CONTENT OF HIGH BIO AVAILABLE CAROTENOIDS IN TOMATO - BASED PRODUCTS

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Abstract

The most abundant carotenoids present in tomato-based products were taken into account. Several samples coming from Italian stores have been analysed by using an RP – HPLC method. These products show a free carotenoids content proportional to the intensity of the manufacturing processes to which their are subjected. Among the carotenoids lycopene isomerises in various cis forms under different chemical – physical conditions. The lycopene antioxidant activity appears to be related to its *trans – cis* isomerisation degree, and the stability and the relative abundance of the isomers were investigated by applying heating and illumination. The cooking process seems to increase the total lycopene availability, whereas a prolonged exposure to a light source appears to be the best way to obtain the cis isomers. β-carotene is partially subjected to degradative processes, and do not shows an appreciable isomerisation degree under the same conditions.

Riassunto

In questo lavoro vengono studiati i più importanti carotenoidi presenti nei prodotti a base di pomodoro. I campioni dei diversi prodotti, reperiti sul mercato italiano, sono stati analizzati usando una metodica RP-HPLC. La quantità dei carotenoidi liberi riscontrata, appare proporzionale all'intensità del trattamento tecnologico impiegato. Tra i carotenoidi il licopene isomerizza in diverse forme cis a seconda delle diverse condizioni chimico-fisiche del trattamento.

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L'attività antiossidante del licopene appare correlabile al grado di isomerizzazione *trans – cis*; e sono state studiate la stabilità e la relativa abbondanza degli isomeri nei trattamenti di cottura ed illuminazione. Nel processo di cottura è stato evidenziato un incremento della quantità di licopene disponibile, mentre una prolungata esposizione alla luce determina una maggiore quantità di cis isomeri. Il β-carotene è parzialmente soggetto a processi degradativi, e non mostra un apprezzabile grado di isomerizzazione nelle stesse condizioni.

Keywords: carotenoids, isomerisation, lycopene, technological treatment tomato-based products

Introduction

The carotenoids constitute an abundant group of naturally occurring pigments responsible for a range going from light yellow to dark red colouring vegetables and fruits. They belong to one of the main classes of isoprenoids, the tetraterpenoids, having a central skeleton made up of 22 carbon atoms and two ends with an additional 9 carbon atoms each. The carotenoids may be acyclic (e.g. lycopene) or contain 5- or 6-membered rings at one or both ends of the molecule (e.g. *β*-carotene, lutein).

In raw tomato (*Lycopersicum esculentum*) lycopene is by far the most abundant carotenoid (80-90 % of the total carotenoids), while the phytoene, second in abundance (5-10 %), is also an *in vivo* precursor of lycopene (1). Because of long-chain conjugated double-bond presence, lycopene has been reported to possess the highest antioxidant activity of all the carotenoids and to be responsible for protecting cells against oxidative damage and thereby decreasing the risk of chronic diseases (2-4). As an antioxidant, it has the ability, to quench singlet oxygen (more than *β*-carotene), to trap peroxyl radicals, to inhibit the oxidation of DNA, to inhibit lipid peroxidation and to inhibit the oxidation of low-density lipoprotein (LDL) (5-6). It may also show suppression of the proliferation of human cancer cells and therefore contribute to lower risk especially for prostate, pancreas, intestine, and lung cancer (7-9).

Like other carotenoids, lycopene occurs in various geometrical isomers. In most raw foods, the thermodynamically more stable all-*trans* configuration is quantitatively the most important, and within the relatively small portion of *cis*-isomers, 5, 9, 13 *cis*-lycopene usually predominate.

In red tomato fruits, typically 94-96% all-*trans*, 3-5% 5 *cis*, 1% 13 *cis* and 0.1% 9-*cis* can be found, while in Tangerine-type tomatoes the tetra *cis*-isomers (7, 9, 7′, 9′) were shown to account for more than 90% of the lycopene content due to a lack of carotenoid isomerise (10-11).

The all-*trans* configuration may be converted in some different *cis* configurations during food processing (12), and several reports have demonstrated that the isomerisation process strongly increases the bio-assimilation (13-14).

β-carotene, formed via the action of the enzyme lycopene *beta*-cyclase, is present at a lower percentage (3-5%) in raw tomato. This molecule, as is well known, is an *in vivo* precursor for vitamin A (its provitaminic activity is approximately twice that of α and γ isomers activity) and exhibits a high antioxidant activity, by scavenging peroxyl radicals, although it depends on the oxygen tension present in the system (15).

β-carotene, naturally present in the all-*trans* form, is partially converted into its different *cis*-isomers after processing, under effects of heating and illumination (16-17), and it has been found, for example, that all-*trans β*-carotene was lower in canned carrots and other processed vegetables, as compared to the fresh samples (18). In addition, some authors reported that all-*trans*-*β*-carotene had higher antioxidant activity than 13-*cis β*-carotene (19), and others recently demonstrated the contribution of both the main *β*-carotene isomers, 9-*cis* and all-*trans* isomers, in a synergistic way (20).

The aim of the present study was to examine the presence and the relative abundance of *cis*-isomers of lycopene and *β*-carotene in some tomato based products, and to test the isomerisation degree under treatments such cooking and light exposure.

The tomato based products, according to Italian Regulation (D.P.R. 11.4.1975) are the following: peeled, sauces, semi-condensed (at least 12% of dry residue), condensed (18%), double condensed (28%), triple condensed, (36%) sextuple condensed (55%). Carotenoids analysis was carried out using a highly sensitive diode array detector (DAD) coupled with reversed-phase HPLC (21-23).

Materials And Methods

Chemicals

Acetone, hexane, methanol and methylene chloride were purchased by Merck (Darmstadt, Germany). Lycopene of 98% purity and all-

trans-*β*-carotene with a purity greater than 95% were supplied by Sigma - Aldrich (St. Louis, MO, USA).

Samples

The most representative Italian trademarks were taken into account, by examining sixteen samples of tomato based foods purchased in retail stores across Italy. The samples were grouped in three categories of product: peeled tomatoes, sauces and condensed sauces (condensed, double and triple). The condensed, double and triple contain dry residual superior to 18%, 28% and 36 % respectively. Seven batches for each sample of tomato based product were purchased.

About 10 g of homogenised sample were extracted with 50 mL of hexane-acetone-methanol (2:1:1) solution (24). The homogenate was subsequently washed and filtered in a Büchner funnel under vacuum. The extract was dried in a rotary vacuum evaporator at room temperature in nitrogen atmosphere. Then, methylene chloride was added to the residue and the solution was made up to a final volume of 25 mL.

HPLC analysis

The equipment consisted of a Spectra System P2000 pump with an SCM 100 vacuum membrane degasser and a Spectra System UV6000LP detector set at 476 nm (Thermoquest, San Jose, CA, USA). Samples were injected with a model 7725i sample valve equipped with a 20 μL loop (Rheodyne, Rohnert Park, CA, USA); the volume injected was 10 μL. Peak areas were calculated by means of Chromquest software (Thermoquest). Separations were carried out with a Supelco Discovery, RP-C18 (25cm × 4,6 mm; 5≒m i.d.) reversed phase column (Supelco, Bellefonte, PA, USA) equipped with a Supelco Pelliguard LC-18 2cm precolumn (Supelco). Although C30 columns have been reported to be the first choice for lycopene isomers analysis in physiological matters, C18 columns have been already adopted for simultaneous determination of lycopene and β-carotene isomers in vegetables extracts (25-26).

The column was eluted with two mobile phase solvents. Solvent A contained methylene chloride, acetonitrile, methanol (10:73:17 v/v/v) and solvent B contained methylene chloride, acetonitrile, methanol (26:65:9 v/v/v). Each solvent was prepared fresh before use. The gradient elution was: 100% A for 10 min, then increased to 100% B in 1 min; 100% B was hold for 10 min and return to 100% A in 1 min, then 100% A was hold for 3 min. The flow rate was 1 mL/min. An example of a chromatogram obtained by applying the above mentioned conditions is reported in the Figure 1. HPLC standards were prepared by dissolving 10 mg *β*-carotene standard into 10 mL methylene chloride (1:10 mL, p/v) and 5 mg lycopene standard into 50 mL methylene chloride (1:10 mL, p/v). The identification and the purity of the chromatographic peaks were estimated using a UV6000LP photodiode array detector (DAD) (Thermoquest). The identification of the main carotenoids, all-*trans β*-carotene and lycopene, was carried out by comparing the retention times and absorption spectra with reference standards. The peaks attribution of carotenoid *cis*-isomers, has been performed by on-line spectral analysis, by comparing retention times and relative quantities and by the characteristics of the absorption spectra (12,22,24).

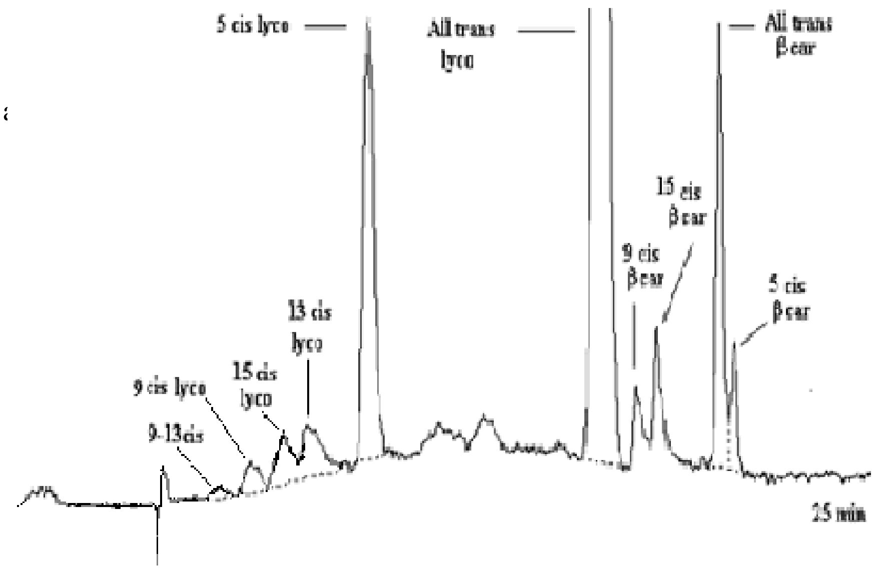

Fig. 1 - Reversed phase HPLC chromatogram of a double condensed sample.

Light exposure and thermal treatment

To evaluate the possible isomerisation, degradation and the related changes in bioavailability of carotenoids in tomato products, some samples have been undergone to different and extreme conditions such as to the light exposure (a) and to the thermal treatment at high temperature (b).

a) A sample of double condensed, prepared as previously described, was subjected to light exposure. A halogen lamp of 100 W as light source, providing an illumination intensity in the range 1,000 – 2,000 lux, at a distance of 30 cm, has been used. The total exposition period of the extract added up to 250 hours.

b) A sauce has been put in an Erlenmeyer flask and boiled with an electrical isomantel under nitrogen atmosphere.

Afterwards, both samples have been treated as previously described for the crude samples.

Statistical analysis

The extraction procedure and the HPLC analysis of each sample have been repeated for three times. All data were statistically processed using the SPSS 10 (SPSS, Chicago, IL, USA) and the Microsoft Excel software package (Microsoft Corp).

Analysis of variance was used to determine significant differences ($P < 0.05$) in the isomers content of different tomatoes products. The repeatability of the method (relative standard deviation) was found to be less than 6%.

Results and Discussion

On the basis of the HPLC data reported in Table 1, we can state that the total content of both lycopene and β-carotene in condensed is higher than in sauces and peeled tomatoes.

This fact clearly indicates a positive correlation between the concentration of the carotenoids found in the dry matter and the technological treatments which the tomatoes are subjected to. In fact, although the same production method (approximately consisting in trituration, purification, concentration, pasteurisation and packing) is applied, the magnitude of these treatments increases from peeled to sauces and then onto condensed.

An exception to this trend can be observed in the case of the triple condensed. In spite of the fact that it generally takes 7-8 kg of fresh tomatoes to obtain just 1 kg, compared to 6 kg of fresh tomatoes per each 1 kg of double tomato concentrate, a lower content of carotenoids was noticed in the two examined samples (C4 and C5). This lower content could be explicated by a different degree of thermal stress inducted by the treatment used to obtain the finished product.

TABLE 1

LYCOPENE AND B-CAROTENE CONTENTS OF COMMONLY CONSUMED COMMERCIAL TOMATO PRODUCTS (VALUES ARE IN mg/kg OF DRY MATTER)

	Lycopene							β-carotene				
Peeled	**All-trans**	**5-cis**	**9-cis**	**9,13-cis**	**13-cis**	**15-cis**	**Tot.**	**All-trans**	**5-cis**	**9-cis**	**15-cis**	**Tot.**
P1	126.4	0.59	0.15	0.14	0.38	0.15	127.8	2.44	0.53	0.38	0.61	3.96
P2	114.6	3.01	0.33	0.14	0.42	0.14	118.6	2.28	0.54	0.40	0.54	3.76
P3	97.76	3.18	0.33	0.16	0.33	0.14	101.9	2.49	0.40	0.19	0.61	3.69
P4	117.9	3.29	0.48	0.08	0.53	011	122.4	2.41	0.61	0.24	0.65	3.76
P5	154.5	4.18	0.47	0.18	0.62	0.20	160.1	2.71	0.53	0.35	0.85	4.44
P6	109.3	3.01	0.45	0.09	0.42	0.07	113.3	2.30	0.47	0.40	0.52	3.69
Sauces												
S1	158.2	4.20	0.42	0.12	0.80	0.12	163.9	3.58	0.48	0.65	0.70	5.41
S2	164.9	4.68	0.43	0.15	1.05	0.20	171.4	4.15	0.70	0.90	1.02	6.77
S3	174.8	4.32	1.08	0.22	0.55	0.40	181.4	4.22	0.72	0.68	0.70	6.32
S4	221.9	5.94	0.22	0.25	1.31	0.38	230.0	4.75	0.65	0.81	0.96	7.17
S5	204.2	5.06	0.72	0.16	1.50	0.22	211.9	4.36	0.69	1.28	1.59	7.92
Condensed												
C1	754.0	15.73	1.46	n.d	1.96	0.53	773,6	14.24	4.36	2.22	3.70	24.52
C2	905.0	18.94	1.74	0.50	2.15	6.64	928.9	16.84	5.74	2.74	5.30	30.62
C3	1176	24.72	5.48	0.68	6.72	1.40	1215	20.28	6.04	3.40	6.88	44.52
C4	833.9	13.41	3.22	0.50	4.85	0.94	856.8	14.27	3.29	1.85	4.13	23.54
C5	828.0	18.48	2.92	0.52	3.52	0.80	854.2	15.56	4.70	2.48	4.96	27.60

The products, as reported in table 1, are the following: Peeled [*Pelati Cirio* **(P1)**, *Pelati Conad* **(P2)**, *Pelati De Rica* **(P3)**, *Pelati Marechiaro* **(P4)**, *Pelati Pomodoro* **(P5)**, *Pelati Rustichella* **(P6)**]; Sauces [*Passata Bertolli* **(S1)**, *Passata de Cecco* **(S2)**, *Passata la Palma* **(S3)**, *Passata Ortolina* **(S4)**, *Passata Valfrutta* **(S5)**]; Condensed (*Concentrato Cirio* **(C1)**]; Double [*Doppio concentrato Coop* **(C2)**, *Doppio concentrato Mutti* **(C3)**); Triple (*Triplo Concentrato Hero* **(C4)**, *Triplo Concentrato Mutti* **(C5)**].

Moreover, the data in Table 1 show that the percentages of lycopene *cis*-isomers in the commercial products appear to be variable roughly in the range 1-5% of the total amount while for *β*-carotene this percentage is in the range of 34-43%. The higher percentages of lycopene *cis*-isomers (9, 9-13, 13) and of 5-*cis* *β*-carotene found in the condensed (more treated) products has led us to the decision to study the possible isomerisation and degradation of these carotenoids in different conditions.

During the first 50 hours of light exposure, there was not a relevant reduction of total lycopene. After about 250 hours, the total lycopene amount was degraded of 46.7%, while the *cis*-isomers quantity increased strongly. This process seems to be oriented to some specific types of *cis*-isomers (9, 9-13, 13, 15, as shown in Figure 2). In the case of *β*-carotene, no isomerisation was pointed out, while after 250 hours 80% of degradation was detected (data are reported in Table 2).

The total *β*-carotene submitted to thermal treatment (cooking) was decreased after 12 hours, while the quantity of total lycopene was increased, suggesting that this treatment can enhance the lycopene availability. In this case no appreciable degree of isomerisation was observed, except for 15-*cis* (data are reported in Table 3).

In addition, spontaneous isomerisation was casually noticed in a sample stocked at –25 °C for approximately 20 days. The values shown in Table 4 highlight the fact that after this period the total quantity of lycopene decreased about of 22%. This fact is a consequence of the decrease of the all-*trans*-isomer quantity, subjected both to degradation and to isomerisation. Under the same conditions, *β*-carotene total quantity increased about of 9%.

It must be stressed that, for practical reasons related to the sample preparations, the cooking has been performed on the product as such (sauce), while the light exposure and the spontaneous isomerisation was found out detected in the methylene chloride extracts of the corresponding product (double condensed).

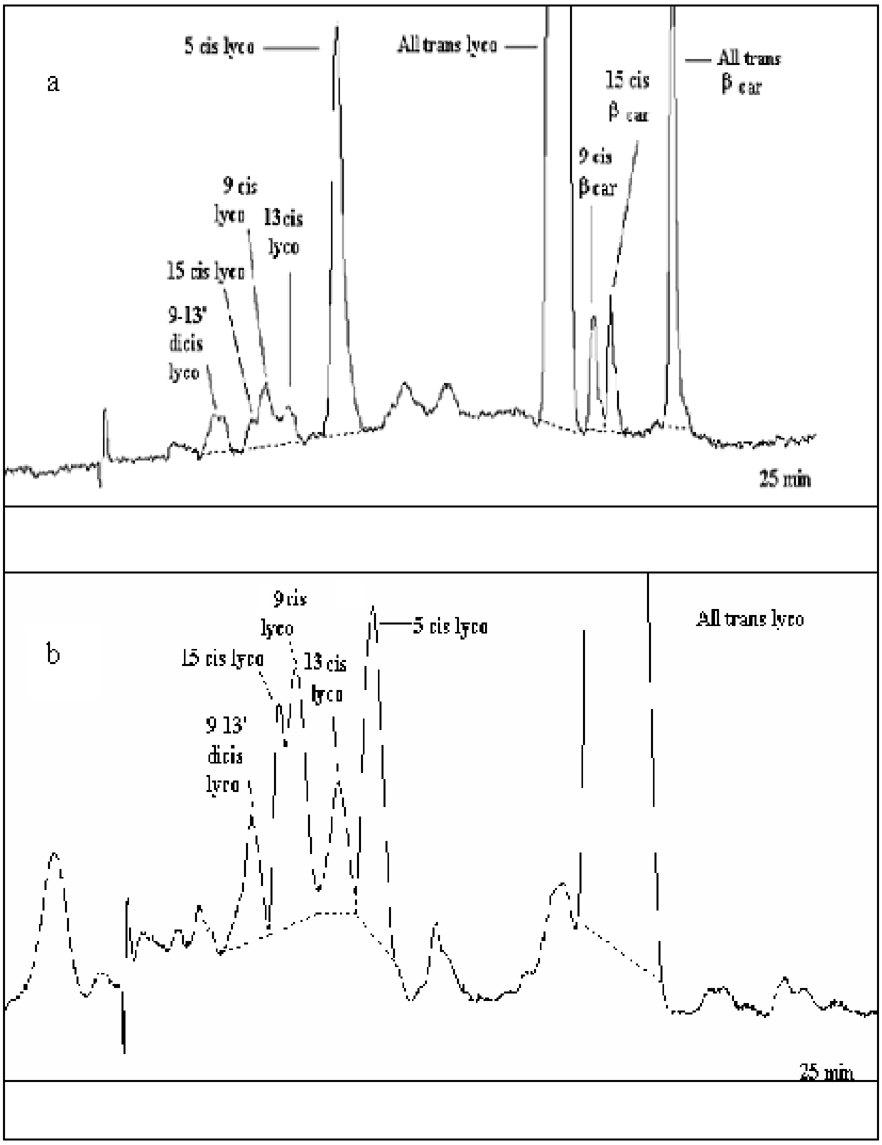

Fig. 2 - Reversed phase HPLC chromatogram of a double condensed sample, after 25 hours (a) and 250 hours (b) of light exposure

TABLE 2

DOUBLE CONDENSED EXTRACT SUBMITTED TO PROLONGED LIGHT EXPOSURE (VALUES EXPRESSED IN mg/kg OF DRY MATTER)

	Lycopene								***β*-carotene**				
Time h	**All-*trans***	**5-*cis***	**9-*cis***	**9,13-*cis***	**13-*cis***	**15-*cis***	**Tot.**		**All-*trans***	**5-*cis***	**9-*cis***	**15-*cis***	Tot.
0	1176	24.72	5.48	1.40	6.72	0.68	1215		20.28	6.04	3.40	6.88	36.60
25	1084	25.52	3.87	4.05	2.84	1.41	1122		23.16	0.89	6.77	6.22	37.04
50	1143	26.10	6.99	5.16	2.53	1.95	1186		24.62	1.28	7.44	6.88	40.22
100	862.0	25.62	13.94	5.66	2.51	4.80	914.6		22.08	1.16	4.87	4.08	32.19
250	581.7	19.57	18.37	9.38	10.27	8.85	648.1		2.26	1.35	2.39	1.13	7.13

TABLE 3

A SAUCE SAMPLE SUBMITTED TO THERMAL TREATMENT (VALUES EXPRESSED IN mg/kg OF DRY MATTER)

	Lycopene								***β*-carotene**				
Time h	**All-*trans***	**5-*cis***	**9-*cis***	**9,13-*cis***	**13-*cis***	**15-*cis***	**Tot.**		**All-*trans***	**5-*cis***	**9-*cis***	**15-*cis***	Tot.
0	163.3	4.04	0.575	0.125	1.20	0.175	169.4		3.48	0.55	1.02	1.27	6.32
1	170.4	4.30	0.486	0.109	1.12	0.085	176.5		2.62	0.85	0.84	1.20	5.51
4	179.3	4.13	0.375	0.100	1.38	0.404	185.7		2.43	0.83	0.45	0.79	4.50
12	182.5	4.51	0.608	0.083	1.29	0.479	189.5		2.76	0.79	0.68	0.89	5.12

TABLE 4

SPONTANEOUS ISOMERISATION IN A DOUBLE CONDENSED EXTRACT (VALUES EXPRESSED IN mg/kg OF DRY MATTER)

	Lycopene								**β-carotene**				
Days	**All-*trans***	**5-*cis***	**9-*cis***	**9,13-*cis***	**13-*cis***	**15-*cis***	**Tot.**		**All-*trans***	**5-*cis***	**9-*cis***	**15-*cis***	**Tot.**
0	919.5	16.1	1.45	0.80	1.13	0.61	939.7		4.83	1.34	1.24	1.37	8.78
20	675.3	22.9	5.11	5.95	11.35	8.41	729.1		5.80	1.72	1.39	0.65	9.56

Conclusions

Our study has demonstrated that the most common commercial tomato based products have a free carotenoids content proportional to the magnitude of their technological treatment. In fact, if the detected quantities of carotenoids are expressed in mg/kg of dry matter, the content appears to be in the following order: in the condensed > in the sauces> in the peeled.

Other results of this study put in evidence the tendency of lycopene to isomerise in various cis forms under different chemical – physical conditions. This transformation could be associated to a better in vivo bioavailability. Whereas the cooking process seems to increase the availability of the total lycopene, a prolonged exposure to a halogen lamp appears to be the best way to achieve elevated quantities of *cis*-isomers. Among these, 9-*cis*, 15-*cis* and 9-13 di-*cis*, above all, seem to be easy to obtain by this way.

β-carotene shows no tendency to isomerisation in the conditions taken in account in this study, whereas a strong degree of degradation is present after a long time of light exposure. Anyway, the isomerisation should be avoided (unlike lycopene), as the bioconversion of *β*-carotene to the active form, retinol, is higher for the all-*trans* isomer than for the *cis* isomers (27).

In addition, the preliminary data concerning the spontaneous isomerisation, pointed out in a double condensed extract, appear to be of great interest and further studies are needed to evaluate this phenomenon under different experimental conditions and its exact evolution in the time.

Finally, this work confirms that in some cases the technological treatments positively influence the nutraceutical properties of the food products, if compared with those of the crude matter. Progresses in this field could give indications in order to find out the best characters for the foodstuff and for improving the treatments to be adopted in its transformation.

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