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# Research Article
# Production and characterization of nanodispersions of β-Carotene extracted from carrot waste and incorporated in functional processed cheese

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**Abstract**

**Background and Objective:** β -carotene is one of the important bioactive compounds for human health. **Materials and Methods:** β-carotene is one of the important bioactive compounds for human health. In this study, β-carotene was extracted from dried carrot waste using Soxhlet extraction methods and was quantified by HPLC. β-carotene nano-dispersed (β-CND) was prepared using different ratios of whey protein isolate (WPI) (1.50, 3.00, and 4.50%). The particle size, surface charge, stability, and encapsulation efficiency of β-CND were evaluated. Processed cheese was fortified with β-CND. **Results:** The lowest particle sizes of β-CND occurred at a ratio of 1.50 %, which also achieved a zeta potential of -34.6 ± 2.25 mV. The highest polydispersity index (PDI) values were observed with 4.5% β-CND. The encapsulation efficiency of all samples was more than 81.45%. β-CND was more stable after exposure to pasteurization or sterilization treatments than β-carotene (unencapsulated). The fortified processed cheese with β-CND (10%) gives high sensory acceptability and structural properties, followed by β-CND (15%). **Conclusion**: this research recommended using β-CND in the fortification of processed cheese with the indicated successful ratios and researching its application in other dairy products due to its high nutritional and functional value.

**Keywords:** Processed cheese, β-Carotene, Nanoencapsulation, Carrot waste, Solvent extraction.

## INTRODUCTION:

The carrot (Daucus carrot L.), a popularly eaten vegetable, contains various beneficial nutrients, including minerals, vitamins, tocopherol, dietary fibre, and ascorbic acid[1]. In addition, this root is one of the most important dietary sources of carotenoids, with reported quantities ranging from 42 to 150 mg of carotenoids per kilogram of carrot[2]. Carotenoids are naturally occurring pigments that have been connected to a biological role in the prevention of cancer, macular degeneration, and cardiovascular disease. This is mostly attributable to the anti-inflammatory and antioxidant qualities that carotenoids possess[3,4].

Many "functional food ingredients" can't be introduced into food systems by simple mixing because they are incompatible with the food matrix. Before being used in food systems, they often need to be appropriately enclosed utilizing specialized delivery methods[5]**.** The pharmaceutical industry has paid more attention to nanoparticles and nanodispersions during the last 10 years. This is primarily owing to the nanoparticles' and nanodispersions' capacity to regulate medication release and distribution and alleviate the inherent drawbacks of water-insoluble medicines' sluggish and partial disintegration[6]. Nanodispersions of water-insoluble active ingredients are desirable food components in the food business because they allow for more food formulations and increase the active compounds' bioavailability[7]. Nanodispersions, which include particles in the nanoscale range, are physically more stable than traditional dispersions containing molecules in the micrometer range. Solvent displacement, called nanoprecipitation, is the easiest way to make water-insoluble bioactive chemical nanodispersions. The process involves mixing an aqueous phase containing an emulsifier with ethanol or acetone. The rapid diffusion of the organic solvent in the aqueous phase causes interfacial nano-deposition of the bioactive molecule[8,9]. The polymer diffuses with the organic solvent and is stranded at the interface.

Processed cheese is an essential dairy product because of its strong ability for preservation. Because of this quality, processed cheese may be made and handled without the requirement for particular handling procedures. Because of its delicious taste and distinctive consistency, it's also a highly well-liked item in the culinary world, especially among adolescents. Natural cheese of varying ages and stages of maturation is used in the production of processed cheese. This cheese is then combined with emulsifying salts and other dairy and non-dairy components before being heated and continuously mixed to produce a consistent product and has a lengthy shelf life [10,11]. Despite the product's low bioactive chemicals and antioxidant content, its structure and properties allow for the inclusion of bioactive substances. So, adding more bioactive and functional ingredients to processed cheeses may improve their functionality [12].

The main goal of this research is to maximize carrot waste's recovery using the Soxhlet extraction and enhance the carotenoid extract's ability to withstand the conditions of manufacturing processes by nanodispersion in whey protein isolate. β-carotene nano-dispersed were characterized by particle size, zeta potential, and encapsulation efficiency. Finally, successfully incorporating carotenoids in processed cheese saves good sensory acceptability and economic value.

## MATERIAL AND METHODS

**Materials:** Carrots (*Daucus carota L.*) were obtained from a local market in Cairo, Egypt. β-carotene standard and n-hexane obtained from Sigma-Aldrich, USA. Whey protein isolate (WPI) (91% protein, 5% moisture, 3% ash, and 1% fat) was obtained from Fonterra (Darnum, Victoria, Australia). Sodium azide was from Sigma-Aldrich (Castle Hill, NSW, Australia). Double distilled water was used to prepare all solutions and emulsions. Matured cheddar cheese (6 months old) was imported from New Zealand by Khaled Khoshala Co. for Food Industries & Cooling, Egypt. Ras cheese (1 month old) was obtained from Mariam Co., Giza, Egypt. The Fonterra butter was obtained from Sakr Group Co., Egypt. Spray process grade A, nonfat dry milk made from pasteurized milk distributed by dairy America, made in the U.S.A. Egy Phos S2 emulsifying salts were obtained from The Egyptian Company for Dairy Products and Food Additives "EGYdairy" Egypt.

### Methods

**Preparation of Carrot waste powder:** Carrot wastes were placed uniformly as a thin layer on an aluminum plate. Then placed uniformly as a thin layer on an aluminum plate subjected to dehydration. Drying experiments were performed at temperatures of 45°C. The required drying time to reach the final desired moisture content (≤ 10%) was determined by[13]. Immediately after, the dried material was ground using a hammer mill with a 0.8 mm sieve.

**Extraction of β-carotene:** The β-carotene extraction is performed by using the Soxhlet extraction method. Fifty grams of carrot waste powder is placed in a porous cellulose thimble. The thimble is placed in the extraction chamber of the Soxhlet extractor, located between the boiling flask at the bottom and the condenser at the top. The round boiling flask is filled with 250 ml of solvent hexane: acetone (1:1). Then, the water source is opened and channeled from the bottom condenser and exits at the top of the condenser. The extraction process is performed at 63°C, which is its boiling point. The extraction time for the process is 20 hours for each run[14].

**Quantification of β-carotene by HPLC:** Using a Waters™ HPLC system, managed by the Empower software, with the column oven set to 33 °C and a photodiode array detector, profiles of the carotenoids were discovered in an acetone extract by HPLC[15]. (PDA). By using a gradient elution of methanol and methyl tert-butyl ether, carotenoid separation was achieved in a C30 column (S-3 Carotenoid, 4.6 mm 250 mm, YCMTM). 20% methyl tert-butyl ether and 80% methanol were used to begin the elution process. The concentration of ether was raised to 25% at 0.5 min, 85% at 15 min, and 90% at 15 min. The ether concentration was held constant at 90% until 16.50 minutes, at which point it returned to the initial condition (20%) and stayed there until 28 minutes had passed. Running time was 28 min at a flow rate of 0.8 mL min [1.] The samples were injected in a volume of 15 L. Based on their retention times and UV/Vi's absorption spectra in comparison to the retention times of the carotenoid standards, carotenoids were identified.

**Preparation of Biopolymer**: whey protein isolate (WPI, 8% w/v) solution (w/v) was developed with Milli-Q ultra-pure water and continuously stirred (1500 rpm) at room temperature for 2 h as an aqueous phase. Then, the solution was refrigerated for 24 h for complete hydration. The solution was adjusted by 1 M NaOH solution to pH 7.0, followed by heating to 80 ◦C for 15 min with constant mechanical stirring to denature WPI, and then equilibrated at ambient temperature.

**Preparation of β-carotene nano-dispersed (β-CND):** β-CND was prepared by modifying the method described by [16] based on interfacial nano deposition of carotenoid at the interface of acetone and aqueous phase after solvent displacement. The organic phase, which was acetone containing carotenoid, was added drop-by-drop in different ratios, 1.5, 3, and 4.5 g carotenoid/ 100 ml biopolymer, to obtain desired ratios, with continuous stirring for 2 h. The ratio of organic phase to aqueous phase was set at 1:9 by volume. Acetone was evaporated off; Then, the mixture was homogenized for 5 min with high-intensity ultrasound at an amplitude of 30% in an ice bath using a VCX800

(Vibra Cell, Sonics, Newtown, CT, USA) with a 13mm diameter probe (high-grade titanium alloy).

**Characterization of β-CND:** Characterization of β-CND in terms of mean particle size (Z-average), PDI, and zeta potential was assessed using a Zeta sizer Nano ZS 90 (Malvern Instruments, Worcestershire, UK). Fresh nanoparticle suspensions were used for the analysis of nanoparticle characterization. Briefly, samples were diluted to (0.01%) with deionized water and poured1 mL of each sample into a measuring cell for analysis. All the samples were analyzed for three independent measurements at 25 ◦C.

**Encapsulation Efficiency% (EE%):** EE% was measured according to the method as explained with some modifications[17]. To assess the EE, hexane extracted carotenoids from the WPI–Carotenoid nanoparticles suspension. Briefly, 2 ml of the sample was mixed with 4 mL of hexane, vortexed for 10 min, and centrifuged at 2200g for 10 min. The carotenoids in the upper supernatant were transferred to a 10 mL brown volumetric flask. The extraction was repeated 3 times with 2 mL hexane until the aqueous layer was clear and then measured with a UV-visible spectrophotometer (UV-2550, Shimadzu) at 450 nm using an established standard curve (R2 = 9966). The EE was calculated as follows:

EE% = Total carotenoids amount – Free carotenoids amount / Total Carotenoids amount

**Stability of β-CND**: β-CND were exposed to simulated standard sterilization and pasteurization processes for aqueous samples (with pH around 7), as described by[18] with adaptations. In simulating the sterilization, the autoclave was preheated to 100 °C. The Test tubes (made of borosilicate and with screw thread) containing the samples were then placed in the equipment and heated to 121 °C (1.1 atm) for 9 min. In the pasteurization process, the bath was preheated to 100 °C. The samples were then placed in the apparatus and maintained in this condition for 14 min. After both heat treatments, the nanodispersion was left to cool until 30 °C and kept at 4 °C, protected from light until the moment of analysis.

To evaluate the stability in light, the samples were placed in Petri dishes and stored in a refrigerator at 4 ° C with a relative humidity of 85%. The refrigerator was fitted with a 25 W lamp 20 cm above the samples.

**Manufacture of processed cheese analog (PCA):** Processed cheese was manufactured as described by[19] with some modifications. The full-fat experimental PCS treatments were formulated to contain 58 % moisture and 45% fat-in-dry-matter. Ras cheese and Cheddar cheese curd were milled and mixed with water and emulsifying salt in addition to β-carotenoid extract (βCE) and (β-CND). The chemical analysis of ingredients used in formulating the processed cheese spread blends is shown in Table (1). Processed cheese and PCA treatments were prepared by blending the ingredients with previously warmed. The blends were heated to 82°C, then treatment adds to the blend and processed by ultra-homogenizer price for 5 – 10 min on 10 pars, heated again to a final temperature of 82°C in approximately 4 min, then packaged and stored at 5-7°C and analyzed for chemical, rheological and microbiological properties, and sensory evaluation.

**Table (1): shows the composition of the raw materials required to make processed cheeses**

| Parameters / Ingredients | T. S | Fat | T. P | Ash |
|---|---|---|---|---|
| **Ras cheese** | **54.82** | **24.77** | **21.33** | **5.76** |
| **Cheddar cheese** | **65.8** | **34.8** | **24.37** | **5.42** |
| **Skim milk powder** | **96** | **1.5** | **37.13** | **7.98** |
| **Butter** | **84** | **82** | **-** | **-** |
| **βCE** | **2** | **-** | **-** | **-** |
| **β-CND** | **10** | **-** | **8** | **-** |

βCE: β-carotene extract**.** β-CND: β-carotene nano-dispersed. T. S: Total solids. T. P: Total protein

**Physicochemical and Rheological analysis of PCA:** The total solids, ash, titratable acidity, and total protein of PCA were determined as described by[20]. Total protein content was determined by the Semi Micro-Kjeldahl method. Fat content was determined using the Gerber method as described by[21]**.** The salt content was determined as described by[22]**.** The pH values were measured using a laboratory digital pH meter model Adwa 1030. The Texture Profile Analysis (TPA) of processed cheese was performed using a multi-test 1-d texture analyzer, (mecmesin limited, Slinfold, West Sussex, UK). Experiments were carried out by a compression test that generated a plot of force (N) versus time (sec). Samples were double compressed at a compression speed of 2 cm/min. The analysis was carried out at room temperature. Hardness (N), springiness (mm), chewiness (N*mm), gumminess (N), and cohesiveness were evaluated as described by[23]**.**

**Organoleptic properties evaluation:** All samples of processed cheese were evaluated organoleptically using a hedonic scale of 1-5, which was designed based on the hedonic scales provided by[24,25,26]**.** The sensory evaluation was carried out by 15 persons of staff members of the Dairy Department, Al-Azhar University.

**Microbiological analysis:** The microbiological methods outlined in the standard methods for the examination of

dairy products[22] were employed for the determination of the following specific bacterial groups:
(a) Total bacterial count.
(b) Aerobic and anaerobic spore-forming bacterial count.
(c) Total Coliform bacterial count.
(d) Yeasts and molds count.

**Statistical analysis:** Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS version 20 (IBM)) according to[27]. Each preparation and measurement were conducted in triplicate. The experimental data were subjected to analysis of variance for a completely random design. Duncan's multiple range tests were used to determine the difference among means at the level of 0.05**.**

## RESULTS AND DISCUSSION:

**High-performance liquid chromatography (HPLC) of Carrot waste extract by Soxhlet:** High-performance liquid chromatography (HPLC) for quantifying lutein and β-carotene the primary lipophilic antioxidants in carrot waste extract are carotenoids. The retention time for lutein and β-carotene were 4.673 and 154.437 minutes, respectively (Fig. 1). Approximately 0.097 ± 0.007 mg/g of lutein and 49.75 ± 3.15 mg/g of β-carotene were present in carrot waste extract, respectively. On the other hand, according to[28], carrot pomace extract by sonication contains around 44.75 mg of β-carotene.

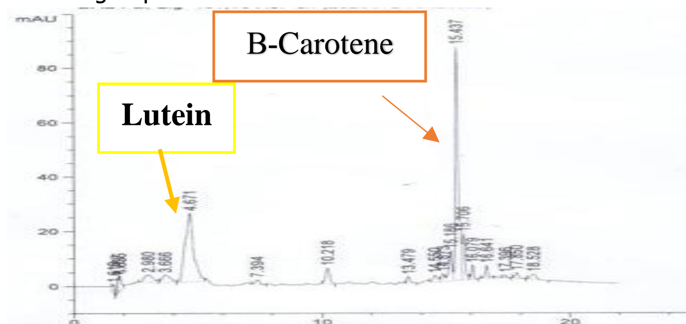



**Figure1.** HPLC Of carrot waste extract

**Nanoparticles of carotenoid:** The use of (WPI) for encapsulating different bioactive components is a promising approach to developing nanoparticles. Many hydrophobic substances, such as curcumin and beetroot juice powder, have been studied in our lab because of the success of whey protein isolate nanoparticles in protecting and improving their physicochemical, microstructural, and rheological characteristics[29,30]. In the present study, nanoparticles made from whey protein isolate were used to encapsulate carotenoids with the goal of improving their physicochemical qualities and providing effective protection while in storage. For this aim, three formations were prepared with different WPI/carotenoid ratios, 1.50, 3.00, and 4.50% (w/w), using a high-intensity ultra-sound for 7 min, and were subsequently evaluated for physicochemical characterization

**Physicochemical characterization of β-CND:** The solvent displacement approach was used to create carotenoid nanodispersions. As a result of mutual diffusion between the two phases, there was fast interfacial spreading and turbulence when the organic phase was introduced to the aqueous phase. According to[31]**,** the organic phase's unpredictable and quick pulsations may have resulted in a localized reduction of the interfacial tension, creating interfacial turbulence. The free energy produced when the solvent was redistributed to reach its equilibrium condition provided the energy required for these jerky motions[8]**.**

Particle size, polydispersity index (PI), and zeta potential are important characteristics that may provide vital information on the creation of stable formulations from nano-encapsulated substances. Table 1 shows the particle size of WPI-only and carotenoid-encapsulated whey protein isolate nanoparticles treated with ultrasound. The mean particle sizes of carotenoids encapsulated whey protein isolate nanoparticles were 148.34 8.73, 194.09 14.52, and 221.70 9.59 nm for samples with WPI to carotenoids ratios of 1.50, 3.00, and 4.5%, respectively, which were substantially greater than native WPI (102.30 4.68 nm) (p 0.05). This increase in particle size might be attributed to carotenoids being entrapped in or absorbed on β-lactoglobulin (the primary component of whey protein isolate) due to improved hydrophobic interaction. The three binding sites of β-lactoglobulin allow it to bind hydrophobic substances. The loop EF (strands) is open at pH 7.0, allowing ligands to enter the hydrophobic core and interact large numbers of tiny hydrophobic molecules, causing particle size to grow. Nanoparticles prepared with WPI exhibited a smaller particle size compared to other proteins due to their good emulsification property, which acts as a surfactant because of their hydrophilic and hydrophobic regions[17,32,33]**.** [16]found that depending on the DIM to WPI ratio utilized in the preparation, it was possible to adjust the mean particle size of the nanoparticles down to 96-157 nm.

A good emulsifier should inhibit particle agglomeration in the nanodispersion during storage. The zeta potential may be used to forecast the storage stability of nanodispersions. In general, if the particles in a nanodispersion have a high surface charge, the particles are less agglomerate. A minimum zeta potential of 30 mV is necessary for a physically stable nanodispersion maintained by electrostatic repulsion by an emulsifier[34]**.** However, when the protein's purity increased, the nanodispersion stability was expected to increase accordingly, as indicated by the increase in the zeta potential of WPI-stabilized nanodispersion[35]. The zeta potential values of carotenoid encapsulated WPI

nanodispersions are presented in Table 2. The zeta potential value of native WPI (-31.9 ± 2.15 mV) significantly increased to -34.6 ± 2.25, -41.4 ± 4.73, and -44.3 ± 3.89 mV after WPI encapsulated carotenoids coated ($p < 0.05$), indicating that carotenoid may be attached to WPI nanoparticles. Similar results with an increase in zeta potential values after encapsulation were shown by [35], who reported that the zeta potential value was -48.40 mV after encapsulation of algae oil. The zeta potentials of all samples were significantly negative, resulting in stronger repulsive interactions between particles and potentially improving the physical stability of diverse systems[34].

**Table 2. Particle size, (PDI), zeta potential, and (EE%) of whey protein isolate only and carotenoids encapsulated WPI nanoparticles.**

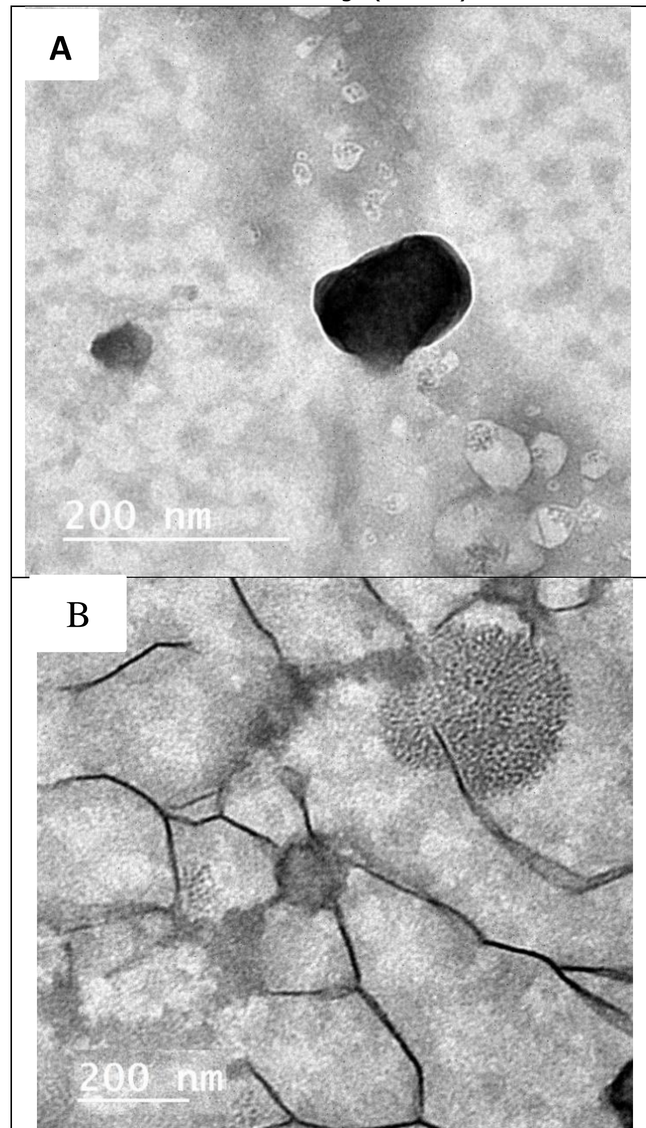
| Samples | Size (nm) | PDI | Zeta-potential | EE % |
|---|---|---|---|---|
| WPI | 102.30 ± 4.68 | 0.359 | -31.9 ± 2.15 | -- |
| 1.50% Carotenoids-WPI | 148.34 ± 8.73 | 0. 495 | -34.6 ± 2.25 | 90.08 ± 1.95 |
| 3.00 %Carotenoids-WPI | 194.09 ± 14.52 | 0.508 | -41.4 ± 4.73 | 87.89 ± 2.05 |
| 4.50% Carotenoids-WPI | 221.70 ± 9.59 | 0.558 | -44.3 ± 3.89 | 81.45 ± 3.75 |

PDI: polydispersity indexes. EE%: encapsulation efficiency

The polydispersity index (PDI) values for all treatments are shown in Table (2). Nanoparticles with higher PDI values have wider size dispersion, making them more vulnerable to Ostwald ripening. A lower PDI value, on the other hand, indicates a relatively narrow distribution with strong physical stability[16,36]. As carotenoid was added to WPI nanoparticles, the PDI value rose when compared to native WPI. However, the differences were not statistically significant (p 0.05). The greater the carotenoid content, the higher the PDI value, perhaps owing to more interacted WPI and carotenoids, which became more uniform with a restricted size distribution[17]. Furthermore, all the formulations' polydispersity indices (PDI) values were less than 0.6, indicating a narrow distribution with high stability.

The encapsulation efficiency of carotenoids distributed in WPI nanoparticles is shown in Table 2. Encapsulation efficiency was reported to decrease with increasing carotenoid content to WPI nanoparticles, indicating that a tiny quantity of carotenoid was not entrapped in the protein matrix instead of being embedded.[29]discovered this property as well, revealing that EE reduced with increasing curcumin content in protein nanoparticles, resulting in precipitation. The encapsulation efficiency of all samples was more than 81.45%. In prior research, it was discovered that the encapsulation efficiency (EE) of carotenoid encapsulated with whey protein isolate was 97%[37].

**Transmission electron microscopy:** Transmission electron microscopy (TEM) was used to examine micrographs of native WPI and WPI nanoparticles covered with carotenoids (Figure 2). Micrographs of nanoparticles showed striking variation based on carotenoids' relative abundance in the WPI system. These findings are consistent with dynamic light scattering DLS data, which shows that an increase in the carotenoid ratio with WPI leads to bigger particle sizes (Table 2). The micrograph of heated WPI alone revealed a polymeric network (unfolding of protein particles happened). Also, the WPI particles were discovered to be smaller than those of other encapsulated carotenoid ratio nanoparticles (Figure 2). After encapsulation with carotenoid, the morphology of WPI nanoparticles was more uniformly smooth, spherical, and noticeable in all three formulations tested (WPI: carotenoid ratios of 1.5%, 3.5%, and 4.5%)[38,39]. In contrast, WPI: carotenoid mixtures with 4.5% were found to have larger particles coated by a dense-rigid polymeric network of WPI that lacked a porous character. These findings are consistent with the DLS findings (Table 2).



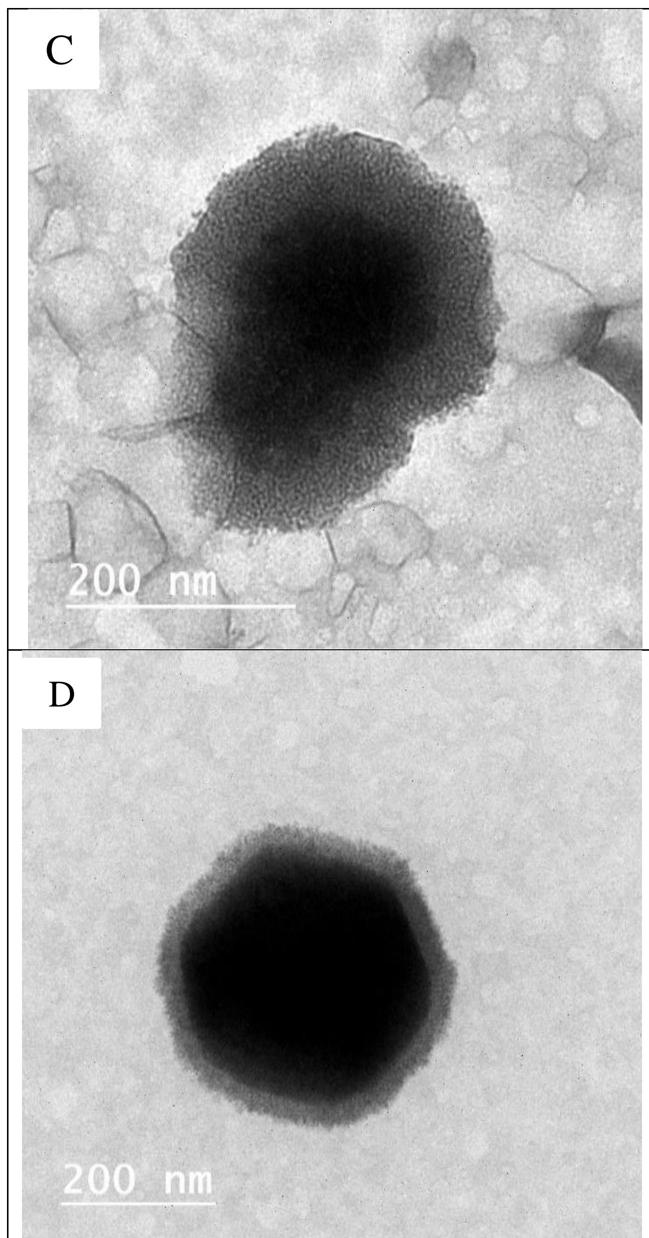



Figure 2. Transmission electron microscopy of carotenoids nanoencapsulation A.: nanoencapsulation containing 0 % carotenoids; B: nanoencapsulation containing 1.5 % carotenoids; C: nanoencapsulation containing 3 % carotenoids; D: nanoencapsulation containing 4.5 % carotenoid respectively.

**Stability of carotenoids**

**Photodegradation**: Many phytochemicals undergo isomerization, oxidation, or oligomerization in response to light. Several mechanisms have been proposed to explain how carotenoids deteriorate when light exposure. Also, carotenoid radical cations are a putative species generated during photooxidation[40]**.** beta-carotene radical cations may be formed through a slower degradation process after excited beta-carotene molecules return to the ground state and are attacked by radical by-products formed during the light reaction, as found by[41]**.**

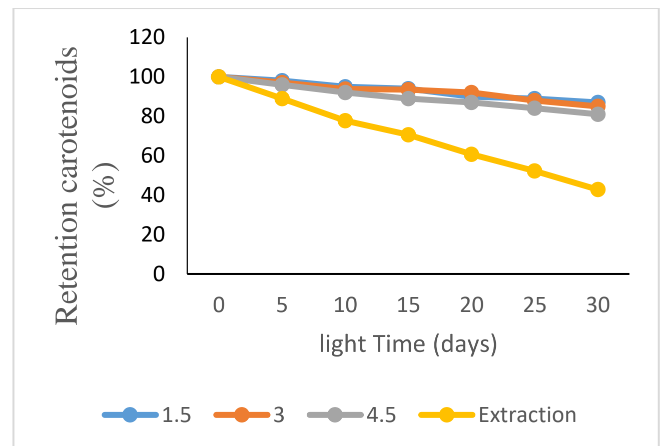



**Figure 3.** Photochemical stability of native carotenoids and WPI: β-CND (1.5, 3, and 4.5%(w/w) against light during storage for 30 days.

Figure 3 illustrates the outcomes of light illumination stability of carotenoids. Compared to the control sample, whey protein isolate nanoparticles kept their carotenoid more intact after being exposed to light for 30 days (carotenoids in solvent). After adding 1.5, 3, and 4.5%(w/w) carotenoids, more than 87.32%, 85.15%, and 81.47% of carotenoids remained unchanged in WPI nanoparticles, respectively, demonstrating that WPI nanoparticles give greater protection for carotenoids against the light. But, on day 30, only 42.78 % of carotenoids were in the control sample. To prevent damage from environmental factors, carotenoids should be encased in protein nanoparticles. The incorporation of carotenoids into nanocapsules has previously been shown to improve scavenger activity and preserve carotenoids against light-induced alterations [42]**.**

**Thermal Degradation**

When carotenoids are heated in the presence of oxygen, volatile molecules and large non-volatile components develop[43,44]presented a beta-carotene degradation sequence based on the products identified after beta-carotene heating at 97°C for up to 3 hours in the presence of air as assessed by GC-MS and absorption spectrophotometry. [45]noticed that at 60 °C with oxygen, the beta-carotene in toluene yielded a diverse combination of compounds, including but not limited to epoxides. Due to these byproducts, the researchers concluded that under these oxidation circumstances, a variety of radical species are produced and are capable of combining with oxygen to generate peroxyl radicals, which are then propagated by other carotenoids [45]**.**

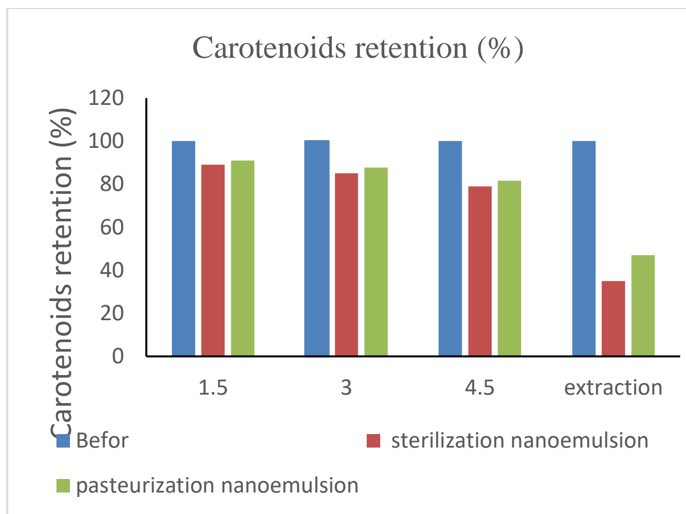



**Figure 3** illustrates the outcomes of heat treatment stability of carotenoids.

Compared to the control sample (carotenoids in solvent), whey protein isolate nanoparticles-carotenoids 1.5, 3, and 4.5% (w/w) kept their carotenoid more intact after being exposed to pasteurization or sterilization. The remains of carotenoids with sterilization were 89.21%, 85.27%, and 79.47% of carotenoids unchanged in WPI nanoparticles, respectively. While the mimic pasteurization treatment was 91.00, 87.75, and 81.63%, carotenoids remained unchanged in WPI nanoparticles, but the carotenoid in the solvent was high degradation. The results demonstrate that WPI nanoparticles protect carotenoids more against mimic pasteurization and sterilization.[46]provided evidence that beta-carotene terminal double bonds could be broken at lower temperatures (60°C), producing various epoxides as determined by chromatography followed by the determination of absorption spectra. This was done by passing a stream of oxygen through beta-carotene in toluene. This study disproved autoxidation as a degradation process by demonstrating no lag phase in the breakdown of beta-carotene.

**The influence of adding β-carotene on the properties of processed cheese:**

Within three months of cold storage (5- 7 °C), processed cheese analog products manufactured with βCE and β-CND were assessed for their chemical, rheological, microbiological, and sensory qualities and compared to the control sample (without beta-carotene). The mixes are displayed in Table (3).

**Table 3: Recipe for various processed cheese analogs with β-carotene, (gm/1 kg).**

| Ingredients | Control | processed cheese treatments | | |
|---|---|---|---|---|
| | | βCEP | β-CND | |
| | | | 10% | 15% |
| **Cheddar cheese** | **128** | **128** | **128** | **128** |
| **Ras cheese** | **384.4** | **384.4** | **384.4** | **384.4** |
| **Skim milk powder** | **51.2** | **51.2** | **51.2** | **51.2** |
| **Butter** | **102.6** | **102.6** | **102.6** | **102.6** |
| **Egy Phos S2** | **25** | **25** | **25** | **25** |
| **βCE** | **--** | **3** | **--** | **--** |
| **NCβC** | **--** | **--** | **2** | **3** |
| **Water** | **308.8** | **305.8** | **306.8** | **305.8** |
| **Total** | **1000** | **1000** | **1000** | **1000** |

βCEP: processed cheese with β-carotene extract. β-CND P: processed cheese with Nanoencapsulation of β-carotene

**Sensory perspective of processed cheese prepared with or without β-carotene:** Assessment of the organoleptic properties of food products is one of the most important monitoring manners that indicate food's satisfaction to consumers. The sensory assessment was carried out to assess of surface appearance, spreading quality, smoothness of texture firmness of body, and stickiness. Furthermore, evaluation of breakdown properties, saltiness, oiling off, flavor, and overall desire of the resultant products. The obtained data of βCE and β-CND processed cheese samples to the sensory properties were recorded in the table (4). Herein, the products with the βCE, and 10 and 15% β-CND addition didn't exhibit a significant difference in terms of flavor, firmness of the body, Oiling off, and saltiness. Overall preference scores for 10 and 15% β-CND cheese samples were 3.43 ±0.53 and 3.14 ±0.69 from 5, respectively, compared to the control sample (4.86 ± 0.38from 5). Meanwhile, the βCEP cheese samples were 2.00 ±0.822 from 5. These significantly low values for mouth feel and flavor scores for βCEP which were not commonly accepted by some panelists, hence, this sample was rejected. We found that the best treatments of analog cheese were the cheese with 10% β-CND which was the nearest to the control sample followed by a 15% addition β-CND.

**Table 4:** Sensory perspective of processed cheese prepared with or without β-carotene

| Qualities | Control | processed cheese treatments | | |
|---|---|---|---|---|
| | | βCEP | β-CND | |
| | | | 10% | 15% |
| **Flavor (5)** | 3.00 ± 0.58 | 2.00 ± 0.58 | 3.14 ± 0.69 | 3.00 ± 0.58 |
| **Surface appearance (5)** | 2.86 ± 0.69 | 3.86 ±0.69 | 3.43 ±0.53 | 3.57 ± 0.53 |
| **Firmness of body (5)** | 2.71 ± 0.49 | 2.86 ± 0.38 | 2.00 ± 0.58 | 2.14 ± 0.69 |
| **Smoothness of texture (5)** | 1.86 ± 0.38 | 3.14 ± 0.38 | 2.43 ± 0.53 | 3.86 ± 0.69 |
| **Spreading quality (5)** | 2.86 ± 0.38 | 2.14 ± 0.69 | 3.14 ± 0.69 | 2.86 ± 0.38 |
| **Breakdown properties (5)** | 2.14 ± 0.38 | 2.86 ± 0.69 | 3.43 ± 0.79 | 3.29 ± 0.49 |
| **Oiling off (5)** | 1.14 ± 0.38 | 1.21 ± 0.39 | 1.29 ± 0.49 | 1.36 ± 0.48 |
| **Stickiness (5)** | 3.00 ± 0.58 | 2.00 ±0.58 | 2.14 ± 0.38 | 2.29 ± 0.49 |
| **Saltiness (5)** | 1.14 ± 0.38 | 1.29 ± 0.76 | 1.43 ± 0.53 | 1.43 ± 0.79 |
| **Overall preference (5)** | 4.86 ± 0.38 | 2.00 ±0.82 | 3.43 ±0.53 | 3.14 ±0.69 |

βCEP: processed cheese with β-carotene extract. β-CND: processed cheese with Nanoencapsulation of β-carotene

**Chemical composition of β-carotene processed cheese:** Table displays the results of the chemical analysis (5). In terms of total proteins, fat, salt, and total solids, the results show that there are no significant variations between the control sample and the treatments. There are also no significant differences between treatments and some of them with of β-carotene added. The chemical characteristics of processed cheese are therefore unaffected by the addition of β-carotene. The results agree with the results of [47]**.**

Except for the processed cheese sample at a level of 10% addition of β-CND, there were no discernible differences between the control sample and the treatments for ash. The components of the primary mixture are shown by all obtained data, and the range for the fat/dry matter ratio is 47 to 50%. These results agree with[48].

**Table 5: Chemical composition of processed cheese made with β-carotene**

| Sample Parameters | Control | processed cheese treatments | | |
|---|---|---|---|---|
| | | βCEP | β-CND | |
| | | | 10% | 15% |
| **Total solid %** | **45.30±0.70$^{bc}$** | **45.74±0.36b** | **44.79±0.31$^{c}$** | **46.61±0.24$^{a}$** |
| **Fat%** | **22 ± 1$^{a}$** | **22±1a** | **21±1.73$^{a}$** | **22±1.73$^{a}$** |
| **F/ DM%** | **48.62±2.96$^{a}$** | **48.09±2.03$^{a}$** | **46.90±4.17$^{a}$** | **47.20±3.94$^{a}$** |
| **Total protein %** | **11.6±0.41$^{a}$** | **11.7±0.40$^{a}$** | **11.9±0.31$^{a}$** | **12.1±0.10$^{a}$** |
| **Salt %** | **1.47±0.01$^{a}$** | **1.28±0.14$^{b}$** | **1.40±0.07$^{ab}$** | **1.48±0.12$^{a}$** |
| **Ash %** | **4.34±0.50$^{a}$** | **4.20±0.11$^{a}$** | **3.90±0.27$^{b}$** | **4.29±0.90$^{a}$** |

* Means with the same letters in a row are not significant at the 5 % level. βCEP processed cheese with β-carotene extract. β-CNDP: processed cheese with Nanoencapsulation of β-carotene. F/ DM: Fat/dry matter ratio

**PH and titratable acidity:** Figure 4 display the pH of samples of processed cheese enhanced with β-carotene. The pH values of the mixes were affected by the type of raw materials, th it was ranged from 5.7 - 5.85. this decrease in pH in all mixes may be due to the lower pH of the cheese used in preparing the mixtures, where the processed cheese is affected by the qualities of the cheese inside the manufacturing process these results agree with[49].
Compared with the control sample (5.71), the pH values of the treatments have been increased were 5.86, 5.86, and 5.84, respectively (pH range of carrot extract 5.88-6.40). These results are consistent with[50]**.** On the other side, the pH value decreased as the storage duration increased, that when all treatments were stored at 7° C. This could be attributed to the acidity increase of the cheese during storage as shown in Figure (4). These results are conforming to previous reports by [51,52].

**Titratable Acidity (TA)**: Figure 5 displays the fresh and after 30, 60, and 90 days of cold storage titratable acidity values for the processed cheese analog PCA. The findings revealed an increase in acidity values, which increased linearly during the storage time. The increased acidity of samples could be due to cheddar cheese in the blends. However, both when the samples were fresh and during the cold storage period, there was a considerable increase in titratable acidity between the samples. Typically, the increase of titratable acidity of all samples during cold storage was attributed to the changes in the emulsifying salt form, lactose, and soluble nitrogen content. These data are agreed with the findings of [51].

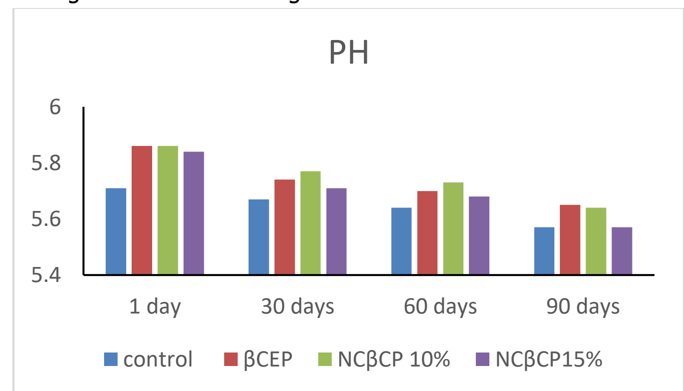



Figure 4 shows the PH changes for stored processed cheese made with βCEP: processed cheese with β-carotene extract.

β-CND: processed cheese with Nanoencapsulation of β-carotene

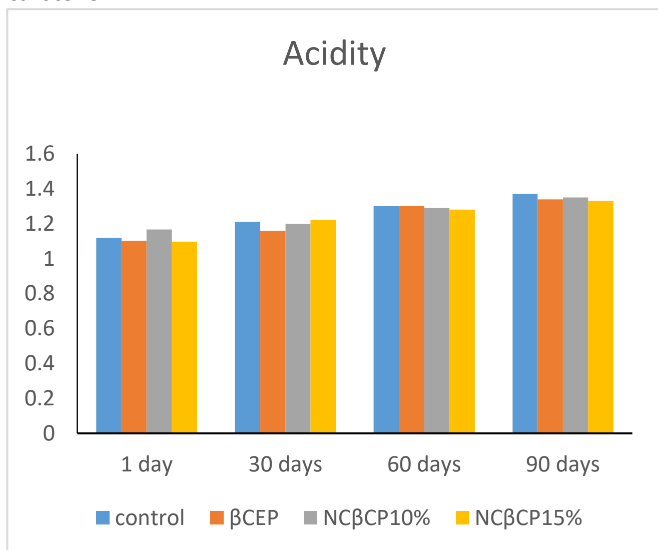



Figure 5 shows the acidity changes for stored processed cheese made with βCEP: processed cheese with β-carotene extract. β-CND: processed cheese with Nanoencapsulation of β-carotene. βCEP: processed cheese with β-carotene extract. β-CNDP: processed cheese with Nanoencapsulation of β-carotene.

**Texture profile:** Texture has an important effect on the consumer acceptance of foods. International Organization for Standardization defines texture as a sensory characteristic perceived largely by way of the senses of movement and touch. Both sensorial and instrumental methods can be used to identify the textural properties of foods. Instrumental texture profile analysis (ITPA) imitates the actions of the human mouth. Due to limitations of time, panel training, panelist psychology, and the labor-intensive nature of sensory analysis, instrumental methods have been designed to measure food properties that relate to relevant sensory characteristics. Texture profile analysis is used as a common instrumental measurement for cheese-texture evaluation[53]**.** To investigate the effect of β-carotene addition on the texture properties of the processed cheese, the following properties were measured, as presented in Table (6), Hardness, Adhesiveness, Cohesiveness, Springiness, Gumminess, and Chewiness.

The results exhibited that the TPA values of the processed cheese were affected by the processing factors to various degrees, including raw cheese composition, water content, emulsifying salts, and protein to fat ratio. Where a relative increase in the hardness of the processed cheese was found, this may be due to the use of high temperatures from 80-100°c, and it may also be due to the use of cheddar cheese of one month old in the mixture. The increase in the proper protein increases the hardness somewhat[54]**.** A fluctuation in the TPA values was observed for the processed cheese with the changes in the parameters. Parameters Where textural properties change significantly when water content varies during cheese production. In addition, the processing time and temperature play major roles in controlling the emulsion formation, determining the final properties of processed cheeses[55,56]. The results illustrated that the processing conditions influenced the TPA parameters of processed cheeses and that the water content played the most significant role in comparison to the other factors. Hardness and chewiness were more susceptible to the change of processing conditions in comparison with the other TPA parameters.

**Table (6): Texture properties of processed cheese treatments with different ratios of β-carotene:**

| Sample | Hardness (N) | Springiness (mm) | Cohesiveness | Gumminess (N) | Chewiness (N*mm) |
|---|---|---|---|---|---|
| **Control** | **9.1** | **0.36** | **0.42** | **3.80** | **1.38** |
| **[1]βCEP** | **7.700** | **1.020** | **.44** | **3.36** | **3.44** |
| **[2] β-CNDP 10%** | **5.800** | **.700** | **.46** | **2.65** | **1.85** |
| **[2] β-CNDP 15%** | **12.6** | **.9100** | **0.31** | **3.92** | **3.59** |

Means with the same letters in a row are not significant at the 5 % level. 1: processed cheese with β-carotene extract 2: processed cheese with Nanoencapsulation of β-carotene

**Microbiological analysis of processed cheese:** Results in Table (7) show the hygienic properties of the studied processed cheese. The processed cheese samples tested immediately as fresh were of excellent microbiological quality. The total bacterial count in processed cheese samples with beta carotene was as little as possible, while the other samples were acceptable[57] mentioned that 5 ×104 represents an acceptable level for the total plate count of processed cheese. These scarce counts may be due to contamination of products after cooking during filling or circulation because the heat temperature during the process was higher than 80˚C.

Spore formers count was not detected in all samples at zero time and after 1 month, as well coliform bacteria and yeast & molds were not detected in all samples. This may be due to the high-temperature processing in addition to the pH of blends. The blends were heated to a final temperature of 82°C at approximately 8 min. the pH of processed cheese was Range between 5.57 – 5.90. The results of our study seem to confirm that the application of a well-standardized and mechanized procedure, which minimizes the manual handling of the product, combined with proper hygiene practices during manufacturing will help to reduce the risk of developing food

safety and/or spoilage problems in these kinds of dairy products. These results were like the results obtained by[57,58]. These results agree with [48].

**Table 7** shows the microbiological examination of processed cheese containing β-carotene (log CFU/g)

| Microbial groups | Cold period Storage | Control | processed cheese treatments | | |
|---|---|---|---|---|---|
| | | | βCEP | β-CNDP | |
| | | | | 10% | 15% |
| Total bacterial count | Fresh | 4.28 | 4.53 | 4.43 | 4.00 |
| | 30 days | 4.32 | 4.78 | 4.71 | 4.04 |
| | 60 days | 4.54 | 4.96 | 4.83 | 4.85 |
| | 90 days | 4.67 | 4.06 | 4.08 | 4.15 |
| Spore formers count | Fresh | ND | ND | ND | ND |
| | 30 days | ND | ND | ND | ND |
| | 60 days | 2.04 | 2.08 | 2.23 | 2.67 |
| | 90 days | 2.04 | 2.18 | 2.32 | 2.79 |
| Coliform group | Fresh | ND | ND | ND | ND |
| | 30 days | ND | ND | ND | ND |
| | 60 days | ND | ND | ND | ND |
| | 90 days | ND | ND | ND | ND |
| Molds & Yeast | Fresh | ND | ND | ND | ND |
| | 30 days | ND | ND | ND | ND |
| | 60 days | ND | ND | ND | ND |
| | 90 days | ND | ND | ND | ND |

βCEP: processed cheese with β-carotene extract. β-CNDP: processed cheese with Nanoencapsulation of β-carotene**.**

## CONCLUSION:

This study concluded it was demonstrated that beta-carotene could be successfully extracted from carrot waste using a solvent mixture of hexane and acetone at a ratio of (1/1) by Soxhlet extract, as well as that using whey protein isolate for encapsulation could help beta-carotene be more resistant to manufacturing conditions that are unsuitable for its physical and chemical properties. By including beta-carotene nanocapsules (β-CND in processed cheese combinations, processed cheese is improved with great nutritional, practical, sensory acceptability, and economical advantages.

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