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Effect of water activity on carotenoid degradation in dehydrated carrots

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Abstract

The effect of water activity on the stability of carotenoids in dehydrated carrots was studied.

Freeze-dried carrots from blanched and unblanched batches were placed in air-tight glass jars containing saturated salt solutions with water activity ranging from 0.052 to 0.75, at 40 °C. The equilibrium moisture, water activity, α- and β-carotene, and lutein contents were analysed at different storage times.

The Guggenheim–Anderson–de Boer (GAB) equation was applied to model experimental data for moisture content as a function of water activity, and to calculate the monolayer water activity, where the oxidative reactions are expected to be at minimum. Estimated monolayer water activity was 0.33 (confidential limits at 95%: 0.26 and 0.38). α- and β-carotene, and lutein degradation followed pseudo-first-order kinetics in all dehydrated carrots, with rate constants ranging from 0.031 to 0.374 days^{-1}. Similar rate constants were found between α- and β-carotene, whereas lutein degraded faster.

In both blanched and unblanched batches the rate of carotenoid degradation was at a minimum over the water activity range 0.31–0.54. Blanching resulted in a higher initial carotenoid content, but it accelerated carotenoid decrease during storage of dehydrated carrots.

Keywords: Carrot; Carotenoid; Water activity

1. Introduction

Vitamin A deficiency is a major cause of blindness in children particularly in developing countries. Dietary intervention with foods rich in pro-vitamin A, such as carrots, has been suggested as one of the solutions to this problem (El-Arab, Khalil, & Hussein, 2002). In addition carotenoids may also be beneficial in preventing major health problems such as cancer, cardiovascular/coronary heart diseases, and other diseases due to their antioxidant activity (Yeum & Russell, 2002).

Carrots are a good source of α- and β-carotene, and lutein. Up to now, β-carotene has been the most studied carotenoid. Beside its provitamin A activity other physiological roles such as cell-to-cell communication, immunomodulatory effect, and UV skin protection have been documented. Knowledge on the other carotenoids is rapidly expanding. The xanthophylls lutein and zeaxanthin are the only carotenoids present in the macula region of the retina, probably functioning as blue light filters and singlet oxygen quenchers (Van den Berg et al., 2000). Previous studies in our laboratory demonstrated that the carotenoid content of minimally-processed carrots did not decrease during storage, however, these products are degraded by microbial spoilage and accelerated metabolic activity (Lavelli, Pagliarini, Ambrosoli, Minati, & Zanoni, 2006; Zanoni et al., 2007).

Reduction of water activity (a_w) is reported to result in a longer shelf-life of carrots, though carotenoids degrade faster in dehydrated systems, through autocatalytic oxidation (Goldman, Horev, & Saguy, 1983). The influence of a_w on oxidation is complex (Gloria, Vale, & Bobbio, 1995; Jayathilakan, Sharma, Radhakrishna, & Bawa, 2007; Obara,

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Obiedzinski, & Kolczak, 2006). Increasing the water content in dry matrices may increase the rate of oxidation by enhancing the mobility of reactants and bringing catalysts into solution. As the solid matrix swells, new surfaces for catalysts are exposed. However, water may also slow down the oxidation process by hydrating or diluting heavy metal catalysts or precipitating them as hydroxides. Water may also counteract peroxide decomposition by hydrogen bonding with hydroperoxides, and encourage radical recombination which could interrupt the oxidation reaction chain. The net result of all these actions is that, in many foods, the rate of oxidation reaches a minimum in the a_w corresponding to the monomolecular moisture content (Brennan, 1994). Therefore, it is suggested that dehydrated foods should be stored at a monolayer a_w to decrease oxidative degradations and thus extend their shelf-life.

Knowledge on the water sorption properties of different food matrices would be helpful in order to predict the relative rate of oxidative degradations. Literature data on the sorption properties of carrots are incomplete and contradictory results have been reported on the monolayer a_w (Iglesias & Chirife, 1982; Kiranoudis, Maroulis, Tsami, & Marinos-Kouris, 1993). While studies on carotenoid stability have been mainly carried out on model systems simulating dehydrated foods, little information is available on carotenoid stability in carrots at intermediate a_w values.

This work was focused on dehydrated carrots with the aim to study: (a) the water sorption properties, and (b) the rate of carotenoid degradation as a function of a_w.

2. Materials and methods

2.1. Materials

Two batches of carrots were obtained from the local fruit and vegetable distribution center of Milan (Italy). Manually, they were sorted, peeled, washed with cold water, dripped, discarded of upper and lower ends and half-sliced. Half-carrots were cut in sticks "Julienne type" by a vegetable cutter (Mouli Julienne mod. A44506, Moulinex, Milan, Italy). The first batch (sample 1 UB) and a part of the second batch (sample 2 UB) of sticked carrots were not blanched whereas the other part of the second batch was blanched in boiling water for 1 min (sample 2 B). Blanched and unblanched carrots were dehydrated by freeze drying in a Lyoflex Edwards (Crawley, UK) apparatus.

2.2. Methods

2.2.1. Storage study

About 0.5 g of freeze-dried samples were placed into Petri dishes (4 cm diameter) to allow for a high surface area between air and powder during storage, and then into thermostated air-tight glass jars containing saturated salt solutions kept at 40 °C (Table 1) (Greenspan, 1977), since room temperature in a warm climate is 40 °C. The equilibrium moisture content was reached within 2 days.

Table 1
Water activity values for saturated salt solutions at 40 °C

Saturated salt solution	a_w
LiBr	0.0580 ± 0.0039
$ZnBr_2$	0.0754 ± 0.0020
LiCl	0.1121 ± 0.0021
KF	0.2268 ± 0.0081
$MgCl_2$	0.3160 ± 0.0013
NaBr	0.5317 ± 0.0041
KI	0.6609 ± 0.0023
NaCl	0.7468 ± 0.0013

As reported previously (Greenspan, 1977).

It may be hypothesized that prior to the equilibrium the water content was not homogeneous in carrots despite of a large surface area between carrots and air. Water gradients within the samples resulted in different carotenoid degradation rates, therefore, the non-equilibrium period was not studied. Samples were analyzed after two days of incubation, for zero time, and periodically for 30 days. Duplicate Petri dishes were removed from the jars for each measurement.

2.2.2. Moisture content and a_w

Moisture contents of carrots (n_s, kg of water/kg dry solids) were determined using a vacuum oven at 70 °C and 50 Torr for 6 h (AOAC, 1980). The a_w of carrots and saturated salt solutions was measured by a dew point hygrometer (Aqualab, Decagon Devices, WA, USA). Triplicate determinations were made for each sample.

2.2.3. Modelling of sorption isotherm

The Guggenheim–Anderson–de Boer (GAB) equation was applied to model experimental data for n_s as a function of a_w, as recommended by Spiess and Wolf (1987). The GAB model is expressed as indicated in Eq. (1):

$$n_s = n_{sm} C k a_w / [(1 - k a_w)(1 - k a_w + C k a_w)] \quad (1)$$

where n_s is the equilibrium moisture content on dry basis; n_{sm} is the monolayer moisture content on dry basis; C and k are related to the temperature effect.

2.2.4. Carotenoids

Carrots were blended by a Braun AG 4261 instrument, and 0.125 g (on dry weight basis) were added to 10 mL of tetrahydrofuran (THF) stabilized by the addition of 0.1% butylated hydroxytoluene (2,6-di-*tert*-butyl-*p*-cresol) (BHT). The mixture was kept refrigerated in an ice bath and mixed by an Ultra-Turrax homogenizer (T25 Janke & Kunkel IKA Labortechnik) under nitrogen at moderate speed for 2 min. The extract was centrifuged (12000*g* at 5 °C for 10 min), and residual solids were re-extracted with 10 mL of stabilized THF. The second extract was centrifuged (12000*g* at 5 °C for 10 min). The clarified THF extracts were quantitatively transferred into a volumetric

flask, and brought up to 25 mL with stabilized THF. Extractions were carried out in duplicate. Carotenoid content was analyzed by HPLC as described previously (Lavelli, Peri, & Rizzolo, 2000). In brief, a Vydac 201TP54 C18 column (250 mm × 4.6 mm), equipped with a C18 precolumn, was used. Chromatographic separation was performed with methanol/stabilized THF (95:5) as an eluent under isocratic conditions, 1.0 mL/min flow rate, at room temperature. UV–Vis detector was set at 454 nm. α- and β-carotene were quantified from a calibration curve built with pure β-carotene, and expressed as milligram β-carotene equivalents/kg carrots (on dry weight basis). Lutein was quantified from a calibration curve built with pure standard and expressed as milligram/kg carrots (on dry weight basis).

2.2.5. Statistical analysis of data

Experimental data were processed by one-way ANOVA using the least significant difference (LSD) as a multiple range test, and by regression analysis using Statgraphics 5.1 (STCC Inc.; Rockville, MD).

3. Results and discussion

3.1. Modelling of sorption isotherm

The experimental data for a_w as a function of n_s are shown in Fig. 1. GAB model among other mathematical model is recommended by the European COST 90 project on a_w to describe the sorption isotherm modelling of food material (Spiess & Wolf, 1987).

The experimental data of this study fitted well with the GAB equation ($R^2 = 99.65\%$). The values of n_{sm}, C, and k are calculated by regression analysis of Eq. (1), and shown in Table 2. The estimated value of n_{sm} was 0.066 kg/kg on dry weight basis, corresponding to the mean a_w of 0.33 and the confidence interval of 0.26–0.38 (on the 95% probability level).

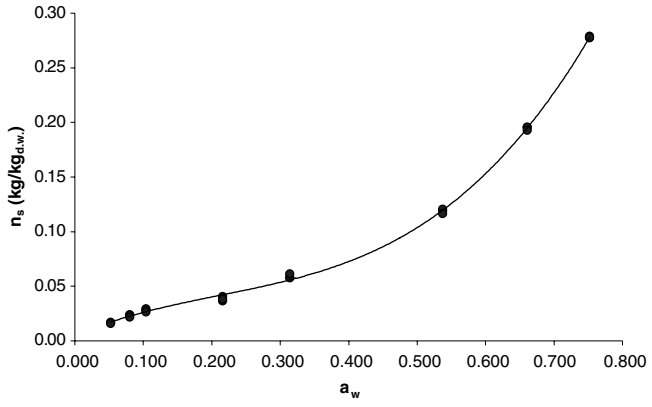

Fig. 1. Water sorption properties of carrots: (●), experimental data for freeze-dried carrots equilibrated over saturated salt solution in desiccator at 40 °C; (—), adsorption isotherm obtained by fitting experimental data with GAB model.

Table 2
Mathematical modelling of carrot sorption properties

Product specifications	n_{sm}	C	k	Source
Freeze-dried carrots at 40 °C	0.066 ± 0.04	3.6 ± 0.5	1.04 ± 0.02	This work
Air-dried carrots at 37 °C	0.052	n.d.	n.d.	Iglesias and Chirife (1982)
Fresh carrots at 37 °C	0.052	n.d.	n.d.	Iglesias and Chirife (1982)
Fresh carrots at 45 °C	0.21	3.9	0.66	Kiranoudis et al. (1993)

Carrots were equilibrated over saturated salt solutions in static desiccators. n_{sm} is the monolayer moisture content on dry basis, calculated either using the GAB equation (in this study and in that by Kiranoudis et al., 1993), or by using the Iglesias and Chirife equation (in the study by Iglesias and Chirife, 1982). C and k are GAB parameters related to the temperature effect.

Carrot sorption properties obtained in previous studies under similar temperature conditions are reported in Table 2 and compared with our data. Iglesias and Chirife (1982) studied carrot sorption properties under both desorption and adsorption conditions. Their equation was used to model experimental data and a monolayer moisture content of 0.052 kg/kg on dry weight basis was calculated, in both conditions. These data are in agreement with our data. On the contrary, in a third study reported by Kiranoudis et al. (1993), carrots were studied under desorption conditions only, and data were modelled by the GAB equation. A monolayer moisture content of 0.21 kg/kg on dry weight basis was calculated. Therefore carrots were found to be more hygroscopic than in our study. Since this difference is noticeable, we do not believe that is attributable to different maturity stage or to the carrot variety. We can conclude that more research is necessary to reach an universal consensus on how to evaluate the monolayer moisture content, which is reputed to be valuable to predict food oxidative stability.

3.2. a_w Dependence of the rate of carotenoid degradation

α and β-carotene, and lutein contents of freeze-dried carrot lots at the beginning of the storage study are reported in Table 3. These values were within the concentration range observed in fresh carrots (i.e. α-carotene: 259–654 mg/kg$_{d.w.}$; β-carotene: 303– 1005 mg/kg$_{d.w.}$; lutein:

Table 3
Initial content of α- and β-carotene in dehydrated carrots

Carrots	α-Carotene (mg/kg$_{d.w.}$)	β-Carotene (mg/kg$_{d.w.}$)	Lutein (mg/kg$_{d.w.}$)
1 UB	251 ± 5	578 ± 15	9.1 ± 0.5
2 UB	396 ± 5	475 ± 7	31.8 ± 1.5
2 B	599 ± 11	838 ± 16	59.4 ± 5.2

Two different carrot lots were used, identified as 1 and 2; UB, unblanched; B, blanched.

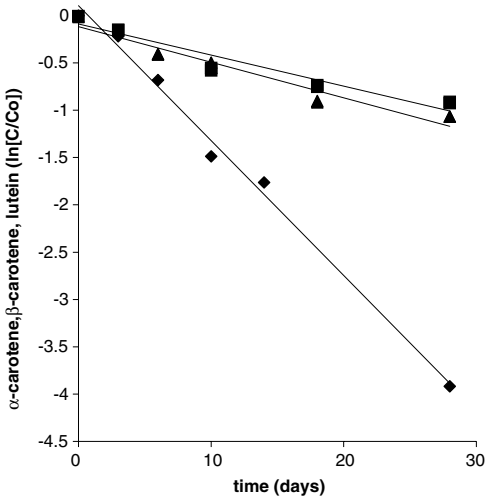

Fig. 2. Time course of the degradations of α-carotene (■), β-carotene (▲), and lutein (◆) in the carrot lot 1 UB, at a_w 0.537, at 40 °C. Data were fitted to first-order kinetics.

17–30 mg/$kg_{d.w.}$) (Hart & Scott, 1995; Sant'Ana, Stringheta, Brandao, & Cordeiro de Azeredo, 1998).

During storage of dehydrated carrots at 40 °C, carotenoid decreased by following pseudo-first-order kinetics. An example of the behavior of carotenoid is provided as illustrated in Fig. 2. A similar behaviour has also been found between α- and β-carotene, whereas lutein was found to degrade faster. The rate for lutein degradation was determined only at a_w 0.54 because of the rapid loss at other a_w values.

It was demonstrated that carrots retained about >90% of their initial α- and β-carotene content when stored under anaerobic conditions at 37 °C for 7 weeks. This suggests that the mechanism of degradation was likely direct oxidation of the carotenes without destabilization through isomerization (Wagner & Warthesen, 1995). The kinetic parameters for α- and β-carotene decrease are reported in the Tables 4 and 5, respectively.

First-order rate constants for α- and β-carotene, plotted against a_w showed an U-shaped curve typical of most oxidative reactions (Fig. 3). In fact a decrease in rate constants was observed with an increase in a_w up to about 0.314. In the a_w range of 0.341–0.537 carotenoids showed the maximum stability. In this a_w range microbial growth is arrested, enzymatic activity and non-enzymatic browning are at minimum (Labuza, 1971). Above these a_w values carotenoid stability decreased with a further increase in a_w up to 0.754. In the a_w range of 0.537–0.754 the microbial growth rate and the enzymatic activity are still at minimum; however the occurrence of non-enzymatic browning cannot be ruled out.

The a_w range corresponding to maximum carotenoid stability was next to the monolayer a_w, however it was not symmetrically located with the mean estimated value for monolayer; in fact, it showed a shift towards higher a_w values.

Table 4
Rate constants for α-carotene degradation in carrots stored at 40 °C as calculated by assuming pseudo-first-order kinetics

a_w	Lot 1 UB		Lot 2 UB		Lot 2 B	
	$k_{40\,°C}$ ($days^{-1}$)	R	$k_{40\,°C}$ ($days^{-1}$)	R	$k_{40°C}$ ($days^{-1}$)	R
0.052 ± 0.003	0.123 ± 0.023	−0.94	0.119 ± 0.021	−0.92	0.171 ± 0.015	−0.98
0.080 ± 0.003	0.115 ± 0.019	−0.95	0.107 ± 0.020	−0.91		
0.104 ± 0.003	0.101 ± 0.008	−0.98	0.087 ± 0.007	−0.98	0.173 ± 0.021	−0.96
0.216 ± 0.003	0.061 ± 0.005	−0.97	0.070 ± 0.009	−0.95	0.121 ± 0.008	−0.99
0.314 ± 0.003	0.055 ± 0.008	−0.97	0.053 ± 0.005	−0.97		
0.537 ± 0.003	0.031 ± 0.004	−0.94	0.042 ± 0.007	−0.93	0.057 ± 0.006	−0.97
0.661 ± 0.003			0.162 ± 0.017	−0.97		
0.752 ± 0.003			0.257 ± 0.021	−0.99	0.349 ± 0.016	−0.99

$\ln(C) = \ln(C_0) - kt$; R, correlation coefficient; $P < 0.01$. Two different carrot lots were used, identified as 1 and 2; UB, unblanched; B, blanched.

Table 5
Rate constants for β-carotene degradation in carrots stored at 40 °C as calculated by assuming pseudo-first-order kinetics

a_w	Lot 1 UB		Lot 2 UB		Lot 2 B	
	$k_{40\,°C}$ ($days^{-1}$)	R	$k_{40\,°C}$ ($days^{-1}$)	R	$k_{40\,°C}$ ($days^{-1}$)	R
0.052 ± 0.003	0.130 ± 0.023	−0.94	0.137 ± 0.019	−0.95	0.197 ± 0.016	−0.98
0.080 ± 0.003	0.121 ± 0.017	−0.96	0.126 ± 0.023	−0.91		
0.104 ± 0.003	0.123 ± 0.007	−0.99	0.104 ± 0.007	−0.99	0.189 ± 0.020	−0.97
0.216 ± 0.003	0.089 ± 0.003	−0.99	0.082 ± 0.009	−0.96	0.133 ± 0.010	−0.98
0.314 ± 0.003	0.060 ± 0.007	−0.97	0.062 ± 0.006	−0.97		
0.537 ± 0.003	0.040 ± 0.002	−0.98	0.048 ± 0.007	−0.95	0.065 ± 0.007	−0.97
0.661 ± 0.003			0.153 ± 0.015	−0.97		
0.752 ± 0.003			0.271 ± 0.026	−0.98	0.374 ± 0.021	−0.99

$\ln(C) = \ln(C_0) - kt$; R, correlation coefficient; $P < 0.01$. Two different carrot lots were used, identified as 1 and 2; UB, unblanched; B, blanched.

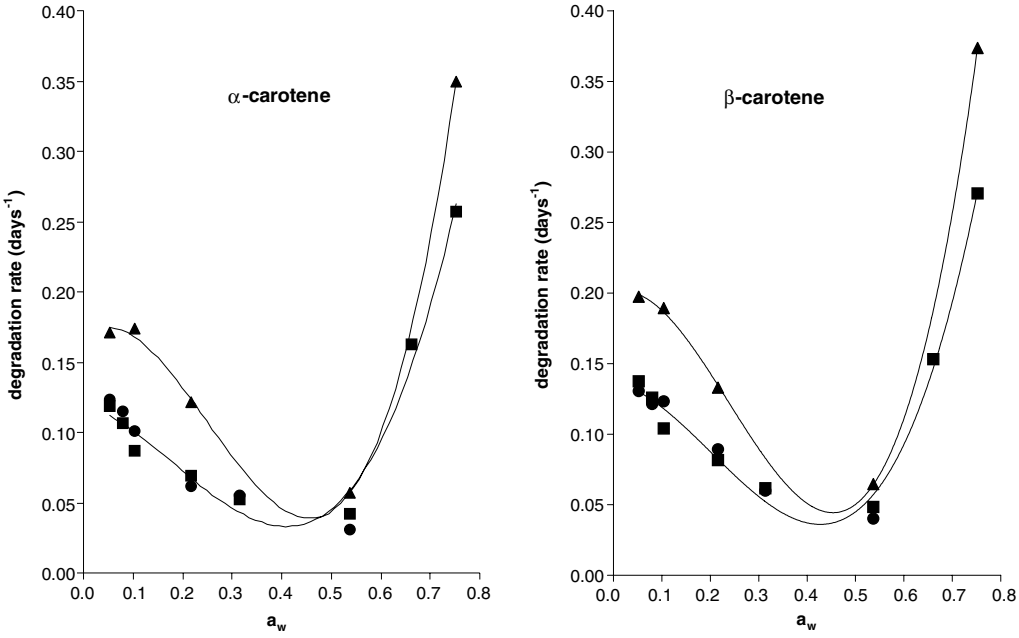

Fig. 3. Observed and fitted values of the first-order rate constants for α and β-carotene degradation in dehydrated carrots at various a_w, at 40 °C. Symbols represent lots 1 UB (●), 2 UB (■), and 2 B (▲).

Arya, Natesan, Parihar, and Vijayaraghavan (1979) reported that in dehydrated carrots, stored in the a_w range 0.0–0.73, total carotenoids, as measured spectrophotometrically, were more stable in the a_w range of 0.32–0.57. The rate constants for carotenoid degradation and the monolayer a_w were not reported in this latter study. Similarly, in microcrystalline cellulose: β-carotene model system, β-carotene degradation was at minimum at the monolayer a_w of 0.31 and at a_w of 0.51 compared to a_w of 0.11; other a_w values were not assayed (Baloch, Buckle, & Edwards, 1977). Using the same model system, Goldman et al. (1983) found that carotenoid degradation was lower at a_w 0.33 than in "dry conditions" (a_w not specified).

3.3. Blanching effect on carotenoid content

As shown in Table 3, the initial carotenoid content of lot 2 UB was lower than that of lot 2 B, due to the stabilizing effect of blanching on carotenoids, which had already been observed (Arya et al., 1979). This effect is generally believed to be due to the inactivation of peroxidase and lipoxidase activity. These enzymes can act during the dehydration process until substrate mobility becomes a limiting factor for catalytic activity. However, the rate of carotenoid degradation was higher in lot 2 B than in lot 2 UB (Fig. 2). This has suggested that some substances which are responsible to stabilize the carotenoids are either degraded or leached during blanching (Arya et al., 1979). Alternatively, it may also be argued that blanching causes physical damage to tissues by which it became highly proned for oxidation (Gomez, Toledo, Wadso, Gekas, & Sjoholm, 2004). However, despite the mechanism reported further investigations are necessary to optimise blanching in order to maximize carotenoid retention in dehydrated carrots.

3.4. Rate of carotenoid degradation in carrots as compared to other matrices

The degradation of pure β-carotene is reported to follow pseudo-zero-order kinetics in cyclohexane and ethanol, with rate constants 19.1 and 22.1 days^{-1}, respectively, at 35 °C (Minguez-Mosquera & Jaren-Galan, 1995) and indicating that β-carotene is found to be very unstable.

In microcrystalline cellulose powder, β-carotene is found to have enhanced stability (Baloch et al., 1977). In fact, at a_w of 0.31, under 75% N_2 and 25% O_2 as a storage atmosphere, the first-order rate constant for β-carotene degradation is 0.070 days^{-1} at 37 °C. The stability of β-carotene is considerably enhanced by SO_2 addition (k at 37 °C = 0.0036 days^{-1}) or by O_2 exclusion in the atmosphere (k at 37 °C = 0.022 days^{-1}).

Encapsulation of β-carotene with maltodextrin is another means to protect carotenoids from oxidation (Desobry, Netto, & Labuza, 1998; Wagner & Warthesen, 1995). Studies indicated that the higher dextrose equivalent (DE) starch forms a tighter and more gas impermeable matrix and provides a greater carotenoid stability. In fact, the first-order rate constants for encapsulated β-carotene in the a_w range 0.154–0.178, under air, at 40 °C, are 0.031 and 0.014 days^{-1} in 4 DE and 36.5 DE powders, respectively (Wagner & Warthesen, 1995). On the other hand, hygroscopicity is also dependent on the DE. In general, the higher the DE, the higher the hygroscopicity, which can lead to moisture uptake during storage. Therefore, it was decided to use a 25 DE maltodextrin as an encapsulat-

ing agent (Desobry, Netto, & Labuza, 1997). The first-order rate constants of β-carotene in this latter system ranged from 0.027 to 0.044 $days^{-1}$, depending on the drying technique (Desobry et al., 1998).

Our data showed that in the maximum stability a_w range (0.341–0.537) the first-order rate constants for carotenoid degradation in freeze-dried carrots were comparable with those observed by the other authors with maltodextrin or microcrystalline cellulose as matrices. It may be hypothesized that the protein–carotenoid complexes which are present in raw carrots (Desobry et al., 1998) could be unaffected by freeze-drying and thus account for this stabilizing effect.

4. Conclusions

The results of this study lead to some practical points about processing and storage conditions required to maintain high carotenoid contents in dehydrated carrots. Partial dehydration of carrots to intermediate moisture levels could be proposed instead of removing water completely, according to the following protocols:

(a) reduction of a_w values to 0.31–0.54, corresponding to 6–11% of moisture (on wet weight basis). In this a_w range microbial growth is arrested, enzymatic activity and non-enzymatic browning are at minimum, and our data indicate maximum carotenoid stability;
(b) alternatively, reduction of a_w values to 0.54–0.75, corresponding to 11–22% of moisture (on wet weight basis). In this a_w range the microbial growth rate and the enzymatic activity are still at minimum; however, the most effective factors which account for carotenoid stability are still to be investigated. Furthermore, the occurrence of non-enzymatic browning cannot be ruled out.

Both criteria should be combined with optimised packaging conditions, which reduce exposure of product to air and light during storage.

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