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DETERMINATION OF THE ACTIVITY OF NANO COATED CONJUGATED LINOLENIC ACID ON THE CHEMICAL COMPOSITION OF CHEESE

Shahba Rafe Abdullah[1] Mohammad Ahmad Jassim [2] Rafid Khalil Abdul Rezzaq[3]
Department of Food Sciences, College of Agriculture, Tikrit University, Salah al-Din, Iraq
shahbaarafiaabbdullah@gmail.com
kkk.aa91@yahoo.com
rafidkhaleel@tu.edu.iq

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Abstract:

This study was conducted to evaluate the effect of conjugated Nano- and Non-Nano-coated linolenic acid on the chemical composition of cheese during different storage periods. C1 = control and cheese sample with added linolenic acid at a concentration (100 mg/kg), C2 and , cheese sample with added linolenic acid with a concentration of (200 mg / kg) C3 and cheese sample with added uncoated linolenic acid at a concentration (100 mg / kg) C4 and cheese sample with added cheese Uncoated linolenic acid at a concentration of (200 mg/kg) C5, and the results of storing cheese showed that the moisture content decreased significantly ($p<0.05$) until the end of the storage period, and the treatment C3 was less in moisture loss (58.96%). It was also found that the percentages of protein, fat and ash did not show significant differences at the time of one day compared with the control sample C1, then these percentages gradually increased as the preservation period was prolonged 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 (6.30- 6.45) compared with the control sample was 6.05 and it was found that the values of fatty acids for all treatments were significantly lower compared with the control treatment C1 at the time of one day, and the percentage of acids increased The free fatty acids reached their highest values between (0.55-0.13)% for all treatments compared to the control treatment C1 at the end of the storage period, which amounted to 1.25%.

Keywords: linolenic acid, cheese, nanotechnology, chemical composition, control sample.

INTRODUCTION:

Conjugated linolenic acid(Omega-3), 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 (Silwah, 2018). Nanotechnology, where nanotechnology has made a new revolution in scientific fields, especially in recent times, 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 nm) (Filippon and Sutherl, 2013) and differs from its counterpart from the raw material used by changing the chemical and physical properties of nanomaterial's such as changing solubility, dispensability of particles, change of color and other characteristics. There are three ways to We get Nano-sized particles which are - 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 (Neethirajan and Jayas, 2011; Sozer and Kokini, 2009) and reduce the percentage of fat use in foods and increase the stability and absorption of nutrients (Weiss et al, 2006; Chaudhry; et al, 2008) and other benefits such as masking undesirable odors and flavor compounds associated with foodstuffs and increasing the degree of consumer acceptance (Neethirajan and Jayas 2011). Packaging to be edible and biodegradable, and its characteristics have been improved in terms of high ability to retain moisture, gases and undesirable odors (Silwah, 2018), in addition to its effectiveness against microorganisms, yeasts and molds that cause food spoilage and

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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:

Nano-encapsulation of Conjugated Linolenic Acid by Poly Lactic Acid-PLA and gelatin using Emulsion-Diffusion method and using these Nano capsules to fortify some commonly consumed dairy products (soft cheese) to fill part of the consumer's required alpha-acid needs Conjugated linolenic Nano-coated and uncoated from conjugated linolenic acid and studying 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 soft cheese: The cheese was prepared according to the method of Fox et al. (2017), where fresh cow's milk collected from a milk processor in Salah al-Din Governorate was pasteurized at a temperature of 63 °C for 30 minutes and the addition of Nano capsules with the studied concentrations of 100 mg / kg and 200 mg/kg coated and uncoated of conjugated linolenic acid in addition to making the control sample without any addition and then cooling directly to a temperature of 37°C and adding microbial rennet (renin enzyme) in a ratio (1 g/25 liters of milk) according to the company's instructions Meito Sangyo CO. LTD and calcium chloride was added at 0.02% and left for 30-30 minutes until the required cheese was obtained. The curd was placed in a piece of sterile gauze for the purpose of getting rid of the largest amount of whey and getting the caseinate pieces. The curd was cut and salt was added at a rate of 2% of the weight of the curd And packing in special molds and kept in the refrigerator at a temperature of (5±2)ºC until chemical, microbial and sensory tests are performed during the storage periods.

Cheese 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 stock cheese that has been coated with Nano-coated and unwrapped linolenic acid. The table shows a significant decrease ($P<0.05$) in the percentage of moisture since the beginning of experiment time until the end of the storage period at the time 21 days at a temperature (5±2) ºC. It is noted from the results that the percentage of moisture immediately after manufacturing for the treatments C1, C2, C3, C4, C5 at one day was is 61.91, 60.75, 60.61, 61.87, 61.79 The results converged and were

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not affected by the type of Nano-encapsulation or the additives. The few differences in the moisture content are attributed to the fact that the process of compressing the curd was manual, and the whey proteins were not completely disposed of, and the moisture content was not less than 50% in all treatments, as mentioned in the Iraqi standard specification for soft cheese. , (1988) and the decrease in the percentage of moisture contain as the storage period increased until reaching the lowest moisture percentage at the end of the storage period for the treatments C1, C2, C3, C4, C5 which are 55.52, 58.82, 58.96, 56.46, 57.31 %, respectively.

Table (1) Effect of different treatments on the percentage of moisture (%) in packaged cheese stored for 21 days at a temperature of (5±2)ºC

Treatments	1 day	7 day	14 day	21 day
C1	61.91^{e}±0.01	58.76^{n} ±0.01	56.79^{o}±0.01	55.52^{p}±0.01
C2	60.75^{f} ±0.00	59.88^{gh} ±0.01	59.10^{hi}±0.01	58.82^{i}±0.01
C3	60.61^{gh}±0.01	59.92^{i} ±0.01	59.25±0.01	58.96^{g}±0.00
C4	61.87^{a}±0.01	59.72^{c}±0.01	57.63^{g}±0.01	56.46^{jk}±0.01
C5	61.79^{b}±0.01	59.53^{d}±0.01	58.44^{jk}±0.04	57.31^{m}±0.01

- The numbers in the table are averaged for three replicates and express the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between the studied groups.
- C1 = control, C2= cheese sample with added CALANPs (100 mg/kg), C3= cheese sample with added CALANPs (200 mg/kg), C4= from cheese sample with added CALA (100 mg/kg), C5= cheese sample with added CALA (200 mg/kg).

The above results are in agreement with Ahmed, (2020), who stated that all unwrapped cheese samples that were coated with gelatin films or gelatin added to nanoparticles showed a significant decrease ($P<0.05$) in the moisture content from the beginning of the experiment time until the end of the storage period at the time of 20 days. The moisture ratios of the treatments ranged between (53.50-58.66%) in the different treatments and it also coincided with Al-Jubouri, (2017), who mentioned the existence of a large variation in the moisture percentage in the rural cheese(57.98-67.4) %.

Percentage of fat: Table (2) shows the effect of different treatments on the percentage of fat (%) in packaged cheese stored for 21 days at a temperature of (5±2)ºC to which linolenic acid was added Nano-coated and unwrapped. For fat immediately after manufacturing, the transactions C1, C2, C3, C4, C5 at one day are 15.56,16.11, 16.18, 15.72, 15.83 %, respectively, and a gradual increase occurred with the progression in the storage periods until reaching the highest percentage at the end of the storage period for the transactions C1, C2, C3, C4, C5 at 21 days, where they were18.13, 17.55, 17.40, 17.77,17.90 %, respectively.

Table (2) Effect of different treatments on the percentage of fat (%) in packaged cheese stored for 21 days at a temperature of (5±2)ºC

Treatments	1 day	7 day	14 day	21 day
C1	15.56^{p}±0.01	16.81^{o}±0.01	17.90^{m}±0.02	18.13^{k}±0.02
C2	16.11^{gh}±0.01	16.66^{fg}±0.01	17.12^{ef}±0.01	17.55^{e}±0.01
C3	16.18^{d}±0.01	16.43^{c}±0.01	17.06^{b}±0.01	17.40^{a}±0.01
C4	15.72^{o}±0.02	16.92^{m}±0.02	17.55^{j}±0.01	17.77^{i}±0.01
C5	15.83^{n}±0.01	16.97^{l}±0.01	17.68^{h}±0.01	17.90^{gh}±0.01

- The numbers in the table are averaged for three replicates and express the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between the studied groups.
- C1 = control, C2= cheese sample with added CALANPs (100 mg/kg), C3= cheese sample with added CALANPs (200 mg/kg), C4= from cheese sample with added CALA (100 mg/kg), C5= cheese sample with added CALA (200 mg/kg).

The results above were close to what was mentioned by Ahmed, (2020), who stated that
the percentage of fat for soft cheeses covered with gelatin membranes and to which some nanoparticles were added in all treatments of soft cheese was close after the end of the manufacturing process and ranged between (16.16-16.50%), then The percentage of fat increased after 20 days of storage to reach between (17.23-18.76%) and it also coincided with Al-Jubouri, (2017), who mentioned that the percentage of fat in soft cheese ranged between (14.21-17.73)%, at a rate of 15.59%, and explained the reasons for the difference in the percentage The fat in cheese is the type of milk used because some producers remove part of the fat or add sorted milk to the milk prepared for the manufacture of cheese. The percentage of fat

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is also affected by the method of manufacture. Part of the fat may be lost with whey when it is drained. In addition, the percentage of fat is affected by other contents in soft cheese, especially the presence of percentage of moisture.

Protein percentage: The results of Table (3) show the effect of different treatments on the percentage of protein (%) in the packaged cheese stored for 21 days at a temperature of (5±2)ºC, as the percentage of protein in all treatments C1, C2, C3, C4, C5 was at one day, they are 18.50, 18.25, 18.36, 18.11, 18.46 %, respectively. These percentages are considered among the required rates in soft cheese, and a gradual increase occurred with the progression in the storage periods until reaching the highest percentage at the end of the storage period for the treatments C1, C2, C3, C4, C5 at the time of 21 days, which were 19.77,19.50,19.34,19.56,19.61 %, respectively.

Table (3) Effect of different treatments on the percentage of protein (%) in packaged cheese stored for 21 days at a temperature of (5±2)ºC

Treatments	1 day	7 day	14 day	21 day
C1	18.50^{m}±0.00	18.79^{e}±0.01	19.46^{d}±0.02	19.77^{a}±0.02
C2	18.25^{jk}±0.01	18.41^{j}±0.01	19.28^{h}±0.01	19.50^{g}±0.02
C3	18.36^{l}±0.01	18.78^{f}±0.02	19.10^{d}±0.02	19.34^{b}±0.01
C4	18.11^{k}±0.02	18.67^{g}±0.02	19.44^{d}±0.02	19.56^{c}±0.01
C5	18.46^{j}±0.01	18.80^{i}±0.01	19.32^{hi}±0.01	19.61^{g}±0.02

- The numbers in the table are averaged for three replicates and express the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences (p<0.05) between the studied groups.
- C1 = control, C2= cheese sample with added CALANPs (100 mg/kg), C3= cheese sample with added CALANPs (200 mg/kg), C4= from cheese sample with added CALA (100 mg/kg), C5= cheese sample with added CALA (200 mg/kg).

The reason is due to the effect of added linolenic acid in limiting the growth of microorganisms producing proteolytic enzymes, the difference in moisture content, and the chemical composition of the antimicrobial materials added in the Nano capsules. Soft cheese coated with gelatin and some nanoparticles added to it at a temperature of 7º C in all treatments of soft cheese were close after the end of the storage period of 20 days and ranged between (18.90-19.53 %), and the above results also converged with Al-Jubouri, (2017) where It was found that the protein content ranged between (15.05-21.69%) and an average of 18.71%, in the soft cheese samples.

Ash percentage: Table (4) shows the effect of different treatments on the percentage of ash (%) in packaged cheese stored for 21 days at a temperature of (5±2)ºC to which linolenic acid was added, both Nano-coated and unwrapped. The percentage of ash immediately after manufacturing for transactions C1, C2, C3, C4, C5 in one day is: 1.56,1.29, 1.38, 1.40, 1.43 %, respectively, until reaching the highest percentage at the end of the storage period for treatments C1, C2, C3, C4, C5 at the time of 21 days, where they were 1.88,1.56,1.55,1.73,1.78 % respectively.

Table (4) Effect of different treatments on the percentage of ash (%) in packaged cheese stored for 21 days at a temperature of (5±2)ºC

Treatments	1 day	7 day	14 day	21 day
C1	1.56^{e}±0.01	1.63^{d}±0.01	1.77^{bc}±0.02	1.88^{a}±0.02
C2	1.29^{i}±0.01	1.36^{h}±0.01	1.43^{g}±0.01	1.56^{e}±0.01
C3	1.38^{h}±0.02	1.43^{g}±0.01	1.48^{f}±0.01	1.55^{e}±0.01
C4	1.40^{gh}±0.00	1.50^{f}±0.00	1.61^{d}±0.01	1.73^{c}±0.01
C5	1.43^{g}±0.01	1.65^{d}±0.01	1.73^{c}±0.01	1.78^{b}±0.01

- The numbers in the table are averaged for three replicates and express the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences (p<0.05) between the studied groups.
- C1 = control, C2= cheese sample with added CALANPs (100 mg/kg), C3= cheese sample with added CALANPs (200 mg/kg), C4= from cheese sample with added CALA (100 mg/kg), C5= cheese sample with added CALA (200 mg/kg).

The above results converged with what Ahmed ,(2020) mentioned about a gradual increase in the percentage of ash with an increase in the storage period in samples of soft cheese coated with gelatin and some nanoparticles added to it after 21 days of storage, where the percentage of ash ranged (1.70-1.84%) as it gradually rises as it lasts The storage period, due to the decrease in the percentage of moisture, and the results converged with Al-Jubouri (2017), who stated that the percentage of ash in soft cheese ranged

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between (2.67-4.72%) due to the difference in moisture content and the difference in the composition of soft cheese from mineral elements in addition to the effect of The percentage of table salt added to cheese in different treatments.

pH values: Table (5) shows the effect of different treatments on the pH values of packaged cheese stored for 21 days at a temperature of (5±2) ºC, to which linolenic acid was added Nano-coated and unwrapped. The pH immediately after manufacturing the treatments C1, C2, C3, C4, C5 on one day is: 6.67, 6.81, 6.82, 6.67, 6.68 respectively. The pH is one of the most important factors in determining the extent of the development of the casein network. Casein particles and whey proteins are the component. The main component in the composition of cheese until reaching the lowest pH at the end of the storage period for the parameters C1, C2, C3, C4, C5 at the time of 21 days, which were: 6.05, 6.36, 6.30, 6.45, 6.34 respectively. The Iraqi standard specification for soft cheese states, 1988) provided that the pH of soft cheese does not exceed (6.4±0.2).

Table (5) Effect of different treatments on the pH of packed cheese stored for 21 days at a temperature of (5±2)ºC

Treatments	1 day	7 day	14 day	21 day
C1	6.67^{c}±0.02	6.57^{de}±0.02	6.38^{hi}±0.03	6.05^{ab}±0.04
C2	6.81^{ab}±0.00	6.68^{c}±0.02	6.52^{ef}±0.03	6.36^{ghi}±0.04
C3	6.82^{a}±0.02	6.72^{bc}±0.02	6.66^{cd}±0.04	6.30^{de}±0.02
C4	6.67^{c}±0.04	6.56^{ef}±0.04	6.48^{efg}±0.02	6.45^{i}±0.02
C5	6.68^{c}±0.02	6.52^{ef}±0.03	6.46^{fgh}±0.02	6.34^{ghi}±0.02

- The numbers in the table are averaged for three replicates and express the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between the studied groups.
- C1 = control, C2= cheese sample with added CALANPs (100 mg/kg), C3= cheese sample with added CALANPs (200 mg/kg), C4= from cheese sample with added CALA (100 mg/kg), C5= cheese sample with added CALA (200 mg/kg).

The reason for the low pH of cheese is due to the use of primitive methods in the manufacturing process and the lack of control of the pH when manufacturing it, in addition to the presence of a percentage of contamination with microorganisms that cause their growth to raise the acidity and reduce the pH in cheese during storage, in addition to the fermentation of the excess lactose sugar with Cheese samples after whey separation affected by the microbial content and the percentage of moisture that affect the activity of microorganisms. These results are consistent with what Ahmed, (2020) found that with the continuation of the storage period, the pH values gradually decrease until reaching the lowest values at the end of the 20-day storage period. Where it ranged between (6.19 - 6.29) in all treatments coated with gelatin and some nanoparticles were added to it. Rural tender.

Percentage of free fatty acids: Table (6) shows the effect of different treatments on free fatty acids in packaged cheese stored for 21 days at a temperature of (5±2)ºC, to which linolenic acid was added, both Nano-coated and unwrapped. Free fatty immediately after manufacturing for C1, C2, C3, C4, C5 at one day: 0.10, 0.02, 0.01, 0.08, 0.05 % respectively until reaching the end of the storage period for C1, C2, C3, C4, C5 at time 21 days To be:1.25, 0.21, 0.13, 0.55, 0.34% respectively. These results show that the release of free fatty acids was lower in the cheese treatments to which the encapsulated linolenic acid and the unwrapped linolenic acid were added compared to the no-addition control treatment. C1 contains the highest percentage of free fatty acids and it amounted to 1.25% compared to the best sample is C3, where the percentage of free fatty acids reached 0.13% and the reason for this is attributed to the role of Nano capsules in prolonging the preservation periods in cheese.

Table (6) Effect of different treatments on free fatty acids in packaged cheese stored for 21 days at a temperature of (5±2)ºC

Treatments	1 day	7 day	14 day	21 day
C1	0.10^{ghi}±0.00	0.19^{def}±0.01	0.32^{c}±0.07	1.25^{a}±0.03
C2	0.02^{j}±0.00	0.07^{hij}±0.01	0.13^{fgh}±0.01	0.21^{de}±0.01
C3	0.01^{j}±0.00	0.03^{j}±0.00	0.05^{ig}±0.00	0.13^{fgh}±0.01
C4	0.08^{hij}±0.01	0.19^{def}±0.01	0.24^{d}±0.02	0.55^{b}±0.01
C5	0.05^{ij}±0.01	0.16^{efg}±0.01	0.20^{de}±0.01	0.34^{c}±0.01

- The numbers in the table are averaged for three replicates and express the values of the averages ± standard deviation.
- Different letters in the same column indicate significant differences ($p<0.05$) between the studied groups.

- C1 = control, C2= cheese sample with added CALANPs (100 mg/kg), C3= cheese sample with added CALANPs (200 mg/kg), C4= from cheese sample with added CALA (100 mg/kg), C5= cheese sample with added CALA (200 mg/kg).

The above results converged with Ahmed ,(2020), who mentioned that the use of gelatinous membranes with some nanoparticles and determining their effect on the values of the percentage of free fatty acids, which is an indicator of the viability of the gene during the storage period of 20 days, that the values of fatty acids for all treatments were low and high when At the end of the storage period, it was recorded between (0.5-0.52)% for all treatments. The results also converged with Soleimani et al., (2018), who mentioned a lower rise in the percentages of free fatty acids for cheddar cheese coated with xanthan gum compared to the uncoated control sample.

CONCLUSIONS:

The addition of linolenic acid Nano-encapsulated at a concentration (100 and 200 mg/kg) helps the processed cheese retain 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 required quantities to meet part of the daily needs of the adult human being because of its Fatty acids 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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