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Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices

K. Selim[a], M. Tsimidou[b], C.G. Biliaderis[c,*]

[a]Mediterranean Agronomic Institute of Chania (MAICH), Alsillion Agrokipiou, Chania, 73100 Crete, Greece
[b]Laboratory of Food Chemistry and Technology, Faculty of Chemistry, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece
[c]Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54006, Thessaloniki, Greece

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Abstract

Kinetic studies on degradation of saffron water soluble carotenoids (mainly crocins) encapsulated in three different amorphous matrices (pullulan and two polyvinylpyrrolidone, PVP, samples differing in their molecular weight) were carried out at different water activity (a_w) conditions (0.43, 0.53, 0.64 and 0.75) in the dark at 35°C. Degradation of the polar pigments was monitored by periodic measurements of the coloring strength. Among the polymeric matrices used as wall materials, which largely decreased the oxidation rates of crocetin glycosides, PVP 40 was the most effective carrier under all storage conditions. In the vicinity of the glass transition temperature (T_g) zone, where pullulan and PVP360 undergo state transformations, there was a change in the reaction rate. The lower degradation rates were observed for PVP40 under conditions where this matrix was fully plasticized (i.e. rubbery) and "collapsed", implying that the degradation kinetics are not governed by factors related to the physical state and molecular mobility of the inert matrix. Carotenoid losses have been observed even at temperatures below the T_g of the polymeric matrices. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Saffron; Crocins; Degradation; Encapsulation; Glass transition temperature

1. Introduction

In recent years, despite of its high price, the use of saffron is steadily increasing, due to changes of consumer preference towards natural products having functional properties. Saffron is typically used as a spice with coloring properties at levels of 1–260 ppm in a wide range of culinary, bakery and confectionery preparations as well as in alcoholic and non alcoholic beverages (Basker & Negbi, 1983; Ghorpade, Deshpande & Salunkhe, 1995; Sampathu, Shivashankar & Lewis, 1984). Many therapeutical properties have been also attributed to saffron so that its current demand may be partially related to the presence of certain biologically active compounds (Abdullaev, 1993; Nair, Pannikar & Pannikar, 1991; Rios, Recio, Giner & Manez, 1996). Among these compounds, the water soluble carotenoids, which include the crocins, a family of glycosylated derivatives of crocetin, are most effective (Li, Lin, Kwan & Min, 1999; Pfander & Rychener, 1982; Tarantilis, Tsoupras & Polissiou, 1995; van Calsteren et al., 1997). According to van Calsteren et al. the following compounds are found in saffron (Fig. 1): all-*trans*-crocetin di-(β-D-gentiobiosyl) ester (i.e. crocin), all-*trans*-crocetin β-D-gentiobiosyl-β-D-glucosyl ester, all-*trans*-crocetin di-(β-D-glucosyl) ester, all-*trans*-crocetin mono-(β-D-gentiobiosyl) ester, 13-*cis*-crocetin di-(β-D-gentiobiosyl) ester and 13-*cis*-crocetin β-D-gentiobiosyl-β-D-glucosyl ester. A minor crocetin glycoside which has three and two glucose moieties on each end has been also isolated from saffron stigmas (Tarantilis et al.). The shelf life of saffron is greatly related to the fate of its pigments (Raina, Agarwal, Bhatia & Gaur, 1996). As for other carotenoids, crocins, being highly unsaturated, are prone to degradation during processing and storage. Contrary to the non-polar carotenoids the stability of crocins is largely affected by water activity ($a_w \sim p/p_o$ or 'relative vapor pressure of water') and pH (Alonso, Varón, Gómez, Navarro & Salinas, 1990; Amelotti & Mannino, 1977; Morimoto, Umezaki, Shoyama, Saito, Nishi & Irino, 1994; Raina et al., 1996; Tsimidou & Biliaderis, 1997; Tsimidou and Tsatsaroni, 1993).

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* Corresponding author. Tel.: +30-31-998785; fax: +30-31-998789.
E-mail address: biliader@agro.auth.gr (C.G. Biliaderis).

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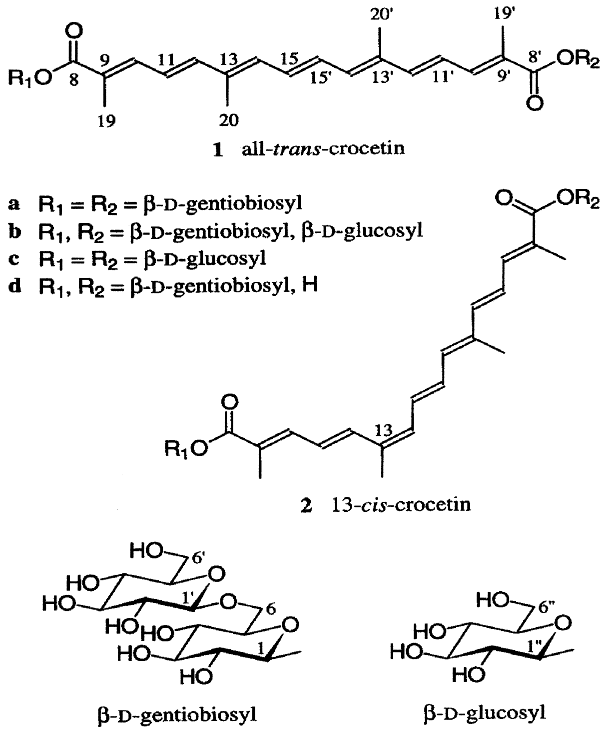

Fig. 1. Molecular structures of polar crocetin glycosides.

Encapsulation has found numerous applications in the food and pharmaceutical industries for coating colorants, flavors and other bioactive ingredients in an effort to increase their shelf life (Balassa & Fanger, 1971; Dziezak, 1988; Jackson & Lee, 1991; Kibry, 1991; Shahidi & Han, 1993). There are several encapsulation techniques that have been used commercially or developed on a small scale by the food industry. These techniques include spray-drying, freeze-drying, air suspension coating, extrusion, spray-cooling and spray-chilling, centrifugal extrusion, rotational suspension separation, coacervation and inclusion complexing. Encapsulation of carotenoids makes them easier to handle and improves their stability to oxidation (Dziezak, 1988). Among four different hydrocolloids examined as wall materials (15 D.E. maltodextrin, gum arabic, gelatin and sodium caseinate), maltodextrin was found the most effective in protecting paprika carotenoids from oxidation (Beatus, Raziel, Rosenberg & Kopelman, 1985). Wagner and Warthesen (1995) have studied the effect of various dextrose equivalent (D.E.) starch hydrolyzates on the stability of α- and β-carotenes of spray-dried carrot powder. They have reported that all four carriers increased the shelf-life by 72–220 times, with the 36.5 D.E. hydrolyzate being superior to the products with D.E. 4, 15, and 25. It was also shown that carotene retention increased with increasing maltodextrin level. Desobry, Netto and Labuza (1997) studied the effects of three different drying processes (spray-drying, freeze-drying and drum-drying) on the retention of encapsulated β-carotene in a 25 D.E. maltodextrin. Drum-drying was the most effective technique for retention of β-carotene; at all temperatures studied there was at least 50% retention during storage. On the other hand, spray-dried powders showed the fastest degradation ($\sim$20% retention) for β-carotene, whereas the freeze-dried material exhibited intermediate protection ($\sim$35% retention).

To our knowledge, there is no information about the effect of encapsulation on the biologically active carotenoids of saffron. Therefore, the kinetics of freeze-dried saffron carotenoids under varying water activity conditions using three different encapsulating agents was investigated in the present study. The selection of three different polymers, pullulan, PVP40 and PVP360, was based on their good water solubility and film-forming properties, and their ability to form an amorphous state on dehydration. Moreover, these materials do not readily crystallize upon hydration. This is important since encapsulated compounds may become lost from amorphous carriers as a result of crystallization of the matrix (Roos, 1995). Pullulan, a linear polysaccharide of glucose, consisting of maltotriose units linked via α-1→6 linkages, has the ability to form strong films which are impermeable to oxygen (Deshpande, Rale & Lynch, 1992; Yuen, 1974), whereas polyvinylpyrrolidone (PVP) is a polyamide that possesses unusual complexing and colloidal properties and is physiologically inert (Blecher, Lorenz, Lowd, Wood & Wyman, 1980). The two types of PVP used, with molecular weights 360 and 40 kDa, provide different physical properties to the matrix and its responses to plasticization by water, as has been noted by Bell and Hageman (1994) and Karmas, Buera and Karel (1992) on degradation of aspartame and browning reactions in model systems, respectively. Kinetic studies reported herein may be useful for minimizing the undesired losses of saffron carotenoids due to oxidation, and thereby extend the application of saffron extracts in food (as mutraceutical) or pharmaceutical products. The rates of many deteriorative changes in foods at reduced water contents have been given increasing attention as they were shown to largely depend on molecular mobility (Karel, 1985; Roos, 1995; Slade & Levine, 1991). Molecular mobility is governed by the physical state of food solids. Therefore, a chemical reaction that is affected by diffusion may proceed with an extremely slow rate in a glassy matrix. A significant increase in the rate constant could then occur as the material is transformed into a liquid-like viscous state above the glass transition temperature (T_g) due to increasing molecular mobility. Consequently, a further objective of this work was to examine whether the degradation rates of encapsulated saffron carotenoids are dependent on water activity or on molecular mobility associated with the glass transition of the amorphous matrix.

2. Experimental

2.1. Samples and chemicals

The saffron sample was a representative mixture of saffron stigmas which was kindly donated by the Cooperative of Saffron Producers (Crocos, Kozani, Greece). The sample was air-dried in the dark and kept in a desiccator at 4°C before use. Stigmas were used for preparation of aqueous extracts within a month from the production date. Two polyvinylpyrrolidone samples (M.W. 40 000 and 360 000 Da) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pullulan IP 20 was kindly donated by Hayashibara Biochemical Lab. Inc. (Okayama, Japan). All other chemicals were of analytical grade and purchased from Sigma Chemical Co. or Merck KGaA (Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of freeze-dried aqueous saffron extract

Saffron (12 g) was extracted with distilled water (500 ml) under continuous shaking in an incubator at 25°C for 16 h. The water-soluble extract was filtered, concentrated under vacuum and freeze-dried (freeze-drier Leybold-Heraeus Gmbh & Co., Koln, Germany). The freeze-dried powders were kept in the dark at −18°C until used.

2.2.2. Encapsulation of water soluble saffron carotenoids in various matrices by freeze-drying

Polyvinylpyrrolidone or pullulan (5.0 g) were dissolved in distilled water (100 ml) along with 250 mg of freeze-dried saffron extract, under stirring for 30 min and the pH was adjusted at 7.0 with 0.1 N NaOH. Aliquots, 5 ml, were transferred in non-transparent plastic containers, which were frozen at −18°C before freeze drying. Freeze-dried samples were kept at the same temperature until used.

2.2.3. Kinetic studies of saffron carotenoid degradation

Relative humidity environments were prepared using saturated solutions of K_2CO_3, $Mg(NO_3)_2{\cdot}6H_2O$, $NaNO_2$, and NaCl, which provide water activity (a_w) levels (25°C) of 0.43, 0.53, 0.64 and 0.75 (Labuza, 1984). Control samples (saffron carotenoid extracts alone) and encapsulated materials were kept in these a_w conditions at 35°C in the dark using a Gallenkamp incubator. Degradation of carotenoids was followed by periodic measurements of the coloring strength (Tsimidou & Biliaderis, 1997). This is expressed as $E^{1\%}_{440\mathrm{nm}}$, where $E = A_{440\mathrm{nm}}/C_{(1\mathrm{g}/100\mathrm{cm}^3)}$ according to the ISO 3632-2 method (ISO, 1993). Measurements were carried out in triplicate and first order reaction rate constants (ks) and half-life periods ($t_{1/2}$) were calculated using a first-order reaction model (Tsimidou & Tsatsaroni, 1993).

2.2.4. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (Polymer Labs Ltd, Epsom, UK) was used to determine the glass transition temperature, T_g (midpoint temperature of endothermic baseline shift), of saffron carotenoid extracts, free or encapsulated in different matrices. Freeze-dried samples were stored over P_2O_5, at a temperature below their T_g. Portions of these materials (10–15 mg) were placed in Mettler medium-pressure, stainless-steel pans (ME 29990, Mettler-Toledo AG Greifensee, Switzerland) and rehumidified at various relative humidities over saturated salt solutions in vacuum desiccators at 25 °C; triplicate samples were tested at each relative humidity environment. The pans were then hermetically sealed and analyzed by DSC. The samples were scanned over the glass transition region at 5°C/min under a continuous nitrogen flushing. Temperature and heat flow calibration of the calorimeter were carried out as described by Biliaderis, Lazaridou and Arvanitoyannis (1999). The moisture content of the samples was determined by oven drying at 103°C (1 h). The data were fit to the Gordon–Taylor model (Gordon & Taylor, 1952):

$$T_g = \frac{W_1 T_{g1} + KW_2 T_{g2}}{W_1 + KW_2}$$

where W_1: dry solids, T_{g1}: glass transition temperature of the sample in zero moisture content, W_2: moisture content, T_{g2}: glass transition temperature for glassy water, and K: a constant related to the strength of polymer–diluent interaction (the larger the K, the greater the plasticization effect). A T_g of −138°C was used for water (Sugisaki, Suga & Seki, 1968).

2.3. Statistical analysis

Linear regression analysis was used to obtain the degradation rate constants for all materials studied. Standard errors of the rate constant k were calculated using the Statistical Graphics System Computer Package (Statgraphics version 5.0, Statistical Graphics Corporation). Significant differences among the rate constants were determined using the t-test (95% confidence level) and (n_1-2+n_2-2) degrees of freedom (Steel & Torrie, 1980).

3. Results and discussion

3.1. Storage stability of encapsulated saffron carotenoids

Because of the presence of a large number of crocetin glycosides in saffron stigmas, the degradation kinetics of crocins, as a family of structurally related compounds, were monitored by absorption measurements at 440 nm. According to ISO specifications (ISO, 1993), absorbance values at this region of the spectrum are used to determine the coloring strength index of saffron. The protection effect of the three encapsulating matrices on saffron carotenoids was studied at four a_w levels (0.43, 0.53, 0.64 and 0.75) in the dark at 35°C. Conditions were selected according to previous studies on the rate of carotenoid degradation in relation to temperature and water activity (a_w) level (Tsimidou & Biliaderis, 1997) for aqueous saffron extracts. The results of these studies are illustrated in Fig. 2. A linear relationship was observed from the plots of the [$\ln(E_{440}^{1\%})$] vs time implying first-order reaction kinetics for pigment degradation. Similar kinetic responses have also been reported for saffron pigment oxidation in ground samples at 40°C and high relative humidities (a_w 0.75) (Alonso et al., 1990), for aqueous extracts studied in the pH range 3–7 (Tsimidou & Tsatsaroni, 1993), and for aqueous saffron extracts at a_w of 0.11–0.75 and temperatures of 25–60°C (Tsimidou & Biliaderis, 1977). The rate constant values, standard errors of the slope (s_k), half-life periods and coefficients of determination at each humidity level are summarized in Table 1; significant differences between the rate constants are also presented.

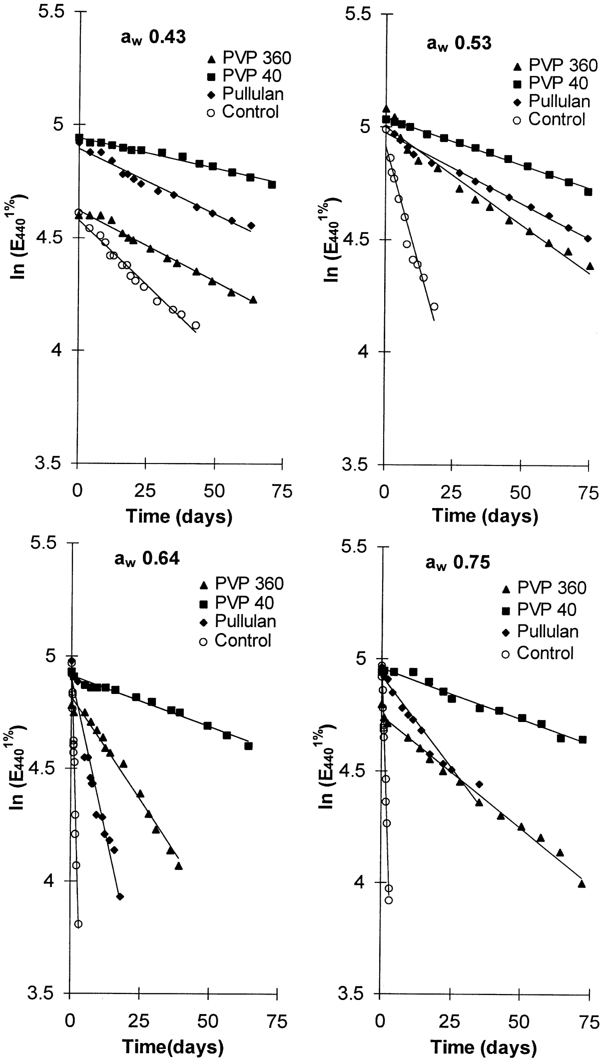

Fig. 2. First-order degradation plots for saffron carotenoids encapsulated in different matrices (coloring strength at $\lambda_{max} = 440$ nm) during storage in the dark at 35°C and different water activity environments.

When samples were stored at a higher relative humidity level (a_w 0.53 and 0.64) the differences in the rate constants between the polymer matrices and the control became more pronounced. With increasing a_w there was an increase in the rate constant, particularly above the zone corresponding to the intermediate moisture regime. A change in the kinetic responses was observed for samples stored in a_w 0.75. In this environment the rate constants for all systems were lower than those found for a_w 0.64 but certainly higher than those of the less humid environments. As shown in Table 1, these trends in the rate constants were more prominent for the control samples and less distinct in the case of PVP40. The data for the PVP40 strongly point to an effective matrix in retaining the saffron coloring strength on storage.

In general, the observed kinetic responses are somewhat different from those reported in most studies concerning oxidation of nonpolar carotenoids in low-moisture systems (Goldman, Horev & Saguy, 1983; Rodriguez-Amaya, 1993; von Elbe, 1987). Such a behavior may be related

Table 1
Rate constants, s_k values, half life periods and coefficients of determination (r^2) for degradation of saffron carotenoids encapsulated in different matrices during storage in the dark at 35°C and different a_w environments[a,b]

Sample	$k \times 10^3 \pm s_k \times 10^3$ (days^{-1})	$t_{1/2}$ (days)	r^2 (n)[c]
$a_w = 0.43$			
Control	11.6±0.5 a 1	59.7	0.98 (14)
Pullulan	5.8±0.3 b 1	119.5	0.99 (14)
PVP360	6.4±0.2 c 1	108.3	0.99 (14)
PVP40	2.7±0.1 d 1	256.7	0.97 (14)
$a_w = 0.53$			
Control	43.0±2.8 a 2	9.3	0.98 (14)
Pullulan	6.3±0.2 b 1	110.0	0.97 (14)
PVP360	8.6±0. 5c 2	80.6	0.98 (14)
PVP40	4.1±0.1 d 2	169.0	0.99 (14)
$a_w = 0.64$			
Control	417±11.1 a 3	1.7	0.99 (14)
Pullulan	55±3.7 b 2	12.6	0.95 (14)
PVP360	18.5±0.7 c 3	37.5	0.98 (14)
PVP40	4.6±0.2 d 3	150.6	0.98 (14)
$a_w = 0.75$			
Control	347.6±5.1 a 4	2.0	0.99 (14)
Pullulan	16.4±0.1 b 3	42.3	0.96 (14)
PVP360	10.0±0.3 c 2	65.3	0.99 (14)
PVP40	4.5±0.2 d 3	154.0	0.98 (13)

[a] Different letters for k values (columns) in each a_w level indicate significant differences among the matrices ($P < 0.05$).

[b] Different numbers for k values (columns) indicate significant differences for the same matrix at different a_w environments ($P < 0.05$).

[c] n = degrees of freedom.

to the higher water solubility of saffron carotenoids compared with non-polar carotenoids, favoring a greater access of dissolved oxygen to the former. In fact, the moisture-content dependence of the oxidation rates of encapsulated saffron carotenoids resembled more that of reactions controlled by the mobility of reactants in foods at low a_w (von Elbe, 1987). In dry environments water is tightly bound to surface polar sites and is generally unavailable for reaction either as a reagent or as a diluent (Leung, 1987; von Elbe, 1987). On the other hand, reactant dilution effects become predominant in the high a_w range. As a result, the rate may reach a maximum value at intermediate a_w (~0.40–0.64) and then decrease with further increase in $a_{w.}$ The kinetic responses observed at 35°C (rate maximum at a_w 0.64) are in agreement with this view.

3.2. *Reaction rates and physical state of the matrix*

All amorphous matrices used largely increased the half-life of saffron carotenoids (Table 1). The most prominent protective effect was with PVP40, whereas pullulan was the least effective among the three matrices. On storage, a high degree of collapse was visually observed for PVP40 at all a_w levels studied. PVP360 was more stable and exhibited structural collapse to a lesser extent at 0.64 and 0.75 than PVP40. Collapse is usually defined as the readily visible deformation (or shrinkage) of the sample under the force of gravity during storage of a dried amorphous matrix. This phenomenon has been attributed to the glass–rubber transition of amorphous food powders as a result of heating and/or plasticization of the matrix by water or small molecular weight additives (Roos, 1995; Slade & Levine, 1991). Pullulan had a rather stable structure without visible collapse at all a_w levels examined, presumably because of its high molecular weight (Roukas & Biliaderis, 1995).

Fig. 3 shows the DSC scans for a selected system (pullulan/saffron carotenoids) having different moisture contents. In these traces the heat flow as a function of temperature is recorded while the samples are heated at a constant rate. As expected the T_g of each matrix–carotenoid system was found to decrease with increasing moisture content due to plasticization by the solvent. The DSC scans of the samples with 11–13% moisture contents exhibited endothermic transitions. Such endothermic events have been attributed to enthalpy relaxation of the glassy state on aging of water- and/or polyol-plasticized polysaccharides (Appelqvist, Cooke, Gidley & Lane, 1993; Biliaderis et al., 1999). Polysaccharide glasses are non-equilibrium structures which may relax through local motions towards a lower energy state (enthalpy and volume relaxation) on aging at sub-T_g temperatures; i.e. a very 'open' glass produced by rapid thermal quenching densifies through volume reduction on annealing (there is a decrease in entropy of the solid). The T_g–water content relationships (Gordon–Taylor plots) given in Fig. 4 indicate the sensitivity of T_g to increasing amounts of water in the freeze-dried systems. The pullulan matrix seems to be more sensitive to water plastization compared to the PVP samples as it exhibited a steeper decline in the T_g with increasing moisture. Moreover, the T_g curve of the PVP40 was shifted to lower temperatures than that of PVP360, as expected from the molecular size difference between the two samples. Estimated values of T_g for the dried polymers were $T_{g\text{pullulan}} = 151°\text{C}$, $T_{g\text{PVP }40} = 127°\text{C}$ and $T_{g\text{PVP }360} = 138°\text{C}$.

The estimated constants for saffron carotenoid in different matrices using the Gordon–Taylor model are summarized in Table 2. The PVP40, because of its lower molecular size, has a lower glass transition temperature (T_g) than PVP360 and, therefore, it would enter the rubbery domain at much lower water contents assuming a constant storage temperature. It can be suggested that

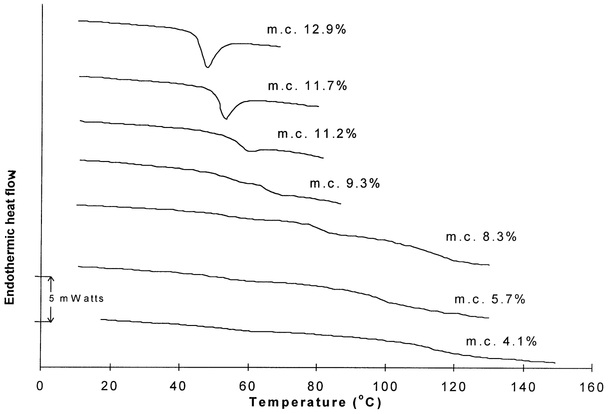

Fig. 3. DSC thermal curves for pullulan/saffron carotenoids freeze dried materials at several moisture contents (m.c.).

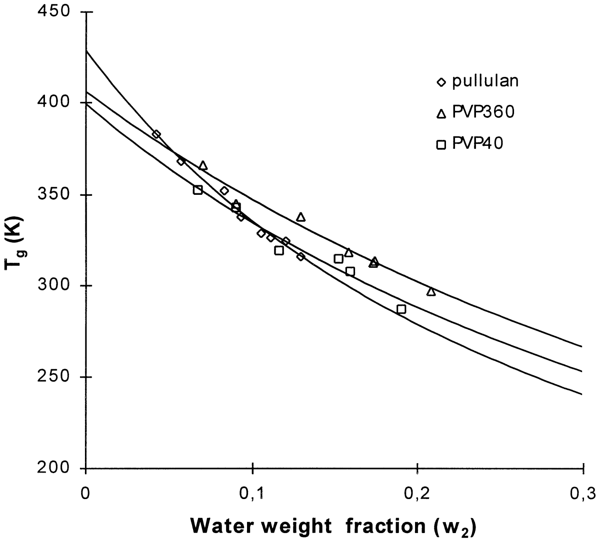

Fig. 4. Relationship between water weight fraction (W_2) and T_g, according to the Gordon–Taylor model.

Table 2
Estimated constants of the Gordon-Taylor model (K, T_{g1}) and coefficient of determination (r^2) for saffron carotenoids encapsulated in different matrices

Matrices	K	T_{g1}	r^2 (n)[a]
Pullulan	4.18	428.6	0.998 (9)
PVP40	2.91	399.3	0.994 (7)
PVP360	2.49	406.1	0.998 (7)

[a] n = degreees of freedom.

PVP40 may provide high protection for saffron carotenoids because it collapses rapidly during storage and, thereby, it may become a more effective barrier to oxygen permeation. Collapse of freeze-dried materials is associated with the disappearance of micropores and cavities through which oxygen enters the amorphous matrix. On the other hand, pullulan as wall material, being relatively stable in storage, could be more permeable to oxygen and thus less effective than the polyvinylpyrrolidone systems in protecting saffron carotenoids. Nevertheless, pullulan did offer a high degree of protection to saffron pigments (by a factor of 2 at a_w 0.43; of ~7 at intermediate a_ws; and of more than 20 at the most humid environment, a_w 0.75) compared to the freeze dried saffron extract degradation (control).

The degree of protection offered by a matrix, besides its dependence on the chemical characteristics, is also related to the physical state and its macrostructure (i.e. the extent of its surface, the size, shape and distribution of pores) (Goldman et al., 1983; O'Boyle, Aladain-Kassam, Rubin & Diosady, 1992). For example, at a_w 0.64 the degradation rate for PVP40 was reduced by a factor of 4 compared to that for PVP360. This finding should be attributed to differences in the physical state and morphology of the two matrices which have similar chemical characteristics. The decline in degradation rate was much higher when it was compared to that for pullulan (by a factor of more than 11). Such a reduction may be not only be related to the differences in chemical composition of these matrices but also to the physical properties and macrostructure of the wall material. Porosity characteristics and macrostructure of spray-dried microcapsules were shown to be affected by the molecular weight and chemical nature of the solutes used as wall materials (Sheu & Rosenberg, 1998; Moreau & Rosenberg, 1999). Also, Moreau and Rosenberg (1996, 1998) have demonstrated that permeability of wall matrix to oxygen is affected by porosity and this determines the oxidative stability of the core.

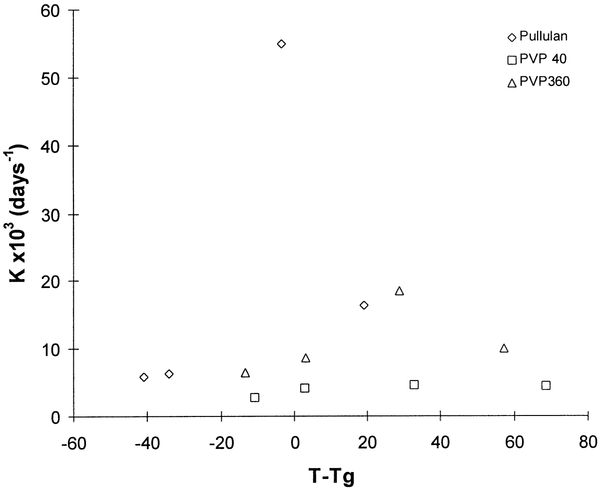

Fig. 5. Dependence of oxidation rates of saffron carotenoid encapsulated in different matrices on $\Delta T = T - T_g$.

The relationships between reaction rate constants, k, and $T - T_g$ for the various systems examined are presented in Fig. 5. It would appear that substantial carotenoid degradation occurs in the freeze-dried systems even at temperatures below the T_g of the amorphous matrices. In general, with increasing $\Delta T\left(T - T_g\right)$ there was an increase in the rate constant for the PVP360 and pullulan up to a point where a maximum is reached and this was followed by a decline in the reaction rate. In the vicinity of T_g, where the pullulan and PVP360 undergo state transformations (as evidenced by structural collapse of the matrix), there seems to be a change in the reaction rate. After the maximum there is a drop in the rate constant, probably due to the structural collapse of the matrix which would affect oxygen diffusivity and, thereby, reactivity of the system. For PVP40 the state transformation is rapidly observed under most storage conditions as expected from the much higher $\Delta T = T - T_g$ values found in this case. As a result, very little variation in the k values is observed over the limiting ΔT range examined in this study. In contrast, for dried powders of saffron stigmas, Tsimidou and Biliaderis (1997) have reported much stronger dependence of reaction rate constants on temperature above the T_g ($> T_g + 40°\mathrm{C}$).

In conclusion, encapsulation of saffron carotenoids greatly improves their stability to oxidation. However, it is obvious from the results of Table 2 and Fig. 5 that estimation of the T_g of the polymer used as wall material is not a useful predictor of colorant stability. It is plausible that molecular mobility of the reactants is not rate limiting even when the encapsulating amorphous matrix exists in the glassy state. Other factors such as microstructure and porosity of the polymeric matrix may be more important as modifiers of reaction kinetics.

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