



## **DETERMINATION OF THE ACTIVITY OF NANO COATED CONJUGATED LINOLENIC ACID ON THE CHEMICAL COMPOSITION OF LABNEH**

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### **Abstract**

This study was conducted to evaluate the effect of the Nano- and Non-Nano-coated conjugated linolenic acid on the chemical composition of brick during different storage periods. and the sample of Labneh with added linolenic acid at a concentration of (200 mg/kg) (M3) and the sample for cheese to which was added uncoated linolenic acid at a concentration (100 mg/kg) (M4) and the sample of Labaneh to which was added with non-Nano-coated linolenic acid at a concentration (200 The results of storage of labneh showed that the moisture percentage decreased significantly ( $p < 0.05$ ) until the end of the storage period, and the treatment (M3) was less in the percentage of moisture loss (75.14%), and it was also found that the percentages of protein, fat and ash did not Significant differences appear at the time of one day compared with the control sample (M1)), then these percentages gradually increased with the length of the preservation period and reached their highest values at the time of 21 days. As for the pH values, it decreased significantly to be at the end of the storage period between (4.21 - 4.30) compared with the control sample was 4.53 and it was found that the values of fatty acids for all treatments were significantly lower compared to the control treatment (M1)) at the time of one day, and the percentage of acids increased The free fatty acids reached their highest values between (0.25-0.88)% for all treatments compared to the control treatment (M1)) at the end of the storage period, which amounted to 1.70%.





**Keywords:** Linolenic acid, Lactic acid, Nanotechnology, Chemical composition, Control sample, Labneh.

## Introduction

Conjugated Linolenic Acid is one of the essential fatty acids necessary for human health (Omega-3), which the body cannot synthesize, so it must be compensated by eating food sources that contain it (Silwa, 2018). One of the important modern technologies is Nanotechnology, where Nanotechnology has brought about a new revolution in scientific fields, especially recently, especially in the field of food, from the stage of manufacturing and production to the stage of processing, storage and development of innovative and modern materials and products. Nanotechnology includes production, processing and application of materials with sizes less than 100 nanometers (Sozer and Kokini, 2009). The size in nanotechnology is between (1-100 nanometers) (Filippon and Sutherl, 2013) and differs from its counterpart from the raw material used, and that By changing the chemical and physical properties of nanomaterial's, such as changing solubility, diffusion of molecules, change of color and other characteristics, and there are three ways to get Nano-sized particles, namely - physical methods - chemical methods - biological methods (Gupta et al., 2016). One of the advantages of nanotechnology is to extend the shelf life of foodstuffs, improve their qualities, and manufacture safe, high-quality foods (Jayas and; 2011, Neethirajan Sozer and Kokini, 2009) and reduce the percentage of fat use in foods and increase the stability and absorption of nutrients (Weiss) and others, 2006; Chaudhry; and others, 2008 (and other benefits such as masking undesirable odors and flavor compounds associated with foodstuffs and increasing the degree of consumer acceptance of them, Jayas, 2011), and (Neethirajan, 2011) the process of food packaging in recent years has witnessed a wide interest by academics and researchers in food safety and preservation, where food materials have been manufactured Packaging to be edible and biodegradable, and its characteristics have been improved in terms of high ability to retain moisture, gases and undesirable odors (Silwa, 2018), in addition to its effectiveness against microorganisms, yeasts and molds that cause food spoilage and spoilage for the purpose of prolonging storage life and avoiding the use of preservatives harmful to human health Baswal et al. (2020) One of the modern methods of packaging and protecting foodstuffs from different conditions is the use of Nano capsules to encapsulate active biological compounds to obtain stable foods during storage (Silwah, 2018). Most of the materials used in packaging or Nano capsule are gelatin, albumin, alginate and Collagen and alpha-lactalbumin (Reis et al., 2011). Dairy products are among the most consumed foods,



and because they are poor in conjugated linolenic acid, which is of great importance to human health, so the aim of this study is:

Conjugated Linolenic Acid Nano-encapsulation by Poly Lactic Acid-PLA and Gelatin Using Emulsion-Diffusion Method and the use of these Nano capsules in fortification of some commonly consumed dairy products (labneh) to fill part of the required consumer needs of Co-CLA Nanotechnology and uncoated conjugated linolenic acid and a study of its chemical, sensory, biological and microbial properties.

## **Materials and Methods**

### **Preparation of the Nano capsules**

The Nano capsules of conjugated linolenic acid were made using the emulsion-diffusion method as indicated by Salam et al. (2012). Some changes were made in the proportions of the materials after several practical experiments, as 180 ml of polylactic acid with a molecular weight (30000 g / mol) was taken and dissolved in 12 ml of acetone organic solvent with stirring at a temperature of 35 ° C to facilitate dissolution, then 140 mg of conjugated linolenic acid (molecular weight 278.44 g / mol) and dissolved in 12 ml of acetone, the above components were mixed to make a mixture called the organic phase, which is added to the aqueous phase called (continuous phase) consisting of a solution of 1% gelatin with a percentage of (5 :1) (organic phase: aqueous phase) and by a mechanical homogenizer of the type (Rotor-Sterile) at a speed of (3000 rpm) for 5 hours, and the volume was completed after homogenization to 500 ml by adding distilled water and leaving it for an hour for the purpose of spreading nanoparticles and it is called This phase is in the dilution phase, and the organic solvent and distilled water were disposed of by a rotary evaporator at a temperature of 35 °C until the final volume reached 60 ml, which is a transparent color center where the Nano capsules began to form automatically in the continuous phase when adding the organic phase Containing polylactic acid (PLA) and causing Clear dispersion in the medium and these Nano capsules are formed due to the differences in the surface tension between the organic and aqueous phase, which results in inter-disorders in the system leading to the continued flow of the solvent away from the areas of low surface tension and the polymer collects on the hydrophobic surface, thus forming the Nano capsules as well. The shell of the Nano capsules is formed From the adsorbed surface layer is a stabilizer and an emulsion, while the linolenic fatty acid and the polymer (PLA) are dissolved in the inner sphere of the medium ( Siloh ,2018).





## **The Method of Making Labneh**

The fresh cow's milk collected from one of the milk processors in Salah al-Din Governorate was heated at a temperature of 90 ° C for 5 minutes in a water bath, and then gradually cooled to a temperature of 45 ° C and the initiator consisting of strains was added Ready-made bacteria in the form of dry cells after activation (*Streptococcus thermophiles* and *Lactobacillus bulgaricus*) produced from the French company Danisco at a percentage of (3%) and adding the Nano capsules at the studied concentrations of 100 mg/kg and 200 mg/kg coated and uncoated conjugated linolenic acid in addition to making the control sample without Any addition and incubated at a temperature of 45-42° C for 3-4 hours) and after the coagulation process, the samples were left to cool at room temperature for an hour, and then (0.5%) salt was added to them and then placed in a sterile white cloth bag in the refrigerator on Temperature of 5 ° C for 24 hours, after which the contents of the bag were emptied after removing the whey completely and placed in sterile containers, and the samples were stored at a temperature of (5 ± 2 ° C) until chemical, microbial and sensory tests are performed during the storage periods (Al-Jubouri, 2021).

## **Labneh Chemical Tests**

moisture was estimated according to Ling method, and Kerber's method mentioned by Min and Ellefson, (2010). The percentage of fat, protein and pH were estimated as mentioned by Hool et al, (2004), and ash and free fatty acids were estimated by direct burning method described in AOAC (2010).

## **Statistical Analysis**

The results of the experiments were analyzed using the Linear Model General within the ready-made statistical program SAS to study the effect of factors on the complete random design (CRD) ( $P < 0.05$ ).

## **Results and DISCUSSION**

### **Moisture percentage**

Table (1) shows the effect of different treatments on the percentage of moisture (%) in the stored coated Labneh to which the Nano-coated and unwrapped linolenic acid was added. The time is 21 days at a temperature of (5±2)°C. It is noted from the results that the percentage of humidity immediately after manufacturing for the treatments M1, M2, M3, M4, and M5 on one day is 62 77, 75.65, 76.52, 75.90, 76.72 %, respectively. The results converged and were not affected by the type of Nano-encapsulation or additives, and the gradual decrease continued as the storage period increased until



reaching the lowest moisture percentage at the end of the storage period at the time 21 days at a temperature of  $(5 \pm 2) ^\circ\text{C}$  for the above treatments, which were 71.11, 74.10, 75.14, 72.02, 73.07%, respectively.

Table (1) Effect of different treatments on the percentage of moisture (%) in the Labneh stored for 21 days at a temperature of  $(5 \pm 2) ^\circ\text{C}$ .

Treatments	1 day	7 day	14 day	21 day
M1	77.62 <sup>i</sup> ±0.07	75.34 <sup>n</sup> ±0.11	72.18 <sup>o</sup> ±0.07	71.11 <sup>p</sup> ±0.03
M2	75.65 <sup>bc</sup> ±0.03	75.20 <sup>cd</sup> ±0.02	74.89 <sup>e</sup> ±0.01	74.10 <sup>fg</sup> ±0.01
M3	76.52 <sup>cd</sup> ±0.01	76.98 <sup>de</sup> ±0.01	75.77 <sup>ef</sup> ±0.01	75.14 <sup>g</sup> ±0.01
M4	75.90 <sup>a</sup> ±0.02	74.33 <sup>i</sup> ±0.02	73.82 <sup>k</sup> ±0.04	72.02 <sup>m</sup> ±0.04
M5	76.72 <sup>b</sup> ±0.02	75.90 <sup>h</sup> ±0.02	74.83 <sup>j</sup> ±0.04	73.07 <sup>l</sup> ±0.02

- The numbers in the table are averaged for three replicates and express the values of the averages  $\pm$  standard deviation.
- Different letters in the same column indicate significant differences ( $p < 0.05$ ) between the studied groups.
- M1 = control, M2= Labneh sample with added CALANPs (100 mg/kg), M3= Labneh sample with added CALANPs (200 mg/kg), M4= Labneh sample with added CALA (100 mg/kg), M5= Labneh sample with added CALA (200 mg/kg).

The above coincided with what Kawther et al. (2018) stated that the moisture content of labneh ranged (73.12%-72.74) in the control treatment and in the treatments added to it alginate capsules with a concentration of (2%), where the moisture percentage was at (73.13-72.89) ) After 15 days of refrigerated storage.

The results of labneh also converged with that of al-Jubouri (2021), where he mentioned a decrease in the moisture content after 14 days of storage that ranged from -70.38 (72.32%) due to the gradual decrease in the moisture percentage with the progression in the storage periods to the depletion of the shrub from the labneh.

### Percentage of Fat

Table (2) shows the effect of different treatments on the percentage of fat (%) in the coated labneh stored for 21 days at a temperature of  $(5 \pm 2) ^\circ\text{C}$  to which the Nano-coated and uncoated linolenic acid was added. It is noted from the results the percentage of fat after Direct manufacturing Transactions M1, M2, M3, M4, M5 were at 7.15, 7.52, 7.30, 7.18, 7.42%, respectively, and there was a gradual increase with the progress in the storage periods until reaching the highest percentage at the end of the



storage period for the above transactions at time 21 days 8.20 , 7.75, 7.48, 7.71 and 7.82%, respectively.

Table (2) Effect of different treatments on the percentage of fat (%) in the coated Labneh stored for 21 days at a temperature of  $(5\pm 2)^{\circ}\text{C}$ .

Treatments	1 day	7 day	14 day	21 day
M1	$7.15^{o\pm 0.02}$	$7.37^{lm\pm 0.02}$	$7.85^{hi\pm 0.01}$	$8.20^{gh\pm 0.02}$
M2	$7.52^{kl\pm 0.02}$	$7.65^{ig\pm 0.03}$	$7.70^{fg\pm 0.01}$	$7.75^b\pm 0.01$
M3	$7.30^{n\pm 0.02}$	$7.36^{m\pm 0.01}$	$7.42^{jk\pm 0.03}$	$7.48^a\pm 0.04$
M4	$7.18^{p\pm 0.02}$	$7.29^f\pm 0.01$	$7.48^c\pm 0.02$	$7.71^c\pm 0.02$
M5	$7.42^{m\pm 0.03}$	$7.58^f\pm 0.02$	$7.69^e\pm 0.01$	$7.82^d\pm 0.02$

- The numbers in the table are averaged for three replicates and express the values of the averages  $\pm$  standard deviation.
- Different letters in the same column indicate significant differences ( $p < 0.05$ ) between the studied groups.
- M1 = control, M2= Labneh sample with added CALANPs (100 mg/kg), M3= Labneh sample with added CALANPs (200 mg/kg), M4= Labneh sample with added CALA (100 mg/kg), M5= Labneh sample with added CALA (200 mg/kg).

These results are in agreement with Mohamed et al. (2021), where they mentioned a high percentage of fat in labneh after 21 days of storage due to the low percentage of moisture for transactions upon storage, and these results were also consistent with Al-Jubouri, (2021), where he mentioned a decrease in the percentage of fat in labneh after 14 days of storage. Storage was (8.93-8.53%).

### Percentage of Protein

The results of Table (3) show the effect of different treatments on the percentage of protein (%) in the coated Labneh stored for 21 days at a temperature of  $(5\pm 2)^{\circ}\text{C}$ , as the percentage of protein in all treatments M1, M2, M3, M4, M5 on one day were at 9.73, 9.77, 9.78, 9.74, 9.76%, respectively, until reaching the highest percentage at the end of the storage period for the above transactions at the time of 21 days, which were 10.21, 10.01, 10.12, 10.20, 10.18%, respectively .



Table (3) Effect of different treatments on the percentage of protein (%) in the coated labneh stored for 21 days at a temperature of  $(5 \pm 2)^{\circ}\text{C}$

Treatments	1 day	7 day	14 day	21 day
M1	9.73 <sup>ef</sup> ±0.02	9.89 <sup>c</sup> ±0.01	10.03 <sup>b</sup> ±0.01	10.21 <sup>a</sup> ±0.02
M2	9.77 <sup>j</sup> ±0.03	9.81 <sup>i</sup> ±0.02	9.90 <sup>gh</sup> ±0.02	10.01 <sup>de</sup> ±0.02
M3	9.78 <sup>gh</sup> ±0.03	9.86 <sup>fg</sup> ±0.02	9.95 <sup>cd</sup> ±0.01	10.12 <sup>e</sup> ±0.04
M4	9.74 <sup>ij</sup> ±0.02	9.94 <sup>gh</sup> ±0.01	10.08 <sup>ef</sup> ±0.01	10.20 <sup>c</sup> ±0.02
M5	9.76 <sup>hi</sup> ±0.02	9.90 <sup>g</sup> ±0.02	10.11 <sup>cdf</sup> ±0.02	10.18 <sup>cd</sup> ±0.02

- The numbers in the table are averaged for three replicates and express the values of the averages  $\pm$  standard deviation.
- Different letters in the same column indicate significant differences ( $p < 0.05$ ) between the studied groups.
- M1 = control, M2= Labneh sample with added CALANPs (100 mg/kg), M3= Labneh sample with added CALANPs (200 mg/kg), M4= Labneh sample with added CALA (100 mg/kg), M5= Labneh sample with added CALA (200 mg/kg).

The results also agreed with what was found with Al-Jubouri, (2021), where he stated that the percentage of protein in labneh after storage for 14 days was 10.48-10.31 (10.31 percent), and the reason is due to the effect of added linolenic acid in limiting the growth of microorganisms producing proteolytic enzymes. And the difference in the percentage of moisture and to the chemical composition of the anti-microbial materials added in the Nano capsules.

### Percentage of Ash

Table (4) shows the effect of different treatments on the percentage of ash (%) in the coated Labneh stored for 21 days at a temperature of  $(5 \pm 2)^{\circ}\text{C}$ , to which linolenic acid was added Nano-coated and unwrapped. It was noted from the results that the percentage of ash Immediately after manufacturing transactions M1, M2, M3, M4, M5, they were at 1.84, 1.49, 1.57, 1.69, 1.81, 1.43 %, respectively, until reaching the highest percentage at the end of the storage period for transactions at the time of 21 days, which was 2.15, 1.71, 1.80, 1.91 , 2.02 % respectively .





Table (4) Effect of different treatments on the percentage of ash (%) in the packed labneh stored for 21 days at a temperature of  $(5\pm 2)^{\circ}\text{C}$ .

Treatments	1 day	7 day	14 day	21 day
M1	$1.84^{ef}\pm 0.04$	$1.95^{d}\pm 0.02$	$2.08^{b}\pm 0.03$	$2.15^{a}\pm 0.02$
M2	$1.49^{j}\pm 0.03$	$1.55^{i}\pm 0.02$	$1.62^{hi}\pm 0.03$	$1.71^{fg}\pm 0.01$
M3	$1.57^{h}\pm 0.01$	$1.63^{gh}\pm 0.01$	$1.70^{fg}\pm 0.01$	$1.80^{e}\pm 0.03$
M4	$1.69^{h}\pm 0.02$	$1.75^{f}\pm 0.02$	$1.82^{e}\pm 0.02$	$1.91^{d}\pm 0.03$
M5	$1.81^{f}\pm 0.02$	$1.89^{e}\pm 0.05$	$1.96^{d}\pm 0.02$	$2.02^{c}\pm 0.02$

- The numbers in the table are averaged for three replicates and express the values of the averages  $\pm$  standard deviation.
- Different letters in the same column indicate significant differences ( $p < 0.05$ ) between the studied groups.
- M1 = control, M2= Labneh sample with added CALANPs (100 mg/kg), M3= Labneh sample with added CALANPs (200 mg/kg), M4= Labneh sample with added CALA (100 mg/kg), M5= Labneh sample with added CALA (200 mg/kg).

These results are in agreement with Al-Jubouri (2021), who mentioned a decrease in the ash percentage after 14 days of storage from -2.20 (2.42%) in the samples of labneh coated with gelatin membranes and reinforced with nanoparticles, where the percentage of ash in labneh and cheese increases with the increase in storage periods. The results also agreed with what It was found by Mohamed et al. (2021), who mentioned that the ash percentage ranged between (1.70-0.80)% in the different treatments of brick stored for 21 days. The difference in the mineral content of labneh and cheese, the exudation of whey proteins and the high total solids.

### pH Values

Table (5) shows the effect of different treatments on the pH values of the coated Labneh stored for 21 days at a temperature  $(5\pm 2)^{\circ}\text{C}$  to which the nano-coated and uncoated linolenic acid was added. Manufacturing directly for the treatments M1, M2, M3, M4, M5 were at 4.78, 4.40, 4.38, 4.50, 4.45, respectively. The pH is one of the main factors in determining the extent of the development of the casein network. Casein particles and whey proteins are the main component in the composition of labneh and cheese. And even reaching the lowest pH at the end of the storage period for the above transactions at the time of 21 days was 4.53, 4.25, 4.21, 4.30 and 4.29, respectively.





Table (5) Effect of different treatments on the pH of the coated Labneh stored for 21 days at a temperature of  $(5\pm 2)^{\circ}\text{C}$ .

Treatments	1 day	7 day	14 day	21 day
M1	$4.78^{a\pm 0.00}$	$4.64^{b\pm 0.02}$	$4.49^{o\pm 0.02}$	$4.53^{c\pm 0.01}$
M2	$4.40^{e\pm 0.01}$	$4.35^{fg\pm 0.00}$	$4.29^{i\pm 0.00}$	$4.25^{j\pm 0.01}$
M3	$4.38^{ef\pm 0.00}$	$4.34^{g\pm 0.01}$	$4.29^{i\pm 0.01}$	$4.21^{k\pm 0.00}$
M4	$4.50^{d\pm 0.00}$	$4.38^{ef\pm 0.00}$	$4.33^{gh\pm 0.00}$	$4.30^{hi\pm 0.01}$
M5	$4.45^{d\pm 0.00}$	$4.33^{gh\pm 0.01}$	$4.31^{hi\pm 0.00}$	$4.29^{i\pm 0.00}$

- The numbers in the table are averaged for three replicates and express the values of the averages  $\pm$  standard deviation.
- Different letters in the same column indicate significant differences ( $p < 0.05$ ) between the studied groups.
- M1 = control, M2= Labneh sample with added CALANPs (100 mg/kg), M3= Labneh sample with added CALANPs (200 mg/kg), M4= Labneh sample with added CALA (100 mg/kg), M5= Labneh sample with added CALA (200 mg/kg).

The above results are in agreement with Al-Jubouri (2021), who stated that the pH value gradually decreases as the storage period is prolonged from 4.16 to 4.36 due to the high percentage of lactic acid in it due to the initiator bacteria in the 14-day labneh treatments. The results were in agreement with what was found by Mohamed and others (2021), who stated that the pH ranged between (4.14-3.97)% in the different labneh treatments stored for a period of 21 days. Its manufacture is in addition to the presence of a percentage of contamination with microorganisms whose growth causes an increase in acidity and a decrease in the pH of yoghurt and cheese during storage, in addition to the fermentation of the excess lactose sugar with samples after whey separation affected by the microbial content and the percentage of moisture that affect the activity of microorganisms.

### Percentage of Free Fatty Acids

Table (6) shows the effect of different treatments on the free fatty acids in the coated labneh stored for 21 days at a temperature  $(5\pm 2)^{\circ}\text{C}$  to which the Nano-coated and uncoated linolenic acid was added. Immediately after manufacturing transactions M1, M2, M3, M4, M5, they were at 0.93, 0.24, 0.13, 0.69, 0.54 %, respectively, until reaching the end of the storage period for the above transactions at the time of 21 days, they were at 1.70, 0.35, 0.25, 0.88, 0.83 % respectively.



Table (6) Effect of different treatments on free fatty acids in coated labneh stored for 21 days at  $(5 \pm 2)^{\circ}\text{C}$

Treatments	1 day	7 day	14 day	21 day
M1	$0.93^{d \pm 0.01}$	$1.25^{c \pm 0.00}$	$1.56^{b \pm 0.01}$	$1.70^{a \pm 0.02}$
M2	$0.24^{kl \pm 0.01}$	$0.27^m \pm 0.01$	$0.31^{ij \pm 0.00}$	$0.35^i \pm 0.01$
M3	$0.13^m \pm 0.01$	$0.17^g \pm 0.01$	$0.21^{kl \pm 0.01}$	$0.25^{kl \pm 0.00}$
M4	$0.69^g \pm 0.01$	$0.69^g \pm 0.03$	$0.78^f \pm 0.01$	$0.88^e \pm 0.02$
M5	$0.54^h \pm 0.01$	$0.73^g \pm 0.02$	$0.79^f \pm 0.01$	$0.83^f \pm 0.01$

- The numbers in the table are averaged for three replicates and express the values of the averages  $\pm$  standard deviation.
- Different letters in the same column indicate significant differences ( $p < 0.05$ ) between the studied groups.
- M1 = control, M2= Labneh sample with added CALANPs (100 mg/kg), M3= Labneh sample with added CALANPs (200 mg/kg), M4= Labneh sample with added CALA (100 mg/kg), M5= Labneh sample with added CALA (200 mg/kg).

The above results converged with Al-Jubouri (2021), who mentioned that the percentage of free fatty acids gradually increases with the long storage period (0.39-0.29)%, due to the role of these coatings in trapping moisture and then providing a suitable growth environment for the activity of the initiator bacteria, especially in the presence of water activity ( $a_w$ ), especially lipolytic bacteria that increase the degree of lipolysis in labneh stored for 14 days.

## Conclusions

The addition of the Nano-encapsulated linolenic acid at a concentration (100 and 200 mg/kg) helps to preserve the manufactured yogurt with its chemical properties and for longer storage periods compared to the treatment without it, in addition to the role of Nano-encapsulation, which works to provide the quantities required to meet part of the daily needs of the adult human being because it is a fatty acid Important for human health while maintaining the quality of milk products without affecting the sensory qualities.

## Suggestions

The use of Nano coated linolenic acid in other food products.



## Recommendations

The addition of nano-encapsulated linolenic acid in dairy products for the purpose of increasing the shelf life as long as possible.

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