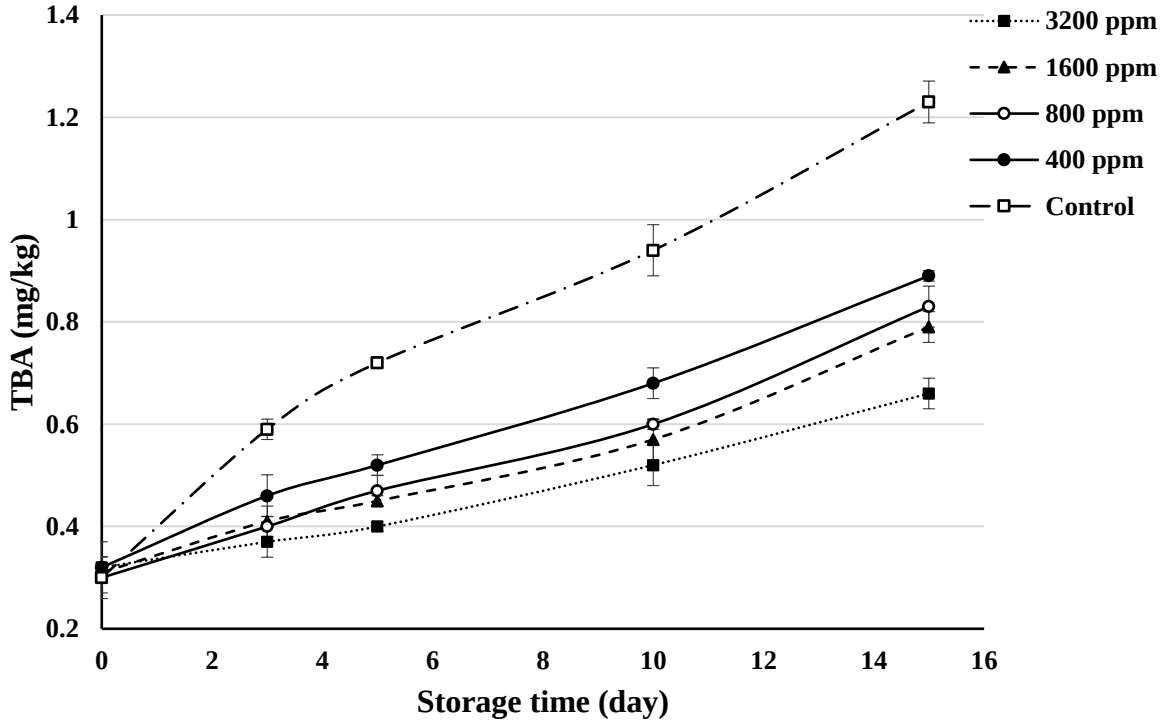

Figure 2- Changes in thiobarbituric acid value of beef burger containing ferula extract microliposome during refrigerated storage

Release of phenolic compounds during the storage period

The effect of polyphenols in solvents is often higher than in the food matrix. In addition, polyphenols show limited stability and solubility and unpleasant flavors, which must be modified before adding to food products [30]. Also, phenolic compounds can be connected with other food components such as proteins, which causes a decrease in its performance. For example, polyphenol compounds of green tea in food products usually do not maintain their antioxidant function [31]. In addition, the sensitivity of catechins to different pH during storage, as well as digestion in the gastrointestinal tract and low availability, have limited its use [32]. Therefore, microliposome with controlled release can not only increase the shelf life and nutritional value of extracts, but also prevent unpleasant taste in food [33]. The results in Figure 3 show that the release of phenolic compounds increases with increasing storage time. The stability of

phenolic compounds has decreased during storage time, and the release of Ferula extract over time is done, because the microliposome wall is destroyed due to the oxidation and permeability of the liposome wall membrane. Also, the increase in the fluidity of the lipid membrane due to storage causes an increase in the leakage of phenolic compounds out [34]. Aala et al. (1401) investigated the free and nanoliposome extracts of Laurus nobilis and rosemary leaves extracts in silver carp fish at refrigerator. The results showed that phenolic and flavonoid compounds in rosemary extract was more favorable than Laurus nobilis extract. Addition of 1.5% extract of Laurus nobilis and rosemary preserves the quality of silver carp fish [35].

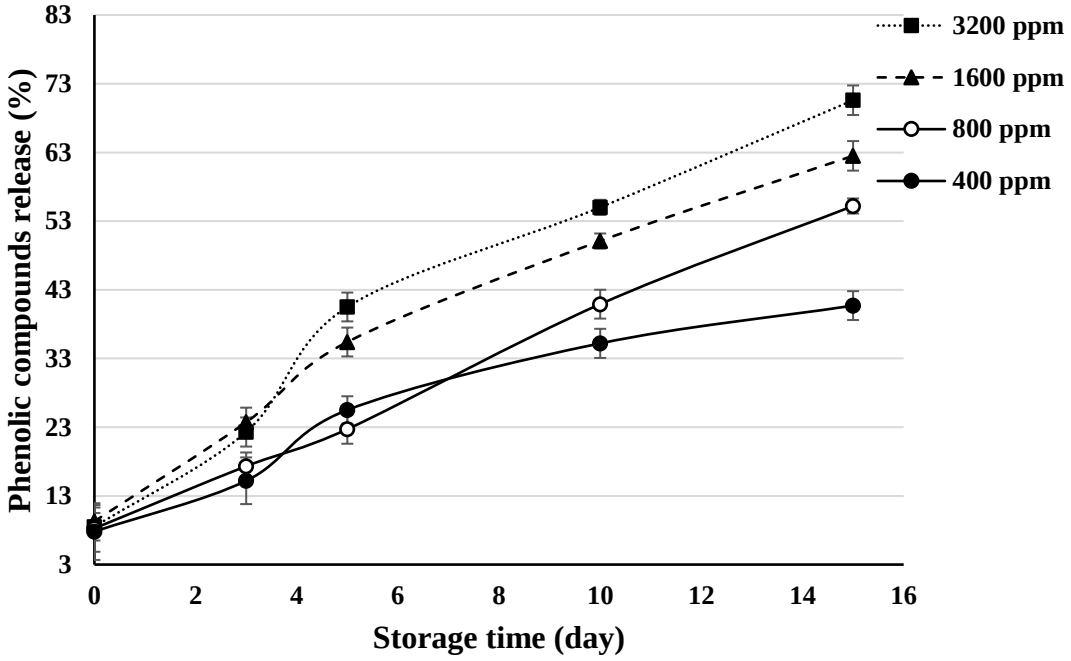

Figure 3- Phenolic compounds release from microliposomes containing Ferula extract in beef burger during refrigerated storage

Microbial changes

Food spoilage is one of the main problems that limits their shelflife. Due to the high protein and fat, hamburger is very susceptible to spoilage and has a short shelf life. The use of chemical preservatives is one of the important ways to prevent the pathogenic activity and spoilage microorganisms. However, these preservatives have carcinogenic and mutagenic effects, and their residues also have toxic effects. Therefore, the consumption is limited. The use of natural antimicrobial compounds increases its shelf life. Extracts and essential oils are generally known to be safe and have significant effects on spoilage and pathogenic microorganisms, thereby increasing the shelf life of food products [36]. The results related to the changes in the total bacteria count during the storage period are shown in Figure 4. It can be seen that the highest bacteria count is shown in the control sample. In all treatments, until the 15th day, the bacteria count increased and this increase in the sample containing 3200 PPM of Ferula extract (6.5 ± 0.13 log CFU/g) compared to the control sample (8.7 ± 0.15 log CFU/g) was lower after the storage period.

Plant extracts are compounds with antioxidant and antimicrobial properties against a wide range of microorganisms. These natural compounds have several

antimicrobial mechanisms. Based on the studies, it has been determined that the extracts that have strong antimicrobial activity against food pathogens contain high phenolic content [37]. The results are in agreement to other studies [38, 39]. Shahbazi et al. (2016) researched the effect of using Ziziphora clinopodioides essential oil, nisin and their combination on the microbial properties of raw beef patty. Based on the results, with the increase of storage time, the total count of microorganisms increased, and the increase in the essential oil and nisin concentration caused a decrease in the microbial count compared to the control sample. Also, the use of a mixture of nisin and Ziziphora clinopodioides more effectively prevented the growth of microorganisms. Also, the Nano encapsulation technique is effective in increasing the antibacterial activity of bioactive compounds [40]. In the research of phycocyanin pigment extracted from Spirulina platensis algae was encapsulated with sodium maltodextrin caseinate, this result was confirmed [41].

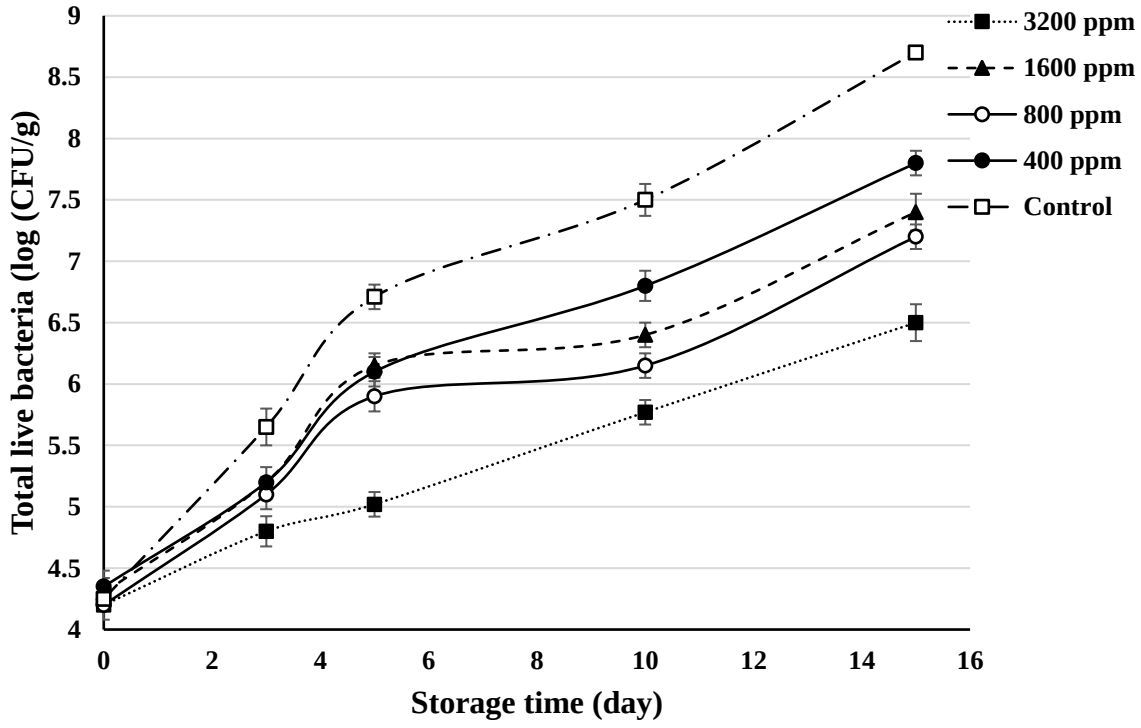

Figure 4- Total viable count of beef burger containing ferula extract microliposome during refrigerated storage

The results of the changes in the psychrotrophic bacteria count during the storage time are shown in Figure 5. As can be seen, psychrotrophic bacteria count at the beginning and end of the storage period has significant difference. The reason for the decrease in the bacteria count during the storage period is related to the antibacterial effects of Ferula extract and its controlled release from the microliposome. Tometri et al. (2020) extracted and microencapsulated Laurus nobilis leaf extracte and its effect on oxidative, microbial and sensory properties of hamburger at refrigerator for 16 days. The results showed that the most phenolic and flavonoid compounds, antioxidant and antimicrobial properties were observed in the hydroalcoholic extract. To evaluate the shelf life of hamburger, different concentration (1000 ppm extract, 1000 ppm microliposome and 1500 ppm

microliposome) were evaluated. The results showed that microencapsulated extract at 1500 ppm has the least microbial spoilage and oxidation [14]. Also, Homayounfar et al. (2021) used nanoencapsulated garlic essential oil to increase the shelf life of hamburgers at refrigerator. The results of the physical, microbial and sensory evaluation of the hamburger showed that the extract in the nanoliposome was able increase hamburger shelf life [21]. Emami et al. (1401) also investigated the effect of free and encapsulated of cinnamon on *Listeria monocytogenes* and *Escherichia coli* inoculated into ground beef. The results showed that although the antibacterial activity of cinnamon was more than those in nanoliposomes, both cinnamon nanoliposomes showed high antibacterial activity against *Escherichia coli* and *Listeria monocytogenes* bacteria. In hamburger showed that nanoliposome containing cinnamon extract was used to increase the shelf life of hamburgers without causing adverse effects and low oxidation and microbial spoilage [36].

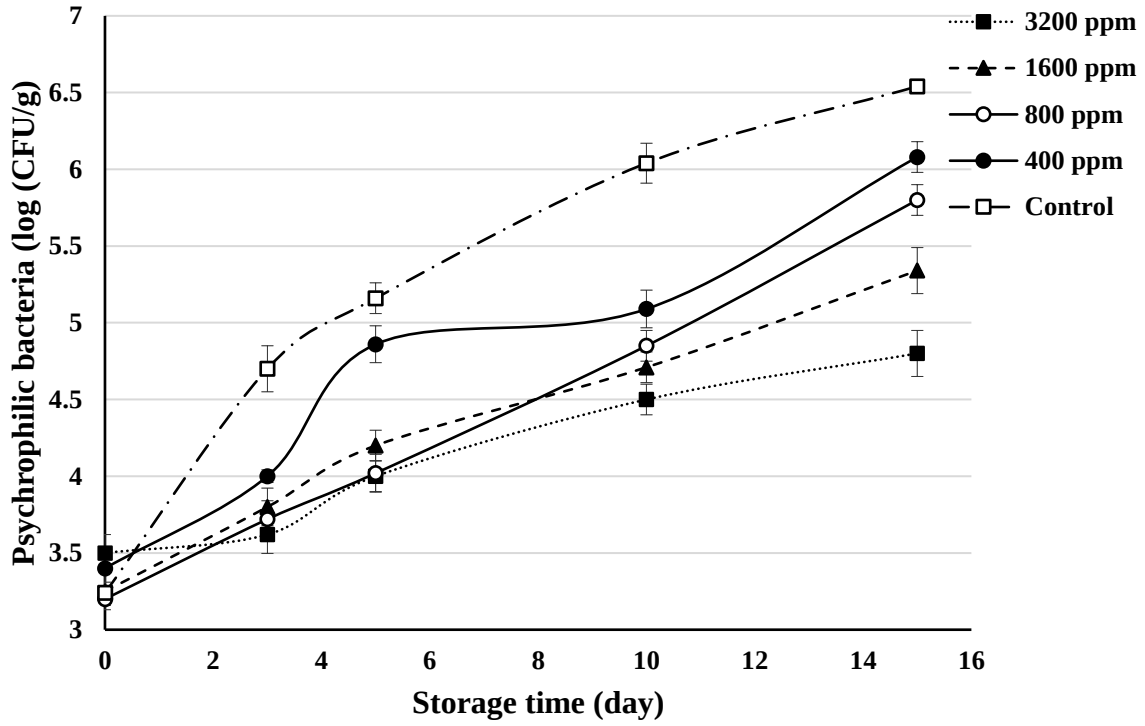

Figure 5- Psychrotrophic bacteria count of beef burger containing ferula extract microliposome during refrigerated storage

Sensory evaluation results

One of the important sensory changes of hamburger is unpleasant changes in color, odor, texture and taste, which is due to microbial growth and oxidation [20]. The results of the sensory evaluation immediately after cooking are shown in Table 3. The results of the hamburger containing 3200 PPM Ferula extract got a lower score due to the spicy taste. But the odor, color, texture and taste are favorable in the samples containing 1600 and 800 PPM. Also, Rather et al. (2016) investigated the effect of guar gum as a fat substitute on the quality characteristics mutton goshtaba (a traditional Indian product) and reported the samples containing 0.5% gum and the control sample showed no significant difference in overall acceptance [42]. The use of Ferula extract in the microliposome can be a suitable solution for the production of a new, useful and desirable product that is effective in improving the nutritional

quality due to its significant antioxidant properties and increasing its shelf life.

Table 3 - Sensory evaluation of minced beef containing ferula extract microliposome

	Control	Ferula extract (ppm) in microliposome			
		400	800	1600	3200
Color	10.0±0.0[a]	10.0±0.1[a]	9.5±0.3[b]	9.5±0.0[b]	9.0±0.0[c]
Odor	10.0±0.2[a]	9.9±0.2[a]	9.0±0.1[b]	8.8±0.3[b]	8.5±0.2[c]
Taste	9.7±0.3[a]	9.6 ±0.2[a]	9.7±0.2[a]	9.0±0.1[b]	7.8±0.2[c]
Texture	9.8 ±0.3[a]	9.9±0.1[a]	9.8±0.0[a]	9.7±0.1[a]	9.6±0.4[a]
Overall acceptance	9.7 ±0.3[a]	9.7±0.2[a]	9.5±0.2[a]	9.3 ±0.3[a]	8.6 ±0.1[b]

Different small letters in the same column represent significant difference ($p < .05$).

4- Conclusion

The purpose of this research was to evaluate microliposome containing Ferula leaves extract on quality characteristics of hamburger during 15 days at refrigerator. The results showed that the oxidative stability of hamburger during 15 days in the presence of microliposome containing Ferula extract was significantly higher than the control sample, which indicates the protective role of the microliposome in the controlled release of phenolic compounds. The antimicrobial properties of Ferula extract were also significant at different concentration in the microliposome. The sensory evaluation results showed that the overall acceptance of microliposome containing 800 and 1600 PPM of Ferula extract is not different with the control sample and the microliposome was able to cover the flavor of the extract.

5- References

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کاربرد میکرولیپوزوم حاوی عصاره برگ فرولا بر ماندگاری همبرگر طی دوره نگهداری در یخچال

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چکیده

با وجود توسعه روش‌های افزایش ماندگاری و ایمنی فرآورده‌های غذایی، همچنان زیان اقتصادی ناشی از فساد مواد غذایی از چالش‌های اصلی محسوب می‌شود. با توجه به فرهنگ سازی استفاده از مواد طبیعی و غذاهای فراسودمند، تمایل مصرف‌کننده به مواد غذایی طبیعی با ماندگاری بالا افزایش یافته است. ترکیبات فنلی همچون بسیاری از ترکیبات زیست فعال به تدریج غیر فعال می‌گردد و معمولا پس طعم تلخی را در مواد غذایی ایجاد می‌کنند. میکرولیپوزوم یکی از راهکارهای موثر برای افزایش پایداری و کاهش طعم نامطلوب ترکیبات بیواکتیو محسوب می‌شود. در این پژوهش، عصاره برگ فرولا با اتانول استخراج شد و خصوصیات فنلی و فلاونوئیدی آن تعیین شد. سپس خصوصیات آنتی اکسیدانی عصاره به روش DPPH و بتا کاروتن-اسید لینولئیک در سطوح غلظتی مختلف (٤۰۰، ۸۰۰، ۱٦۰۰ و ۳۲۰۰ PPM) تعیین شد. سپس، به صورت لیپوزوم به همبرگر اضافه شد و خصوصیات اکسایشی، میکروبی و حسی و میزان رهایش ترکیبات فنلی در همبرگر طی دوره ۱۵ روز مورد بررسی قرار گرفت. نتایج حاصل نشان داد که محتوی فنل و فلاونوئید کل عصاره برگ فرولا به ترتیب ۵/۸ ± ۲۷۰/٦۷ میلی‌گرم اسید گالیک بر گرم عصاره و ۵/٦۵ ±۱٦۰/۸۱ میلی‌گرم کوئرستین در هر گرم از عصاره بود. با افزایش غلظت عصاره فرولا، میزان مهار رادیکال آزاد DPPH از ۳۳/۷۳ به ۸٤/٤۲ درصد و بتا کاروتن- اسید لینولئیک از ۳۲/۵٦ به ۷٤/۹۰ درصد از غلظت ٤۰۰ تا ۳۲۰۰ PPM افزایش می‌یابد. نتایج بدست آمده بر روی ماندگاری همبرگر نشان داد بیشترین رشد میکروبی و اکسایش لیپیدی در نمونه شاهد مشاهده شد و کمترین مقدار رشد در تیمار حاوی عصاره ۳۲۰۰ PPM عصاره فرولا دیده شد. براساس آزمون اکسایشی و حسی، در صورت افزودن میکرولیپوزوم عصاره برگ فرولا در غلظت ۱٦۰۰ PPM می‌توان زمان ماندگاری همبرگر را به طور قابل توجهی در یخچال افزایش داد.