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ORIGINAL ARTICLE

Evaluation of Color Stability of Yogurt Pigmentation with Β-Carotene Nanodispersions

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ABSTRACT
The formation of stearic acid nanoemulsiones containing Tween 80 as nonionic surfactant and β-carotene as active compound using homogenization-emulsification technique was studied in terms of physicochemical properties with the aim to incorporate in a semi-solid dairy product to simulating orange color. The mean droplet diameter observed in nanoemulsions ranged from 180.10 to 343.40 nm.The particle size decreased with increases the homogenization time. Moreover, higher particle sizes were observed when increased the oil phase concentration. The color stability was determined by colorimetry each 7 days for a period of 28 days. Chromaticity coordinates L, a* and b* for the different yogurt samples pigmented with β-carotene nanoemulsions showed a low variation of their values during storage time, with ΔE values ≤ 3.0 indicating a good stability of β-carotene into the matrix.*
Keywords: *β-carotene, color, homogenization-emulsification, nanoemulsion, stearic acid, yogurt.*

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INTRODUCTION

Carotenoids are one of the most important groups of natural pigments [1]. β-carotene is among the carotenoids of orange coloured pigment that has received particular attention owning to its high provitamin A activity and antioxidant capacity, furthermore can be used as an alternative to replace synthetic red and yellow colorants, which have been banned by the Food and Drug Administration (FDA) since the 80s [2]. Research have shown that β-carotene may provide protection against such serious diseases as lung cancer, heart disease, colorectal adenomas and so on [3,4,5]. However, β-carotene is insoluble in water, this makes it difficult to be incorporated in food formulations [6]. Additionally, β-carotene is sensitive to oxygen, heat and light, which further limits its applications in food, nutraceutical and pharmaceutical products [7]. Preparation of functional lipids in the forms of nanoemulsions is expected to greatly improve their solubility and bioavailability because the ultra fine particles reduce the inherent limitations of slow and incomplete dissolution of the functional lipids [8]. In addition, with a particle size in the nanometer ranges, nanodispersions are physically more stable compared to the conventional dispersions which contain particles in micrometer sizes [9]. Nanoemulsions containing lipophilic bioactive substances can be produced using various emulsification processes. Homogenization-emulsification technique is the simplest method to prepare nanodispersions of water insoluble bioactive compounds. Specifically, to form a fine stable emulsion, the lipid is melted at temperature of 5-10ºC above its melting point and the bioactive compound is dissolved or dispersed in the melted lipid. Then a hot aqueous surfactant solution (preheated at the same temperature) is added to the first mix and homogeneously dispersed by a high shear mixing device and a nanoemulsion takes place [10]. These systems have a droplet size ranging from 50 to 1,000 nm and they offer great potential to encapsulate many active agents into a wide range of foodstuffs [11].
In this work, β-carotene nanodispersions were prepared with stearic acid as lipid matrix using homogenization-emulsification technique. The main objective of this study was incorporate these systems

into a dairy product (yogurt) simulating peach color; the chromaticity coordinates were measured using the color space CIEL*a*b*. Based in these measurements, color stability was determined in cold storage (4 ± 2 ºC) for a period of 28 days.

MATERIALS AND METHODS

Materials

β-carotene (30% in sunflower oil) was purchased from Roche. Stearic acid was used as lipid core and was purchased from Sigma-Aldrich, Malaysia,Tween 80 a nonionic surfactant was purchased from Sigma-Aldrich, USA. Milli-Q (Millipore Corporation) water was used for all the experiments throughout the study. A commercial brand yogurt (4.13 g protein, 3.25 g fat and 17.18 g carbohydrate per 125 mL) was purchased from a local market in the Mexico city.

Preparation of β-carotene nanoemulsions and pigmentation of natural yogurt

Oil in water (O/W) nanoemulsions were prepared using stearic acid containing β-carotene as the dispersed phase and Milli-Q water as the continuous phase. Tween 80 was preferred as surfactant owing to its high hydrophilic-lipophilic balance (HLB) value which is favorable for formulating O/W nanoemulsions. Initially, the two phases were prepared separatly, first β-carotene (30% in sunflower oil) was mixed with an equal volume of stearic acid at 80 ºC, and the aqueous phase was prepared with Milli-Q water with a concentration of 2% of Tween 80. The ratio of the oil and aqueous phases was different for each formulation. The mixture of both phases was homogenized using a high speed homogenizer (Ultra-Turrax T25, IKA, Germany) at different times and stirring speeds (Table 1). Subsequently, β-carotene nanoemulsion was added in a ratio 1:3 to natural yogurt of a selected brand (without flavor and color) with the purpose of obtain a soft peach color. Then, was mixed using the same high speed homogenizer at 8,000 rpm for 2 min. The samples were stored at 4 ºC for 28 days.

Physicochemical characterization

pH, electrical conductivity and surface tension of β-carotene nanoemulsions

The pH of the dispersions was measured using a pH meter (510 pH Oakton, Malaysia). Conductivity was measured using the same equipment with a conductive plastic electrodecalibrated with 0.01 M KCl solution being the specific conductivity (κ) of 1413 μS cm^{-1}. The surface tension of the emulsions was determined using a tensiometer Fisher Surface Tensiomat (Model 21, Iowa) with a platinum ring circumference of 5.983 cm, calibrated with distilled water. The samples were analyzed putting 30 mL into the measuring vessel, the ring was immersed 3 to 6 mm below the sample surface, the mechanism system was activated and subsequently reading (dyne cm^{-1}). All experiments were done in triplicate.

Particle size analysis and polydispersity index (PDI) of β-carotene nanoemulsions

The mean particle diameter of nanodispersions was determined using dynamic light scattering (DLS) technique employing a Zetasizer Nano-ZS (Malvern instruments, UK). The scattering intensity was measured a scattering angle of 173º. Samples for the size measurement were prepared by diluting all the generated nanoemulsions with Milli-Q water. All measurements were performed in triplicate. Therefore, droplets sizes were obtained from the correlation function calculated using algorithm of the dispersión technology software (DTS). The PDI was a dimensionless measure of the width of size distribution calculated from the cumulant analysis ranging from 0 to 1.

Color determination in β-carotene nanoemulsions (CIELAB a*, b* and L*)

50 mL of the dispersion was placed in a glass beaker, then the color parameters of the CIELAB scale L*, a* and b* were measured using a colorimeter (Hunter-Lab, MS/B, USA) over a period of 28 days.All measurements were performed in triplicate.

Color analysis in yogurt(ΔE)

Color analysis in time of yogurt pigmentation with the five formulation nanoemulsions was conducted based on the variation of L* (measure of the lightness), a* (measure of redness, +a* = red, -a* = green) and b* (measure of yellowness, +b* = yellow, -b = blue) and the color differences (ΔE) were determined using the following equation:

$$(\Delta E) = \sqrt{(L_i^* - L_o^*)^2 + (a_i^* - a_o^*)^2 + (b_i^* - b_o^*)^2}$$

Where L^*_o, $a^*_o$and b^*_o were the values of the samples at zero time (this time was taken after concluded the process of preparation of the samples) and L^*_i, a^*_i and b^*_i were the values of each sample in time (each 7 days for a period of 28 days). Sasaninejad *et al*

Statistical analysis

Experimental results were expressed as mean values ± standard deviations. Statistical analysis was conducted using SAS (Statistical Analysis System Version9.0), one way variance analysis (ANOVA) was applied to determine the significance of differences between assays. A comparative mean Duncan test ($p < 0.05$) was applied to establish the significance of differences found between each group.

RESULTS AND DISCUSSION

Physicochemical characterization

Physicochemical characterization included pH, conductivity and surface tension measurements of all formulated nanoemulsions were done. The Variance analyses of pH show significant differences ($p< 0.05$) in all emulsion samples at 0 and 28 storage days. Furthermore, the changes in this parameter from 0 to 28 storage days were below 2% in samples, natural and yogurt pigmented (Table 2). This suggests the ratio 1:3 emulsion: yogurt was no relevant and the pH was maintained like control. The conductivity in nanoemulsions was ranged between 32.3 to 36.0 µS/cm. This parameter is affected for oil: water ratio mainly [12]. The storage time had an important effect in conductivity increase values, is probably due to oil drops coalesce over time allowed electricity conduction. A slight decrease in surface tension was observed in all nanoemusions samples (Table 2) during storage time, surfactant concentration play an important role innanoemulsions formation because help to the process decreasing the free energy required for the formation on nanoemulsion by lowering interfacial tension at oil/water interface [13]. In this work the surfactant concentration was kept constant (2%).

Particle size analysis

Impact of processing conditions on droplet size was studied. Table 1 shows the droplet size and polydispersity index (PDI) of different nanoemulsiones systems developed for the entrapment of β-carotene. With an increase in homogenization time, decrease in droplet size was observed. Lowest droplet size of 180.10 nm was obtained for F1 formulation.Ghosh*et. al*[14,15] reported the similar behavior in cinnamon and sesame oil reaching a minimum droplet size by increasing the emulsification time. The effect of oil phase concentration also was studied and it was found that mean droplet diameter of the emulsion increases as the stearic acid concentration increases. On the other hand, variations in the stirring speed have no outstanding effect in the particle size and PDI.

The PDI was a dimensión less measure of the width of size distribution calculated from the cumulant analysis ranging from 0 to 1. A small value of PDI indicates a monodispersed population, while a large PDI indicates a broaderdistribution of dropletsize [16].PDI valueswere obtained in the range of 0.448 to 0.642 (F4 to F3 respectively). These results suggest a polydisperse system.

Color stability of β-carotene nanoemulsionsand yogurt during storage

Chromaticity coordinates value L*, a* and b* and color differences (ΔE)obtained during the storage of 28 days for the β-carotene nanoemulsions and yogurt pigmented are showed in Figure 1 and 2, respectively. In the CIELAB color space, the lightness coefficient L* ranges from 0 = black to 100 = white [17]. The highest L* values (71.2)at 28 storage days were obtained with the control (natural yogurt) while for the samples of yogurt pigmented the F5 formulation showed a L* value of 68.13. As can be seen in Figure 2 the values of L* were stable from 0 to 28 days. The parameter L* for nanoemulsions were 30.2 to 36.3 at the end of storage period,showing a good stability. The difference between L* values for emulsions and yogurt is due to the lightness provided by the yogurt.

As the coordinate a* the nanoemulsions range was 1.23 (F5) to 12.33 (F1), all of these values of chromaticity coordinate a* with positive values were located in the red zoneindicating that the pigment is well embedded in the lipid matrix used as support for the generation of nanoemulsions (Figure 3). However the yogurt pigmented formulations F4, F5 and control presented negative a* values (Figure 4), which represents a great effect induced by the yogurt composition causing lowest values.

In the case of chromaticity coordinate b* the mean values were 4.77 (F5) – 10.10 (F1) and 5.17 (F5) – 9.73 (F2) for nanoemulsions and yogurt pigmented, respectively and they are all located on the positive side of the axis, which represents the yellow zone.

ΔE value variation was higherin nanoemulsions (Figure 1) respect yogurt samples (Figure 2). It is well known that ΔE values less than 3.0 cannot be easily detected by the human eye [18]. This parameter can be used as indicative of the color stability of some pigments entrapped in lipid matrices as well as the application of these in a model food.

Table 1. Formulation and mean droplet diameter (expressed through *z*-diameter and PDI) of β-carotene nanoemulsions tested.

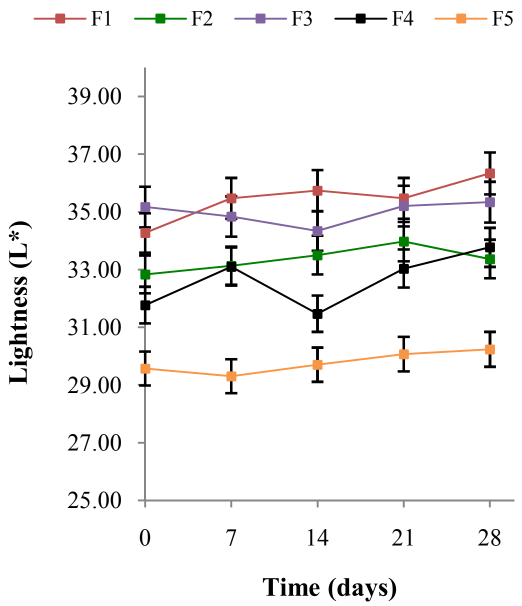
Sample	Oil phase (mL)	Aqueous phase (mL)	Homogenization time (min)	Stirring speed (rpm)	*z*-Diameter (nm)	PDI
F1	0.275	49.725	6.8	10,000	180.10 ± 11.29^{d}	0.526± 0.08^{ab}
F2	0.500	49.500	6.0	5,000	313.27 ± 9.30^{ab}	0.451 ± 0.05^{b}
F3	0.275	49.725	4.0	10,000	224.83 ± 19.06^{c}	0.642 ± 0.14^{a}
F4	0.500	49.500	2.0	15,000	343.40 ± 30.12^{a}	0.448 ± 0.05^{b}
F5	0.275	49.725	1.17	10,000	300.33 ± 13.13^{b}	0.617 ± 0.08^{a}

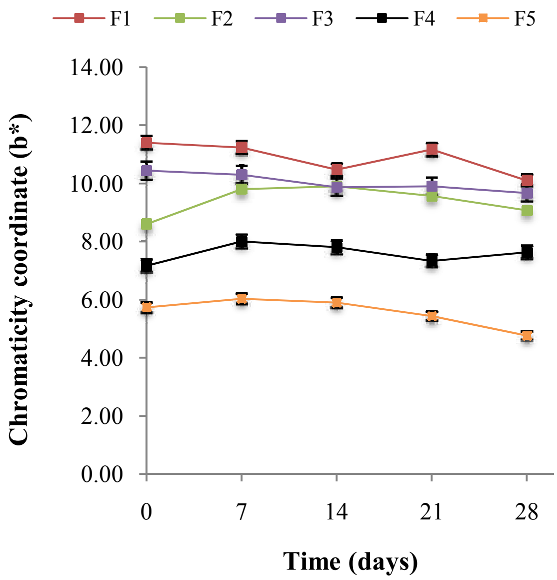
Data of *z*-Diameter and PDI represents the means ± deviation standard for each sample. Different superscript letters in the same column indicate statistical significance ($p< 0.05$) according to the Duncan least significant different test.

Table 2. Physicochemical characterization of β-carotene nanoemulsions and yogurt pigmented

Sample	**pH yogurt pigmented**		**pH emulsion**		**Conductivity (μS/cm)emulsion**		**Surface tension (Dinas/cm)emulsion**	
	0 days	28 days	0 days	28 days	0 days	28 days	0 days	28 days
Natural	4.19 ± 0.01^{ed}	4.07 ± 0.02^{d}	---	---	---	---	---	---
F1	4.25 ± 0.01^{a}	4.30 ± 0.01^{a}	4.56 ± 0.01^{b}	4.79 ± 0.01^{e}	34.13 ± 0.15^{b}	54.47 ± 0.40^{a}	43.17 ± 0.15^{a}	39.03 ± 0.06^{c}
F2	4.22 ± 0.01^{bc}	4.18 ± 0.02^{c}	4.46 ± 0.01^{c}	5.27 ± 0.01^{b}	36.00 ± 0.53^{a}	51.60 ± 0.17^{b}	39.07 ± 0.12^{d}	39.97 ± 0.25^{a}
F3	4.21 ± 0.01^{cd}	4.06 ± 0.01^{d}	4.80 ± 0.01^{a}	5.00 ± 0.02^{c}	32.30 ± 0.17^{c}	46.33 ± 0.38^{c}	40.87 ± 0.15^{b}	39.60 ± 0.10^{b}
F4	4.21 ± 0.01^{b}	4.27 ± 0.02^{b}	4.58 ± 0.02^{b}	4.86 ± 0.03^{d}	32.37 ± 0.15^{c}	40.77 ± 0.25^{d}	40.63 ± 0.67^{c}	38.87 ± 0.15^{c}
F5	4.19 ± 0.01^{e}	4.03 ± 0.01^{e}	4.44 ± 0.02^{c}	5.42 ± 0.01^{a}	30.30 ± 0.20^{d}	35.50 ± 0.20^{e}	40.27 ± 0.21^{c}	39.33 ± 0.15^{b}

Data represents the means ± deviation standard for each sample. Different superscript letters in the same column indicate statistical significance ($p< 0.05$) accordingtothe Duncan least significant different test.





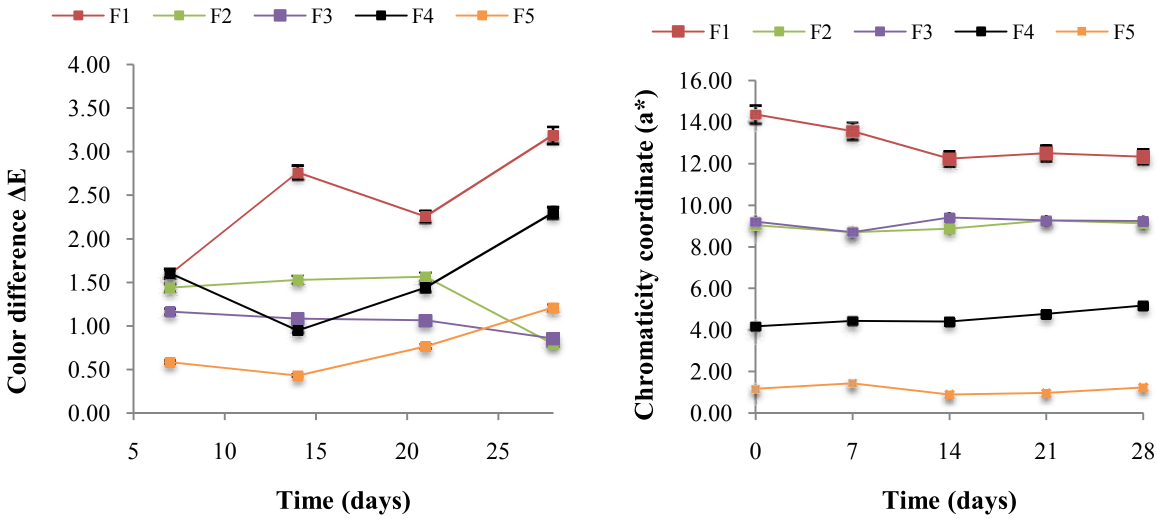


Figure 1. Changes in instrumental color values of five formulations ofβ-carotene nanoemulsions as a function of storage time (n = 3).

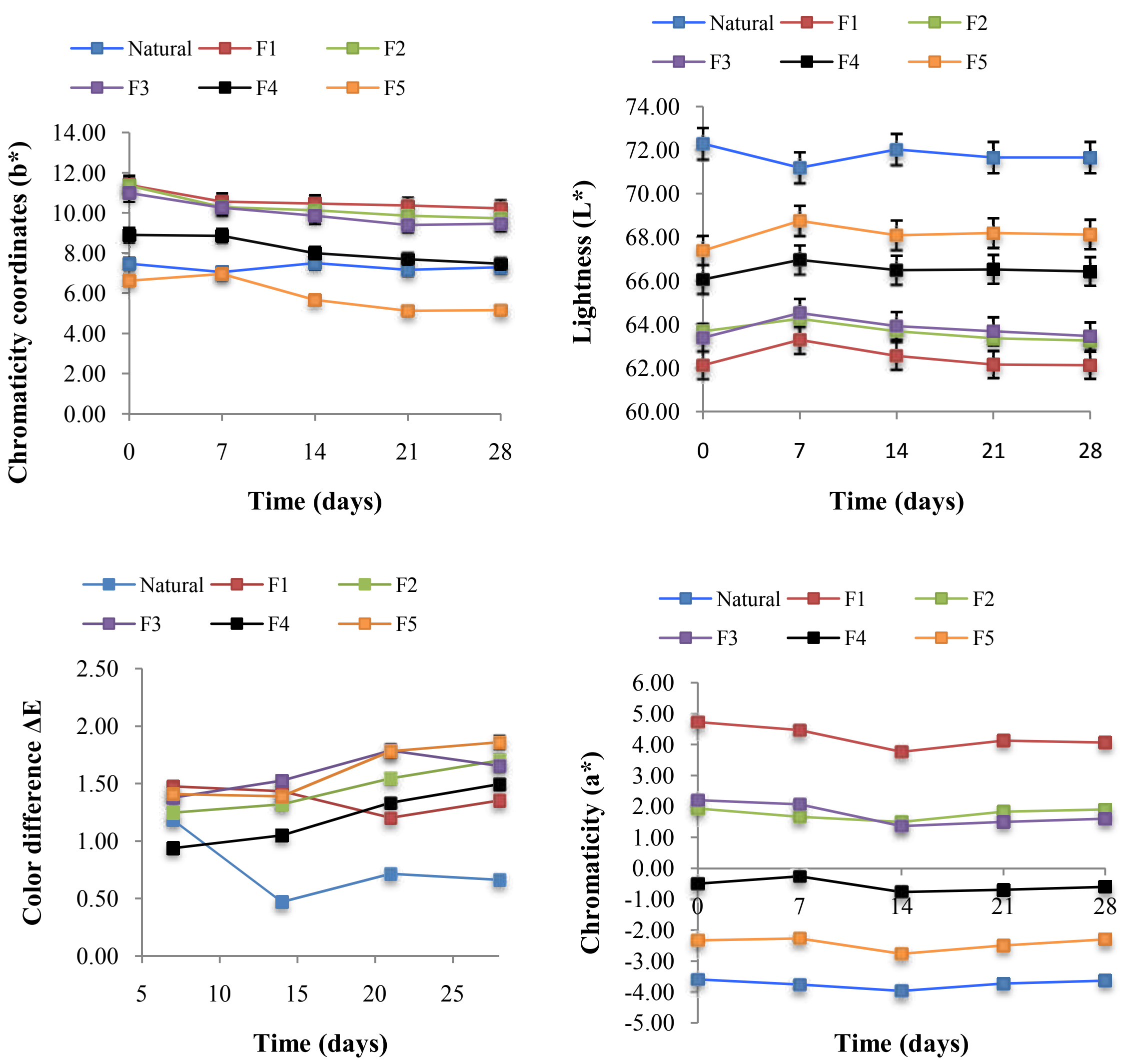


Figure 2. Changes in instrumental color values of yogurt pigmented with β-carotene nanoemulsions to simulate peach color as a function of storage time (n = 3).

0 Days

7 Days

14

21

28

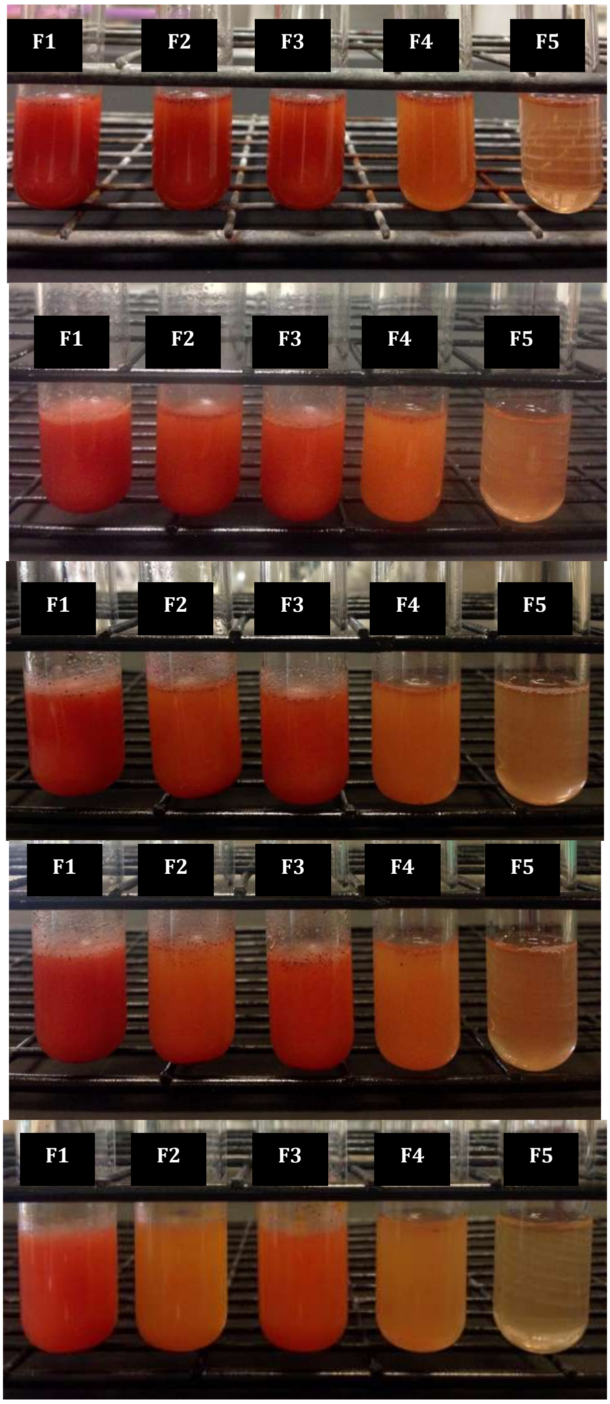
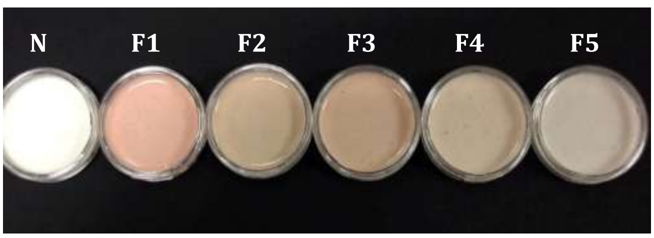


Figure 3.Changes in visual appearance color of β-carotene nanoemulsions during 28 days of storage.

0 Days



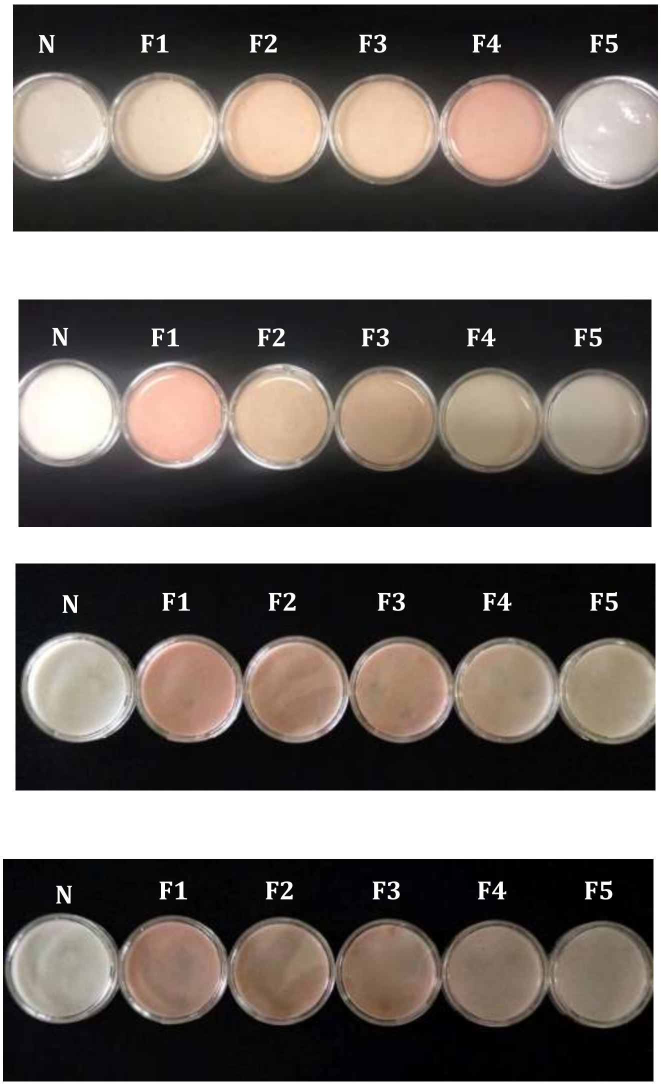


Figure 4. Changes in visual appearance color of yogurt pigmented with β-carotene nanoemulsions during 28 days of storage.

CONCLUSIONS

This study confirmed that homogenization-emulsification is a relatively simple and effective technique for obtaining β-carotene nanoemulsions in the nanometer range (180.10 – 343.40 nm) and it is possible to use these systems to simulate the peach color in a natural yogurt. The particle size nanoemulsions decreased with increases the homogenization time. Moreover, higher particle sizes were observed when increased the oil phase concentration. The results of colorimetric analysis allowed concluding that this carotenoid pigment is well-packed in the lipid matrix of the final product. In general, the color parameters remained very stable over 28 days of storage. The results of this study contribute to promote the use of β-carotene nanoemulsions by way of natural pigmentation in semi-solid dairy products.

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