Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Feasibility of producing a functional energy drink containing tannin-linoleic acid conjugated chitosome

Morteza Jamshid Eini[1], Hamid Tavakolipour[*2], Rezvan Mosavi Nadoshan[3], Mohsen Mokhtarian[4]

1-Department of Food Science and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran.

2 -Department of Food Science and Technology, North Tehran Branch, Islamic Azad University, Tehran, Iran.

3 -Department of Food Science and Technology, Faculty of Biological Science, North Tehran Branch, Islamic Azad University, Tehran, Iran.

4 -Department of Food Science and Technology, Roudehen Branch, Islamic Azad University, Roudehen, Iran.

ARTICLE INFO

Article History:
Received:2024/5/25
Accepted:2024/9/30

Keywords:

conjugated linoleic acid,
tannin,
chitosome,
pomegranate peel extract,
sports drink

DOI: **10.22034/FSCT.22.158.99.**

*Corresponding Author E-tavakolipour@gmail.com

ABSTRACT

Nowadays, due to the interest in consuming extra-beneficial foods and the increasing spread of cardiovascular diseases, the desire to consume extra-beneficial products has increased. Therefore, researchers are looking for the optimization of new formulations of products with practical features in this direction. The study aimed to produce a functional energy drink containing tannin-linoleic acid-conjugated chitosome. After extracting bioactive compounds (especially tannin) from pomegranate peel, they were included in the structure of chitosomes along with conjugated linoleic acid. The results showed the significant effect of different concentrations of pomegranate biological extract on the oxidative stability of linoleic acid. The results showed the significant effect of different concentrations of pomegranate biological extract on the oxidative stability of linoleic acid. The examination of nano-chitosome groups showed the presence of structures in pomegranate bioactive along with linoleic acid in chitosomes. The uniformly chitosan spherical particles were observed with sizes of 77.66 and 79.90 nm. The addition of nano chitosomes showed a significant increase in pH (decrease in acidity), increase in turbidity, viscosity, two phases, and phenolic content. Also, a decrease in the L* value and an increase in a* and b* values were reported. The present results showed that biopolymers play a key role in the stability of the liposome membrane structure. They have a stable release of molecules trapped by a spatial barrier on the surface. This nano chitosome will provide a potential platform for the carrier's design for nutrients or preservatives, to increase the shelf life and safety of food matrices.

[DOI: 10.22034/FSCT.22.158.99]

1-Introduction

Today, researchers have found that foods, in addition to providing essential nutrients, contain components that may be useful in maintaining health and preventing chronic diseases. The components in animal products are each associated with a specific effect on human health, which is why the interest in conjugated acids has increased significantly [1]. CLA is a dietary supplement used to reduce body mass, muscle damage, and inflammatory responses. Therefore, it has attracted considerable scientific attention in the past few decades. Linoleic acid is a family of polyunsaturated fatty acids, which refers to a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds, and it can be placed in any position of the carbon chain - usually between 8 and 13 - in a cis or trans configuration [2]. The most common isomers are cis-9, trans-11 (c9, t11) and trans-10, cis-12 (t10, c12), which are naturally present in dairy products (milk, cheese and yogurt) and ruminant meat (beef and lamb) [3]. Studies have shown that linoleic acid, in addition to being used for the treatment of cardiovascular diseases, metabolic syndrome and asthma, can have various health-giving effects such as anti-cancer properties, inhibiting arteriosclerosis, increasing the function of the immune system, liver metabolism and blood sugar. But the most reliable findings about the health-giving effects of linoleic acid include anti-cancer and reducing the effects of body fat [4]. More recent research studies show that both isomers of linoleic acid have very different health effects. t10, c12-CLA is an anti-cancer, anti-obesity and anti-diabetic agent, while c9, t11-CLA is mainly anti-inflammatory. It is also possible that some of the effects are due to the synergistic action of these isomers [1].

Punica granatum is a native of Iran to the Himalayas in northern India, which is cultivated in the climate and climate of semi-arid desert, dry, semi-arid, semi-humid and cold climates in western and humid regions. In the central regions of the country, there are more than 300 species in the form of trees and shrubs. This plant is also found naturally in very hot areas where rainfall occurs only in winter and dry summers. Nowadays, distribution of pomegranate tree in Central Asia, Mediterranean areas, Southeast Asia and North America has been reported [5]. In Iran, 670,000 tons [6] and approximately 218,000 tons are cultivated in the world. After proving the antimicrobial and antiviral properties of pomegranate, its cultivation has increased significantly [5]. Pomegranate skin makes up 40% of the whole fruit. Pomegranate skin is rich in polyphenols. Since polyphenols are antioxidant agents, they can show the medicinal ability of pomegranate. Many researchers have confirmed that pomegranate skin is a rich source of bioactive compounds such as ellagitannins, catechins, rutin and epicatechins [7]. Also, studies have shown that pomegranate skin is rich in hydrolysable tannins in ellagitannins. It has been shown that the content of tannin in pomegranate peel is higher than the content of flavonoids (mainly anthocyanins) [5]. The tannins in pomegranate are mainly condensed tannins and hydrolysable tannins. Hydrolysable tannins make up approximately 85% of tannins in pomegranate peel [8]. Many biological activities of tannins, including antioxidant, antibacterial, antifungal, antitumor, etc., have also been shown. Ortho-dihydroxyl groups present in tannin are responsible for chelating metal ions. High degree of polymerization and molecular weight play an important role in the antioxidant activity of tannins. When tannins are consumed as part of human or animal diet, tannins may affect protein utilization by forming complexes with protein and iron. They can form strong bonds with proteins by forming hydrogen bonds between phenolic groups and -NH groups of peptides that are not broken by digestive enzymes [9].

[DOI: 10.22034/FSCT.22.158.99]

Liposomes are spherical particles (vesicles) with one or more double-layered membranes, with amphipathic (amphipathic) properties, which are formed by the accumulation of fat or phospholipid molecules (especially phosphatidylcholine) in aqueous medium. Despite the many advantages of liposomes (especially the possibility of producing healthy and harmless compounds for human health), only a few unmodified liposomes are stable. Most of them have a high tendency to aggregate, which over time may be trapped by leakage of contents [10]. To overcome these defects and for more stability, modification of the surface of liposomal carriers using biopolymers (such as pectin or chitosan) has been done to design more effective delivery systems. By reducing the reaction with the external environment, encapsulation protects polyphenols against degradation, adverse effects of light, moisture and oxygen. As a result, it helps to increase their useful life. In addition, encapsulation will result in reduced core evaporation, controlled core release, core dilution (in cases where only small amounts of phenolic compounds are required) and increased half-life [10, 11]. Ramli et al. (2019) showed an increase in the encapsulation efficiency of propolis extract with chitosome and the stability of chitosome after one month of storage [12]. Silva et al. [13] also showed that the total extraction efficiency increased from 35 to 48.1% with increasing chitosan concentration (0.4 to 1%).

The Food and Drug Administration defines energy drinks as a category of non-alcoholic beverages, usually containing caffeine, or without other additives. These drinks usually contain large amounts of caffeine, added sugars, other additives and legal stimulants such as guarana, taurine and L-carnitine [14]. In addition, low-alcohol beverages also have potential health benefits. Benefits such as lowering cholesterol and increasing high-density lipoprotein, which helps prevent heart disease and reduce the risk of alcohol-related diseases [15]. Therefore, this study describes the possibility of producing a healthy and useful energy drink. Linoleic acid-tannin chitosomes have the potential to improve product quality by increasing the enrichment of bioactive compounds.

2- Materials and methods

2-1 Materials: fresh pomegranate fruit from a garden in Saveh in Markazi province. Iran (in the north of Central province between latitude (34 degrees and 45 minutes latitude) Wonderful, canola oil from Laden company (Iran), CLA from HSF company (China) And the chemicals needed for the research were purchased from Merck (Germany).

2-2 Extraction of bioactive compounds (especially tannin) from pomegranate skin: First, the outer leathery skin and other biological wastes (mesocarp and dividing membrane) were removed from the seeds (aryls). Then fresh pomegranate biological waste was dehydrated at 65°C and dried in a freeze drier (Operon-Korea) (55°C, 0.15 mm Hg) for 48 hours. until its initial humidity decreased from 69.93 ± 0.5% in moisture basis to a safe moisture level of approximately 4.76 in moisture basis [16]. After the drying stage, the final product was pulverized to a size of 1 mm using a laboratory mill (LB20ES, Waring, USA). The produced powder was sieved through a stainless-steel mesh sieve. Particles with about 1 mm were selected [17]. To extract tannin from pomegranate skin, water solvent and ultrasonic device (ZE 1031 DT, Heinrichstrabe, Germany) were used. The sample to solvent ratio was 1 to 20, and the temperature for the ultrasound method was set to 45°C. About 3 grams of skin powder was added in separate 125 ml bottles of reagent and 60 ml of solvent. The reagent bottles were placed in the sonicator bath for 1 hour. Filtration of the samples was done with the help of Whatman No. 41 filter paper and centrifugation (HB320, Brea, USA) was performed for about 10-12 minutes at 5000 rpm. The solvent was evaporated under a vacuum of 140 mbar and a temperature of 45 °C

[DOI: 10.22034/FSCT.22.158.99]

until drying. The extracts were collected in amber colored glass bottles and refrigerated at 4-6°C [18].

2-3 Drying of the extracted bioactive compounds: The isolated bioactive compounds of pomegranate peel (especially tannin) were mixed in 250 ml of 70% ethanol by immersion method. Then the resulting mixture was kept at 37°C for 4 days and was shaken manually. In the next step, the ethanol extract was passed through Whatman No. 4 filter paper. The ethanol in the extract was removed by a rotary evaporator (B-480, Flawil, Swiss) under vacuum at a temperature of 35 °C. For the additional drying process of the concentrated extract, first the concentrated extract was mixed with 75% maltodextrin. Then it was dried by a freeze dryer (VACO5, ZIRBUS, Germany) at a temperature of 40 °C and a pressure of 0.1 MPa. Then, the dried extract was sealed in a dark colored glass container and stored at -20°C until the next experiments [19].

2-4 Production of chitosome microcapsules containing bioactive cores (tannin - CLA): with the aim of preventing the oxidation of linoleic acid, different amounts of dried extract (0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 mg/ml) was added to CLA. 10% of linoleic acid was added to canola oil without antioxidants. The rate of oxidation of CLA (by the method of accelerated shelf-life test or (ALST) in a fixed period of time (60 minutes)) was monitored through the peroxide index, until the optimal amount (That is, the concentration that has the highest level of inhibition of linoleic acid oxidation) natural antioxidants of the dried biological extract was determined [19]. Finally, chitosomes were produced using thermal method. For this purpose, powdered soybean lecithin was first dissolved in ethanol solvent (1% by weight/volume). In the next step, in the rotary evaporator under vacuum, the solvent was removed until a thin layer (formation of multi-layer lipid liposome vesicle) was created at the bottom of the balloon. Then the balloon was placed in a desiccator to separate the remaining solvent. In edema, using a magnetic heater (MS300H, Glassco, Netherlands), the lipid layer formed in phosphate buffer containing bioactive compounds was dissolved. Ultrasound treatment was performed at 90% range for 10 minutes [20].

2-5 Preparation of a useful energy drink: First, the components of the basic formulation were prepared. The components used in the production of the drink include the following: sodium citrate (0.045%), potassium sorbate (0.003%), sodium benzoate (0.005%), Tutti frutti flavoring (0.02%), Natural caramel color (0.02 percent), vitamin B1 (0.002 percent), vitamin B2 (0.014 percent), vitamin B5 (0.0002 percent), vitamin B6 (0.0004 percent), sucrose (7.7 percent) 8 percent), citric acid (0.55 percent), maltodextrin (0.06 percent), gum Arabic (0.05 percent), taurine (0.037 percent), glucuronolactone (0.025 percent), caffeine (0.03 0.0%), the rest of the formulation is made up of water. In order to produce a drink containing chitosome microcapsules, 75.5 grams of chitosome was added to the volume of 250 ml of the base formulation [21].

2-6 Chitosome tests

2-6-1 Determination of peroxide index: The amount of 5 grams of each sample of linoleic acid along with the dried biological extract was completely mixed in a 5 ml beaker with 0.2 grams of heptadecanoic acid - which was added as an internal standard. 20 mg of each mixture sample was oxidized in the vicinity of air under continuous stirring at 50 degrees Celsius in 0, 12, 14, 16, 18, 20, 22, 28, 35, 44 and 48 hours, respectively. To determine the peroxide of the samples, 5 grams of the prepared sample was weighed in a 250 ml Erlenmeyer flask and 30 ml of solvent (a mixture of acetic acid and chloroform) was added to it. Then about 0.5 ml of potassium iodide was added to it. The mixture was allowed to stand for one minute and stirred occasionally. Then about 30 ml of distilled water was added to it and the mixture was allowed to stand still for one minute and a

[DOI: 10.22034/FSCT.22.158.99]

few drops of starch glue were added to the solution and titrated with 0.1 normal thiosulfate solution. When the color of the sample reached a transparent state, the titration was stopped and the peroxide number was calculated in terms of milliequivalents per kilogram through formula 1 [22]:

Proxied Value = (100*N*V2)/V1

where: N: Normality of thiosulfate/ V1: Consumption titration volume/ V2: Sample volume

2-6-2-Analysis test of functional groups (bond formation) with FTIR: Functional groups were determined by FTIR spectroscopy at a wavelength of 400-4000 cm^{-1} using an FTIR device (IRSprit, Shimadzu, Japan). In order to investigate the creation of electrostatic interactions between chitosan and liposome, as well as to evaluate the encapsulation of bioactive compounds, this device - that is, FTIR infrared Fourier transform spectroscopy - was used [20].

2-6-3 Morphology characteristics of the produced chitosome: The microscopic structure of the optimally produced microcapsule, its monomorphism or polymorphism, and particle size were investigated using a field emission scanning electron microscope (EVO 15, ZEISS. Germany) at 15 kW. For this purpose, a drop of the prepared sample was poured on a laboratory slide and dried for 1 hour at ambient temperature, and then the sample was covered with a gold layer using an ion sputtering device to be observed under an electron microscope. These steps were performed for uncoated AFM testing. For this purpose, a drop of the prepared sample was poured on a laboratory slide and dried for 1 hour at ambient temperature, and then the sample was covered with a gold layer using an ion sputtering device to be observed under an electron microscope. These steps were performed for uncoated AFM testing. For TEM test, a drop of the diluted sample (5 times with 7.4 phosphate buffer) was placed on a copper grid containing a protective film. The sample was stained with phosphotungstic acid for 5 minutes. Then the excess liquid was removed using filter paper and after drying the mesh, the sample was examined by TEM [20].

2-7- Energy drink tests

2-7-1 pH and acidity test: The pH of the samples was tested with the help of a pH meter at ambient temperature and acidity by titration method with 0.1 normal sodium hydroxide until the appearance of a stable pale pink color [23].

2-7-2 Turbidity test: To evaluate the turbidity, first, in order to equalize the concentration of the extract, the dry substance concentration of the final extract was diluted to the level of (4.3 mg/l). The amount of turbidity change after 24 hours was measured by spectrophotometric method (SpectroDirect, Lovibond, Germany) by measuring the amount of light absorption at the wavelength of 800 nm [20].

2-7-3 Color test: The color indices of the energy efficient samples were determined using a colorimeter (Hunter Lab, Colorflex EZ, USA) and indices like L*(Transparency-darkness index), a* (redness-greenness index) and b* (yellow-blue index) [22].

2-7-4 Viscosity test: The rheological properties of the useful energy drink were investigated using a capillary flow viscometer (YR-1, Brookfield, USA) at a temperature of 5 degrees Celsius. The time required for a standard volume of fluid to pass through a capillary tube of known length is measured. The viscosity of drinks was determined in the temperature range of 30 to 80 degrees Celsius with temperature intervals of 10 degrees Celsius. The reference fluid used in this study was distilled water [20].

2-7-5 Bypassing test: To check the degree of bypassing, the samples of energy drinks were poured into 50 ml flasks. And they were kept at a temperature of 4 °C for 30 days. In order to calculate the amount of bypassing of the samples, the amount of the upper phase was measured by a ruler and divided by the total

[DOI: 10.22034/FSCT.22.158.99]

amount of useful energy drink contained in the tube and multiplied by 100 [24].

2-7-6 Total phenol test: First, a standard solution of dried biological extract (concentration 10 mg/g) was prepared in methanol (purity 96%) at room temperature. Then, a 10 ml Falcon tube was used to mix 0.75 ml of the dried biological extract solution with 0.75 ml of Folin-Ciocalteu reagent. The resulting mixture was stirred in a 25 °C water bath (including a reciprocating shaker at 250 rpm). For 30 seconds after resting for 3 minutes, 0.75 ml of sodium carbonate (Na_2CO_3 with 20% concentration) was added to each sample and its volume was adjusted to 10 ml by adding distilled water. In the next step, each tube of the sample was stirred again at the same time and kept in a dark warehouse at 25°C for 1 hour. Then, the absorbance of the test sample and the resulting white (contains 0.75 ml of distilled water, 0.75 ml of Folin-Ciocalteu reagent and 0.75 ml of 20% Na_2CO_3) was measured by a spectrophotometer (Spectro Direct, Lovibond, Germany) at 725 nm. The total phenol of each sample was obtained from the standard curve of gallic acid (drawn from 25, 50 to 100 µg/g) and expressed as mg GAE (gallic acid equivalent)/g dry matter of the dried biological extract [25].

2-8 Statistical analysis: All data analysis of this research was done using SAS software version 9/4. Also, one-way ANOVA and Tukey's test were used to check the difference between the samples.

3-Results and discussion

3-1 Oxidative stability of CLA

The results of oxidative stability of CLA containing different concentrations of pomegranate biological extract are shown in Figure 1. The results showed a significant effect of different concentrations of the extract on the oxidative stability of the sample ($p<0.05$). As expected, the highest peroxide index (that is, the lowest oxidative stability) was observed in the sample without pomegranate biological extract ($p<0.05$). The addition of extracts decreased the peroxide index (that is, increased oxidative stability) of the samples ($p<0.05$). Studies have shown that CLA is generally very unstable in air. The stability results of CLA isomers are at least partly determined by their cis or trans configuration, not by the position of their double bonds. Because the cis double bond is chemically more unstable than the trans double bond. because the former has a higher free energy level and is more vulnerable to oxygen attack [26]. Pomegranate skin is a valuable raw material due to its chemical compounds and biological activities. It contains various phenolic compounds such as anthocyanins, gallotannins, hydroxycinnamic acids, hydroxybenzoic acids, ellagitannins and galagyl esters. These phenolic compounds are responsible for various biological activities of pomegranate skin such as anti-mutagenic, antioxidant, antimicrobial and apoptosis [27]. The addition of frozen pomegranate bio-extract decreased the peroxide index (i.e., increased oxidative stability) of the samples. This decreasing trend was more significant with increasing concentration of pomegranate extract. This can be attributed to the presence of phenolic compounds in pomegranate extract. And with the increase of the added extract, the concentration of phenolic compounds increases. There is a positive correlation between total phenolic compounds and antiradical activity [28]. It should be noted that from the concentration of 1400 ppm and above, no statistically significant difference was observed in the biological extract of pomegranate in terms of oxidative stability. The reason for this can be due to the peroxidation effect of frozen pomegranate biological extract [29]. They also introduced the presence of CLA in pomegranate extract as an antioxidant agent. However, studies have shown that, like phenolic antioxidants, CLA can easily donate an electron or a hydrogen due to resonance displacement. But unlike phenolic antioxidants, its intermediate free radical may not be stable. and exposed to further oxidative degradation. In fact, it has been shown that CLA is rapidly degraded to form furan fatty acids [30].

[DOI: 10.22034/FSCT.22.158.99]

Carvalho Filho (2014), also introduced the presence of CLA in pomegranate extract as an antioxidant agent [31]. However, studies have shown that, like phenolic antioxidants, CLA can easily donate an electron or a hydrogen due to resonance displacement. However, unlike phenolic antioxidants, its free radical intermediate may not be stable and may be subjected to more oxidative degradation. In fact, it has been shown that CLA is rapidly degraded to form furan fatty acids [32]. Shayanmehr et al. (2021) reported that the optimal conditions for the production process of CLA isomers with high stability are: solvent concentration 6.74 ml, reaction temperature 100 °C and reaction time 140 minutes. In optimal conditions, the amounts of trans10-cis12 and cis9-trans11 isomers were 8.44% and 7.62%, respectively [33].

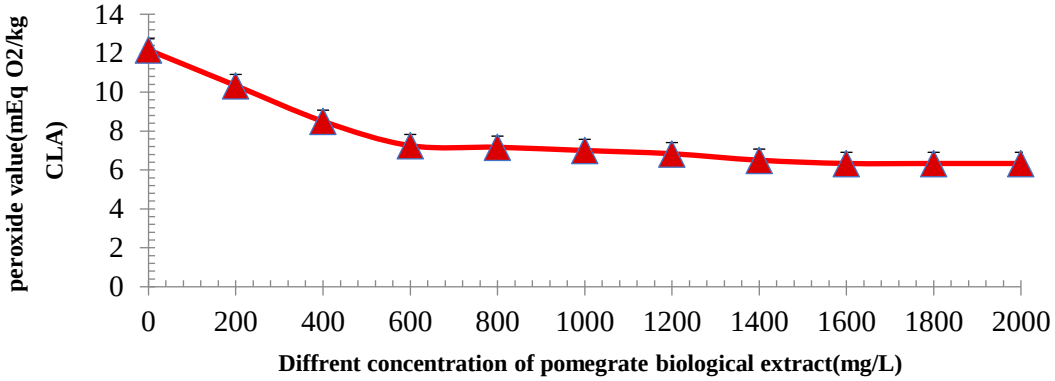


Fig 1. Results of linoleic acid peroxide index containing frozen pomegranate biological extract.

3-2 Analysis of functional groups (bond formation) with FTIR

In FTIR spectroscopy, the energies of infrared rays coincide with the vibrational energies of molecules, and this adaptation causes the absorption of electromagnetic radiation energy by the sample. Therefore, by changing the frequency of the radiation in a specific range (infrared), a spectrum is obtained whose transmission value is reduced in some wavelengths, or in other words, it is absorbed by the molecules of matter. Therefore, by examining the absorption frequency of each spectrum, it is possible to understand the bonds in that material. The studies of Shahmoradi et al. (2023), Nandiyanto et al. (2019), Jung et al. (2018) and Gholizadeh et al. In the FTIR spectrum of chitosan sample (Figure 2-a), the peak created at 3361 cm^{-1} wave number is related to stretching vibrations of O-H and N-H bonds in hydroxyl and amide structures. The peak placed in the wave number cm^{-1} 1646 and cm^{-1} 1612 is related to the bending vibrations of these links. The peak corresponding to C-H stretching and bending vibration is located at 2884 cm^{-1} and 1395 cm^{-1} wave number, respectively. Also, the peaks appearing in the wave numbers of 1163 cm^{-1} and 1041 cm^{-1} are related to the stretching vibrations of C-N and C-O bonds, respectively. The shape of the FTIR spectrum related to the pomegranate sample (Figure 2-b), showed that in the FTIR spectrum related to the pomegranate sample, the peak that appeared at the wave number cm^{-1} 3454 is related to the stretching vibrations of the O-H bond and the peaks that appeared at the wave number 2983 cm^{-1} and 2899 cm^{-1} are related to the asymmetric and symmetric stretching vibrations of the C-H bond in the aliphatic structure of the substance, respectively. The peaks related to the vibrations of alkene, carbonyl and lactone groups are located at 2508 cm^{-1}, 2406 cm-1 and 1838 cm^{-1} wave numbers, respectively. Also, the peaks appearing in the wave numbers of 1713 cm^{-1} and 1527 cm-1 are attributed to the stretching vibrations of C=O and C=C bonds, respectively. The peaks

[DOI: 10.22034/FSCT.22.158.99]

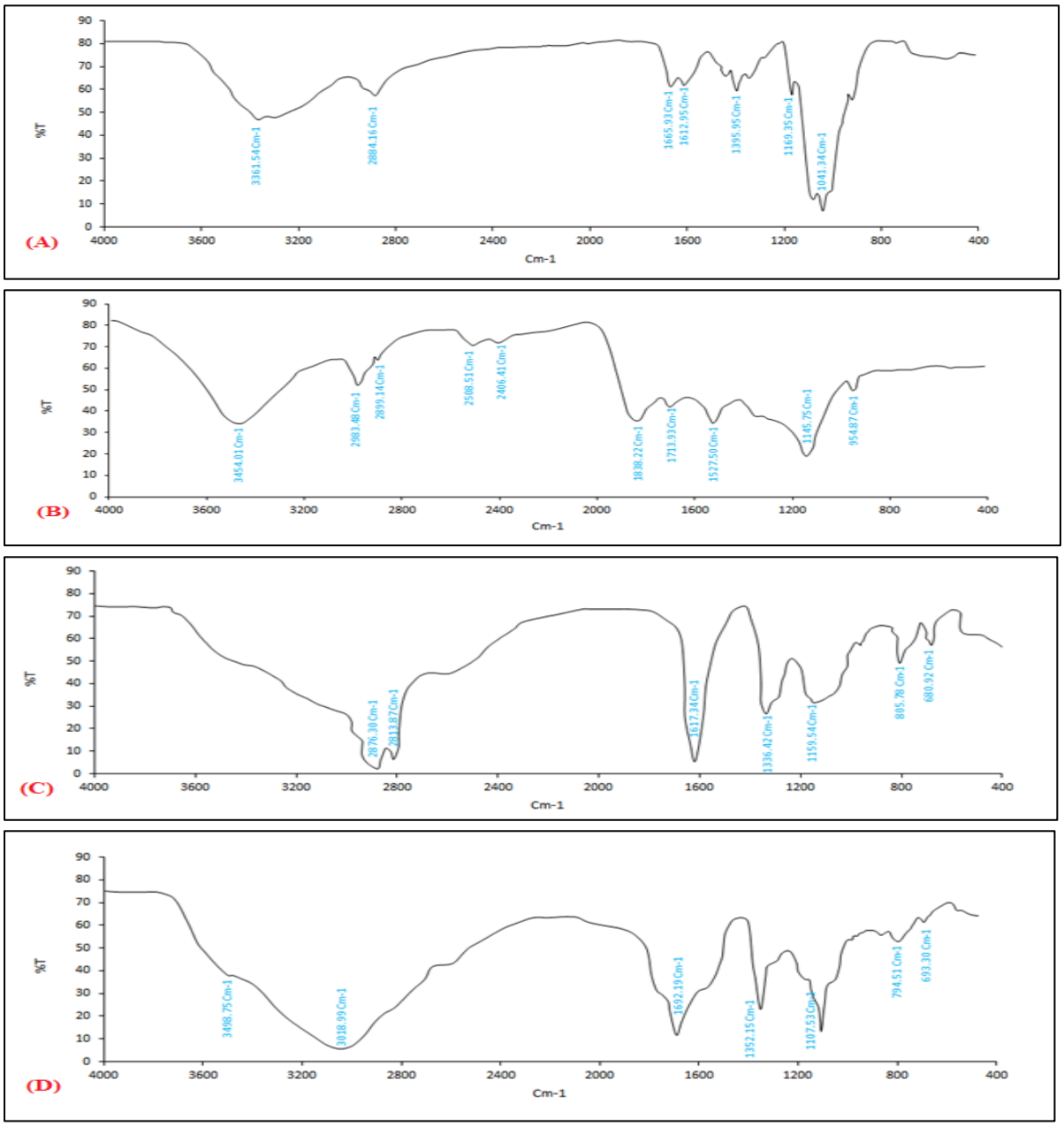
appearing in the wave numbers of 1713 cm^{-1} and 1527 cm^{-1} are attributed to the stretching vibrations of C=O and C=C bonds, respectively. The peaks appearing in the wave number of 1145 cm^{-1} and 954 cm^{-1} are attributed to the stretching vibrations of C-OH and C-O-C bonds, respectively. Figure 2-c and Figure 2-d showed that in the spectrum related to CLA, the peaks are located in the wave number of about 3500 cm^{-1} and 1617 cm^{-1}. And in the spectrum related to the sample of pomegranate bioactive compounds, the peaks located in the wave number of 3498 cm^{-1} and 1692 cm^{-1} are again respectively related to the stretching vibrations of the O-H bond in the hydroxyl structure and the stretching vibration of the C=O bond in the carboxyl structure of linolenic acid. Also, the peaks located at 2876 cm^{-1} and 2813 cm^{-1} wavenumbers in the CLA sample and the peak located at 3018 cm^{-1} in the pomegranate bioactive sample are respectively related to the asymmetric and symmetric stretching vibrations of C-H bonds and the bending vibration of the C=C bond in the aliphatic structure of this acid. the peaks placed in the wave number of 1336 cm^{-1} and 1352 cm^{-1} respectively in the spectra related to the sample of CLA and pomegranate bioactive are related to the bending vibration of the C-H bond. The peaks placed in the wave number of 1107 cm^{-1} and 1159 cm^{-1} respectively in the spectra of CLA sample and pomegranate bioactive are related to the stretching vibration of the C-OH bond. The peaks located in the wavelengths of 680 cm^{-1} and 805 cm^{-1} in the CLA sample as well as 693 cm^{-1} and 794 cm^{-1} in the pomegranate bioactive sample are also common in both samples and are related to Rocking vibration and Wagging vibration of C-H bonds in the aliphatic structure of this fatty acid. Therefore, the presence of these commonalities in both spectra indicates the successful interaction of linoleic acid with pomegranate bioactive compounds. FTIR test was used to check the accuracy of chitosome formation. It was observed that in the FTIR spectrum of the chitosome sample (Figure 2-e), the peak appearing at the wave number cm^{-1} 3464 is related to the stretching vibration of the O-H and N-H bonds, which are present in the structure of chitosan and active zeit compounds in pomegranate. The peaks appearing in the wave number of 2945 cm^{-1} and 2840 cm^{-1} are respectively related to the asymmetric and symmetric stretching vibrations of the C-H bond in the aliphatic structure of linolenic acid and other structures in pomegranate active oil and chitosan. The peaks related to the vibrations of alkene, carbonyl and carboxyl groups are located at 2505 cm-1, 2391 cm^{-1} and 1764 cm^{-1}, respectively, which were also observed in the pomegranate spectrum. The peaks placed in the wave number of 1629 cm^{-1} and 1515 cm-1 are related to the bending vibrations of the O-H and N-H bonds in the chitosan structure, respectively. Also, the peaks located at the wave number of 1071 cm^{-1} and 896 cm^{-1} are respectively related to the stretching vibration of the C-O bond and the dancing vibrations of the C-H bond, which are present in the structures of linolenic acid and tannin in pomegranate bioactive. Therefore, the existence of structures in pomegranate bioactive along with chitosan in this composite can be proved.

3-3 Morphological features

The morphology of dried extract of pomegranate peel by sublimation method (Figure 3) showed that the use of ultrasonic waves caused the formation of small particles in nanometer size (between 48.581 and 62.214 nm). Also, the glass-like amorphous shape is characteristic of the freeze dryer, it was observed on the surface of the extracts. Freeze-dried pomegranate biowaste extracts showed flaky forms with smooth surfaces. The results of Febriyenti et al. (2014) also showed that the freeze-dried Haruan extract had a smooth surface and flaky amorphous areas [38]. The morphology of the final chitosome produced is shown in Figure 4. It showed the presence of almost spherical particles with nanometer size (77.66 and 79.90 nm). Also, the uniformity observed in the size of the particles confirms the proper distribution of the size of the particles.

[DOI: 10.22034/FSCT.22.158.99]

Also, integrity was observed in nanocarrier systems, without phase separation, accumulation in nanocarriers, and the results showed that the shape of nanochitosomes was irregular, which could be related to the presence of chitosan absorbed on the surface of bioactive compounds. In addition, free chitosan may form aggregates during the drying process. Free chitosan can create a dynamic equilibrium between the "aggregated form" of the biopolymer and its "adsorbed form" around the bioactive compounds. In fact, chitosan self-aggregates. Therefore, when diluted in aqueous solution, it forms nanoparticles and microparticles, and its aggregation process strongly depends on the molecular weight of chitosan and the degree of acetylation [39]. The obtained results were consistent with the results of Nerli et al. (2023). During the investigation of the morphology of chitosomes coated with chitosan, they reported the spherical and homogeneous shape of chitosans and their nanometer size [40]. Hashemi et al. (2020) showed that chitosomes containing linoleic acid reported a small size of spherical and homogeneous particles with a smooth surface [41].



[DOI: 10.22034/FSCT.22.158.99]

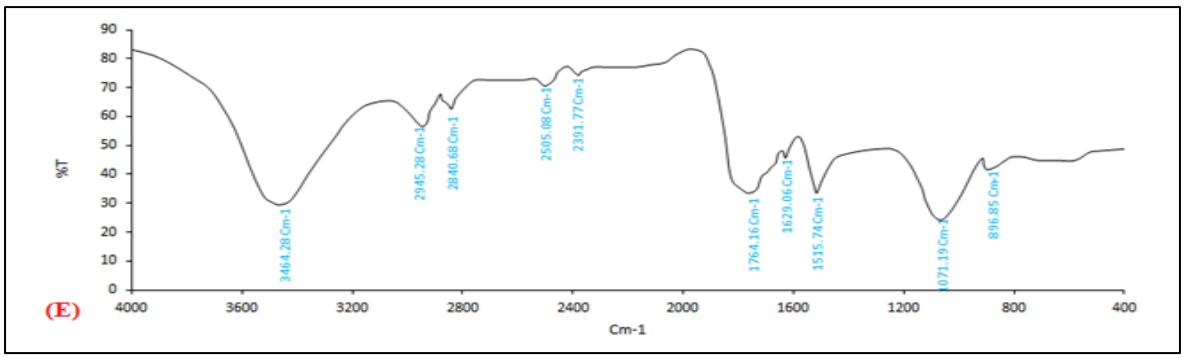


Fig 2. FTIR spectrum and approximate areas of different identified functional groups (A): Chitosan sample/ (B): Pomegranate sample/ (C): Linoleic acid/ (D): Pomegranate aqueous extract/ (E): Chitosome

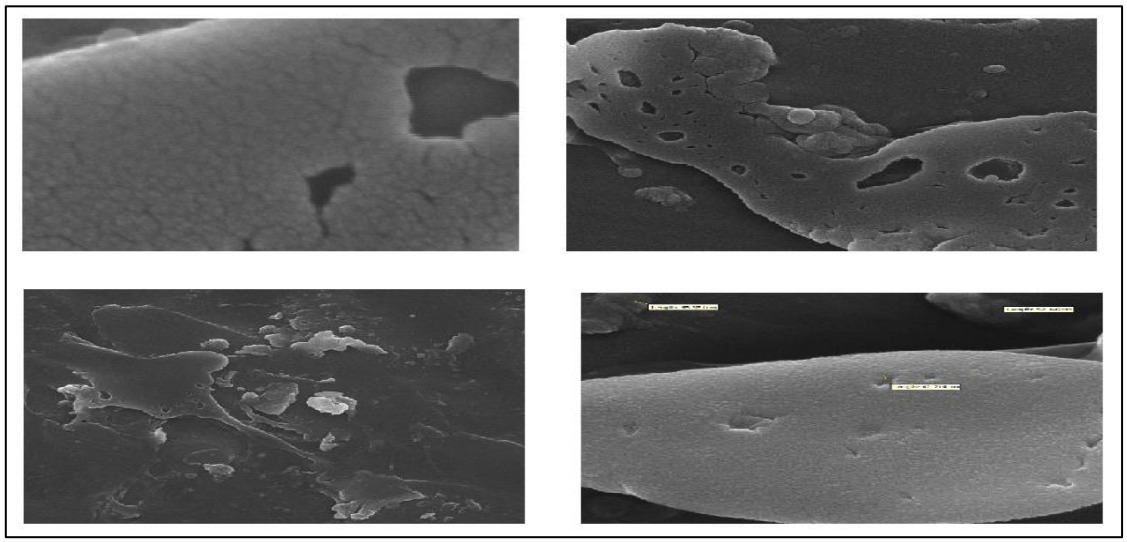


Figure 3. Morphology of dried pomegranate peel extract by sublimation method

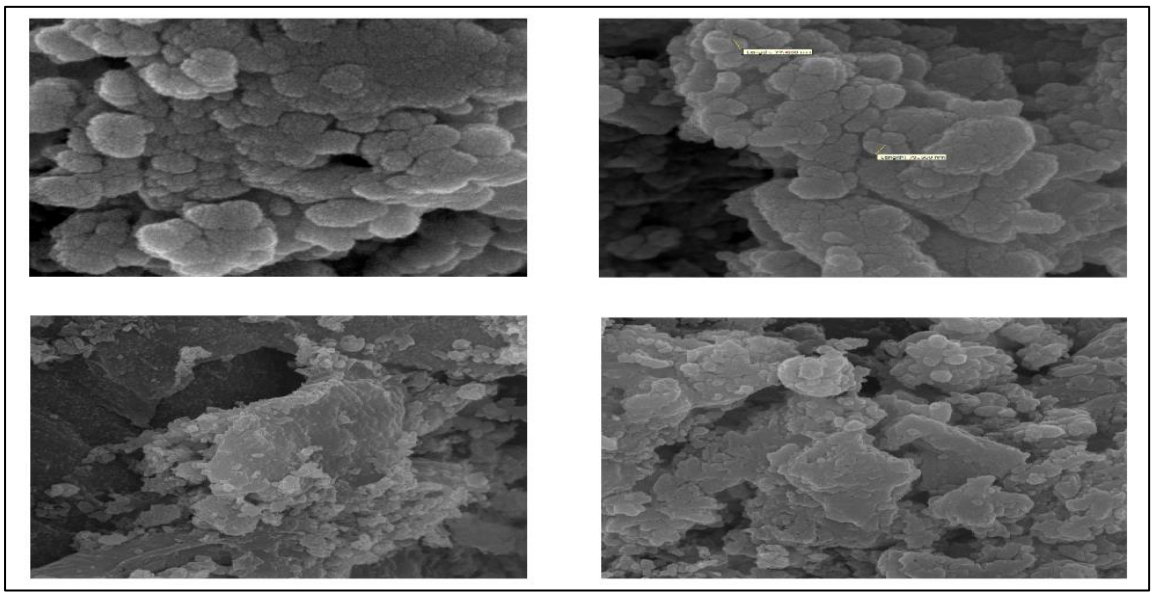


Figure 4. Morphology of produced chitosome

3-5 Physico-chemical characteristics of energy drink

3-5-1 pH and acidity

Energy drinks are drinks that, apart from calories, contain caffeine with other potential energy-increasing compounds such as plant extracts and B group vitamins. Studies have

[DOI: 10.22034/FSCT.22.158.99]

shown that pH plays an important role in efficient production, quality control, growth of microorganisms and shelf life of products because it can directly affect the sensory characteristics of beverages [42]. Table 1 shows the pH and acidity of energy drinks. According to the results, the addition of produced chitosomes caused a significant increase in the pH (or a significant decrease in acidity) of the drinks compared to the control sample. However, during 45 days of storage, a significant decrease in pH (or a significant increase in acidity) was reported in the two investigated groups. Studies have shown that the decrease in pH in energy drink samples can be due to the formation of acidic products resulting from the Maillard reaction and microbial growth during storage [43]. The lower pH (higher acidity) of the samples enriched with chitosomes can be attributed to the acidity of linoleic acid and pomegranate extract. which can be attributed to the increase in the release of hydrogen ions during long-term storage [44]. Fikry et al. (2023), also showed the similar trend of this study [42]. Selahvarzi et al. (2021) also showed that pomegranate seed extract in caffeinated drinks decreased the pH and increased the acidity of the drinks [45].

Table 1. The results of the physicochemical characteristics of the functional energy drink during 45 days

parameter	Treatment	Shelf-life (Day)	
		1Day	45 Day
PH	Control	3.81 ± 0.00^{Aa}	3.45 ± 0.00^{Bb}
	Contain chitosome	3.30 ± 0.00^{Ab}	3.51 ± 0.03^{Ba}
Acidity	Control	0.55 ± 0.00^{Ba}	0.71 ± 0.00^{Aa}
	Contain chitosome	0.50 ± 0.00^{Bb}	0.69 ± 0.01^{Ab}

*Different lowercase letters indicate significant differences between treatments ($p<0.05$).

*Different capital letters indicate significant differences between days ($p<0.05$).

3-5-2 Turbidity

One of the most important effective factors in the use of colloidal nanocarriers for the enrichment of all kinds of food and especially clear drinks is to check the turbidity of the nanocarrier. The turbidity is determined by the amount of light scattered by the particles in the suspension medium and depends on the nature, number, colloidal size of the particles in the liquid phase and the difference in the reflection coefficient of the particles and the suspension medium and the size distribution of the particles [46]. The results of the turbidity of energy drinks showed (Figure 5) that the addition of chitosome in the drinks caused a significant increase in the turbidity compared to the control sample. Also, a significant increase in turbidity was observed in these samples during 45 days of storage. This increase in turbidity can be due to the release of compounds from the chitosome structure. This means that chitosan is an efficient coagulant/coagulant agent for clarifying beverages [47]. In this regard, Mokhtarian et al showed that caffeine-

polyphenol interaction is a main factor in the formation of green tea cream, which is associated with increasing the appearance of cloudiness in the extract [19]. Keshksera et al. (2023) during the study of the production of practical drinks based on the bioactive compounds of green tea and green coffee intertwined in the chitosome structure also showed similar results to this research [25].

[DOI: 10.22034/FSCT.22.158.99]

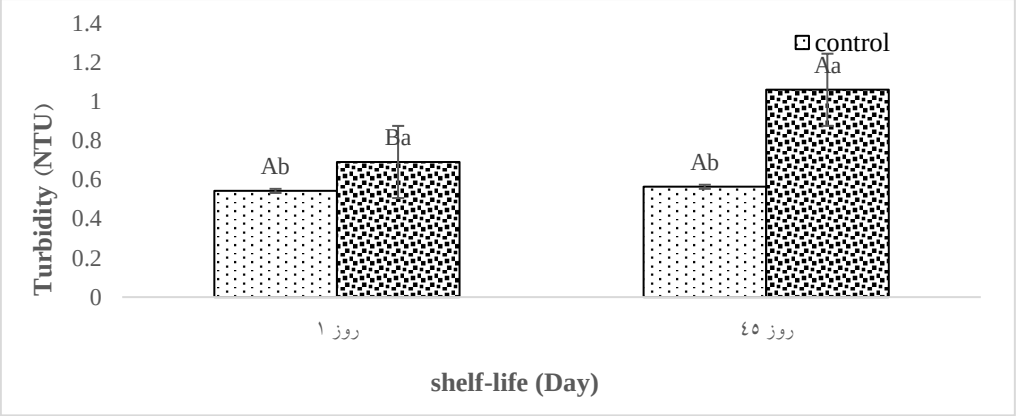


Fig 5. The results turbidity in the energy drink during 45 days
*Different lowercase letters indicate significant differences between treatments (p<0.05).
*Different capital letters indicate significant differences between days (p<0.05).

3-5-2 Viscosity

The results of the viscosity of energy drinks (Figure 6) showed that the addition of chitosomes significantly increased the viscosity of the drinks in the energy drink sample during storage. This increase in viscosity can be caused by the leakage of chitosomes in drinks during storage. This is probably due to the presence of carboxyl groups that produce negative charges, which leads to electrostatic repulsion between chains, which is manifested in the increase in the viscosity of the drink [42]. Zindabudi et al. (2019) showed similar results during the production of an energy drink containing xanthan hydrocolloid and date extract [49]. Elkot et al. (2024) also showed an increase in the viscosity of sports energy drink enriched with Spirulina platentis rose [50].

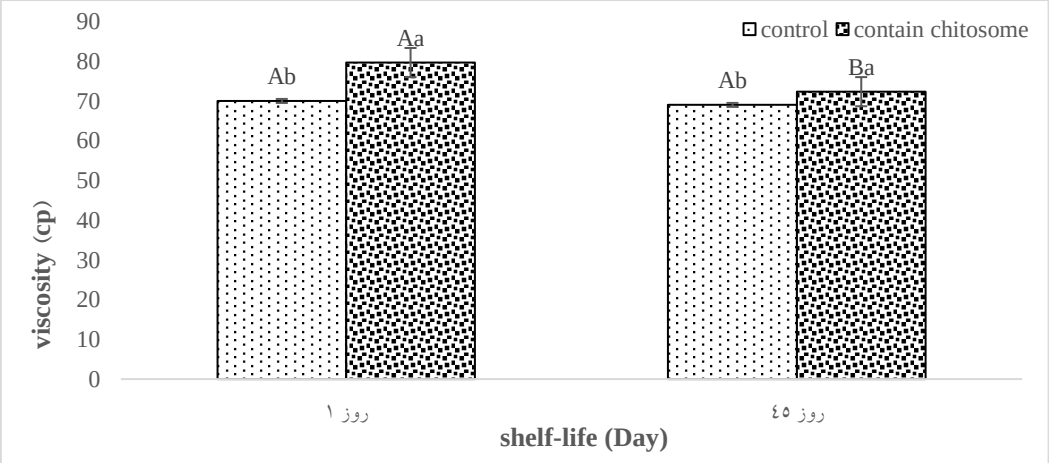


Fig 6. The results viscosity in the energy drink during 45 days
*Different lowercase letters indicate significant differences between treatments (p<0.05).
*Different capital letters indicate significant differences between days (p<0.05).

3-5-3 *Two phases*

The results of the Two-phase rate of energy drinks (Figure 7) showed that the addition of produced chitosome caused a significant increase in the Two-phase rate of the drinks compared to the control sample. While no Two-phase rate was observed in the control samples. The results show the instability of chitosomes over time. These results can be explained as a consequence of sonication of liposomes that had been dried in the absence of a lyophilizer, thus creating weak structures that fragmented over time as a result of aggregation and fusion of destabilized vesicles [51]. Studies have shown that the surface coating of nanoliposomes is one of the efficient solutions

[DOI: 10.22034/FSCT.22.158.99]

to improve the stability of nanoliposomes and increase the residence time of compounds enclosed inside nanocarriers, which is of great interest to increase the quality of formulated food products. Katouzian et al. (2021) reported that the reason for the decrease in the stability of liposomes is the extract loaded into chitosomes, which may help the Ostwald process, in which the hydroxyl groups in the extract are changed to groups containing O with a negative charge, and in turn, they form hydrogen ions. It connects with positively charged groups, the head part of phospholipids, and leads to Ostwald ripening process. A possible explanation for the enhanced stability may be the formation of a positively charged barrier by chitosan on the nanoliposomes, which increases both the oxidative stability and the physical stability of the network. This fact limits the formation of free radicals and thus prevents the degradation of phospholipids. Another reason for the instability of chitosomes can be attributed to the size of the obtained nanoparticles. During the formation of nanoparticles with a small diameter, it leads to the formation of a relatively homogeneous network. As a result, the increase in stability can be attributed to the electrostatic repulsion between positively charged chitosomes and the low tendency of nanoparticles to precipitate - due to the decrease in their molecular weight [52]. Peng et al. (2018) also showed that when nanocarriers are used in food and beverages, they may stimulate interference reactions between food components. In addition, leakage of core materials and also accumulation can occur during storage time and cause a decrease in the quality of the food matrix [53].

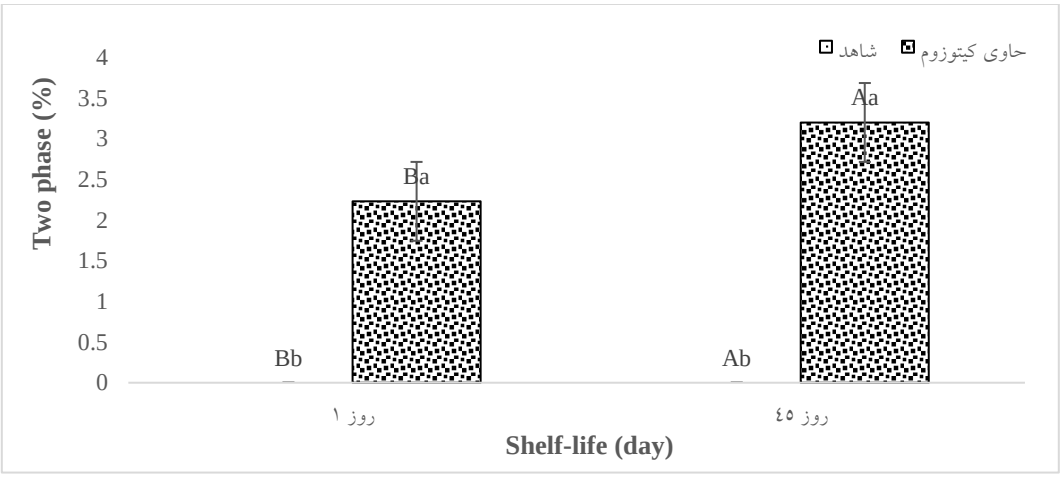


Fig 7. The results of two phase in the energy drink during 45 days

*Different lowercase letters indicate significant differences between treatments ($p<0.05$).

*Different capital letters indicate significant differences between days ($p<0.05$).

3-6 Color

Color index is one of the most important parameters in product acceptance by consumers. The results of the brightness index of the energy drink samples (Table 2) showed a decrease in the brightness of the samples with the addition of chitosome, which could be due to the yellow color of soy lecithin used in these products. This decrease in brightness index over time can be due to temperature and ambient light, which usually flavonoids destroy the components related to the color of the drink. The increase in the redness index can be due to the color of the pomegranate peel extract - as a substance that affects the color of the drink, i.e., the anthocyanins of the extract. This can be explained by their inhibitory effect on enzymes, especially on O-diphenol oxidase [54]. Also, the results showed that during 45 days of storage, the color changes were significantly higher in the energy drink sample containing chitosome. Sami et al. (2021) also showed

[DOI: 10.22034/FSCT.22.158.99]

similar results when adding pomegranate peel extract to milk color [54]. An increase in the yellowness of ultra-beneficial energy drinks during 45 days of storage was also observed.

Table 2. The results of the color value of the functional energy drink during 45 days

parameter	Treatment	Shelf-life (Day)	
		1Day	45 Day
L*	Control	54.43 ± 0.25 ^{Aa}	53.89 ± 0.16 ^{Ba}
	Contain chitosome	52.54 ± 0.32 ^{Ab}	51.38± 0.05 ^{ABa}
a*	Control	14.88 ± 0.02 ^{Ba}	15.12 ± 0.09 ^{Aa}
	Contain chitosome	13.10 ± 0.07 ^{Bb}	15.469 ± 0.30 ^{Ab}
b*	Control	16.56 ± 0.04 ^{Ba}	17.04 ± 0.10 ^{Ab}
	Contain chitosome	15.77 ± 0.04 ^{Bb}	17.70 ± 0.23 ^{Aa}
ΔE	Control	0.00 ± 0.00 ^{Ba}	0.75 ± 0.27 ^{Ab}
	Contain chitosome	0.00 ± 0.00 ^{Bb}	3.26 ± 0.38 ^{Aa}

*Different lowercase letters indicate significant differences between treatments ($p<0.05$).
*Different capital letters indicate significant differences between days ($p<0.05$).

3-7 Total phenol

Bioactive compounds (including carotenoids, essential oils, antioxidants or flavors) are a group of chemicals with health-giving, medicinal and nutritional properties, which are widely used in food products in order to improve sensory characteristics or develop pharmaceutical properties [55]. According to the results presented in Figure 8, the storage time and temperature had a significant effect on the phenol content of the samples. The highest amount of total phenolic compounds (corresponding to the highest release rate of phenolic compounds) in the beverage environment was observed in beverages stored at 45°C during 45 days of storage. This is probably due to the decrease in the resistance of the cytosome wall (corresponding to the decrease in membrane fluidity) and as a result the release of phenolic compounds [20]. Also, the results of the total content of the beverage samples stored at 5 and 25 °C showed the lowest phenolic content in the sample stored at 5 °C and after that in the samples stored at 25 °C. became. Also, the results showed that the phenolic content decreased during 45 days of storage in all investigated groups. This state is probably due to the destruction of polyphenolic compounds released in the drinking environment and the destructive effects of environmental storage conditions [58]. In line with the obtained results, Seyedabadi et al. (2021), while investigating the amount of caffeine release at 37°C from chitosome in the simulated environment of juice, showed that the sudden release of caffeine from the structure of schistosome in the simulated environment of juice, it is due to the swelling of the structure caused by the penetration of the simulating environment and the subsequent release of caffeine on the surface of the nanoparticles [20]. Also, Liu et al. (2017) investigated the release of chitosan nanoparticles containing tea polyphenols mixed with gelatin film in 50% and 95% ethanol fat simulating environments. They showed that the release of polyphenolic compounds in 50% environment was faster than in 95% environment due to the doubling of the diffusion coefficient [59].

[DOI: 10.22034/FSCT.22.158.99]

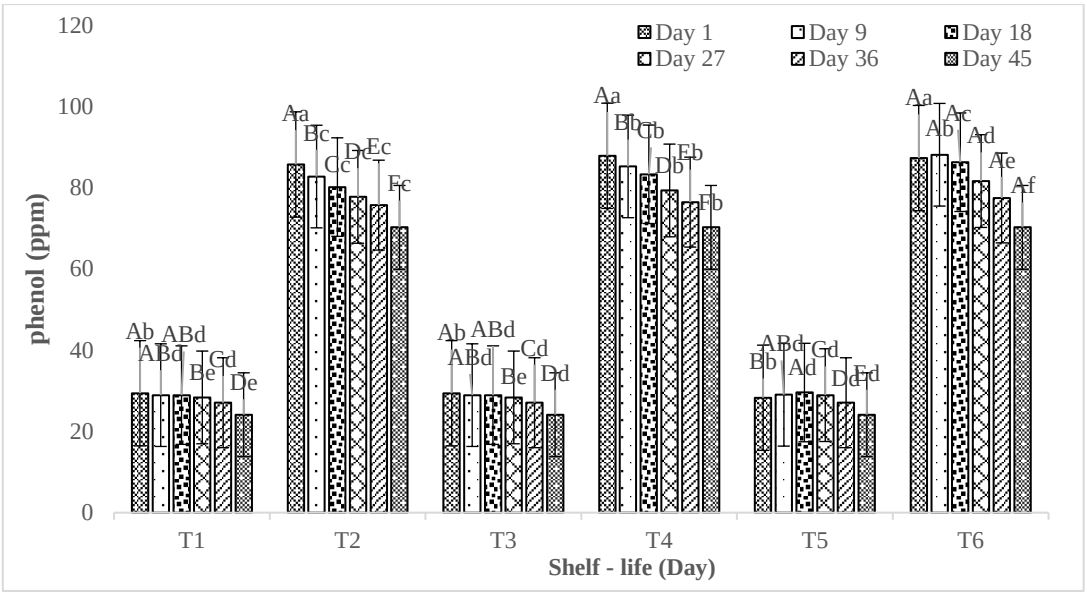


Fig 9. The results of phenol in the energy drink during 45 days

*T1: control sample kept at 5°C/ T1 sample containing chitosome kept at 5°C/ T3: control sample kept at 25°C/ T4: sample containing chitosome kept at a temperature of 25°C/T5: control sample kept at a temperature of 45°C/T6: a sample containing chitosome kept at a temperature of 45°C

*Different lowercase letters indicate significant differences between treatments ($p<0.05$).

*Different capital letters indicate significant differences between days ($p<0.05$).

4- Conclusions

In this research, the energy drink contains microparticles of chitosome intertwined with bioactive tannin-CLA compounds. Oxidative resistance of linoleic acid, functional groups with FTIR spectroscopy, X-ray diffraction of chitosomes and their morphology were investigated. Finally, the physicochemical characteristics(pH, acidity, turbidity, biphasing and viscosity) and color index (L*, a* and b*) and phenol content of all beverages were monitored at 5 and 25 °C during 45 days of storage. The qualitative evaluation of the optimal chitosome microcapsule and the presence of similarities in the FTIR spectra indicated the successful coating of bioactive tannin-linoleic acid in chitosan. Morphological examination of the frozen extract of pomegranate biological waste extracted with the help of ultrasonic waves showed the formation of small particles in nanometer size (between 48.58 and 62.214 nm) similar to glass. Also, the presence of almost spherical chitosan particles with nanometer size (77.66 to 79.90 nm) was uniformly observed, so the size of the particles confirms the proper distribution of the particle size. During the review of the results of ultra-beneficial energy drinks, a significant increase in pH (or a significant decrease in acidity) was observed with the addition of nano chitosomes, a significant increase in viscosity and turbidity in ultra-beneficial energy drinks. Also, the instability of chitosomes during the storage period showed a significant increase in biphasing of drinks. A significant decrease in brightness, a significant increase in the redness and yellowness index of beverages containing nanochitosomes, and a significant increase in the phenolic content of enriched energy drinks were observed. Therefore, nanochitosomes containing tannin-CLA can be a potential release system for bioactive molecules as food supplements and increase food safety.

[DOI: 10.22034/FSCT.22.158.99]

5-References

[1] Arjeh, E., Khodaei, S. M., Barzegar, M., Pirsa, S., Karimi Sani, I., Rahati, S., & Mohammadi, F. (2022). Phenolic compounds of sugar beet (Beta vulgaris L.): Separation method, chemical characterization, and biological properties. *Food Science & Nutrition*, *10*(12), 4238-4246.

[2] Badawy, S., Liu, Y., Guo, M., Liu, Z., Xie, C., Marawan, M. A., ... & Martínez, M. A. (2023). Conjugated linoleic acid (CLA) as a functional food: Is it beneficial or not? *Food Research International*, 113158.

[3] Nasrollahzadeh, A., Tavani, S. M., Arjeh, E., & Jafari, S. M. (2023). Production of conjugated linoleic acid by lactic acid bacteria; important factors and optimum conditions. *Food Chemistry: X*, 100942.

[4] Costantino, A., Maiese, A., Lazzari, J., Casula, C., Turillazzi, E., Frati, P., & Fineschi, V. (2023). The dark side of energy drinks: a comprehensive review of their impact on the human body. *Nutrients*, *15*(18), 3922.

[5] Ain, H. B. U., Tufail, T., Bashir, S., Ijaz, N., Hussain, M., Ikram, A., ... & Saewan, S. A. (2023). Nutritional importance and industrial uses of pomegranate peel: A critical review. *Food Science & Nutrition*, *11*(6), 2589-2598.

[6] Tehranifar. and weaponization, Y. (2006). Location of pomegranate in Iran. *The second desert ruby pomegranate festival*. [in Persian].

[7] Ko, K., Dadmohammadi, Y., & Abbaspourrad, A. (2021). Nutritional and bioactive components of pomegranate waste used in food and cosmetic applications: A review. *Foods*, *10*(3), 657.

[8] Akuru, E. A., Chukwuma, C. I., Oyeagu, C. E., Mpendulo, T. C., & Lewu, F. B. (2023). Potential Relevance of Pomegranate (Punica granatum) Peel in the Nutrition, Health, and Quality Products of Poultry. *Iranian Journal of Applied Animal Science*, *13*(4), 627-646.

[9] Gomes, P. W. P., Gomes, E. R. S., Carvalho, A. R., Mannochio-Russo, H., Leão, T. F., Reis, J. D. E., ... & da Silva, M. N. (2023). Characterization of a tetrameric proanthocyanidin in Stryphnodendron pulcherrimum and an overview on potential health benefits of condensed tannins via interaction with gut microbiota. *Journal of Functional Foods*, *109*, 105801.

[10] Tavakolipour, H., & Mokhtarian, M. (2023). Production of functional beverage based on cross-linked bioactive compounds of green tea and green coffee in chitosome structure. *Journal of food science and technology (Iran)*, *19*(133), 79-89.

[11] Shaddel, R., & Rajabi-Moghaddam, S. (2024). Encapsulation of caffeine in chitosan-coated nanoliposomes and its application in drink formulation. *Food Hydrocolloids*, *149*, 109598.

[12] Ramli, N. A., Ali, N., & Hamzah, S. (2019). Indo-Malayan Stingless Bees' Propolis Extract-Loaded Chitosomes: Characterization and Storage Stability Evaluation. *International Journal of Recent Technology and Engineering*, *7*(6), 560-567.

[13] da Silva, N. C., Assis, O. B. G., de Oliveira Sartori, A. G., de Alencar, S. M., & Martelli-Tosi, M. (2022). Chitosan suspension as extractor and encapsulating agent of phenolics from acerola by-product. *Food Research International*, *161*, 111855.

[14] Amiri, S., Mokarram, R. R., Khiabani, M. S., Bari, M. R., & Khaledabad, M. A. (2020). In situ production of conjugated linoleic acid by Bifidobacterium lactis BB12 and Lactobacillus acidophilus LA5 in milk model medium. *LWT*, *132*, 109933.

[15] Kodchasee, P., Pharin, N., Suwannarach, N., Unban, K., Saenjum, C., Kanpiengjai, A., ... & Khanongnuch, C. (2023). Assessment of Tannin Tolerant Non-Saccharomyces Yeasts Isolated from Miang for Production of Health-Targeted Beverage Using Miang Processing Byproducts. *Journal of Fungi*, *9*(2), 165.

[16] AOAC. (1998). Official Methods of Analysis of the Association of Official Analytical Chemists (17th ed.). Association of Official Analytical Chemists. Washington, DC, USA.

[DOI: 10.22034/FSCT.22.158.99]

[17] Tavakolipour, H., Mokhtarian, M., & Kalbasi-Ashtari, A. (2017). Lipid oxidation kinetics of pistachio powder during different storage conditions. *Journal of food process engineering*, *40*(3), e12423.

[18] Rajha, H. N., Koubaa, M., Boussetta, N., Maroun, R. G., Louka, N., Lebovka, N., & Vorobiev, E. (2020). Selective ultrasound-assisted aqueous extraction of polyphenols from pomegranate peels and seeds. *Journal of Food Processing and Preservation*, *44*(7), e14545.

[19] Mokhtarian, M. O. H. S. E. N., Tavakolipour, H., Jafari Savareh, S., & Amiri, M. (2014). Determination of optimal parameters to extraction and formulation of functional drink from green tea and determining its physicochemical and rheological properties. *Research and Innovation in Food Science and Technology*, *3*(1), 51-66. [in Persian].

[20] Seyedabadi, M. M., Rostami, H., Jafari, S. M., & Fathi, M. (2021). Development and characterization of chitosan-coated nanoliposomes for encapsulation of caffeine. *Food bioscience*, *40*, 100857.

[21] Meng, R., Wu, Z., Xie, Q. T., Cheng, J. S., & Zhang, B. (2021). Preparation and characterization of zein/carboxymethyl dextrin nanoparticles to encapsulate curcumin: Physicochemical stability, antioxidant activity and controlled release properties. *Food Chemistry*, *340*, 127893.

[22] Yang, L., Cao, Y., Chen, J. N., & Chen, Z. Y. (2009). Oxidative stability of conjugated linolenic acids. *Journal of agricultural and food chemistry*, *57*(10), 4212-4217.

[23] ISIRI. (2007). Fruit juices – Test methods. Institute of Standards and Industrial Research of Iran No2685. [In Persian]

[24] HOJJATI, M., Jafari, S., & Noshad, M. (2018). Effect of Xanthan Gum and Soluble Soybean Polysaccharide on Characterictics of Functional Flixweed (Descurainia Sophi L.) Syrup. *Food Processing and Preservation Journal*, *10*(2), 63-80. [in Persian].

[25] Kashksara, K. M., Tavakolipour, H., & Mokhtarian, M. (2023). Effects of atmospheric-thermosonication process on phenolic compounds extraction, extraction productivity and antioxidant activity of freeze-dried green tea and green coffee aqueous extracts. *Journal of Agriculture and Food Research*, *12*, 100582.

[26] Yang, L., Leung, L. K., Huang, Y., & Chen, Z. Y. (2000). Oxidative stability of conjugated linoleic acid isomers. *Journal of Agricultural and Food Chemistry*, *48*(8), 3072-3076.

[27] Drinić, Z., Mudrić, J., Zdunić, G., Bigović, D., Menković, N., & Šavikin, K. (2020). Effect of pomegranate peel extract on the oxidative stability of pomegranate seed oil. *Food Chemistry*, *333*, 127501.

[28] Burits, M., & Bucar, F. (2000). Antioxidant activity of Nigella sativa essential oil. *Phytotherapy research*, *14*(5), 323-328.

[29] Keshvari Fard, F., Mokhtarian, M., & Tavakolipour, H. (2020). Evaluation of the effects of peppermint essential oil (Mentha piperita) on oxidative stability of soybean oil. *Journal of Food Processing and Preservation*, *12*(1), 81-94. [in Persian].

[30] Zhang, A., & Chen, Z. Y. (1997). Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. *Journal of the American Oil Chemists' Society*, *74*, 1611-1613.

[31] Carvalho Filho, J. M. (2014). Pomegranate seed oil (Punica granatum L.): a source of punicic acid (conjugated α-linolenic acid). *J Human Nutri Food Sci*, *2*(1), 1-11.

[32] Zhang, A., & Chen, Z. Y. (1997). Oxidative stability of conjugated linoleic acids relative to other polyunsaturated fatty acids. *Journal of the American Oil Chemists' Society*, *74*, 1611-1613.

[33] Shayanmehr, M. R., Elhamirad, A. H., & Armin, M. (2021). Optimization of conjugated linoleic acid (Cla) synthesis conditions applying it in milk enrichment. *J Res Innov Food Sci Technol*, *9*, 375-88.

[DOI: 10.22034/FSCT.22.158.99]

[34] Shahmoradi, A. R., Bejandi, M. S., Rasanani, E. H., Javidparvar, A. A., & Ramezanzadeh, B. (2023). Graphene oxide nano-layers functionalized/reduced by L-Citrulline/Pectin bio-molecules for epoxy nanocomposite coating mechanical properties reinforcement. *Progress in Organic Coatings*, *178*, 107493.

[35] Nandiyanto, A. B. D., Oktiani, R., & Ragadhita, R. (2019). How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science and Technology*, *4*(1), 97-118.

[36] Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez, V., Beers, K. L., Balazs, G. H., ... & Lynch, J. M. (2018). Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine pollution bulletin*, *127*, 704-716.

[37] Gholizadeh, E., Belibel, R., Bachelart, T., Bounadji, C., & Barbaud, C. (2020). Chemical grafting of cholesterol on monomer and PDMMLA polymers, a step towards the development of new polymers for biomedical applications. *RSC advances*, *10*(54), 32602-32608.

[38] Febriyenti, M. N., Mohamed, N., Hamdan, M. R., Salleh, S. N. M., & Baie, S. B. B. (2014). Comparison of freeze drying and spray drying methods of haruan extract. *Int J Drug Deliv*, *6*(3), 286-91.

[39] Scurti, E., Martins, J. P., Celia, C., Palumbo, P., Lombardi, F., Iannotta, D., ... & Viitala, T. (2023). In Vitro Characterization and Real-Time Label-Free Assessment of the Interaction of Chitosan-Coated Niosomes with Intestinal Cellular Monolayers. *Langmuir*, *39*(23), 8255-8266.

[40] Nerli, G., Robla, S., Bartalesi, M., Luceri, C., D'Ambrosio, M., Csaba, N., & Maestrelli, F. (2023). Chitosan coated niosomes for nose-to-brain delivery of clonazepam: formulation, stability and permeability studies. *Carbohydrate Polymer Technologies and Applications*, *6*, 100332.

[41] Hashemi, F. S., Farzadnia, F., Aghajani, A., Ahmadzadeh NobariAzar, F., & Pezeshki, A. (2020). Conjugated linoleic acid loaded nanostructured lipid carrier as a potential antioxidant nanocarrier for food applications. *Food Science & Nutrition*, *8*(8), 4185-4195.

[42] Fikry, M., Yusof, Y. A., Al-Awaadh, A. M., Baroyi, S. A. H. M., Ghazali, N. S. M., Kadota, K., ... & Al-Ghamdi, S. (2023). Assessment of Physical and Sensory Attributes of Date-Based Energy Drink Treated with Ultrasonication: Modelling Changes during Storage and Predicting Shelf Life. *Processes*, *11*(5), 1399.

[43] Davídek, T., Robert, F., Devaud, S., Vera, F. A., & Blank, I. (2006). Sugar fragmentation in the Maillard reaction cascade: Formation of short-chain carboxylic acids by a new oxidative α-dicarbonyl cleavage pathway. *Journal of agricultural and food chemistry*, *54*(18), 6677-6684.

[44] Akubor, P. I. (1996). The suitability of African bush mango juice for wine production. *Plant Foods for Human Nutrition*, *49*, 213-219.

[45] Selahvarzi, A., Ramezan, Y., Sanjabi, M. R., Mirsaeedghazi, H., Azarikia, F., & Abedinia, A. (2021). Investigation of antimicrobial activity of orange and pomegranate peels extracts and their use as a natural preservative in a functional beverage. *Journal of Food Measurement and Characterization*, *15*, 5683-5694.

[46] Saberi, A. H., Fang, Y., & McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. *Journal of colloid and interface science*, *391*, 95-102.

[47] Bindes, M. M. M., Reis, M. H. M., Cardoso, V. L., & Boffito, D. C. (2019). Ultrasound-assisted extraction of bioactive compounds from green tea leaves and clarification with natural coagulants (chitosan and Moringa oleífera seeds). *Ultrasonics sonochemistry*, *51*, 111-119.

[DOI: 10.22034/FSCT.22.158.99]

[48] Cardozo, C. J. M., Castañeda, C. A. J., & Ripoll, C. S. S. (2017). Development of mango (Mangifera indica L.) energy drinks. *Revista Facultad Nacional de Agronomía Medellín*, *70*(1), 8115-8121.

[49] Zendeboodi, F., & Ghollan, H, M. (2019). Optimization of formulation of energetic soft drink containing natural sweetener and whey protein using mixture design-extreme vertices. *Journal of food science and technology (Iran)*, *16*(89), 113-123. [in Persian].

[50] Elkot, W. F., Elmahdy, A., Talaat, H., Alghamdia, O. A., Alhag, S. K., Al-Shahari, E. A., ... & Ismail, H. A. (2024). Development and characterization of a novel flavored functional fermented whey-based sports beverage fortified with Spirulina platensis. *International Journal of Biological Macromolecules*, *258*, 128999.

[51] Iida, A. S. L., Luz, K. N., Barros-Alexandrino, T. T., Fávaro-Trindade, C. S., Pinho, S. C. D., Assis, O. B. G., & Martelli-Tosi, M. (2020). Investigation of TPP-Chitosomes particles structure and stability as encapsulating agent of cholecalciferol. *Polímeros*, *29*.

[52] Katouzian, I., & Taheri, R. A. (2021). Preparation, characterization and release behavior of chitosan-coated nanoliposomes (chitosomes) containing olive leaf extract optimized by response surface methodology. *Journal of Food Science and Technology*, *58*, 3430-3443.

[53] Peng, S., Zou, L., Liu, W., Liu, C., & McClements, D. J. (2018). Fabrication and characterization of curcumin-loaded liposomes formed from sunflower lecithin: Impact of composition and environmental stress. *Journal of agricultural and food chemistry*, *66*(46), 12421-12430.

[54] Sami, R., Khojah, E., Alharbi, M., AM Al-Mushhin, A., Saeed Alkaltham, M., Mohammad Salamatullah, A., ... & Helal, M. (2021). Functional effects of pomegranate peel extracts on milk: Antibacterial measurements, antioxidant activities, and photochemical characterizations. *Journal of Biobased Materials and Bioenergy*, *15*(5), 571-579.

[55] Shabani, M., Mokhtarian, M., Kalbasi-Ashtari, A., & Kazempoor, R. (2021). Effects of extracted propolis (Apis mellifera) on physicochemical and microbial properties of rainbow-trout fish burger patties. *Journal of Food Processing and Preservation*, *45*(12), e16027.

[58] Tai, K., Rappolt, M., Mao, L., Gao, Y., Li, X., & Yuan, F. (2020). The stabilization and release performances of curcumin-loaded liposomes coated by high and low molecular weight chitosan. *Food Hydrocolloids*, *99*, 105355.

[59] Liu, F., Avena-Bustillos, R. J., Chiou, B. S., Li, Y., Ma, Y., Williams, T. G., ... & Zhong, F. (2017). Controlled-release of tea polyphenol from gelatin films incorporated with different ratios of free/nanoencapsulated tea polyphenols into fatty food simulants. *Food Hydrocolloids*, *62*, 212-221.

[DOI: 10.22034/FSCT.22.158.99]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

امکان‌سنجی تولید نوشیدنی انرژی‌زا فراسودمند حاوی کیتوزم تانن- اسیدلینولئیک کانژگه

مرتضی جمشید عینی[۱]، حمید توکلی پور*[۲]، رضوان موسوی نادوشن[۳]، محسن مختاریان[۴]

۱- گروه علوم و صنایع غذایی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران

۲- گروه علوم و صنایع غذایی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران

۳- گروه علوم و صنایع غذایی، دانشکده علوم زیستی، واحد تهران شمال، دانشگاه آزاد اسلامی، تهران، ایران

۴- گروه علوم و صنایع غذایی، واحد رودهن، دانشگاه آزاد اسلامی، رودهن، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۳/۵

تاریخ پذیرش: ۱۴۰۳/۷/۹

کلمات کلیدی:

اسید لینولئیک کنژوگه،

تانن،

کیتوزوم،

عصاره پوست انار،

نوشیدنی انرژی‌زا

DOI:10.22034/FSCT.22.158.99.

* مسئول مکاتبات:

tavakolipour@gmail.com

چکیده

امروزه با توجه به علاقه برای مصرف مواد غذایی فراسودمند و گسترش روز افزون بیماری‌های قلبی و عروقی، تمایل به مصرف محصولات فراسودمند افزایش یافته است. بنابراین محققان به دنبال بهینه‌سازی فرمولاسیون‌های جدید محصولات با ویژگی‌های عملگرا هستند و در این راستا. مطالعه حاضر با هدف تولید نوشیدنی انرژی‌زا فراسودمند حاوی کیتوزم تانن- اسیدلینولئیک کانژگه انجام شد. پس از استخراج ترکیبات زیست فعال (بویژه تانن) از پوست انار همراه با اسید لینولئیک کنژوگه در ساختار کیتوزوم‌ها گنجانده شدند. پس از بررسی پایداری اکسیاشی اسید لینولئیک، بررسی طیف‌سنجی FTIR و ریخت‌شناسی کیتوزوم‌ها، به میزان ۰/۷۵ گرم در فرمولاسیون نوشیدنی‌ها افزوده شدند. نتایج تأثیر معنادار غلظت‌های مختلف عصاره زیستی انار بر پایداری اکسیداتیو اسید لینولئیک را نشان داد. بررسی طیف‌سنجی FTIR بیانگر وجود ساختارهای زیست فعال انار و اسیدلینولئیک در کیتوزم‌ها بود. ذرات تقریباً کروی کیتوزان با اندازه nm ۷۷/٦٦ و ۷۹/۹۰ با یکنواختی مشاهده شد. افزودن نانو کیتوزم‌ها سبب افزایش معنادار pH (کاهش اسیدیته)، افزایش کدورت، ویسکوزیته، دوفاز شدن و محتوی فنلی را نشان داد. همچنین کاهش شاخص L* و افزایش a* و b* در نوشیدنی‌ها گزارش شد. نتایج حاضر نشان داد که پلیمرهای زیستی نقش کلیدی در پایداری ساختار غشای لیپوزومی و انتشار پایدار مولکول‌های به دام افتاده توسط یک مانع فضایی بر روی سطح دارند. این یک سکوی بالقوه را جهت طراحی مناسب حامل‌ها برای مواد مغذی یا نگهدارنده‌ها به منظور افزایش ماندگاری و ایمنی ماتریس‌های غذایی فراهم خواهد کرد.