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Comparative study of carotenoid composition in three mexican varieties of *Capsicum annuum* L

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Abstract

Normal and reverse phase high-performance liquid chromatography have been used in order to identify and separate the carotenoid pigments present in the commercial varieties ancho, guajillo and mulato of *Capsicum annuum*, since there is considerable variation in carotenoid composition. In the reverse phase 13 common carotenoids out of 24 were found. In normal phase there were 14 common carotenoids out of 22. These varieties are highly appreciated and used because of their distinctive flavour and colour in Mexican cuisine. Among the major identified carotenoids, were β-carotene and β-cryptoxanthin which have provitamin A activity.

Keywords: *Capsicum annuum*; Mexican chile peppers; Carotenoid pigments; Pepper constituents; Provitamin A

1. Introduction

Capsicum annuum L., popularly known in Mexico as chile, has a very large number of varieties, and only a few have been studied in detail. Although *C. annuum* is originally from the American Continent, its use has spread to many countries all around the world. Some of the applications relate to its varied flavours while others are related to colour. The red colour of different spices, such as paprika, pimenton, is mainly due to the biosynthesis of keto carotenoids, such as capsanthin and capsorubin, which are characteristic of the genus (Curl, 1962; Davies, Susan, & Kirk, 1970), although other carotenoids may contribute to the red colour as well.

The colour of *C. annuum* fruits is variable, starting from green, yellow or white for the unripe fruit, and turning to red, dark red, brown and sometimes almost black in the ripe state (Long-Solis, 1998). The colour of each *Capsicum* variety in the full-ripe stage depends on its capacity for synthesizing carotenoids and even for retaining chlorophyll pigments. The dark red stage is the most common in commerce, followed by the brown. These commercially available varieties are valued, mainly for their characteristic aroma and flavour, although they also give a strong colour to food prepared using them. However, these varieties are not processed to obtain paprika or pimenton-like spices. The concentration of capsaicins, which are responsible for the peppery, burning sensation in chile peppers, is variable but low and they are not valued solely by their capsaicins content. The capsaicins content is unrelated to colour. The name chilli or ''chile'' is only applied in the current literature to those that are pungent as are most of the Longum group. However, there is a gradation of pungency and some of the varieties are known to show slight pungent taste or no pungency at all. This property has been manipulated by selection in order to obtain extremely pungent varieties, such as chile Habanero or sweet non-pungent varieties, such as pimenton. Traditionally in Hungary, paprika is appreciated for its pungent taste.

Carotenoids are usually fairly stable in their natural environment; however, they become much more labile once they are separated and purified or dissolved in organic solvents. The hydroxycarotenoids are frequently found as esters of long chain fatty acids and this fact

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also contributes to their stability. Due to the unsaturation of carotenoids, they must be carefully handled and kept in the absence of light and oxygen; pure samples must be kept at low temperatures (Kanner & Mendel, 1976; Malchev, Ioncheva, Tanchev, & Kalpakchieva, 1982). Most of the degrading reactions involve oxidation and formation of free radicals. The acidity of silica columns, especially when using normal phase HPLC, may cause some isomerization and other reactions, such as the transformation of carotenoid 5,6-epoxides to the 5,8 (furanoid) epoxides (Goodwin & Britton, 1988). The concentrations of compounds such as β-carotene and β-cryptoxanthin are specially important since they have provitamin-A activity (Minguez-Mosquera & Hornero-Mendez, 1994; Howard, Smith, Wagner, Villalon, & Burns, 1994). It is also noteworthy that α-carotene, lutein and β-cryptoxanthin have shown antitumor initiating activity (Nishino et al., 1999). The purpose of this paper is to compare the carotenoid composition of the three selected varieties as determined by normal and reverse-phase chromatography.

2. Materials and methods

2.1. Samples

Commercial samples from dried whole fruit of the three varieties, Ancho, Mulato and Guajillo, were purchased from the market (San Lazaro brand). One kilogramme was selected and examined for integrity of the fruit and absence of dust and insect contamination. From 800 g each of Ancho, Mulato and Guajillo (whole fruit), the seeds and peduncules were eliminated, and they were cut into small pieces.

2.2. Pigment extraction

Ten grammes were taken from representative samples of minced and homogenized material from each variety and each sample was extracted with acetone, using stirring and in the absence of light. The treatment was repeated until no more colour was extracted. The extracts of each variety were pooled, placed in a separating funnel and mixed with 100 ml of ethyl ether and treated with 100 ml of a 10% NaCl water solution; the phases were separated and washed with a saturated Na_2SO_4 solution. The ether layer was further dried over anhydrous Na_2SO_4 to remove the remaining water. The ether phase, containing carotenoid esters, was saponified by using 100 ml of a solution containing 20% potassium hydroxide and 20% MeOH. The mixture was allowed to stand for 1 h, with shaking in the dark. Afterwards, the aqueous phase was removed and the organic phase washed with distilled water until neutral, and then evaporated to dryness at 35 °C in a rotary evaporator.

The pigments were kept at −20 °C in acetone solution prior to the HPLC separation. General work-up methods, such as sample preparation and chlorophyll determination, were done according to Harold, Benjamin, and Walter (1971).

2.3. Separation and quantification of pigments by HPLC

Two different techniques were used in order to separate and quantify the carotenoid pigments by HPLC. A normal phase separation was carried out, following the method by Almela, López-Roca, Candela, and Alcazar (1990), with some minor modifications. The column used was a Millipore, Waters Div. (Milford, Massachusetts, USA) μ-porasil 125 Å, 10 μm, 3.9×150 mm, under the conditions described in the above reference, using a gradient of hexane and acetone (9.5:0.5). For the reverse-phase HPLC, which is more frequently used (Deli, Matus, & Tóth, 1996), we employed a Waters μ-bondapak C_{18} column 3.9×150 mm, using a 1.5 ml/min flux. The solvents used were all of HPLC grade and filtered through a 0.45 μm membrane and degassed before use. The instrument used was a Waters Delta prep 4000, with tunable absorbance detector at 450 nm and integrator system.

Other apparatus used included a Büchi rotavapor RE/A, Flawil, Switzerland and a UV lamp UVP, Inc. Model UVGL-25 Mineral light 254/366 nm, San Gabriel, CA, USA.

Some response factors were taken from Minguez-Mosquera and Hornero-Méndez (1993) and transformed to β-carotene base as explained in the same reference using the formula:

$$F_{i/\beta\text{-car}} = f_i / f_{\beta\text{-car}},$$

where i represents each pigment and $F_{i/\beta\text{-car}}$ is the factor of each pigment relative to β-carotene. The additional factors used were derived from the relative areas of peaks and weights described by Minguez-Mosquera and Hornero-Mendez (1994), Deli, Matus, and Szaboles (1992, 1996) and Almela et al. (1990). The response factors in this paper were calculated with respect to β-carotene and are shown in Table 1.

2.4. Identification of carotenoids

Pure samples of β-cryptoxanthin, capsorubin and capsanthin, were kindly provided by Dr. Fernando Walls Armijo (Instituto de Química, México, DF); pure β-carotene was obtained from Sigma Chemical Co., St. Louis, MO, USA. The above mentioned carotenes, together with anteraxanthin, were isolated by thin-layer chromatography from the three varieties of *C. annuum* and identified by UV–Vis and infrared spectra and melting point. Lutein and canthaxanthin were only isolated from Mulato, while neoxanthin was isolated

Table 1
Response factors with respect to β-carotene

Pigment	Response factors[a]
Latoxanthin	1.068
Capsorubin	1.676
Neoxanthin	1.899
Capsanthin 5,6-epoxide	1.690
Violaxanthin	1.124
Capsanthin 3,6 epoxide	1.598
Luteoxanthin	0.429
Cucurbitaxanthin B	1.800
Cucurbitaxanthin A[b]	1.088
Capsanthin	1.175
Cycloviolaxanthin	1.005
Antheraxanthin	0.774
Mutatoxanthin 2	0.734
Mutatoxanthin 1	0.493
9-*cis*-Capsanthin	1.891
13-*cis*-Capsanthin	2.294
Lutein	1.100
Zeaxanthin	1.021
9-*cis*-Zeaxanthin	1.309
13-*cis*-Zeaxanthin	1.620
15-*cis*-Zeaxanthin	2.330
Cryptocapsin	1.288
Cryptoflavin	2.480
α-Cryptoxanthin	0.699
β-Cryptoxanthin	1.010
9-*cis*-Cryptoxanthin	1.360
α-Carotene	2.303
β-Carotene	1.000
9-*cis*-β-Carotene	1.166

[a] Response factors were determined at 450 nm, using β-carotene as reference.
[b] From normal phase chromatography data.

Table 2
Carotenoid composition in three varieties of *C. annuum* in reverse-phase HPLC (% of total carotenoids)

Pigment (Order of elution)	Ancho	Guajillo	Mulato
1. Latoxanthin	5.55	1.56	1.53
2. Capsorubin[a]	2.17	0.35	4.20
3. Neoxanthin[a]	–	0.28	2.20
4. Capsanthin 5,6-epoxide	–	–	1.43
5. Violaxanthin	14.5	13.2	22.0
6. Capsanthin 3,6-epoxide	–	–	11.6
7. Luteoxanthin	–	–	2.10
8. Cucurbitaxanthin B	10.5	–	–
9. Capsanthin[b,a]	9.69	12.6	11.2
10. Cycloviolanxanthin	–	0.51	–
11. Anteraxanthin[a]	traces	7.07	traces
12. Mutatoxanthin 2	0.30	0.31	1.23
13. Mutatoxanthin 1	0.33	0.30	–
14. 9-*cis*-Capsanthin	10.1	10.0	9.93
15. Lutein[a]	–	–	0.88
16. Zeaxanthin[b]	4.29	1.88	3.56
17. 9-*cis*-Zeaxanthin	–	0.50	–
18. 15-*cis*-Zeaxanthin	–	4.79	–
19. Canthaxanthin	–	–	0.78
20. Cryptocapsin	0.24	10.0	0.62
21. α-Criptoxanthin	5.28	2.24	0.72
22. β-Criptoxanthin[b]	9.70	9.53	6.10
23. β- Apo-8′-carotenal[a,b]	ref.	ref.	ref.
24. α-Carotene[b]	2.1	1.27	2.98
25. β-Carotene[a,b]	20.9	17.9	14.9
Non-identified compounds	4.39	5.74	2.00
weight (%)	100%	100%	100%
Total carotenoids (mg/100 g dw)	7.52	6.76	7.24

Most carotenoids were assigned only by their retention times in HPLC and UV–Vis spectra.
[a] Compounds isolated and identified by UV, visible, infrared and mp.
[b] Compared with reference standards.

from Guajillo and Mulato and identified by UV–Vis and infrared spectra and melting point.

Most carotenoids were assigned only by their retention times in HPLC and UV–Vis spectra. For this purpose we followed, as closely as possible, the conditions described by Deli et al. (1996) in the case of reverse phase chromatography and in the case of normal phase those of Almela et al. (1990).

The provitamin A activity was calculated as retinol equivalents (RE) according to WHO (1982) guidelines (Mejia, Hudson, Gonzalez de Mejia, & Vazquez (1988); Simpson, 1983). For this purpose, β-carotene weight was divided by six, while β-cryptoxanthin and α-carotene weights were divided by twelve. The weight percent of carotenoids was obtained considering the total carotenoid dry weight for each variety, together with the weight percents from Table 2.

3. Results and discussion

The composition of carotenoids present is similar, in qualitative terms, to that observed in other *C. annuum* varieties examined in the literature (Deli et al., 1992, 1996). However, there are some important differences in the presence or absence of certain carotenoids, as well as in the relative concentration. This is a first time report for the carotenoids in these three chile pepper varieties.

We first direct attention to the pattern of carotenoids present. When using reverse phase HPLC (see Table 2 and Fig. 1), the last five non-polar components show a similar pattern for the three varieties. Capsanthin together with its 9-*cis* isomer and violaxanthin represent a major part of the carotenoid composition. There is also a similar pattern in normal phase HPLC (see Table 3 and Fig. 2) when comparing the non-polar portion between 1.8 and 7.63 min, especially when observing Mulato and Guajillo. In normal phase HPLC there were 14 common carotenoids out of a total of 22. In reverse phase there were 13 common carotenoids out of total of 24 identified carotenoids. In Ancho and Guajillo, β-carotene is the main component present. The next most abundant carotenoid is violaxanthin. This is at variance with respect to other *C. annuum*, in which the most abundant carotenoid is capsanthin. In Ancho and Guajillo, the next

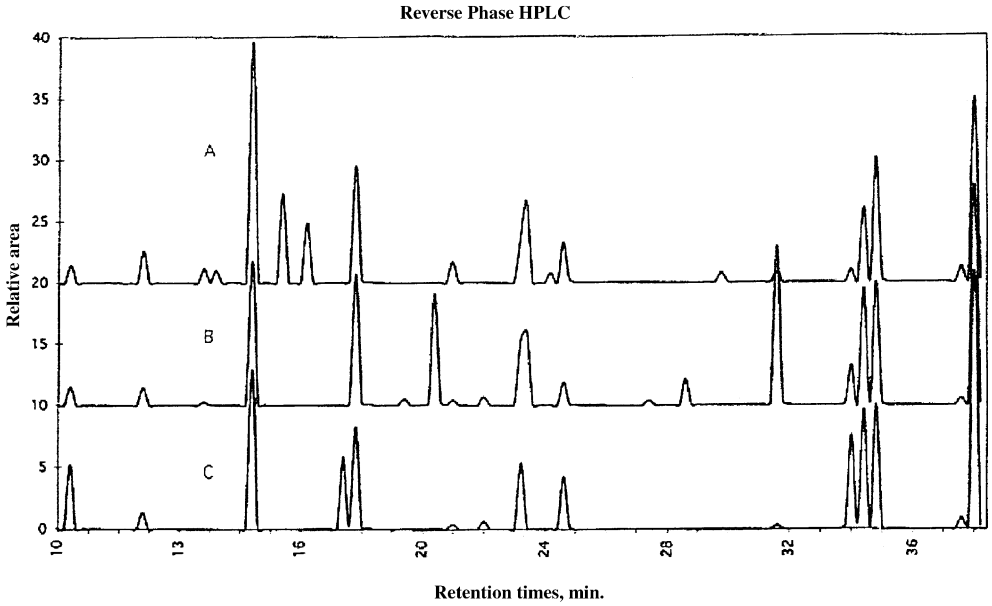

Fig. 1. Reverse phase HPLC. The relative areas of carotenoids (retention time) are shown for: latoxanthin (9.7), capsorubin (11.4), neoxanthin (13.1), capsanthin 5,6-epoxide (13.25), violaxanthin (14.4), capsanthin 3,6-epoxide (15.3), luteoxanthin (16.1), curcurbitaxanthin B (17.3), capsanthin (17.8), cicloviolaxanthin (19.3), antheraxanthin (19.3), mutatoxanthin (20.9), mutatoxanthin (21.9), 9-*cis*-capsanthin (23.0), lutein (24.0), zeaxanthin (24.3), 9-*cis*-zeaxanthin (27.2), 15-*cis*-zeaxanthin (28.4), canthaxanthin (29.6), cryptocapsin (31.5), α-cryptoxanthin (33.9), β-crytopxanthin (34.4), β-apo-8′-carotenal (34.9), α-carotene (40.4), β-carotene (40.8) A: Mulato, B: Guajillo, C: Ancho.

Table 3
Carotenoids identified in normal phase HPLC in three varieties of *C. annuum* (% of total carotenoids)

Pigment (order of elution)	Ancho	Guajillo	Mulato
1. β-Carotene[a,b]	19.7	16.2	11.0
2. *cis*-β-Carotene	–	4.29	8.80
3. α-Carotene	13.8	7.65	8.50
4. Cryptocapsin	–	6.87	5.63
5. Cryptoflavin[c]	8.06	4.13	3.50
6. β-Cryptoxanthin[a]	–	4.43	0.33
7. α-Cryptoxanthin	–	10.8	7.08
8. Antheraxanthin[b]	Traces	Traces	Traces
9. Lutein[b]	Traces	Traces	0.02
10. Cucurbitaxanthin A	–	–	2.75
11. Luteoxanthin	2.67	–	2.75
12. Zeaxanthin	8.63	4.41	0.03
13. *cis*-Zeaxanthin	8.40	–	1.79
14. Mutatoxanthin	Traces	3.42	7.30
15. Cycloviolaxanthin	–	1.15	0.29
16. 9-*cis*-capsanthin[b]	17.7	7.88	0.12
17. Capsanthin[a,b]	5.82	Traces	0.05
18. Capsanthin 3,6 epoxide	Traces	0.85	1.16
19. Violaxanthin	Traces	Traces	11.1
20. Capsorubin[b]	1.40	0.30	0.67
21. Neoxanthin[b]	Traces	6.00	5.93
22. Latoxanthin	0.71	0.88	2.96
Non-identified compounds	15.7	20.8	20.3
weight (%)	100%	100%	100%

[a] Compared with reference standards.
[b] Compounds isolated and identified by UV–Vis, infrared and mp.
[c] Carotenoids which were assigned only by normal phase HPLC.

most abundant carotenoid is violaxanthin. In Mulato, the main component is violaxanthin when using reverse phase HPLC; the next most abundant carotenoid is β-carotene. In Guajillo and Mulato, *cis*-β-carotene is found when normal phase HPLC is used.

The detection of cryptoflavin in normal phase HPLC (see Table 3 and Fig. 2) in all three varieties and its absence in all three varieties, in reverse phase HPLC, is noteworthy and intriguing. To our knowledge, cryptoflavin has only been found in *C. annuum* when normal phase HPLC is used. One possible explanation is that peak resolution may not be high enough. Another possible explanation is that cryptoflavin may be an artifact, produced as a result of a catalytic effect by silica in normal phase HPLC. Also noteworthy is that the three varieties which we examined differ in colour. Mulato is quite dark (almost black) and this may be explained in part by its relatively large chlorophyll content (9.78 μg/g), together with the large number of carotenoids present, including a high concentration of violaxanthin and capsorubin, capsanthin 5,6-epoxide (Matus, Deli, & Szaboles, 1991), and capsanthin 3,6-epoxide (Parkes Kevin & Pattenden, 1986) (total carotenoid content 7.24 mg/100 g). Ancho is reddish brown (total carotenoid content 7.52 mg/100 g) and has a small amount of chlorophyll (0.96 μg/g). Finally, Guajillo has a red colour (total carotenoid content 6.76 mg/100 g) and has no chlorophyll.

Three of the carotenoids found have provitamin A activity, (β-carotene, α-carotene and β-cryptoxanthin) (Howard et al., 1994; Mejia et al., 1988). The provitamin A activity is highest in Ancho (335 μg RE/100 g d.w.) mainly due to β-carotene (262 μg RE/100 g d.w.) and to β-cryptoxanthin (69.3 mg RE/100 g d.w.); this variety is

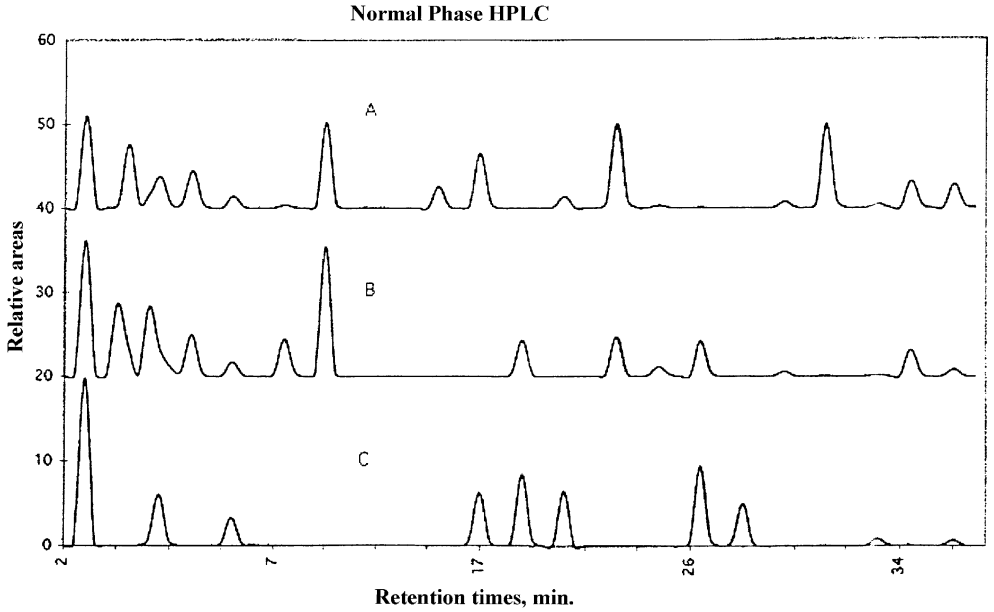

Fig. 2. Normal phase HPLC. The relative areas for carotenoids (retention time) are shown for: β-carotene (1.85), *cis*-β-carotene (2.35), α-carotene (2.68), cryptocapsin (3.32), cryptoflavin (4.05), β-cryptoxanthin (7.38), α-cryptoxanthin (7.63), anteraxanthin (9.33), lutein (11.55), curcurbitaxanthin A (13.47), luteoxanthin (17.27), zeaxanthin (19.56), *cis*-zeaxanthin (20.25), mutatoxanthin (23.67), cycloviolaxanthin (24.60), 9-*cis*-capsanthin (26.08), capsanthin (27.50), capsanthin 2,6 epoxide (28.75), violaxanthin (30.82), capsorubin (33.12), neoxanthin (33.63), latoxanthin (34.83) A. Mulato, B: Guajillo, C: Ancho.

followed by Guajillo (263 μg RE/100 g d.w.), mainly due to β-carotene (201 μg RE/100 g d.w.) and to β-cryptoxanthin (53.7 μg RE/100 g d.w.). The lowest provitamin A activity is calculated for Mulato (235 μg RE/100 g d.w.) also mainly due to β-carotene (180 μg RE/100 g d.w.) and to β-cryptoxanthin (36.8 μg RE/100 g d.w.).

4. Conclusions

We found considerable differences in the carotenoid compositions of the selected varieties of *C. annuum* L. All the studied varieties show relatively high provitamin A carotenoid contents. The Ancho variety showed the highest provitamin A value (335 μg RE/100 g dry weight), followed by Guajillo (263 μg RE/100 g dry weight); the lowest is Mulato (235 μg RE/100 g dry weight).

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