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Effects of Sodium Alginate Bead Encapsulation on the Storage Stability of Durum Wheat (*Triticum durum* Desf.) Bran Oil Extracted by Supercritical CO_2

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ABSTRACT: The aim of this study was to investigate the influence of encapsulation on the storage stability of oil extracted by supercritical carbon dioxide from a micronized durum wheat bran fraction. Wheat bran oil was encapsulated in 2% (w/v) sodium alginate beads. Encapsulated and unencapsulated oil samples were stored at 4 or 25 °C, in daylight or darkness, over 90 days, and, at defined time points, subjected to stability evaluation based on fatty acid hydroperoxide production and tocopherol (α, β, and γ forms), tocotrienol (α, β, and γ forms) and carotenoid (lutein, zeaxanthin, and β-carotene) degradation. The encapsulation of the oil into alginate beads significantly increased stability, optimally when stored at 4 °C, maintaining high levels of isoprenoids and low content of fatty acid hydroperoxides over 30 days of storage.

KEYWORDS: *carotenoids, oil encapsulation, hydroperoxides, supercritical fluids, tocopherols, tocotrienols, Triticum durum*

INTRODUCTION

Wheat milling industry byproducts are a rich source of valuable natural nutrients for human consumption. Recently, durum wheat (*Triticum durum* Desf.) bran has been used to extract high quality oil by supercritical carbon dioxide (SC-CO_2).[1] SC-CO_2 is an alternative technique to conventional organic solvent extraction; it has the advantage of being nontoxic, nonflammable, cheap, and recyclable. SC-CO_2 is gaseous at ambient temperature and pressure, providing a solvent-free high quality product. SC-CO_2 has traditionally been used to extract bioactive compounds from many plant materials.[2−5]

Wheat bran oil is rich in polyunsaturated fatty acids, vitamin E, carotenoids, and quinones.[1,6−9] Vitamin E and carotenoids have gained relevance and attracted consumers, food producers, and cosmetics manufacturers due to their antioxidant properties and nutritional benefits for humans. Vitamin E comprises two groups, tocopherols and tocotrienols, each made up of four members differing in the number and position of methylation on the aromatic ring, namely, the α, β, γ, and δ forms. There is a large body of evidence that vitamin E could play a role in preventing chronic, oxidant-related diseases, such as cardiovascular diseases, atherosclerosis, and cancer.[10] Likewise, carotenoids are thought to play an important antioxidant role in protecting the cell against lipid peroxidation and photooxidative damage.[11,12] Many carotenoids, including β-carotene and β-cryptoxanthin, have pro-vitamin A activity, while others, such as the xanthophylls lutein and zeaxanthin, protect against age-related macular degeneration by quenching the singlet oxygen and blue light at the retina layer level.[13] As Vitamin E and carotenoids are very unstable due to their highly conjugated structure, these molecules can be easily degraded when exposed to oxygen, heat, or light during processing or storage, causing loss of nutritive and biological value.[14] Besides, oils with high contents of unsaturated fatty acids, especially polyunsaturated ones, are much more susceptible to oxidation. Therefore, the potential use of durum wheat oil as an ingredient in the preparation of innovative products for human health and wellness is conditional upon improving oil stability over time.

Microencapsulation is one of the most frequently employed techniques used to overcome the stability problems of oils and their components. It has been applied by the food and cosmetics industries to control the delivery of molecules and to protect them from oxidation. Various methods have been used, including liposome entrapment, spray drying, extrusion, or inclusion complexation.[15] For these purposes, different food-grade polymers, i.e., β-cyclodextrins, chitosan, or alginate, have been applied.[16−18] Particularly, alginate gels have been used to improve the qualitative properties of numerous functional edible oils such as fish, primrose, and olive oils.[19,20] With this encapsulation technique, based on an ionic cross-linking method, dropping a sodium alginate solution into a calcium chloride solution produces an effective controlled release carrier in the form of beads or microcapsules.[18]

In previous studies, we reported the biochemical composition of the oil extracted by SC-CO_2 from durum wheat bran, in terms of its isoprenoid and lipid contents.[1] In this study, we investigated oil stability over 90 days of storage and the effect of encapsulation into sodium alginate beads on fatty acid hydroperoxide (HP) production and degradation of lipid soluble antioxidants (vitamin E forms and carotenoids).

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MATERIALS AND METHODS

Wheat Debranning. The micronized durum wheat bran fraction used in this work was prepared at the Tandoi S.p.a. milling industry (Corato, Ba, Italy) as described by Rizzello et al.[21] Durum wheat caryopses were debranned by a combination of friction and abrasion using the PeriTec process to obtain three different fractions named 1, 2, and 3. Fraction 1, containing the most external teguments, was eliminated, while fractions 2 and 3 were mixed together, micronized, and fractionated through a Separ Micro System apparatus (Flero, BS, Italy) to obtain a coarse and a fine fraction with granulometry comprised between 180 and 500 μm. Approximately 500 g of the coarse fraction, thereafter simply called wheat bran, was oven dehydrated at 60 °C, for 3 days (residual moisture ~3%), and this fraction was used for SC-CO_2 extraction.

SC-CO_2 Extraction. Wheat bran oil extraction was carried out using a laboratory scale apparatus (Spe-ed SFE system, Applied Separations, Allentown, PA, USA) fitted with a 25 mL stainless-steel extraction vessel (ø = 1 cm^2; h = 25 cm). In each extraction, 25 g of wheat bran was packed into the vessel and extracted statically (no fluid flow) for 15 min and subsequently dynamically, for 1 h, by flowing CO_2 at a rate of ~0.5 kg/h. The other operative parameters were pressure = 300 bar, and temperature = 60 °C.

Extraction of Vitamin E, Carotenoids, and HPs. Vitamin E, carotenoids, and HPs were extracted by mild saponification as described by Rosa et al.[22] Briefly, SC-CO_2 extracted oil (0.1 g) was dissolved in 5 mL of absolute ethanol with subsequent addition of 100 μL of a 2.5% (w/v) Desferal (deferoxamine mesylate salt, Sigma-Aldrich, Milan, Italy) aqueous solution, and 1 mL of a 25% (w/v) ascorbic acid aqueous solution, to which 500 μL of 10 M KOH was added. The mixture was left in the dark at room temperature for 16 h with magnetic stirring. After the addition of 10 mL of *n*-hexane and 7 mL of distilled water, the mixture was centrifuged (900*g*; 1 h). The upper phase, containing the isoprenoids, was collected and dried under nitrogen flux. The dried material was redissolved in 1 mL of ethyl acetate, filtered through a 0.45 μm syringe filter (Millipore Corporation, Billerica, MA, USA), and assayed by HPLC as described below.

The lower phase had a further 10 mL of *n*-hexane added, was acidified to pH 3.0 with 37% HCl, and centrifuged (900*g*; 1 h). The newly formed upper phase, containing the HPs, was collected, dried under nitrogen flux, and redissolved in 1 mL of acetonitrile plus 0.14% acetic acid (v/v). The solution was filtered through a Millipore filter (0.45 μm) and assayed by HPLC as described below.

HPLC Analysis of Vitamin E and Carotenoids. HPLC analyses were carried out using an Agilent 1100 Series HPLC system as described by Fraser et al.[23] but slightly modified. Isoprenoids were separated using a reverse-phase C_{30} column (5 μm, 250 × 4.6 mm) (YMC Inc., Wilmington, NC, USA) with mobile phases consisting of methanol (A), 0.2% ammonium acetate aqueous solution/methanol (20/80 v/v) (B), and *tert*-methyl butyl ether (C). The isocratic elution was as follows: 0 min, 95% A and 5% B; 0 to 12 min, 80% A, 5% B, and 15% C; 12 to 42 min, 30% A, 5% B, and 65% C; 42 to 60 min, 30% A, 5% B, and 65% C; 60 to 62 min, 95% A, and 5% B. The column was re-equilibrated for 10 min between runs. The flow rate was 1.0 mL/min, and the column temperature was maintained at 25 °C. The injection volume was 10 μL. Absorbance was registered by diode array at wavelengths of 475 nm for carotenoids and 290 nm for vitamin E. Isoprenoids were identified by comparing their retention times and UV−vis spectra to authentic standards. Tocopherol, tocotrienol, and carotenoid standards were purchased from Sigma-Aldrich (Milan, Italy), Cayman Chemicals (Ann Arbor, MI, USA), and Extrasynthese (Genay CEDEX, France), respectively.

HPLC Analysis of HPs. HPs were resolved as described by Rosa et al.[22] with a minor modification, using a Phenomenex Luna C18 column (5 μm, 250 × 4.6 mm) with a mobile phase of acetonitrile/water/acetic acid (70/30/0.12 v/v/v) at 1.5 mL/min flow rate and 25 °C column temperature. The injection volume was 20 μL. The detection was performed at 234 nm wavelength. Quantification of HPs was performed using a standard reference curve. HP standards (*cis,trans*-13-hydroperoxydeoctadecadienoic acid and *cis,trans*-9-hydroperoxydeoctadecadienoic acid) were purchased from Larodan (Malmö, Sweden).

Oil Encapsulation into Sodium Alginate Beads. Sodium alginate beads were prepared as described by Bleve et al.[24] with some modifications, using 2% (w/v) sodium alginate dissolved in boiling hot water under vigorous mechanical stirring. The alginate/oil emulsion was obtained by mixing the sodium alginate aqueous solution with the SC-CO_2 extracted wheat bran oil (1:5 v/v) and stirring at 350 rpm for 60 min. The alginate/oil emulsion was excluded drop-by-drop from a hypodermic needle into 100 mL of 0.05 M $CaCl_2$ gelling solution with constant stirring, at room temperature, from approximately 15 cm above the surface of the gelling bath. The spherical beads (ø ≈2.7 mm) obtained were washed in distilled water, freeze-dried overnight (Labconco, Kansas City, MO, USA), and stored in different temperature and light conditions. At different time intervals, the oil was extracted from the sodium alginate beads and assayed for HPs and isoprenoid content as described above.

Extraction of the Encapsulated Oil from Sodium Alginate Beads. Freeze-dried alginate oil beads were dissolved in 20 mL of 0.1 M sodium citrate buffer at pH 7.2. The mixture was mechanically stirred for 1 h while sparging with nitrogen. After adding 30 mL of *n*-hexane, the mixture was stirred for a further 5 min and centrifuged at 5000*g* for 10 min. The organic phase was collected, dried under nitrogen, and immediately analyzed.

Statistical Analysis. Results are presented as the mean value ± standard deviation of three independent experiments (n = 3). Statistical analysis was based on a one-way ANOVA test. Tukey's post hoc method was applied to establish significant differences between means ($p < 0.05$). All statistical comparisons were performed using SigmaStat version 11.0 software (Systat Software Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effect of Storage on the Content of HPs, Vitamin E, and Carotenoids in Unencapsulated Oil. The oxidation stability of the oil extracted by SC-CO_2 from durum wheat bran was evaluated over 30 days by measuring possible changes in HPs, vitamin E (tocopherols and tocotrienols), and carotenoids under different storage conditions: light or dark and room (~25 °C) or low (4 °C) temperature.

HPs. Lipid oxidation is a major problem during oil storage. Qualitative characteristics of wheat bran oil are strongly affected by triglyceride hydrolysis, resulting in the production of free fatty acids, mostly polyunsaturated, which in turn can be converted into HPs by autoxidation, enzymatic, or photosensitized oxidations. HPs are highly unstable, reactive molecules leading to the formation of mainly volatile compounds responsible for rancid off-flavors in oils and which are potentially toxic.[25] HPs have been shown to act as pro-oxidants in soybean oil and have the ability to initiate the degradation of carotenoids to colorless compounds, in addition to affecting other nutritionally important isoprenoids.[26,27]

In order to evaluate the storage stability of wheat bran oil lipids, we measured the production of HPs derived from linoleic acid (*cis,trans*-13-hydroperoxydeoctadecadienoic acid and *cis,trans*-9-hydroperoxydeoctadecadienoic acid), the most abundant unsaturated fatty acid therein. The formation of HPs is one of the most common indicators of oil quality.[28] The rate of HP formation at 4 °C was significantly lower than that in the oil stored at room temperature ($p < 0.05$). After 30 days at 4 °C (dark), the amount of HPs was only 1.6-fold higher than in the freshly extracted oil (Figure 1). By contrast, oil stored for 30 days at 25 °C, in daylight or darkness, enabled the HP level to increase to 2.3- and 1.6-fold, respectively, in comparison to the amount measured at 4 °C (3.6- and 2.6-fold the initial content).

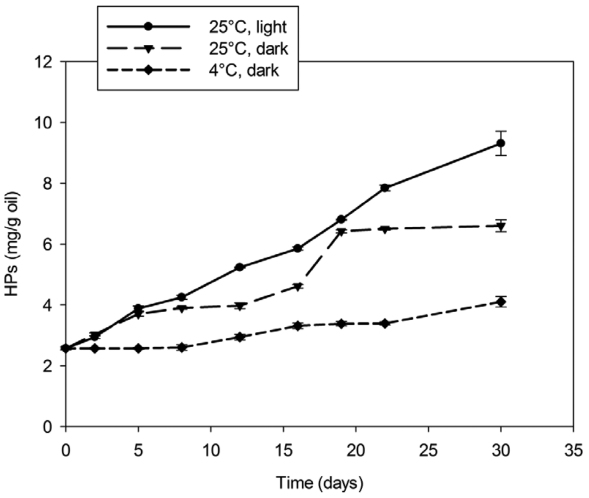

Figure 1. Time course of the production of fatty acid hydroperoxides (HPs) during storage of unencapsulated oil in different conditions. Data, expressed as mg/g oil, are the mean ± standard deviation of three independent replicates (n = 3).

Temperature therefore is one of the factors that most affect HP production during SC-CO_2 extracted wheat bran oil storage and must be kept low to preserve the oil's qualitative characteristics. Since, as suggested by Anguelova and Warthesen,[29] the increase in HP content possibly correlates with vitamin E and carotenoid oxidative degradation, we monitored over time the content of these important nutraceuticals in the oil.

Vitamin E. At room temperature, a rapid decrease in α-T concentration in the oil was observed, independently of the presence or absence of light (Figure 2a). In these conditions, the maximal reduction rate was obtained within the first 8 days of storage, and α-T was undetectable after 20 days. Storage at 4 °C, in the dark, greatly improved α-T stability in the oil samples. After 22 days of storage, α-T concentration was still 72% of the initial amount but decreased to 32% after 30 days. β- and γ-T forms (which comigrate as a single peak) showed a trend similar to that of α-T (Figure 2b). When stored at 25 °C, either in daylight or in the dark, β,γ-T concentrations fell to 3% and 8%, respectively, while, at 4 °C, after a 20% initial reduction (within the first 2 days of storage), concentrations remained relatively stable over 30 days.

Tocotrienols (α-, β-, γ-, and δ-T3) are considered even more effective than tocopherols in protecting oils against lipid peroxidation,[30,31] probably due to the presence of multiple double bonds in their farnesyl isoprenoid tail, which readily quench free radicals.[32] The extracted tocotrienol forms (α-T3 and β,γ-T3) showed a stability trend similar to that of tocopherols (Figure 2c,d). α-T3 was undetectable after 20 days of storage at 25 °C, whether in daylight or in the dark. At 4 °C, in the dark, the amount of α-T3 slowly decreased by 20% during the first 22 storage days, after which a dramatic decrease to less than 40% of the initial content occurred between 22 and 30 days of storage (Figure 2c).

When stored at 25 °C, whether in daylight or in the dark, β,γ-T3 content decreased almost linearly along the entire time interval (Figure 2d). After 22 days, the β,γ-T3 amount was approximately 94% lower than that in the freshly extracted oil.

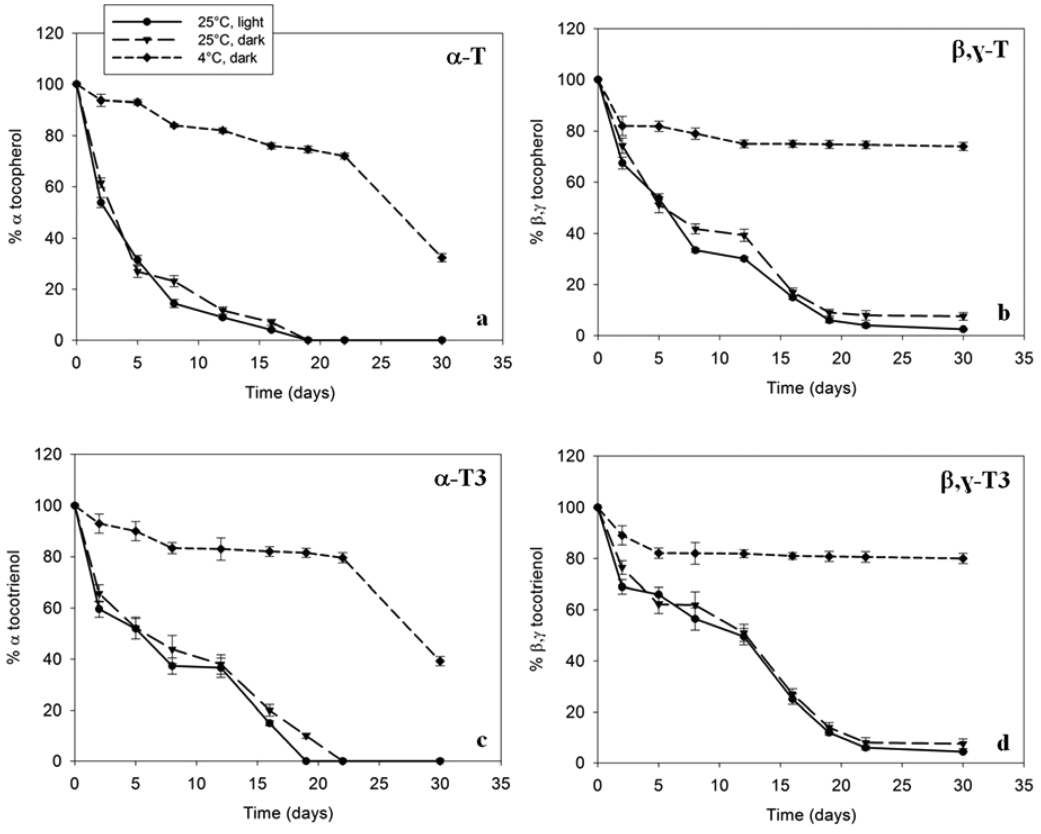

Figure 2. Time course of the amounts of tocopherols (T) and tocotrienols (T3) during the storage of unencapsulated oil in different conditions. Data, expressed as percentage of the amounts in the freshly extracted oil, are the mean ± standard deviation of three independent replicates (n = 3).

A limited reduction (20% of the initial content) was also observed in β,γ-T3 forms after 30 days of storage at 4 °C in the dark.

In this study, α forms of vitamin E (α-T and α-T3) were more susceptible to degradation than others. It has been reported that fully methylated structures can increase their ability to act as effective hydrogen donors thus making α-T and α-T3 particularly susceptible to oxidation.[33,34]

Carotenoids. In durum wheat bran oil, lutein is the predominant carotenoid followed by β-carotene and zeaxanthin.[1,7] Lutein and zeaxanthin are positional isomers, which differ only in the position of one double bond. Lutein can isomerize to zeaxanthin during kernel aging and storage or when exposed to heat generated during milling.[35] At 25 °C, the amount of lutein in the oil progressively decreased over time and was undetectable after 16 days of storage, regardless of light exposure. In oil stored at 4 °C, lutein content was relatively stable up to 22 days, not significantly different ($p = 0.426$) from the initial value, but slightly decreasing by approximately 20% after 30 days (Figure 3a).

In contrast, zeaxanthin rapidly decreased under all the conditions tested. After 2 days of storage, zeaxanthin levels had reduced by approximately 70%, regardless of light and temperature. Thereafter, zeaxanthin levels remained almost unchanged up to the end of the storage period when the oil was maintained at 4 °C, while levels were below the detection limit after 16 days at 25 °C, regardless of the presence or absence of light (Figure 3b). The higher stability of lutein compared to zeaxanthin indicates that no spontaneous lutein−zeaxanthin isomerization occurs during oil storage at 4 °C in the dark.

β-Carotene content also rapidly decreased during storage at 25 °C. No significant differences in β-carotene levels ($p < 0.05$) were observed between light and dark conditions, and levels were undetectable after 16 days. At low temperature, β-carotene had reduced by 25% after 5 days of storage and remained stable over the next 14 days but decreased to 36% after 30 days (Figure 3c).

It is widely accepted that tocopherols can protect carotenoids from oxidative damage.[36,37] Furthermore, many studies reported that tocopherols extend the inhibitory effect of β-carotene on singlet oxygen-initiated photo-oxidation by preventing β-carotene decay.[38] We can, therefore, hypothesize that at lower temperatures carotenoids (in particular lutein) are relatively stable in oil due to their high tocopherol content. As the temperature increased, tocopherols decreased, most likely as a consequence of HP formation caused by lipid peroxidation.

Effect of Storage on the Content of HPs, Vitamin E, and Carotenoids in the Encapsulated Oil. Alginate encasement was chosen because it has proven to be an effective, safe, and cheap barrier to entrap active ingredients, and it is widely applied in the food, cosmetic, and pharmaceutical industries.[39] The beads were freeze-dried to slow down microbial growth and stored in the dark at 4 °C or light at 25 °C, over 90 days.

To evaluate the influence of the encapsulation process on the quality of the oil, it was extracted at regular intervals from the freeze-dried beads. HPs, vitamin E, and carotenoid contents in the extracted oil were determined and compared to the values obtained from unencapsulated wheat bran oil.

HPs. The production of HPs during encapsulated and unencapsulated oil storage is reported in Figure 4. After 90 days of storage at room temperature, the HP content in encapsulated oil was 2.3-fold higher than in freshly extracted

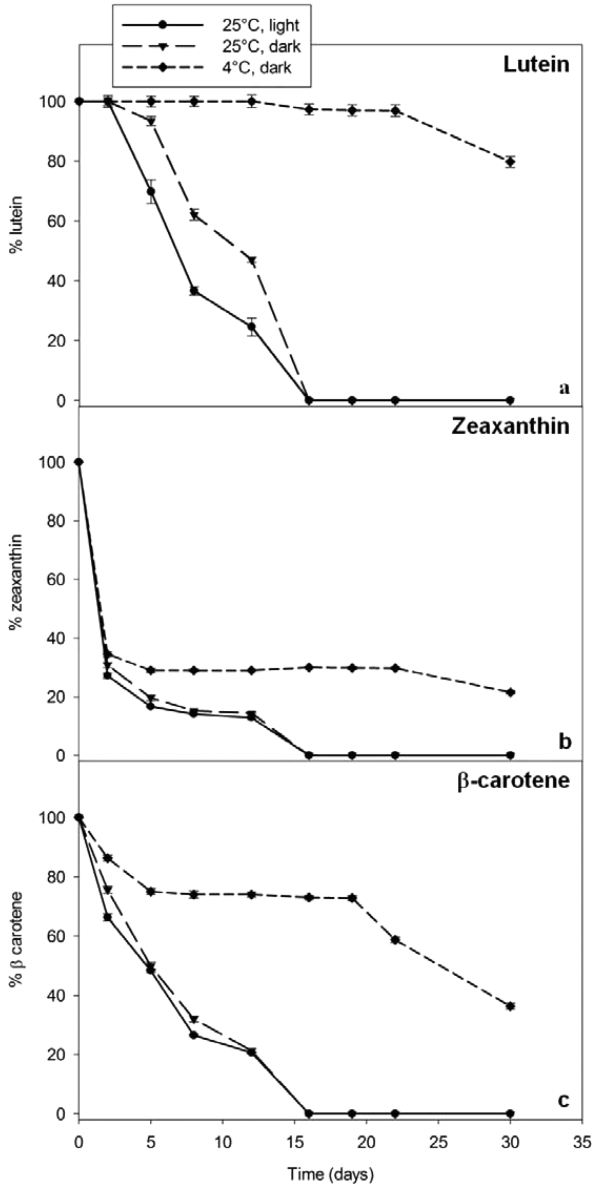

Figure 3. Time course of the amounts of lutein, zeaxanthin, and β-carotene during storage of unencapsulated oil in different conditions. Data, expressed as percentage of the amounts in the freshly extracted oil, are the mean ± standard deviation of three independent replicates ($n = 3$).

oil, while at 4 °C, it increased only by 1.4-fold. The increase in HP levels observed in encapsulated oil samples was, however, significantly lower ($p < 0.05$) than that in the corresponding unencapsulated oil sample stored at room temperature. The chromatographic profile of HPs of unencapsulated oil, stored either in the light or in the dark at 25 °C, revealed unknown peaks, possibly secondary oxidation products that may result from decomposition reactions of HPs, in accordance with the reports of Kamal-Eldin et al.[40] These unknown peaks were not observed in the samples stored at 4 °C, neither unencapsulated nor encapsulated (data not shown). This confirms the finding that temperature accelerates the oxidation process of fatty acids,[41] while encapsulation seems to retard the process.

Vitamin E. After 90 days of storage at 4 °C, a statistically significant ($p < 0.05$) increase in vitamin E stability (with the only exception of α-T3), was observed in encapsulated oil

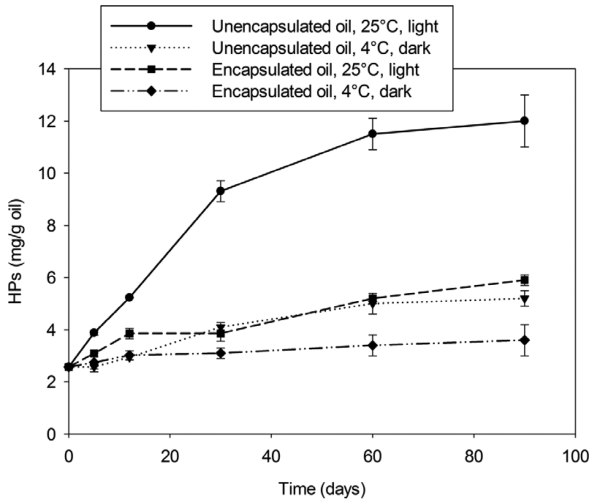

Figure 4. Time course of the production of fatty acid hydroperoxides (HPs) during storage of encapsulated and unencapsulated oil in different conditions. Data, expressed as mg/g oil, are the mean ± standard deviation of three independent replicates (n = 3).

compared to unencapsulated control samples (Figure 5). At low temperature, the amount of α-T, the most biologically active form of vitamin E, was stable (90% of the initial value) up to 30 days of storage. After 60 days, α-T levels decreased to about 50%, and at the end of the storage period (90 days), only 21% of the initial content remained (Figure 5a). In the encapsulated oil stored at 4 °C, β,γ-T levels decreased by 23% and 50% after 30 and 90 days, respectively (Figure 5b). α-T3 was stable for 30 days of storage, showing no significant differences in its content (p = 1) compared to the initial amount (Figure 5c). The amount of β,γ-T3 was reduced to 14% and 72% after 30 and 90 days, respectively (Figure 5d). In agreement with Zhou and Roos[42] and Aripin et al.,[43] these results indicate that encapsulation or storage at 4 °C increased vitamin E stability. Furthermore, these two conditions were synergistically effective.

Carotenoids. In encapsulated oil stored at 4 °C, lutein did not decrease over 30 days, but a 27% decrease was observed after 90 days (Figure 6a). In the conditions tested, zeaxanthin was less stable than lutein, decreasing by 44% and 78% after 10 and 90 days of storage at 4 °C, respectively (Figure 6b). β-Carotene, stored at 4 °C, decreased by 10% and 80% after 30 and 90 days (Figure 6c). All carotenoids tested were better protected from oxidation when oil was encapsulated in the gelatin matrix.[44]

The results reported here strongly suggest that encapsulation of SC-CO_2 extracted wheat bran oil in alginate beads resulted in a protective effect on nutritionally important compounds such as vitamin E, lutein, zeaxanthin, and β-carotene. Moreover, in encapsulated oil samples, lower levels of HPs were observed with respect to unencapsulated control samples.

Storage in the dark at 4 °C was shown to be very effective in preventing oxidative degradation of wheat bran oil. Encapsulation in alginate beads and storage in the dark at 4 °C seem to

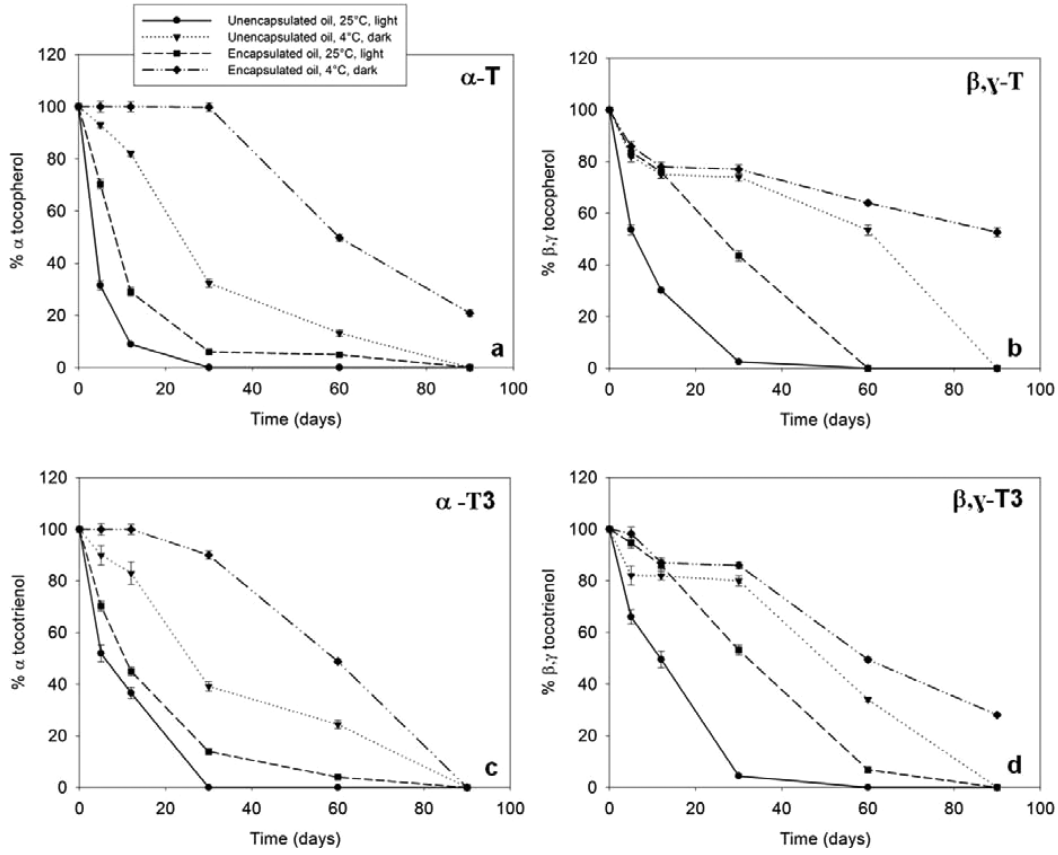

Figure 5. Time course of the amounts of tocopherols (T) and tocotrienols (T3) during storage of encapsulated and unencapsulated oil in different conditions. Data, expressed as the percentage of the contents in the freshly extracted oil, are the mean ± standard deviation of three independent replicates (n = 3).

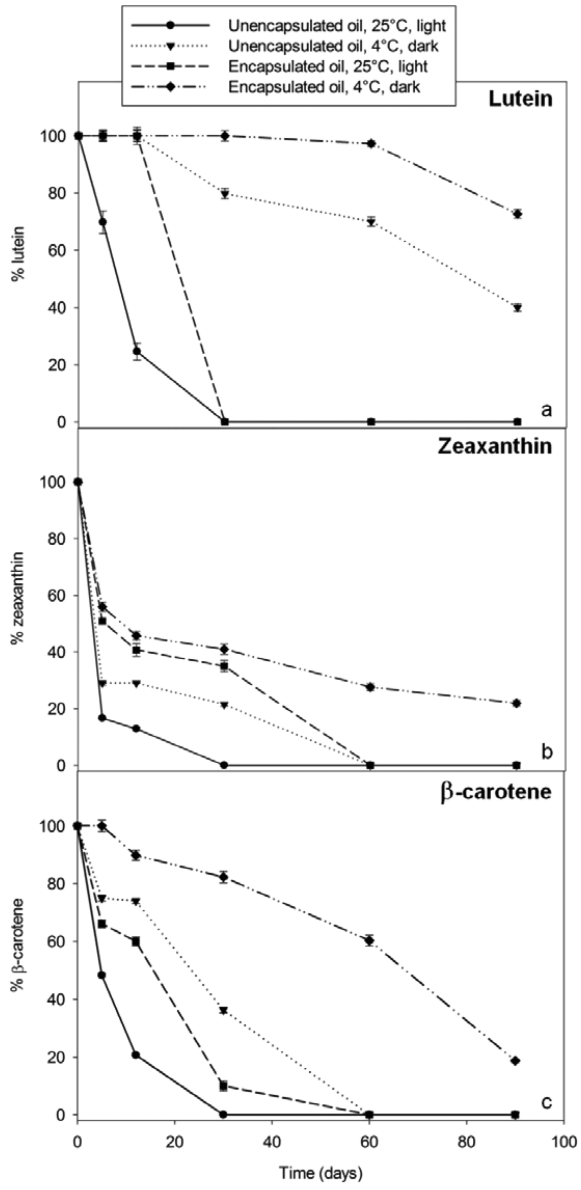

Figure 6. Time course of lutein, zeaxanthin, and *β*-carotene amounts during the storage of encapsulated and unencapsulated oil in different conditions. Data, expressed as the percentage of the contents in the freshly extracted oil, are the mean ± standard deviation of three independent replicates (n = 3).

have a synergistic effect in preventing the degradation of vitamin E and carotenoids.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

HP, fatty acid hydroperoxide; SC-CO_2, supercritical carbon dioxide; T, tocopherol; T3, tocotrienol

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