Extending the shelf life of soft cheese using nanoencapsulation

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Abstract

The study conducted to use the encapsulation of Nigella sativa oil to increase its thermal stability and making it more resistant to oxidation, lipolysis and use it to enrich soft cheese. Nigella Sativa oil particles was prepared by encapsulating oil with chitosan nanoparticles (NSP) by using the ionotropic gelation technique. The prepared size of the nanoparticles was 70.93 nm. This study included Four treatments to produce soft cheese. In the 1st treatment(M1) 0.5% of the solution was added to NSP particle pasteurized milk (m1), while the 2th treatment(M2) treatment 1% of liquid solutions to NSP was added to pasteurized milk. A 0.5% and 1% of liquid solutions NSP were added to cared treatments (M3 and M4), respectively. The 5th treatment (M5), the soft cheese was used as a control and left without additions. All treatments were kept in cooling at a temperature of 7 ± 2o C for 14 days for conduct the physiochemical and microbiological examinations. The results showed that the coating of Nigella sativa oil with chitosan and in the nano capsulate form increase the resistance of this oil to the manufacturing conditions, which led to an increase in its resistance to heat and oxidation processes. Also, the addition of nanoparticles to soft cheese in two percentages 0.5 & 1% caused increase the preservation ability of cheese during the 14 days of cooling storage.

Keywords: Nanotechnology, Coating, Nanoencapsulation, Nigella Sativa oil, Soft cheese.

Introduction

Nigella sativa is a spice plant of family Ranunculacea, commonly known as black cumin or black seed. It is an erect herbaceous annual plant. It grows in Mediterranean countries and Asian countries including India, Pakistan, Indonesia. The seeds of N. sativa are used by the Indian people in pickles as spice and food preservative, while in Egypt these are used as carminative and flavoring agents in bread. Black cumin oil prepared by compressing the seeds of N. sativa is also used for cooking. For centuries, the seeds have been used for medicinal purpose [1,2,3,4]. Nigella sativa has many different chemical ingredients including thymoquinone (TQ) (30- 48%), flavo-

noids, anthocyanins, alkaloids and essential fatty acids, particularly linoleic and oleic acid (5). Previous studies have indicated many medical properties of black seeds, including immunomodulatory activities as well as anti-inflammatory, antimicrobial and antioxidative effects [6]. Research results indicated that there are no toxicity or side effects when using black seed oil in medical treatments [4, 5]. Nanoparticles were produced from natural polymers have been widely used in the pharmaceutical and food industries. These systems are biocompatible, biodegradable, and low toxic including chitosan. Chitosan is a molecule similar to cellulose which could improve the dissolution of drugs with low solubility in water, controlling drug release, improving drug targeting and increasing absorption, as well as protecting against degradation of the encapsulated compound (7). Chitosan polymers have gained more attention from chemists, physicists and engineers to develop a variety of structures containing chitosan nanomaterials. The biological arrangement of chitosan is the second most abundant in nature after cellulose, and it is a functionally versatile biopolymer due to the presence of amino groups responsible for the various properties of the polymer. It has been used in many industrial applications. In recent studies, it has been used as a biodegradable food packaging material (8), Chitosan can be used to prepare nanoparticles due to the fact that chitosan is a natural polymer that is degradable, readily available, cheap and non-toxic. Chitosan nanoparticles can be used in the pharmaceutical industries as an antimicrobial agent and a means of transporting and delivering the drug into the body in a safe way (9). In recent years, chitosan nanoparticles as Nano transmitters have gained great attention due to their biodegradability, biocompatibility, and nontoxicity. Chitosan nanoparticles containing vitamins, flavors and enzymes that could maintain their activity. The present study aim is to encapsulate Nigella Sativa oil for protecting from the effects o manufacturing processes and develop its thermal stability as well as use these nanocapsules to enrich the processed soft cheese with Nigella Sativa oil with protecting from oxidation processes, thus extend its storage life without affecting consumer acceptance. cheese with Nigella Sativa oil with protecting from oxidation processes.

Materials and Methods

Preparation of Nigella Sativa oil Coated with Chitosan Nanoparticles:

Nano chitosan (supplier from the Chinese company Sagherb) was used with a molecular weight of 161.2 kD and the percentage of acetylcholine removed was 95.7%. Nigella sativa was obtained from the local markets of Baghdad city, and the oil was extracted from it by a cold hydraulic press. The method of Deng *et al*., (10) was used to prepare nanoparticles of Nigella sativa oil and chitosan.

Diagnosis of Nigella sativa oil particles coated with chitosan nanoparticles

Determination of the size of Nigella sativa oil particles coated with chitosan nanoparticles was estimated by the technology of atomic force microscopy (AFM), which showed the size and dimensions of the nanoparticles using the method of Grobelny *et al*., (11). The Zeta Potential was estimated using the Zeta Plus device. Determination of the surface shape by using SEM microscopy.

Measurement of encapsulation efficiency

To determine the encapsulation efficiency of nigella oil after being coated with nano-chitosan, the amount of encapsulation chitosan of nigella oil was estimated in light of comparing the difference in optical absorption of the total solution containing only nano-chitosan and the solution containing nigella oil. The absorbance using a UV − VIS Spectrophotometer Genesys10 − (Thermo Fisher Scientific, Walthman, MA, USA) at a wavelength of 244nm, then the encapsulation efficiency was calculated using the following equation:

% efficiency = absorbance of the first solution - absorbance of the second solution / absorbance of the first solution × 100

Manufacture of soft cheese treatments

Method of Al-Dhaan (1983) was adopted in the manufacture of soft cheese treatments, in which the prepared Nigella sativa nanoparticles were added to the pasteurized milk at rates of 0.5 and 1% in the treatments M1 and M2, and the particles were added in the same proportions to the curd resulting from milk coagulation before Adding salt to it to prepare the two treatments M3 and M4, and the fifth treatment (M5) was left without adding Nigella sativa particles to it to be a control. The cheese treatments were stored in refrigeration at a temperature of 7 ± 2 C for the purpose of studying the effect of adding black seed nanoparticles on the shelf life of the cheese through the necessary testing procedures.

Chemical, microbiological analysis and Sensory evaluation

The acidity of fat in the processed soft cheese was determined according to the Frankel and Tarassuk (13) method, and the peroxide value was determined according to the method reported by Pearson (14).The method of pouring dishes mentioned in APHA (15) was followed by using the solid nutrient media(Nutrient agar) in estimating the total number of microorganisms (total plate count), and the number of Psychotropic bacteria was estimated using Nutrient agar according to the aforementioned method (15), and The pH was estimated using a pH meter as reported in A.O.A.C (16).

Results and Discussion

Determination of Nigella sativa oil nanoparticles

The results of measuring the size of the particles of Nigella sativa oil coated with chitosan nanoparticles prepared by the ionotropic gelation method, showed that the size of the prepared particles was 70.93 nanometers, which indicates that these particles were within the nanoscale. The preparations processes of the nanoparticles including homogenization, mechanical mixing and treating with high sound waves (Sonication) at intervals of 6 minutes to break nanoparticles into smaller sizes, and this process aims to enhance the oil packaging process more. Cai *et al*., (17) showed that Ultrasound waves have an effect in reducing the size of nanoparticles due to the physical bonding between nanoparticle (figure1).

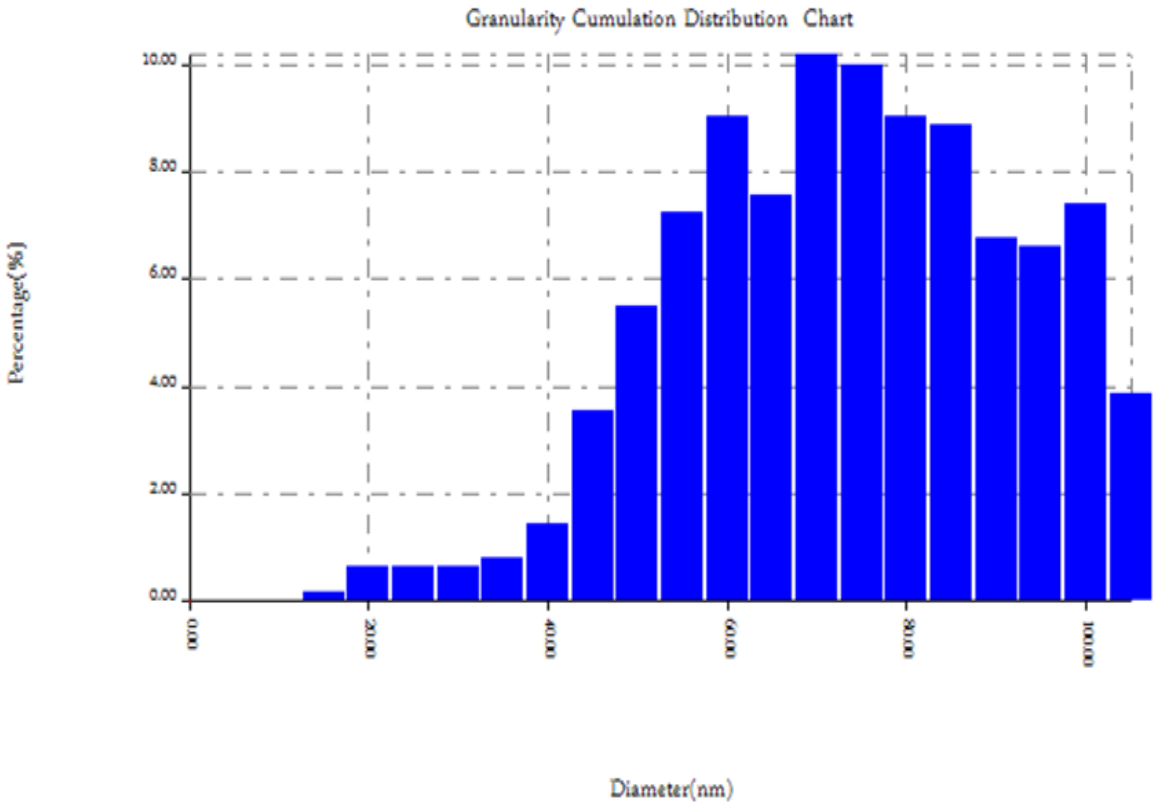

Figure (1): A graph showing the relationship between the percentage of nanoparticle size to diameter in nanometers that were prepared using (200 µl TPP + 600 µl nano-chitosan) using AFM atomic force microscopy

Encapsulation efficiency

The results showed that the coating efficiency was 68%., as the coating efficiency reached 67.2%. These results are in agreement with the results of Zhang et.al. (18) which indicated that the encapsulation efficiency of insulin ranged between 58-80%.

Xu and Du(19) mentioned that the viscosity of chitosan affected the encapsulation efficiency, which makes encapsulation more difficult because the increased viscosity may impede the encapsulated material to diffuse in solution Chitosan, so that the encapsulation efficiency is reduced.

Determining of the shape of the nanoparticles using a scanning electron microscope

The shape of the Nigella sativa oil particles coated with chitosan nanoparticles was investigated using a Scanning Electron Microscope (SEM) (Figure 2).The figure shows that the dominant shape is spherical granules . which is due to The presence of chitosan and TPP had a great effect on the size and shape of the nanoparticles.

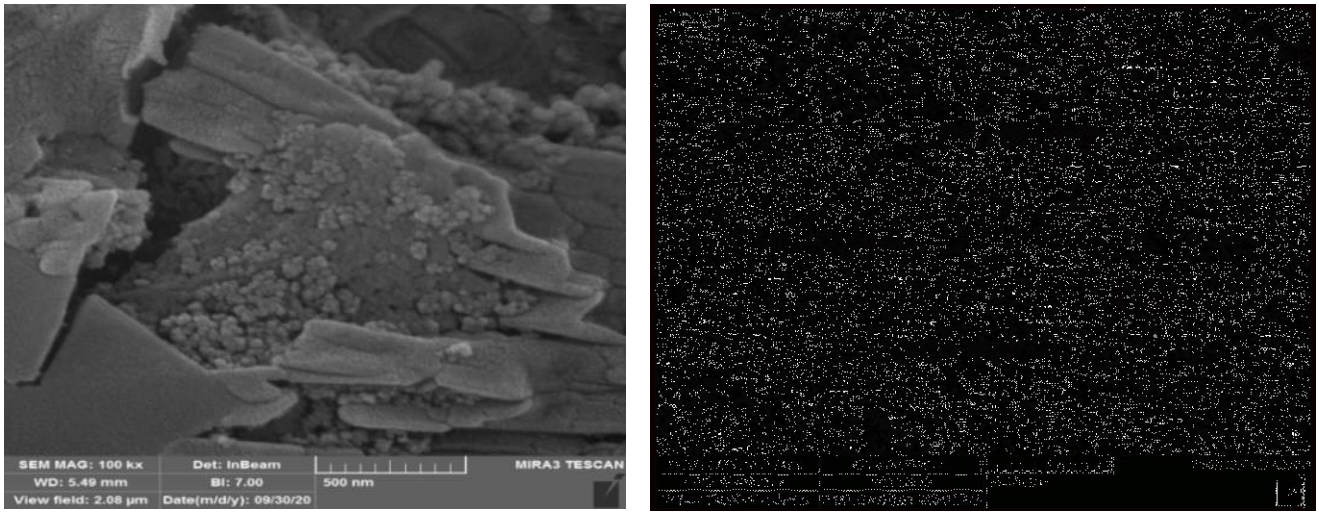

Figure (2): shows the shape of Nigella sativa oil particles coated with chitosan nanoparticles using SEM microscopy with magn ification power of 200 and 500 nm

Figure (3) shows the value of the zeta potential was milli 47.55 volts ,The appearance of positive charges on the surface of nanoparticles is due to the amine groups in chitosan. According to Singh and Lillard (20) nanoparticles whose zeta potential is above ± 30 millivolts are stable in suspension, while the surface charge reduces its pooling. Similar charges cause the particles to repel each other, while different charges cause the particles to be attracted to each other.

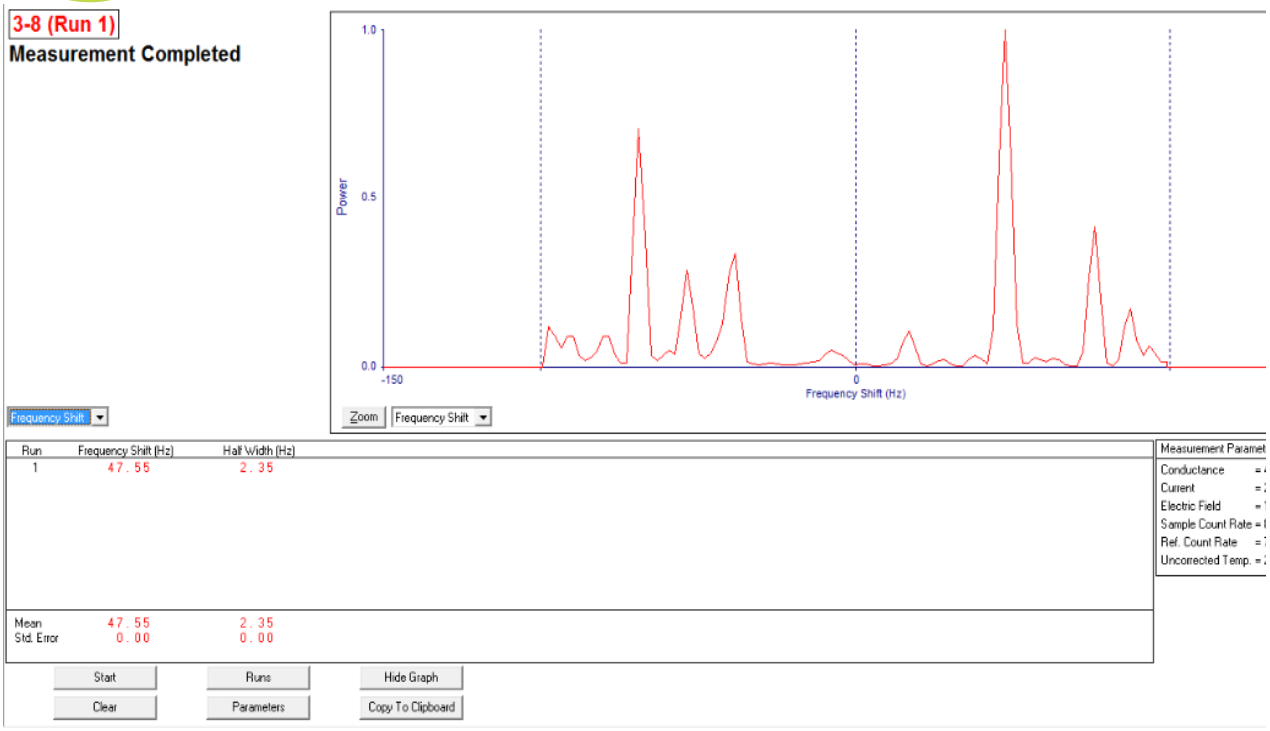

Figure (3): shows the value of the zeta potential (millivolts) measured by the Zeta Potentia Analyzer

pH ranges

The results of pH for all soft cheese treatments were ranged between 6.4 – 6.5 ,immediately after processing. There was a gradual decrease in the pH after the treatments were refrigerated (7±°C / 14 days) with the duration of preservation and that the least evolution in acidity was in the treatment of cheese at zero time that treated NSP, The highest development in the proportion of acidity In the M5 treatment These results can be explained that Nigella sativa nanoparticles has an effect on the work of bacteria that ferment lactose, which leads to reduce the production of lactic acid, which in turn is reflected in low values pH with increasing storage age. the work of bacteria that ferment lactose, which leads to reduce the production of lactic acid.

Table (1): Results of pH for soft cheese treatments treated at 0.5 and 1% of for different times during the preservation period at 7±2°C and 14 days

Treatment	days			
	0	5	10	14
M1	6.4	6.2	6.1	5.5
M2	6.4	6.2	6.0	5.7
M3	6.4	6.3	6.1	6.0
M4	6.5	6.5	6.3	6.1
M5	6.5	5.8	5.3	-

Lipolysis

Table (2) shows that the ADV values of soft cheese enriched with NSP (0.5 -1)% ranged from 0.71 to 1.87 mMm for 100 g fat, which indicates that there are no high differences between them. All treatments show high ADV values after storage at 7 ± 2 °C for 14 days due to the effect of lipolytic factors caused by fat-producing enzymes. These results are in agreement with Abdel-Baqi et al. (21) who reported that ADV for fats advances the storage period where the source as well as lipolysis by decomposing the lipase enzymes produced by bacteria and the initiator and lactose fermentation which is mainly responsible for the primary synthesis of acetic acid and propionic acid. The results also show that the higher percentage of nanoparticles added the lower the amount of evolution in the ADV values, it was also observed from the table that there were significant differences in the ADV values between the NSP-added treatments and the control treatment at the end of the preservation period, but no such differences were observed between the treatments The four (M1 , M2. M3 and M4) factors during the conservation period. that refers the role of utilization Nigella sativa oil which coated with Nano chitosan in maintaining from lipolysis developments of soft cheese and thus its shelf life.

The results (table 2), indicated that the control treatment show highest increase in Peroxide Value (POV) ASP ,and less developed was in the treatments that supplemented with ASP indicating the active effect of using chitosan to coat the NS by forming a protective layer for Nigella Sativa oil against oxidation and thus the formation of peroxides. The results reveled that the addition of Nigella sativa oil coated with Nano chitosan to some treatment gave a good peroxide value and slowly with slightly high which indicates that the coating process was contributed for preserving the processed cheese and giving its resistance to oxidation processes throughout the storage period.

Table (2): Acid degree value ADV (mMm for 100 g fat) and POV (meq/ kg fat) for soft cheese treatments treated at 0.5 and 1% of Nigella sativa nanoparticles for different times during the preservation period at 7±2°C and 14 days

Treatment	test	Days			
		0	5	10	14
M1	ADV	0.80	1.01	1.11	1.35
	POV	0.29	0.39	1.18	2.05
M2	ADV	0.81	1.07	1.16	1.87
	POV	0.30	0.35	1.09	1.65
M3	ADV	0.80	1.05	1.31	1.59
	POV	0.30	0.35	1.29	1.54
M4	ADV	0.73	1.o4	1.27	1.49
	POV	0.30	0.33	1.19	1.43
M5	ADV	0.71	1.01	1.19	1.29
	POV	0.31	0.44	1.37	2.45

Table (3) shows increase in the ratio of non-protein nitrogen to total nitrogen in the all treatments of cheese. It is noted that the ratio of NPN to total nitrogen was higher especially in the final stages in the treatment M5 compared to the treatments M1, M2, M3 and M4 The same table also shows increase in NPN ratios in M1 and M2 treatments compeer. This is due to the properties of the NSP against microorganisms especially proteolytic bacteria.

Table (3): Percentage of non - protein nitrogen to total nitrogen for soft cheese treatments treated at 0.5 and 1% of Nigella sativa nanoparticles for different times during the preservation period at 7±2°C and 14 days

Treatment	Days			
	0	5	10	14
M1	14.21	15.31	20.38	25.01
M2	14.21	15.28	20.11	23.08
M3	14.21	15..05	19.48	20.01
M4	14.21	14.58	16..44	19.30
M5	14.21	15.43	23.21	-

Microbiological tests

To identify the role of lipolytic and proteolytic bacteria and follow the development in the growth of Psychrophilic bacteria, which also have a role in the production of lipoproteins and proteins in milk and milk products (22). Refrigerated, the results of these tests showed that soft cheese that was treated with ASP, which is made from

pasteurized milk, the numbers of lipolytic , proteolytic and Psychrophilic bacteria (m1, m2) ranged in numbers 2.1.2 and 6×10^2 (cfu gm) respectively after manufacturing, but As the preservation period progressed, a clear development was observed The development of these three bacteria continued to the end of the preservation period of 14 days, while we find that the development in the preparation of these three types of bacteria was more in cheese treatments that were treated with ASP during the period of preservation, as the results of these tests showed that the increase in the proportion of ASP in cheese helped to reduce the number of lipophilic bacteria and proteolytic bacteria more, with the complete elimination of Psychrophilic bacteria in treatments m3 and m4 whish that added of 0.5 and 1% from ASP to their curd.

The results showed that the treatments with added Nigella sativa oil particles coated with chitosan nanoparticles were the least compared to their counterparts without addition due to the fact that the use of the technology of encapsulating the oil with chitosan nanoparticles had distinct characteristics represented by improving the stability of this oil as well as the role of chitosan as an anti-microorganism agent with prolonging the shelf life of the cheese fortified with those nanoparticles.

Table (4): Results of tests for proteolytic and lipolytic bacteria for soft cheese treatments treated at 0.5 and 1% of Nigella sativa nanoparticles for different times during the preservation period at 7±2°C.

Treatment	days	Count of bacteria (cfu gm)		
		Psychrophilic	Protolytic	Lipolytic
M1	1	6× 10 2	1.2× 10 2	2× 10 2
	5	7× 10 2	1.8× 102	4× 10 2
	10	16× 10 2	4× 10 2	16×10 3
	14	21× 10 2	8× 10 3	8× 10 3
M2	1	4× 10 2	1.3× 10 2	5× 10 2
	5	6× 10 2	1.5× 10 2	7× 10 2
	10	9× 10 2	2× 10 2	6× 10 3
	14	15× 10 2	6× 10 3	25× 10 3
M3	1	Nill	3× 10 2	4 10 2
	5	Nill	8× 10 2	13×10 2
	10	Nill	13× 10 2	25×10 2
	14	Nill	9× 10 2	13×10 3
M4	1	Nill	5× 10 1	6× 10 2
	5	Nill	9× 10 1	6× 10 2
	10	Nill	6× 10 2	6× 10 2
	14	Nill	13× 10 2	6× 10 2
M5	1	6× 10 2	1.2× 10 2	2× 10 2
	5	17× 10 2	1.3×10 2	5× 10 2
	10	92× 10 2	6×10 2	6.8×10 2
	14	98× 10 2	5×10 3	2× 10 3

The results indicated that the coating of Nigella sativa oil with chitosan in the form of nano helped to increase the resistance of oil to the manufacturing conditions, which led to an increase in its resistance to heat and oxidation processes. Also, the addition of nanoparticles to soft cheese in two percentages 0.5 & 1% helped to increase the preservation ability of the cheese, so that it could be storage for up to 14 days.

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