



International Journal of
Dairy Science

ISSN 1811-9743



Academic
Journals Inc.

www.academicjournals.com



Research Article

Preparation and Characterization of Functional Ice Milk Incorporated with Curcumin Nano-Encapsulated in Conjugated Whey Protein-Maltodextrin

¹Tarek Nour Soliman and ²Samera H. Shehata

¹Department of Dairy Sciences, Food Industries and Nutrition Research Division, National Research Centre, El-Buhouth St., Dokki, Cairo, Egypt

²Department of Dairy Science and Technology, Menoufia University, Shibin Elkom, Cairo, Egypt

Abstract

Background and Objective: Curcumin (CCM) is a bioactive component because it is a natural polyphenol compound with wide therapeutic potential. This work aimed to investigate and characterization the ability of curcumin encapsulation by Whey Protein Isolate-maltodextrin (WPI-MD) WPI-MD complex and WPI-MD conjugate Then, the impact of adding free CCM and encapsulated CCM on the quality of the ice milk was also investigated by evaluating the texture, melting resistance and sensory quality of the resultant ice milk. **Materials and Methods:** The curcumin was encapsulated in whey protein isolate with maltodextrin complex or conjugate. The antioxidant activity and encapsulation efficiency of encapsulated CCM were measured. Dynamic laser scattering characterized the size and zeta potential was determined. The 30 mg CCM loaded to WPI-MD conjugate was incorporated in ice milk and analyzed for physicochemical properties, viscosity and sensory evaluation. **Results:** The WPI-MD conjugate has the advantage over the WPI-MD complex of higher stability and better binding of curcumin, exhibiting bigger binding and high encapsulation efficiency (>91.98%) of different levels of CCM (15-45 mg) in WPI-MD conjugate. The resultant ice milk from the addition of encapsulated curcumin has better sensory and technological properties than control and fortified with free CCM. **Conclusion:** Thus it could be concluded that functional ice milk containing CCM encapsulated by WPI-MD conjugate is of acceptable quality and high antioxidant activity.

Key words: Whey protein isolates, maltodextrin, glycate, curcumin, Nano-encapsulation, functional ice milk, viscosity, sensory evaluation

Citation: Soliman, T.N. and S.H. Shehata, 2021. Preparation and characterization of functional ice milk incorporated with curcumin nano-encapsulated in conjugated whey protein-maltodextrin. *Int. J. Dairy Sci.*, 16: 58-66.

Corresponding Author: Tarek Nour Soliman, Department of Dairy Sciences, Food Industries and Nutrition Research Division, National Research Centre El-Buhouth St., Dokki, Cairo, Egypt Tel: +20237082628, +201155945511

Copyright: © 2021 Tarek Nour Soliman and Samera H. Shehata. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

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 is a natural polyphenolic compound extracted from the rhizome of *Curcuma longa*, being commercially turmeric extract¹. Also, curcumin is a food additive (E100), it can act as a natural dye in food industries². Increasing awareness has been given to curcumin for its pharmacological and biological activities such as antioxidant, antitumor and anticancer properties³.

Nowadays, treatment by drugs was effective and available for human diseases such as obesity, diabetes and cancer but very costly. Alternatively, people are looking forward to naturally bioactive ingredients in plants, which have effects like drugs. However, using curcumin as food additives confront two base limitations. Firstly, curcumin is poor availability and solubility in water (11 ng mL⁻¹) at acidic and neutral pH values. Secondly, the stability of curcumin at acidic pH is good but unstable (breaks down) at pH 7 and alkali pH. Hence, using curcumin in functional foods deeply depends on methods that overcome these limitations, triggering their incorporation into some foods⁴. Extended previous literature has shown that curcumin can be binding with proteins such as whey proteins⁵, casein⁶, β -lactoglobulin⁷ and soy protein^{8,9}, which may significantly improve curcumin stability and solubility. However, proteins head for to submit evident aggregations at the pH isoelectric point of there, when increased ionic strength above a certain level and when denatured by thermal. Thus, it is very hard to monitoring, the stability, bioavailability and release of protein encapsulated bioactive components in sundry environmental conditions.

Protein/polysaccharides complexes formation gives stability for proteins against aggregation or dissociation on a wide range of pH and temperature¹⁰. Recently, researchers finding the bovine serum albumin/carrageenan complexes and particles give good encapsulation efficiency for polyphenolic compounds by successfully binding and more properties compared to native proteins^{11,12}. Even so, protein/ polysaccharide complexes formation based on electrostatic interaction without noncovalent interactions are sensitive to environmental conditions Reaction of Maillard is natural and nontoxic. Proteins conjugate with polysaccharides such as whey proteins¹³, casein¹⁴, bovine serum albumin¹⁵ and soybean protein¹⁶, giving remarkably increased functional properties such as stability, solubility.

Curcumin Nano-encapsulated can be incorporated into food systems such as ice cream for the development of health-promoting functional foods. Ice cream is one of the dairy-based frozen products. Generally, it combined with

milk, sweeteners, stabilizers, emulsifiers and flavourings¹⁷. The quality of ice cream is evaluated by its structure, texture and resistance to melting¹⁸.

The present study aims to investigate and characterization the ability of curcumin encapsulation by WPI-MD Complex and WPI-MD Conjugate. Then, the impact of adding free CCM and encapsulated CCM on the quality of the ice milk was also investigated by evaluating the structure, texture, melting resistance and sensory quality of the resultant ice milk.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Dairy Sciences, National Research Centre and Department of Dairy Science and Technology, Menoufia University Shibin El Kom, Egypt from March-September, 2020).

Chemicals: Curcumin (CCM) was purchased from Merck Schuchardt (8011 hohennbrunn be münchen) with a purity of 94% w/w, according to the vendor. Whey Protein Isolate (WPI, BiPro[®]) was obtained from Davisco Foods International, Le Sueur, MN, USA). Maltodextrin (MD), with a dextrose equivalent value of 6, containing 4.1% moisture and 0.1% ash, was obtained from Corcoran Chemicals Ltd, Dublin, Ireland. Fresh buffalo's milk obtained from the Dairy Industry Unit, Animal Production Research Institute, Ministry of Agriculture (Dokki, Cairo, Egypt) with an average composition of 17.12% total solids, 4.15% protein, 0.81% ash, 0.15% acidity. Mango fruit containing 32.33% total solids, 3.09% total protein, 1.58% ash and 1.70% fibre was obtained from the local market in Cairo, Egypt. Other chemicals were obtained from Sigma-Aldrich or Thermo Fisher Scientific, Pittsburgh, PA. All chemicals used were of annular grade.

Preparation of WPI-MD complex and WPI-MD conjugates:

Preparation of the WPI (10%, w/v, protein) and MD (10%, w/v), were prepared in ultrapure water to obtain a 10% WPI-MD solution, with low-speed magnetic stirring and allowed to solubilize for 2 hrs at 22 °C. The solution was adjusted to pH 7.0 using 0.1 M HCl or 0.1 M NaOH, stored at 4 °C for 18 hrs and readjusted to pH 7.0 as necessary. Then, the complex solutions were prepared by mixes the WPI and MD (1:1). The WPI-MD solution was frozen initially at -20 °C for 12 hrs and then tempered at -80 °C for 3 days. Freeze-drying was carried out under vacuum at a pressure of <0.1 m bar for 72 hrs. The resulting material was milled and divided into two portions, the first portion WPI-MD complex and the second portion

transferred to a plastic petri dish (150×15 mm) and then placed on a perforated plate in a desiccator that contained a pre-equilibrated saturated potassium bromide solution generating an atmosphere with relative humidity (RH) of 79% at 60°C/20 hrs to prepared WPI-MD Conjugate¹⁹.

Determination of available amino groups: The concentration of available amino groups in solutions, prepared from 0.1% dry heated WPI and WPI-MD, was measured by the θ -phthalaldehyde (OPA) method²⁰ as detailed by Mulcahy *et al.*²¹. The concentration of available amino groups in the WPI and WPI-MD conjugate solutions at 70°C for 20 hrs expressed by a percentage of available amino groups in the respective unheated solutions.

Preparation of CCM Loaded WPI-MD complex and WPI-MD conjugate: For the preparation of curcumin-loaded WPI-MD complex and WPI-MD conjugate, the powder them was dissolved in distilled water with a concentration of 100 mg mL⁻¹. Curcumin was dissolved in anhydrous ethanol and then added to the WPI-MD complex or WPI-MD Conjugate solution at a concentration of 15, 30 and 45 mg mL⁻¹ (curcumin to shell ratio of 15:100, 30:100 and 45:100). The mixture was stirred at room temperature for 1 h in the dark and then homogenized for 5 min with high-intensity ultrasound at an amplitude of 40% for 5 min in an ice bath using a VCX800 (Vibra Cell, Sonics, Newtown, CT, USA) with a 13 mm diameter probe (high-grade titanium alloy). A part of the nanoparticles was kept as a solution to measure the particle size and zeta-potential, while the rest was dried.

Determination of Encapsulation Efficiency (EE): The encapsulation efficiency of curcumin for the WPI-MD complex and the WPI-MD conjugated process was measured according to Mohammadian *et al.*²².

Particle size, Polydispersity Index (PDI) and zeta potential measurements: The particle size, PDI and zeta potential measurements were carried out on a Malvern Zeta-sizer Nano ZS (Malvern Instruments, London, England) at 25°C according to Jones and McClements¹⁰.

Antioxidant activity: The antioxidant activities of WPI, whey protein conjugated, curcumin loaded conjugate and the aqueous solution of curcumin was measured using DPPH radical scavenging activity test according to the method of Lin *et al.*²³ with minor adjustments. Different samples were diluted with distilled water to a protein concentration of 5 mg mL⁻¹ and a curcumin concentration of 0.1 mg mL⁻¹ before the measurements.

Ice milk-making: Buffalo's milk, skim milk powder, sugar and Lacta 9050 as a stabilizer, were used to prepare a plain ice milk mix with a composition of 4.0, 14.0, 16.0 and 0.4% for fat, milk solids non-fat, sugar and stabilizer, respectively. The mixture was divided into three equal parts, the first portion without CCM to create a control (C). Both Free CCM and encapsulated CCM in WPI-MD conjugate were added at a rate of 0.09% (w/w) to created T₁ and T₂, respectively. After preheating at 65°C and mixing all the ingredients, the mixture was homogenized using a laboratory homogenizer ((EURO TURRAXT 20b, IKA Lobo Technik 27000 min G1), pasteurized at 81°C and cooled then aged overnight at 5±1°C. Just before freezing in a batch freezer (Staff Ice System, BTM 10, Rimini Italy), 4% mango pulp was added to the aged ice cream mixture. Overrun was calculated for all formulated ice cream using the weight-volume method as described by Adapa *et al.*²⁴. The resultant ice milk was poured into plastic cups, covered and hardened in a deep freezer at -20°C for 24 hrs before analysis. Three replicates were done for each batch.

Ice milk mixture properties: The pH value of all ice milk mixtures was measured using a laboratory pH meter with a glass electrode (HANNA, Instrument, Portugal). The acidity content was determined by adding 0.1N sodium hydroxide to the phenolphthalein endpoint as described by Ar buckle²⁵. The structural viscosity of the ice cream mixtures was determined using a Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden) attached to a work station loaded with software V88 viscometry program. The viscosity was expressed as mill Pascal (mPa•s). The whipping abilities of the ice cream mixtures were determined using a mixer with 2.6 cm blades (Heidolph No. 50 111, Type RZRI, Germany) according to the method described by Baer *et al.*²⁶. The colour analysis of mango ice milk samples was conducted via Hunter colorimeter (Hunter Ultra Scan VIS) according to Hunter and Harold²⁷.

Ice milk properties: The melting properties of the ice cream were determined according to Naeem *et al.*²⁸.

Sensory evaluation: The ice milk samples were evaluated for appearance, melting quality, body and texture and flavour by a regular taste panel of 21 staff members from The Department of Dairy Sciences, National Research Centre, Egypt. The ice cream was sensorial evaluated using the nine-point hedonic scale, ranging from extremely (9 points) through like or dislike (5 points) to dislike extremely (1 point) according to the method of Naeem *et al.*²⁸.

Statistical analysis: Analysis of variance (ANOVA) and Duncan's multiple comparison procedure used to compare the means using software SPSS version 15.0 (SPSS Inc. Chicago, Illinois). A probability of $p < 0.05$ was used to establish statistical significance.

RESULTS AND DISCUSSION

Characteristics of WPI-MD conjugate: As shown in Fig. 1, the absorbance of free amino groups at 340 nm. The free amino groups decreased for WPI-MD from 93.75-81.45% according to heat-treatment time from 10-20 hrs respectively. But this decreases unnoticeable with the WPI of 99.8% after 10 hrs decreased to 96.75% after 20 hrs of heat treatment. Maillard's reaction in its given light yellow colour occurred in the product of conjugate. Conjugation by Maillard reaction gives different products, which are dependent upon pH, temperature, time, concentration, relative humidity and type of reactants (WPI and MD) as an alteration in the contents of secondary structure²⁹. The decreased in available amino groups in native WPI is most likely due to the heat-induced reaction of whey proteins with a low concentration of lactose (0.3%) which present in WPI or structural changes within the protein causing blockage of available amino groups³⁰. Similar results were obtained by previous research³¹. While the free amino groups decreased for WPI-MD from 93.75% to 81.45 % according to heat-treatment time from 10 to 20 h respectively.

Physical characterization of curcumin Nano-encapsulated Effect of Type WPI-MD on Droplet Size, PDI, Zeta Potential and Encapsulation Efficiency: The particle size distribution, PDI value and zeta-potential are important properties to provide valuable information about nanoparticles regarding the formation of stable formulations. As shown in Table 1, the particle size was higher in the WPI-MD conjugate ($p < 0.05$) than in the WPI-MD complex, while the polydispersity

indexes (PDI) and ζ potential were higher in the WPI-MD ($p < 0.05$) complex than in WPI-MD conjugate. Also, the particle size increased ($p < 0.05$) but ζ potential decreased as a concentration of curcumin (CCM) in WPI-MD increased ($p < 0.05$). The PDI of WPI-MD conjugate decreased, while that of the WPI-MD complex increased on the addition of CCM, up to 45 mg mL^{-1} . The increase in particle size may be due to the presence of curcumin adsorbed on or entrapped in β -lactoglobulin (the major component of WPI) due to boost hydrophobic interaction. Hydrophobic components can bind β -lactoglobulin the loop EF is open at pH 7, which permits ligands to inter till hydrophobic core and increase in amount hydrophobic molecules binding causing increases in particle size^{21,31}. Also, conjugates exhibited a higher binding capacity for CCM these changes have been attributed to the presence of more hydrophobic microdomains in the conjugate³². The surface charge (ζ potential) of all samples was negative, which cause more stability of solution due to more repulsive forces between particles. Similar results with a decrease in ζ potential values after encapsulation was shown by Khan *et al.*³³. Also, the glycation of whey proteins alters the distribution of values after encapsulation was shown by Khan *et al.*³³. Also, the glycation of whey proteins alters the distribution of protein surface charge, by the decrease in the content of basic

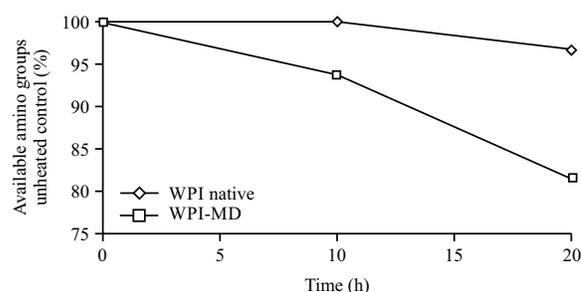


Fig. 1: Percentage of available amino groups in solutions prepared from Native WPI and dry heated WPI-MD pH of 7.0, 60 °C and 79% relative humidity for up to 20 hrs

Table 1: Size (nm) and ζ potential (mV), polydispersity index (PDI) and encapsulation efficiency for WPI-MD complexes, WPI-MD conjugated and CCM ratio encapsulated after sonicated

Samples	Size (nm)	Calculated PDI	ζ potential (mV)	Encapsulation efficiency (%)
WPI-MD conjugate after sonication	94.35 ± 6 ^d	0.45	-29.11 ± 1.42 ^e	--
15 CCM	102.34 ± 19 ^c	0.39	-30.90 ± 1.97 ^f	95.24 ± 1.2 ^a
30 CCM	139.30 ± 28 ^b	0.19	-34.20 ± 2.74 ^g	94.45 ± 3.1 ^a
45 CCM	168.90 ± 17 ^a	0.14	-39.50 ± 3.45 ^h	91.98 ± 2.9 ^b
WPI-MD complexes	43.78 ± 9 ^g	0.62	-12.60 ± 0.24 ^a	--
15 CCM	45.34 ± 8 ^g	0.63	-13.60 ± 0.89 ^b	81.57 ± 3.4 ^c
30 CCM	51.69 ± 9 ^f	0.67	-21.10 ± 1.02 ^c	75.38 ± 2.7 ^d
45 CCM	66.95 ± 7 ^e	0.67	-23.30 ± 2.41 ^d	69.65 ± 2.5 ^e

^aNumbers are Mean ± Standard deviation from triplicate samples. Different superscripts indicate differences in means ($p < 0.05$)

Table 2: Effect of added free CCM, or CCM nano-encapsulated on Physico-chemical properties of mango ice-milk mixture

Parameters	Formulation		
	Control	T ₁	T ₂
Total solids	34.83±0.35 ^a	34.85±0.30 ^a	36.90±0.27 ^b
Protein (%)	7.49±0.20 ^a	7.48±0.25 ^a	8.53±0.35 ^b
Fat (%)	4.00±0.05 ^a	3.95±0.10 ^a	3.90±0.15 ^a
Ash (%)	1.24±0.08 ^a	1.25±0.13 ^a	1.24±0.07 ^a
Acidity (%)	0.35±0.06 ^a	0.36±0.07 ^a	0.35±0.04 ^a
pH	6.38±0.14 ^a	6.36±0.09 ^a	6.35±0.17 ^a
Antioxidant activity (%)	29.78±1.24 ^a	37.35±2.53 ^b	52.57±3.49 ^c

^{a,b,c}Show the significant increase and no effect ($p>0.05$), CCM (T₁), CCM encapsulated (T₂)

lysine on protein surface after glycation with MD, which is supported by the lower value of zeta potential of glycated whey protein isolate. That indicates the significant changes in the protein structure by glycation. The CCM encapsulated WPI-MD conjugate showing narrow distribution and good stability of the particle size. A significant difference was observed for PDI values of the CCM-encapsulated WPI-MD conjugate and WPI-MD complex nanoparticles ($p<0.05$, Table 1). Repulsive forces caused by the utmost values of zeta potential for nanoparticle dispersion cause physical stability³³.

Decrease the encapsulation efficiency with an increase in CCM addition to both WPI-MD complex and WPI-MD conjugates, referring to the small quantity of CCM entrapped with protein matrix as a replacement of being embedded. Loading efficiency was greater when CCM entrapped with the WPI-MD conjugate, which reached 95.24% than CCM entrapped with the WPI-MD complex, which reached 81.57%. Due to the conjugates exhibited a higher binding capacity for curcumin, these changes have attributed to the presence of more hydrophobic micro-domains in the conjugate³².

Therefore, these results suggested that the WPI-MD complex or conjugated/CCM nanoparticle size were dependent on the addition of CCM to WPI-MD complex or conjugated nanoparticles (core-coating ratios and increased by increasing the CCM content in the solutions³².

From the results obtained, it was found that the best source for CCM encapsulated that can be incorporated in ice milk was 45 mg CCM loaded to WPI-MD conjugate, In terms of loading efficiency, diffusion rate and stability.

Mango ice milk mix properties: The chemical properties and antioxidant activity of mango ice milk mix fortified with free CCM (T₁) or CCM loaded WPI-MD conjugates (CCM encapsulated, T₂) are presented in Table 2. The addition of free CCM had no significant effect ($p>0.05$) on total solids, proteins, fat, ash and acidity content as well as the pH value of mango ice milk mix. However, the addition of CCM encapsulated caused a significant increase ($p<0.05$) in total solids and

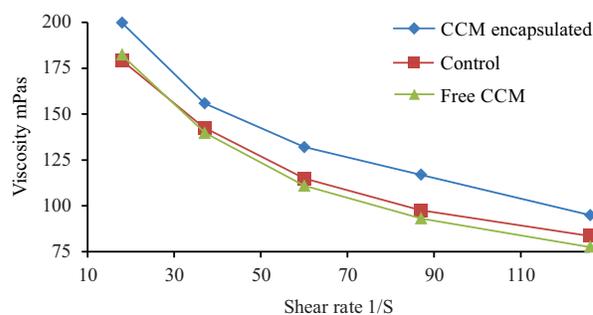


Fig. 2: Viscosity of mango ice milk fortified with free CCM or CCM encapsulated

proteins compared to control and T₁ mixes due to the addition of 2 g of powdered CCM encapsulated at a concentration of 30 mg CCM g⁻¹ WPI-MD conjugate. The total solids and proteins increased from 34.83±0.35 and 7.49±0.20% in the control mix to 36.90±0.27 and 8.53±0.35% in T₂, respectively. These results are consistent with Lima *et al.*³⁴ who's using Beta-carotene encapsulated good alternatives to reduce the application of artificial dyes to the products.

In particular, the DPPH radical scavenging activity of ice milk mix fortified with CCM encapsulated was 52.57±3.49%, followed by that of fortified with free CCM (37.35±2.53%) and control mix (29.78±1.24%). Li *et al.*³¹ found that the Synergist between the β-Lg and CCM in the mixture does the antioxidant activity not only dependent on the concentration of CCM but also the structure of β-Lg and the interaction with CCM. So the high activity of entrapped CCM may be contributed by both CCM concentrations and the structure of WPI-MD conjugates.

Mango ice milk properties

Viscosity of ice milk mixture: The apparent viscosity is important proper to evaluate ice milk for overrun, texture smoothness. The effect of added free CCM and CCM encapsulated on the viscosity of the ice milk mixes was represented in Fig. 2. Generally, when increasing the shear rate

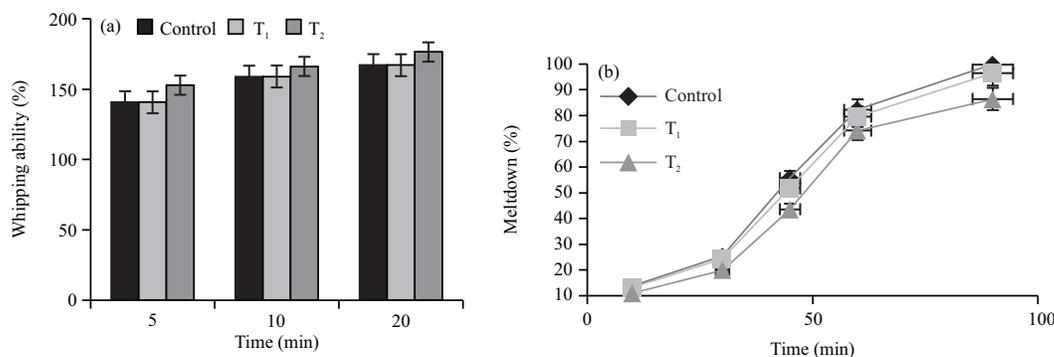


Fig. 3(a-b): Effect of added free CCM, CCM nano-encapsulated on (a) Whipping ability and (b) Melting resistance loss of the mango ice-milk mixes

Table 3: Effect of add free CCM, CCM nano-encapsulated on some properties of the mango ice milk

Samples	Overrun (%)	Colorimetric parameters		
		L*	a*	b*
Control	55.93	88.16	-2.63	22.13
T ₁	65.00	82.69	-1.24	59.51
T ₂	68.30	81.62	-1.57	69.305

L*: Value of the lightness, like 0-100 representing dark to light, a*: Value of the degree of red and green colour where higher positive indicating redder and b*: Value of the degree of the yellow and blue colours, where higher value indicating more yellow

the viscosity decreased in all the ice milk mixtures which showed shear-thinning behaviours. Bajad *et al.*³⁵ notified that the mixture of ice-cream isn't a Newtonian fluid, pseudoplastic behaviour, which the viscosity decreased with increases the shear rate. Furthermore, the addition of the CCM type had a significant effect on the mixture viscosity. Prepared ice milk mixture with CCM nano-encapsulated had a significantly higher viscosity than any others ($p < 0.05$). This is possibly due to an increase in total solids by 2% of the coating in the curcumin nano-capsules. But there was no significant difference ($p > 0.05$) in the viscosity of prepared ice milk with free CCM as compared to the control.

Overrun and colour properties: The colour properties of the ice milk samples were shown in Table 3. The incorporation of both free CCM and CCM encapsulated caused a significant change in the colour of the final ice milk ($p < 0.05$), especially when added CCM encapsulated. The L* (lightness) values of samples decreased from 88.16 in control ice milk to 82.69 and 81.62 in ice milk containing free CCM or CCM encapsulated, respectively. Similarly, the redness degree of ice milk (a*) decreased when free CCM and CCM encapsulated was added. Inversely, yellowness degree markedly increased from 22.13 in ice milk to 59.51 and 69.31 in ice milk containing free CCM or CCM encapsulated, respectively.

Overrun is an important physical characteristic of the ice-milk, it was defined by an increase of the product's volume

to the liquid mix used to produce it, in percentage. This is related to the amount of air incorporated into the ice cream during the production and, consequently, interferes with the texture and physical properties of the melting and hardness of the product³⁶.

Whipping ability and meltdown: As shown in Fig. 3a the whipping ability (increase the volume with the time of whipped) of mango ice milk mixes containing free CCM or CCM encapsulated. The mix fortified with CCM encapsulated showed the highest whipping ability after 5 min and followed the same pattern at 10 and 20 min ($p < 0.05$) compared with control and T₁ mixes. However, there was no significant difference between the control mix and that fortified with free CCM ($p < 0.05$) at 5, 10 and 20 min. That is maybe due to the shell material of CCM capsules, whey protein isolates conjugate maltodextrin, which improves their functional properties. The addition of WPI-MD conjugate may have helped increase the air incorporation within the ice milk matrix by increasing the binding in the air-cell membrane²¹. Medrano *et al.*³⁷ found the β -Lactoglobulin with lactose and glucose conjugates improvements in the foaming property.

Meltdown is the important desired parameters of ice milk quality is god shape retention and slow melting³⁸. The meltdown of mango ice cream (g/100 g) formulated with or without free CCM, or CCM encapsulated is shown in Fig. 3b.

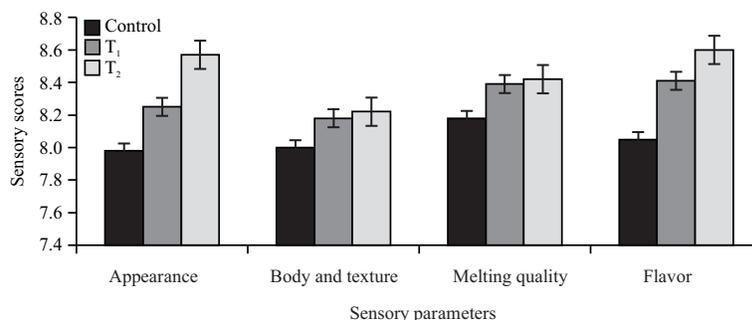


Fig. 4: Sensory properties of ice-milk affected by CCM virgin and CCM encapsulated

After 10 min, the addition of free CCM had no significant effect ($p > 0.05$) on the amount of melted ice cream compared with the control. However, the melted ice cream was the lowest when added CCM encapsulated ($p > 0.05$) compared to other treatments.

Sensory evaluation: Figure 4 illustrated the sensory quality of ice milk samples. The addition of CCM increased the sensory properties scores of mango ice milk and acceptability of the product compared with control, especially when using CCM encapsulated.

The Appearance properties of the samples changed significantly and the colour of ice milk was fortified with free CCM higher than the control, however, the highest change in colour showed with ice milk fortified with CCM encapsulated. Manoharan *et al.*³⁹ found that the CCM in the preparation of the butterscotch flavour ice cream will have good sensory acceptability and health promotion.

CONCLUSION

The WPI-MD conjugated has an advantage over the WPI-MD complex of higher stability at different pHs and better hydrophobic binding such as curcumin, exhibiting a bigger binding continual and stability. Curcumin is a bioactive component because it is a natural polyphenol compound with wide therapeutic potential. But, curcumin is poor availability and solubility in water and unstable (breaks down) at pH 7 and alkali pH. So, WPI-MD conjugated bind CCM gave the power to applicable in neutral pH products. Wherefore, ice cream was selected as an ideal food system for the delivery of curcumin Nano-encapsulated and found to be stable under the different processing conditions of production ice milk. Accordingly, curcumin encapsulated is open the way to its application in therapeutic applications and functional dairy products such as ice milk.

SIGNIFICANCE STATEMENT

This study discovers that curcumin can bind with WPI-MD conjugate which has stability at different pH, which can be beneficial for using curcumin in functional dairy products at neutral pH 7 and alkali pHs with stability and solubility and good functional properties during manufacture conditions. This study will help to use curcumin as a natural colourant with wide therapeutic potential that can be applied in functional dairy industries.

REFERENCES

1. Alavi, F., Z. Emam-Djomeh, M.S. Yarmand, M. Salami, S. Momen and A.A. Moosavi-Movahedi, 2018. Cold gelation of curcumin loaded whey protein aggregates mixed with k-carrageenan: Impact of gel microstructure on the gastrointestinal fate of curcumin. *Food Hydrocolloids*, 85: 267-280.
2. Raj, S. and D.R. Shankaran, 2016. Curcumin based biocompatible nanofibers for lead ion detection. *Sens. Actuators, B: Chem.*, 226: 318-325.
3. Aggarwal, B.B., A. Kumar and A.C. Bharti, 2003. Anticancer potential of curcumin: Preclinical and clinical studies. *Anticancer Res.*, 23: 363-398.
4. Pinheiro, A.C., M.A. Coimbra and A.A. Vicente, 2016. *In vitro* behaviour of curcumin nanoemulsions stabilized by biopolymer emulsifiers-Effect of interfacial composition. *Food Hydrocolloids*, 52: 460-467.
5. Awad, R., Z.M.R. Hassan, A.F. Farrag, M.M. El-Sayed and T.N. Soliman, 2015. The use of whey protein isolate in microencapsulation of curcumin. *Int. J. Food Nutr. Sci.*, 4: 125-131.
6. Benzaria, A., M. Maresca, N. Taieb and E. Dumay, 2013. Interaction of curcumin with phosphocasein micelles processed or not by dynamic high-pressure. *Food Chem.*, 138: 2327-2337.

7. Sneharani, A.H., J.V. Karakkat, S.A. Singh and A.A. Rao, 2010. Interaction of curcumin with β -lactoglobulin: Stability, spectroscopic analysis and molecular modeling of the complex. *J. Agric. Food Chem.*, 58: 11130-11139.
8. Tapal, A. and P.K. Tiku, 2012. Complexation of curcumin with soy protein isolate and its implications on solubility and stability of curcumin. *Food Chem.*, 130: 960-965.
9. Teng, Z., Y. Luo and Q. Wang, 2012. Nanoparticles synthesized from soy protein: Preparation, characterization and application for nutraceutical encapsulation. *J. Agric. Food Chem.*, 60: 2712-2720.
10. Jones, O.G. and D.J. McClements, 2011. Recent progress in biopolymer nanoparticle and microparticle formation by heat-treating electrostatic protein-polysaccharide complexes. *Adv. Colloid Interface Sci.*, 167: 49-62.
11. Yang, M., Y. Wu, J. Li, H. Zhou and X. Wang, 2013. Binding of curcumin with bovine serum albumin in the presence of κ -carrageenan and implications on the stability and antioxidant activity of curcumin. *J. Agric. Food Chem.*, 61: 7150-7155.
12. Li, J. and X. Wang, 2015. Binding of (-)-epigallocatechin-3-gallate with thermally-induced bovine serum albumin/ κ -carrageenan particles. *Food Chem.*, 168: 566-571.
13. Zhu, D., S. Damodaran and J.A. Lucey, 2010. Physicochemical and emulsifying properties of whey protein isolate (WPI)-dextran conjugates produced in aqueous solution. *J. Agric. Food Chem.*, 58: 2988-2994.
14. Xue, J., C. Tan, X. Zhang, B. Feng and S. Xia, 2014. Fabrication of epigallocatechin-3-gallate nanocarrier based on glycosylated casein: stability and interaction mechanism. *J. Agric. Food Chem.*, 62: 4677-4684.
15. Xia, S., Y. Li, Q. Zhao, J. Li, Q. Xia, X. Zhang and Q. Huang, 2015. Probing conformational change of bovine serum albumin-dextran conjugates under controlled dry heating. *J. Agric. Food Chem.*, 63: 4080-4086.
16. Takano, K., M. Hattori, T. Yoshida, S. Kanuma and K. Takahashi, 2007. Porphyran as a functional modifier of a soybean protein isolate through conjugation by the maillard reaction. *J. Agric. Food Chem.*, 55: 5796-5802.
17. Chen, Y., Y. Lu, R.J. Lee and G. Xiang, 2020. Nano encapsulated curcumin: And its potential for biomedical applications. *Int. J. Nanomedicine*, 15: 3099-3120.
18. Haque, Z.U. and T. Ji, 2003. Cheddar whey processing and source: II. Effect on non-fat ice cream and yoghurt¹. *Int. J. Food Sci. Technol.*, 38: 463-473.
19. O'Regan, J. and D.M. Mulvihill, 2009. Preparation, characterisation and selected functional properties of sodium caseinate-maltodextrin conjugates. *Food Chem.*, 115: 1257-1267.
20. Nielsen, P.M., D. Petersen and C. Dambmann, 2001. Improved method for determining food protein degree of hydrolysis. *J. Food Sci.*, 66: 642-646.
21. Mulcahy, E.M., C.W. Park, M. Drake, D.M. Mulvihill and J.A. O'Mahony, 2016. Improvement of the functional properties of whey protein hydrolysate by conjugation with maltodextrin. *Int. Dairy J.*, 60: 47-54.
22. Mohammadian, M., M. Salami, S. Momen, F. Alavi and Z. Emam-Djomeh, 2019. Fabrication of curcumin-loaded whey protein microgels: Structural properties, antioxidant activity and *in vitro* release behavior. *LWT*, 103: 94-100.
23. Lin, S., W. Tian, H. Li, J. Cao and W. Jiang, 2012. Improving antioxidant activities of whey protein hydrolysates obtained by thermal preheat treatment of pepsin, trypsin, alcalase and flavourzyme. *Int. J. Food Sci. Technol.*, 47: 2045-2051.
24. Adapa, S., H. Dingeldein, K.A. Schmidt and T.J. Herald, 2000. Rheological properties of ice cream mixes and frozen ice creams containing fat and fat replacers. *J. Dairy Sci.*, 83: 2224-2229.
25. Arbuckle, W.S., 1986. *Ice Cream*. 4th Edn., Springer, ISBN: 978-1-4615-7222-0 Pages: 483.
26. Baer, R.J., N. Krishnaswamy and K.M. Kasperson, 1999. Effect of emulsifiers and food gum on nonfat ice cream. *J. Dairy Sci.*, 82: 1416-1424.
27. Hunter, R.S. and D.L. MacAdam, 1976. The measurement of appearance. *Physics Today*, Vol. 29. 10.1063/1.3024412.
28. Naeem, M.A., L.K. Hassan and M.A. El-Aziz, 2019. Enhancing the pro-health and physical properties of ice cream fortified with concentrated golden berry juice. *Acta Scientiarum Polonorum Technol. Aliment.*, 18: 97-107.
29. Sonu, K.S., M. Bimlesh, S. Rajan and K. Rajesh, 2017. Preparation and functional characterization of whey protein-maltodextrin conjugates. *Res. Rev. J. Food Dairy Technol.*, 5: 7-16.
30. Nacka, F., J.M. Chobert, T. Burova, J. Léonil and T. Haertlé, 1998. Induction of new physicochemical and functional properties by the glycosylation of whey proteins. *J. Protein Chem.*, 17: 495-503.
31. Li, M., Y. Ma and M.O. Ngadi, 2013. Binding of curcumin to β -lactoglobulin and its effect on antioxidant characteristics of curcumin. *Food Chem.*, 141: 1504-1511.
32. Meng, J., T.T. Kang, H.F. Wang, B.B. Zhao and R.R. Lu, 2018. Physicochemical properties of casein-dextran nanoparticles prepared by controlled dry and wet heating. *Int. J. Biol. Macromol.*, 107: 2604-2610.
33. Khan, A., C. Wang, X. Sun, A. Killpartrick and M. Guo, 2019. Physicochemical and microstructural properties of polymerized whey protein encapsulated 3,3-Diindolylmethane nanoparticles. *Molecules*, Vol. 24. 10.3390/molecules24040702.
34. de Lima, J.G., T.C. Brito-Oliveira and S.C.D. Pinho, 2016. Characterization and evaluation of sensory acceptability of ice creams incorporated with beta-carotene encapsulated in solid lipid microparticles. *Food Sci. Technol.*, 36: 664-671.

35. Bajad, D.N., S.D. Kalyankar, S.D. Dehmukh, P.R. Bachanti and G.S. Bajad, 2016. Impact of physico-chemical properties of mix on the final quality of ice-cream. *Asian J. Dairy Food Res.*, 35: 293-297.
36. Cruz, A.G., A.E.C. Antunes, A.L.O.P. Sousa, J.A.F. Faria and S.M.I. Saad, 2009. Ice-cream as a probiotic food carrier. *Food Res. Int.*, 42: 1233-1239.
37. Medrano, A., C. Abirached, L. Panizzolo, P. Moyna and M.C. Añón, 2009. The effect of glycation on foam and structural properties of β -lactoglobulin. *Food Chem.*, 113: 127-133.
38. Goff, H.D., K.B. Caldwell, D.W. Stanley and T.J. Maurice, 1993. The influence of polysaccharides on the glass transition in frozen sucrose solutions and ice cream. *J. Dairy Sci.*, 76: 1268-1277.
39. Manoharan, A., D. Ramasamy, B. Dhanalashmi, K.S. Gnanalashmi and D. Thyagarajan, 2012. Studies on sensory evaluation of Curcumin powder as natural color for butterscotch flavor ice cream. *Indian J. Drugs Dis.*, 1: 43-46.