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BASE FORMULATION PROSPECTION OF A COLLOIDAL BEVERAGE MIMIC SYSTEM ADDED WITH LUTEIN

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Abstract

Lutein daily intake is related to the maintenance of visual and cognitive health. The incorporation of this lipophilic compound into an aqueous matrice requires the use of an emulsion as a carrier system. Lutein was extracted from marigold petals by supercritical fluid extraction, using sunflower seed as co-solvent at three different temperatures. Extraction efficiency was evaluated through the total carotenoid content. A colloidal beverage mimic system was prepared with an oil-in-water emulsion formulated with the carotenoid extract and its physical and chemical stability were evaluated during a 15-day period over accelerated storage conditions.

Key words: Carotenoids; supercritical extraction; emulsion.

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Introduction

Projections of population growth show a constant change in the age structure of the population, with a regular increase in the amount of citizens above 65 years old [5]. In developed countries, the main cause of irreversible visual acuity loss in the elderly population is the Age-related Macular Degeneration (AMD) – an anomaly in the retinal pigment epithelium that leads to a progressive degeneration of the photoreceptor nerve cells (macula) responsible for a high resolution vision [2].

The carotenoids of the retina, lutein and zeaxanthin, are selectively concentrated in the macula [1, 4]. The Lutein is a potent antioxidant; according to Dagnelie et al. (2000), lutein protects the photoreceptors by filtering the blue light – which has the highest energy amongst the visible light spectrum – known to induce photooxidative stress through the formation of reactive oxygen and nitrogen species [3].

Studies shows that concentrations of lutein in the blood and tissues are often low and, since humans are not able to synthesize carotenoids, lutein is exclusively obtained through the diet – its source of greater commercial importance are the flowers of the *Tagetes*genus (marigold*)* [10, 12, 13]. Several authors have found a relationship between lutein supplementation and a risk reduction of AMD and considering its low bioavailability, some authors have proposed its dietary supplementation through the additionto dairy beverages, which usually are the main products for addition of functional compounds [3, 6, 7, 11].

However, a technical difficult is faced to incorporate carotenoids,lipophilic compounds, in water based matrices, so, it is necessary to use carrier systems, such as emulsions, to facilitate the addition of these compounds [8, 9]. Carotenoids are conventionally extracted by organic solvents, but the residues originated from this type of extraction may cause environmental and health problems. In face of this, there has been an interest in alternative technologies such as supercritical fluid extraction.In this work, we presented the base formulation of a colloidal beverage mimic system and the quantification of the total carotenoids of the beverage and of the supercritical extracts added in its preparation, as well as a physical and chemical stability monitoring of the beverage during a 15-day period over accelerated storage (45ºC).

Methods

Obtention of the carotenoid extract rich in lutein

Carotenoid was obtained from marigold petals. The petals were pulled off from the flowers and lyoplhilized – the process pressure was approximately 35 µHg, with a runtime of 72 to 96 hours depending on the amount of petals. Afterwards, the petals were milled in a food processing machine and stored on a freezer at –20ºC.

The carotenoid extract rich in lutein was obtained through extraction with supercritical fluid, using CO_2 and sunflower seed as solvent and co-solvent, respectively. Three temperature conditions were evaluated (30°C, 40°C and 60°C) at a constant process pressure of 250 bar. The extracts were characterized by their carotenoid content, spectrophotometrically quantified. The extracts obtained at 30°C and 40°C were used to prepare the emulsion because of their higher lutein content.

Emulsion and colloidal beverage mimic system preparation

The oil-in-water emulsion was prepared by homogenization in Ultraturrax T25 follwerd by a microfluidization (3 cycles, 10,000 psi), with a 5% oil phase (0.4% of carotenoid extract and 4.6% of sunflower oil) and a 95% aqueous base (1.5% of Tween 20 and ultrapure water). The colloidal beverage mimic system was prepared with an isotonic beverage base containing: citric acid, sodium benzoate, sodium citrate, potassium chloride, sodium chloride, potassium phosphate monobasic, fructose, glucose, whey protein isolate and sucrose, besides 10% oil-in-water emulsion. The mixture was prepared under magnetic stirring, followed by homogenization in Ultraturrax T25.

Chemical and Physical Stability Monitoring

Total Carotenoid Content

For the beverage, quantification of carotenoids was carried out on the days 0, 7 and 15. Briefly, methanol was added to the sample, followed by freezing, centrifugation and the upper layer was discarded for the removal of non-carotenoid lipid components that hinder the subsequent extraction phases. After that, ethyl ether, hexane and 10% NaCl were added, followed by centrifugation. The procedure was repeated until exhaustive extraction and the carotenoid content was determined spectrophotometrically.

Colour

The colour was evaluated using the CIELAB system (colorimeter ColorQuestII, HunterLab) – Light source D_{65} with an observation angle of 10º. Results were reported in terms of the Chroma (C*), that represents the colour intensity – $C^* = \sqrt{(a^*)^2 + (b^*)^2}$,a* (red-green) and b* (yellow-blue) parameters.

Particle Size and Zeta Potential

The particle size was measured through laser light scattering on a Mastersizer 2000(Malvern) and the zeta potencial was measured through Electrophoretic mobility in a Zetasizer Nano-Z, Malvern.

Gravity Separation

The sedimentation was used to determinate the gravitacional separation. The height of the two phases was measured and photographed over the 15 days.

Results and Discussion

Carotenoid extract rich in lutein

The extracts obtained at 30° C and 40°C showed higher total carotenoid contents when compared to the extract obtained at 60°C (Chart 1), which is related to the carotenoids thermodegradability,resulting in a decrease in the content of carotenoids with an increasein temperature.

Chart 1. Total carotenoid content in the extracts rich in lutein (mg lutein/g extract).

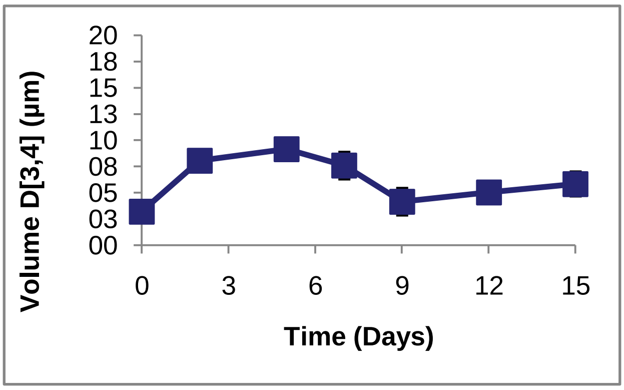
Extract	Carotenoidcontent
30°C	0.57
40°C	0.52
60°C	0.42

Colloidal beverage mimic system

Physical Stability

Particle Size and Zeta Potential

The particle size was express in therms of the particle volume moment mean (D[4,3]). In the beginning, some particle agglomeration took place, until approximately day 7 and afterwards, there was a slight decrease in the particle volume (Graph 2). However, when the day 0 and the day 15 are compared, there is no significant change in the particle volume (α< 0,05), indicating a good physical stability concerning the particle agglomeration.



Graph 1 – D[4,3] values over the storage period.

The zeta potential can be used to predict and control the stability of colloidal emulsions or suspensions. Larger values (in module) indicate greater stability, since the charged particles present mutual repulsion and this repulsion force overcomes the natural tendency to aggregate. On the characterization of emulsion the result obtained for the zeta potential was -36 mV on day 0 and -36 mV on day 15, so the emulsion presented a range of zeta potential considered "moderate stability" (within ± 30 and ± 40 mV).

For the beverage positive zeta potential values were observed, which are characteristic of isolated whey protein in an acid medium (beverage pH between 3.5 and 3.7). The zeta potencial values remained between ±10 and ± 30 mV, indicating a beginning of instability.

Gravity Separation

Gravitational separation occurs due to the action of gravity on the droplets as a result of the difference between the density of liquid droplets and solid phase. In this case, as the droplets have greater density than the continuous phase, they present a propensity to sedimentation.

After the evaluated period, the overall *Sedimentation Index* was approximately 5% (Image 1). In addition to phase separation, the formation of aggregates in suspension (flocculation) in the lower phase, caused by isolated whey protein (IPS) added in formulation of the beverage,was observed from day 5.

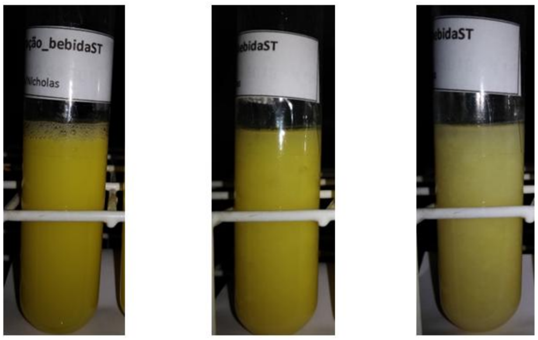
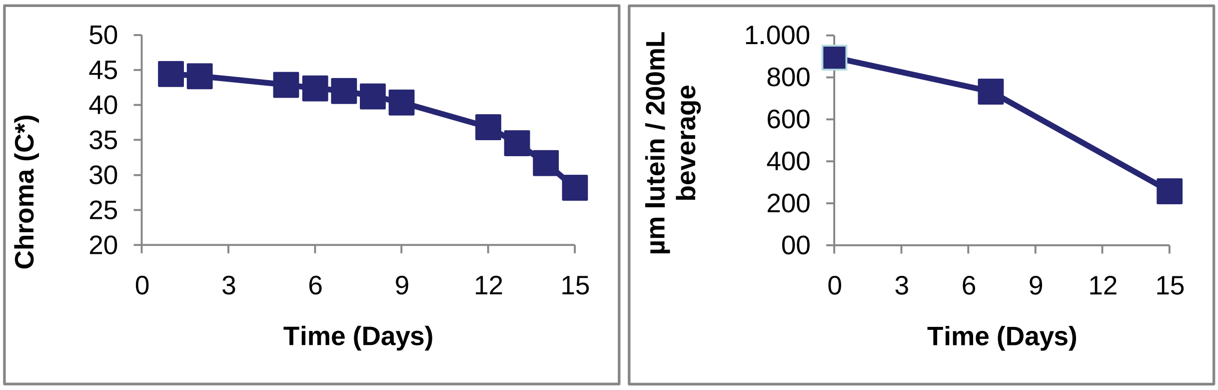


Image 1 – Gratity Separaton analysis result on the days 0, 7 and 15, respectively.

Chemical Stability

Total Carotenoid Content and Colour

The colour and total carotenoid content analysis showed a constant degradation of the carotenoids over the 15-day period (Graph 1).



Graph 2 – Values of Chroma (left) and total carotenoid content expressed in lutein (right) over the storage period.

The total content of carotenoids suffered a reduction of 71% when compared to the full content on the day 0. Since the only source of pigment in the beverage comes from the lutein added, the degradation in the carotenoid content is closely related to the decrease in the colour intensity (Chroma).

Conclusions

The supercritical fluid extraction carried out at lower temperatures was more efficient due to the thermal degradation of the lutein.

The accelerated storage conditions caused a continuous degradation of the colloidal beverage mimic system, what can be related to the decreases in the Chroma parameter and the total carotenoid content. The beverage presented a satisfactory stability concerning the sedimentation and phase flocculation and further studies will be carried out to evaluate the effects of thermal treatments over physical and chemical stability.

References

[1] Bandello F. AMD Book [e-book]. 1st ed. Theá Portugal, SA; 2010.
[2] Chen Y, Bedell M, Zhang K. Age-related macular degeneration: genetic and environmental factors of disease. Mol Aspects Med. 2010;10(5):271-81.
[3] Dagnelie, G.; Zorge, I.; Mcdonald, T.M. Lutein improves visual function in some patients whith retinal degeneration: a pilot study via the internet. Optometry, v. 71, p. 147-164, 2000.
[4] Holz F, Pauleikhoff D, Spaide R, Bird A. Age-related Macular Degeneration [e-book]. 2nd ed. Springer; 2013.
[5] IBGE (2013). Projeções da População: Brasil e Unidades da Federação. Série Relatórios Metodológicos, volume 40.
[6] Koh, H.H. et al. Plasma and macular responses to lutein supplement in subjects with and without age-related maculopathy: a pilot study. Exp. Eye Res., v. 79, p.21-27, 2004.
[7] Kruger, C.L. et al. An innovative approach to the determination of safety for a dietary ingredient derived from a new source: case study using a crystalline lutein product. Food Chem. Toxicol., v. 40, p. 1535-1549, 2002.
[8] McClements, D. J. (2005). Food emulsions: Principles, practice, and techniques (2nd ed.). Boca Raton: CRC Press Inc.
[9] Salvia-Trujillo, L., &McClements, D. J. (2016).Influence of Nanoemulsion Addition on the Stability of Conventional Emulsions.*Food Biophysics*, *11*(1), 1–9. https://doi.org/10.1007/s11483-015-9401-8
[10] Schalch, W. A Importância dos carotenóides. Disponível em: <http://nutricaoempauta.locaweb.com.br/lista_artigo.php?cod=345>. Acessoem: 16 abr. 2017.
[11] Seddon, J.M., Ajani, U.A., Sperduto, R.D. (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. JAMA, 272, 1413 – 1320.
[12] Southon, S.; Faulks, R. Carotenoids in food: bioavailability and functional benefits. In. Phytochemical functional foods. Chicago: Woodhead CRC Press LLC, 2003. cap. 7.
[13] Wenzel, A.J., Sheehan, J.P., Burke, J.D., Lefsrud, M.G., Curran-Celentano, J. (2007) Dietary intake and serum concentrations of lutein and zeaxanthin, but not macular pigment optical density, are related in spouses. Nutr. Res., 27, 462–469.