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Storage and degradation kinetics of physicochemical and bioactive attributes in microalgal-derived fucoxanthin-rich microcapsules

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ABSTRACT

Microencapsulation can improve carotenoid stability by slowing down degradation. Studies on the production and processing effects of microencapsulated carotenoids were reported in the past however long-term storage studies on fucoxanthin stability remains limited. This study investigated the effects of an eighteen-week storage period across four conditions on fucoxanthin derived from the diatom, *Chaetoceros calcitrans*. The fucoxanthin powders were prepared using two microencapsulation methods i.e., freeze drying and spray drying. Briefly, the microcapsules produced were stored in amber bottles under room temperature (25 °C) or refrigerated (4 °C) in the dark or in the presence of light. Samples were collected every two weeks where the physicochemical characteristics, carotenoid stability and antioxidant activity were evaluated. It was found that the freeze-dried microcapsule stored in 4 °C showed significantly ($p < 0.05$) better carotenoid retainment (7.5 times more) and antioxidant outcomes (3.5 times higher), as compared to the spray-dried microcapsule stored in 25 °C light. All microcapsules were found to be mainly comprised of the carotenoids fucaxanthin, dehydro fucoxanthin acetate, capsanthone, antheraxanthin, and celaxanthin. The major carotenoid identified was fucoxanthin where correlation studies showed it was responsible for the antioxidant activities and stability of the produced microcapsules. Overall, both freeze-dried and spray-dried fucoxanthin microcapsules followed a first-order kinetic degradation reaction and the recommended storage condition for fucoxanthin microcapsules was ranked as follows 4 °C (dark) > 25 °C (dark) > 40 °C (dark) > 25 °C (light). This finding offer useful insights into optimizing fucoxanthin microencapsulation methods, maintaining product quality during storage and distribution, and ensuring compliance with quality standards of fucoxanthin-based products available to consumers across the production and distribution chain.

1. Introduction

Improved functional foods that efficiently balances oxidative status are highly demanded by consumers [1]. Microalgae, a producer of unique carotenoids are emerging sustainable antioxidant sources. Carotenoids from microalgae confer cell protection through the maintenance of oxidative balance as reflected by good antioxidant properties in its extracts [2]. In particular, the diatoms *Chaetoceros calcitrans* photosynthetically manufacture fucoxanthin, an exclusive marine carotenoid that holds promises not only in antioxidant activities [3], but also in the treatment and prevention of life-style related diseases such as anti-inflammation [4], anti-obesity [5], anti-diabetes [6] and anti-cancer [7].

However, the same double bonds that confer these bioactivities unfortunately also make the carotenoid structure susceptible to degradation by light, temperature, pH or oxygen exposure in the long run [8]. This can affect colour, flavour and bioactivity [9] which is not ideal when incorporated in food and commercialized products. Previous studies on the production of antioxidant rich fractions consisting of one or more bioactive compounds were found to confer higher antioxidant efficacies as compared to single active compounds. This is likely due to the additive or synergism among bioactive compounds that contributes to the total antioxidant capacity [10]. Antioxidant rich fractions are beneficial for bioactives that are intended for long term storage (at least

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3 months) to enhance product benefit per-cost, for instance avoiding forfeiture and product replacement for easily perishable products while assuring the claims and health effect of the product.

Other alternative to slow down carotenoid degradation is, microencapsulation by spray or freeze drying. Microencapsulation have shown to improve stability [11] and bioaccessibility [12] of many bioactives, including carotenoids [13,14]. Added advantages of microencapsulation include increased solubility that makes it easy and convenient to be incorporated as bioactive ingredients in water-emulsion systems or solid food products [15–17]. Also, the weight and bulk volume of dried products are significantly reduced, which facilitates easy handling and logistics during storage and distribution. Spray drying is an established and cheap food processing technology suitable for the protection of bioactive compounds. In comparison, freeze drying remove water from frozen products through the principle of sublimation. An obvious advantage of freeze drying over spray drying is that it minimizes thermal degradation [18].

Unlike past studies focusing on effects of carotenoid processing [19] or common carotenoids like lutein [20] or beta carotene [21]; only a handful focused on fucoxanthin stability studies. Fucoxanthin is an under-discovered xanthophyll with proven antioxidants [2], anti-obesity [5] and anti-cancer activities [7]. This work contributes to the understanding of the lesser-accessible fucoxanthin and can be used as a antecedent to promote its use as an emerging bioactive ingredient for the food, nutraceuticals and cosmeceuticals industry. The purpose of this study was to evaluate the stability of microencapsulated fucoxanthin-rich fraction (FxRF) from the microalgae, *Chaetoceros calcitrans* by comprehending the behaviour and degradation kinetics of their physicochemical and bioactive components. The effect of four storage conditions on the microcapsules physicochemical characteristics, fucoxanthin quantification and antioxidant activity were investigated over a period of 18-weeks.

2. Materials and methods

2.1. Materials

Reagents and chemicals utilized were either analytical or HPLC grade. All solvents used for LCMS analysis was of LCMS grade. Tween 20, soy lecithin, dichloromethane and methanol were procured from Merck KGaA (Darmstadt, Germany). Maltodextrin 10 DE (MD) was provided by San Soon Seng Food Industries Sdn. Bhd. (Selangor, Malaysia), whereas gum arabic (AG) was purchased from Acros Organics (New Jersey, USA). Fucoxanthin standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS radical), potassium persulphate, formic acid, $CoCl_2.6H_2O$, $FeCl_3.6H_2O$, $MnCl_2.4H_2O$, Na-EDTA, $NaH_2PO_4.2H_2O$, $NaNO_3$, $ZnCl_2$, $(NH_4)_6Mo_7O_{24}.4H_2O$, $CuSO_4.5H_2O$, $Na_2SiO_3.9H_2O$, H_3BO_3, ammonium formate and vitamin B_{12} were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Fucoxanthin-rich fraction (FxRF) preparation and characterization

The crude extract from *Chaetoceros calcitrans* biomass was obtained following the specific procedure outlined by Foo et al. [22] was followed. To isolate the FxRF, the methodology described in Foo et al. [3] was followed and the extracts were stored at −80 °C freezer (Thermo Fisher Scientific, USA) prior to analysis. The FxRF produced was pooled and subjected to chromatographic analysis by LC-MS/MS-QTOF for the identification of major compounds in the microalgal-derived rich fraction. This separation of analytes was carried out using an Agilent 1290 Infinity liquid chromatography mass spectroscopy (LCMS) system (Agilent Corp., Milford, MA, USA) on a ZORBAX Eclipse XDB-C18, Analytical 4.6 × 150 mm, 5-μm (PN:993,967–902) column maintained at 25 °C. The mobile phase consisted of water (A, 0.1 % formic acid) and methanol (B, 0.1 % formic acid). A mobile phase gradient was applied as follows: starting from 100 % B 0 min, 100 %–0 % B in 4 min, 0 %–50 % B in 6 min, 50 %–75 % B in 8 min, 75 %–90 % B in 12 min, 90 %–95 % B in 16 min and 95 %–100 % B in 30 min. Before injection, each sample (1 mg mL^{-1}) was filtered through a 0.22 μm PTFE syringe filter. The injection volume was 1 μL, and the flow rate was set at 0.5 $mLmin^{-1}$. For detection and identification of fucoxanthin in the samples, an Agilent 6520 accurate-mass quadruple time of flight (Q-TOF) mass spectrometer with dual electro spray ionization (ESI) source was used. The positive electrospray mode was utilized for ionization, with a capillary voltage of 4000 V and a skimmer voltage of 65 V. The nebulizer pressure was set at 45 psi, and the nitrogen flow rate was 10 L/min. The drying gas temperature was maintained at 300 °C. In the full scan mode, the mass range covered was from m/z 100 to 1000. Reference ions for fucoxanthin were used at 121.0508 and 922.0097. The acquisition rate was set at 1.03 spectra/s, and the fragmentor voltage was 125 V. Finally, the compounds were identified with the aid of the METLIN database at the positive ionization mode. Past literatures were used for cross validation purposes.

2.3. Preparation of microcapsules

2.3.1. Formulation and drying

The FxRF was added to each blend at a ratio of 1 % w/v following previously optimized formulation by Foo et al. [23]. A total of 5 L of this mixture was prepared, stirred and homogenized at 17,500 rpm for 5 min (WiseTis® HG-15s digital homogenizer, Daihan Scientific, Korea). This homogenization process was repeated three times to ensure a homogeneous suspension. The final suspension was filtered through a 40 μm cheese cloth. From the resulting mixture, 2.5 L were subjected to freeze-drying, while the remaining 2.5 L were spray-dried.

2.3.1.1. Freeze drying. The feed liquids were frozen at −80 °C for 24 h and placed into Fast-Freeze flasks® attached with adaptors to valve ports on the drying chamber. The freeze dryer (Labconco 12 L Freeze Dryer, Kansas, USA) was operated at a pressure of $25 \pm 3 \times 10^{-2}$ Pa and −40 °C collector temperature. After 48 h of drying, powders were ground to microcapsules and sieved through a 125-μm mesh screen.

2.3.1.2. Spray drying. The mixture was spray-dried using a Büchi mini spray dryer model (Model 290, Büchi Labortechnik AG, Switzerland). Feed liquids were pumped to the atomizer with a pump and atomization performed with a fluid nozzle using compressed air at pressure (6.5 bar) and flow rate (8.5 mL min^{-1}). The following was kept constant where air flow was 30 m^3 h^{-1}; feed temperature (25 °C), pump rate (10 %), aspirator rate (100 %) and atomization air rotameter (35 mm). The inlet temperature 100 °C, outlet temperature of 70 ± 2 °C was selected for minimal temperature affecting extracts.

2.4. Stability study

The stability experiment following the method by Kang et al. [24], was designed to span a duration of four and a half month, equivalent to 18-weeks. During the study period, a comprehensive set of physicochemical characterization studies was conducted bi-weekly. The study involved precisely 3 g of powder placed in 240 individual 30 mL amber specimen bottles, and sealed with Bakelite screw caps. To create a diversified test environment, the prepared amber bottles were allocated and stored at three temperatures in the dark i.e., 4 °C, 25 °C and 45 °C. For the last storage condition, amber specimen bottles were placed at 25 °C light intensity (35 ± 5 μmol m^{-2}. s^{-1}) with a fluorescent tube placed 30 cm away from the sample bottles. At two-week intervals, three bottles from each storage condition were randomly selected, and physicochemical characterization studies were executed.

For the sake of clarity throughout the manuscript, the following

denotations were adopted: S25L represent spray dried capsules stored under 25 °C light conditions; S25D correspond to spray dried microcapsules stored under 25 °C dark conditions; S04 represent spray dried microcapsules stored under 4 °C dark; and S40 is spray dried microcapsules stored under 40 °C dark. In parallel, F25L is freeze dried microcapsules stored under 25 °C light conditions; F25D is freeze dried microcapsules stored under 25 °C dark conditions; F04 is freeze dried microcapsules stored under 4 °C dark; and F40 is freeze dried microcapsules stored under 40 °C dark.

2.5. Physicochemical characterization

2.5.1. Moisture content

The moisture content of microcapsules was performed according to AOAC 2005 method 930.15 [25].

2.5.2. Water activity (a_w)

Water activity (a_w) is a measure of the amount of water vapor pressure present in a substance compared to the vapor pressure of pure water under the same conditions. In this study, triplicate samples, each containing 1 g of microcapsules, were subjected to a water activity meter (Aqualab Series 3 TE, Pullman, WA, USA). The water activity measurements were obtained by recording the mean a_w of samples. Before taking these readings, a calibration process was performed using saturated potassium sulphate (K_2SO_4) and potassium chloride (KCl) to ensure the accuracy of the instrument's measurements.

2.5.3. Particle size distribution

The mean particle size and particle size distribution of microcapsules were determined using a light-scattering particle size analyser (Mastersizer 2000, Malvern, Worcestershire, UK) fitted with a small volume sample presentation unit and integration software (Malvern Instruments Ltd., UK). The microcapsules were placed into the provided unit and a laser beam was directed through them. As light was scattered by the sample with regards to powder sizes, photodiodes situated behind the cuvette detects the characteristic patterns of each powder and sends information to the computer. Calculation of the particle size distributions for pre-storage and post storage microcapsules stored in different conditions were based on a relative particle refractive index of 1.1500 and particle absorption of 1.0000.

2.5.4. Scanning electron microscopy (SEM) microstructure

The pre- and post-storage powder structure and morphology were evaluated using a LEO 1455 variable pressure SEM (JEOL, Japan) with Oxford Inca EDX at an accelerating voltage of 15–20 kV and working distance of 7–15 mm. Microcapsules were uniformly scattered onto SEM stubs with double-sided adhesive tape. The specimens were sputter coated with gold at 20 mA for 180 s using a Bal-Tec SCD 005 sputter coater. Sample images at selected magnification were captured.

2.5.5. Water solubility index (WSI)

The WSI of the powders were determined following Kha et al. [26]. An amount of 0.5 g of microencapsulated powders was added with 6 mL of distilled water at 25 ± 4 °C) and vigorously mixed in a 15 mL centrifuge tube and centrifuged (Sigma 4–15 Laborzentrifugen, GmbH, Germany) for 20 min at 10,000 rpm. The supernatant was carefully collected into a pre-weighted petri dish and oven dried at 120 ± 2 °C for 4 h until a constant weight. The WSI (%) was calculated as percentage of dried supernatant with respect to the amount of initial 0.5 g of powder.

2.5.6. Colour

The colour of microcapsules was measured using the Hunterlab colour difference meter (Minolta Chroma meter, CR-300, USA) calibrated with a white standard tile. The results were expressed as Hunter values L, a and b values, where L denotes lightness, a* redness and greenness, and b* yellowness and blueness. Chroma, indicating colour intensity, was calculated by the formula $(a^{*2} + b^{*2})^{1/2}$ whereas hue angle (H°) was calculated by the formula $H^{\circ} = tan^{-1}\left(\frac{b^*}{a^*}\right)$.

2.6. Bioactivity analyses

2.6.1. Total carotenoid content

An amount of 0.5 g of microencapsulated fucoxanthin powders was dispersed into 2 mL of 0.9 % saline to break microcapsules followed by another 2 mL of methanol added to the test tube. Sealed tube was sonicated for 10 min and centrifuged (Sigma 4–15 Laborzentrifugen, GmbH, Germany) for 5 min at 10,000 rpm. The supernatants recovered were passed through a 0.45 μm nylon filter and the concentration of extractable carotenoids was determined by a UV spectrophotometer at 445 nm [27]. Freshly extracted sample from the powders were immediately measured and the values interpolated to a fucoxanthin standard curve. The blank used consisted of equal volumes of 0.9 % saline and methanol.

2.6.2. Fucoxanthin quantification by HPLC-DAD

To accurately measure the amount of fucoxanthin, high performance liquid chromatography-diode array detector (HPLC-DAD) were performed using the method by Foo et al. [3]. The recovered pigments were injected with an Agilent G1301A autosampler into an Agilent 1300 series HPLC series (Agilent Technologies Inc., Alpharetta, GA, USA) equipped with a DAD 1400 diode array detector. The chromatographic separations were executed on a Merck Chromolith RP-18e (3 mm × 4.6 mm i. d. 2 μm pore size) with detection set at 445 nm. The mobile phase opted was a gradient of 100 % water (A) and 100 % methanol (B): starting from 0 % to 100 % A in 2 min, 100 %–50 % A in 3 min, 50 %–25 % A in 4 min, 25 %–10 % A in 6 min, 10 %–5 % A in 8 min, and 0 %–100 % B in 15 min. A flow rate of 1 mL min^{-1} was used with an injection volume of 20 μL. Samples and mobile phase were filtered through PTFE syringe filters (0.22 μm pore size) prior to HPLC injection. Each chromatogram was generated using the Agilent Chemstation enhanced integrator. The standard curve and retention times were calibrated using fucoxanthin standard purchased from Sigma-Aldrich Co., St. Louis, MO, USA. Linearity was calculated with five different concentrations in triplicates. All samples were analysed in triplicates and results were expressed as milligram fucoxanthin per gram powder (mg FX. g^{-1} DW).

2.6.3. Total antioxidant capacity

2.6.3.1. ABTS$^{\cdot+}$ scavenging assay. ABTS$^{\cdot+}$ scavenging activity was determined according to method by Khong et al. [28]. Briefly, ABTS radical cation (ABTS$^{\cdot+}$) was prepared by reacting 50 mL of 7 mM ABTS stock solution with 50 mL of 2.45 mM potassium persulfate for 24 h in the dark. Next, 200 μL of ABTS$^{\cdot+}$ working solution (0.70 ± 0.05 absorbance at 734 nm) was added to 20 μL sample/Trolox standard (Sigma-Aldrich Co., St. Louis, MO, USA). Antioxidant activity were expressed as mg TE.g^{-1} powder.

2.6.4. Degradation kinetic analysis

Calculation of kinetic degradation was done according to past studies [13,18]. Concentration of total carotenoid content vs. storage time for each microencapsulate were plotted to yield a first-order kinetic model, $In\ A = InA_0 - kt$. Rate constant, k was calculated as the negative slope of the straight line and subsequently, half-life ($t_{1/2}$) was obtained for each sample using equation, $t_{1/2} = \frac{In2}{k}$.

2.7. Statistical analysis

The analysis was conducted using one-way analysis of variance (ANOVA) neswith a significance level of 5 %. Normal distribution was checked with Levene's test ($p > 0.05$). When necessary, data were transformed to avoid violation of assumption underlying ANOVA test.

Table 1
Top 50 major compounds in FxRF from *C. calcitrans* in the LC-MS/MS-QTOF positive ion mode.

No.	RT	*m/z* ratio	Metabolite name	Formula	Compound class
1	21.199	681.414	Dehydro fucoxanthin acetate	C42H58O6	Xanthophylls
2	23.42	566.413	Diatoxanthin	C40H54O2	Triterpenoids
3	21.199	581.400	Pectenolone	C40H52O3	Triterpenoids
4	20.475	681.412	Dehydro fucoxanthin acetate	C42H58O6	Xanthophylls
5	22.068	583.413	Diadinochrome	C40H54O3	Triterpenoids
6	16.598	542.325	(3E,7E,11E,15E)-5,9,13,17,18-pentahydroxy-4,6,8,10-tetramethyl-2-[(E)-2-methylbut-2-enyl] nonadeca-3,7,11,15-Tetraenedioic acid	C28H44O9	Very long-chain fatty acids
7	2.486	581.399	Pectenolone	C40H52O3	Triterpenoids
8	21.602	583.414	Diadinochrome	C40H54O3	Triterpenoids
9	2.39	151.145	Thymol	C10H14O	Aromatic monoterpenoids
10	27.893	566.410	Diatoxanthin	C40H54O2	Triterpenoids
11	24.112	582.407	Capsanthone	C40H54O3	Xanthophylls
12	26.606	553.282	Euphorbiasteroid	C32H40O8	Diterpenoids
13	25.946	767.471	Scabioside C	C41H66O13	Triterpenoids
14	3.001	321.168	[5-hydroxy-3-(hydroxymethyl)-2-oxo-6-propan-2-ylcyclohex-3-en-1-yl] 3-methylpentanoate	C16H26O5	Menthane monoterpenoids
15	2.454	422.329	AgelasineE/F	C26H40N5	Diterpenoids
16	21.087	584.421	Antheraxanthin	C40H56O3	Xanthophylls
17	3.355	673.341	(3S,4S,6aR,6bS,8R,8aR,12aS,14bR)-8-hydroxy-4,6a,6b,11,11,14b-hexamethyl-3-[(2S,3R,4S,5R)-3,4,5-trihydroxyoxan-2-yl]oxy-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicene-4,8a-dicarboxylic acid	C35H54O10	Triterpenoids
18	2.116	367.271	Scalarin	C27H40O5	Scalarane sesterterpenoids
19	3.001	577.245	3,7-O-diacetyl-5-O-benzoyl-13,17-oxy-14-oxopremyrsinol	C31H38O9	Bicyclic monoterpenoids
20	21.022	637.266	5,6,7-triacetoxy-3-benzoyloxy-14,15-dihydroxy-9-oxojatropha-6(17),11E-diene (8)	C33H42O11	Jatrophane and cyclojatrophane diterpenoids
21	19.156	299.196	5-amino-3-[(2E)-2-(3,5-dimethyl-2-oxocyclohexylidene)ethyl]-5-oxopentanoic acid	C15H23NO4	Medium-chain fatty acids
22	2.132	277.222	FA 18:3+1O	C18H30O3	Medium-chain fatty acids
23	18.207	627.427	Ginsenoside Rh3	C36H60O7	Triterpenoids
24	28.826	566.414	Diatoxanthin	C40H54O2	Triterpenoids
25	3.066	355.189	Carnosic acid	C20H28O4	Diterpenoids
26	27.603	566.413	Diatoxanthin	C40H54O2	Triterpenoids
27	21.264	658.427	Fucoxanthin	C42H58O6	Xanthophylls
28	3.452	365.188	(2E,4E)-12-hydroxy-13-(hydroxymethyl)-3,5,7-trimethyltetradeca-2,4-dienedioic acid	C18H30O6	Long-chain fatty acids
29	22.873	582.408	Capsanthone	C40H54O3	Xanthophylls
30	22.068	581.400	Pectenolone	C40H52O3	Triterpenoids
31	2.116	381.259	5-(4-acetyloxy-3-hydroxy-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydronaphthalen-1-yl)-3-methylpentanoic acid	C22H36O5	Diterpenoids
32	19.156	595.356	(2R,6R)-6-[(3R,10S,12S,13R,17R)-3-(2-carboxyacetyl)oxy-12-hydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-methyl-3-methylideneheptanoic acid	C34H52O7	Triterpenoids
33	2.132	411.289	4-hydroxy-3-tetratrenylbenzoic acid	C27H38O3	Diterpenoids
34	2.342	487.294	(2R,2aR,2′R,4R,5R,5′R,6aR,8aS,8bR,9S,11aS,12bS)-5′,6a,8a,9-tetramethyldocosahydrospiro [naphtho[2′,1′:4,5]indeno[2,1-b]furan-10,2′-pyran]-2,2a,4,5-tetraol	C27H44O6	Triterpenoids
35	2.277	485.361	Poricoic acid B	C30H44O5	Triterpenoids
36	2.132	469.329	(2S,4aS,6aS,6bR,12aS,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid	C30H44O4	Triterpenoids
37	2.712	171.063	Penicillic Acid	C8H10O4	Medium-chain keto acids and derivatives
38	2.213	443.396	(3beta)-lup-20(29)-ene-3,28-diol	C30H50O2	Triterpenoids
39	21.602	551.432	Celaxanthin	C40H54O	Xanthophylls
40	16.211	431.312	Hecogenin	C27H42O4	Triterpenoids
41	3.066	389.239	5-(5-methoxycarbonyl-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl)-3-methylpentanoic acid	C21H34O4	Diterpenoids
42	26.155	793.483	(2S,3R,4S,5R)-2-[(2R,3R,4S,5S,6R)-2-[[(3S,6S,8R,10R,12R,13R,14R,17S)-3,12-dihydroxy-17-[(2S)-2-hydroxy-6-methylhept-5-en-2-yl]-4,4,8,10,14-pentamethyl-2,3,5,6,7,9,11,12,13,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-6-yl]oxy]-4,5-dihydroxy-6-(hydroxymethyl)oxan-3-yl] oxyoxane-3,4,5-triol	C41H70O13	Triterpenoids
43	19.397	441.203	Diferuloyl putrescine	C24H28N2O6	Hydroxycinnamic acids and derivatives
44	20.507	658.425	Fucoxanthin	C42H58O6	Xanthophylls
45	2.358	459.268	Ganolactone B	C27H38O6	Triterpenoids
46	19.397	329.240	Incensole	C20H34O2	Sesquiterpenoids
47	2.47	376.250	(1S,3R,6S,6aR,6bR,8S,9S,11R,11aR,12R,12aR,14R)-1-ethyl-6,8,11-trihydroxy-3-methyl-10-methylenetetradecahydro-3,6a,12-(epiethane[1,1,2]triyl)-9,11a-methanoazuleno[2,1-b]azocine 1-oxide	C22H33NO4	Kaurane diterpenoids
48	19.172	320.243	Dihydropiplartine	C17H21NO5	Cinnamic acids and derivatives
49	21.602	979.604	18-b-Glycyrrhetinic acid	C30H46O4	Triterpenoids
50	2.438	438.324	(1S,4S,5R,9S,10R,13R,14R)-14-hydroxy-5,9-dimethyl-14-{[(3-methylbutanoyl)oxy]methyl} tetracyclohexadecane-5-carboxylic acid	C25H40O5	Kaurane diterpenoids

Note: RT denotes retention time and *m/z* is the mass-to-charge.

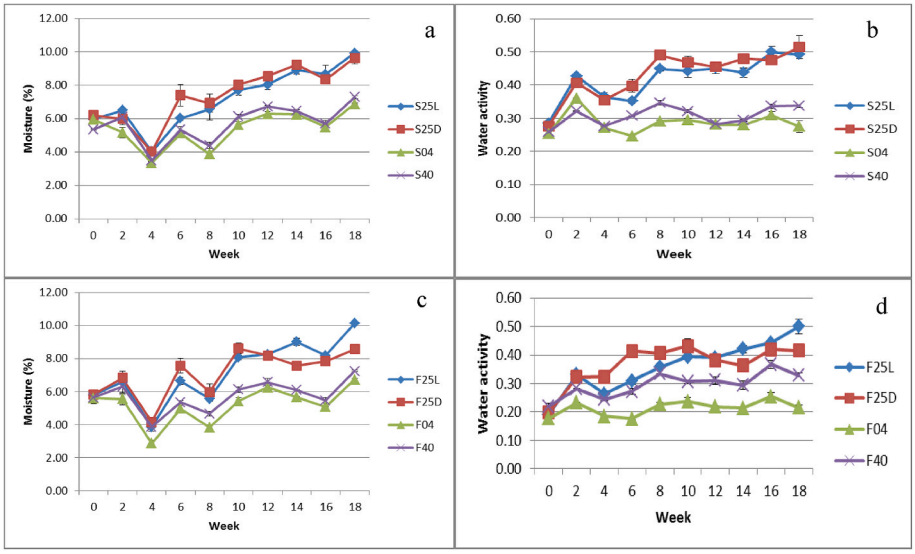


Fig. 1. Increasing moisture content (%) and water activity value (a_w) of spray dried (a,b) and freeze dried microcapsules (c,d) during storage.
**S25L is spray dried capsules stored under 25 °C light conditions, S25D is spray dried microcapsules stored under 25 °C dark conditions, S04 is spray dried microcapsules stored under 4 °C dark, S40 is spray dried microcapsules stored under 40 °C dark; F25L is freeze dried microcapsules stored under 25 °C light conditions, F25D is freeze dried microcapsules stored under 25 °C dark conditions, F04 is freeze dried microcapsules stored under 4 °C dark and F40 is freeze dried microcapsules stored under 40 °C dark. Error bar denotes standard error of the means (n = 3).

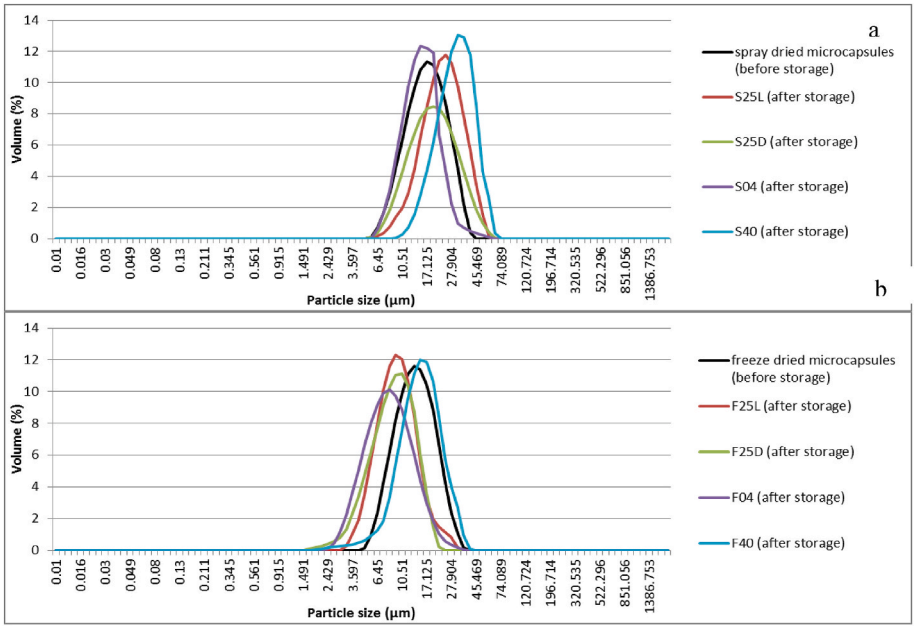


Fig. 2. Particle size distribution in spray dried (a) and freeze dried (b) microcapsules before and after storage.

Tukey test was selected to analyse significant differences ($p < 0.05$) between samples using the statistical program, SPSS Version 22.0 (SPSS Inc., Chicago, USA). Linear regression analysis was used to determine model adequacy describing carotenoid degradation kinetics. Pearson correlation studies were applied to investigate relationships between variables. The results are presented as the means ± standard deviation (SD) of three replicates (n = 3).

3. Results and discussion

3.1. Fucoxanthin-rich fraction (FxRF) composition analysis by LC-MS/MS-QTOF and HPLC

Study findings offer insights into the establishment of a carotenoid profile aimed at providing baseline data of standardized *C. calcitrans* extracts for eventual commercialization purposes. Primarily, the LC-MS/MS-QTOF analysis revealed the composition of FxRF, as detailed in Table 1. The compounds were categorized into seven major categories i.

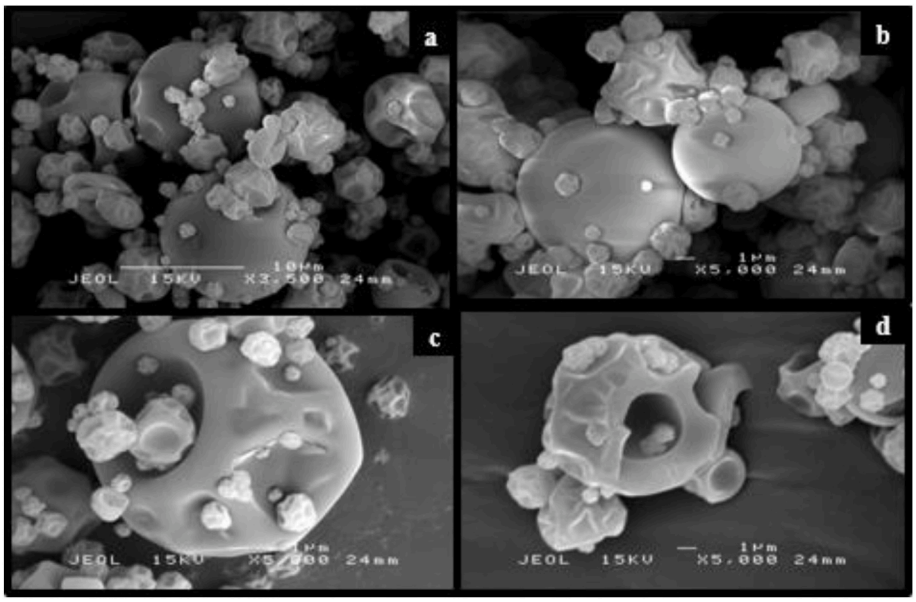


Fig. 3. Representative SEM micrographs of spray dried microcapsules stored at (a) 4 °C dark refrigerator; (b) 25 °C dark; (c) 25 °C 12:12 h L:D cycle; (d) 40 °C dark oven after storage.

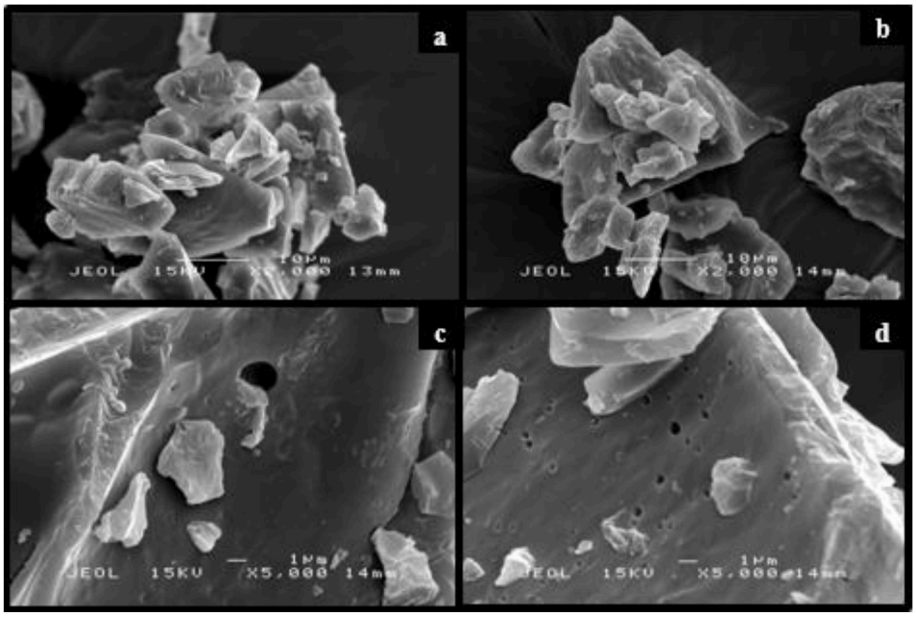


Fig. 4. Representative SEM micrographs of freeze dried microcapsules stored at (a) 4 °C dark refrigerator, (b) 25 °C dark, (c) 25 °C 12:12 h L:D cycle (d) 40 °C dark oven after storage.

e., terpenoids, cinnamic acid and derivatives, long chain fatty acids, medium chain fatty acids, very long chain fatty acids, keto acids and xanthophylls. Notably within the cohort of top 50 compounds in the FxRF, the xanthophylls identified were fucaoxanthin, dehydro fucoxanthin acetate, capsanthone, antheraxanthin, and celaxanthin.

Subsequently, the HPLC analysis was selected to quantify any reduction in fucoxanthin content, a reasonable proxy to reflect the intrinsic quality of FxRF. Although this approach inherently constrained the investigation to a singular compound standard, this approach was inevitable, given our objective involving a time-lapse study. In addition, we have also demonstrated that fucoxanthin is the major compound responsible for the bioactivity of the FxRF in our previous study (2, 3, 7). Thus, we determined that a focus on fucoxanthin, the compound of interest, took precedence. It is worth acknowledging that further studies of enhanced quantitative analysis would benefit from the inclusion of a broader array of standards.

3.2. Physicochemical characteristics of microcapsules

3.2.1. Moisture content and water activity

There was a significant correlation between moisture and water activity ($R^2 = 0.867$, $p < 0.05$) in the studied microcapsules. This was expected given that moisture and water activity complement each other and each is important on its own [13,18]. Water activity is defined as ratio of water vapor pressure in a food system to pure water vapor pressure at the same temperature [29] and is considered an important factor that influences shelf life of food products.

It was observed that microcapsules revealed a gradual increment in

Table 2
Water solubility index (%) of microcapsules before and after storage study. Water solubility index (%) of microcapsules before and after storage study.

Sample	WSI (%) on week 0	WSI (%) on week 18
S25L	50.83 ± 2.59^{a}	27.13 ± 2.39^{e}
S25D	55.07 ± 1.89^{a}	27.67 ± 2.85^{de}
S04	56.35 ± 2.10^{a}	24.33 ± 1.23^{bc}
S40	51.22 ± 2.65^{a}	23.15 ± 2.08^{f}
F25L	50.67 ± 0.67^{a}	20.57 ± 1.54^{ab}
F25D	53.03 ± 3.50^{a}	16.61 ± 1.76^{a}
F04	46.32 ± 3.68^{a}	18.33 ± 3.11^{a}
F40	54.02 ± 4.02^{a}	17.60 ± 2.50^{cd}

Note**:** S25L is spray dried capsules stored under 25 °C light conditions; S25D is spray dried capsules stored under 25 °C dark conditions; S04 is spray dried capsules stored under 4 °C dark; S40 is spray dried capsules stored under 40 °C dark, F25L is freeze dried capsules stored under 25 °C light conditions; F25D is freeze dried capsules stored under 25 °C dark conditions; F04 is freeze dried capsules stored under 4 °C dark and; F40 is freeze dried capsules stored under 40 °C dark. Different letters within the same column indicates significant difference ($p < 0.05$). Error bar denotes standard error of the means ($n = 3$).

Table 3
Colour parameter differences of powder microcapsule after 18 weeks storage studies.

Sample	L*	a*	b*	Hue angle (h°)	Chroma	TCD
S25L	−11.40 ± 0.63^{c}	21.58 ± 0.36^{cd}	−16.17 ± 0.44^{c}	21.51 ± 0.66^{e}	−13.45 ± 0.29^{d}	−12.89 ± 0.59^{c}
S25D	−21.19 ± 0.73^{a}	19.83 ± 0.43^{e}	−11.71 ± 0.09^{b}	76.07 ± 1.16^{b}	−16.00 ± 0.36^{c}	−22.93 ± 0.78^{a}
S04	−20.75 ± 1.06^{a}	17.03 ± 0.38^{f}	−7.01 ± 0.38^{a}	122.23 ± 1.31^{a}	−13.75 ± 0.39^{d}	−22.36 ± 1.04^{a}
S40	−21.01 ± 0.11^{a}	21.80 ± 0.50^{b}	−15.17 ± 0.31^{c}	39.15 ± 1.00^{d}	−13.75 ± 0.27^{d}	−22.56 ± 0.11^{a}
F25L	−14.76 ± 0.55^{b}	27.68 ± 0.37^{a}	−24.78 ± 0.31^{e}	−5.54 ± 0.56^{f}	−19.08 ± 0.36^{b}	−17.91 ± 0.58^{b}
F25D	−17.97 ± 0.65^{ab}	28.11 ± 0.50^{a}	−16.35 ± 0.30^{c}	62.84 ± 2.93^{c}	−23.00 ± 0.41^{a}	−21.59 ± 0.59^{a}
F04	−17.20 ± 0.58^{b}	28.18 ± 0.20^{a}	−15.04 ± 0.37^{c}	72.82 ± 1.19^{d}	−20.70 ± 0.57^{b}	−20.58 ± 0.55^{ab}
F40	−17.12 ± 0.59^{b}	26.99 ± 0.02^{a}	−19.96 ± 0.54^{d}	45.90 ± 0.85^{d}	−23.25 ± 0.46^{a}	−20.76 ± 0.48^{ab}

Note: TCD denotes total colour difference. S25L is spray dried capsules stored under 25 °C light conditions; S25D is spray dried capsules stored under 25 °C dark conditions; S04 is spray dried capsules stored under 4 °C dark; S40 is spray dried capsules stored under 40 °C dark, F25L is freeze dried capsules stored under 25 °C light conditions; F25D is freeze dried capsules stored under 25 °C dark conditions; F04 is freeze dried capsules stored under 4 °C dark and; F40 is freeze dried capsules stored under 40 °C dark. Values are means of three determinations ±SD ($p < 0.05$) and the different letters within the same column indicates significant difference ($p < 0.05$).

moisture content with time but at different extents depending on storage conditions (Fig. 1). For example, microcapsules that were stored at extreme temperatures (4 °C and 40 °C) had significantly lower moisture and a_w values than the microcapsules that were stored at ambient temperature (25 °C) ($p < 0.05$). Lower temperature (4 °C), generally slow down the rate of moisture migration and water vaporization. This can help preserve the product's dryness and reduce the overall moisture content, which is also the reason most refrigerator temperature setting adopts temperature at this degree. On the other hand, higher temperature (40 °C) promotes water evaporation and can lead to a decrease in water activity. It was worth noting that established guidelines for maintaining microbiological stability was values, less than 6 % for moisture content and less than 0.6 for a_w [30]. Remarkably by the end of the 18-weeks storage study, all microcapsules except for those stored at ambient temperature had a_w values of less than 0.6 by end of the storage study. This meant the produced microcapsules were suitable for long term storage as they contained less free water for biochemical reactions to take place. Findings are parallel to astaxanthin microcapsules with 0.36 a_w [13].

3.2.2. Particle size distribution

The employment of particle size measurement is widely used in food unit operations as a quality control technique to investigate changes in product characteristics as it directly implicates any physical, chemical or mechanical process [31]. Before the commencement of storage studies, spray-dried and freeze-dried microcapsules had particle sizes of 16.10 ± 0.17 μm and 12.61 ± 0.54 μm respectively. After 18-weeks of storage in four different conditions, respective microcapsules showed differences in particle sizes.

The Gaussian (bell-shaped) curve in Fig. 2 and represented by different colours shows how different heat and light treatment conditions influences the volume percentages and particle size over time. An intriguing trend revealing a not so straightforward relationship between particle size and volume percentage. For instance, in the case of S25D, despite having a larger particle size, the volume percentage was lower compared to its state before storage. A possible explanation for this could be related to changes in particle packing, agglomeration, as well as material characteristics. For example, materials with higher densities tend to have lower volume percentages, while porous materials may have higher volume percentages for the same particle size. Parallel results were reported by Masum et al. [32] where they observed the inlet and outlet temperature combinations used in spray drying alone could affect stability of powders during storage. Furthermore, SEM findings observed freeze-dried particles may have experienced structure collapse rendering them to become smaller in size. The reason for structure collapse could be related to factors like moisture absorption over time, temperature, or mechanical forces experienced during storage.

3.2.3. Scanning electron microscopy (SEM) microstructure

In the SEM analysis, it was evident that spray-dried microcapsules exhibited a spherical shape, featuring shallow dents from drying and cooling processes. In contrast, freeze-dried microcapsules displayed a structure with sharp, fragmented, glass-like surfaces. A similar observation was reported by Haque et al. [33], where their spray dried products were spherical, while freeze-dried products resembled fractured glass pieces, showing clear differences in material characteristics i.e., physical and thermal behaviours over time.

Furthermore, after storage, the SEM micrographs confirmed notable changes. Spray-dried microcapsules exhibited an increase in size (Fig. 3), whereas freeze-dried microcapsules displayed size reduction (Fig. 4). These observations underscored the distinct particle rearrangement and compaction resulting from each production processes. Spray drying, characterized by the rapid evaporation of liquid droplets, yielded spherical or near-spherical microcapsules. In contrast, freeze drying process involved the sublimation of water crystals, leaving voids in the structure and resulting in porous and irregularly shaped products. That said, structural differences from the distinct production processes of spray drying and freeze drying respectively, can potentially impact the exposure of carotenoids to external degradation factors.

Notably, the study revealed some intriguing findings concerning carotenoid degradation. Over an 18-week period, the extent of degradation for S25L was 91 %, and for F25L, it reached 87.1 %. Similarly, S40 (92.5 %) and F40 (84.6 %) showed a similar extent of fucoxanthin degradation after 18 weeks. The same trend was observed for S25D (76.4 %) and F25D (52.9 %). Under these instances, results indicated that the spray drying method was as effective as the freeze-drying method in protecting carotenoids. A justification for this is, while the freeze-drying process does not involve high temperatures, the inherent porosity of the structure and particle shape can expose carotenoids to more surface area that can lead to increased susceptibility to

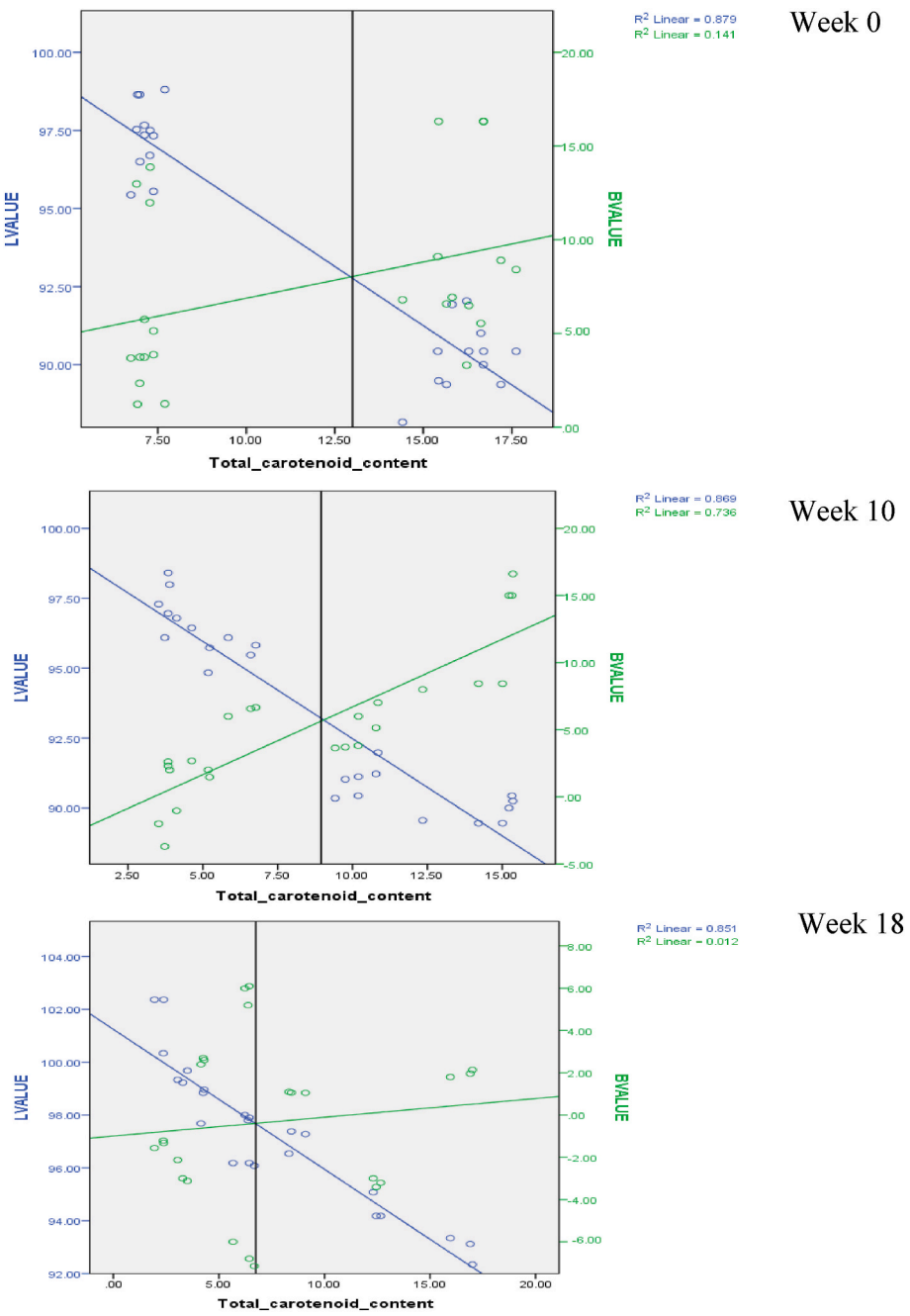


Fig. 5. Correlation of colorimetry parameters L* and b*, and carotenoid retention at week 0, week 10 and week 18. It is observed there is a considerable decrease in carotenoid content over time.

degradation factors by oxygen, light, and moisture. Conversely, the spray dried spherical particles are cheaper to produce and can pack more densely which minimizes gaps between particles. This can further reduce exposure to external factors and provide better protection for the encapsulated carotenoids. These findings hold significant implications for industries seeking cost-effective drying methods to preserve carotenoids.

Furthermore, post 18-weeks storage, the S04 microcapsules maintained their initial integrity, while the S25L microcapsules increased in size with sunken surfaces, potentially accelerating carotenoid degradation with time. The S40 microcapsules showed the occurrence of cracking and revealing of the inner surface of empty microcapsules, which could expose the inner surface of the capsules, leading to potential carotenoid exposure and degradation. The S25D microcapsules remained intact, suggesting that storage at 25 °C in the dark to be the next best method after 4 °C. For freeze dried microcapsules, F25D and F04 microcapsules showed no significant morphological differences, while F25L and F40 microcapsules displayed increased porosity and indentations, indicative of particle breakdown. Overall, the findings suggest that certain storage conditions, such as 4 °C and 25 °C in the dark, may better maintain microcapsule integrity and carotenoid stability. However, further in-depth studies are warranted to assess the direct impact of these structural changes on carotenoid degradation and its bioavailability.

3.2.4. *Microcapsule solubility*

Table 2 reports the effect of different storage conditions on the water solubility index (WSI) in freeze and spray-dried microcapsules. Initially,

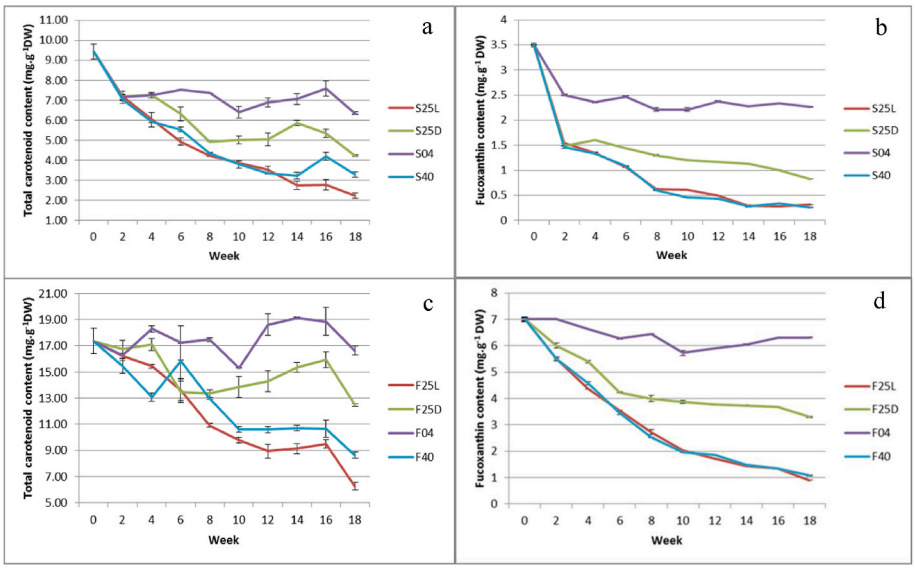


Fig. 6. The decline in total carotenoid and fucoxanthin content of spray dried (a,b) and freeze dried microcapsules (c,d) with time at different storage conditions. **S25L is spray dried capsules stored under 25 °C light conditions, S25D is spray dried microcapsules stored under 25 °C dark conditions, S04 is spray dried microcapsules stored under 4 °C dark, S40 is spray dried microcapsules stored under 40 °C dark; F25L is freeze dried microcapsules stored under 25 °C light conditions, F25D is freeze dried microcapsules stored under 25 °C dark conditions, F04 is freeze dried microcapsules stored under 4 °C dark and F40 is freeze dried microcapsules stored under 40 °C dark. Error bar denotes standard error of the means (n = 3).

all microcapsules were not significantly from each other ($p > 0.05$) but post storage, there was a change in WSI of microcapsules ranging from 17.6 % to 27.67 %. Notably, spray-dried microcapsules had a significantly higher ($p < 0.05$) WSI as compared to freeze-dried microcapsules. WSI is an important criterion where a quick and complete reconstitution is highly desired in the commercialization cycle [34] and WSI in this study acts as an indication of solubility of biomolecules (lipid, pigments i.e. fucoxanthin and related carotenoids, proteins and/or sugars etc.) before or after various storage condtions in the presence of excess water. Values reported in spray-dried powders in this study were parallel to spray-dried tomato powders (17.65–26.73 %) reported by D'Sousa, Borges, Magalhães, Ricardo and Azevedo [35]. In addition, a significantly positive correlation between WSI and particle size ($R^2 = 0.703$; $p < 0.05$) was observed. This is reflected in the 40 °C storage condition where particle size and WSI for spray-dried microcapsules were significantly higher ($p < 0.05$) than freeze-dried microcapsules. The reason for this is powder solubility are affected by parameters including drying aid, carrier agent, nature of material to be encapsulated or type of drying technology [26].

3.2.5. Colour

Overall, freeze-dried microcapsules exhibited a lesser change in respective colour parameters as compared to spray-dried microcapsules. For example, L value (lightness) in freeze-dried microcapsules did not differ much as compared to spray-dried microcapsules along the storage duration suggesting better carotenoid retainment ($p > 0.05$). This observation was further corroborated by chroma values and total colour difference (TCD) which showed similar patterns (Table 3). Besides that, the chromatic coordinate b was a good indicator for carotenoid retention as it characterizes positive b values as yellow and negative b values as blue. With gradual carotenoid losses over time, the intensity of yellow colour reduces in powders, leading to more negative b values. Fig. 5 presents the relationship between the colorimetry parameters L and b with the carotenoid content at three time points. The intersection point corresponded to the average amount of carotenoids remaining in samples. Here, the gradual decrease of carotenoid content could be observed from week 0 (13.0 mg g^{-1} DW), week 8 (8.95 mg g^{-1} DW) to week 18 (6.75 mg g^{-1} DW). The carotenoid remaining in samples corresponded to b values and L values with time. Indeed, this finding corroborated with past work reporting that an increase in L value in systems coloured with carotenoids were a discoloration indicator attributed by carotenoid degradation (Estupiñan et al., 2011). Overall, colour measurement is a satisfactory method to complement carotenoid content and could potentially be a rapid quantitative method useful for food and nutraceutical applications in quality assurance and control.

3.2.6. Carotenoid and fucoxanthin content in microcapsules

Carotenoids in microcapsules degraded over time but at different rates (Fig. 6). At the end of storage study, microcapsules ranked from highest carotenoid content were F04>F25D > F40>S04 and these significantly different ($p < 0.05$) from F25L > S25D > S40 > S25L. This aligned to fucoxanthin content F04 > F25D > S04 > F40 which was significantly different ($p < 0.05$) from F25L > S25D > S25L > S40. Further validation with Pearson correlation between carotenoid and fucoxanthin yielded a significantly positive relationship of $R^2 = 0.881$ at $p < 0.05$ level.

The percentage carotenoid losses were calculated after 18-weeks (Fig. 6) and it was found that freeze-dried microcapsules experienced significantly lesser carotenoid losses as compared to spray-dried microcapsules ($p < 0.05$). In terms of storage conditions, the F04 microcapsules experience the least amount of carotenoid losses at 5.5 % whereas the S25L microcapsules lost the most carotenoids at 72 %. Figs. 7 and 8 illustrates the extent of fucoxanthin degradation with time. From here, it was clear that the type of storage conditions can have a profound effect on carotenoid degradation with findings showing that 4 °C dark, 25 °C dark and 40 °C were better than 25 °C light conditions. Therefore, minimizing light exposure reduces carotenoid degradation and subsequently prolongs product shelf life.

In this study, we employed both spectrophotometric and HPLC, for its high precision and specificity [36] to provide a comprehensive dataset that revealed interesting trends.

It was noteworthy to observe some fluctuations in total carotenoid content in both the spray-dried and freeze-dried samples from week 14–18. This could be attributed to complex physicochemical processes involving moisture, which directly impacts the stability of carotenoids in the matrix. During the transition from week 14–16, there was an increase in total carotenoid content. This phenomenon can be partially explained by the presence of moisture in the storage environment.

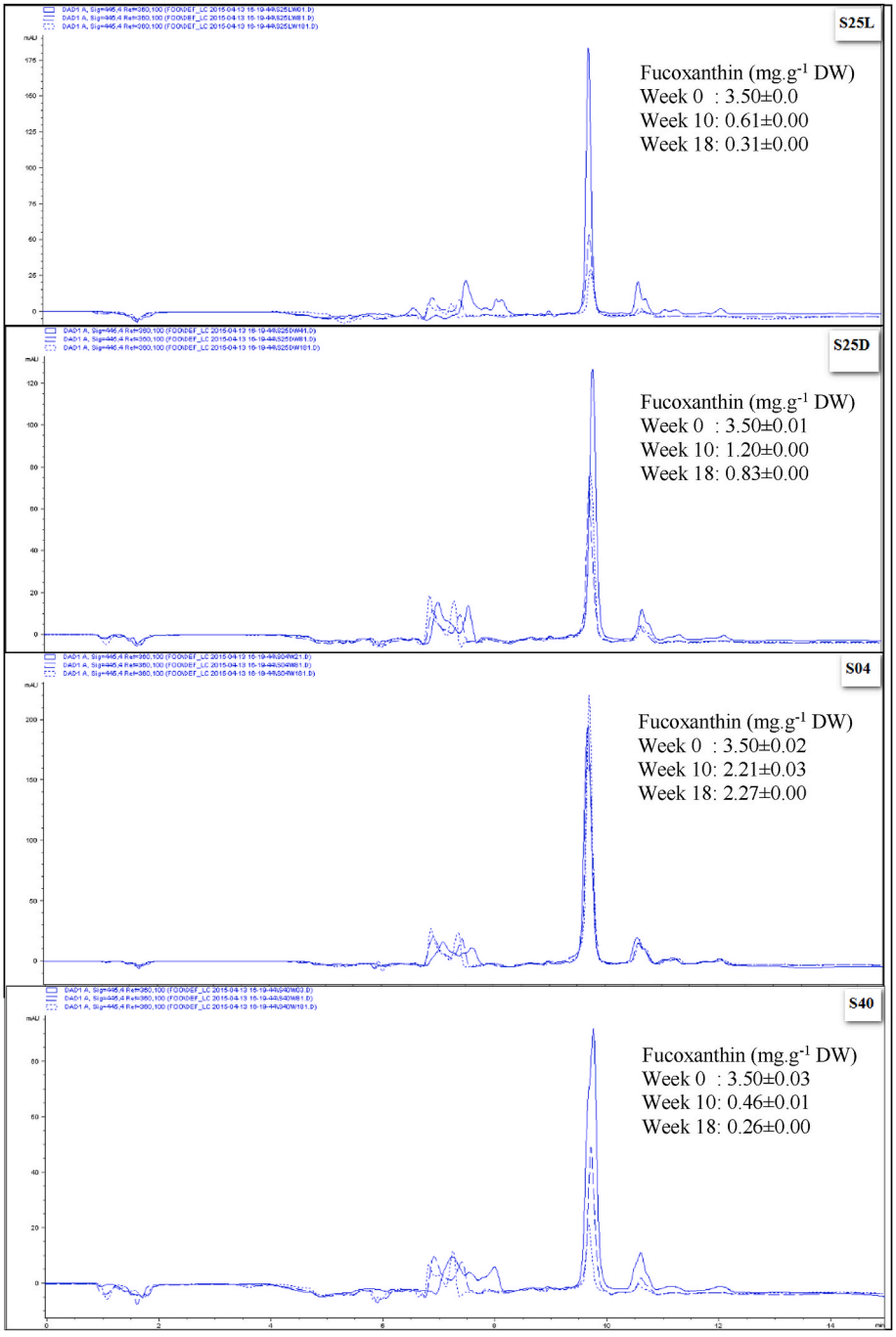


Fig. 7. Representative HPLC chromatogram overlay of spray dried microcapsules kept at selected storage conditions. A gradual reduction in fucoxanthin quantity is observed from week 0 (solid line), week 10 (dashed line) to week 18 (dotted line).

Moisture, particularly in the form of water vapor, can act as a protective barrier by limiting the exposure of carotenoids to degradation factors such as oxygen and light. In a slightly humid environment, carotenoids may be less prone to oxidation and photodegradation, resulting in the preservation of their content. This was demonstrated by Ramakrishnan et al. [37] explaining moisture at a certain level could exert protective influence by forming a barrier due to the extension of monomolecular rate. Indeed, the moisture content within the samples might have influenced the diffusion and migration of carotenoids within the microcapsules, potentially leading to an increase in their availability for carotenoid analysis during this period. Conversely, the subsequent decrease in total carotenoid content from week 16 to week 18 can be attributed to several factors, primarily the cumulative effects of moisture exposure. While moisture can initially have a protective effect as shown in week 14 to week 16, extended exposure can lead to undesirable consequences. Both this study and Beta et al. [38] observed a direct correlation between moisture and carotenoid degradation. By week 16, prolonged moisture contact facilitated the release of more carotenoid from cell wall structures, promoting chemical reactions that degrade carotenoids, possibly through hydrolysis and oxidation processes. These reactions may result in the breakdown of carotenoid molecules, leading to a decrease in the total carotenoid concentrations. It is also crucial to point out method limitations. The spectrophotometric method for quantifying total carotenoids is based on the principle of measuring the absorption of light by carotenoid molecules in a sample. While spectrometric techniques have their advantages, including simplicity and

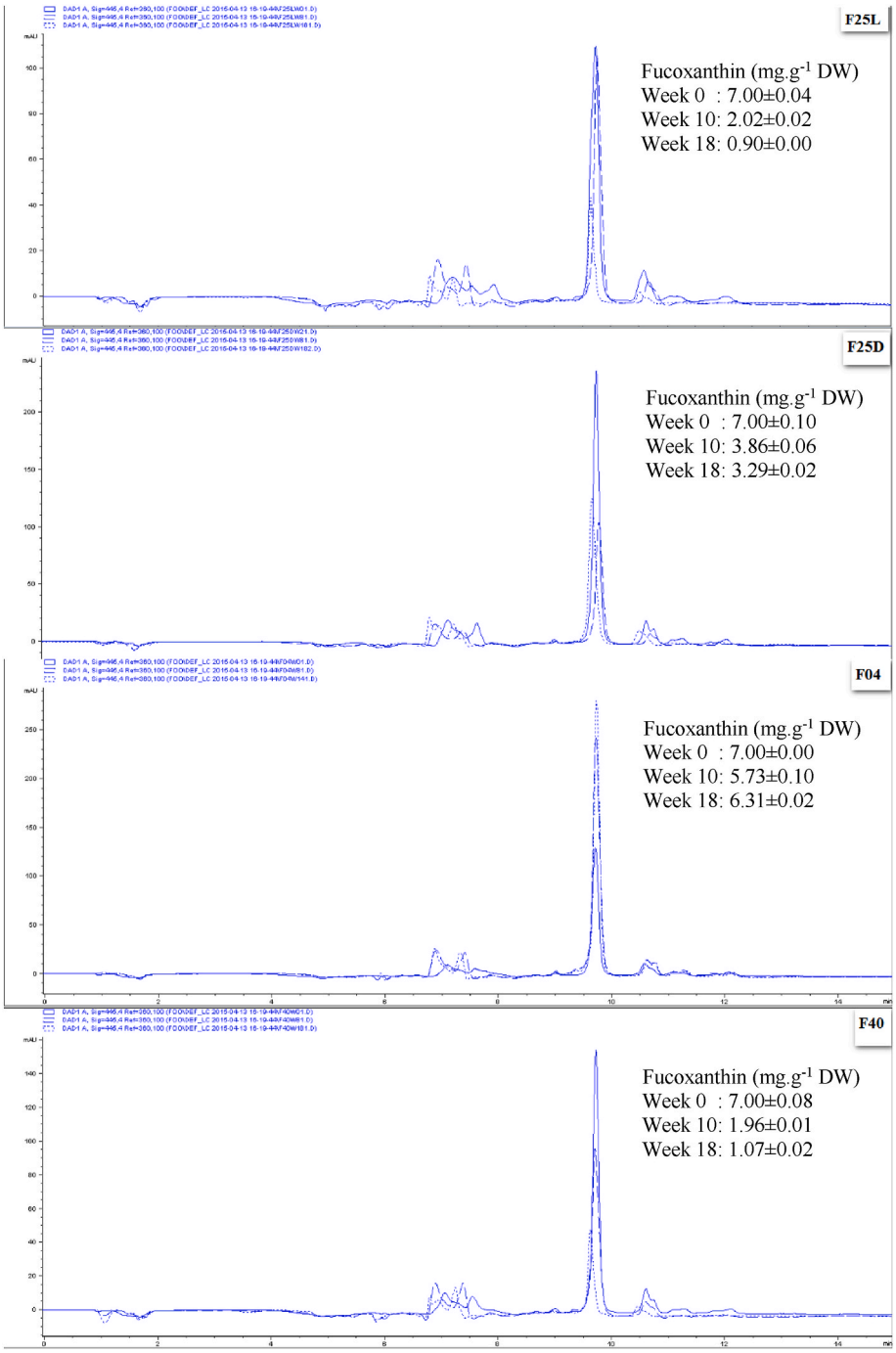


Fig. 8. Representative HPLC chromatogram overlay of freeze-dried microcapsules kept at selected storage conditions. A gradual reduction in fucoxanthin quantity is observed from week 0 (solid line), week 10 (dashed line) to week 18 (dotted line).

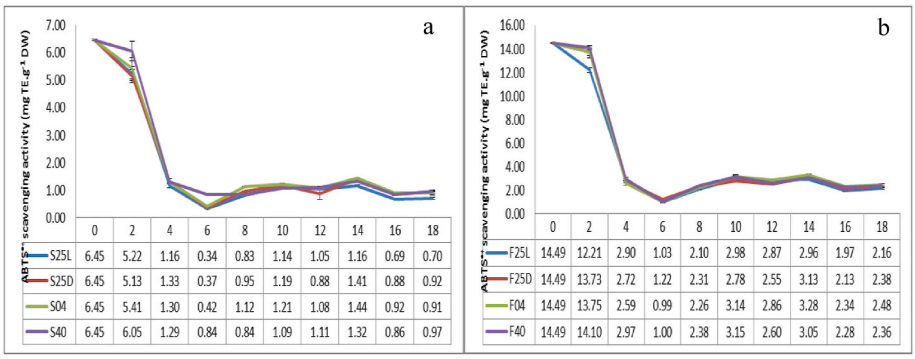
speed, they also come with limitations, particularly in terms of specificity, sensitivity, and the ability to quantify individual carotenoids accurately as compared to HPLC studies.

3.2.7. *Antioxidant activity*

Post storage, it was found that freeze-dried microcapsules exhibited significantly higher $ABTS^{\cdot+}$ scavenging activities compared to spray-dried microcapsules ($p < 0.05$) (Fig. 9). Firstly, S04 microcapsules showed the least reduction ($p < 0.05$) in radical scavenging activities than S40, S25D or S25L microcapsules (Fig. 9a). Secondly, F25D, F40 or F04 microcapsules experienced the least reduction in radical scavenging activities ($p < 0.05$) as compared to the F25L microcapsules (Fig. 9b). Notably, F04 microcapsules (0.70 mgTE.g^{-1} DW) exhibited 3.5 times higher ABTS radical scavenging activity as compared to S25L microcapsules (2.48 mgTE.g^{-1} DW). Once again, the type of storage conditions is an important factor in prolonging carotenoid shelf life and its' activities. Overall, the decrease of $ABTS^{\cdot+}$ scavenging activities could be attributed to the reduction of fucoxanthin over time, as reflected by the highly significant Pearson relationship between fucoxanthin and antioxidant activity ($R^2 = 0.954$, $p < 0.05$) (Fig. 10).

3.2.8. *Degradation kinetic studies*

The degradation kinetics of studied microcapsules followed a first order kinetics reaction order, except for the microcapsules stored at 4 °C



	0	2	4	6	8	10	12	14	16	18
S25L	6.45	5.22	1.16	0.34	0.83	1.14	1.05	1.16	0.69	0.70
S25D	6.45	5.13	1.33	0.37	0.95	1.19	0.88	1.41	0.88	0.92
S04	6.45	5.41	1.30	0.42	1.12	1.21	1.08	1.44	0.92	0.91
S40	6.45	6.05	1.29	0.84	0.84	1.09	1.11	1.32	0.86	0.97

	0	2	4	6	8	10	12	14	16	18
F25L	14.49	12.21	2.90	1.03	2.10	2.98	2.87	2.96	1.97	2.16
F25D	14.49	13.73	2.72	1.22	2.31	2.78	2.55	3.13	2.13	2.38
F04	14.49	13.75	2.59	0.99	2.26	3.14	2.86	3.28	2.34	2.48
F40	14.49	14.10	2.97	1.00	2.38	3.15	2.60	3.05	2.28	2.36

Fig. 9. Progression of antioxidant activities (ABTS·$^{+}$ radical scavenging) in spray dried (a,b) and freeze dried microcapsules (c,d) at different storage conditions. **S25L is spray dried capsules stored under 25 °C light conditions, S25D is spray dried microcapsules stored under 25 °C dark conditions, S04 is spray dried microcapsules stored under 4 °C dark, S40 is spray dried microcapsules stored under 40 °C dark; F25L is freeze dried microcapsules stored under 25 °C light conditions, F25D is freeze dried microcapsules stored under 25 °C dark conditions, F04 is freeze dried microcapsules stored under 4 °C dark and F40 is freeze dried microcapsules stored under 40 °C dark. Error bar denotes standard error of the means (n = 3).

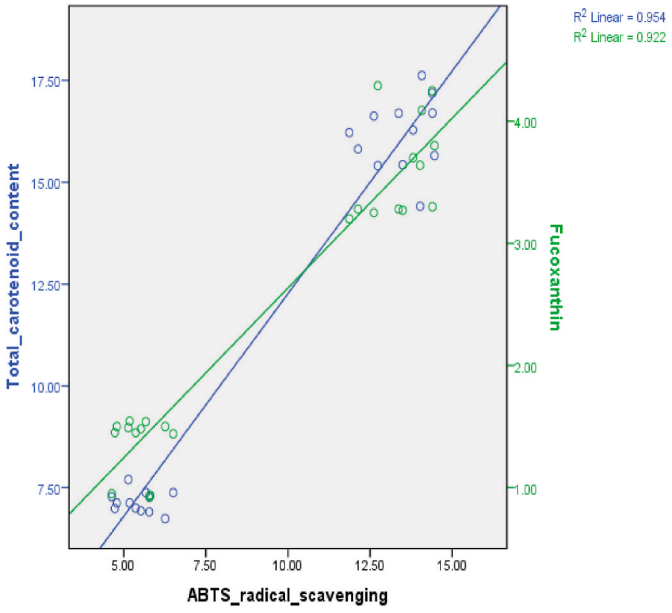


Fig. 10. Strong correlation between total carotenoids (R^2 = 0.954) and fucoxanthin (R^2 = 0.922) to ABTS·$^{+}$ scavenging activity indicates positive relationship.

(S04 and F04). This was because S04 and F04 microcapsules did not undergo significant degradation to considerably lower their carotenoid content. First order degradation kinetics has been reported in numerous carotenoids including astaxanthin [13], beta-carotene [18] and fucoxanthin [39]. Rate constant, K and half-life, $t_{1/2}$ (day) of each microcapsule were tabulated in Table 4. Half-life, $t_{1/2}$ is a measure of number of time (in days) needed for the carotenoid sample to reach half of its original concentration. It was found S25L microcapsules exhibited highest degradation with rate constant (day^{-1}) and half-life ($t_{1/2}$) of 0.009 ± 0.001 and 76.40 ± 7.08 days respectively. This was significantly different ($p < 0.05$) from F25L microcapsules which accounted a lower rate constant, 0.007 ± 0.001 day^{-1} and longer half-life of 93.69 ± 9.21 days. As such when microcapsules were ordered from least to highest in terms of kinetic degradation, it was found F04>S04>F25D > S25D > F40>S40>F25L > S25L. From here, it was proposed spray-dried microcapsules showed faster degradation rate than freeze-dried microcapsules. The better carotenoid retention in freeze-dried microcapsules could be explained by its processing conditions. The low temperature employed in the end to end freeze-drying process were effective in

Table 4
Linear regression parameters for first order kinetics degradation, rate of degradation (day^{-1}) and half life, $t_{1/2}$ (day) of samples for carotenoids and fucoxanthin respectively.

	Carotenoids				
Sample	Y = mx + c	R^2	Reaction order	Rate constant (day^{-1})	Half life, $t_{1/2}$ (day)
S25L	Y = −0.0087x+4.3848	0.9774	First	0.0091	76.22
S25D	Y = −0.0036x+4.347	0.7231	First	0.0045	155.40
S04	–	–	–	0.0016	442.75
S40	Y = −0.00059x+4.2594	0.7963	First	0.0063	110.70
F25L	Y = −0.0065x+4.4274	0.9298	First	0.0074	93.69
F25D	Y = −0.0016x+4.36	0.5155	First	0.0025	280.84
F04	–	–	–	0.0004	1705.66
F40	Y = −0.0041x+4.319	0.8841	First	0.0051	135.44

Note: S25L is spray dried capsules stored under 25 °C light conditions; S25D is spray dried capsules stored under 25 °C dark conditions; S04 is spray dried capsules stored under 4 °C dark; S40 is spray dried capsules stored under 40 °C dark, F25L is freeze dried capsules stored under 25 °C light conditions; F25D is freeze dried capsules stored under 25 °C dark conditions; F04 is freeze dried capsules stored under 4 °C dark and; F40 is freeze dried capsules stored under 40 °C dark.

reducing fucoxanthin's exposure to various degradative elements. Fig. 11 illustrates the first-order kinetics plot of spray-dried microcapsules and freeze-dried microcapsules. When ranked from least to most carotenoid degradation, storing microcapsules at 4 °C dark retained the most carotenoids followed by 25 °C dark and 40 °C dark. Storing fucoxanthin microcapsules at 25 °C light regardless if prepared by freeze drying or spray drying were not recommended as reflected by highest amount of carotenoid losses.

In summary, this study findings on the effects of long-term storage of microencapsulated fucoxanthin powders will prove highly valuable to various stakeholders involved in the production, manufacturing, distribution, quality assurance, and consumption of products containing fucoxanthin. Firstly, the study highlights that freeze-drying (F04) is a superior method for microencapsulation compared to spray drying (S25L) in terms of retaining fucoxanthin and antioxidant activity. This information can guide manufacturers in choosing the most effective production method for fucoxanthin-based products. Secondly, affirming that fucoxanthin microcapsules should be stored at 4 °C in the dark will help distributors maintain the quality and stability of their products

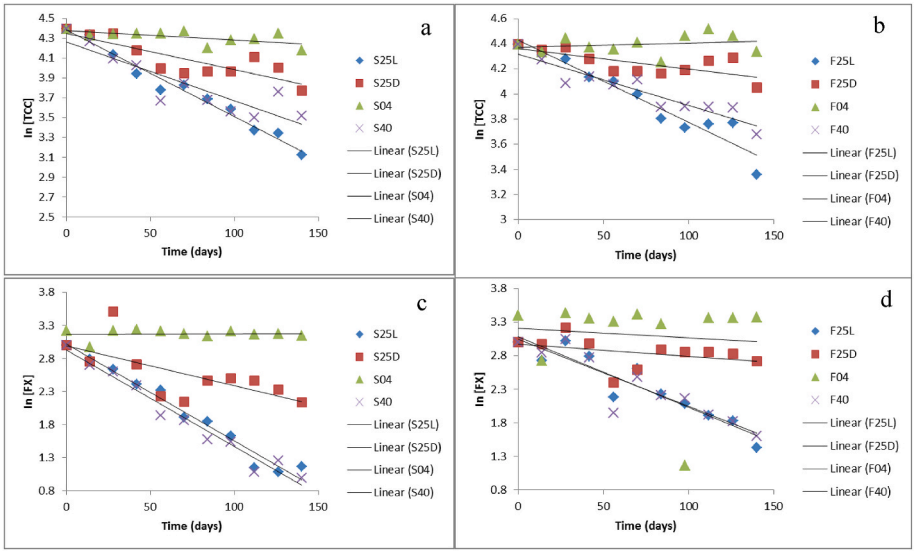


Fig. 11. First order kinetics plot of total carotenoids (a,b) and fucoxanthin (c,d) degradation in spray dried and freeze dried microcapsules respectively. Microcapsules stored at 4 °C refrigeration conditions experienced the least carotenoid loss. Whereas, microcapsules stored at 25 °C (light) lost the most carotenoids.

during transportation and storage. Also, quality assurance and regulatory bodies can benefit from understanding the optimal storage conditions and degradation kinetics, thereby assisting the establishment of guidelines and standards for fucoxanthin-containing products. This ensures that products on the market meet safety and quality requirements. Ultimately, consumers will benefit from higher quality fucoxanthin products that retain their carotenoid content and antioxidant activity for a longer period, providing them with the intended health benefits.

4. Conclusions

Overall, the employment of spray and freeze-drying techniques for the microencapsulation of FxRF was effective in slowing down degradation of the investigated carotenoid, fucoxanthin. Although all the microcapsules studied were found to be compliant to the recommended food powder standards in terms of the water content and activity, it was found that the recommended storage condition for carotenoid-rich microcapsules were ranked from best to least favourable as: 4 °C (dark) > 25 °C (dark) > 40 °C (dark) > 25 °C (light). Indeed, encapsulation improved the water solubility facilitating easy incorporation for food and nutraceutical applications. The fucoxanthin microcapsules' colour was found to be significantly affected following storage and degradation ($p<0.05$) and thus, Hunterlab values could be used as a rapid tool for carotenoid evaluation studies. SEM studies facilitated the understanding of morphological changes for surface of the microcapsules when stored under different conditions. Although spray-dried microcapsules had a higher WSI than freeze-dried microcapsules, the freeze-dried microcapsules showed better fucoxanthin and antioxidant retainment, as validated in the kinetic degradation studies. Pearson correlation studies confirmed that fucoxanthin was mainly accountable for the antioxidant activities in the microcapsules. Finally, this study open avenues for the exploration of new formulations in emerging food application from sustainable resources like microalgae.

Credit author statement

FSC: Conceptualization, Investigation, Data curation, analysis and software visualization, Writing-original draft preparation and editing; FMY: Writing-reviewing and editing; NMHK: Writing-reviewing and editing. All authors take full responsibility for the integrity of the work, from conception to finished article.

Submission declaration and verification

We declare that this manuscript is original, has not been published before nor is it currently under consideration for publication elsewhere.

Declaration of Generative AI and AI-assisted technologies in the writing process

Nothing to disclose.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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