

Carotenoid Retention In Canned Pickled Jalapeño Peppers and Carrots As Affected by Sodium Chloride, Acetic Acid, and Pasteurization

M. GUERRA-VARGAS, M. E. JARAMILLO-FLORES, L. DORANTES-ALVAREZ, AND H. HERNÁNDEZ-SÁNCHEZ.

ABSTRACT: The combined effect of salt, acetic acid, and pasteurization temperature on the retention of carotenoids in canned pickled carrots and green jalapeño peppers was studied by a central composite design. The results were analyzed by response surface methodology. The carotenoid standards were obtained by open column chromatography and the quantitation was done by HPLC. Only the main carotenoids were quantified: α - and β -carotene in carrots and α -, β -carotene, lutein, and violaxanthin in peppers. After analyzing the experimental results and the restrictions of the Mexican Regulations, 2% NaCl and 2% acetic acid concentrations were recommended. The optimal pasteurization conditions were 70 °C/12.45 min for carrots and 83 °C/5.2 min for peppers.

Key words: carrots, jalapeño peppers, salt, carotenoids

Introduction

CAROTENOIDS ARE PIGMENTS WIDELY DISTRIBUTED IN NATURE and are always found in photosynthetic tissues (Simpson 1983). Some carotenoids are well known for their provitamin A activity, especially β -carotene, and to a lesser extent, α -carotene and β -cryptoxanthin. This activity is essential for human life (Olson 1989).

Carotenoids have been shown to be effective antioxidants in vitro, however, because the in vivo antioxidant behavior depends on many factors: concentration; localization in the actual target cells or tissues; the nature of the reactive oxygen species, and so on, the situation is less clear (Van den Berg and others 2000), although antioxidation has been generally related to the deactivation of free radicals in biological systems (Krinsky 1993). A correlation has also been observed between an increase in fruit and vegetable consumption and a decrease in the incidence of cardiovascular disease and cancer (Bendich 1989; Gerster 1991; Gerster 1993; Ziegler 1989).

The consumption of peppers (*Capsicum annum*) has been part of Mexican tradition and culture for thousands of years (SARH 1982). Pickled peppers and carrots are very popular in Mexico because of their attractive flavors and colors, and are often added to other foods to increase palatability. Traditionally, peppers have been brined at concentrations of salt (2%) high enough to prevent fermentation. More recently, vinegar is added to brines to reduce the salt concentration necessary to keep the product from spoiling (Fleming and others 1993) and also to reduce the pH to less than 4.6 according to acidified food regulations to allow for pasteurization. This thermal treatment is commonly used to preserve pickled items (like cucumbers) at relatively low concentrations of salt (0.6%) and acetic acid (0.6%). Pickled cucumbers retain much of the characteristic crispness and appearance of the natural product (Monroe and others 1969). Firmness of pasteurized whole cherry and jalapeño peppers is not influenced significantly by acetic acid concentrations of 2, 3, or 4% (Fleming and others 1993). However, in the case of jalapeño pepper rings, acetic acid concentrations > 1.5% resulted in extensive softening due to acid hydrolysis of pectic substances. CaCl_2 at a concentration of 0.2% was rec-

ommended as a firming agent (Howard and others 1994a)

Jalapeño peppers and carrots have a high level of carotenoids and, therefore, are very good dietary sources of provitamin A and antioxidants (Davies and others 1970; Desobry and others 1998; Heinonen 1990; Howard and others 1999; Mejía and others 1988).

Data on the effects of processing on the carotenoid content of foods are controversial because of several factors. The effect is generally different depending on the specific carotenoid assessed, and may be dependent not only on the type (such as β -carotene and lycopene in tomato products) and conditions used (time, temperature, pressure cooking, covered pots, and so on) but also on other constituents of the product. In addition, the ways of calculating the retention are not often comparable from one study to another (Van den Berg and others 2000).

Thermal processing has been reported to increase carotenoid concentration, perhaps because of greater chemical extractability and loss of moisture and soluble solids that concentrate the sample. Heat treatment also inactivates some oxidative enzymes and breaks some structures, leading to a higher bioavailability as in the case of microwave cooking (Howard and others 1999; Van den Berg and others 2000). Heat induces cis/trans isomerisation and different carotenoid by-products, like 13-cis- β -carotene can be formed (Chandler and Schwartz 1987, 1988).

In the specific case of canning, controversial results have been obtained. Some authors report a decrease in the carotenoid concentration after processing (Chandler and Schwartz 1987) while others report an increase in the content of these compounds (Edwards and Lee 1986). Canning is also reported to increase the cis/trans isomerisation and to reduce the carotene content because of thermal destruction but prevents oxidation by means of air removal. (Desobry and others 1998; Chandler and Schwartz 1988). However, canning does not only involve a heat treatment, but also the effect of the salt and the acetic acid of the brine in the case of pickled products. The combined effect of these factors in the carotenoid retention has not been studied yet.

The purpose of this study was to determine the effect of

sodium chloride, acetic acid, and pasteurization conditions on the carotenoid retention in canned pickled jalapeño peppers and carrots.

Materials And Methods

Raw material and pretreatments

Jalapeño peppers (*Capsicum annuum* L.) and carrots (*Daucus carota* L.) were obtained from a local market in México City.

Peppers and carrots were washed under tapwater; after washing, surface water was removed with a paper towel, and then both vegetables were kept at $-18\text{ }^{\circ}\text{C}$. When the experiments were done, the 2 products were allowed to thaw to room temperature ($20 \pm 1\text{ }^{\circ}\text{C}$) and then the peppers were sliced (approximately 1.5 cm width and 5 cm length) and the carrots were cut to give disks (approximately 2 cm diameter and 0.4 cm height).

Thermal processing

Eighty grams of peppers were placed into a 211×300 can and 100 mL solution of sodium chloride and/or acetic acid was added. The same treatment was performed for carrots. The cans were submitted to different thermal treatments in a water bath following the experimental design. The time of processing was calculated from the equation described by Stumbo (1973), as follows:

$$B = f_h (\log j_{ch} I_h - \log g_c)$$

where B = Time of processing (min),

f_h = Reciprocal of the slope of the heating curve (min),

j_{ch} = lag heating factor,

I_h = bath temperature- initial temperature of the food ($^{\circ}\text{C}$),
and

g_c = bath temperature- maximal temperature reached by
the food ($^{\circ}\text{C}$).

The temperature inside the cans was determined using an Ecklund copper-constantan thermocouple (Techni CAL, Inc., New Orleans, La.) inserted in the geometric center. The cans were put in a water bath with a heating temperature of $85\text{ }^{\circ}\text{C}$ in order to obtain the heating curve and to determine the values of f_h and j_{ch} . After this, cooling water was introduced at $20\text{ }^{\circ}\text{C}$ to obtain the cooling curve and to be able to calculate the lag cooling factor (j_{cc}). Considering that the commodities inside the cans were acid foods, according to Stumbo (1973) the pasteurization parameters for this kind of foods were: $D_{65^{\circ}\text{C}} = 1\text{ min}$; $z = 5.6\text{ }^{\circ}\text{C}$; $F = 15D$.

Experimental design

A central composite design was used and the results were analyzed by response surface methodology (RSM) with the aid of the Design Expert (v 5) software (Stat-Ease Inc., Minneapolis, Minn., U.S.A.). The independent variables were: pasteurization conditions calculated by the Ball (Stumbo 1973) method (at $64\text{ }^{\circ}\text{C}$, $74\text{ }^{\circ}\text{C}$ and $83\text{ }^{\circ}\text{C}$), and sodium chloride (0, 3.5 and 7%) and acetic acid (1.33, 2.67, and 4%) concentrations. These last values were selected after considering the recommended contents of salt and acetic acid of the Official Mexican Regulations (SECOFI 1982) for canned pickled green jalapeño peppers. The response variables were the retention amounts (%) of the more important carotenoids in each case.

Physical and chemical analysis

Moisture was determined according to the AOAC method 4.1.03 (Windham 1995). A minimum of 3 replicates was made and the average was calculated.

The pH of the canned products was measured with an Orion model 920A glass electrode pH meter according to the AOAC method 42.1.04 (Mulvaney 1995). A minimum of 3 replicates was made and the average was calculated.

Sample preparation.

The methodology used was essentially that of the open column described by Mercadante and Rodríguez-Amaya (1990). Triplicate samples of 3 g were crushed in a mortar with 20 mL of cold acetone ($5\text{ }^{\circ}\text{C}$) and vacuum filtered through a Buchner funnel, using filter paper No. 1 of 10 cm diameter (Whatman International Ltd., Maidstone, England). Nitrogen was bubbled in the solvent to prevent carotenoid oxidation. The residue was re-extracted with more cold acetone (20 mL) and the pigments in the combined filtrates were transferred to petroleum ether by adding water in a separatory funnel. The 2 layers were allowed to separate without stirring and the lower phase was discarded. The ether phase was washed 4 or 5 times with water to eliminate all the residual acetone. The carotenoid-containing ether phase was separated from the water-acetone phase and concentrated to a volume of 5 mL using a stream of nitrogen. This sample was used for the open column chromatography. In the case of the HPLC analysis, the ether phase was evaporated to dryness with nitrogen and kept frozen at $-18\text{ }^{\circ}\text{C}$. The powder was dissolved in 20 mL of acetone before being injected into the HPLC. The whole procedure was carried out in the dark.

Open column chromatography (OCC).

The carotenoids were separated on a MgO:hyflosupercel column (1:1) developed with increasing concentration of acetone in petroleum ether to elute α -carotene (0%) and β -carotene (2%) in carrots and peppers, and lutein and violaxanthin (30-40%) in peppers. Identification was based on chromatographic behavior on column and thin layer chromatography (TLC), absorption spectra (350-550 nm) and group chemical reactions (acetylation of primary and secondary hydroxyl group and methylation of allylic hydroxyl groups). In the first technique, the carotenoid (about 0.1 mg) was dissolved in 2 mL pyridine and 0.2 mL of acetic anhydride were added. The reaction mixture was left in the dark at room temperature for 21 h. The carotenoid was transferred to petroleum ether in a separatory funnel with the addition of water. The ether phase was washed with water, collected, dried with sodium sulfate, concentrated, and applied on a silica thin-layer plate next to unreacted carotenoid. The development was done with 5% methanol in toluene. The reaction was considered positive if the resulting product had an R_f much higher than that of the original carotenoid. In the second technique, the carotenoid (about 0.1 mg) was dissolved in 5 mL methanol. A few drops of 0.2 N HCl were added and the reaction allowed to proceed at room temperature in the dark for 3 h. The carotenoid was transferred to petroleum ether and submitted to thin-layer chromatography as described above. A positive reaction was also shown by an increase in R_f (Rodríguez-Amaya 1999). Two TLC systems were used. Silica gel plates developed with 3% methanol in benzene were used to follow the course of the chemical reactions and to distinguish the carotenes which ran with the solvent front, and the xanthophylls which were distributed in

Table 1. Absorption values in UV-visible and absorption coefficients of carotenoids in petroleum ether

Carotenoid	λ_{\max} (nm)	$A^{1\%}_{1\text{ cm}}$
α -Carotene	422, 445, 473	2800 a 444 nm
β -Carotene	425, 450, 477	2592 a 450 nm
Violaxanthin	416, 440, 465	2550 a 440 nm ^a
Lutein	421, 445, 474	2600 a 445 nm ^b

Britton (1991).

^a: in ethanol.^b: in ethyl ether.

the plate according to the nature and number of substituents. As an additional parameter to make a distinction between dihydroxy- and trihydroxycarotenoids, silica gels were also developed with 30% acetone in petroleum ether (Gross 1980). The existence of the hydroxyl substituents was confirmed by acetylation with acetic anhydride (Aasen and Liaasen-Jensen 1966) and location at an allylic position by methylation with acidified methanol (Liaasen-Jensen and Hertzberg 1966). In both reactions a positive response was perceived as an increase in the R_f value, proportional to the number of hydroxyl groups present. Quantitative measures were spectrophotometric and 5, 6-epoxide groups were detected as spots on TLC plates which changed from yellow to blue after being exposed to HCl vapor (Davies 1976).

High-performance liquid chromatography (HPLC)

The HPLC equipment consisted of a Beckman System Gold Liquid Chromatograph with a Model 168 UV-Vis detector (Beckman Coulter, Fullerton, Calif., U.S.A.) and a Gold Nouveau V 1.72 Chromatographic Data System (Beckman Coulter). The equipment included also a Prodigy 5 ODS (2) column 25 cm X 4.6 mm ID (Phenomenex, Torrance, Calif.) along with a solvent system of acetonitrile: methanol: tetrahydrofuran (THF) (58:35:7) pumped at a flow rate of 1 mL / min for the analysis of α -carotene, β -carotene, lutein, and violaxanthin (Bushway 1985). All runs were carried out at 20 ± 1 °C and visible detection was achieved at 450 nm using an 8 nm slit. The HPLC quantification was done using a linear calibration plot (area against concentration) obtained with external standards whose concentration was determined spectrophotometrically (as in the open column method) and later injected into the HPLC and the area measured. Sample extracts were spiked with a given standard (the standards of the 4 carotenoids were obtained by the same open column method) to determine its simultaneous appearance on the chromatogram in relation to the sample peak being identified. When this was done, standard and sample peaks were perfectly superimposed confirming sample peak identification. Confirmation of the identity was done by comparing the obtained peak profile with the one reported by Bushway (1985) for carotenoid separation using the same conditions and a similar C_{18} column (Mejía and others 1988).

Results and Discussion

Carotenoid composition

The carotenoid extracts had an orange color in the case of carrots and green in the case of the peppers. Five bands were observed in the OCC method in the case of carrots and 9 in the case of peppers (see Figure 1). Only the more concentrated bands were considered for isolation and identification. In the case of carrots the 1st and 2nd bands were identified as α - and β -carotene by their typical absorption spec-

Table 2—Carotenoid content of carrots (fresh weight) determined by HPLC

Author	α -Carotene ($\mu\text{g}/100\text{ g}$)	β -Carotene ($\mu\text{g}/100\text{ g}$)
Bhaskarachary and others (1995)	-	6500
Bureau and Bushway (1986)	3789	7602
Bushway (1986)	2045	7293
Edwards and Lee (1986)	3577	8001
Rodríguez-Amaya (1997)	-	3400
Granado and others (1992)	2895	6628
Heinonen and others (1989)	530	7600
This study	830	3078

tra (Table 1) and chromatographic behavior. Similarly, in the pepper extract, the 2nd, 3rd, 7th and 8th bands were identified as α -carotene, β -carotene, violaxanthin, and lutein, respectively. The confirmation of the identity of the carotenoids was done by the TLC techniques described before and in both cases (carrots and peppers) the R_f values and reactions were similar to those reported by other authors (Davies and others 1970). Table 2 shows the carotenoid content (α - and β -carotenes) of the carrots used in this study compared with the results obtained by other authors (Bhaskarachary and others 1995; Bureau and Bushway 1986, Bushway 1986, Edwards and Lee 1986, Granado and others 1992, Heinonen and others 1989, Rodríguez-Amaya 1997). It can be observed that the variation among data is high. This can be explained in terms of differences in the harvesting time, variety of carrot and agronomic conditions. There are also important differences in the techniques for extraction and quantitation of the carotenoids used by the different authors, including different columns and solvent mixtures for HPLC. This problem has also been pointed out by Howard and others (1999). Similarly, Table 3 shows the carotenoid content (α - and β -carotenes, lutein, and violaxanthin) of the green jalapeño peppers used in this research compared with the content reported by other authors (Bureau and Bushway

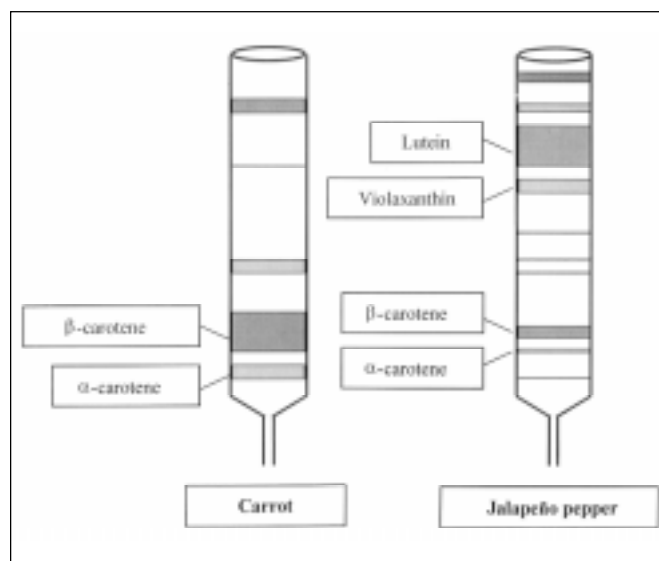
**Figure 1—Bands of carotenoids observed in open column chromatography of the extracts of carrots and green jalapeño peppers.**

Table 3—Carotenoid content of green peppers (fresh weight) determined by HPLC

Author	Green pepper type	α -Carotene ($\mu\text{g}/100\text{ g}$)	β -Carotene ($\mu\text{g}/100\text{ g}$)	Lutein ($\mu\text{g}/100\text{ g}$)	Violaxanthin ($\mu\text{g}/100\text{ g}$)
Bureau and Bushway (1986)	N.S.	34	217	n.d.	N.D.
Bushway (1986)	N.S.	n.d.	129	n.d.	N.D.
Granado and others (1992)	N.S.	n.d.	205	341	N.D.
Heinonen and others (1989)	Sweet bell	n.d.	240	700	N.D.
Howard and others (1994)	Jalapeño	60	305	N.D.	N.D.
Mejía and others (1988)	Jalapeño	53	377	n.d.	N.D.
This study	Jalapeño	9	381	836	225

n.d.: none detected at a detection limit of 1 $\mu\text{g}/100\text{ g}$ product for all compounds.

N.D.: not determined

N.S.: not specified

Table 4—Effect of freezing at -18°C on the amount of extractable carotenoids from carrots and jalapeño peppers (dry basis)

Vegetable	Duration of freezing (days)	α -Carotene ($\mu\text{g}/100\text{ g}$)	β -Carotene ($\mu\text{g}/100\text{ g}$)	Lutein ($\mu\text{g}/100\text{ g}$)	Violaxanthin ($\mu\text{g}/100\text{ g}$)
Carrot	0	6350 \pm 685	34659 \pm 2832	-	-
	45	19769 \pm 1203	69876 \pm 4217	-	-
Peppers	0	146 \pm 18	6374 \pm 95	13975 \pm 558	3768 \pm 368
	25	179 \pm 12	8576 \pm 270	15888 \pm 2543	3015 \pm 47

1986; Bushway 1986; Granado and others 1992; Heinonen and others 1989; Howard and others 1994b; Mejía and others 1988). Again, a great variability in the data can be observed, which can also be explained in terms of differences in the degree of ripeness, type and variety of pepper (in many studies the type is not specified), agronomic conditions and analytical procedures.

Effect of storage at -18°C on the carotenoid content

Table 4 shows the variation in the carotenoid content of carrots and green jalapeño peppers during the storage at -18°C . It was observed that the concentration of α - and β -carotene in carrots and of β -carotene in peppers increased significantly ($p < 0.05$) with the storage time (111.43, 101.61 and 34.55 % respectively). This could be explained considering that the exposure to freezing temperatures and subsequent thawing can cause damage in the vegetable tissues, improving the extraction efficiency of some of the carotenoids present in the carrots. The fact that the concentrations of β -carotene, lutein and violaxanthin remained constant during the freezing and thawing of the peppers could be attributed to differences in the composition of the solid matrix in these commodities (the structure of a root is very different from that of a fruit); nevertheless this hypothesis remains to be worked out in future investigations, since there are few studies regarding the extractable carotenoid content after freezing. In the case of the Guku leaves from Africa, there was no appreciable changes in the levels of β -carotene after the leaves were kept frozen at -18°C for 5 wk (Benhura and Chitsiku 1997). Similar results were obtained for frozen broccoli, carrots and green beans, however in this particular case the freezing process was done at -40°C in an air blast freezer which favored the retention of nutrients (Howard and others 1999). The relative strength of binding of the different caro-

tenoids to the proteins in the tissues is also to be considered. Ruban and others (1999) studied the strength of binding of carotenoids to the photosystem II light harvesting complexes and found 2 types of binding sites: internal for lutein and peripheral for violaxanthin. This last carotenoid was easier to remove from the complex in the presence of detergents than the first. It is possible then, that in this study, the β -carotene has a looser binding site than the other carotenoids or that the binding site is peripheral, and freezing could have release it in a higher proportion.

Thermal processing

The pH values of the canned products were in the range of 3.12 to 3.74 for the carrots and of 2.68 to 3.50 for the green jalapeño peppers. The values of f_h , j_{ch} and j_{cc} were 9.95 min, 0.6 and 0.74 for carrots and 8.1 min, 0.69 and 0.7 for the peppers. The values of g_c and the times of processing (at different temperatures) to assure pasteurization of canned carrots and peppers were calculated using the Ball equation described by Stumbo (1973) in Methods and are shown in Table 5. As expected the time of processing decreased with the heating temperature and the values of B were used in the experimental design.

Optimization

Using the RSM and the Design Expert software, the retentions of α - and β -carotene of carrots could be fitted to a quadratic model ($p < 0.05$). The following equation shows the effect of the temperature of processing (T) and NaCl content (S) on the α -carotene content.

$$\% \text{ retention } \alpha\text{-carotene} = -1220.7 + 39.27 T - 5.15 S - 0.29 T^2 \quad (1)$$

Here, a negative effect of the salt concentration on the reten-

Table 5—Values of g_c and times of processing (B) of canned carrots and green jalapeño peppers at different temperatures

Temperature (°C)	g_c (°C) (carrot)	g_c (°C) (pepper)	B (min) (carrot)	B (min) (pepper)
64	5×10^{-3}	7.97×10^{-4}	40.2	40.9
74	6.24	5.58	9.1	10.1
83	19.39	18.53	4.8	5.2

tion of this carotenoid can be observed. It is also worth noticing that acetic acid, at the concentrations used in this work, had no effect on the retention of α -carotene. The effects of pasteurization temperature and sodium chloride can be observed easily in Figure 2, where the region of higher content of α -carotene is located at 0 % salt and near 70 °C, decreasing above and below this temperature. In this figure the content of acetic acid was constant at 2% (the maximal concentration recommended by the Official Mexican Regulations (SECOFI 1982) which is sensorially acceptable). If the value of $S = 0\%$ is substituted in equation 1, the derivative of this equation with respect to T can be obtained and equated to zero in order to obtain the temperature of maximal retention of α -carotene. In the case of the carrots, a value of $T = 67.7$ °C was calculated and a retention of 108.7% was obtained for α -carotene.

The influence of S (NaCl concentration in %), A (acetic acid concentration in %) and T (temperature of processing in °C) on the content of β -carotene in carrots is summarized in the next equation:

$$\% \text{ retention } \beta\text{-carotene} = -733.42 + 25.27 T - 6.34 S + 10.47 A - 0.19 T^2 \quad (2)$$

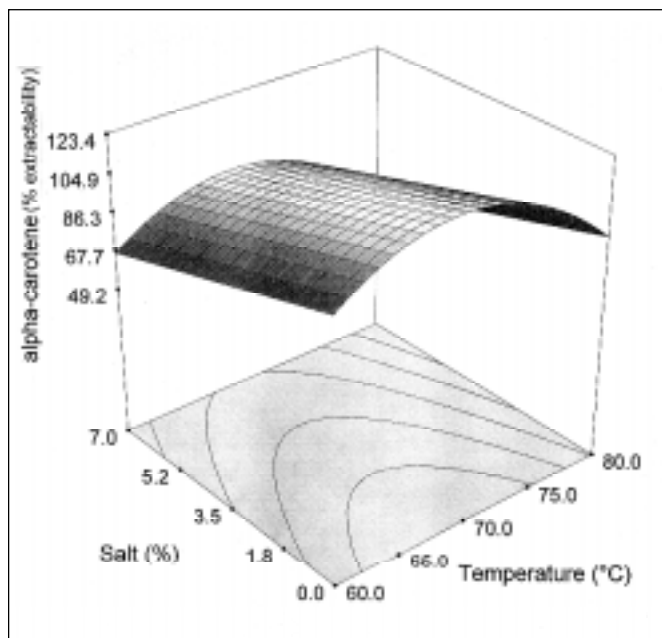
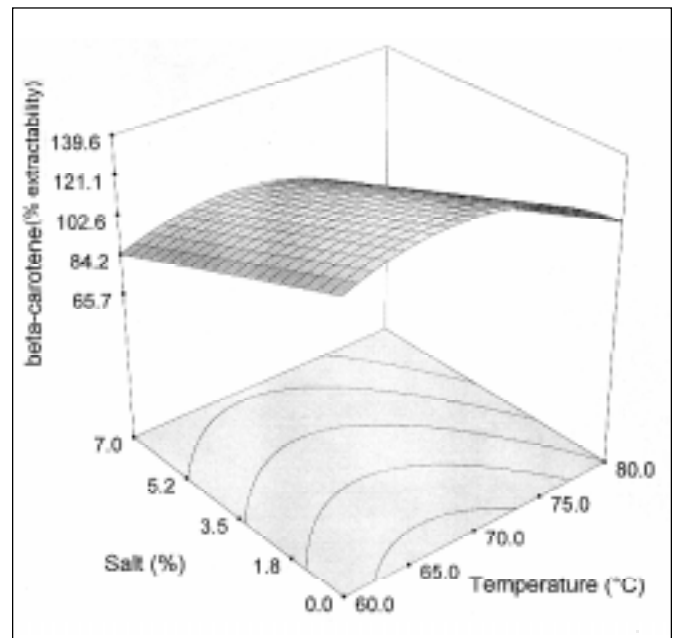
Again, it can be observed that the salt content had a negative

effect, whereas acetic acid was beneficial for the retention of β -carotene. Figure 3 shows the response surface for β -carotene retention where it can be seen that a concentration of salt of 0% and pasteurization temperatures of around 70 °C are the best conditions for the retention of this carotenoid in carrots. If the same mathematical procedure used on equation 1 is applied to equation 2 (using values of $S = 0\%$ and $A = 2\%$), a temperature of 66.5 °C is calculated for a maximal retention of 127.75% of β -carotene in carrots. Since the 2 calculated pasteurization temperatures are very close, the average of them (67.1 °C) can be used as the recommended processing temperature for canning carrots. After considering this, the conditions for a maximal simultaneous retention of α - and β -carotenes are: 0% salt, 2% acetic acid and a pasteurization temperature of 67.1 °C (20.9 min according to the equation of Ball) (Stumbo 1973). Under these conditions, very adequate retentions of 108.6 and 127.7% for α - and β -carotenes were obtained. Besides, since no salt was added to the product, it could be considered as a low-sodium food which could be included in the diet of people with high blood pressure.

In the very particular case of Mexico, according to the Official Mexican Regulations (SECOFI 1982), the minimal recommended content of NaCl is 2 % and the maximal recommended concentration of acetic acid is 2 % for canned vegetables. Substituting the above conditions and the temperature of 67.1 °C in equations 1 and 2, retention percentages of 98.32 and 115 were obtained for α - and β -carotenes. These retentions, although considered adequate for canned products, are inferior to those obtained in the case of canned carrots with no salt added.

In the case of green jalapeño peppers, the following equations were obtained for the retention of α - and β -carotenes, lutein and violaxanthin:

$$\% \text{ retention } \alpha\text{-carotene} = 149.6 - 9.81 S \quad (3)$$

**Figure 2—Response surface plot of α -carotene retention (%) in canned carrots with 2% acetic acid.****Figure 3—Response surface plot of β -carotene retention (%) in canned carrots with 2% acetic acid.**

$$\% \text{ retention } \beta\text{-carotene} = 116.36 - 5.95 S \quad (4)$$

$$\% \text{ retention lutein} = 111.67 - 5.64 S \quad (5)$$

$$\% \text{ retention violaxanthin} = 24.12 + 1.02T - 5.11 S \quad (6)$$

It can be noted that, as in the case of the carrots, the addition of sodium chloride had a negative influence on the retention of the 4 carotenoids in the jalapeño peppers. The acetic acid had no influence on the retention of the carotenoids. In the case of violaxanthin there is a beneficial effect of the temperature, so high temperatures and short times are recommended for a maximal retention of this carotenoid. This particular case can be observed in Figure 4 where its response surface is shown. Then, for jalapeño green peppers, a salt concentration of 0 %, 2 % acetic acid and a temperature of 83 °C (for 4.82 min according to the equation of Ball) (Stumbo 1973) are recommended for the maximal retention of the four main carotenoids in green jalapeño peppers (149.6 % α -carotene, 116.36% β -carotene, 111.67% lutein and 108.78% violaxanthin according to equations 3, 4, 5 and 6). Again, in the special case of Mexico, the Official Regulations are to be considered. Substituting this restriction ($S = 2\%$) and $T = 83\text{ }^{\circ}\text{C}$ in equations 3, 4, 5 and 6, retentions of 129.98, 104.46, 100.39 and 98.56% for α -, β -carotene, lutein and violaxanthin, respectively, were obtained. As in the case of carrots, the retentions were adequate but smaller than the ones obtained for the low-sodium green jalapeño peppers.

In Mexico it is very common to pickle and can green peppers, carrots, onions and spices together to elaborate a product called "chiles en escabeche". In this case and considering the previous results, it would be necessary to choose the best temperature (between 67.1 and 83 °C) to pasteurize the product. Using equations 1,2 and 6, a temperature of 67.1 °C would be recommended for this last product, since at this temperature the violaxanthin retention in the peppers de-

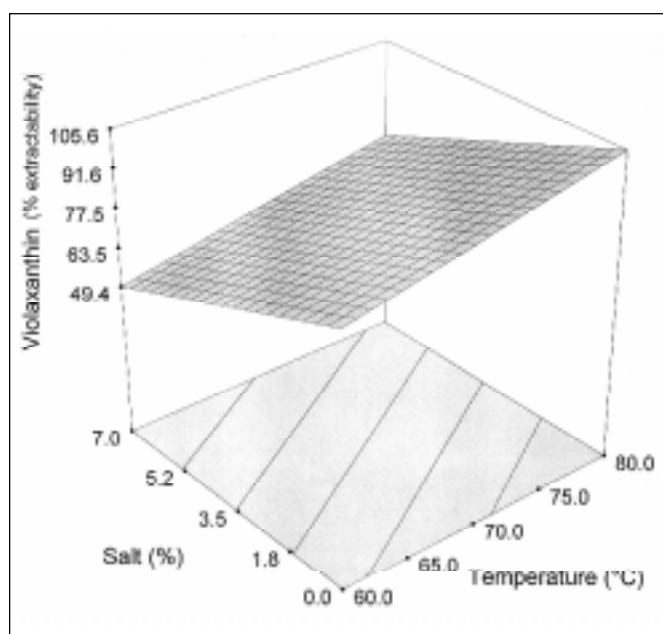


Figure 4—Response surface plot of violaxanthin retention (%) in canned green jalapeño peppers with 2% acetic acid.

creases only to 92.56%, whereas at 83 °C, the retention of α - and β -carotene in carrots would drop to 40.9 and 76.02%, respectively, for the product with 0% salt. In the case of the Mexican product with 2% salt, the violaxanthin retention in the jalapeños would decrease to 82.34% at 67.1 °C and the retention of α - and β -carotenes in the carrots would be reduced to 30.6 and 63.34% respectively. Again, the negative effect of the salt can be observed. Even when there is no salt in the brine, the product is shelf stable, since the low pH and the thermal processing are enough to guarantee that no spoiling would occur.

There are several studies about the effect of heating on carotenoids. Chen and Huang (1998) studied the degradation and isomerization of β -carotene as affected by oven-heating and found that a first-order model could fit these processes. They also found that 13-*cis*- β -carotene was formed preferentially during heating, however, this study was done using pure standards and not foods. Different authors (Bao and Chang 1994, Chen and others 1995) have concluded that the canning process decreased α - and β -carotene levels in carrot juice. The differences in carotenoid contents found in these studies between the solid vegetables and the juices support the idea that the presence of a solid matrix is very important.

In the case of carrots, several authors (Edwards and Lee 1986; Granado and others 1992; Howard and others 1999) have reported that cooking increased the levels of carotenoids in the product. This has been explained in terms of a loss of soluble solids into the brine during processing, a loss of carotenoid-degrading enzymes or an increase in tissue degradation, which allowed greater accessibility of the carotenes to the extracting solvents. In our case the presence of the salt and the acetic acid may have altered this behavior. Edwards and Lee (1986) did their study in carrots canned in water. In our study, the negative effect of the sodium chloride on the carotenoid retention is a very important factor which deserves to be studied separately, since it was observed in the two vegetables studied. It is possible that the salt, in some form, could help to dissociate the carotenoid-protein complexes and that the released carotenoid migrated into the brine. Also, the osmotic gradient induced by salt could promote leaching of the carotenoids out of the vegetative tissues. The actual mechanism remains to be studied.

Conclusion

IT WAS FEASIBLE TO SET A RANGE OF CONDITIONS TO OPTIMIZE the canning process for pickled carrots and green jalapeño peppers in order to secure a maximal retention of carotenoids by using the RSM. It was observed that sodium chloride had a negative influence on the retention of this kind of compounds in both vegetables.

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Departamento de Graduados e Investigación en Alimentos. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Carpio y Plan de Ayala, C.P. 11340, México, D.F., Mexico. Corresponding author: María Eugenia Jaramillo-Flores, Departamento de Graduados e Investigación en Alimentos. Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Carpio y Plan de Ayala, C.P. 11340, México, D.F., MEXICO. Tel: (52)-5729-6000 ext. 62464. Fax: (52)-5729-6000 ext. 62359. email: jaramillo_flores@hotmail.com