American Journal of Food and Nutrition, 2021, Vol. 9, No. 3, 96-105
Available online at http://pubs.sciepub.com/ajfn/9/3/1
Published by Science and Education Publishing
DOI:10.12691/ajfn-9-3-1

Production of Processed Cheese Supplemented with Curcumin Nanoemulsion

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Received June 09, 2021; Revised July 11, 2021; Accepted July 29, 2021

Abstract In this study, curcumin nanoemulsion (CR-NE) was used in the preparation of processed cheese as a functional and healthy ingredient. Curcumin nanoemulsion showed higher antibacterial activity against *Staphylococcus aureus*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas marginalis* strains. Curcumin nanoemulsion was added to processed cheese formula by ratio 2.5% and 5% (T1 and T2) to reach a final concentration of 25 mg and 50 mg of nanocurcumin/kg processed cheese, respectively, and physicochemical, textural, microstructure, and organoleptic properties were tested. The results showed that the addition of CR-NE improved the physicochemical, microstructure, and textural properties of processed cheese during cold storage at 4°C for 60 days. Curcumin nanoemulsion decreased the loss of moisture during storage. The hardness of cheese containing CR-NE was slightly changed during storage compared with control samples. The T1 samples recorded the best sensory evaluation scores compared with T2 samples. No significant differences were found in acceptability between T1 samples and control samples during storage. The T1 processed cheese samples obtained represent high quality and healthy product that adds value to the dairy sector.

Keywords: *processed cheese, nanocurcumin, nanoemulsion, microstructure, antimicrobial*

Cite This Article: Mahmoud Ibrahim El-Sayed, and Thanaa I. Shalaby, “Production of Processed Cheese Supplemented with Curcumin Nanoemulsion.” *American Journal of Food and Nutrition*, vol. 9, no. 3 (2021): 96-105. doi: 10.12691/ajfn-9-3-1.

1. Introduction

Processed cheese is a popular and traditional gel-like dairy product consumed in many countries; their demand by the consumers is on a steady rise [1]. Their success is due to a combination of factors such as they are more economical than most natural cheeses since they usually contain inexpensive non-dairy ingredients; they are commercially available in a wide variety of flavors, consistency, and functional properties [2,3]. Processed cheese is a dairy product which differs from natural cheese in the fact that process cheese is not made directly from milk [4]. The fortification of processed cheese types with bioactive components has increased in recent years, such as the incorporation of dried materials, essential oils, carrot paste, apricot pulp, tomato juice and medicinal herbs extract into cheeses which resulted in improvement of nutritional value and sensory properties and decreased the deterioration process of quality parameters in different cheese types [5,6,7,8,9]. The addition of bioactive compounds in processed cheese could affect not only the taste but also the consistency [10].

Curcumin, a phenolic pigment found in the rhizome of turmeric (*Curcuma Longa*), and widely used as an ingredient in foods (seasoning and coloring agent) and medicines in many countries [11]. Studies investigated the physicochemical characteristics and pharmacological effects of curcumin on various diseases like diabetic nephropathy, cardiovascular diseases, cancer (pancreatic cancer, colon cancer, multiple myeloma, psoriasis, oral cancers, pre-cancerous lesion), rheumatoid arthritis, inflammatory bowel disease (IBD), Alzheimer’s, periodontal disease and wound healing [12,13,14,15]. The wonderful nutritional and medicinal impacts of curcumin made it a good substitutional to some conventional drugs and food flavouring or colouring materials. But, the low solubility of curcumin is a challenging hindrance [16]. Also, The poor solubility of curcumin in aqueous solvents leading to low absorption, fast metabolism, and quick systematic elimination [17]. To enhancing the bioavailability and biological activity of curcumin, the structural modifications are recommended [18].

In the last few years, many studies investigated the increased of curcumin solubility via nano-sizing, recrystallization and loading in a biodegradable polymeric [19]. The physicochemical properties of nanocurcumin (NC) are playing an important role in the alteration of normal curcumin into the nanoform [20]. Particle size, surface charge, surface area, and hydrophobicity are very important physicochemical properties that make nanocurcumin more effective than native curcumin [21]. Curcumin nanoparticles exhibit higher have more antioxidant activities in vivo and in vitro than its native curcumin [22,23]. The increase in antimicrobial activity of NC than curcumin due to the increase in aqueous-phase solubility and simple dispersibility [20].

There are bioactive compounds (such as poly phenols) that are difficult to incorporate into foods; these compounds are non-polar with poor solubility in water and high melting points. The use of nanoemulsions is an excellent method used to incorporate these bioactive compounds into foods [24,25,26,27]. The nanosystems have a greater surface area, reactivity, solubility, and availability of compounds, and they have the capability to react with the food components to decrease the physiological and enzymatic reactions, and producing novel products [28]. Nanoemulsions appear as one of the systems that have shown the greater interest for utilization as ingredients in the food industry, generally because of the different methods for their preparation, as well as being considered as stable systems for the encapsulation of bioactive substances [29,30]. Nanoemulsions and nanosized ingredients act as a viable alternative in the development of new products for including components with specific functions [31,32]. Nanoemulsion was used to increase the solubility and bioavilabilty of curcumin [33,34]. The aim of this study was to investigate the effect of curcumin nanoemulsion as a functional ingredient on the texture, microstructure, and organoleptic properties of processed cheese during cold storage at 4°C for 60 days.

2. Material and Methods

2.1. Raw Materials

Curcumin powder was obtained from Sigma- Aldrich, St. Louis, MO, USA. Cheddar cheese (6 months old, 62.4% DM, 25% protein and 34% fat) and Butter 82% fat were obtained from Al Sakr for food products (Rotana), Alexandria, Egypt. Milk protein concentrate (70% protein) and cow skim milk powder (34% protein) were obtained from Fonterra Ltd, Auckland, New Zealand. Emulsifying salt (Egy Phos S20) was obtained from EGY-Dairy Company, 10^{th} of Ramadan City, Egypt.

2.2. Methods

2.2.1. Preparation of Curcumin-nanoemulsion (CR-NE)

CR-NE was prepared according to the method of [35] with some modifications. Curcumin was entrapped in an oil phase (coconut oil) at a final concentration of 1mg/mL. The organic phase (acetone) was prepared, containing medium-chain-triglycerides and natural phospholipids (Lipoid Co. Nattermannallee, Germany) at 55°C. Subsequently, this organic solution was added to the aqueous phase, containing an anionic surfactant, poloxamer 188 (Sigma-Aldrich Co., St. Louis, MO, USA). In the final step, the organic solvent was fully removed using a rotary evaporator (STRIKE®201 rotary evaporator instruments KENTRON) under reduced pressure at 60°C. All samples were prepared in aseptic conditions and in the absence of contaminants and chemicals interference.

2.2.2. Characterization of CR-NE

1- **Transmission electron microscopy (TEM)**

The size and shape of the prepared CR-NE were determined by TEM (JEOL-100 CX), NE was diluted with distilled water (1:4), then sonicated for 10 minutes. A drop of the suspension was placed on a 200-mesh carbon-coated copper grid at room temperature. Grids containing the samples were dried in the air, negatively stained using 2% uranyl acetate, and allowed to dry at room temperature. The grids containing samples were used for TEM examination.

2- Particle size analysis and zeta potential measurements

Hydrodynamic particle size, size distribution (polydispersity index, PDI), and zeta potential of CR-NE were measured by Zetasizer (particle size and zeta potential analyzer) based on laser light scattering technique.

3-Stability of the CR-NE

Stability of CR-NE was assessed by monitoring the change in phase separation, creaming, and discoloration after storage for 2 months at room temperature. A formulation was kept in a dry and dark place at room temperature.

2.2.3. Antimicrobial Activity of CR-NE

1. Microorganisms and culture conditions

Five pathogenic strains were used to scan CR-NE antimicrobial potentials; two Gram-positive strains; *Staphylococcus aureus* NCTC10788 and *Clostridium sporogenes* ATCC3584, three Gram-negative strains; *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* EMCC1637 and *Pseudomonas marginalis* EMCC1271. All strains were obtained from Microbiological Resources Center (MERCIN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The test was held and the strains were maintained in 60% glycerol/ LB culture at -80°C by; the Department of Food Technology, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Egypt.

2. Minimum Inhibitory Concentration (MIC) determination

To examine antimicrobial activity of curcumin nanoemulsion, agar well diffusion assay was used [36], against pathogenic bacteria. The bacterial strains were grown in nutrient broth at 37°C for 24 h. Briefly, 100 µL of overnight activated culture of each pathogen strain (10^6 CFU/mL) were aseptically spread over Nutrient agar plates. About 100 µL of nanoemulsion (100 µg/mL) was transferred into each agar well individually. The plates were then incubated at 37°C for 18 h and the formed clear zones (if found) were measured and recorded. A set of 4 concentrations of CR-NE (100, 50, 25 and 12.5 µg curcumin/mL), were examined to determine the minimum inhibitory concentration (MIC) of each against a specific pathogenic strains [37]. DEMSO was used for dilutions and a control well was applied. The zones of inhibition were calculated by measuring the diameter of the inhibition zone around the well 5 (mm), including the well diameter. The readings were taken in three different fixed directions in all duplicates and the average values were tabulated.

Table 1. The formula of processed cheeses

Treatments	Ingredients (%)						
	Cheddar cheese	SMP*	MPC**	Butter 82%	Curcumin Nanoemulsion	Emulsifying salts	NaCl
C	15	8	4.8	21	-	3	0.4
T2	15	8	4.8	18	2.5	3	0.4
T3	15	8	4.8	15	5.0	3	0.4

SMP: Skimmed Milk Powder; **MPC: Milk Protein Concentrate.
C: Control, T1: processed cheese containing 2.5% CR-NE (25mg NC /kg cheese), T2: processed cheese containing 5% CR-NE (50mg NC/kg cheese).

2.2.4. Preparation of Processed Cheese

Processed cheese was prepared according the method of [38] with some modifications, by blending the dry ingredients with previously warmed milk fat (60°C) Table 1, into a processing kettle. The first treatment was kept as control (C). Curcumin nanoemulsion was added to treatment T1 and T2 by ratio 2.5 and 5.0% to reach final concentration 25 and 50 mg NC/kg cheese, respectively, depending on the MIC of CR-NE. Warmed distilled water (60°C) was added to assure that the moisture content of the processed cheese is controlled at approximately 60 ± 1.0% (w/w). Cooking was carried out at 86°C with continuous agitation for 4 minutes. The processed cheese was cooled to 70°C and packed. Processed cheese samples were stored at 4±1°C for 60 days.

2.2.5. Physicochemical Analysis of Processed Cheese

Chemical analysis of processed cheese (protein %, fat % and dray matter % (DM %)) was carried out according the AOAC procedures [39]. The pH of homogenized processed cheese was determined using pH meter (Jenway, UK).

2.2.6. Texture Profile Analysis

The textural analysis of processed cheese samples was performed using a texture analyzer (Stable Micro Systems Ltd. Vienna court, Lammas TA.XT. Plus) based on the procedure stated by [40]. Cone Probe with a 35 mm diameter was used in the test. The optimized test condition was 60 mm back-off distance to sample, deformation ratio 35%, speed of test 60 mm per min, and force of trigger 0.15N. The Hardness (N), adhesiveness (N/s), springiness (m), cohesiveness, gumminess, and chewiness of processed cheese were measured at 20°C.

2.2.7. Microstructure of Processed Cheese

The microstructure of the processed cheese was evaluated by scanning electron microscopy according to the method of [41]. Small pieces of fresh specimens of processed cheese samples were removed and fixed by immersing them immediately in 4F1G (fixative, phosphate buffer solution) pH 7.4 at 4°C for 3 hours. Specimens were then postfixed in 2% OsO4 in the same buffer at 4°C for 2 hours. Samples were washed in the buffer and dehydrate at 4°C through a graded series of ethanol. Samples of processed cheese were dried by means of a critical point method, mounted using carbon paste on an AL-stub and coated with gold up to a thickness of 400 A in a sputter-coating unit (JFC-1100E). The observation of processed cheese morphology in the coded specimens was performed in a Jeol JSM-5300 scanning electron microscope operated between 15 and 20 KeV, and an irradiation current of 10 µm.

2.2.8. Organoleptic Properties

Processed cheese samples were evaluated for organoleptic characteristics (color, taste, texture and overall acceptability), by 12 trained panelists (3males and 9 females) from Food Technology Research Institute, Agricultural Research Center, Alexandria, with ages ranging from 30 to 49 years, they have a good experience in the sensory evaluation of food and dairy products. Processed cheese samples (20 g) were presented in white small dishes coded randomly with 3-digit numbers, at a temperature of 20°C. Water was provided between evaluations of samples for mouth rinsing. Nine points system was used for evaluation based on the method of [42] with minor modification. The evaluation was identified using a 9-point hedonic scale (1 = dislike extremely, 3 = dislike moderately, 5 = neither like nor dislike, 7 = like moderately, and 9 = like extremely).

2.2.9. Statistical Analysis

The data were analyzed by a general linear model procedure (GLM) using SAS statistical analysis software package (SAS Procedure Guide "Version 6.12 Ed." SAS Institute Inc., Cary, 2004). The statistical analysis was performed using one-way analysis of variance (ANOVA). Means were compared by Duncan's test at the significance level of $P \leq 0.05$. Pearson's correlation coefficient was used to calculate the correlation.

3. Results and Discussion

3.1. Characterization of CR-NE

1- Transmission electron microscope (TEM)

The size and morphology of CR-NE were performed using TEM. The particles are spherical with an average size of 30 nm Figure 1. According to [21], the good properties and functionality of NC are due to its particle size, surface charge, surface area, and hydrophobicity. Also, [43] reported that curcumin nanoparticles with the size of 2-40 nm processed by a wet-milling technique, showed higher antimicrobial activity compared with native curcumin.

2. Zetasizer measurements

The hydrodynamic particle size, polydispersity index (PDI) and, zeta potential *(ζ)* of both blank CS NPs and spiramycin-loaded CS NPs were determined using Zetasizer, Figure 2 & Figure 3.

In the present study, the mean hydrodynamic particle size of CR-NE was 40 nm and with polydispersity index (PDI) of 0.33, thus indicating a narrow and favorable particle size distribution (PDI < 0.5). The respective zeta potential value of CR-NE was 37 mV. The zeta potential values were highly negative which can influence the stability in suspension by means of electrostatic repulsion between the similarly charged negative particles. Furthermore, high stability of the current CR-NE was observed over a storage period of 2 months at room temperature. This stability can be linked to the high negative zeta potential of the current CR-NE. High positive or negative zeta potentials have been shown to contribute greatly to the stability of microemulsions and NEs due to the highly charged surfaces, which resist droplet aggregation [44].

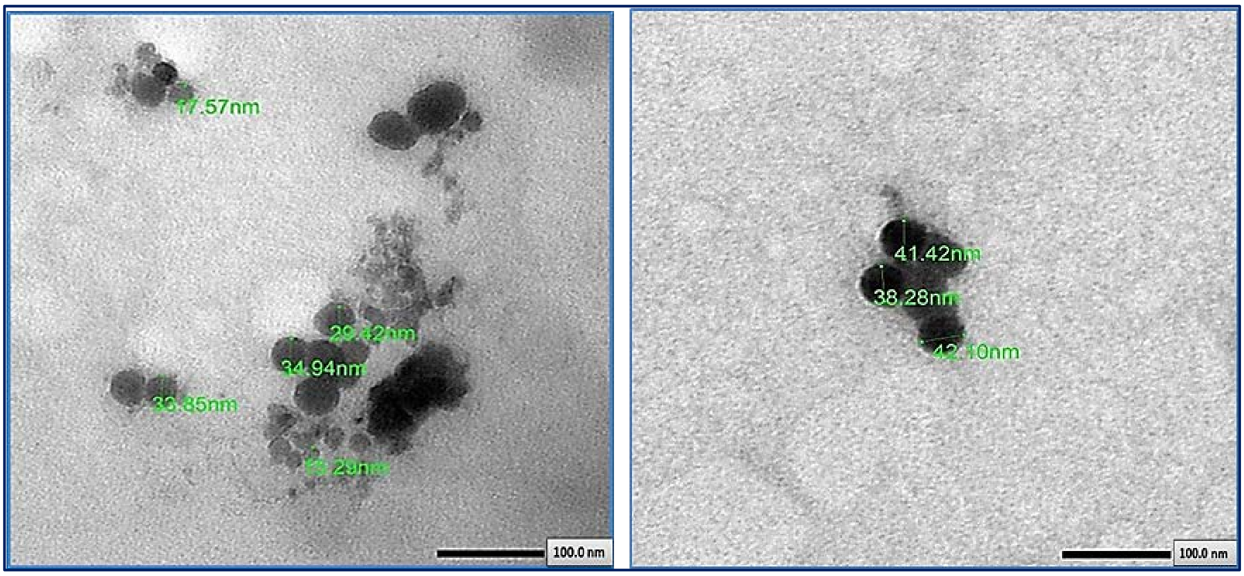


Figure 1. TEM image of CR-NE studied by TEM ((JEOL-100 CX)

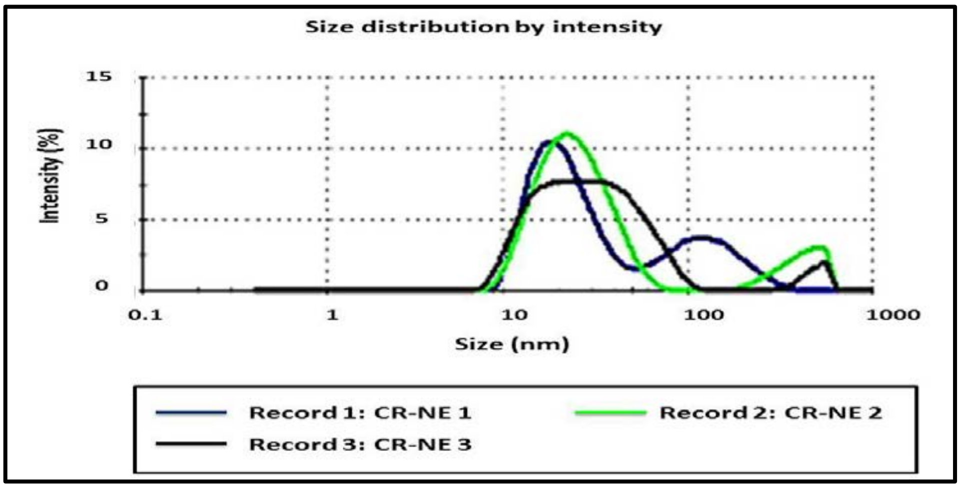


Figure 2. The hydrodynamic particle size of CR-NE as measured by Zetasizer

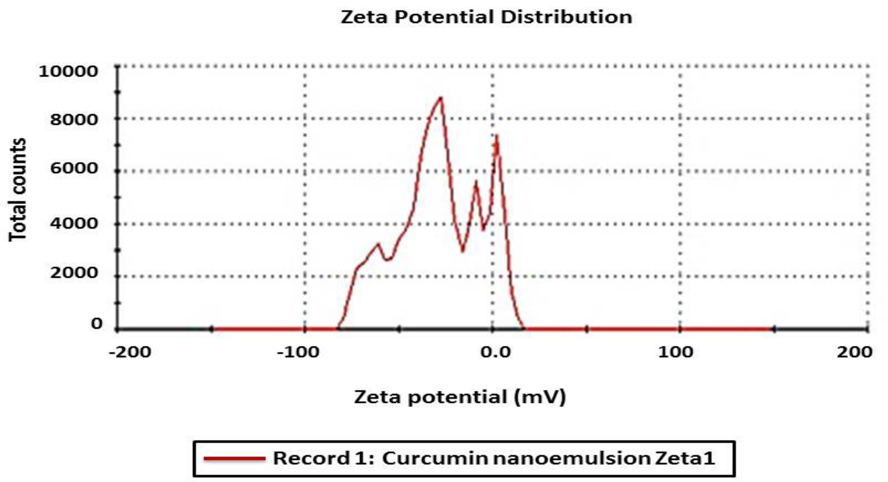


Figure 3. The zeta potential of CR-NE as measured by Zetasizer

3.2. Antimicrobial Activity of CR-NE

The results of the antibacterial activity of CR-NE against pathogenic bacteria strains are shown in Table 2. Curcumin nanoemulsion showed antibacterial activity against *Staphylococcus aureus* NCTC10788, *Clostridium sporogenes* ATCC3584, *Escherichia coli* ATCC25922, *Klebsiella pneumonia* ATCC12296 and *Pseudomonas marginalis* EMCC1271 at concentration 100 and 50 µg /mL, while at concentration 25 µg /mL the nanoemulsion exhibited antibacterial activity against all previous strains except *Clostridium sporogenes* not detected. The minimum inhibitory concentration (MIC) of nanoemulsion was 25 µg /mL with all strains except with *Clostridium sporogenes* strain was 50 µg /mL. These results are in agreement with [45], who found that NC (60 mg/mL) caused inhibition activity against *E. coli* ATCC25922, *S. aureus* ATCC6538, and *E. faecalis* ATCC29212. According to previous studies, curcumin nanoparticles showed antimicrobial activity than normal curcumin because of its enhanced aqueous-phase solubility and dispersibility [43,46]. In our study, the MIC was ranged between 25-50 µg/mL according to the type of bacteria strain. The antimicrobial activity of any nanoparticles depends on their physicochemical properties (size, shape, and surface properties) and the amount used [47,48]. According to [49] and [45] the nanoparticles with a size below 100 nm can disrupt the functions of the cell membrane by binding to the surface of cell membranes with a high affinity compared to larger nanoparticles.

Table 2. Antimicrobial activity and minimum inhibitory concentration (MIC) of curcumin nanoemulsion (CR-NE) against pathogenic strains

Pathogenic strains	Inhibition zone diameter (mm)**				MIC
	100*	50*	25*	12.5*	
Gram-positive bacteria					
***Staphylococcus aureus* NCTC10788**	23	17	10	ND	25
***Clostridium sporogenes* ATCC3584**	19	13	ND	ND	50
Gram-negative bacteria					
***Escherichia coli* ATCC25922**	23	19	12	ND	25
***Klebsiella pneumonia* ATCC12296**	21	15	11	ND	25
***Pseudomonas marginalis* EMCC1271**	20	18	10	ND	25

MIC: Minimum Inhibition Concentration
*Concentrations of CR-NE and MIC are in percent (%) as follows *100= 100 µg NC/mL, *50= 50 µg NC/mL, *25= 25 µg /mL and *12.5= 12.5 µg NC/mL . ND; Not detected.

3.3. Physicochemical Analysis of Processed Cheese

The physicochemical characteristics of foods play a vital role in determining their quality and shelf life [50]. Table 3 presents the physicochemical characteristics of the processed cheese supplemented with CR-NE during 60 days of storage at 4°C. The results showed that the addition of CR-NE (T1 and T2 samples) had no effect on moisture (%) and DM (%) compared with control samples. Meanwhile, at the end of storage (60 days at 4°C) the moisture and DM of C samples were significantly (P<0.05) decreased, while the samples of T1 and T2 were not affected. On 1st day of storage (fresh samples), no significant (P>0.05) differences were found among all treatments in fat, protein, and ash contents, while, after 60 days of cold storage, C samples showed a significant (P<0.05) increased in fat, protein and ash contents, whereas, T1 and T2 samples not changed during storage. The significant (P<0.05) increase in fat, protein and ash contents of C samples lead to the significant (P<0.05) decrease in moisture percentage. These results are in agreement with that found by [51]. On the other hand, the presence of nanoemulsion decreased the loss of moisture in T1 and T2 samples. Many previous studies suggested that nanoemulsion-based delivery systems can positively impact the sensory and physicochemical properties of processed foods [52,53]. Also, pH values of all treatments not changed during storage, and no significant (P<0.05) differences were observed in pH values among processed cheese treatments in fresh samples and after 60 days of storage at 4°C. these results in agreement with El-Sayed et al. [4] who found that the pH value of UHT-processed cheese made from cheddar cheese, MPC and SMP did not changed during cold storage at 4°C for 120 days.

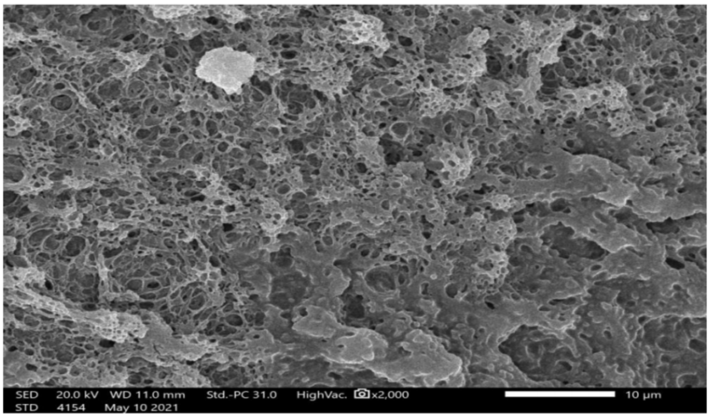
3.4. Microstructure of processed cheese

Scanning electron micrographs (X5000, 10 µm, 20 kV) showed the sizes of fat globules in the processed cheeses containing CR-NE at different concentrations Figure 4. Differences in the protein structure between T1 and T2 were less compared with control. The cheese fat and protein clusters appear densely packed and there some free fat is present. Protein and fat clusters in the control sample appear smaller than T1 and T2 samples. Free fat globules are significantly more prominent in the control sample than observed in other treatments. The globules of fat-free in the control sample were smaller in size than that in T1 and T2 samples. In this study, we found that when adding CR-NE the firmness was decreased and the casein network structure became softer, this means that the adding of CR-NE caused changes in the structure of protein-fat network. In this study, the addition of CR-NE with emulsifying salts increased the dispersion of fat globules. Therefore, it can be suggested that the cheese processed supplemented with CR-NE has become like particle-filled gel networks where fat globules act as filler molecules in the protein network [54]. According to [30], nanoemulsions are used in foods to improve the performance of the ingredients of the formulations. Also, nanoemulsions can be used as texture modulators. Depending on internal-phase proportion, oil composition, stabilizer (type and concentration), and size of the droplet, nanoemulsions can exhibit different rheological behaviors from those of viscous liquids in viscoelastic solids [55].

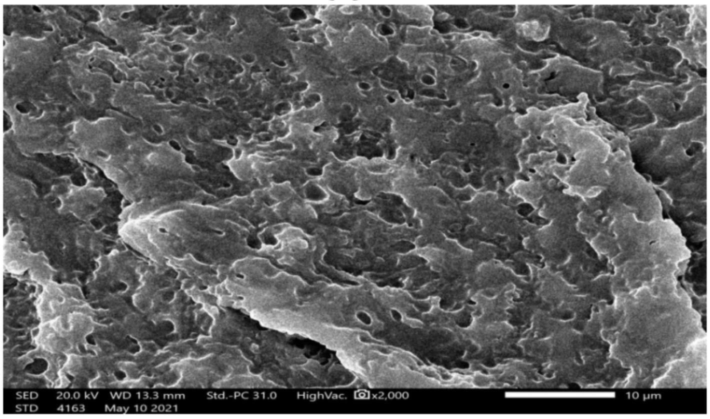
Table 3. Physicochemical analysis of processed cheese supplemented with curcumin nanoemulsion (CR-NE) during cold storage

Characteristics	Storage time	Treatments		
		C	T1	T2
Moisture%	Fresh	59.63±0.32^{aA}	59.22±1.16^{aA}	59.61±0.42^{aA}
	60 days	56.69±0.18^{bB}	58.69±0.45^{aA}	58.83±0.62^{aA}
DM%	Fresh	40.36±0.32^{aB}	40.77±1.16^{aA}	40.38±0.43^{aA}
	60 days	43.31±0.08^{aA}	41.30±0.45^{bA}	41.16±0.62^{bA}
Fat %	Fresh	22.88±0.07^{aB}	22.95±0.05^{aA}	22.98±0.02^{aA}
	60 days	24.27±0.50^{aA}	23.11±0.12^{bA}	23.05±0.15^{bA}
Protein %	Fresh	9.83±0.55^{aB}	9.81±0.25^{aA}	9.80±0.15^{aA}
	60 days	10.77±0.25^{aA}	9.81±0.30^{bA}	9.83±0.19^{bA}
Ash %	Fresh	4.09±0.066^{aB}	4.04±0.057^{aA}	4.02±0.092^{aA}
	60 days	4.67±0.158^{aA}	4.13±0.050^{bA}	4.05±0.058^{bA}
pH	Fresh	5.76±0.012^{aA}	5.77±0.015^{aA}	5.80±0.010^{aA}
	60 days	5.74±0.050^{aA}	5.75±0.050^{aA}	5.74±0.052^{aA}

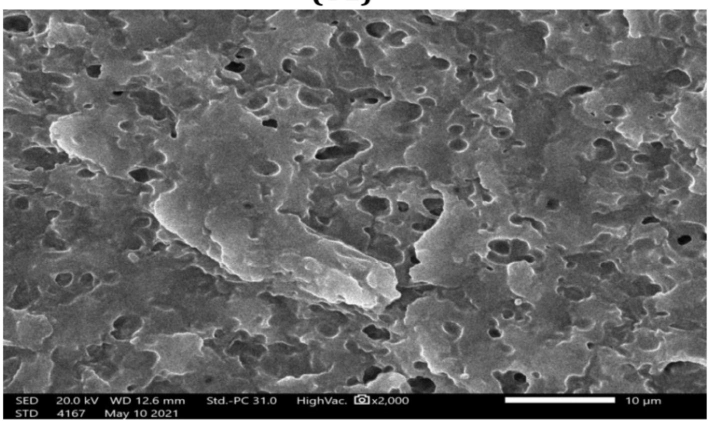
C: Control, T1: processed cheese containing 2.5% CR-NE (25mg NC /kg cheese), T2: processed cheese containing 5% CR-NE (50mg NC/kg cheese). **Values are mean ± SD (n = 3). The level of significant was present at $p < 0.05$. Means with the same letter(s) are not significant. Small letters refer to differences among treatments in the same row. Capital letters: refer to differences among storage times in the same column.*



(C)



(T1)



(T2)

Figure 4. Scanning electron micrographs of processed cheese supplemented with CR-NE. C: Control; T1: processed cheese containing 2.5% CR-NE (25mg NC /kg cheese), T2: processed cheese containing 5% CR-NE (50mg NC/kg cheese)

3.5. Texture Profile Analysis of Processed Cheese

To test the impact of CR-NE addition on the properties of processed cheese samples, the texture profile analysis (TPA) of the cheese was exploited. The result in Table 4, illustrated that the hardness of all processed cheese samples was significantly (P<0.05) increased during cold storage (at 4 °C for 60 days), but the higher increase was observed with the control sample (C) than the samples containing CR-NE (T1 and T2). The T2 samples showed less hardness at the end of storage time (60 days). The results in Table 4 clearly show that the Adhesiveness was significantly (P < 0.05) decreased with the increase of CR-NE content in cheese formula (T1 and T2 samples). The Adhesiveness of C and T1 samples were significantly (P < 0.05) increased during cold storage, while, the Adhesiveness of T2 samples was significantly (P < 0.05) decreased by the end of storage. The cohesiveness values were significantly (P < 0.05) increased during storage with C and T1 samples, while, T2 sample not changed. On 1st day of storage, the highest cohesiveness was observed with T2, meanwhile, the control sample recorded the highest value followed by T1 samples, and T2 was the lowest. The springiness was significantly (P < 0.05) increased in samples containing CR-NE (T1 and T2), whereas, control sample was significantly (P < 0.05) decreased. The highest value of springiness was observed with C in the fresh sample, and with T1 at the end of storage. Gumminess value was significantly (P < 0.05) increased with all treatment during cold storage. The order of the samples in terms of Gumminess value was as follows: C>T1>T2.

All processed cheese treatments showed a significant (P < 0.05) increase in chewiness value during storage. The order of the samples in terms of chewiness value at the beginning and end of the storage period was as follows: C>T1>T2.The decrease in moisture content and increase of DM% especially protein content results in the increase in the hardness of cheese. This result agrees with [56] who reported that the hardness of processed cheese, in general, is an attribute that directly correlates with the moisture content, pH value, emulsifying salt, fat content, and the type and properties of natural cheese used in the blend. Also, [57] reported that the increase of hardness of stored cheese may be due to a decrease in moisture content and less water availability during storage, and consequently change of texture properties. While, [58] reported that increasing the protein content of cheese results in significant increases in hardness during storage. Similarly, [59] confirmed the positive correlations between casein content and firmness of cheddar processed cheeses. Nanoemulsions were used in the production of low-fat foods such as ice cream and mayonnaise without immolating their texture, but offering a healthier option to consumers [60].

Table 4. Texture profile analysis of processed cheese supplemented with curcumin nanoemulsion during cold storage

Characteristics	Time	C	T1	T2
Hardness (N)	Fresh	1.500±0.15^{aB}	0.863±0.057^{bB}	0.712±0.020^{bB}
	60 days	3.121±0.12^{aA}	1.260±0.15^{bA}	0.810±0..07^{cA}
Adhesiveness (mJ)	Fresh	3.87±0.080^{aB}	1.93±0.040^{bB}	1.35±0.011^{cA}
	60 days	5.62±0.055^{aA}	2.14±0.016^{bA}	1.07±0.020^{cB}
Cohesiveness	Fresh	0.750±0.200^{bB}	0.646±0.035^{cB}	0.850±0.026^{aA}
	60 days	1.310±0.030^{aA}	1.070±0.026^{bA}	0.913±0.035^{cA}
Springiness (mm)	Fresh	6.14±0.035^{aA}	6.04±0.066^{bB}	5.96±0.230^{cB}
	60 days	5.71±0.095^{cB}	6.28±0.080^{aA}	6.21±0.060^{bA}
Gumminess (N)	Fresh	1.100±0.050^{aB}	0.606±0.025^{bB}	0.506±0.009^{cB}
	60 days	4.100±0.062^{aA}	1.400±0.070^{bA}	0.703±0.020^{cA}
Chewiness (mJ)	Fresh	6.86±0.045^{aB}	3.43±0.030^{bB}	3.02±0.075^{cB}
	60 days	23.29±0.319^{aA}	8.83±0.050^{bA}	4.20±0.010^{cA}

C: Control, T1: processed cheese containing 2.5% CR-NE (25mg NC /kg cheese), T2: processed cheese containing 5% CR-NE (50mg NC/kg cheese). **Values are mean ± SD (n = 3). The level of significant was present at p < 0.05. Means with the same letter(s) are not significant. Small letters refer to differences among treatments in the same row. Capital letters: refer to differences among storage times in the same column.*

3.6. Organoleptic Evaluation of Processed Cheese

Consumers' demands for new and foods with higher health benefits are encouraging the food and dairy industry to research novel strategies to supplement food products with bioactive compounds and turning foods into products that promote health and wellness [61,62]. Figure 5 shows the organoleptic evaluation of processed cheese made with CR-NE during 60 days of storage at 4°C. There were no significant differences (P>0.05) between the control and T1 samples in terms of color, taste, texture, and overall acceptability scores during cold storage. Meanwhile, T2 samples recorded the lowest (P<0.05) color, taste, texture, and overall acceptability scores compared to C and T1 samples during storage. On the other hand, no significant (P>0.05) changes were observed in terms of color, taste, texture, and overall acceptability of all treatments during cold storage at 4°C for 60 days. The higher concentration of CR-NE (50µg NC/g cheese) in processed cheese formula (T2) caused a decrease in sensory properties, while, the addition of CR-NE (25 µg/g cheese) had no effect on the sensory properties of the T1 cheese sample compared to control sample. The T1 samples showed higher acceptability than the T2 samples at the beginning and end of storage. Nonetheless, T2 samples are also sensory acceptable but less than the T1 sample. Although the T2 sample is the highest in terms of health benefits, consumer acceptance of the product is the most important criterion for the success of this product. The results of T1 samples are agree with [63] who reported that the nanoemulsion-based delivery system should have compatibility with the food matrix and minimal effect on the organoleptic properties of the food such as its flavor, appearance, and texture. The quality and acceptability of the product are directly related to the quality of the raw material and manufacturing [59]. Newly, consumers have shown a preference for minimally processed products with the most natural ingredients and additives that have a health benefit, such as prebiotics and probiotics, enzymes, antimicrobials, and antioxidants that exist naturally as extracts or essential oils obtained in general from plants [64,65], and this is where nanotechnology allows improvement of the functionality of various ingredients, decreasing the concentration of substances, modifying their solubility, and potentiating their effectiveness or controlling their release [66]. Therefore, food companies have been using nanoemulsion in the development of many food products, to improve their properties like color, flavor, odor, texture, solubility, stability, bioavailability, and efficient utility of many bioactive compounds edible/food-grade and to extend shelf life [67,68,69,70].

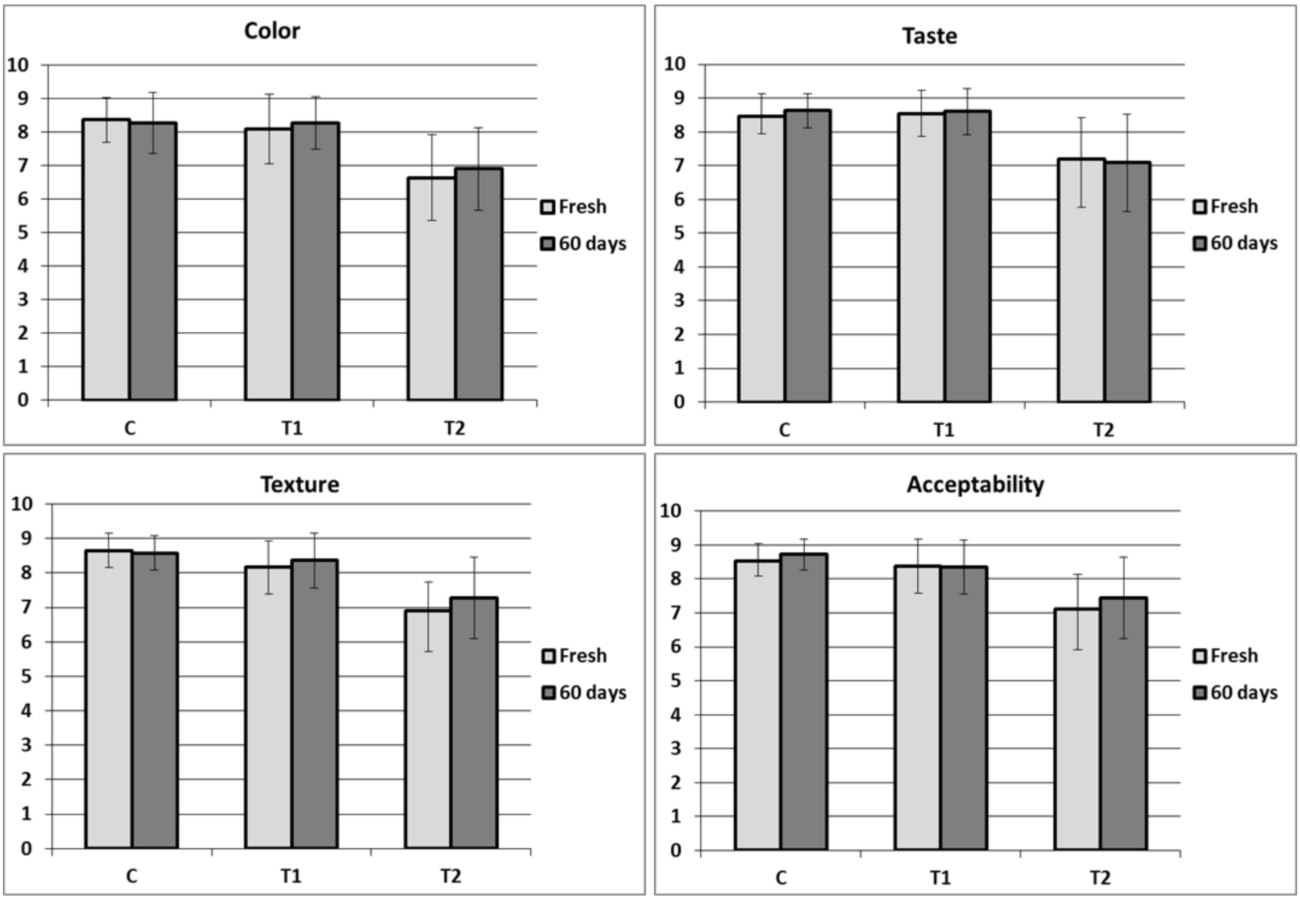


Figure 5. Sensory evaluation of processed cheese supplemented with CR-NE during 60 days of storage at 4°C. C: Control, T1: processed cheese containing 2.5% CR-NE (25mg NC /kg cheese), T2: processed cheese containing 5% CR-NE (50mg NC/kg cheese)

4. Conclusion

This study aimed to investigate the effect of curcumin nanoemulsion (CR-NE) on physicochemical, textural, microstructure and organoleptic properties of processed cheese. Curcumin nanoemulsion showed higher antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and, *Pseudomonas marginalis* at a concentration of 25 µg NC/mL and 50 µg NC/mL with *Clostridium sporogenes.* Curcumin nanoemulsion improved the physicochemical, microstructure, textural and microstructure properties of processed cheese during cold storage at 4°C for 60 days. Moreover, the sensory evaluation of processed cheese containing 2.5% CR-NE not affected in compared with control, while, T2 samples containing 2.5% CR-NE were slightly changed during storage. The acceptance level and excellence properties obtained of T1 represent a very important result, and this acceptance is an indicator that the dairy factories could introduce such a product into the local market.

Acknowledgments

The authors would like to thank Dr. Esam Othman, Quality Manager, Mansour Group, Alexandria, Egypt, for his help during the preparation of the processed cheese. The authors appreciate the assistance of Dr. Rehab Mohamed Ibrahim, Special Food and Nutrition Department, Food Technology Research Institute, Alexandria, Egypt, during the conduct of the research.

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