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Rate of Carotenoid Degradation in Dehydrated Carrots

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Abstract

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 water activity.

Freeze-dried carrots from blanched and not blanched 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, and carotenoid content were analysed at different storage times.

Results showed that the adsorption isotherm fitted the Guggenheim-Anderson-de-Boer (GAB) equation (R^2 = 99.65%). Estimated monolayer water activity was 0.33 (confidential limits at 95%: 0.26 and 0.38).

α- and β-Carotene contents decreased in all dehydrated carrots following pseudo-first-order kinetics, with rate constants ranging from 0.031 to 0.374 $days^{-1}$. Similar rate constants were found between α- and β-carotene.

In all carrot batches the rate of carotenoid degradation was at a minimum within the water activity range 0.31 - 0.54. Below and above this range the rate of carotenoid degradation increased significantly.

Blanching resulted in a higher initial carotenoid content, but it accelerated carotenoid decrease during storage of dehydrated carrots.

Based on these results we concluded that two strategies could be useful to increase carotenoid stability during storage:

a) the development of intermediate moisture carrots (water activity in the range 0.31 - 0.54);
b) the identification of protective factors that can increase carotenoid stability at water activity above 0.54.

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

Keywords: carrot, water activity, GAB model, carotenoid

Introduction

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

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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 et al., 2006; Zanoni et al., 2006).

Reducing the water activity (a_w) results in a longer shelf-life of carrots, though carotenoids degrade faster in dehydrated systems, through autocatalytic oxidation (Goldman et al., 1983). The influence of a_w on oxidation is complex. 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 up oxidation 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 (Kiranoudis et al., 1993; Iglesias and Chirife, 1982). 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.

Materials and Methods

Carrots. Two batches of carrots were obtained from the wholesale 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 NB) and a part of the second batch (sample 2 NB) 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 not-blanched carrots were dehydrated by freeze drying in a Lyoflex Edwards (Crawley, UK) apparatus.

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 (Table 1), at 40°C. The equilibrium moisture content was reached within 2 days.

It may be hypothesized that prior to the equilibrium the water content was not homogeneous in the carrots, in spite of a large surface area between the carrots and the air. Water gradients within the samples result in different carotenoid degradation rates, therefore, the non-equilibrium period was not studied. Samples were analyzed

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after two days of incubation, for zero time, and periodically for 30 days. Duplicate Petri dishes were removed from the jars for each measurement.

Table 1. Water Activity Values for Saturated Salt Solutions at 40°C

Saturated salt solution	a_w[a]
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

[a]As reported by Greenspan (1977)

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

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 follows:

$$n_s = n_{sm}Cka_w/[(1 - ka_w)(1 - ka_w + Cka_w)] \quad (1)$$

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 through the equations:

$$C = C_o\exp[(H_m - H_n)/RT] \quad (2)$$

$$k = k_o\exp[(H_l - H_n)/RT] \quad (3)$$

where T is the absolute temperature (K), R is the universal gas constant (J/molK), H_m and H_n are heat of condensation of mono-and multi-layers of water (J/mol), respectively, H_l is the heat of condensation of water vapour (J/mol), C_o and k_o are constants.

n_{sm}, C, and k were obtained by nonlinear regression analysis of experimental data using eq. (1).

The GAB equation was also used by Kiranoudis et al. (1993) to process sorption isotherms of carrots at different temperatures. Eqs. (2) and (3) were applied to calculate the values of C and k at 40°C, according to the values of C_o, k_o, H_l, H_m and H_n reported by Kiranoudis et al. (1993). C, K, and the value of n_{sm} reported by Kiranoudis et al. (1993) were then processed by eq. (1) and the resulting desorption isotherm was plotted in Figure 1.

The Iglesias and Chirife's equation was used by Iglesias and Chirife (1982) to process sorption isotherms. This equation is expressed as follows:

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$$\ln[n_s + (n_s^2 + n_{s0.5})^{1/2}] = B_1 a_w + B_2 \quad (4)$$

where B_1 and B_2 are parameters to be calculate by nonlinear regression analysis, and $n_{s0.5}$ is the moisture content at $a_w = 0.5$. The values of $n_{s0.5}$, B_1, and B_2 reported by Iglesias and Chirife (1982) were processed by eq. (4) and resulting desorption isotherm was plotted in Figure 1.

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 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 (12,000×*g* at 5°C for 10 min), and residual solids were re-extracted with 10 mL of stabilized THF. The second extract was centrifuged (12,000×*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 et al., 2000). Briefly, 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 by a calibration curve built with pure β-carotene, and expressed as mg β-carotene equivalents/kg carrots (on dry weight basis).

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).

Results

Modelling of sorption isotherm

The experimental data for a_w as a function of n_s are shown in Figure 1. Several mathematical models have been reported in literature to describe water sorption isotherms of food materials. Among them the GAB model (equation (1) under the Materials and Methods session) was recommended by the European COST 90 project on water activity to standardize sorption isotherm modelling (Spiess and Wolf, 1987). Indeed, the experimental data fitted well the GAB equation (R^2 = 99.65%). The values of n_{sm}, C, and k, calculated by regression analysis of eq. (1), are shown in Table 2. 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).

Carrot sorption isotherms obtained in previous studies under similar temperature conditions are plotted in Figure 1 to allow comparison with our data, and the corresponding parameters of isotherm equations are reported in Table 2. An agreement between our absorption isotherm and that reported by Iglesias and Chirife (1982) to describe both absorption and desorption phenomenon in carrots was found, particularly at the higher a_w values. On the contrary, according to Kiranoudis et al.

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(1993) carrots were more hygroscopic, i.e. at each a_w more water removal was required with respect to our data.

Table 2 Mathematical Modelling of Carrot Sorption Properties[a]

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	observed
Air-dried carrots at 37°C	0.051	n.d.	n.d.	Iglesias and Chirife, 1982
Fresh carrots at 37°C	0.051	n.d.	n.d.	Iglesias and Chirife, 1982
Fresh carrots at 45°C	0.21	3.9	0.66	Kiranoudis et al., 1993

[a]Carrots were equilibrated over saturated salt solutions in static desiccator

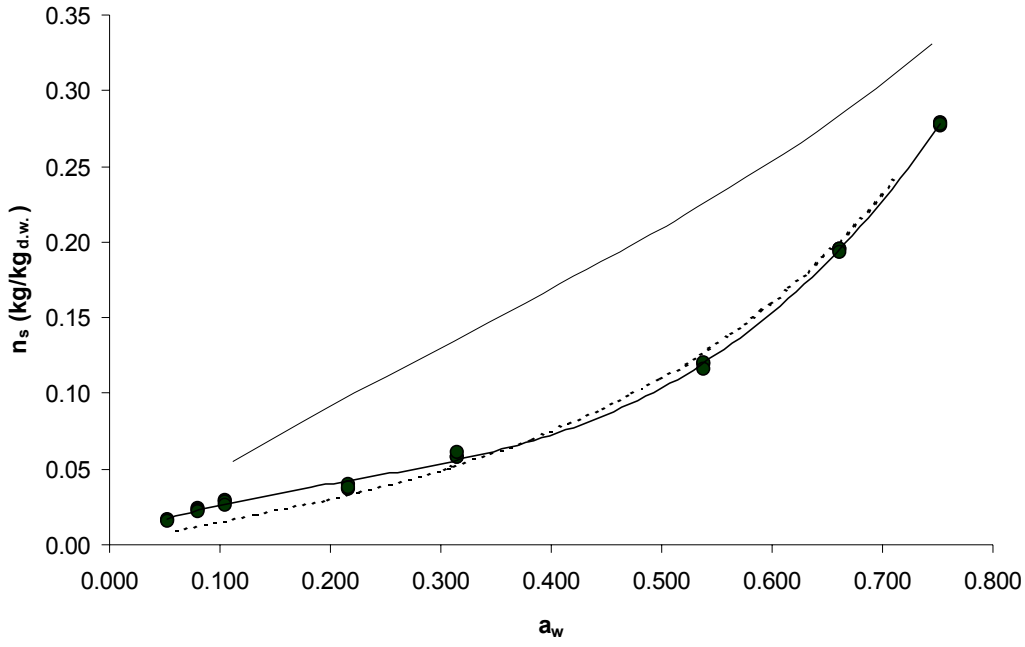

Figure 1. Water sorption properties of carrots: (•), experimental data for freeze dried carrots equilibrated over saturated salt solution in desiccator at 40°C; (▬), adsorption isotherm obtained by fitting experimental data with GAB model; (—) desorption isotherm of carrots at 40°C based on data by Kiranoudis et al., 1993; (---) absorption and desorption isotherm of carrots at 37°C based on data by Iglesias and Chirife, 1982.

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A_w dependence of the rate of carotenoid degradation

α e β-carotene 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/$kg_{d.w.}$ (Sant'Ana et al., 1998).

Table 3. Initial Content of α- and β-Carotene in Dehydrated Carrots.

Carrots[a]	α- carotene (mg/$kg_{d.w.}$)	β-carotene (mg/$kg_{d.w.}$)
1 NB	251 ± 5	578 ± 15
2 NB	396 ± 5	475 ± 7
2 B	599 ± 11	838 ± 16

[a]1,2 are to two different carrot batches; NB, not blanched; B, blanched.

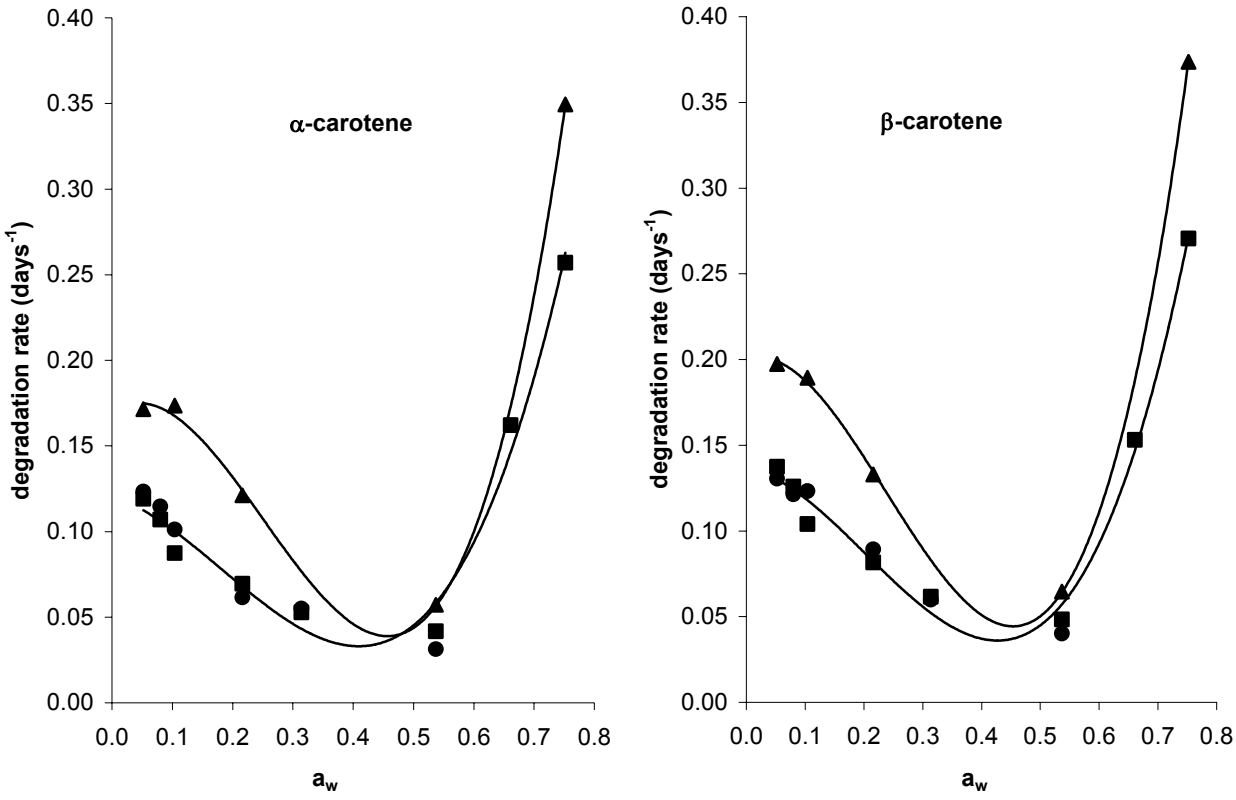

Figure 2. Observed and fitted values of the first-order rate constants for α and β-carotene degradation in dehydrated carrots at various a_w, at 40°C. Symbols represent lots 1 NB (●), 2 NB (■) , and 2 B (▲).

During storage of dehydrated carrots at 40°C, α- and β-carotene decreased following pseudo-first-order kinetics. Similar behaviour was found between α- and β-carotene. First-order rate constants for α- and β-carotene, plotted against a_w showed

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an U-shaped curve typical of most oxidative reactions (Labuza, 1971) (Figure 2). 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. Above these a_w values carotenoid stability decreased with a further increase in a_w up to 0.754. 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. Accordingly, Arya et al. (1979) found 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.

Blanching effect on carotenoid content

As shown in Table 3, the initial carotenoid content of lot 2 NB 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 NB (Figure 2). This suggests that some substances which stabilize 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 which become more exposed to oxidation (Gomez et al., 2004). Whatever be the mechanism, further investigations are necessary to optimise blanching in order to maximize carotenoid retention in dehydrated carrots.

Rate of carotenoid degradation in carrots as compared to other matrices

Table 4 presents the results obtained by several authors on the kinetics of β-carotene degradation in solvents and in some dehydrated systems, in darkness at about 40°C. Pure β-carotene is very unstable; the pseudo-zero-order rate constants in cyclohexane and ethanol and the pseudo-first-order rate constant in water, at 35°C are 19.1, 22.1, and 0.12 $days^{-1}$, respectively (Minguez-Mosquera and Jaren-Galan, 1995).

In microcrystalline cellulose powder, β-carotene stability is enhanced (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 (Wagner and Warthesen, 1995; Desobry et al., 1997). 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 and 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 encapsulating agent (Desobry et al., 1998). The first-order rate constants of β-carotene in this latter system range from 0.027 to 0.044 $days^{-1}$, depending on the drying technology (Desobry et al., 1997).

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Our data showed that carrots naturally provide protection for carotenoids. In fact, in the maximum stability a_w range (0.341 - 0.537) the first-order rate constants for carotenoid degradation were comparable with those observed by the other authors with maltodextrin or microcrystalline cellulose as matrices.

Table 4. Rate Constants for β-Carotene Degradation in Solvents and Dehydrated Systems in Darkness

Model systems		
β-carotene in solvents (Minguez-Mosquera and Jaren-Galan, 1995)		
	Storage conditions	k ($days^{-1}$)
cyclohexane	35°C	19.1[a]
ethanol	35°C	22.6[a]
water	35°C	0.12[b]
β-carotene in microcrystalline cellulose (Baloch et al., 1977)		
	Storage conditions	($days^{-1}$)
non sulphited	a_w 0.31, 75% N_2 25% O_2, 37°C	0.070[b]
non sulphited	a_w 0.31, 100% N_2, 37°C	0.022[b]
sulphited (2050 ppm)	a_w 0.31, 75% N_2 25% O_2, 37°C	0.0036[b]
Spray-dried carrot juice + 20% hydrolyzed starch (Wagner and Warthesen, 1995)		
	Storage conditions	k ($days^{-1}$)
4 DE	a_w 0.154 - 0.178, air, 40°C	0.031[b]
25 DE	a_w 0.154 - 0.178, air, 40°C	0.025[b]
36.5 DE	a_w 0.154 - 0.178, air, 40°C	0.014[b]
β-carotene in 25 DE maltodextrin (Desobry et al., 1997)		
	Storage conditions	k ($days^{-1}$)
spray-dried	a_w 0.32, air, 40°C	0.044[b]
drum-dried	a_w 0.32, air, 40°C	0.027[b]
freeze-dried	a_w 0.32, air, 40°C	0.044[b]
Freeze-dried carrots (observed)		
	Storage conditions	k ($days^{-1}$)
not blanched	a_w 0.537, air, 40°C	0.044[b]
not blanched	a_w 0.314, air, 40°C	0.061[b]
blanched	a_w 0.537, air, 40°C	0.065[b]

[a] pseudo zero-order kinetics: $C/C_0 = 1 - kt$
[b] pseudo first-order kinetics: $\ln(C/C_0) = - kt$

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 rules:

a) reducing 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 (Labuza, 1971), and our data indicate maximum carotenoid stability;
b) alternatively, reducing 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 can not be ruled out (Labuza, 1971).

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

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