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Manufacturing of processed cheese that is enhanced with β-Carotene that is derived from waste carrots

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ABSTRACT

Emulsions of oil in water have shown to be successful in carrying lipophilic bioactive substances. In this research, β-Carotene has been extracted from dried carrot waste by ultrasound-assisted sunflower oil at different levels from carrot powder/oil (1, 3, and 5% carrot powder) to oil (w/w). After comparing the oil's stability with Rancimat and estimating its carotenoids, Whey protein isolate (WPI) was next used as a biopolymer to form a nanoemulsion with β-carotene. The nanoemulsion was then added to processed cheese blends and evaluated for microbiological, chemical, and physical characteristics. Results showed an increase in oxidative stability, small droplet sizes, and retention of 80-70% of β-Carotene when exposed to pasteurization and sterilization temperatures. The emulsion also had no effect on chemical attributes. The most effective treatment was 15% NEβC, receiving a 4 out of 5 score in terms of sensory evaluation. The research recommends using NEβC in cheese manufacturing and exploring its use in other dairy products.

Keywords: β-Carotene, Sunflower oil, Nanoemulsion, Processed cheese, green extraction.

INTRODUCTION

Carrots, a popular vegetable, were high in minerals, vitamins, tocopherol, ascorbic acid, and dietary fiber (Idrovo *et al*., 2016). Furthermore, research indicates that this root is one of the major food sources of carotenoids, including 42–150 mg of these compounds per kilogram of carrot (Lee, *et al*., 2018). Natural colourants known as carotenoids have been linked to a biological function in preventing cancer, macular degeneration, and cardiovascular disease because of their potent anti-inflammatory and antioxidant properties (Rygula *et al*., 2018 and Saini *et al*., 2015).

Lower amounts of lutein and β-carotene can be detected in carrots; β-carotene accounts about 45–80% of the overall carotenoid content (De Oliveira, *et al*., 2010). These compounds were essential to human nutrition because they function as vitamin A supplements (Lee *et al*., 2018 and Wang *et al*., 2018). In considering the positive benefits of β-carotene, it will be interesting to observe how this material is reduced or concentrated in the next application. Vegetable oils may be used for this purpose, according to the research of Purohit and Gogate (2015); Goula *et al*. (2017) and Baria *et al*. (2019), who extracted carotenoids using linseed oil, sunflower oil, and coconut oil, respectively.

Processed cheese is an essential dairy product that may be made and handled without the need for special care because of its exceptional potential for preservation. The delicious flavour and special texture make it a highly popular product as well, especially among kids. In processed cheese, emulsifying salts, non-dairy additives, and natural cheeses of different ages and maturities are blended and cooked to produce a homogenous product with a long shelf life

(Meyer, 1973 and Guinee *et al*., 2004). The product's structure and attributes facilitate the assimilation of bioactive elements, despite its small concentration of antioxidants and bioactive compounds. Thus, processed cheeses may perform better if additional bioactive and functional components are added (Bachmann, 2001).

The objective of this study was to use carrot-extracted β-carotene as a green extraction method for bioactive components and to enhance sunflower oil with this β-carotene. The β-carotene was then used in processed cheese as a nano-emulsion of β-carotene (NEβC). The purpose of this research is to optimize the utilization of agricultural waste that contains some bioactive components but cannot withstand specific food manufacturing conditions by encapsulating the waste and developing processed cheese by adding specific chemicals with functional qualities. To this end, the following research topics were studied: Part I: Preparing Powder from Carrot Waste. Part II: collecting the carotenoids from the residual carrots and enhancing their chemical and thermal stability One carotenoids nanoemulsion. Part III: Manufacture functional cheese by the addition of β-carotene.

MATERIALS AND METHODS

Materials:

The carrots (Daucus carota L.) were purchased from a local market in Cairo, Egypt. The sample's β-carotene content was determined using the following reagents: n-hexane (Sigma-Aldrich, 99.9%) and β-carotene standard. The sunflower oil (SO) was obtained from Arma Oil Co. on the 10th of Ramadan, Egypt. As declared by the manufacturer, the composition of the sunflower oil was refined, bleached, and deodorized (RBD) oil (free of antioxidants). The amount of fatty acids in the oil was measured, and quality indicators like acidity percentage and peroxide value (PV) were evaluated. The oil characterization analysis used: potassium hydroxide, methanol, sodium hydroxide, ethanol, ethyl ether, and phenolphthalein (Sigma-Aldrich, 99.9% purity). Fonterra provided the whey protein isolate (WPI) (5% moisture, 91% protein, 1% fat, and 3% ash). Sigma-Aldrich was the source of sodium azide. All the solutions and emulsions were made with the highest purity of water. For Food Industries & Cooling, Egypt, Khaled Khoshala Co. imported mature cheddar cheese that was six months old from New Zealand. Mariam Company, Giza, Egypt, provided the one-month-old RAS cheese. From Sakr Group Co. in Egypt, Fonterra butter was purchased. Dairy America distributes pasteurized milk used in the production of spray process grade A nonfat dry milk, which is produced locally. The Egyptian company for dairy products and food additives, EGYdairy, is where the emulsifying salts Egy Phos S2 were obtained.

Drying of Carrot peel and Pomace carrot:

Carrot peel and pomace were dried in compliance with the previously published methods by Basuony *et al.* (2022).

Peroxide value determination:

The peroxide value, a measure of the quantity of peroxides in the oil, is obtained by measuring the amount of iodine released from potassium iodide. According to the AOAC (2019).

Fatty acid composition:

Fatty acid methyl esters were made according with the AOAC 2019 standards.

Preparation of oil enriched with carotenoids:

The edible oil filled with carotenoids was prepared in two steps: A) Sunflower oil is used to extract carotenoids from the dried matrix using a blender running at 10,000 rpm and

room temperature. B) An ultrasonic bath (Ultronique, Q 5.9/40A, Eco-Sonics) operated at 25 kHz and 165 W of power has been used. Ultrasound was used extensively in view of the results (Mirheli and Dinani, 2018). After extraction, the solid material that had been dispersed across the oil was extracted using vacuum filtering and quantitative filter paper (rapid filtration) in a Buchner funnel with a 150 mm diameter. The carrot mass/oil volume (%M/V) was extracted at three different rates: 1%, 3%, and 5%. The agitation and sonication times were 15 and 30 minutes, respectively. The extraction was placed in a 100 mL total volume. For shelf-life testing, the improved oil was kept dry and at room temperature in amber glass bottles.

Determination of carotenoids in sunflower oil (not enriched and enriched):

The total carotenoid content of enriched and unenriched sunflower oil was measured using spectrophotometry. To 25 mL volumetric flasks containing 0.5 g of each sunflower oil (which was not enhanced or supplemented with carotenoids), petroleum ether was added. The total amount of carotenoids in the oil was measured using spectrophotometry at 450 nm (Pacheco *et al*., 2014).

Oxidative stability (Accelerated Rancimat test):

The Rancimat technique (Professional Rancimat 892, Metrohm Corporation, Switzerland) (Zahran and Najafi in 2020).

Quantification of β-carotene by HPLC:

Using an Empower software-controlled Waters HPLC system with a photodiode array detector and a column oven set to 33 °C, the profiles of the carotenoids in an acetone extract were ascertained by HPLC (Pacheco *et al.,* 2014). (PDA).

Nanoemulsion preparation:
(a) Preparation of Biopolymer:

Using Milli-Q ultra-pure water, an 8% whey protein isolate (WPI) solution (w/v) was created. At room temperature, the aqueous phase was constantly stirred for two hours at 1500 rpm. After that, the mixture cooled for a full day to ensure full hydration. Using a 1 M NaOH solution, the solution was raised to pH 7.0. It was then heated to 80 °C for 15 minutes, stirring continuously with a mechanical stirrer, to denature WPI. Finally, it was allowed to equilibrate at room temperature (Khan *et al*., 2019).
(b) Nano-emulsion of carotenoids preparation:

To prepare the primary nano-emulsions, two processes were used. The 10% oil and 90% aqueous phases were initially combined in a high-shear mixer (JRJ300-D-I, Shanghai Specimen and Model Factory, Shanghai, China) set at 7000 rpm for 10 minutes to produce coarse oil-in-water emulsions. Second, ultrasonic treatment utilized a titanium probe (0.636 cm in diameter; Washin Instrument Co. Ltd., Wuxi, Jiangsu, China) for ultrasonic cell disruption. The coarse emulsion was placed in a beaker with two walls surrounding it and a cooling water jacket to reduce temperature rises. 20 kHz ultrasonic treatment with varying treatment durations (10 min), output powers (400W), pulse lengths (4 s), and off-times (2 s). It was used fresh (O/W) emulsion (Li, *et al*., 2015).

Particle size and ζ-potential measurement for nanoemulsion:

The ζ-potential, globule size distribution (expressed as polydispersity index, or PDI), and mean particle size of the nanoemulsion were determined using a Zetasizer (Nano-ZS, Malvern Instruments, UK) in compliance with the previously published methods by Hamed *et al.* (2019). Before measurement, the samples were diluted with a pH 7 phosphate buffer solution (10 mM).

Measurement of carotenoids stability:

The physical stability of nanoemulsions was monitored under different environmental conditions in compliance with the previously published methods by Basuony *et al.,* (2022).

DPPH Radical scavenging activity:

The antioxidant activity of carotenoid extract was measured using stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), according to Darwish *et al*. (2021).

Production of processed cheese analog (PCA):

Processed cheese was produced in compliance with the previously published methods by Basuony, *et al*. (2022). Table (1) shows the combinations.

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

Ingredients	Control	βCEP-oil	NEβCP	
			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	102.2	101.8	101.4
Egy Phos S2	25	25	25	25
βCE-oil	--	3	--	--
NeβC	--	--	6.7	10
Water	308.8	306.2	302.9	300.0
Total	1000	1000	1000	1000

βCE-oil: β-carotene extract in oil, NEβC: nano-emulsion of β-carotene

The chemical analysis of the ingredients used to make the processed cheese spread blends is shown in Table (2).

Table (2): Composition of raw materials used in the manufacture of processed cheese spreads.

Ingredients/ Parameters	Total solids %	Total protein %	Fat%	Ash%
Ras cheese	54.81	21.33	24.77	5.76
Cheddar cheese	65.8	24.37	34.8	5.42
Skim milk powder	96	37.13	1.5	7.89
Butter	84	-----	82	-----
βCE-oil	12	-----	10	-----
NeβC	20	8	10	------

βCE-oil: β-carotene extract in oil, NEβC: nano-emulsion of β-carotene

Physicochemical and Rheological analysis of PCA:

According to AOAC (2019), the total solids, ash, titratable acidity, and total protein content of PCA were measured. The Semi Micro-Kjeldahl technique was used to calculate the total protein and soluble nitrogen content. 6.38 was the conversion factor. The fat content was measured using the Gerber method, according to Ling (1963). The procedure Marshall (1992) outlined was used to determine the salt content. A digital pH metre type Adwa 1030 was used in the laboratory to test the pH values.

Organoleptic properties evaluation:

All processed cheese samples were analyzed organoleptically using a hedonic scale that ranges 1 to 5, which was developed based on the hedonic scales provided by Caul (1957), Brandt *et al*. (1963), and Larmond (1977). A total of fifteen staff members from the dairy department at Al-Azhar University completed the sensory analysis.

Statistical analysis:

SAS, (2001) indicates that the Statistical Package for the Social Sciences (SPSS version 20 (IBM)) was used to perform statistical analyses. Every preparation and measurement occurred three times. The experimental data was subjected to an analysis of variance for a completely random design. To determine the significance level of the mean differences, we used Duncan's multiple range tests.

RESULTS AND DISCUSSION

Fatty acid composition and quality properties of sunflower oil (SO):

Table (3) presents the qualitative parameters of SO that were described. The acquired data showed that SO's acidity percentage was $0.05 \pm 0.001\%$. On the other hand, 0.16 ± 0.01 mEq.O2/kg oil was the peroxide value. These findings showed that the RBD sunflower oil utilized in this investigation met the standard values specified by the edible vegetable oil codex. The amount of all primary oxidation products in edible oils is known as the peroxide value. It is frequently used to assess the quality of oil and determine how well fats and oils store. In the early phases of lipid oxidation, it quantifies the concentration of peroxides and hydroperoxides (Metwally *et al*., 2022 and Harkat *et al*., 2022).

Table 3: Quality properties of sunflower oil (SO).

Parameters	Result
Acidity (%)	0.05 ± 0.001[*]
Peroxide value (mEq.O2/kg oil)	0.16 ± 0.01

[*]Value = mean ±standard deviation.

Table (4) indicates that the predominant fatty acid composition of the SO sample (88.90%) is made up of unsaturated fatty acids, specifically oleic acid (28.43%) and linoleic acid (59.64%). However, linolenic acid content was lower, at 0.27%. The saturated fatty acid was palmitic acid (6.96%).

Table 4: Fatty acid composition of sunflower oil (SO).

Fatty acids	Common name	percentage (%)
(C14:0)	Myristic acid	0.21 ± 0.01[*]
(C16:0)	Palmitic acid	6.96 ± 0.12
(C16:1)	Palmitoleic acid	0.28 ± 0.03
(C18:0)	Stearic acid	3.68 ± 0.72
(C18:1n9c)	Oleic acid	28.43 ± 1.05
(C18:2n6c)	Linoleic acid	59.64 ± 1.17
(C18:3n3c)	Linolenic acid	0.27 ± 0.05
(C20:0)	Arachidic acid	0.25 ± 0.02
(C20:1)	Gadoleic acid	0.28 ± 0.01
Σ of saturated fatty acids (SFA)		11.10 ± 0.85
Σ of unsaturated fatty acids (UFA)		88.90 ± 2.24

However, myristic acid was shown to be present at a low concentration (0.21%). 11.10% of the total fatty acids were made up of saturated fatty acids. The results obtained correspond with what Chowdhury *et al*. (2007) found.

High-performance liquid chromatography (HPLC) of Carrot waste extract:

High-performance liquid chromatography (HPLC) can be used to quantify the carotenoids, which are lipophilic antioxidants present in carrot waste extract and mostly consist of lutein and β-carotene. The retention durations for lutein and β-carotene were 14.43 and 14.85 minutes, respectively, as shown in Figure 1. The amount of β-carotene and lutein in carrot waste extract was 45.75 ±3.15 mg/g and 0.085 ±0.003 mg/g, respectively. Mustafa *et al*. (2012) report that the amount of β-carotene in fresh carrots is approximately 71 parts per million.
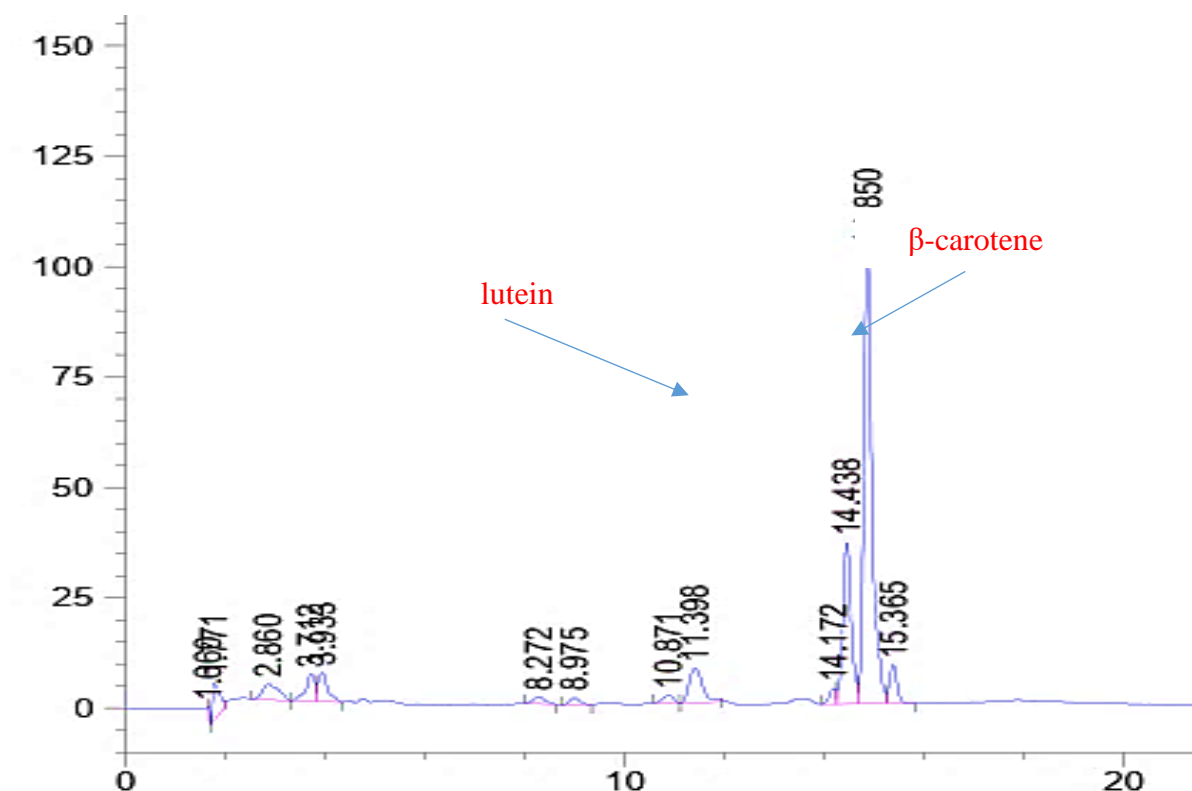

Fig. 1. HPLC chromatogram of carrot waste extract.

Carotenoid content of sunflower oil samples enriched and not enriched:

Table 5 displays the information collected, which showed that the samples enhanced with 5% (carrot to oil, w/w) carrot extract during extraction were substantially greater than the 1% (80.85 µg/g) and 3% (112.34 µg/g) samples. Furthermore, there was a significant difference in the results obtained when compared to the 33.41 µg/g control sample, which was not enriched. In 2020, da Silva *et al*. investigated the effects of temperature, time, and ultrasonic-assisted extraction on the amount of carotenoids in carrots enriched with sunflower oil. They discovered that the highest removal of β-carotene was promoted by a maximum temperature of 55 °C, a time of 60 min, and an oil-to-carrot ratio of 30 ml/g. Our findings differed slightly from the authors' findings in this investigation. Specifically, there is a 3% oil to carrot ratio.

The samples for evaluation showed significant (p≤0.05) variations in oxidative stability when compared to the control sample and other ratios (1%). The induction time of the oil samples enriched with carotenoids in a ratio of 189.47 µg/g was 7.87 hours, and there was no discernible difference between it and the 3% (7.55 hours) sample. Carotenoids derived from

carrots are useful antioxidants that can reverse the stability of oil by adding more dose (Zahran and Najafi, 2020).

According to the data presented in Table 5, the oil samples that were enhanced with carotenoids at a concentration of 189.47 µg/g of oil showed the highest percentage of antioxidant activity (47.89%). In contrast, for the oil sample enriched with 189.47 µg/g oil of carotenoids extracted by 5% ratio, the peroxide values increased from 0.22 mEq.O2/kg for the control sample to 1.87 mEq.O2/kg. The obtained results obviously show that the oxidative stability of enhanced oils is correlated with their carotenoid concentration. The value of photovoltaic (PV) in oils can be naturally increased by high temperatures, light, and oxygen. This could be the reason for the larger value of ESO achieved after extraction at higher temperatures (de Silva *et al.*, 2020).

Table 5: Characterization of SO samples enriched and not enriched.

Parameter	Treatments			
	Control	**1%**	**3%**	**5%**
Carotenoids content (µg/g oil)	--------	80.65 ± 2.75^a	112.34 ± 3.71^b	189.47 ± 3.28^c
Induction time (h)	5.12 ± 0.27^a	6.24 ± 0.11^b	7.55 ± 0.15^c	7.87 ± 0.22^c
Antioxidant activity (%)	--------	33.14 ± 1.05^a	45.11 ± 2.04^b	47.89 ± 1.12^b
Peroxide value (mEq. O_2/kg)	0.22 ± 0.05^a	0.74 ± 0.09^c	0.86 ± 0.03^b	1.87 ± 0.12^c

Means followed by the same small letters (a, b, c, d) in the same columns ± standard deviation.

Carotenoids nanoemulsion characterization:

Particle or droplet size has a major impact on an emulsion's stability. Nanoemulsions find many applications in the food and pharmaceutical industries, but their various usages depend on their particle size and distribution. Particle size may be precisely regulated, which gives an emulsion its distinctive features. To maximize component performance while forming a nanoemulsion, it is essential to look at particle size. Table 6 displays the effects on particle size, zeta potential, and polydispersity index (PDI) of several carotenoids emulsified with WPI 10% ratios that were investigated for the formation of carotenoid nanoemulsion. Immediately following homogenization, a bimodal particle size distribution (peaks at 163.2 - 229 nm) resulted, regardless of the carotenoid concentrations in oil of 80.65, 112.34, and 189.47 µg/g. According to Table 6, the mean droplet sizes of new nanoemulsions with 1, 3, and 5% carotenoids/g WPI were 235.7 ±31.0, 229.5 ± 27.0, 183.5 ± 55.0, and 163.2 ± 66.0, respectively. This suggests that there is a tendency for smaller particle sizes to increase with the concentration of carotenoids in emulsions at a 10% WPI concentration. Trentin *et al.* (2011) and Mohamad *et al.* (2017) suggest that beta-carotene may be the cause of this because it acts as a co-surfactant in emulsified systems, lowering the interfacial tension between the phases and stabilizing the emulsion. Protein chains with a surface-bound may be longer and more compact, but this could prevent flocculation because of steric and electrostatic repulsion (Yi *et al.*, 2014). A common method to quantify sample heterogeneity depending on size is to use the polydispersity index (PI). A sample's size distribution, accumulation, or aggregation during separation or analysis can all lead to polydispersity. The corresponding PDI indexes were 0.267, 0.345, 0.427, and 0.245.

Table 6: Characterization of prepared nanoemulsions from different enriched oil samples.

Treatments	Size (nm)	PDI	Zeta-potential
WPI	235.7 ±31.0	0.427	-45.7 ±1.30*
1 %	229.5 ±27.0	0.345	-47.7 ±5.53
3 %	183.5 ±55.0	0.267	-49.5 ±7.36
5 %	163.2 ±66.0	0.245	-59.0 ±6.72

*Value = mean ± standard deviation, PDI: polydispersity index.

The zeta potential could be used as an efficient means of characterizing the surface charge of the nanoemulsion particles. Greater values, both positive and negative, produce repulsive forces among the particles, potentially enhancing the multiphase system's physical stability. Mueller (1996) suggested preparing an emulsion with a greater zeta potential. Table (6) indicates a significant drop ($p \leq 0.05$) in the average zeta potential value with an increase in the amount of carotenoids in the nanoemulsion. The ζ-potential of the nanoemulsion decreased to -45.7 ±1.3, -47.7 ±5.53, -49.5 ±7.36, and -59.0 ± 6.72, respectively, as the amount of carotenoid was increased. The hydrophobic properties of the oil may be the cause of this, which can reduce zeta potential values (Moro *et al.* 2001). With WPC-80, a similar pattern was noted. When WPC was utilised to stabilise the emulsion, Chalothorn & Warisnoicharoen (2012) discovered that b-carotene nanoemulsions possessed a zeta potential that ranged from -30 to -45 mV.

According to Qian *et al*. (2012), oil droplets coated with whey protein have negative charge at pH 7, resulting in electrostatic repulsion among the droplets. It is anticipated that after adsorption at the O/W interface, partially unfolded structured globular whey proteins may experience macromolecular rearrangement. The partial denaturation of the structure indicates that the adsorbed protein layer polymerizes because of increased protein-protein hydrophobic interactions and cross-linking (by intermolecular disulfide bonding). The bioactivity in the oil phase has been separated from the other emulsion components by the interfacial layer (Mohamad *et al*., 2017).

Transmission electron microscopy:

Figure 2 displays a TEM diffraction of a carotenoid nanoemulsion. The production of spherical particles and uniform dispersion were confirmed by the surface morphology of the nanoemulsion containing carotenoid ratios. Based on their particle sizes and TEM picture, the carotenoid nanoemulsions appeared to be rather uniform over a range of carotenoid ratios. Particle diameter decreased as the carotenoid content increased, in line with the DLS data trend (Soliman and Nasser, 2022).
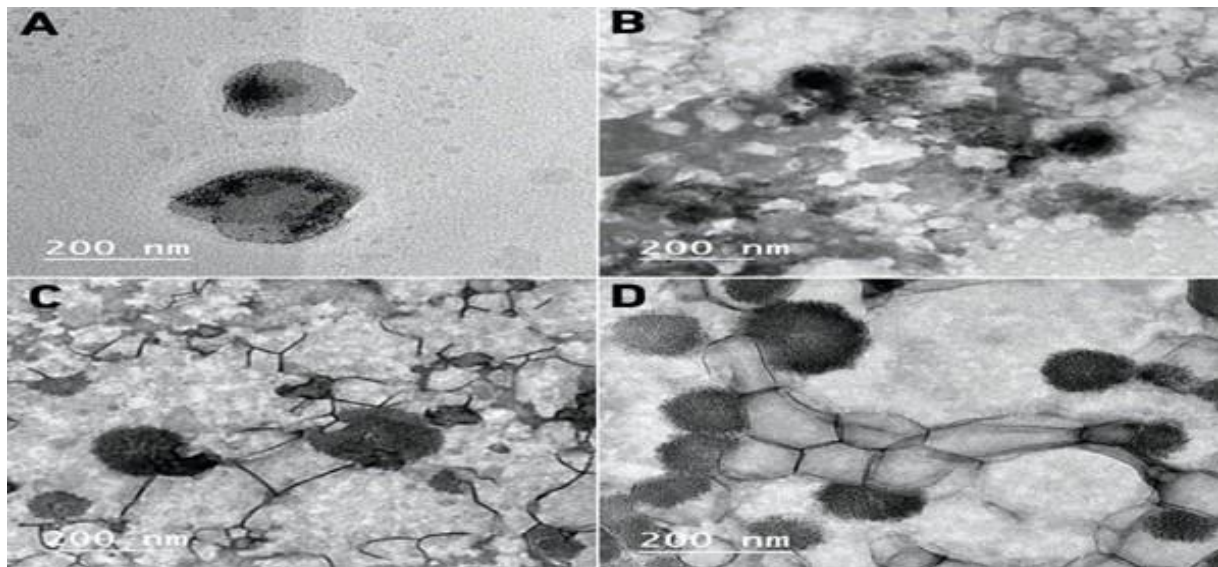
Fig. 2. Transmission electron microscopy of carotenoids nanoemulsion A: nanoemulsion containing 0 % carotenoids; B: nanoemulsion containing 1 % carrot pomace powder; C: nanoemulsion containing 3% carrot pomace powder; D: nanoemulsion containing 5% carrot pomace powder.

Stability of carotenoids:

Nanoemulsions require being stable, both chemically and physically, for commercial uses. This is especially important when the product is being produced, stored, transported, and consumed—all of which includes exposure to environmental conditions. Changes in the characteristics of bioactive substances can affect their appearance, texture, release, and use (McClements & Jafari, 2018). Because the oxidation process of carotenoids is very similar to that of lipids (Hong *et al.*, 2017), exposure to oxygen, heat, and light can cause β-carotene to oxidize and isomerize.
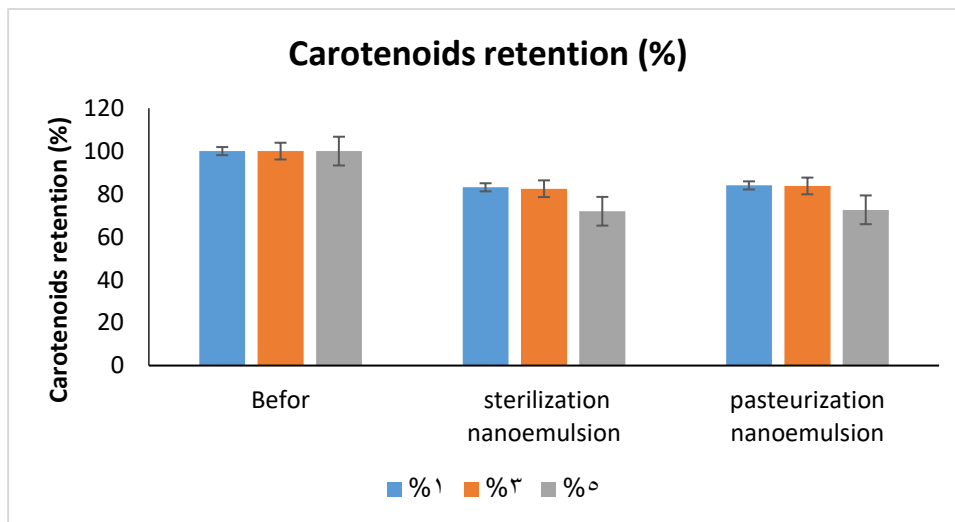
Fig. 3. Stability of carotenoids in oil and nanoemulsions after simulations of pasteurization and sterilization. Means ± standard deviations (n = 3).

Figure 3 displays how, in comparison to the initial concentration, the carotenoid content in the oil and nanoemulsion showed significant degradation after the sterilization or pasteurization simulation. The initial 80.65 and 112.34 ug mL^{-1} carotenoids in the nanoemulsions lost around 17%, and there was no discernible difference (p >.05) between the

sterilization and pasteurization processes. At an initial concentration of 189.47 µg mL1 of carotenoids, the loss averaged about 28%. Additionally, there were no appreciable differences observed in the temperature treatments (p >.05). Nanoemulsions with 1% and 3% concentrations demonstrated significantly less carotenoid degradation (p >.05) during pasteurization and sterilization than nanoemulsions with 5% initial concentrations.

Light Stability:

Figure 4 displays how light exposure affects the stability of carotenoids ratios of 1%, 3%, and 5% carotenoids /g WPI. When compared to sunflower oil and the initial starting concentration, the nanoemulsion showed a considerably (p <0.05) greater retention after five days of storage. Thirty days later, the retention level in 1% carotenoids was 75.12%, followed by 3% with 74.85% and 5% with 71.16% (Fig.4). Nonetheless, sunflower oil exhibited elevated oxidation and isomerization processes, with 47.78 ug of carotenoids remaining after 30 days. Carotenoids have been found to be subjected to oxidation and isomerization during exposure to light by Chen and Huang (1998), Lee and Chen (2002), and Boon *et al*. (2010). The carotenoids have protection from oxidation and isomerization by WPI, an efficient emulsifying agent that decreases the effect of light on the microparticle core. Wang *et al*. (2012) reported that 90% retention levels were observed in microencapsulated lutein using porous starch and gelatin as wall materials after 30 days of exposure to light.
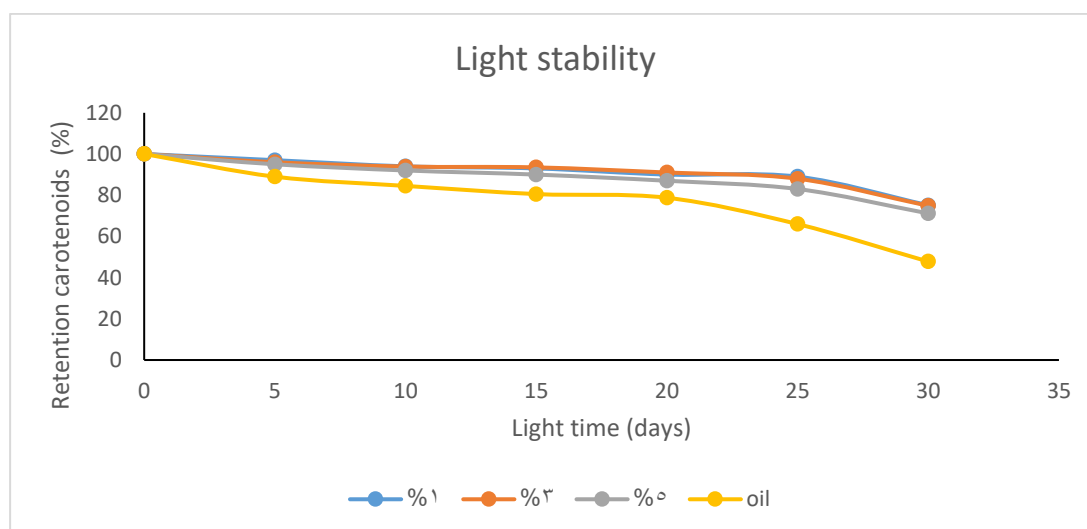

Fig. 4. Effect of illumination on the stability of carotenoids.

Influence of adding β-carotene on the properties of processed cheese:

After processing cheese analogue products with βCE and NEβC, they were evaluated for their chemical and sensory attributes over a 90-day cold storage period (5–7 °C) and contrasted with a control sample (one that didn't include beta-carotene).

Sensory evaluation for processed cheese with or without β-carotene:

The organoleptic qualities of food products provide a crucial means of measuring consumer acceptability of food. A sensory assessment was conducted to appraise the final goods' overall preference, flavour, saltiness, oiling off, surface appearance, firmness, stickiness, spreading qualities, texture smoothness, and breakdown characteristics.

Table (7): Sensory evaluation of processed cheese made by using β-carotene.

Attribute	Control	processed cheese treatments		
		βCE-oil	NEβCP	
			10%	15%
Surface appearance (5)	2.86±0.69*	3.71±0.49	3.71±0.76	3.71±0.49
Firmness of body (5)	2.71±0.49	3.14±0.38	2.57±0.53	1.86±0.38
Spreading quality (5)	2.86±0.38	2.86±0.69	3.29±0.49	3.57±0.53
Stickiness (5)	3.00±0.58	3.29±0.49	3.57±0.98	3.86±0.38
Smoothness of texture (5)	1.86±0.38	2.57±0.53	3.57±0.53	3.14±0.69
Breakdown properties (5)	2.14±0.38	2.43±0.53	3.57±0.53	4.00±0.58
Oiling off (5)	1.14±0.38	1.57±0.53	1.43±0.79	1.71±0.49
Flavor (5)	3.00±0.58	2.00±0.82	3.57±0.79	4.00±0.82
Saltiness (5)	1.14±0.38	1.29±0.49	1.29±0.49	1.14±0.38
Overall preference (5)	4.86±0.38	2.71±0.49	3.86±0.90	4.00±0.58

βCE-oil: β-carotene extract in oil, NEβC: nano-emulsion of β-carotene.

Table (7) contains the obtained results regarding the sensory qualities of cheese samples processed with βCE-oil and NEβC. The products that included βCE-oil and 10% and 15% NEβC addition showed no discernible differences in terms of flavour, oiliness, firmness, or saltiness. The control sample scored 4.86±0.38 out of 5, whereas the NEβCP 10 and 15% cheese samples scored 3.86±0.90 and 4.00±0.58 out of 5, respectively. The βCEP cheese samples scored 2.71±0.49 out of 5. Some panelists did not find it usual to accept the significantly poor mouth feel and flavour values for βCEP. thus, this sample was turned down. The cheese with 10% NEβC addition and 15% NEβC addition, which most closely matched the control sample, were the two best treatments of analogue cheese.

The chemical composition of processed cheese with β-carotene:

The chemical composition findings are displayed in **Table (8)**. Overall proteins, fat, salt, and total solids do not differ significantly between the treatments and the control sample, or between some of the treatments and the β-carotene addition. Thus, adding β-carotene has no effect on the chemical properties of processed cheese. This result is in line with **Biswas's (2016)** research.

Table (8): Chemical composition of processed cheese made with β-carotene.

Component%	Control	processed cheese treatments		
			NEβCP	
		βCE-oil	10%	15%
Fat	22[a]	21[a]	22[a]	21[a]
T. S	45.30[a]	44.35[a]	45.21[a]	44.50[a]
F/ DM	48.62[a]	47.36[a]	48.67[a]	47.19[a]
Salt	1.47[a]	1.20[a]	1.16[a]	1.24[a]
TP	11.6[a]	11.3[a]	11.5[a]	11.2[a]
Ash	4.34[a]	3.97[ab]	4.00[ab]	3.72[b]

* Means with the same letters in a row are not significant at the 5 % level, βCE-oil: β-carotene extract in oil, NEβC: nano-emulsion of β-carotene.

As regards differences in ash levels between the control sample and the treatments, none were apparent, except for the processed cheese sample at the 15% nanoemulsion-β-carotene addition level. The percentage of fat and dry matter ranges from 47 to 50%, and each result reveals the components of the principal combination. The Egyptian Standards (2005) for processed cheese agreed with these findings.

The pH level and acidity of β-carotene cheese:

The pH values of the mixtures, which ranged from 5.7 to 5.85, were affected by the variety of raw materials utilized in them. These findings support those of Olson *et al.* (1958). The decreased pH of the cheese used to make the mixtures—where the qualities of the cheese during the production process affect the processed cheese—could be the reason for the pH drop in all the mixes. The pH range of carrot extract is 5.88–6.40, hence the treatments' pH values increased as compared to the control sample, which had a pH of 5.71. The treatments' pH values were 5.83, 5.87, and 5.82, respectively. Similarly, the titratable acidity (TA) decreased with the addition of β-carotene; it was 1.12 for the control sample and dropped to 1.027, 1.068, and 1.072 for the trades. The results are consistent with Mohamed *et al.* (2016). According to research by Ahmed, (2014) and Mehanna et al., (2017), the pH value decreased with an increase in storage time when all treatments were maintained at 7°C. This may be explained by the cheese becoming more acidic while it was being stored, as Table (9) shows.

Table (9): Changes in PH for processed cheese prepared with β-carotene during storage.

Component%	Control	βCE-oil	processed cheese treatments	
			NeβCP	
			10%	15%
Fresh	5.71[Ac]	5.83[Ab]	5.87[Aa]	5.82[Ab]
30 days	5.67[Bc]	5.72[Bb]	5.72[Bb]	5.78[Ba]
60 days	5.64[Cb]	5.70[Ca]	5.66[Cb]	5.71[Ca]
90 days	5.57[Db]	5.62[Da]	5.57[Db]	5.60[Da]

Means followed by the same capital letters (A, B, C, D) in the same columns; and values followed by the same small letters (a, b, c, d) in the same row are not significantly different (P≤0.05), βCE-oil: β-carotene extract in oil, NEβC: nano-emulsion of β-carotene.

Titratable Acidity TA (%):

Table (10) displays the spreadable processed cheese analogue SPCA values both throughout the product's fresh period and following 30, 60, and 90 days of cold storage. During the cold storage period, TA grew steadily; however, after 90 days, there was a noticeable increase. The cheddar cheese that was added to the blends may have contributed to the samples' higher acidity. However, there was an apparent increase in TA when the samples were still fresh or throughout the cold storage period. The total increase in titratable acidity of all samples during cold storage was caused by modifications in lactose, soluble nitrogen, and emulsifying salt form. These statistics support the findings of Ahmed (2014).

Table (10): Changes in acidity for processed cheese prepared with β-carotene during storage.

Component%	Control	processed cheese treatments		
		βCE-oil	NEβCP	
			10%	15%
Fresh	1.12[Da]	1.027[dD]	1.068[Dc]	1.072[Db]
30 days	1.21[Ca]	1.17[Cb]	1.10[Cb]	1.21[Ca]
60 days	1.30[Ba]	1.27[Bb]	1.19[Bc]	1.27[Bb]
90 days	1.37[Aa]	1.35[Ab]	1.35[Ab]	1.33[Ac]

Means followed by the same capital letters (A, B, C, D) in the same columns; and values followed by the same small letters (a, b, c, d) in the same row are not significantly different (P≤0.05), βCE-oil: β-carotene extract in oil, NEβC: nano-emulsion of β-carotene.

Conclusion

　　　　Through the results of this research, it was found that the process of extraction of beta-carotene from carrot waste using sunflower oil with the help of ultrasound was successful because vegetable oils have a high ability to extract biologically active compounds such as chemical solvents in addition to being non-toxic and therefore can be the product can be used Immediately after extraction, then increasing the ability of beta-carotene to withstand manufacturing conditions that are not suitable for its chemical and physical nature by making a nanoemulsion using whey protein isolate. This led to enhancing the processed cheese with high nutritional, functional, and economic values by adding Nano-emulsion of β-carotene (NEβC) in processed cheese blends.

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