Effect of adding curcumin and betacyclodextrin nanocomplex on some of the physicochemical, microbiological and sensory traits of yogurt

Safa Mohammed Abdul-Hussein[1]
safa.p100519@fosci.uoqasim.edu.iq

Sadeq Muneer Shawkat[2]
sadeq.muneer@fosci.uoqasim.edu.iq

[1,2] Department of Dairy Science and Technology, College of Food Sciences, Al-Qasim Green University, Babylon, Iraq.

- Part of MSc. Dissertation for the first author.
- Date of research received 23/08/2023 and accepted 28/09/2023**.**

Abstract

The aim of this research is to study the effect of adding free curcumin and curcumin loaded with betacyclodextrin to laboratory-made yogurt by conducting a set of tests on the final product. Yogurt was prepared using three treatments. The first treatment was a standard yogurt without any additions, while the second treatment involved adding free curcumin (CU) at concentrations similar to the third treatment, which contained curcumin loaded with betacyclodextrin (B-CU) at concentrations of 100 and 200 µg/ml. which involved measuring the protein percentage, moisture percentage, fat percentage, and ash percentage of the samples studied from day one of production until day 28 of refrigerated storage, which were (4.61, 86.62, 5.64, 0.86) respectively. Various physical and rheological tests were conducted, including total acidity, pH, spontaneous whey separation, viscosity value, and water-holding capacity, which were (0.97, 4.61, 3.1, 2680, 72.36) respectively, up to day 28 of refrigerated storage. Microbiological tests were also conducted on the manufactured yogurt under the same conditions, which showed the efficiency of the active compound, whether in its free form or loaded with β-cyclodextrin, as a microbial inhibitor and preventing contamination of the manufactured product. The tests demonstrated the superiority of the B-CU treatment containing 200 mg/ ml of curcumin-loaded β-cyclodextrin over the other treatments. The sensory evaluation was well-received, ranging from (95-77)% from day one of production until day 28 of refrigerated storage.

Key words: Nanotechnology, Curcumin, Betacyclodextrin, Water holding capacity, Yogurt.
Citation: Abdul-Hussein, S., & Shawkat, S. (2023). Effect of adding curcumin and betacyclodextrin nanocomplex on some of the physicochemical, microbiological and sensory traits of yogurt. *Kirkuk University Journal For Agricultural Sciences*, 14 (4), 28-42. doi: 10.58928/ku23.14403
Correspondence Author: Safa Mohammed Abdul-Hussein - safa.p100519@fosci.uoqasim.edu.iq

Introduction

The issue of food spoilage is one of the challenges faced by both factories and consumers. Spoilage occurs due to various external factors, such as heat, moisture, and oxygen. Nanotechnology offers a solution to increase the shelf life of different types of food and reduce losses caused by microbial contamination [1]. It is considered one of the most promising technologies that can revolutionize the food industry, as it has demonstrated its efficacy in food processing and packaging [2]. Recent studies encourage the use of biodegradable polymers enhanced with nanomaterials in food packaging, ensuring consumer safety [3]. Nanotechnology in food packaging provides enhanced protection to food products by improving mechanical and thermal capabilities well as anti-bacterial properties. Nanotechnology involves dealing with atoms or molecules ranging in size from 1 to 100 nanometers. This allows for the monitoring and processing of materials at the nanoscale. These materials exhibit unique properties due to their high surface-to-volume ratio and other physical and chemical characteristics such as color, solubility, strength, diffusion, toxicity, magnetism, and thermodynamics [4] and [5].

Various factors contribute to food spoilage, including the growth and activity of microorganisms, the action of food enzymes, and chemical reactions within the food itself. To control these factors, preservatives are used [6]. Recently, consumers have become more aware of the importance of a healthy diet for overall well-being, and there is a preference for food products that can improve health. This has led to the development of functional foods and an increase in their consumption in the market [7]. In addition to their high nutritional value, functional foods reduce the risk of certain diseases such as cancer, type 2 diabetes, dyspepsia, and cardiovascular diseases [8]. One of the most important functional compounds with health-promoting and protective properties is curcumin [9], an active compound extracted from the turmeric plant that possesses antioxidant, anti-inflammatory, antimicrobial, and anti-mutagenic properties [10].

This study aimed to use curcumin extract to produce functional foods free of chemical additives as a result of advancements in the food industry and rising demand for health-improving additives, as follows:

1. Investigate the possibility of addition free curcumin and a curcumin and betacyclodextrin nanocomposite to laboratory-made yogurt.
2. Examine the effects of these additions on extending the shelf life of yogurt as well as their impact on its chemical, physical, rheological, microbial and sensory properties.

Materials and Methods:

Curcumin and betacyclodextrin were prepared beforehand following the method described by [11]. The yogurt starter was obtained from Dansco – France, and fresh milk was obtained from the fields adjacent to Al-Qasim Green University.

Yogurt Manufacture:

Before initiating yogurt production, raw whole cow's milk was used, sourced from the fields adjacent to Al-Qasim Green University. The quality of the whole milk was assessed prior to the manufacturing process. The analysis showed the following percentages: moisture (88.20%), fat (3.40%), protein (3.25%), non-fat solids (8.20%), and total solids (11.60%). The pH percentage and total acidity were determined to be 6.67% and 0.15%, respectively. These percentages fall within the normal limits of milk quality as reported by [12] and [13]. The yogurt was prepared according to the method described in [14].

The milk was pasteurized at a temperature of 90°C for 10 minutes, followed by cooling to a temperature of 42±2°C. The milk was divided into three parts. The first part was used for the production of standard C yogurt, while the other two parts was used to add free curcumin and curcumin and betacyclodextrin to the starter culture consisting of *Streptococcus Salivarius subsp thermophiles* and *Lactobacillus delbrueckii subsp bulgaricus*. The quantity of the starter culture was as specified by the producing company, with an average of 0.002% per 1000 ml. The yogurt was then packed in 150ml plastic containers and incubated at 42±2°C for 4-6 hours until the coagulation process was

complete and the pH dropped to 4.6. After the completion of the coagulation process, the yogurt was stored at 5 pm for the necessary tests to be conducted after 1, 7, 14, 21, and 28 days from the manufacturing date.

Chemical Composition of Laboratory-made Yogurt:

Fat Percentage:

The fat content of the laboratory-made yogurt samples was determined using a lactoflash dairy device, which electronically estimates milk components, as described by [15].

Protein Percentage:

The percentage of protein was estimated by determining the total nitrogen content using the Kjeldahl method. The method involved weighing 0.1 g of the yogurt sample and adding 1 ml of copper sulfate ($5H_2O$ $CuSO_4$) (prepared previously by dissolving 5 g of sulfate in a 100 ml volumetric bottle filled with distilled water). Then, 12 g of potassium sulfate (K_2SO_4) and 20 ml of concentrated sulfuric acid were added to the sample. The mixture was thoroughly mixed, followed by a digestion process for half an hour at a temperature of 200°C, and then for an additional hour and a half at a temperature of 420°C. The samples were then distilled and titrated with 0.1 standard HCL acid. The total nitrogen content was calculated using the equation:

$$\textbf{Total Nitrogen\%}=\frac{1.4007\times Volume\ of\ HCl\ consumed\times Standard\ concentration}{sample\ weight}\times 100$$

stated in [17]. The moisture percentage was calculated using the following equation:

Moisture % = (weight of the sample after drying the stalk - weight of the stalk before drying the model)/(weight of the sample) x 100

Physical and Rheological Tests of Laboratory-Made Yogurt:

pH:

The pH of the yogurt samples was determined using a pH meter sensor (HANNA Instruments Microprocessor). The pH measurements were taken on days 1, 7, 14, 21, and 28 during cold storage at 5 ºC for the laboratory-produced yogurt samples.

Estimation of total acidity:

The total acidity was estimated based on the lactic acid content of the yogurt. 9 g of the sample was placed in a glass beaker, and drops of phenolphthalein reagent were added. A sodium hydroxide solution with a concentration of 0.1 N was then added dropwise until a pink color appeared, following the method mentioned in [18]. The percentage of total acidity was calculated using the following equation:

The total protein percentage was calculated by multiplying the total nitrogen content by 6.38, which is the milk nitrogen conversion factor, as mentioned in [16].

Ash Percentage:

For all treatments, 2 g of yogurt samples were taken and placed in a known -weight porcelain jar. The samples were incinerated in an oven at a temperature of 550°C for approximately 6 hours, or until white ash was obtained. The ash was then transferred to a drying container to cool, and the weight was measured. The ash percentage was calculated using the direct burning method mentioned in [17] with the following equation:

Ash% = (the weight of the empty jar - the weight of the jar with the ash)/(the weight of the original model)

Moisture Percentage:

The percentage of moisture in the yogurt was estimated by placing 2 g of the sample in a weighed ceramic jar and drying it in an oven at a temperature of 105°C until a stable weight was achieved, following the method

$$\textbf{Acidity \%}=\frac{The\ volume\ of\ the\ base\ consumed\ (ml)x\ the\ standard\ of\ the\ base\times Weight\ equivalent\ to\ lactic\ acid}{sample\ weight}$$

Viscosity value:

The apparent viscosity of yogurt samples was estimated after 1, 7, 14, 21, and 28 days of cold storage using a Brookfield DVII+ viscometer manufactured by Brookfield Engineering Lab, according to the method

followed by [19]. The viscosity value of a 150ml sample was estimated within a maximum time of 60 seconds after the device broke the gel and stirred it ten times clockwise and ten times counterclockwise. The reading was taken three times.

Spontaneous whey separation:

The spontaneous whey separation was estimated by taking 50 g of yogurt manufactured in a 100ml glass beaker and placing it at a tilted angle of 45 degrees for approximately two hours at a temperature of 5 °C. After this, the spontaneous whey separation was withdrawn using a syringe, and then the weight of the yogurt sample was taken within a maximum of ten seconds to prevent excessive perfusion, according to the method described by [15].

Water-holding capacity:

The water-holding capacity of laboratory-made yogurt was determined by subjecting a 15-gram yogurt sample to a refrigerated centrifuge at 3000 revolutions per hour. Then, the filtrate and the weight of the remaining sediment were neglected, and the percentage of the ability to retain water was estimated using the following equation:

Water holding capacity % = (weight of precipitate)/(initial weight of sample) x 100

Microbiological examinations of laboratory-made yogurt:

After studying the effectiveness of free curcumin extract loaded with betacyclodextrin as antioxidants or microorganisms in the laboratory, their effect on preserving yogurt and prolonging the shelf life of yogurt samples from days 1, 7, 14, 21, and up to 28 days of cold storage at a temperature of 5 ± 1 °C was investigated. 1 ml of the laboratory-made yogurt sample was transferred to a test tube containing 9 ml of previously prepared peptone water. Then, all the contents were mixed with the vortex electrophoresis, and the rest of the dilutions were completed until the desired dilution was reached 0.1 ml of the dilution was transferred to Petri dishes and then poured onto the appropriate media according to the required assay.

Calculating the total number of coliform bacteria:

Total coliform bacteria were estimated using MacConkey Agar culture media, as mentioned in [18] The culture dishes were incubated at 37°C for 24-48 hours.

Estimation of the total number of starter bacteria:

The total numbers of *Streptococcus salivarius subsp thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* were estimated after samples were taken from the decimal dilutions of the previously prepared yogurt treatments, as mentioned in [18]. They were incubated in anaerobic conditions at a temperature ranging between 42-45°C for 48-72 hours.

Calculating the numbers of molds and yeasts:

The numbers of molds and yeasts were estimated using Potato Dextrose Agar, and the dishes were incubated at 22°C for 5 days, as mentioned in [18].

Sensory evaluation of yogurt:

Sensory evaluation tests were carried out for laboratory-manufactured yogurt models within the graduate lab of the Department of Dairy Science and Technology, College of Food Sciences, Al-Qasim Green University, according to the sensory evaluation form. The form included the traits of taste, flavor, texture, color, and external appearance, designed by [20].

Results and Discussion:

Effects of adding free curcumin extract and curcumin and betacyclodextrin nanocomposites to yogurt:

Yogurt was made from whole milk and fortified with different percentages of free curcumin extract and curcumin and betacyclodextrin to determine the appropriate concentration for the best fortified product with unique properties. The manufacturing treatments were as follows: The standard treatment is symbolized by C, to which no concentration of the active compound was added; the second treatment is symbolized by CU, to which 100 and 200 micrograms of free curcumin extract were added per ml of pasteurized milk; and the last treatment is symbolized by B-CU, to which curcumin and betacyclodextrin was added in the same concentrations as mentioned earlier. The three

treatments were stored under refrigeration at a temperature of 1±5°C for 30 days.

Assays for the chemical composition of laboratory-made yogurt:

Fat percentage:

From the table below, we find that the fat percentage in the yogurt for the three aforementioned treatments immediately after the manufacturing process is about 3.35% for the standard sample C, which is consistent with [21]. As for the yogurt supplemented with free curcumin, it ranged from 3.43% to 3.48% for the treated samples of CU (100-200) µg/ml, and for the B-CU sample, it was about 3.38% at the concentration of 100 µg/ml and 3.41% for the concentrations of 200 µg/ml. During refrigerated storage for a period of two and three weeks, we found that the fat percentage differed in relation to the standard sample to 3.45-3.50%, which may be due to the low percentage of moisture and the high percentage of total solids, including fat, as indicated by a previous study. Meanwhile, the CU samples showed a decrease in the fat percentage compared to the sample examined immediately after manufacturing, and this was particularly evident for the concentration of 200 µg/ml, which ranged from 3.59% to 3.62%. This is consistent with the samples of the B-CU treatment (curcumin-betacyclodextrin), which showed a fat percentage close to the standard sample under the same storage conditions, ranging from 3.51% to 3.55%. After a period of 28 days of cold storage, we notice that there is damage in the standard treatment, which may be due to the high percentage of free fatty acids resulting from contamination during the manufacturing process. As for the CU sample (containing free curcumin extract), we observe a slight increase in the fat percentage at modest levels compared to the sample from the third week of cold storage. It remained within the permissible standards at 3.67-3.71% for each of the concentrations used. Under the same conditions for the 28th day of cold storage, we notice that the values of the B-CU treatment remained stable and within the same limits as the sample from the third week of manufacturing, ranging between 3.60% and 3.64%.

Protein Percentage:

The results shown in the table indicate that the percentage of protein in yogurt for the mentioned treatments, specifically for standard treatment C immediately after manufacturing, was about 4.31%. This result is consistent with a previous study on yogurt made from whole milk and stored at 5 °C [22], where the protein content after one to two weeks was 4.33% and 4.35%, respectively. When measuring the change in protein percentage for treatment CU with a concentration of 100 µg/ml from the first day of manufacture until the fourth week, the percentages were 4.33%, 4.35%, 4.37%, 4.46%, and 4.50%, respectively. For a concentration of 200 µg/ml, the percentages were 4.34%, 4.36%, 4.39%, 4.44%, and 4.49% in consecutive order. The high protein percentage is attributed to the decrease in moisture content, which leads to a higher percentage of total solids, including protein. Finally, from the table, we observe that the treatment containing curcumin and betacyclodextrin with concentrations of 100 µg/ml and 200 µg/ml, during the cold storage period at 5 °C, ranged from 4.35% to 4.58% and 4.36% to 4.61%, respectively.

Ash Percentage:

Based on Table 1 the percentage of ash in yogurt for the standard treatment immediately after manufacturing was 0.60%. This result aligns with a previous study [23], that reported a 0.70% ash content in yogurt made from whole milk. Regarding the ash percentage for treatment CU on the first day of manufacturing and during cold storage, it reached 0.67% at a concentration of 100 µg/ml of free curcumin and 0.69% at a concentration of 200 µg/ml. In the second week of cold storage, the ash percentage for the standard yogurt treatment was 0.65%, while for the CU treatment, it was 0.72% and 0.77% for concentrations of 100 µg/ml and 200 µg/ml, respectively. In the third week, the ash percentage for the standard treatment C was 0.68%, while the sample was damaged in the fourth week. The CU treatment showed varying levels of ash percentage for concentrations of 100 µg/ml and 200 µg/ml in the third and fourth weeks, ranging from 0.74% to 0.82% and 0.78% to 0.86%,

respectively. As for the B-CU treatment, from the first day of manufacture until the fourth week, the ash percentage was higher for the concentration of 200 µg/ml compared to the same treatment and manufacturing conditions for the concentration of 100 µg/ml, reaching 0.70%, 0.74%, 0.77%, 0.78%, and 0.83%, and 0.71%, 0.75%, 0.79%, 0.80%, and 0.86%, respectively.

Moisture Percentage:

In the table below, we observe that the moisture percentage of the standard yogurt treatment C was approximately 87.15% on the first day of manufacture, which is consistent with the findings of [24], who reported a moisture content of 87.3% for yogurt made from whole milk during cold storage. For the yogurt treatment (CU with free curcumin) and the B-CU treatment (curcumin-betacyclodextrin) with concentrations that exhibited the highest antimicrobial activity (100-200 µg/ml), the percentages were also close, ranging from 87.28% to 78.35% and 87.17% to 87.20%, respectively, under the same storage conditions. When comparing the moisture percentage between the first and last weeks for all treatments, significant and varied variations were observed. For the standard sample C, the moisture content ranged from 87.03% to 86.59%. For the yogurt treatment CU, the moisture content at a concentration of 100 µg/ml ranged from 87.09% to 86.67%, and at a concentration of 200 µg/ml, it ranged from 87.18% to 86.72%. The values for the B-CU treatment nder the same conditions for the concentrations of 100 µg/ml and 200 µg/ml, ranged from 87.05% to 86.60% and 87.06% to 86.62%, respectively. The LSD value indicates significant differences in moisture, protein, fat, and ash percentages.

Table (1) shows the chemical properties of laboratory-made yogurt for the standard treatment and two treatments containing free curcumin and curcumin loaded with betacyclodextrin during refrigeration at (5±1) ºC for a period ranging from 1 to 28 days.

Treatments	Shelf life of yoghurt (day)	Protein%	Moisture%	Fat %	Ash %
C	1	4.31	87.15	3.35	0.60
	7	4.33	87.03	3.40	0.62
	14	4.35	86.85	3.45	0.65
	21	4.39	86.73	3.50	0.68
	28	4.46	86.59	3.58	0.76
CU 100µg/ml	1	4.33	87.28	3.43	0.67
	7	4.35	87.09	3.49	0.70
	14	4.37	86.91	3.52	0.72
	21	4.46	86.82	3.58	0.74
	28	4.50	86.67	3.67	0.79
CU 200µg/ml	1	4.34	87.35	3.48	0.69
	7	4.36	87.18	3.54	0.73
	14	4.39	86.95	3.59	0.77
	21	4.44	86.88	3.62	0.78
	28	4.49	86.72	3.71	0.82
B-CU 100µg/ml	1	4.35	87.17	3.38	0.70
	7	4.37	87.05	3.44	0.74
	14	4.41	86.87	3.49	0.77
	21	4.51	86.75	3.52	0.78
	28	4.58	86.60	3.60	0.83
B-CU 200µg/ml	1	4.36	87.20	3.41	0.71
	7	4.39	87.06	3.47	0.75
	14	4.44	86.88	3.51	0.79
	21	4.57	86.80	3.55	0.80
	28	4.61	86.62	3.64	0.86
LSD		0.1622	0.2090NS	0.04021	0.02529 NS

- Reading is an average of two repetitions.

Physical and Rheological Tests of Laboratory-Made Yogurt:

pH:

The results mentioned in Table 2, which was shown, the pH evaluations of the laboratory-made yogurt treatments. The pH of the standard treatment after the first day of manufacture was around 4.82. The pH values of the CU-treated samples on the first day of manufacture were approximately 4.79 for a concentration of 100 µg/ml and 4.75 for a concentration of 200 µg/ml. During cold storage at 5 ± 1°C for the same concentrations mentioned above, the pH values for the B-CU treatment ranged from 4.81 to 4.80. With the progression of the storage period from the first week to the second week, the pH values for the standard treatment free of the active compound ranged from 4.79 to 4.76. For the CU treatment at concentrations of 100 and 200 µg/ml, the pH values ranged from 4.64 to 4.59 and from 4.61 to 4.53, respectively. The pH values of the treatment containing curcumin and betacyclodextrin B-CU ranged from 4.79 to 4.75 for a concentration of 100 µg/ml and from 4.77 to 4.73 for a concentration of 200 µg/ml. As the storage period progressed, the pH value of the standard treatment reached its lowest level in the third week of cold storage, at around 4.66. The lowest pH levels for the CU treatment were observed at a concentration of 200 µg/ml, reaching 4.40 in the fourth week. The pH value of the B-CU treatment in the fourth week was around 4.61 for a concentration of 200 µg/ml [25].

Total Acidity:

The results shown in Table 2 display the total acidity percentage for the yogurt treatments during manufacturing and cold storage for up to four weeks. The total acidity values varied among the three treatments and were associated with an increase in the concentration of the active substance, whether in its free form or loaded with betacyclodextrin. This increase is due to the production of lactic acid by the starter bacteria that transform milk sugar into lactic acid. On the first day of manufacturing, the acidity value for the standard treatment C was 0.85%, which is consistent with a previous study [16]. The acidity percentage for the CU treatment ranged from 0.89% to 0.92% for concentrations of 100-200 µg/ml, while for the B-CU treatment, it ranged from 0.86% to 0.85% for the same concentrations used in the CU treatment. With the progression of the storage period, the acidity values tended to converge for the standard control treatment C and the B-CU treatment with concentrations of 200-100 µg/ml from the first week until the third week, ranging from 0.95% to 0.87% and from 0.92% to 0.92%, respectively. The acidity values for the CU treatment during the same time period and under the same storage conditions ranged from 1.01% to 0.92% and from 1.10% to 0.96% for concentrations of 200-100 µg/ml, respectively. The acidity values reached their highest levels for the CU treatment in the fourth week of cold storage, without showing any microbial contamination percentages. The same trend was observed for the control treatment and the B-CU treatment, with values of 1.15% for a concentration of 200 µg/ml and 0.97% for a concentration of 200 µg/ml, respectively. This indicates that the curcumin and betacyclodextrin nanocomposites retain their physical properties and prevent the quick release of curcumin, resulting in an increased rate of acidity development compared to the free curcumin compound.

Viscosity Value:

The viscosity value of the standard yogurt treatment C immediately after manufacturing and during cold storage was about 1852 centipoise. For the CU treatment samples at concentrations of 200-100 µg/ml under the same conditions, the viscosity values ranged from 1890 to 1905 centipoise. As for the B-CU treatment, the viscosity value on the first day after the manufacturing process and during cold storage was 1860 centipoise for a concentration of 100 µg/ml and 1875 centipoise for a concentration of 200 µg/ml. As the products aged in cold storage from the first week to the third week, the viscosity values changed compared to the first day. For treatment C, the viscosity ranged from 2065 to 2389 centipoise, while for the CU treatment at concentrations of 100 µg/ml, it ranged from 2088 to 2411 centipoise. For the

same treatment at a concentration of 200 µg/ml, the viscosity value ranged from 2165 to 2486 centipoise. Under the B-CU treatment, the viscosity values ranged from 2071 to 2380 centipoise for a concentration of 100 µg/ml and from 2085 to 2401 centipoise for a concentration of 200 µg/ml, under the same previous conditions. In the fourth week of cold storage, the viscosity values for the CU treatment and the B-CU treatment reached their highest levels compared to the standard treatment C, which deteriorated during cold storage. The viscosity values for the CU treatment at a concentration of 200 µg/ml ranged from 2682 to 2680 centipoise, while for the B-CU treatment at the same concentration, the viscosity values were in the range of 2682 to 2680 centipoise. This increase in viscosity can be attributed to the active ingredient containing phenols, which enhance the ability of the starter bacteria to increase viscosity by producing exopolysaccharides. These exopolysaccharides form a network with the proteins in the yogurt, thus increasing its viscosity and ensuring its stability of the product [26].

Spontaneous Whey Separation:

According to Table 2, the results of spontaneous whey exudation for laboratory-made yogurt treatments were observed from the first day until the seventh day of control treatment C, with a range of 4.4-4.7 ml/gm. This finding is consistent with [27] study, which also showed whey exudation occurring after the first day of industrialization. For the CU and B-CU yogurt treatments at concentrations of 100 and 200 µg/ml, the ranges were (4.5-4.0), (3.9-4.3), and (4.7-4.0) ml/g, respectively. As the industrial processes progressed, a decrease in spontaneous whey separation was observed. The standard treatment exhibited a range of 4.2-3.9 ml/g from the second week to the third week. For CU and B-CU at concentrations of 100 and 200 µg/ml, the ranges were (3.5-3.2), (3.3-3.1), and (3.8-3.6), (3.6-3.3) ml/g, respectively. During the fourth week, under similar storage conditions, the level of whey separation for the standard treatment was lower than that of the two treatments containing curcumin, whether in its free form or loaded with betacyclodextrin (at both concentrations used during the study). The results were as follows: 3.6, (3.1-3.0), (3.2-3.1), which is consistent with [28] findings. The decrease in whey separation can be attributed to the survival of the starter bacteria and their metabolic activity, as well as the decrease in pressure within the protein formation. Thus, spontaneous whey separation decreased throughout refrigerated storage.

Water-Holding Capacity:

Based on the values provided in Table 2, the water-holding capacity of the standard yogurt treatments, including C, ranged from 53.46% to 55.13% from the first day to the first week of cold storage. This range aligns with the water-holding capacity of yogurt, as reported by [29]. Under similar storage conditions, the water-holding capacity from the second week to the fourth week was as follows: 57.72% - 63.40%. When examining the water-holding values of the CU treatment yogurt from the first day (55.24% - 55.88%) for concentrations of 100 and 200 µg/ml, and under the same manufacturing conditions as the CU treatment and the previous concentrations, the water-holding values ranged from (60.45-65.91-70.58-72.15) to (61.45-66.70-71.68-73.25) from the first week to the fourth week of cold storage. The reason for the increased water-holding capacity may be attributed to the enhancement of curcumin's effect on the starter bacteria and its increased biological activity. Observing the water-holding values for the B-CU treatment at concentrations of 100 and 200 µg/ml under refrigeration, the percentages were (53.50%-54.65%) starting from the first day immediately after manufacture. From the first day to the fourth week of cold storage, the percentages ranged in the following order: (58.26-64.75-69.40-70.15) and (59.78-65.57-69.41-72.36). It can be observed that the values decreased by a similar amount as when treated with free curcumin, but at a slower rate from the first day to the second week for both concentrations used during the study. This slower rate may be attributed to the slow

release of curcumin, which helped enhance the activity of the starter bacteria.

Table (2) physical and rheological tests of yogurt made in the laboratory for the standard treatment and the two treatments containing free curcumin and curcumin loaded with betacyclodextrin during refrigeration at (5±1) ºc for a period ranging from 1 to 28 days.

Treatments	Shelf Life Of Yoghurt (Day	Water Holding Capacity	Spontaneous Whey Separation	Viscosity Centipoise	pH	Total Acidity%
C	1	53.46	4.7	1852	4.82	0.85
	7	55.13	4.4	2065	4.79	0.87
	14	57.72	4.2	2192	4.76	0.89
	21	61.45	3.9	2389	4.66	0.95
	28	63.40	3.6	2645	4.60	0.96
CU 100µg/ml	1	55.24	4.5	1890	4.79	0.89
	7	60.45	4.0	2088	4.72	0.92
	14	65.91	3.5	2242	4.64	0.96
	21	70.58	3.2	2411	4.59	1.01
	28	72.15	3.1	2665	4.51	1.09
CU 200µg/ml	1	55.88	4.3	1905	4.75	0.92
	7	61.45	3.9	2165	4.61	0.96
	14	66.70	3.3	2270	4.53	1.07
	21	71.68	3.1	2486	4.48	1.10
	28	73.25	3.0	2682	4.40	1.15
B-CU 100µg/ml	1	53.50	4.7	1860	4.81	0.85
	7	58.26	4.1	2071	4.79	0.87
	14	64.75	3.8	2214	4.75	0.90
	21	69.40	3.6	2380	4.73	0.92
	28	70.15	3.2	2675	4.62	0.94
B-CU 200µg/ml	1	54.65	4.7	1875	4.80	0.86
	7	59.78	4.0	2085	4.77	0.88
	14	65.57	3.6	2230	4.73	0.92
	21	69.41	3.3	2401	4.63	0.95
	28	72.36	3.1	2680	4.61	0.97
LSD		0.6614	0.3277	0.6614	0.10414NS	0.09358

- Reading is an average of two repetitions.

From the above table, it was noted that there were significant differences in acidity, pH, viscosity, spontaneous exudation, and water holding capacity.

Microbiological Tests of Yogurt:

The microbiological quality of the laboratory-made yogurt treatments was assessed through total microorganism counts, including starter bacteria, total coliform bacteria, as well as yeasts and molds. The counts were performed using the traditional colony counting method during cold storage from the first day to the twenty-eighth day, under hygienic conditions similar to the initial manufacturing day. On the first day of cold storage at 5 ± 1 degrees Celsius, the counts of starter bacteria for the standard treatment C and the two treatments CU and B-CU at concentrations of 100-200 µg/ml were in the range of (12.3×710) to (18.5×710), (20.6×710) to (13.8×710), and (14.2×710) respectively. These findings are consistent with the results mentioned earlier [12]. During the second and third weeks of cold storage, the numbers of live starter bacteria in the CU treatment did not show significant changes at concentrations of 100-200 µg/ml, ranging from (17.3×710) to (13.8×710), and (18×710) to (15.3×710) respectively. The

standard treatment C exhibited a decrease in starter bacteria counts similar to the CU treatment, but not to a significant extent compared to the treatment containing free curcumin. The counts ranged from (11.2×710) to (8.6×710). This decrease in numbers can be attributed to the utilization of phenolic compounds by starter bacteria in their metabolic processes to maintain their viability and activity throughout the preservation period of the yogurt, as confirmed by [30].

For the B-CU treatment, the starter counts during the second and third weeks of cold storage ranged from (16.2×710) to (15.5×710) and (17.3×710) to (15.6×710) at concentrations of 100 and 200 µg/ml respectively. As the storage period continued until the fourth week, the live starter bacteria counts reached their highest level at the concentration of 200 µg/ml for both CU and B-CU treatments, amounting to (5.5×710) to (9.5×710). The slow release of the active compound from betacyclodextrin prolonged the survival of the starter bacteria compared to the standard and free curcumin treatments, leading to increased viscosity of the final product due to the sustained acidity produced by the starter bacteria throughout the cold storage period. No coliform bacteria were observed in both curcumin treatment, whether in its free form or loaded with betacyclodextrin, at the concentrations used during the four weeks of cold storage. This can be attributed to the increased acidity in yogurt due to lactose fermentation and the enhanced activity of the starter bacteria, as well as the antagonistic effect of the active compound against gram-negative colon bacteria. However, the standard treatment C showed no contamination with coliform bacteria starting in the third week, despite refrigerated storage at 5 ± 1 °C, indicating contamination during the manufacturing processes and inadequate sanitary conditions, as indicated by [31]. The absence of curcumin in the product, which is known for its ability to reduce microbial load by interacting with the cell wall components of gram-negative bacteria, further contributed to the contamination, as mentioned by [32].

Regarding yeasts and molds, no visible growth was observed for all treatments from the first day after manufacturing until the second week of storage at 5 ± 1 °C, indicating no contamination. However, live cell growth appeared in the third week for the standard treatment, ranging from (2-4 x 210), indicating a failure to follow proper health conditions during manufacturing operations, including proper sealing. It's worth noting that the Iraqi standard specification for the year 2015 (No. 5/2270) states that microbial quality limits for dairy products should be within one hundred to one thousand colony-forming units per milliliter.

Table (3) results of microbiological tests for yogurt made in the laboratory for the standard treatment and the two treatments containing free curcumin and curcumin loaded with betacyclodextrin when refrigerated at (5±1) °c for a period ranging from 1 to 28 days.

Treatments	Shelf life of yoghurt (day)	Preparation of yeasts and molds (Colony counting unit/g)	Total coliform bacteria count (Colony counting unit/g)	Preparation of starter bacteria (colony counting unit/gm)
C	1	-	-	^{7}10 x 12.3
	7	-	-	^{7}10 x 15.5
	14	-	-	^{7}10 x 11.2
	21	^{2}10 x 2	-	^{7}10 x 8.6
	28	^{2}10 x 4	-	^{7}10 x 1.2
CU 100 µg/ml	1	-	-	^{7}10 x 18.5
	7	-	-	^{7}10 x 20.1
	14	-	-	^{7}10 x 17.3
	21	-	-	^{7}10 x 13.8
	28	-	-	^{7}10 x 4.2
CU 200µg/ml	1	-	-	^{7}10 x 20.6
	7	-	-	^{7}10 x 22.8
	14	-	-	^{7}10 x 18.0
	21	-	-	^{7}10 x 15.3
	28	-	-	^{7}10 x 5.5
B-CU 100µg/ml	1	-	-	^{7}10 x 13.8
	7	-	-	^{7}10 x 17.4
	14	-	-	^{7}10 x 16.2
	21	-	-	^{7}10 x 15.5
	28	-	-	^{7}10 x 7.6
B-CU 200µg/ml	1	-	-	^{7}10 x 14.2
	7	-	-	^{7}10 x 18.9
	14	-	-	^{7}10 x 17.3
	21	-	-	^{7}10 x 15.6
	28	-	-	^{7}10 x 9.5
LSD		0.014151	0 NS	0.08666

- Reading is an average of two repetitions.

Sensory Evaluation of Yogurt:

Sensory tests were conducted for the manufactured yogurt using the different treatments mentioned previously. The table shows that the samples for standard treatment C, immediately after the first day of manufacture, obtained a sensory evaluation score of 92 degrees. These scores were similar to the samples of the B-CU treatment at concentrations of 100 and 200 µg/ml under the same storage conditions, in terms of taste, flavor, texture, acidity, and external appearance. The B-CU treatment obtained scores ranging from 93 to 95 degrees, while the CU treatment with free curcumin at concentrations of 100 and 200 µg/ml received lower scores, indicating that the taste and flavor were not as well-accepted, obtaining scores of 76 and 73 degrees, respectively. The difference in scores was attributed to the external appearance and color values of these three treatments. The standard treatment performed the best, followed by the B-CU treatment containing curcumin loaded with betacyclodextrin at a concentration of 100 µg/ml, then the same treatment with a concentration of 200 µg/ml, and finally the CU treatment. The curcumin orange dye had a negative impact, especially at higher concentrations, which was evident in the evaluations. The treatments mentioned earlier received higher scores for external appearance, while texture and texture were also factors considered. The CU treatment did not differ much from the evaluations of the first day compared to the two standard treatments (C) and B-CU. As the cold storage period progressed until the fourteenth day (the

second week), the B-CU treatment was distinguished from the standard treatment and the CU treatment in terms of taste, flavor, and acidity, which evaluators described as more favorable. The standard treatment (C) followed, and then the CU treatment. In terms of texture and texture, the B-CU treatment, from the lowest to the highest concentration, outperformed the CU treatment, but did not excel in terms of external appearance compared to the standard treatment due to the deposition of some orange pigment from curcumin, especially in samples with a high concentration of 200 µg/ml. This was less apparent in the B-CU treatment, which maintained consistency in external appearance and color, followed by the standard treatment in terms of evaluation. As the cold storage period progressed until the third week of manufacturing, the B-CU treatment obtained the highest sensory evaluation scores for all attributes compared to the standard treatment, which was negatively affected by high acidity and accidental contamination after manufacturing, despite cold storage. The CU treatment followed in terms of sensory evaluations (taste, flavor, acidity, texture, texture, external appearance), maintaining these levels of evaluation until the fourth week of cold storage, despite the concentrations used during the study. Notably, the results showed the effectiveness of the active compound loading process (curcumin) in the B-CU treatment, enhancing the production of flavor compounds and preserving the integrity of the final product, preventing deterioration.

Table (4) sensory evaluation of laboratory-made yogurt for the standard treatment and the two treatments containing free curcumin and curcumin loaded with betacyclodextrin when refrigerated at (5±1) ºc for a period ranging from 1-28 days.

Treatments	Shelf life of yoghurt (day)	Appearance and Color (10º)	Acidity (10º)	Texture (35º)	Taste and Flavor (45º)	Total 100
C	1	10	10	31	41	92
	7	10	9	33	43	95
	14	8	7	27	40	82
	21	6	0	0	0	6
	28	2	0	0	0	2
CU 100 µg/ml	1	8	9	28	31	76
	7	7	7	30	30	74
	14	6	6	29	27	68
	21	5	6	27	25	63
	28	5	5	25	20	55
CU 200µg/ml	1	7	8	30	28	73
	7	6	7	31	25	69
	14	5	5	27	22	59
	21	4	5	25	20	54
	28	4	4	23	20	51
B-CU 100µg/ml	1	10	10	32	41	93
	7	9	8	33	40	90
	14	8	8	31	42	89
	21	8	7	30	37	82
	28	7	6	28	35	76
B-CU 200µg/ml	1	10	10	33	42	95
	7	8	8	34	39	89
	14	6	7	32	39	84
	21	7	7	32	35	81
	28	7	6	31	33	77
LSD		0.2018	0.09358	0.3476 NS	0.3291	0.3206

- Reading is an average of two repetitions.

Conclusion :

Betacyclodextrin had a positive effect on the active compound by masking the yellowish-orange color of curcumin as well as the bitter and sour taste during sensory evaluation. It resulted in a product that was somewhat similar to the standard yogurt treatment. On the other hand, the addition of free curcumin had a clear effect on the final product in terms of color and taste, which was undesirable for consumers.

References :

[1] Pradhan, N., Singh, S., Ojha, N., Srivastava, A., Barla, A., Rai, V., et al. (2015). Facets of nanotechnology as seen in food processing, packaging, and preservation industry. BioMed Res. Int. 2015:365672. Doi: 10.1155/2015/365672.

[2] Jochen Weiss, P. Takhistov, D.J. Mcclements, (2006). Functional materials in food nanotechnology J Food Sci, 71,pp. R107-R116.

[3] Abdollahi, M., Rezaei, M., & Farzi, G. (2012). A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into churnal of Food Engineering, 111(2), 343-350.

[4] Sadeq Muneer Shawkat, Mohammed Al-Jawasim and Luay Salam Khaleefah, Extending Shelf Life of Pasteurized Milk via Chitosan Nanoparticles, J Pure Appl Microbiol., 2019; 13(4):2471-2478. https://doi.org/10.22207/JPAM.13.4.62

[5] Gupta, A., Eral, H.B., Hatton, T.A. and Doyle, P.S. (2016). Nanoemulsions: formation, properties and applications. Soft Matter, 12: 2826-2841.

[6] Mihindukulasuriya, S.D.F. and Lim, L. T. (2014). Nanotechnology development in food packaging: a review. Trends Food Sci. Technol., 40: 149-167.

[7] Dominguez-Diaz, L. D., Fernandez-Ruiz, V. and Camara, M. (2020). An international regulatory review of food health-related claims in Functional food products labeling. Journal of Functional Foods 68: article ID 103896.

[8] Granato, D., Barba, F. J., Bursac Kovacevic, D., Lorenzo, J. M., Cruz, A. G. and Putnik, P. (2020). Functional foods: product development, technological trends, efficacy testing, and safety. Annual Review of Food Science and Technology 11:93-118.

[9] Li, H., Sureda, A., Devkota, H. P., Pittala, V., Barreca, D., Silva, A. S., … and Nabavi, S. M. (2020). Curcumin, the golden spice in treating cardiovascular diseases. Biotechnology Advances 38: article ID 107343.

[10] Patel, S. S., Acharya, A., Ray, R. S., Agrawal, R., Raghuwanshi, R. and Jain, P. (2020). Cellular and molecular mechanisms of curcumin in prevention and treatment of disease. Critical Reviews in Food Science and Nutrition 60(6): 887-939.

[11] Rachmawati, H., Edityaningrum, C. A., & Mauludin, R. (2013). Molecular inclusion complex of curcumin-β-cyclodexetrin nanoparticle to enhance curcumin skin permeability from hydrophilic matrix gel. Aaps Pharmscitech, 14(4), 1303-1312.

[12] Sadiq, Esraa Haider (2019). Study of the effect of fortification of yoghurt with iron-coated iron on its physicochemical, rheological and nutritional properties. Master Thesis - College of Agriculture - University of Baghdad.

[13] Al-Abadi, Muhammad Muzaffar Khalil. (2014). Efficacy of some biostimulants in fermented milk products and soft cheese. Master's Thesis - College of Agriculture - University of Baghdad.

[14] Tamime, A. Y. and Robinson, R. K. (2007). Yoghurt Science and Technology. Pergmon Press Ltd., Oxford.

[15] Amatayakul, T.; Sherkat, F. and Shah, N. P. (2006). Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. Int. J. Dairy Tech. 59. 216-221.

[16] Sharaf A, Al-Shaikh, et al., (2018). Study of the effect of selenium fortification on the physiochemical and sensory properties of functional eugrits. Journal of Karbala Agricultural Sciences, 5(2), 72-90.

[17] AOAC (2005) Official method of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24.

[18] AOAC (2008) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

[19] Koletzoko. B.. Baker. S.. Cleghorn. G.. Neto. U. F.. Gopalan. S.. Hernell. O.. & Pzyrembel. H. (2005). Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. Journal of pediatric gastroenterology and nutrition. 41(5). 584-599.

[20] Donkor, O.N.; Nilmini S.L.I.; Stolic P., Vasiljevic T.; and Shah N.P., (2007). Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int. Dairy J. 17, 657-665.

[21] Wolf IV, Bergamini CV, Perotti MC& Hynes ER (2013). Sensory and flavor characteristics of milk. Milk and dairy products in human nutrition: Production, Composition and Health, pp 310-337.

[22] Remeuf, F., S. Mohammed, I. Sodini, and J. P. Tissier. 2003. Prelimi-nary observations on the effects of milk fortification and heatingon microstructure and physical properties of stirred yogurt. Int.Dairy J. 13:773–782.

[23] Taiwo OS, Afolabi RO, Oranusi SU,Owolabi JB, Oloyede AR, Isibor PO, IgeOJ. Microbiological assessment ofcommercial yogurt sold in Ota Metropolis,Ogun State, Nigeria. IOP ConferenceSeries: Earth and Environmental Science.2018; 210:012019.

[24] Ziena, H., Nasser, S. A., & Dairy, R. (2019). Iron fortified yoghurt: Effect of different iron salts on

the properties of yoghurt. Advances in Dairy Research, 7(2).

[25] APHA (1989) Standard Methods for the Examination of Water and Wastewater, Part 3, Determination of Metals. 17th, American Public Health Association, Washington DC, 164.

[26] Gentès, M.; St-Gelais, D.; Turgeon, S.L. Exopolysaccharide–milk Protein Interactions in a Dairy Model System Simulating YoghurtConditions. Dairy Sci. Technol. 2013, 93, 255–271

[27] Dosh, K.S. and S.K. Mohamed (2017). study of thephysiochemical, rheological and sensory properties of alactoferrin supported yogurt. Euphrates Journal ofAgriculture Science, 9(4): 128-188.

[28] Al-Badrany, Dia Ibrahim Jaru Haider (2017). Effect of milk treatment with different heat treatments on the activity of initiator bacteria and the physicochemical, rheological and sensory characteristics of the product. Al-Furat Journal of Agricultural Sciences, 9 (4): 180-197.

[29] Guler-Akin, M. B., & Akin, M. S. (2007). Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goats milk. Food Chemistry, 100(2), 788-793.

[30] Arslan, S., & Bayrakçi, S. (2016). Physicochemical, functional, and sensory properties of yogurts containing persimmon. Turkish Journal of Agriculture and Forestry, 40, 68-74. http:/dx.doi.org/10.3906/tar-1406-150.

[31] Madrigal-Carballo, S., Rodriguez, G., Krueger, C. G., Dreher, M., & Reed, J. D. (2009). Pomegranate (Punica grantum) supplements: Authenticity, antioxidant and polyphenol cpmosition. Journal of Functional Foods, 1, 324-329.

[32] Ravaei, A., Heshmatti poor, Z., Salehi, T.Z., Tamai,I.A., Ghane, M. and Derakhshan pour, J. (2013)Evaluation of antimicrobial activity of threeLactobacillus spp. against antibiotic resistanceSalmonella typhimurium. Adv.Stud.Biol., 5(2): 61-70.

تأثير إضافة مركب الكركمين والبيتاسايكلودكسترين النانوي على بعض من الخواص الفيزيوكيمياوية، الميكروبية والحسية لليوغرت

صفا محمد عبد الحسين [1] **صادق ضياء منير** [2]

safa.p100519@fosci.uoqasim.edu.iq sadeq.muneer@fosci.uoqasim.edu.iq

[1، 2] قسم علوم وتكنولوجيا الالبان، كلية علوم الاغذية، جامعة القاسم الخضراء، محافظة بابل، العراق.

- البحث تاريخ استلام البحث 23/08/2023 وتاريخ قبوله 28/09/2023
- مستل من رسالة ماجستير للباحث الاول .

الملخص

الهدف من هذا البحث هو دراسة تأثير اضافة الكركمين المحمل بالبيتاسايكلودكسترين على اليوغرت المصنع مختبرياً من خلال اجراء مجموعة الفحوصات على المنتوج النهائي، فقد صنع اليوغرت اعتماداً على ثلاث معاملات الاولى كانت قياسية C خالية من اي اضافة ومعاملة ثانية مضاف اليها الكركمين الحر CU بتراكيز مشابهة للمعاملة الثالثة الحاوية على الكركمين المحمل بالبيتاسايكلودكسترين B–CU (100 و 200 ميكروغرام / مل)، وشملت الفحوصات اختبار التركيب الكيميائي لليوغرت المصنع للمعاملات الثلاثة والتي اشتملت على قياس نسبة البروتين، نسبة الرطوبة، نسبة الدهن، ونسبة الرماد للعينات المدروسة من اليوم الاول للتصنيع والتي بلغت حتى اليوم 28 من الخزن المبرد (4.61، 86.62، 5.64، 0.86) على التوالي وتم اجراء مجموعة من الفحوصات الفيزياوية والريولوجية المختلفة بما في ذلك الحموضة الكلية الاس الهيدروجيني، نضوح الشرش، اللزوجة وقابلية الاحتفاظ بالماء والتي بلغت حتى اليوم 28 من الخزن المبرد (0.97، 4.61، 3.1، 2680 ، 72.36) بالترتيب، وتم ايضاً دراسة الفحوصات المايكروبايولوجية لليوغرت المصنع تحت نفس الظروف والتي اظهرت كفاءة المركب الفعال سواء في شكله الحر او المحمل بالبيتاسايكلودكسترين كمثبط للاحياء المجهرية ومنع تلوث المنتوج المصنع. وقد بينت الفحوصات تفوق المعاملة B–CU الحاوية على 200 ميكروغرام / مل من الكركمين – بيتاسايكلودكسترين على باقي المعاملات الاخرى وتقبل المقيمون الحسيون والتي بلغت بحدود (95 –77)% منذ اليوم الاول للتصنيع والتي بلغت حتى اليوم 28 من الخزن المبرد.

الكلمات المفتاحية: النانوتكنولوجي ، الكركمين، البيتاسايكلودكسترين، قابلية الاحتفاظ بالماء، اليوغرت.