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Original Article

Antioxidant compounds from coriander (*Coriandrum sativum* L.) etheric extract

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Abstract

Five fractions (β-carotene, β-cryptoxanthin epoxide, lutein-5,6-epoxide, violaxanthin and neoxanthin) were isolated from a coriander ether extract using column chromatography and identified according to their chromatographic and spectral characteristics. No significant differences were noted with regard to their antioxidant properties using the β-carotene/linoleic acid model, however they were inferior to the BHT and the crude etheric extract. The β-carotene component represented 61.14% of the carotenoids detected in the extract suggesting that it is a principal component in coriander antioxidant action. The greater antioxidant effect of the crude extract compared to its component fractions suggests a synergistic action between the carotenoids.

Keywords: Carotenoids; Etheric extract; Antioxidant; Coriander

1. Introduction

Experimental and epidemiological evidences have demonstrated the important role of free radicals in the majority of degenerative diseases and the ageing process. As a result, research in the last two decades has been directed towards the search for bioactive phytochemicals. The carotenoids, secondary metabolites of mevalonic acid, are an important part of this group. Their

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antioxidant action has been highlighted amongst other important biological functions (Craig, 1999). This action, attributed to the carotenoids, whether precursors of vitamin A or not, is highly influenced by the chemical structure (Delgado-Vargas et al., 2000). Their efficacy, either in biological systems or in food, seems to be related to the ability to act as singlet oxygen quenchers, the highly reactive form of oxygen, and/or interact with free radicals (Jorgensen and Skibsted, 1993; Palozza and Krinsky, 1992).

Approximately 600 types of pigment, widely distributed in nature principally in the form of leafy vegetables, have been isolated and characterized (Beutner et al., 2001; Rodriguez-Amaya, 1999a). Ramos and Rodriguez-Amaya (1987) and Bhaskarachary et al. (1995) detected the presence of these substances in coriander leaves, *Coriandrum sativum* L., whose antioxidant action in crude extract was detected by Guerra (1975) and confirmed recently by us in a previous study (Melo, 2002). This observation has motivated the continuity of research with the aim of identifying the carotenoids responsible for this action.

2. Material and methods

The coriander (*Coriandrum sativum*), "verdão" cultivar, donated by the Agronomic Department—UFRPE, Brazil, was transported immediately after harvest to the Food Analysis and Experimentation Laboratory of the Nutrition Department of UFPE, where the study was performed. After copious washing in running water, the roots were removed and the leaves and shoots were laid out on a nylon mesh and dried with forced circulating air at 45°C for 48 h. The dried product was ground into a fine power, passed through an 80-mesh sieve and kept frozen at −18°C in polyethylene bags during the study.

2.1. Extract obtention

The powdered coriander (10 g) was submitted to extraction with ethyl ether (100 mL), for 60 min, under agitation at room temperature (25°C ± 2°C), and then the mixture was centrifuged at 3000 g for 10 min. After transferring the supernatant into the flask, the residue was resuspended in ethyl ether (100 mL) and again submitted to the same extraction process. The supernatants were combined and transferred to amber flasks, flushed with nitrogen and stored in a freezer at -18°C until used for analysis.

2.2. Ether extract fractionating

The etheric extract was submitted to saponification with 10% KOH in methanol and left in the dark at room temperature overnight to remove the lipids and chlorophyll, according to the analytical procedure described by Rodriguez-Amaya (1999b). Subsequently, the mixture was exhaustively washed with distilled water in a separatory funnel until free of alkali. The pigments were then concentrated in a rotary evaporator under reduced pressure at 35°C. A 10 mL sample of pigment was applied to a glass column (1.5 × 21 cm), packed with hyflosupercel and magnesium oxide (2:1) to the height of approximately 15 cm. The top of the column had a 1 cm layer of anhydrous sodium sulphate to ensure that no residual water got into the adsorbent. Petroleum

ether (100%), ethyl ether in petroleum ether (1%, 4%, 8%, 12%, 20%, v/v), acetone in petroleum ether (4%, 12%, 20%, 25%, v/v) and acetone (100%) were used for the mobile phase. The fractions obtained using acetone were transferred to the petroleum ether in a separatory funnel and washed exhaustively until free of acetone.

2.3. Carotenoid identification

Identification was undertaken on the basis of the visible absorption spectra, thin-layer chromatography (TLC) and specific chemical reactions. The fractions were adequately diluted in petroleum ether and the visible absorption spectra (350–550 nm) were obtained using the Hitachi U 3200 spectrophotometer and compared with the values obtained by Rodriguez-Amaya (1999b), Britton (1991) and Davies (1976). The concentration of carotenoids in each fraction was determined considering the maximum absorbance and specific absorbance coefficient ($E_{cm}^{1\%}$), and the data obtained in the literature (Britton, 1991; Rodriguez-Amaya, 1999b). The amount of carotenoids was calculated from the equation:

$$\mu g/g = \frac{\text{Volume} \times \text{Absorbance}_{max} \times 10^{-6}}{E_{cm}^{1\%} \times \text{sample weight (g)} \times 100}.$$

The fractions obtained from the column were concentrated in a rotary evaporator under reduced pressure of 35°C and applied on silica gel thin layer (TLC-60-F_{254}, 20 × 20 cm with thickness 0.25 mm—Merck), using 3% methanol in benzene as the mobile phase. The R_f values were calculated for each stain. The plate was then exposed to hydrochloric acid vapour in order to detect the presence of epoxy carotenoids. The test is considered positive when the yellow stains of the chromatogram turn blue or green.

Few drops of 0.1 N hydrochloric acid were then added to the alcoholic pigment solution and after 3 min, the spectrum was recorded in order to verify the presence of the 5,6 epoxide group in the carotenoids' structure. The pigments that demonstrated a hypsochromic effect of 20–40 nm were considered to possess one or two 5,6 epoxide groups, respectively (Davies, 1976).

2.4. Antioxidant activity

The antioxidant activity of the fractions, containing approximately 1.50 μg of carotenoids, was determined by the coupled oxidation of the β-carotene and the linoleic acid, according to the methodology described by Marco (1968) and modified by Hammerschmidt and Pratt (1978). This was subsequently expressed as an oxidation inhibition percentage, calculated in relation to the 100% oxidation that occurs in the control (no antioxidant). The BHT and the crude etheric extract antioxidant activity were determined under the same conditions, as a means of comparison.

2.5. Statistical analysis

All analyses were performed in triplicate. The data obtained were submitted to variance analysis (F test) and the Tukey test, at the 5% significance level, using the Minitab statistical Windows program.

3. Results and discussion

Five fractions were obtained from the coriander etheric extract using column chromatography. The first was eluted with petroleum ether, the second, third and fourth with acetone in petroleum ether (12–25%), whilst the fifth was eluted with acetone (100%). The fractions absorption spectrum (350–550 nm) showed three well-defined peaks (Fig. 1), characteristic of the majority of the carotenoids.

Considering the λ_{max} values 425, 448, 475 for the β-carotene, as determined by Harborne (1973) and Davies (1976), and those referred by Rodriguez-Amaya (1999b) (λ_{max} 425, 450, 477), it was confirmed, through the absorption spectra of the first fraction, that the isolated compound presented the same characteristic as these carotenoids. The remaining fractions (2–5) presented λ_{max} values similar to the xanthophylls: β-cryptoxanthin epoxide, lutein 5,6 epoxide, violaxanthin and neoxanthin, respectively (Table 1). According to Rodriguez-Amaya (1999b) the λ_{max} values of these xanthophylls are: neoxanthin 416, 438, 467; lutein 5,6 epoxide 420, 440, 470 and violaxanthin 416, 440, 465. Britton (1991) used the values 418, 443, 470 for the β-cryptoxanthin epoxide. Hypsochromic effect, 20 nm for the second, third and fifth fractions and 40 nm for the fourth fraction, was noted, confirming the presence of one and two groups of 5,6 epoxide, respectively.

The fractions observed on the TLC chromatogram, with exception of the first, showed blue or green colouring when exposed to HCl vapour, characteristic of the epoxide. The R_f values of the fractions 1, 2, 3, 4, 5 were similar to the β-carotene and the xanthophylls, β-cryptoxanthin epoxide, lutein 5,6 epoxide, neoxanthin and violaxanthin, respectively. The chromatograms demonstrated characteristics similar to those described by Rodriguez-Amaya (1999b) as follows: the β-carotene that has no polar substituents, runs with the solvent front and therefore demonstrated a high R_f; the β-cryptoxanthin, with its single hydroxyl group, showed R_f of around 0.44; the lutein, with two hydroxyl groups, exhibited multizoning on TLC, appearing as two spots, with the principal spot having an R_f of around 0.21; the violaxanthin exhibited R_f less than 0.19; the neoxanthin, possessing three hydroxyl groups, remained near the application point. These findings correlate with those of Ramos and Rodriguez-Amaya (1987) and Rodriguez-Amaya

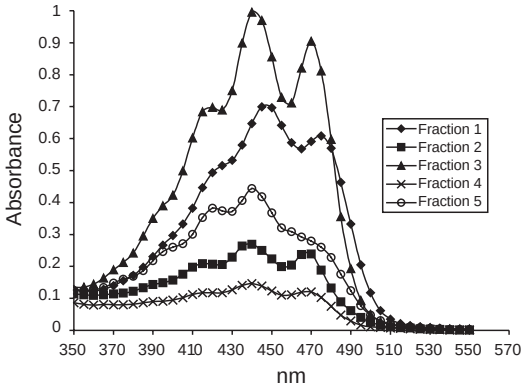

Fig. 1. Carotenoids visible absorption spectra from coriander etheric extract obtained by chromatographic column.

Table 1
Chromatographic characteristics of coriander etheric extract fractions

Fractions	R_f[a]	HCl vapour	Hypsochromic effect	λ_{max} (nm)[b]		
1. β-Carotene	0.99	–	–	(425)	450	475
2. β-Cryptoxanthin epoxide	0.37	+	+	415	440	467
3. Lutein 5,6 epoxide	0.22	+	+	420	441	470
4. Violaxanthin	0.17	+	+	418	442	465
5. Neoxanthin	0.09	+	+	417	438	466

[a] R_f value in 3% methanol in benzene.
[b] Parentheses indicate a shoulder.

Table 2
Carotenoids composition of coriander etheric extract

Fractions	Carotenoids	μg g^{-1}[a]
1	β-Carotene	328.2
2	β-Cryptoxanthin epoxide	14.1
3	Lutein 5,6 epoxide	155.4
4	Violaxanthin	14.3
5	Neoxanthin	24.7
	Total	536.7

Analysis was performed in triplicate ($n = 3$).
[a] μg g^{-1} dry sample.

(1999a) which reported that it is to be generally presumed that green leafy vegetables contain these carotenoids.

The carotenoids levels, calculated according to the absorption coefficients ($E_{cm}^{1\%}$) 2592, 2386, 2400, 2550 and 2243 referring to β-carotene, β-cryptoxanthin epoxide, lutein 5,6 epoxide, violaxanthin and neoxanthin, respectively, cited by Rodriguez-Amaya (1999b) and Britton (1991), are found in Table 2. Ramos and Rodriguez-Amaya (1987) also detected the presence of lutein which, with β-carotene, were the only carotenoids present in the sample that could be effectively quantified.

The antioxidant activity of each fraction was inferior to the BHT and crude etheric extract (61.89%). The first fraction presented 49.66% oxidation inhibition, a value greater than the remaining fractions (42.89%, 42.31%, 42.88% and 41.13%) (Fig. 2). From a statistical perspective, fractions 1, 2, 3 and 4 were similar, whereas the fifth fraction was significantly inferior to the first, although similar to the remaining fractions.

Although the antioxidant action was inferior to BHT, the whole coriander etheric extract and its fractions presented a considerable potential as oxidation inhibitors. These substances can be ingested without limitation, according to the Food and Nutrition Board of the National Academy of Sciences. Data concerning potential adverse effects have been inconsistent and contradictory. Consequently, quantities greater than those used in this study could be used to obtain an even greater antioxidant effect. In addition, the coriander extract with its predominance of β-carotene

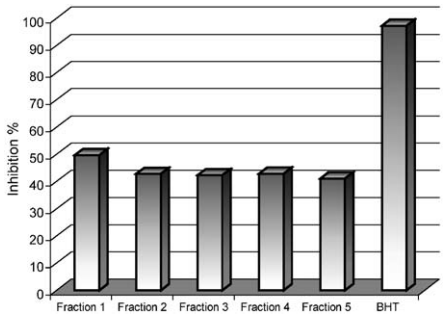

Fig. 2. Antioxidant activity of coriander etheric extract fractions.

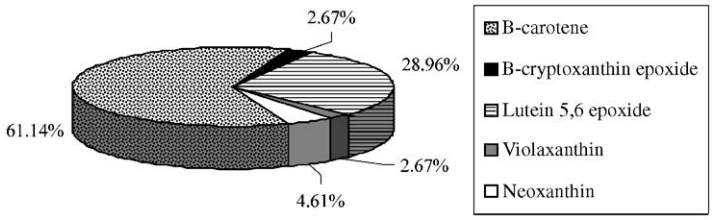

Fig. 3. Carotenoid percentages values in coriander etheric extract.

(61.14%) (Fig. 3), that also demonstrated vitamin A activity, could contribute to reduce the deficiency of this vitamin.

4. Conclusion

In the carotenoids fractions obtained from coriander etheric extract, β-carotene has been identified as the principal antioxidant component. No significant difference in antioxidant activity was found when compared to the other carotenoids. Although inferior to BHT with regard to its protection against lipid oxidation, the carotenoids have the advantage that they can be consumed in unlimited quantity. The greater antioxidant effect of the whole coriander etheric extract in comparison to the component fractions suggests a possible synergistic effect. In the light of these findings and the antioxidant action demonstrated in this study, the coriander etheric extract could be considered as a promising source of bioactive substances.

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