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USE NANOTECHNOLOGY IN CAPSULATION OMEGA-3 FATTY ACID TO IMPROVE ITS THERMAL STABILITY AND USE IT TO ENRICH PASTEURIZED MILK

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ABSTRACT

The study involved the encapsulation of Alpha-Linolenic Acid (ALA) to increasing its thermal stability and making it more resistant to oxidation and lipolysis and use it to enrich pasteurized milk. The Nano Capsules of Alpha-Linolenic Acid (NC-ALA) were prepared using the emulsion-diffusion method, by adding acetone organic solvent containing Poly Lactic Acid (PLA) and ALA to the water phase containing the stabilizer Twain 20. NC-ALA was produced in irregular colloids shapes and nanoparticles ranging from 68.4 to 302.6 nanometers (nm) and the highest ratio was in the suspension at 143.9 nm and its zeta potential was -38.67 mV. Concentrates 5, 10 and 30 mg of NC-ALA and Non-Capsulated ALA (Non-C-ALA) were used to enrich 1 kg of raw milk to prepare pasteurized milk treatments M1, M2, M3, M4, M5 and M6. Mc treatments was left without addition. The results showed that the treatments supplemented with NC-ALA were less developed in the ratio of titration acidity and Peroxide Values (POV) from Non-C-ALA treatments. The PLA coat had a significant role in the protection of ALA against lipolysis by formation of a protective layer protects the ALA from the activity of lipases enzymes, and the addition of ALA to milk was determined the growth of microbial in it. The results of the sensory evaluation showed that M1 and M2 supplemented with 5 and 10 mg of NC-ALA respectively maintained their sensory characteristics during the storage period, with a total grade of 20 of 24 at the end of the storage period which was 7 days at temperature 6±1 ºC, indicating that the capsulated of ALA and its addition to pasteurized milk before the pasteurization process helped preserve it for a longer period without affecting in its various qualities.

Keywords: Nanoencapsulation, Omega-3, Pasteurized milk.

1. INTRODUCTION

Nanotechnology offers many benefits to the food sector, such as providing new sensory properties of products, less fat use, enhanced nutrient stability and absorption, intelligent packaging, improved shelf life, bacterial identification and food quality control using smart sensors (Weiss *et al*., 2006; Chaudhry *et al*., 2008; Neethirajan and Jayas, 2010). A study by Helmut Kaiser Consultancy showed that revenue from the nano food market rose to $ 2.6 billion in 2003 and is expected to rise to $ 7 billion in 2015 and to $ 20.4 billion in 2020 (Helmut Kaiser Consultancy, 2010).

According to Quintanar *et al*., (2005), preparation of nanocapsules by the emulsion-diffusion method requires three phases: organic, aqueous and dilution. Mora-Huertasa *et al*., (2010) mention to prepare nanocapsules using the emulsion-diffusion method, the organic phase is emulsified under high shear agitation in the aqueous phase. The addition of water to the system cases the diffusion of the solvent into the external phase, resulting in nanocapsule formation. The solvent and part of water can be eliminated by evaporation under reduced pressure. Emulsion-diffusion method usually produces nanocapsules in size of approximately 150-200 nm.

Nanoencapsulation has provided bioactive materials with both in vitro and in vivo advantages. In vitro, water solubility, storage and thermal stability, various sensory attributes have been improved by nanoencapsulation. For instance, lipophilic drugs and bioactive food components have been made more water soluble by encapsulation with hydrophilic coating materials. (Aliabadi *et al*., 2007; Byun *et al*., 2011).

Different types of particles can be obtained depending on the physical and chemical properties of the nucleus, the composition of the wall, the microencapsulation technique used as simple particles, particles surrounded by a single thickness, particles of irregular shape, embedded in a continuous matrix of materials, multi-core particles and multi-wall capsules (Gibbs *et al*., 1999).

The long chain unsaturated fatty acids include the two main acids: linoleic (Omega-6 18:2) and linolenic acid (Omega-3 18:3), which are essential for human health as they can't be synthesized in the body, which its requires from food (Ethier, 2010; Ortega, 2007; Burdge and Calder, 2005).

Linolenic Acid ($C_{17}H_{29}COOH$) is the largest part of Omega-3 fatty acids. It is composed of eighteen carbon atoms and contains three double bonds. It is the main source of Omega-3 fatty acids (NIOH,

2004). The Omega-3 fatty acids are oxidized and degraded by heat, so the nutritional value and shelf life of the food will be reduced in foods that have been enriched with acid as a result of the processing and storage processes of these foods (liu *et al*., 2010).

Studies have confirmed that n-3 fatty acids may prevent the onset of arterial stenosis that occurs in 25-40% of patients undergoing catheterization due to the concentration of cholesterol in the blood (Kris-Etherton *et al*., 2002). It also works to reduce triglycerides and blood cholesterol (Davidson *et al*., 2007).

Nanocapsules are a new method to protect nutrients and biologically active food ingredients from different harsh environmental conditions (Habib, 2010). Therefore, there is a need to study the possibility of applying this method to preservation Omega-3 fatty acids in foods that use with it different thermal treatments, and illustration some aspects of Omega-3 nanocapsules related to their degree of degradation, their thermal stability, the effect of time and temperature on the storage viability of each of these compounds and the foods containing them, and because the milk is poor foods from Omega-3 fatty acid multiple benefits, also The use of high temperature in preparation of milk will affect the Omega-3 fatty acid when added it in free form, so the idea of this study was to encapsulate the ALA with PLA to protect it against rancidity and oxidation processes and to develop its thermal stability and then use these nanocapsules to supplemented Pasteurized milk to fill part of the consumer's consumption required of this acid without affecting the consumer's acceptance of it.

2. MATERIALS AND METHODS

2.1. Preparation of the nanocapsules

The ALA nanocapsules were prepared as dispersion by modified emulsion-diffusion method (Quintanar *et al*., 2005). With a change in the proportions of the materials used, taking 90 mg of PLA (molecular weight 30000 g/mol from Nature Work, China) and solvent in 6 ml of organic solvent acetone (from Merck, Germany) with stirring at 35°C to facilitate dissolution of PLA And 70 µl of ALA (molecular weight 278.44 g / mol from Aladdin, China) solvent in 6 ml of the same organic solvent, the solvent content PLA was mixed with the solvent content ALA to prepare a mixture called organic phase It was slowly added drop-wise to the water phase consisting of 1% Tween 20 and with ratio 1:5 (organic phase: aqueous phase) with high shear mixing using rotor-stator device (Silent Crusher M, Heidolph, Germany) rpm = 16000 For 20 minutes, the mixtures was diluted up to 350 ml with distilled water and leave a period of time to allow for the spread of particles in the solution. This phase is called the dilution phase. Organic solvent and some water were removed under vacuum using rotary evaporator at 35°C, nanocapsule dispersions were kept in a refrigerator (6± 1°C) until analysis and characterization.

2.2. Study of thermal stability: The thermal stability of nanocapsules was studied for the purpose of determining the extent of their resistance to the thermal processes used during the manufacture of various dairy products, and used the method cited by (Amr, 1990; Amr and Abu al-rub, 1995) for that, the solution containing the prepared nanocapsules was taking and placed in test tubes 10 ml for every tube, the test tubes were subjected to different temperatures and different times for the purpose of following the thermal stability as in the table below:

Table 1: Shows the thermal treatments of anocapsules.

Tube number	Temperature degree ºC	Time / minutes	Device used
N1	65	30	Water bath
N2	65	60	Water bath
N3	85	30	Water bath
N4	85	60	Water bath
N5	121	15	Autoclave (from Lab Tech, Korea) at pressure 1.5 lb/inch^2

At the end of the specified time, the tubes were cooled and then kept at a temperature of (6±1°C) until the required analysis and characterization tests were performed, which included the determination of the size and shape of the nanocapsules using the Scanning Electron Microscope and particle size analyzer and zeta potential as will be mentioned later.

2.3. Characterization of Nanocapsules

2.3.1. Particle size determination: Particle size was measured by dynamic light scattering technique, using the NanoBrook 90 Plus Particle Size Analyzer, Windows Software Ver. 5.34. A sample of the prepared solution was placed in a special tube of the instrument and the particle size was measured.

2.3.2. Zeta Potential Determination: Zeta Potential was measured by using NanoBrook Zeta

plus Zeta Potential Analyzer. A sample of the prepared solution was placed in a special tube of the instrument and the Zeta Potential was measured.

2.3.3. Scanning Electron Microscope (SEM) Observation: Appearance of the nanocapsule population was visualized by SEM. A drop of the nanocapsule dispersion was deposited on an aluminum slide, let to dry at ambient temperature. Samples were analyzed with a SEM model EFI S50, Holland.

2.4. Pasteurized Milk Manufacture: In the research, cow milk was used as a mixture of the morning meal from the animal field of the Animal Resources Department at the College of Agriculture, University of Baghdad. The milk was transported directly to the laboratory and cooled, tested and manufactured.

Seven treatments of pasteurized milk were manufactured, including M1, M2 and M3, supplemented with 5, 10 and 30 mg of NC-ALA / kg of raw milk respectively and was put in glass bottles. And M4, M5 and M6 supplemented with Non-C-ALA in the same previous concentrations as positive comparison treatments, leaving the treatment Mc without addition (negative control treatment). The pasteurization process was carried out in a water bath at 63 °C for 30 min. After finishing the pasteurization, the bottles were refrigerated and stored at a temperature of 6 ± 1 °C for seven days for check the chemical, microbiological analysis and sensory evaluation.

2.5. Pasteurized Milk Analysis: The percentage of fat was estimated using the Babcock method, total titration acidity and the pH was estimated by using a pH-meter according to A.O.A.C. (2008). The peroxide value was determined by extracting the fat from 35 ml of milk by 10 ml of BDI (30 g of Triton X-100 with 70 g of sodium hexameta phosphate and diluted the volume to 1000 cm^3 with distilled water) and completed the steps as in the Bureau of Dairy Industry-BDI (Deeth and Fiz-Gerald, 1976), the peroxide value was estimated according to the method cited by (Pearson, 1976). The concentration of free fatty acids in milk was estimated according to the method cited by (Frankel and Tarassuk, 1955), the method of total plate count cited by (APHA, 1978) was followed by use Nutrient Agar in estimating the total plate count of micro-organisms. The MacConkey agar medium was used to estimate the count of coliform bacteria and PDA (potato dextrose agar) was used to estimate the count of yeast and molds. These-analysis were used after manufacture and throughout storage period. The sensory evaluation of pasteurized milk samples was conducted by experienced evaluated in the Department of Food Science, college of Agriculture, University of Baghdad, and used the pasteurized milk assessment forms cited by (Horner *et al*., 1980).

3. RESULTS AND DISCUSSION

3.1. Formation of nanocapsules of ALA: The formation of ALA involved the addition of the organic solvent which contains the PLA and ALA to the aqueous phase (continuous phase) which contains the stabilizer Tween 20. The nanocapsules were formed spontaneously in the continuous phase when the organic solution containing PLA was added, causing a transparent dispersion in the solution. These nanocapsules were produced due to variations in surface tension between the aqueous and organic phases, which result in interfacial disturbances in the system, leading to the continuous solvent flow away from regions of low surface tension and the aggregation of polymer on the hydrophobic surface and thus the formation of nanocapsules, The use of stabilizer is also important for the formation of nanocapsules in this method. The coat of nanocapsules consist of an adsorbed surfactant layer (stabilizer / emulsifier) while ALA and polymer are dissolved in the inner spheres (Quintanar *et al*., 1998). Some researchers have experimented about the effect of supplemented by Omega-3 fatty acids on the age of the storage and the stability of different types of foods such as milk, eggs, and spaghetti. They found that supplemented of food by Omega-3 fatty acids cause food to become more susceptible to deterioration and the development of rancidity smell due the oxidation of Omega-3fatty acids (let *et al*., 2005; Betancourt and Diaz, 2009; Verardo *et al*., 2009). The addition of antioxidants or the use of microencapsulation techniques have been suggested to reduce this problem (Kolanowski *et al*., 2004; Jacobsen *et al*., 2008).

3.2. Characterization of Nanocapsulse: The size of capsules was measured by Particle Sze Analyzer device and was between 68.4-302.6 nm (Table 2). The highest percentage of nanocapsules in the solution was in size 143.9 nm. This size was calculated on the basis of the outer coat on the basis that the capsule Consisting of ALA which is inside and component of the nucleus of the capsule and followed by the PLA which is the protective shield of fat and comes after the stabilizer in outer of capsules (Habib, 2010), the results indicate that the nanocapsules obtained are within the nanoscale, it is agreed that for the biological particles to be considered in the nanoscale, they should have a particle size of less than 500 nm (Quintanar *et al*., 1998). The reason that helped to obtain capsules in

these sizes may be due to the use of acetone as an organic solvent and the Tween 20 as stabilizer as well as the ratio between the organic phase and the aqueous phase that used in this study. Moinard-Chécot *et al*., (2008) referred to the effect of organic solvent acetone in determining the final properties of the synthesis of nanocapsules. Acetone superiority on tetrahydrofuran and N, N-dimethylacetamide was recorded when used as an organic solvent to obtain smaller capsules in studies by Aliabadi, *et al*., (2007) and Horing *et al*., 2009). The use of Tween 20 also helped to obtain the nanocapsules size mentioned and this is consistent with (Yin *et al*., 2009), which used Tween 20 to produce smaller capsules during prepared β-Carotene nanodispersions. Aliabadi *et al*., (2007) reported that the rate of cyclosporine nanocapsules diameter (cyclosporine a hydrophobic drug) increased by decreased the ratio of the organic phase to the aqueous phase.

Table 2: show the size of nanocapsules composed of ALA and PLA (nm), measured by dynamic light dispersion technology and by using Particle Size Analyzer.

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
68.4	26	5	128.4	97	40	195.1	80	75
80.6	44	10	135.9	99	45	210.5	70	80
90.1	58	15	143.9	100	50	229.8	58	85
98.4	70	20	152.3	99	55	256.8	44	90
106.1	80	25	161.3	97	60	302.6	26	95
113.6	87	30	171.2	93	65			
120.9	93	35	182.3	87	70			

3.3. Determination of the Zeta Potential of nanocapsules: Figure 1 showed that the Zeta Potential of the nanocapsules was -38.67 mV. This result is an indication of the nanocapsules stability in the solution because of the high repulsion strength resulting from the negative charge on its surface. This result consistent with (Couvreur *et al*., 2002) that the value of the Zeta Potential when were higher than 30 mV or less than -30 mV will help maintain the stability of the nanocapsules in the suspension and will have sufficient repulsion strength to prevent their aggregation.

Nanoparticles with a zeta potential above ±30 mV have been shown to be stable in suspension, while surface charged reduces their aggregation. It may indicate also whether a charged active material is encapsulated within the core of the nanoparticle or on the shell (Singh and Lillard Jr, 2009).

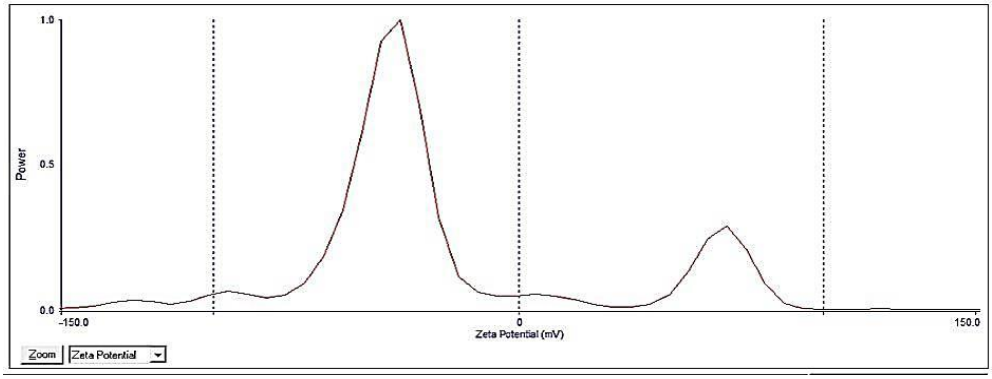

Figure 1: Show the value of the Zeta Potential of the composed of ALA and PLA by using the NanoBrook Zeta plus Zeta Potential Analyzer.

3.4. Determination of the shape of nanocapsules by using SEM: The images in figure (2) showed the shape of the nanocapsules identified by the SEM. The nanocapsules were irregularly shaped. Both the size and shape of the microcapsules formed depend on the materials and methods used to prepare them, like chemical and physical properties for the nucleus materials, the composition of the wall and microencapsulation technique used (Desai & Park 2005, Gouin 2004, Gibbs *et al*., 1999, King 1995, Shahidi & Han 1993).

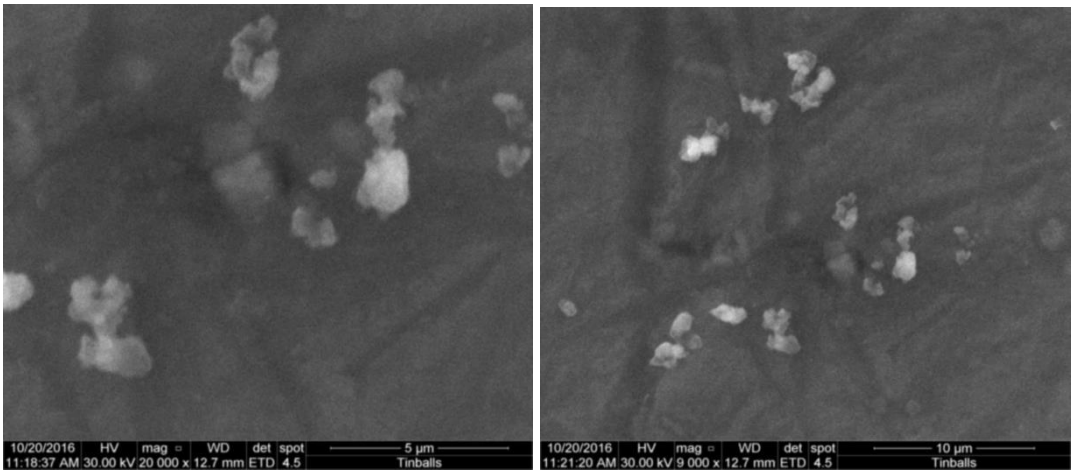

Figure 2: SEM image of nanocapsules composed of ALA capsulated with PLA with the presence of Acetone as organic solvent and Tween 20 as stabilizer.

3.5. Study of Thermal Stability of Nano-particles: The thermal stability of nanocapsules was studied by exposing the solution containing these capsules to a number of heat treatments. The change in the size of the nanocapsules before and after thermal treatments was monitored by measuring the size by the nanometer with the Particle Size Analyzer. As shown in Tables 3, 4, 5, 6, and 7, the thermal treatments that used did not significantly change in the size of the capsules, which ranged between 68.4 to 302.6 nm before exposure to thermal treatments. After exposure to thermal treatments, the size of the capsules ranged between 83.3-340.7, 97.2-295.8, 88.9-335.8 and 82.9-339.5 nm for treatments N1, N2, N3 and N4 respectively, indicating that the nanocapsules have the ability to resistance pasteurization temperatures with no significant change. And that effect is due to PLA shield that used in the capsulation of ALA to maintained the fatty acid from temperature expansion impact, In the treatment of N5, the nanocapsules also maintained their shape with a slight increase in the size of the nanocapsules ranging from 168.7 to 383.4 nm, suggesting an expansion happen in the nanocapsules because the effect of high temperature used. These results are consistent with a study conducted by (Habib, 2010), which showed that ALA capsules that capsulated with PLA had a higher thermal stability than non-capsulated with the percentage of capsules coated, which maintained its size 65% of its original concentration when heated at a temperature of 40ºC For 10 hours compared to 12% for non-capsulated. After 10 hours of heating at 80 °C, the proportion of coated capsules that kept their size were six times more than non-capsulated.

Table 3: showed the size of the nanocapsules (nanometers) is measured by the Particle Size Analyzes of the first treatment (N1) when exposed to temperature 65 °C for 30 minutes.

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
83.2	26	5	151.1	97	40	224.8	80	75
97.3	44	10	159.6	99	45	241.5	70	80
108.1	58	15	168.4	100	50	262.5	58	85
117.4	70	20	177.7	99	55	291.6	44	90
126.2	80	25	187.7	97	60	340.7	26	95
134.6	87	30	198.6	93	65			
142.8	93	35	210.8	87	70			

Table 4: showed the size of the nanocapsules (nanometers) is measured by the Particle Size Analyzes of the first treatment (N2) when exposed to temperature 65 °C for 60 minutes.

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
97.2	26	5	155.6	97	40	213.0	80	75
109.9	44	10	162.4	99	45	225.4	70	80
119.4	58	15	169.5	100	50	240.7	58	85
127.5	70	20	176.9	99	55	261.6	44	90
134.9	80	25	184.7	97	60	295.8	26	95
142.0	87	30	193.1	93	65			
148.8	93	35	202.4	87	70			

Table 5: showed the size of the nanocapsules (nanometers) is measured by the Particle Size Analyzes of the first treatment (N3) when exposed to temperature 85 °C for 30 minutes.

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
88.9	26	5	155.9	97	40	226.8	80	75
102.9	44	10	164.2	99	45	242.7	70	80
113.7	58	15	172.7	100	50	262.5	58	85
122.9	70	20	181.7	99	55	289.9	44	90
131.6	80	25	191.3	97	60	335.6	26	95
139.8	87	30	201.8	93	65			
147.9	93	35	213.4	87	70			

Table 6: showed the size of the nanocapsules (nanometers) is measured by the Particle Size Analyzes of the first treatment (N4) when exposed to temperature 85 °C for 60 minutes.

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
82.9	26	5	150.5	97	40	223.9	80	75
96.8	44	10	158.9	99	45	240.7	70	80
107.6	58	15	167.8	100	50	261.5	58	85
116.9	70	20	177.1	99	55	290.6	44	90
125.7	80	25	187.0	97	60	339.5	26	95
134.0	87	30	197.9	93	65			
142.2	93	35	210.0	87	70			

Table 7: showed the size of the nanocapsules (nanometers) is measured by the Particle Size Analyzes of the first treatment (N5) when exposed to temperature 121 °C for 15 minutes with used pressure 1.5 lb/inch^2 .

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
168.7	26	5	238.8	97	40	300.9	80	75
184.7	44	10	246.4	99	45	313.8	70	80
196.4	58	15	254.3	100	50	329.4	58	85
206.1	70	20	262.4	99	55	350.2	44	90
214.9	80	25	270.9	97	60	383.4	26	95
223.1	87	30	280.0	93	65			
231.0	93	35	289.9	87	70			

3.6. Pasteurized Milk: The chemical, microbial and sensory properties of pasteurized milk were studied during the storage period for 7 days at refrigeration temperature (6±1 ° C) and the results were:

3.6.1. Titration Acidity and pH of Pasteurized Milk: The results indicated in Table (8) that acidity was observed among all pasteurized milk treatments at 1 day of storage, indicating that there were no significant differences between different treatments at this age at the probability level 0.05. This is confirmed by the results of the statistical analysis, at age 3 days showed a slight increase in acidity percentage and continue until the seventh day. The acidity percentage of the milk treatments supplemented with Non-C-ALA and the control treatment was 0.18% and 0.185%, while the development of acidity was lower in the treatments supplemented with NC-ALA it was 0.17% in all treatments.

The pH values of the pasteurized milk treatments were between 6.6 - 6.8 for all treatments at first day, and when storage the treatments at refrigeration temperature, there was a slight decrease in the pH values corresponding to the development of acidity. On day 7, the pH was 6.57 in M1, M2 and M3 treatments and it was 6.52 in the treatments M4, M5 and Mc in the treatment M6 was 6.5, the reason for this simple development in the milk acidity accompanied by low pH values is due to the conversion of part of the milk sugar lactose to lactic acid by lactic acid bacteria (Costa and Conte-Junior, 2015; Costa *et al*., 2016). These results show that the treatments supplemented with NC-ALA were the least developed in acidity of pasteurized milk and thus the possibility to keeping it for longer period.

Table 8: showed the percentage (%) of pasteurized milk acidity was determined as lactic acid and pH during the refrigerated storage period at a temperature of 6±1 °C for 7 days.

The studied character	Storage period (Day)	Treatments							LSD value
		Mc	M1	M2	M3	M4	M5	M6	
Titration acidity (%)	1	0.16	0.16	0.16	0.15	0.16	0.16	0.16	NS
	3	0.17	0.165	0.17	0.16	0.17	0.17	0.17	NS
	7	0.18	0.17	0.17	0.17	0.18	0.18	0.185	NS
LSD value		NS	NS	NS	NS	NS	NS	NS	---
pH	1	6.6	6.6	6.6	6.8	6.6	6.6	6.6	NS
	3	6.58	6.6	6.59	6.6	6.58	6.58	6.58	NS
	7	6.52	6.57	6.57	6.57	6.52	6.52	6.5	NS
LSD value		NS	NS	NS	NS	NS	NS	NS	---

The results represent a repeat rate. NS: No Significant

3.6.2. Peroxide Value of Pasteurized Milk: Table 9 showed the change in Peroxide Value (POV) of pasteurized milk samples during storage at 6±1 °C for 7 days, the results showed no significant differences in the initial POV values for all treatments. After 3 days of refrigerated storage, there was a less increase in POV values for pasteurized milk samples supplemented with NC-ALA it was 0.35, 0.43, and 0.46meq/kg fat (Milli-equivalents (meq)/ kilogram (kg)) for the treatments M1, M2 and M3, respectively, while there was a more increase in the treatments supplemented with Non-C-ALA it was 0.70, 0.74 and 0.79 meq / kg fat for the treatments M4, M5 and M6 respectively. The POV in Mc was 0.38 meq / kg fat, and there was more development in POV values in all treatments after 7 days of storage it was 0.43, 0.70 and 0.80 meq / kg fat in the treatments M1, M2 and M3, and 1.22, 1.37 and 1.45 meq /kg fat for the treatments M4, M5 and M6 respectively compared to 0.66 meq / kg fat in Mc, this development in POV may be due to oxidation in milk fat as well as ALA by the effect of pasteurization temperature (Nawar, 2006).

From these results, we found that the highest increase in POV was in the treatments that supplemented with Non-C-ALA, and less developed was in the treatments that supplemented with NC-ALA indicating the active effect of used PLA to coat the ALA by forming a protective layer protects this acid against oxidation and thus the formation of peroxides.

Table 9: Showed the POV of pasteurized milk treatme-nts during the refrigerated storage period at a tempera-ture of 6±1 °C for 7 days.

Treatment / Age (day)	**POV meq/ kg fat**		
	1	**3**	**7**
Mc	**0.29**	**0.38**	**0.66**
M1	**0.30**	**0.35**	**0.43**
M2	**0.30**	**0.43**	**0.70**
M3	**0.31**	**0.46**	**0.80**
M4	**0.35**	**0.70**	**1.22**
M5	**0.36**	**0.74**	**1.37**
M6	**0.38**	**0.79**	**1.45**
LSD value	**NS**	**0.271 ***	**0.494 ***

The results represent a repeat rate. NS: No Significant, *(P>0.05).

3.6.3. Lipolysis of Pasteurized Milk: Table 10 showed that the Acid Degree Value (ADV) of the lipid at first day of storage was close to all the treatments, and there were no significant differences in the probability level of 0.05, ranging between 0.3-0.4 meq/100g fat, as showed in the same table, there was evolution in ADV With the duration of the storage period up to the seventh day, It was noted that the least evolution in the ADV was 0.44 meq/100g fat in M1, followed by the treatment of M2 which ADV amounted to 0.50 meq/100g fat, while in the rest treatments the ADV increased higher than Mc, with values ranging from 0.67 to 0.75 meq/100g fat compared with Mc which was 0.66 meq/100g fat. These results show the effect of PLA coating in the protection of ALA against lipolysis by forming a protective layer that protects the ALA from the activity of lipase enzymes. The results agree with (AL-Halafi *et al*., 2010) about the increase in the degree of lipolysis of pasteurized milk samples with increased storage periods in temperature at 5 °C and 25 °C. This increase was significant at (p>0.05) Giving the highest rise in the storage period of 6 days.

Table 10: showed The ADV of pasteurized milk treatm-ents during the refrigerated storage period at a tempera-ture of 6±1 °C for 7 days.

Treatment / Age (day)	**ADV meq/100g fat**		
	1	**3**	**7**

Mc	**0.30**	**0.46**	**0.66**
M1	**0.30**	**0.40**	**0.44**
M2	**0.31**	**0.44**	**0.50**
M3	**0.35**	**0.46**	**0.60**
M4	**0.30**	**0.60**	**0.67**
M5	**0.37**	**0.60**	**0.70**
M6	**0.40**	**0.60**	**0.75**
LSD value	**NS**	**0.182 ***	**0.207 ***

The results represent a repeat rate. NS: No Significant, *(P>0.05).

3.6.4. Microbiological Testing of Pasteurized Milk: In table 11, the number of bacteria at first day ranged between 2×10^3 - 8×10^3 cfu./ml (Colony Forming Unit/milliliter), indicating that there were no significant differences in the number of bacteria between the different treatments. Which was set by the Iraqi standard for pasteurized milk (2015), and when follow-up the development in the count of bacteria during the storage period of milk treatments, the number of bacteria ranged between 5×10^3-27×10^3 cfu./ml after 3 days, and 12×10^3 - 35×10^3 cfu./ml at the 7 days. All treatments were kept free from colon bacteria and up to 7 days of refrigerated storage. Coliform bacteria are widespread bacteria and are used as evidence of milk contamination. Their presence indicates insufficient milk thermal treatments or lack of correct methods of manufacturing, and their presence affects the quality of dairy products and causes many problems (Ravaei *et al*., 2013). The Iraqi standard of (2015) stipulates that the number of coliform bacteria should not exceed 10 cfu/ml. The results showed that all the pasteurized milk treatments studied were free from molds and yeast. The results of the total bacterial count, the count of the colon bacteria, yeast and molds indicate the efficiency of the pasteurization process performed for the different milk treatments.

Table (11): showed total bacteria count of pasteurized milk treatments during the refrigerated storage period at a temperature of 6±1 °C for 7 days.

Age (Day) / Treatments	Total bacteria count						
	Mc	M1	M2	M3	M4	M5	M6
1	2×10^3	4×10^3	3×10	6×10^3	2×10^3	7×10^3	8×10^3
3	27×10^3	7×10^3	5×10^3	9×10^3	10×10^3	20×10^3	27×10^3
7	35×10^3	12×10^3	19×10^3	19×10^3	18×10^3	28×10^3	35×10^3
LSD value	11.393 *						

The results represent a repeat rate. *(P>0.05).

3.6.5. Sensory Evaluation of Pasteurized Milk: The treatments of pasteurized milk were evaluated in this experiment to determine the acceptance of the consumer for these treatments from taste, odor and appearance, after supplemented with NC-ALA and Non-C-ALA, the results of the sensory evaluation of pasteurized milk show that the treatments M1 and M2, with a total grade 20 of 24 at the end of the storage period. These treatments maintained their sensory properties throughout the refrigerated storage period, and this due to the presence of PLA coat that limited the oxidative and hydrolysis reactions of the NC-ALA in treatments supplemented with it. The treatment M3 which supplemented with 30 mg of NC-ALA the reason for the low grading rate given to the taste and odor due to the high concentration of NC-ALA used.

Table 12: showed sensory evaluation of pasteurized milk treatments during the refrigerated storage period at a temperature of 6±1 °C for 7 days.

Treatments	Storage period (day)	sensory evaluation			
		Taste (8 degrees)	Odor (8 degrees)	Appearance (8 degrees)	Total (24 degree)
Mc	1	7	7	8	22
	3	6	7	7	20
	7	5	5	7	17
M1	1	7	7	8	22
	3	7	7	7	21
	7	7	6	7	20
M2	1	7	7	8	22
	3	7	7	7	21

	7	7	6	7	20
M3	1	7	7	8	22
	3	6	6	7	19
	7	5	5	6	16
M4	1	7	7	8	22
	3	6	7	7	20
	7	4	3	7	14
M5	1	7	7	8	22
	3	6	7	7	20
	7	3	3	7	13
M6	1	7	7	8	22
	3	6	6	7	19
	7	3	3	5	11
LSD value		1.823 *	2.066 *	2.136 *	4.873 *

The results represent a rate of three replicates. *(P>0.05).

4. conclution

Nanoencapsulation technology can be used by emulsion-diffusion method to encapsulate ALA that belongs to the Omega-3 group by PLA polymers. The method of conservation ALA inside nanocapsules of PLA increases its thermal resistance and stability towards different thermal processes such as pasteurization process, which reaches temperatures to 63 ° C for 30 minutes and sterilization process, which reaches a temperature of 121 ° C for 15 min with pressure also, and gave resistance to this unsaturated fatty acid against the effects of oxidation and hydrolysis of fat rather than Non-C-ALA.

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