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Research Article
Preparation and Characterization of Functional Yoghurt Using Incorporated Encapsulated Curcumin by Caseinate

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Abstract

Background and Objective: Curcumin is a natural polyphenolic and using as a flavour of foods, that has a lot of therapeutic effects. The present work aimed that prepared caseinate-curcumin nanoparticles (CSCCMNPs) designed by desolvation technique and evaluated. Manufacture yoghurt incorporated by CSCCMNPs and study the effect of incorporation of it on properties of yoghurt. **Materials and Methods:** Prepared caseinate curcumin nanoparticles (CSCCMNPs) using a novel method by freeze-drying, for warmed ethanol 40% solution with co-dissolved sodium caseinate (NaCas) and curcumin (CCM). Investigate the effect of Incorporated CSCCMNPs in functional yoghurt on physicochemical, microbiological properties and acceptability evaluation. **Results:** The encapsulation efficiency ranged between 84.95 and 95.10%. The curcumin encapsulated in casein nanoparticles had higher biological activity, as assessed by antioxidant assays than free curcumin, likely due to the improved dispersibility. CSCCMNPs were bigger than those of NaCas processed at encapsulation conditions but were smaller than those of the native NaCas. Fortification of yoghurt with CSCCMNPs had a significant effect on the organoleptic properties of resultant yoghurt. Besides, body and texture were improved and the enhancement of yoghurt acceptability. **Conclusion:** Functional yoghurt of acceptable quality and high antioxidant activity could be prepared by CSCCMNPs.

Key words: Curcumin, sodium caseinate, nanoparticles, functional yoghurt, sensory acceptability, texture profile analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Curcumin, extracted from the rhizomes of turmeric (*Curcuma longa*), is a natural polyphenolic, used as a flavoured food previously but also appears to supply health benefits that are often used in Indian cuisine and traditional medicine[1]. Recently, people are looking towards naturally bioactive compounds in plants and many studies had focused on the protective effect of medicinal plant products which have antioxidant properties as curcumin for curing free radical-induced tissue damage[2,3]. Curcumin has therapeutic potential for treating ageing-associated diseases. Many studies have demonstrated that curcumin possesses anti inflammatory[4], antioxidants[5] and neuroprotective activities[6]. Despite that, curcumin clinical administration is limited due to its poor oral bioavailability and water solubility, low *in vivo* stability, rapid metabolism and clearance[7]. Several papers had been investigated with different carrier materials to enhancement the bioavailability of curcumin such as albumin[8,9], β lactoglobulin[10], phospholipids[11], chitosan[12], cyclodextrins[13], polyvinyl alcohol/polyvinyl alcohol hydrogel[14] and whey protein concentrate[15]. Several techniques have been used (nano-carriers, solid dispersions, amorphous solid form, liposomes and melt-extrusion) to conquer the difficulties with hydrophobic components[16].

Caseins are an excellent source of essential amino acids and calcium. Furthermore, it has unparalleled properties that can use as a delivery system for bioactive ingredients[17]. The heating treatment at 60°C of Casein micelles in aqueous ethanol 40% confers dissociation, because of the enhancement of solvent quality and shifting of pKa values of phosphoserine[18]. Dende *et al.*[19] showed that curcumin nanoparticles (CCMNPs) have a better therapeutic index and oral bioavailability than curcumin. CCMNPs could prevent neuroinflammation and oxidative stress that was induced during status epilepsy[20].

Several diseases have overcome using the role of fortifications of food. Yoghurt is famous fermented milk that has acquired broad acceptance among consumers depended on is a healthy product and a rich source of nutrients such as calcium and proteins high-quality and probiotic starter effects[21]. Recently, the application of nanoencapsulation in food industries had been spread significantly and is very promising[22]. This trend has been paid by the ability of these structures to enhancement the solubility and bioavailability of bioactive components because of their large surface increased volume. That could be done without exposing other food properties[23].

The present work aimed at study caseinate-curcumin nanoparticles (CSCCCMNPs) designed by desolvation prepared and evaluated. Manufacture yoghurt fortified with CSCCMNPs and study the effect of fortification on properties of yoghurt.

MATERIALS AND METHODS

Materials: Curcumin (CCM) was purchased from Merck Schuchardt (8011 Hohenbrunn bei Munchen). The product had a purity of 94% w/w according to the vendor. Sodium caseinate (NaCas) was from Acros (New Jersey, USA). Other chemicals were obtained from either Sigma-Aldrich or Thermo Fisher Scientific (Pittsburgh, PA). Pasteurized caw whole milk (Juhina®, Egypt) and skim milk powder Low heat produced by Dairy America, Inc. California, USA. were used for the preparation of the yoghurt, as well as lyophilized microorganism Choozit® (Danisco, Mexico) containing: *Lactobacillus delbrueckii* spp. *bulgaricus Streptococcus salivarius* spp. *thermophilus*. All employed chemicals exhibited Analar or equivalent quality.

The study was carried out at the Department of Dairy, National Research Centre and Department of Dairy Science and Technology, Menoufia University Shibin El Kom, Egypt from August-October, 2020).

Methods

Preparation of CSCCMNPs: Four gram NaCas was hydrated in 200 mL of 40% v/v aqueous ethanol. After being heated at 60°C in a water bath for 5 min, an excess amount (0.25, 0.50 and 1 g) of curcumin was mixed with the NaCas solution by blending at 10,000 rpm for 4 min using a Cyclone I.Q. microprocessor homogenizer (VirTis, Gardine, NY) according to Pan *et al.*[24] with slight modifications. The mixtures were treated by ultrasonication at 160 W powers, 20 kHz frequency and with 50% pulse (Sonic Vibra cell USA). Centrifugation at 290 g (model 4540 R, Eppendorf, Hamburg, Germany) for 5 min was carried out to remove the excess amount of curcumin. The supernatant was transferred. Finally, the nano-capsules were dried by freeze-dried (LABCONCO, USA) to produce solid powder nano-capsules. A NaCas sample was processed at the same conditions without curcumin, named as NaCas nanoparticles hereafter.

Encapsulation efficiency of curcumin: Five milligram of freeze-dried powder was suspended in 10 mL chloroform and was stirred overnight at room temperature (21°C). After centrifugation at 6,000×g for 10 min (Minispin plus,

Eppendorf, Hamburg, Germany), the supernatant was transferred and filtered through a PTFE syringe filter with 0.45 µm pore size (Fisher Scientific, Pittsburgh, PA). The permeate was diluted 20 times in chloroform and the absorbance at 419 nm was measured using a UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, MA) to determine curcumin concentration based on a calibration curve previously established using standard solutions with different amounts of free curcumin dissolved in chloroform[25]:

$$\text{Encapsulation efficiency (EE)} = \frac{\text{CCM addition-CCM in powder nano-capsules}}{\text{CCM addition}} \times 100$$

Particle size analysis and microstructure by transmission electron microscopy: The size determination and Particle Dispersity Index (PDI) were recorded by Dynamic Light Scattering (DLS) as described by Soliman and Hassan[26]. The samples were examined by TEM using a JEOL JEM-1400 Plus TEM with an accelerating voltage of 100 kV at a magnification of 200,000x[26]. Antioxidant activity determined by The DPPH method was described by Rai *et al.*[27].

Preparation of yoghurt: For the yoghurt preparation, milk was standardized adding 3 g of milk powder per 100 mL of pasteurized milk. Then, a heat treatment was applied by raising the temperature of the milk to 90°C for 20 min and then cooling down to 40-45°C. After cooling at 42°C, the milk was added with the lyophilized culture directly and stirred for 10 min and poured into 100 mL plastic containers, is the same procedure for all samples; then the CCM (both, nanoparticles and free CCM) were added at a 250 mg CCM and stirred for 20 min (120 agitations/min) until complete dissolution. Subsequently, the milk with CCM was incubated at 45°C for 4 hrs until a pH of 4.6 was reached, as well as the control[28] yoghurt samples were stored for 14 days at 5±1°C.

Physicochemical analysis: The pH was measured by a digital potentiometer (Beckman, Denver, CO, USA), previously calibrated, at room temperature. Moisture content was determined through water evaporation method 16.032, AOAC[29]. Acidity was quantified by titration of 9 mL of the sample using phenolphthalein and NaOH (0.1 mol Equi L^{-1}) method 16.023, AOAC[29].

The Texture Profile Analysis (TPA) was determined using a Texture Analyzer (Mult- test 1*d* Memesin, Food Technology Corporation, Slinfold, W. Sussex, UK) Measuring the double compression force (g) in all samples of yoghurt (100 mL, mm height) using a cylindrical body of 4.3 cm in diameter, descending at a speed of 1 mm sec^{-1} and reaching a depth of 20 mm. All measurements were carried out at a temperature of 20°C by triplicate[30].

Evaluation of sensory properties: The yoghurt samples were organoleptically evaluated as described by Hamed *et al.*[31]. Some panellists from the staff members of the Department of Dairy Science, National Research Center, Egypt, were evaluated each yoghurt sample and used a quality rating scorecard for evaluation of flavour (60 points) and body and texture (30 points) and appearance (10 points) as described by Hamed *et al.*[31].

Statistical analysis: The analyses of prepared samples were conducted at least in triplicates; comparisons of the treatments were completed by one-way ANOVA and Tukey's tests by SPSS, ver. 16.0 statistics programs. A 95% minimum confidence level was taken for all statistical analyses[32].

RESULTS AND DISCUSSION

Particle size, polydispersity index and encapsulation efficiency of CSCCMNPs: The result of Table 1 showed that the particle size of dispersions prepared from NaCas nanoparticles and CSCMMNPs containing 0.25, 0.50 and 1.00 g CMM. The particle size of the NaCas nanoparticles 83.27 nm and the size of the dispersions CSCCMNPs with curcumin additions (98.10, 125.70, 184.20 nm) respectively were significantly larger than that of the NaCas nanoparticles. Particle polydispersity of Casein nanoparticle of 0.213, increased to 0.480 by an increase of addition CCM to 1 g/200 mL. de Kruif and Huppertz[33] found bovine casein micelles to be very monodisperse and are a Calculated polydispersity of 0.213-0.480. Encapsulation Efficiency of CCM of (0.25, 0.50 and 1.00 g/200 mL NaCS) CMM was encapsulated by Caseinate of 97.00, 91.25 and 82.90% illustrated in Table 1. This increased with an increase in the CCM ratio. Heat treatment of casein in ethanol 40% at 60°C has changed casein structurally by polarity that affects solvent

Table 1: Casein micelles particle size (nm), polydispersity index (PDI) and encapsulation efficiency of curcumin

Sample	Size (nm)	Calculated PDI	Encapsulation efficiency
NaCas NPs	83.27±4.3^{e}	0.213±0.009^{c}	-
CSCCMNPs 0.25	98.10±3.5^{d}	0.423±0.005^{b}	97.00±2.25^{a}
CSCCMNPs 0.50	125.70±6.4^{c}	0.430±0.008^{b}	91.25±3.50^{b}
CSCCMNPs 1.00	184.20±5.5^{b}	0.480±0.011^{a}	82.90±2.10^{c}

*Numbers are mean±standard error. Different superscript letters represent significant differences in mean. NaCas NPs: Caseinate nanoparticles, CSCCMNPs: Caseinate curcumin nanoparticles

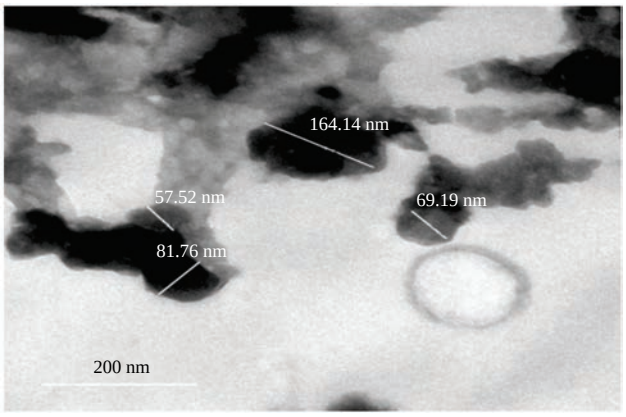

Fig. 1: Transmission electron micrograph of CSCCMNps

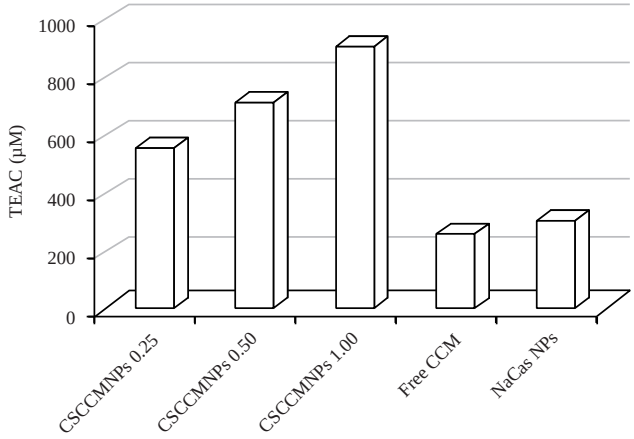

Fig. 2: Trolox Equivalent Antioxidant Capacity (TEAC) of the NaCas nanoparticles, free CCM and encapsulated CCM ratio (0.25, 0.50 and 1 g CCM) CSCCMNPs at 30°C

Table 2: Chemical composition of yoghurt milk-base with CMM fortification

	Percentage			
Test/sample	Total solids	Fat	Total protein	Lactose
Control	14.19±0.09	3.70±0.05	3.80±0.12	4.35±0.06
Free CCM	14.20±0.11	3.70±0.07	3.79±0.10	4.35±0.09
CSCMMNPs	15.27±0.34	3.71±0.03	4.73±0.04	4.19±0.04

Control: Plain yoghurt, Free CCM: Yoghurt fortified with 250 mg free curcumin, CSCMMNPs: Yoghurt fortified with 250 mg curcumin encapsulated in Caseinate

quality and thus dissociation and re-association of caseins[34]. This was consistent with our monitoring of decrease turbidity. The heat treatment of casein in ethanol at 40% increases the availability of hydrophobic ligands of casein for binding with CCM through blending. Also, caseins have open structures containing numerous proline residues evenly distributed throughout their amino acids and are very tendentious as binders of bioactive compounds such as phenolic compounds and antioxidants[35]. The Encapsulation Efficiency (EE) obtained from curcumin in NaCas NPs can be compared with EE of curcumin nanoencapsulation in zein using electrohydrodynamic atomization it's the range of 85-90%[36].

The microstructure of CSCCMNps was examined by TEM as shown in Fig. 1. TEM micrographs revealed that the size of CSCCMNps in the micrograph was not consistent with the results of DLS. We find that the size of the CSCCMNPs is less than the results of DLS because the measurement is done under a vacuum that may lose moisture and thus reduce the size.

Antioxidant activity of CSCCMNPs: Figure 2 showed the TEAC of curcumin before and after encapsulation. The TEAC of the NaCas nanoparticle was also determined, which was 307 µM. Also, the antioxidant activity has been calculated for caseins that are attributing to amino acid remains like tryptophan, cysteine and methionine that can be oxidized by free radicals[37,38]. The antioxidant activity of free curcumin had been low (260 µM TEAC) when thawed in ethanol followed by mitigation in distill water for analysis because the limited solubility of curcumin had effected to bind with free radicals. Although, curcumin probably had phenolic –OH groups which play the main part in oxidation reactions[37]. The antioxidant activity for different ratios from CCM (0.25, 0.50 and 1.00) loaded at NaCS is 553, 713 and 907 µM TEAC respectively. Which, higher than obtained from free CCM and dispersing NaCsNPs. Takahashi *et al.*[39] have been reported that the enhancement of the antioxidant activity of CCM after nanoencapsulation in dispersion colloids such as lecithin and β-casein.

Yoghurt properties: The result of this study has shown significant differences ($p \leq 0.05$) in physicochemical characteristics which increased total solids and protein, in fortified yoghurt by CSCMMNPs of (15.27 and 4.73%) than control yoghurt is (14.19 and 3.80%) respectively (Table 2). While has shown non-significant ($p \leq 0.05$) differences between control yoghurt and fortified with free CCM. Because of the addition of CSCMMNPs nearly 1 g protein but the addition of free CCM nearly 250 mg. The results agree with the obtained by Ghorbanzade *et al.*[40].

In yoghurt, a decrement of pH indicates the release of lactic acid in the medium by Lactic Acid Bacteria (LAB). The data of Table 3 showed yoghurt samples' evaluation of pH and acidity changes during 14 days of storage at 5°C. The pH values showed a trend to decrease ($p<0.05$) during the storage period in all samples but, the acidity values increased significantly through 14 days of storage. Where the control pH decreased from 4.65 at fresh to 4.56 after 14 days of cold

Table 3: Changes in acidity and pH of different yoghurt samples during cold storage

Sample	Storage time	pH	Acidity
Control	0 day	4.65	0.76
	7 days	4.60	0.79
	14 days	4.56	0.82
CSCCMNPs	0 day	4.67	0.75
	7 days	4.62	0.78
	14 days	4.58	0.80
Free CCM	0 day	4.64	0.76
	7 days	4.58	0.81
	14 days	4.55	0.83

Table 4: Texture profile analysis for fresh plain and fortified yoghurt fortified

Sample	Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (g*mm)
Control	79.85±2.72	0.68±0.02	0.58±0.06	46.31±6.34	31.49±5.29
Free CCM	81.58±7.63	0.73±0.04	0.59±0.11	47.54±7.23	34.71±6.45
CSCCMNPs	91.77±6.95	0.77±0.07	0.61±0.09	55.98±8.48	43.10±7.57

Table 5: Sensory properties of control yoghurt and fortification yoghurt at 5±1°C

Sample	Flavour (60)	Body and texture (30)	Colour and appearance (10)	Total (100)
Control	50.00±2.34	25.75±1.61	9.6±0.45	860.00±4.36
Free CCM	51.57±4.27	26.50±2.19	7.8±0.44	87.40±4.03
CSCCMNPs	53.00±3.29	27.50±0.58	9.8±0.44	90.50±4.8

storage. But the acidity increased from 0.76-0.82. We found no significant differences ($p<0.05$) between all treatments for pH and acidity determination. These results also were in agreement with Bonczar *et al.*[41].

Texture profile analyses simulate the conditions of a product in the mouth by compressing. The results in Table 4 showed that the highest hardness, Springiness, Cohesiveness, Gumminess and Chewiness were measured in yoghurt fortified with CSCCMNPs of 91.77 g, 0.77 mm, 0.61, 55.98 g and 43.10 g*mm respectively. Followed by, yoghurt fortified with free CCM is 81.58 g, 0.73 mm, 0.59, 47.54 g and 34.71 g*mm respectively, while the lowest one was observed in control yoghurt is 79.85 g, 0.68 mm, 0.58, 46.31 g and 31.49 g*mm respectively. The physical properties of yoghurt gels including gel stiffness and permeability, rearrangement of protein particles in gel network and structure breakdown of stirred-type yoghurts are important factors that influencing the physical and structural properties of yoghurts[28].

Table 5 shows the sensory properties of control yoghurt and fortification at 5±1°C. There was a significant difference ($p>0.05$) in the Flavour, body and texture, colour and appearance score. The CSCCMNPs scored highest for all parameters, followed by fortified with free CCM and the lowest-scoring control yoghurt.

Finally, the caseinate gives the ability to bind CCM by desolvation methods and freeze-drying for warm caseinate solutions. Also, the results indicated that fortification of yoghurt with CSCURNPs had no adverse effect on the organoleptic properties of resultant yoghurt. Besides, body, taste and texture were improved and the enhancement of yoghurt acceptability. Foda *et al.*[42] noticed that less amount of turmeric in yoghurt given more acceptable and enhancement rheological properties of set yoghurt.

Caseinate gives the ability to bind CCM hence gives the ability to apply in dairy and pharmaceuticals products. CSCURNPs give more acceptable and enhanced rheological properties and texture of yoghurt.

CONCLUSION

Caseinate curcumin nanoparticles were prepared using a novel technique by freeze-drying for warm caseinate solutions. This improves the ability to load and bind curcumin and enhances the solubility of it. The results indicated that fortification of yoghurt with CSCURNPs had an enhancement to the organoleptic properties of resultant yoghurt. Besides, body and texture were improved and the enhancement of yoghurt's sensory acceptability.

SIGNIFICANCE STATEMENT

The present study found the heat treatment of casein in 40% ethanol increases the availability of hydrophobic ligands of casein for binding with CCM through blending. Also, caseins have open structures containing numerous proline residues evenly distributed throughout their amino acids and are very tendentious as binders of hydrophobic components as curcumin. Also, can applicable CSCCMNps in prepared yoghurt, because of her therapeutic properties. Furthermore, CSCCMNps improved texture profile analysis, antioxidant

activity and sensory acceptability and technological properties of the functional yoghurt. Subsequently, we recommended using CSCCMNps in functional dairy products.

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