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Description of volatile compounds generated by the degradation of carotenoids in paprika, tomato and marigold oleoresins

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Abstract

Here we describe the profiles of volatile compounds from the thermal degradation of carotenoids present in oleoresins of marigold, tomato and paprika. The profiles obtained by SPME-GC were complex, with identical compounds, such as toluene and *m*-xylene, being detected in all three oleoresins. Intramolecular cyclisation is proposed as the main reaction mechanism in the formation of the volatile compounds detected; this process is activated by the thermal impact generated during the processing and is followed by a reaction of elimination in the chain or a heterolytic fragmentation reaction. The presence of other compounds, such as various methyl benzaldehydes or isophorone (1,1,3-trimethyl-3-cyclohexene-5-one), also indicates the concurrence of carotenoid oxidation reactions that affect either the central polyenoic chain or the end-groups (β or ε rings). Ethanone,1-(methylphenyl), (4prime-methylacetophenone) not previously reported as a compound resulting from the thermal degradation of carotenoids, was also characterised and the same mechanism is proposed for its formation.

Keywords: Thermal degradation; Carotenoids; Oleoresins; Toluene; *m*-Xylene; Reaction mechanisms; GC-MS; SPME

1. Introduction

Although natural and synthetic colouring additives have been used indiscriminately to colour foodstuffs, pharmaceuticals and cosmetics since the 1990s, there is a tendency in the food industry to replace synthetic colouring agents with naturally occurring compounds, because consumers consider them to be healthier and of higher quality (Wissgott and Bortlik, 1996). Consumers regard colour as an important criterion of selection, hence a natural origin probably reinforces the consumer's positive perception of the foodstuff. This tendency is associated with the concern of governments and regulating bodies to establish norms and criteria of purity for food additives in general and for colouring agents in particular, aimed at ensuring the quality and safety of the foodstuff (Hallagan, Allen, and Borzelleca, 1995).

The carotenoids are pigments in which the association established by the consumer between "natural origin" and "health benefit" is strengthened by the scientific and popular literature (Baker and Günther, 2004). Among the various sources of carotenoid pigments is a noteworthy group of oleoresins obtained from raw materials rich in such pigments, which are responsible for the colour in the final extract. The oily extracts of annatto (*Bixa orellana* L.), marigold (*Tagetes erecta*), tomato (*Lycopersicon esculentum* Mill.) and paprika (*Capsicum annuum* L.) have a very varied carotenoid composition that are able to provide different tonalities from yellow to red and which are sufficiently concentrated, to enable their large-scale commercial use (low doses are sufficient to achieve the desired colour in a large amount of foodstuff). Apart from the carotenoids, these extracts contain components of a lipid nature (e.g.

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waxes, aromas, saturated and unsaturated fatty acids) that are extractable from the raw material. The unsaturated fatty acid composition and the processing conditions – both those used in the extraction of the oleoresin and those applied to the foodstuff in which the oleoresin is used as colouring agent – trigger reactions of lipid oxidation that affect the carotenoid content of the product, thereby decreasing both its the colouring capacity and as well as other properties (e.g. antioxidant activity) with a potentially positive impact (i.e. functional properties) on both the foodstuff and the individual consuming the coloured foodstuff.

However, the oxidation of unsaturated fatty acids also generates a set of substances that provide flavour and aroma and usually have negative repercussions on the quality of the foodstuff and its acceptance by the consumer (Frankel, 1993). Together with the loss of quality there are problems of safety, due to the toxicity of the by-products formed from lipid hydroperoxides (Kubow, 1990). The profiles of volatiles generated by lipid oxidation have been studied in some of the raw materials used to obtain these oleoresins. In the tomato, a very large number of volatile components have been identified and described in studies on different varieties of tomato and under different conditions of growth and processing (Buttery, Teranishi, Ling, Flath, and Stern, 1988; Buttery, Teranishi, Ling, and Turnbaugh, 1990). Whereas in the fresh and processed fruits of the genus *capsicum*, more than 125 volatile compounds have been identified; also available is a description of the changes generated in the aromatic profile by fruit ripening, tissue breakdown and the involvement of Maillard, hydrolytic and oxidative degradation reactions of unsaturated fatty acids (Whitfield and Last, 1991; Cremer and Eichner, 2000). Some studies have been carried out on the oleoresin of pepper with regard to its composition in volatiles and its use to increase the flavour of certain foodstuffs (Teranishi, Keller, Flath, and Mon, 1980), but the effect of thermal degradation has not been determined.

In the case of carotenogenic material and derived products, the aromas generated by the degradation of carotenoids, besides those coming from the oxidative degradation of unsaturated fatty acids, must be taken into account. The degradative oxidation reactions, generally caused by the heat treatment, affect the pigments directly, generating various volatile compounds such as β-ionone, dihydroactinidiolide (2(4*H*)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (*R*)- (9CI)), 2-methyl-2-hepten-6-one (6-methylhept-5-en-2-one) and citral (3,7-dimethyl-trans-2,6-octadienal) (Crouzet and Kanasawud, 1997). Of the oleoresins mentioned, an exhaustive study has been made of the volatile compounds generated by the thermal degradation of carotenoids only in annatto oleoresin. In this case, it has been demonstrated that the formation of products of the thermal degradation of bixin and norbixin, known as C_{17}, is accompanied by the release of aromatic compounds, mainly *m*-xylene and toluene, as well as other minor compounds, such as dimethyldihydronaphthalene, and toluic acid (2-methylbenzoic acid) and its methyl ester (McKeown, 1963; Scotter, 1995; Felicissimo, Bittencourt, Houssiau, and Pireaux, 2004). Scotter, Wilson, Appleton, and Castle (1998, 2000) have reported the presence of *m*-xylene and toluene in different formulations of annatto, finding highly variable levels of *m*-xylene (<5 mg/kg to 200 mg/kg), with toluene in a narrower range (<5–12 mg/kg). The thermal degradation of paprika oleoresins generates carotenoid degradation products that have been identified as *nor*-carotenoids (Pérez-Gálvez, Rios, and Mínguez-Mosquera, 2005). Although this study did not analyse the aromatic profile generated during thermal degradation of the oleoresin, the production of these degradation compounds would also involve the formation of *m*-xylene, since the cyclisation of polyolefins was postulated to be the carotenoid degradation pathway in heat-induced processes. This carotenoid cyclisation reaction would produce the corresponding *nor*-carotenoid and the aromatic hydrocarbon.

The determination of the aromatic profile in oleoresins rich in carotenoid pigments would therefore have considerable importance from two different points of view. First, these compounds would be indicators of the effects of thermal impact, the extraction process and any other subsequent heat process on the product. Obviously, the application of a more vigorous temperature-time regime, aimed at increasing the coefficient of extraction of pigments from the raw material, would result in a greater accumulation of degradation products. The use of a milder processing or an extraction technique with minimal thermal impact (such as extraction with supercritical fluid) is more likely to produce a lower oxidation index. Thus, the presence of aromatic compounds generated by thermal degradation could be used as a quality marker of the processing and even distinguish the type of extraction technique employed.

Secondly, there is the issue of safety due to the toluene and *m*-xylene content of oleoresins. These aromatic hydrocarbons are environmental pollutants and can occasionally be present in foodstuffs that have been in contact with a contaminated source (air, water or packing). The presence of *m*-xylene has been reported in water at concentrations <2 ppb and in various foodstuffs in the range of 1 to 100 ppb (Agency for toxic substances and disease registry, 2005). Levels of toluene in water and foodstuffs appear not to be significant (Environmental protection agency, 1981). Although toluene and *m*-xylene have not been classified as carcinogens, they do have toxic effects of varying seriousness depending on the dose and period of exposure, with *m*-xylene affecting the nervous system and toluene the brain (Agency for toxic substances and disease registry, 2000, 2005). In contrast to such exogenous contamination, this study considers the case in which these aromatic hydrocarbons are endogenous, generated by the thermal degradation of carotenoids.

The aim of the present study was to describe the profile of volatile compounds derived from thermal degradation of carotenoids in oleoresins, obtained from carotenoid-rich materials (marigold, tomato, and paprika). The presence of toluene and *m*-xylene in the aromatic profile would confirm the reaction scheme by which the intramolecular cyclisation and subsequent elimination of an in-chain unit from a carotenoid would generate the mentioned aromatic hydrocarbons. However, volatile components other than these compounds might also be generated from the thermal degradation of carotenoids. The structural description of these compounds was obtained in order to identify the type of reaction that generated them. Each oleoresin contains a specific environment for both its different carotenoid composition and its different composition of other lipid components. A reaction process common to all of the oleoresins could generate different volatile compounds, due to the structural diversity of carotenoids making up the pigment profile in each oleoresin. It is also possible that particular reaction processes are active due to the nature of the molecular environment, in which the carotenoids are immersed, as well as the extraction and processing conditions, favouring some specific reaction that generates specific volatile compounds.

2. Materials and methods

2.1. Raw materials

Paprika, tomato and marigold oleoresins were supplied by Extractos Vegetales (La Línea de la Concepción, Cádiz, Spain), LycoRed (Beer-Sheva, Israel) and Kemin Foods (Des Moines, Iowa, USA), respectively. One sample of each type was available. According to the specification sheet provided by the suppliers, the paprika oleoresin contained 62 g/kg of total carotenoid pigments in an oily matrix obtained from sunflower. The tomato oleoresin contained 64 g/kg of lycopene, diluted in an uncharacterised oily matrix. The marigold oleoresin contained 207 g/kg of lutein, dissolved in corn (maize) oil.

2.2. Headspace-SPME

Volatiles were extracted using the solid phase microextraction (SPME) technique. The SPME device, type 50/30 μm DVB/Carboxen™/PDMS StableFlex™, was supplied by Supelco (Bellefonte, PA, USA). The fiber was conditioned prior to use following the manufacturer's instructions. The oleoresin sample (between 30 and 40 mg weighed exactly) was placed in a 22 × 75 mm (20 ml) vial, and 1 μl of a solution of methyl hexanoate (Sigma–Aldrich, St. Louis, MO, USA) in pentane (0.85 mg/ml) was added. The vial was sealed immediately and placed in a block heater (Labnet Internacional, D1100) at 50 °C, 100 °C or 150 °C. The first temperature (50 °C) was selected to obtain the profile of volatiles of the sample and for their subsequent spectrometric analysis and for the quantitative determination of toluene and *m*-xylene. The temperatures of 100 °C and 150 °C were used for the in situ generation of aromatic hydrocarbons. The vial was placed so that only 50% was inside the block heater, with the fiber remaining in the upper part of the vial to avoid self-desorption, especially when the temperature used for the heat treatment exceeded 50 °C. After 10 min of conditioning, volatiles were extracted from the headspace, exposing the fiber for 15 min at the same temperature. The conditions for determining the profile of volatiles in the oleoresins were adapted from the work of Jia, Zhang, and Min (1998). Desorption of the analytes from the covered fiber was performed in the injection port of the GC, using a specific liner for SPME of 0.8 mm internal diameter, in splitless mode. The desorption time was 5 min at 250 °C. For each oleoresin, four SPME extractions were made, performing the subsequent desorption and analysis by GC-MS for each SPME extraction.

2.3. GC-MS analysis of volatiles

The chromatographic analysis was performed in a Finnigan Trace GC 2000 chromatograph, performing the separation on a Zebron ZB-5 ms fused silica capillary column (Phenomenex, Torrance, CA, USA) of 30 m × 0.25 mm i.d. and 0.25 μm phase thickness. The analytes were analysed by mass spectrometry, using a Polaris-Q ion trap mass spectrometer (ThermoFinnigan, Austin, TX, USA) working in EI+ mode, coupled to the chromatograph. The working conditions were as follows. The injector temperature was 250 °C. The initial temperature of the oven-heating program was 60 °C for 6 min, the temperature was then raised to 220 °C at 4 °C per min and kept at 220 °C for 20 min. The temperatures of the interface and of the ion source were 250 °C and 200 °C, respectively. The carrier gas was helium at a flow of 1 ml/min in constant-flow mode. For the acquisition and processing of the spectrometric data, the Xcalibur software (Thermo Scientific, version 1.4) was used. Sample components derived from carotenoid-thermal degradation process (listed in Table 2) were verified by comparison of the mass spectral data with those of authentic reference compounds, together with the spectral libraries of the National Institute of Standards and Technology and the Wiley Registry of Mass Spectra Data. The quantification of toluene and *m*-xylene was performed on the chromatogram in TIC mode by measuring the areas of the corresponding peaks, using methyl hexanoate as the internal standard. We performed a calibration by injecting mixed standards in pentane, containing a fixed amount of methyl hexanoate as internal standard, and normalising the detector response of toluene and xylene against methyl hexanoate.

3. Results and discussion

Fig. 1 illustrates the profile of volatile compounds obtained by GC-MS for the three oleoresins in accord with the method described. The profiles are complex, with only 5–6 compounds reaching values exceeding 5% of the total and the rest in lower proportions. The compounds can be

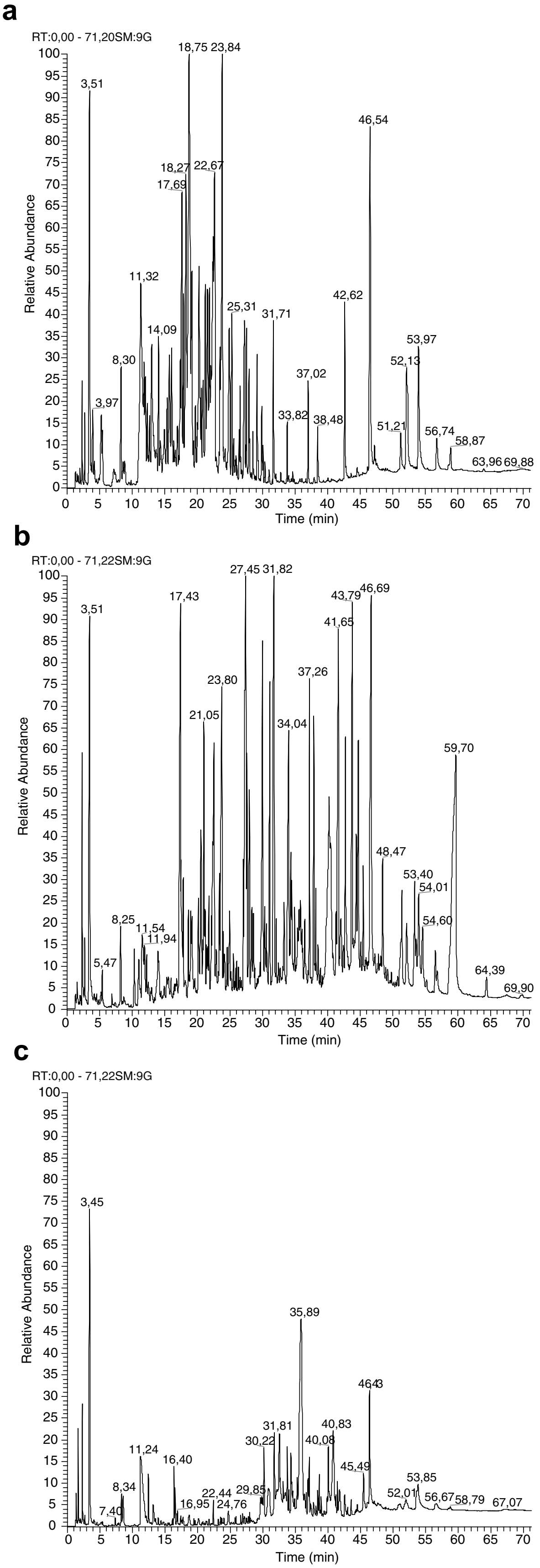

Fig. 1. Gas chromatograms (GC-MS) corresponding to the analysis of the volatile compounds found in paprika (a), marigold (b) and tomato (c) oleoresins. SPME

divided into three broad groups: those derived from fatty acids, volatiles of terpenoid origin, and products derived from the thermal degradation of carotenoids.

The first group contains C_6, C_7, and C_9 lipid-derived volatile flavour compounds such as hexanal, heptanal, 2-heptenal, 2 nonenal, and 2-nonenone, in different percentages in each oleoresin, none exceeding 2% of the total volatile compounds. Table 1 lists the most-representative terpene-derived compounds. Beta-elemene ((1α, 2β, 4β)-1-methyl-2,4-bis(methylvinyl)-1-vinylcyclohexane) appears in the paprika oleoresin, representing 9.24% of the total volatile compounds. This compound has been reported to be responsible for notes of fatty aroma in paprikas (Jun and Kim, 2002). The same oleoresin contains carveol (2-cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)) (1.58% of total volatile compounds), whose presence has not previously been reported in fruits of the genus *capsicum* or its derived products (paprika and oleoresin). This monoterpenoid alcohol has been reported in spearmint oil (*Mentha spicata*). A dominant compound present in marigold oleoresin is pulegone (cyclohexanone, 5-methyl-2-(1-methylethylidene)) (5.33% of total volatile compounds), a monoterpenoid ketone component of various essential oils, such as those obtained from *Nepeta Cataria* (Catnip), *Mentha piperita* and *Mentha pulegium* (Pennyroyal oil). Finally, the tomato oleoresin contains β-citronellol (3,7-dimethyl-oct-6-en-1-ol) (2.58% of total volatile compounds), an acyclic monoterpenoid reported in essential oils of rose and geranium.

The study was focused on the volatile compounds derived from the thermal degradation of carotenoids, particularly toluene and *m*-xylene, which were studied quantitatively; others compounds were evaluated as a percentage of the total volatile components.

Table 2 lists the compounds derived from the thermal degradation of carotenoids. As mentioned above, two groups of compounds are distinguished: cyclic olefins and linear ketones. In the first group, *m*-xylene and toluene stand out. In view of the results from earlier studies (Pérez-Gálvez et al., 2005; Scotter, 1995), the presence of toluene and *m*-xylene was expected in other carotenoid-rich

Table 1
Terpene-derived compounds[a] found in paprika (*Capsicum annuum* L.), marigold (*Tagetes erecta*) and tomato (*Lycopersicum esculemtum*) oleoresins

Compound	Paprika	Marigold	Tomato
Carveol	1.58	–[b]	–
Pulegone	–	5.33	–
β-Citronellol	–	–	2.58
β-Elemene	9.24	–	–

[a] Data are expressed as percentage values with respect to the total volatile profile.

[b] No detected. Systematic names for the compounds listed are: carveol, 2-cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl); pulegone, cyclohexanone, 5-methyl-2-(1-methylethylidene); β-citronellol,3,7-dimethyloct-6-en-1-ol; β-elemene, (1α, 2β, 4β)-1-methyl-2,4-bis(methylvinyl)-1-vinylcyclohexane).

Table 2
Carotenoid-thermodegraded compounds[a] found in paprika (*Capsicum annuum* L.), marigold (*Tagetes erecta*) and tomato (*Lycopersicum esculemtum*) oleoresins

Compound	Paprika	Marigold	Tomato
Toluene (C)	1.08	3.16	3.53
m-Xylene (C)	7.76	8.33	14.9
Methylbenzaldehyde[b] (C)	4.78	3.22	–[c]
6-Methyl-3,5-heptadien-2-one (L)	5.44	–	0.37
6-Methyl-5-hepten-2-one (L)	0.38	–	1.69
Ethanone, 1-(methylphenyl) (C)	6.46	–	0.75
Isophorone (C)	–	1.09	–
Ketoisophorone (C)	–	3.72	–
2,6-Dimethylnaphthalene (C)	–	12.6	–
β-Ionone	3.50	–	–
Loliolide (C)	–	11.1	–

[a] Data are expressed as percentage values with respect to the total volatile profile.

[b] Sum of different methylbenzaldehydes found in the volatile profile.

[c] No detected. Systematic names for the compounds listed are: ethanone, 1-(methylphenyl), (4′-methylacetophenone); isophorone, 3,5,5-trimethyl-2-cyclohexen-1-one; ketoisophorone, 2,6,6-trimethylcyclohex-2-ene-1,4-dione; loliolide, 2(4*H*)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl. C means that the compound is a cyclic olefin. L means that the compound is a linear ketone.

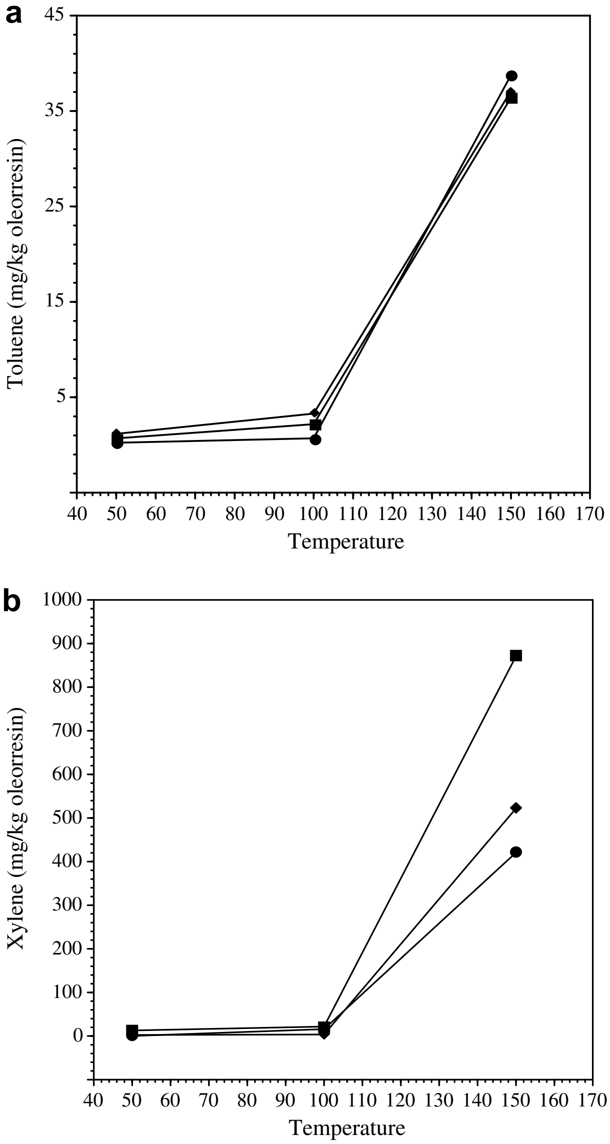

Fig. 2. Concentration of toluene (a) and *m*-xylene (b) in marigold (circles), tomato (diamonds), and paprika (squares) oleoresins after their thermal treatment at 50, 100 and 150 °C.

oleoresins, such as those of paprika, marigold and tomato, due to the thermal degradation of carotenoids. *m*-Xylene is one of the most-representative compounds of the profile in all three oleoresins, reaching percentages of 7.76%, 8.33% and 14.9% in the oleoresin of paprika, marigold, and tomato, respectively; whereas toluene is present in lower percentages, ranging from 1% to 3%. Fig. 2 shows the concentration of toluene and *m*-xylene in mg/kg of oleoresin, determined at 50 °C. The concentrations of these cyclic olefins can be considered low, like those reported in previous studies analysing the toluene and *m*-xylene content in different oleoresins of annatto (Scotter et al., 2000). In those studies, most of the samples had toluene levels below 5 mg/kg whereas the concentration of *m*-xylene was more variable (5–200 mg/kg). In our study, the toluene content was 0.23 mg/kg, 0.69 mg/kg, and 1.17 mg/kg in the oleoresins of marigold, tomato and paprika, respectively. The *m*-xylene concentration was highest in the paprika oleoresin (12.5 mg/kg) whereas values in marigold and tomato oleoresins were each lower than 5 mg/kg.

Assuming that toluene and *m*-xylene are derived from the thermal degradation of carotenoids (ruling out sources of external contamination) and considering the pigment concentration data for each oleoresin, the generation of these aromatic hydrocarbons was very different in each oleoresin. There is no direct correlation between the concentration of toluene and *m*-xylene and the concentration of carotenoids in the oleoresin. The marigold oleoresin, which showed the highest concentration of pigment, contains the lowest concentration of these aromatic hydrocarbons. For example, when the data for *m*-xylene concentration with respect to pigment concentration are normalised (mg of hydrocarbon per kilogram of pigment), values of 1.05 mg/kg, 40.0 mg/kg, and 205 mg/kg of pigment are obtained for the oleoresin of marigold, tomato and paprika, respectively. Upon calculating the relationship of these concentrations to that present in the marigold oleoresin (used as reference because of its lowest concentration), the *m*-xylene concentration in the tomato oleoresin is 38-fold higher whereas the *m*-xylene concentration in the paprika oleoresin is up to 196-fold higher. These data may indicate that the processing conditions used to obtain these two oleoresins and which caused the degradative thermal process affecting the carotenoids – the precursors of toluene and *m*-xylene – were more drastic than those used to obtain the marigold oleoresin. It should also be considered that during or after the procedure to obtain the marigold oleoresin, the concentration of the aromatic hydrocarbons could have been reduced drastically, before extraction of the commercial oleoresin. An alternative explanation of these differences is based on the nature of the oily matrix, in which the pigments are immersed in each oleoresin, that could impact the production of these compounds, either favouring or hindering the reaction process. To determine which of the suggested hypotheses is the most valid, thermal degradation of the three oleoresins was carried out at the temperatures of 100 °C and 150 °C and then toluene and *m*-xylene were quantified.

Fig. 2 shows the values for the concentration of toluene and *m*-xylene obtained for the three oleoresins at the temperatures mentioned, the concentration of aromatic hydrocarbon is expressed per kg of sample. Treatment at 100 °C led to increases in the concentration of toluene and *m*-xylene compared with the values observed at 50 °C. However, the heat treatment at 150 °C caused a marked rise in the concentration of these aromatic hydrocarbons, giving the maximum values for the three oleoresins. The greatest increase (toluene and *m*-xylene concentrations of 169- and 2000-fold, respectively), with respect to the concentration at 50 °C, occurred in the marigold oleoresin. Despite this considerable increase, the concentrations in this oleoresin did not exceed those in the tomato and paprika oleoresins at 150 °C. Under equal conditions of heat treatment, the highest concentration of aromatic hydrocarbons was detected in the paprika oleoresin. The pigment concentration does not determine the extent of formation of toluene and *m*-xylene, that is, there is still no direct correlation detected between the concentration of pigment and the concentration of aromatic hydrocarbons generated. The data for *m*-xylene concentration at 150 °C, expressed as mg/kg of pigment, are 2026, 8173, and 13967 in the oleoresin of marigold, tomato and paprika, respectively. Since the same heat treatment of the three oleoresins generates different concentrations of toluene and *m*-xylene, the most valid hypothesis is that the nature of the oily matrix plays an important role in the quantitative generation of volatile compounds.

This hypothesis is confirmed by the qualitative study of the rest of the volatile compounds detected following the thermal degradation of carotenoids, shown in Table 2. Some of these compounds have been previously described in earlier publications while others are presented for the first time in this work. In the marigold oleoresin, there is an exclusive series of cyclic olefins (compounds C_9, C_{12} and C_{14}). The first is calendin or loliolide (1,3-dihydroxy-3,5,5-trimethylcyclohexylidene-4-acetic acid lactone). This compound is produced from the oxidation of the 3-hydroxy-*β* ring at position 5,6 and its subsequent reorganisation to 5,8-furanoid or by the formation of the 5,8 endoperoxide. These compounds (with oxidation at 5,8) would act as precursors of loliolide, following the reaction scheme suggested in earlier publications (Foote and Brenner, 1968; Mousseron-Canet, Dalle, and Mani, 1968). This is not the first demonstration of the production of loliolide from carotenoids by a degradative process. Klok et al. (1984) suggest that the photo-oxidation of fucoxanthin could be an important source of loliolide. Repeta (1989) describes the degradation of fucoxanthin in marine sediments to bicyclic furanoxides and their subsequent fragmentation to various compounds, including loliolide.

Considering the structure of loliolide and the structural requirements for a carotenoid to be its precursor, it is logical that this compound is not detected in the profile of volatiles in the tomato oleoresin. This is because lycopene cannot generate this oxidation product, since its structure does not contain at least one 3-hydroxy-β ring, as in the case of lutein (a xanthophyll present in the marigold oleoresin). However, loliolide should be detectable in the paprika oleoresin, which contains zeaxanthin (which contains two 3-hydroxy-β rings) and other possible more-direct precursors of loliolide, such as violaxanthin (which contains two 3-hydroxy-5,6-epoxy-β rings).

The cyclic olefin 2,6-dimethylnaphthalene is present exclusively in the marigold oleoresin, and is one of the major compounds in its profile of volatiles (12.6%). The reaction scheme for the formation of this compound from a carotenoid follows the mechanism proposed by Achari, Shaw, and Holleyhead (1973), consisting of double intramolecular cyclisation, with a subsequent heterolysis. This compound might also be expected to be present in the profile of volatiles of the paprika and tomato oleoresins, given that it is formed from the polyenoic chain, a structure common to all the carotenes and xanthophylls present in the oleoresins studied.

Finally and again exclusively in the marigold oleoresin, isophorone (1,1,3-trimethyl-3-cyclohexene-5-one) and ketoisophorone (2,6,6-trimethyl-2-cyclohexene-1,4-dione) are present in the profile of volatiles, each at a concentration lower than 3%. Isophorone has been detected in the volatile fraction of saffron (*Crocus sativus*) during the thermal degradation of crocetin esters (Carmona, Zalacain, Salinas, and Alonso, 2006). The cyclic olefin ketoisophorone has been reported as a product of the thermal degradation of β-carotene in aqueous suspension (Kanasawud and Crouzet, 1990). In the present work, these compounds appear only in the profile of volatiles of the marigold oleoresin.

The various methyl benzaldehydes, which appear in the profile of volatiles of paprika and marigold oleoresins in percentages of 0.87% to 2.45%, also come from carotenoid degradation. In this case, the direct precursors would be 15’-formyl-15-*nor*-carotenoid and 12-formyl-11-*nor*-carotenoid, whose presence during the oxidation of β-carotene by the peroxyl radical, has been reported (Yamauchi, Miyake, Inoue, and Kato, 1993). These authors indicated that these compounds, coming from the oxidation of β-carotene in the oily phase, were unstable and were decomposed into polar products. One mechanism of decomposition is the sequence of reactions proposed in Fig. 3 and by which methyl benzaldehyde is generated, following the intramolecular cyclisation of the formyl-*nor*-carotenoid and its subsequent heterolysis. The already mentioned work of Yamauchi et al. also described the formation of 19-oxomethyl-10-*nor*-β-carotene. This compound or any other oxomethyl-*nor*-carotenoid can be

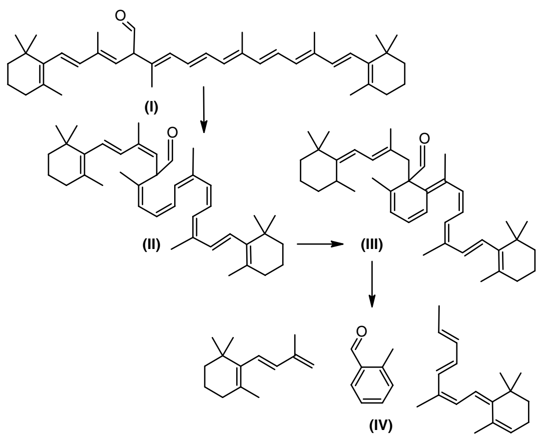

Fig. 3. Scheme of the proposed reaction for the formation of 2-methylbenzaldehyde (IV) from the precursor 12-formyl-11-*nor*-β-carotene (I) through cyclisation (intermediates II and III) and subsequent heterolysis.

the precursor of the compound ethanone,1-(methylphenyl), which appears in the profile of volatiles of paprika and tomato oleoresins (6.46% and 0.75% of the total, respectively). The reaction scheme, shown in Fig. 4, follows a mechanism similar to that described by Edmunds and Johnstone (1965), in which, following the intramolecular cyclisation, a cyclic unit is eliminated, in this case ethanone,1-(methylphenyl).

β-Ionone is detected in the profile of volatiles of the paprika oleoresin obtained at 200 °C, representing 3.5% of the total. The production of this *apo*-carotenoid by heat decomposition has been reported previously as the result of the cleavage of the double bond at position 9,10. It is logical that this compound appears only in paprika oleoresin, since it is the only oleoresin that contains the compound's direct precursor, β-carotene.

The profile of volatiles also includes degradation products of linear carotenoids, such as the ketones 6-methyl-5-hepten-2-one and 6-methyl-3,5-heptadien-2-one, detected in the paprika and tomato oleoresins, with percentages varying between 0.4% and 5.4%. Of these two ketones, the compound containing two double bonds is the major one in the paprika oleoresin. The reaction scheme proposed for the formation of 6-methyl-3,5-heptadien-2-one from β-carotene may consist of a mechanism of addition to the chain at position 13,14 and a subsequent heterolytic fragmentation, generating a ketone that undergoes cleavage at position 7,8, producing 6-methyl-3,5-heptadien-2-one. This ketone is also present in the tomato oleoresin, although in a lower proportion than the monoenoic ketone. The formation of the ketone with two double bonds from lycopene involves addition at position 10,11 and a subsequent fragmentation, generating pseudo-ionone, which undergoes further cleavage to produce 6-methyl-3,5-heptadien-2-one. In the tomato oleoresin, the proportion of the two ketones is reversed, with 6-methyl-5-hepten-2-one being the major one. In this case, the reaction scheme would be addition at position 6,7 and a subsequent cleavage of the chain. Earlier studies have reported this compound as a marker product in the oxidation of lycopene, although γ, δ, and ζ-carotene can also act as precursors (Cremer and Eichner, 2000; Kanasawud and Crouzet, 1990). The appearance of the monoenoic ketone in the profile of volatiles obtained in paprika oleoresin would therefore indicate the presence of lycopene or some other carotenoid precursor of this ketone. However, neither lycopene nor the other previously mentioned carotenes appear in the pigment profile of the oleoresin used for the study. It is therefore possible that there is an alternative route for the formation of the monoenoic ketone from the 6-methyl-3,5-heptadien-2-one.

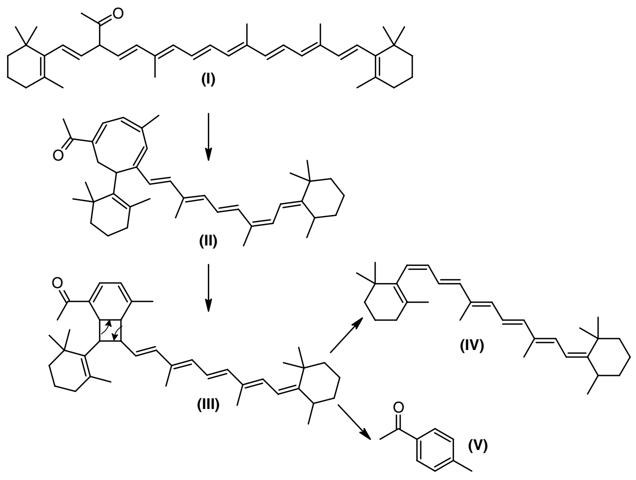

Fig. 4. Scheme of the reaction proposed for the formation of ethanone-1,(methylphenyl) (V) from the precursor 19-oxomethyl-10-*nor*-β-carotene, through intramolecular cyclisation (intermediates II and III) and subsequent in-chain loss unit.

In contrast to biodegradation, a natural process generating volatile compounds from carotenoids, thermal degradation during processing is an artificial process that promotes the decomposition of carotenoids into different volatile components. These include some that are identical to those produced by biodegradation and others that derive exclusively from the thermal degradation process, such as those described here (for example *m*-xylene, toluene, 2,6-dimethylnaphthalene). The use of vigorous processing conditions to obtain oleoresins with a high coefficient of extraction of colour, and the subsequent operations to remove solvent residues, facilitate the reaction processes discussed in the present work. Of these processes, intramolecular cyclisation stands out as the main reaction of structural transformation of carotenoids in all the oleoresins studied, independently of their composition. The *trans–cis* isomerisation reaction is the previous step of the intramolecular cyclisation, so it must be taken into account that the observed degradation product patterns could be a consequence of the different tendency that each carotenoid shows to form poly-cis isomers. This process as well as those that take place subsequently (the reaction of elimination in the chain or heterolytic fragmentation), are promoted by a higher temperature.

The other reaction processes described involve oxidation of the pigment and the subsequent reorganisation and fragmentation of the oxidized intermediate. Considering the structure of the compounds listed in Table 2, it can be seen that the processes of oxidation in the β or ε ring of the pigment have predominated in the case of the marigold oleoresin, so that compounds such as isophorone, ketoisophorone and loliolide were present in the composition of volatiles only in this oleoresin. Although the pigment composition of paprika and tomato oleoresins includes carotenoids that could have generated the aforementioned compounds, they were not detected. The opposite situation is also observed. The paprika and tomato oleoresins include compounds such as 6-methyl-3,5-heptadien-2-one, 6-methyl-5-hepten-2-one. In these cases, oxidation of the polyenoic chain is the promoted process. These compounds could have been generated in the marigold oleoresin as well, since lutein also presents the polyenoic central structure susceptible to oxidation however, the aforementioned ketones were not detected in this oleoresin. That is, different compounds are generated when oxidation takes place at different points of the structure (ring or chain) of structurally very similar compounds. The nature of the environment can promote oxidation at one point or another of the structure of the pigment.

The presence of toluene and *m*-xylene, apart from corroborating the mechanisms of reaction, is also particularly important in the potential toxicity of these compounds. This, together with the lack of regulations on the maximum permitted levels in oleoresins, calls for exhaustive studies on the qualitative and quantitative presence of these products, the processing conditions in which their formation is minimised and – above all – the setting of limits for these volatile components in the specific purity criteria for food colouring agents of carotenoid origin.

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References

Achari, R. G., Shaw, G., & Holleyhead, R. (1973). Identification of ionene and other carotenoid degradation products from the pyrolysis of sporopollenins ferived from some pollen exines, a spore coal and the green river shale. *Chemical Geology, 12*, 229–234.

Agency for toxic substances and disease registry. (2000). *Toxicological profile for toluene*. US Department of Health and Human Services.

Agency for toxic substances and disease registry. (2005). *Draft toxicological profile for m-xylene*. US Department of Health and Human Services.

Baker, R., & Günther, C. (2004). The role of carotenoids in consumer choice and the likely benefits from their inclusion into products for human consumption. *Trends in Food Science and Technology, 15*, 484–488.

Buttery, R. G., Teranishi, R. G., Ling, L. C., Flath, R. A., & Stern, D. J. (1988). Quantitative studies on origins of fresh tomato aroma volatiles. *Journal of Agricultural and Food Chemistry, 36*, 1247–1250.

Buttery, R. G., Teranishi, R. G., Ling, L. C., & Turnbaugh, J. G. (1990). Quantitative and sensory studies on tomato paste volatiles. *Journal of Agricultural and Food Chemistry, 38*, 336–340.

Carmona, M., Zalacain, A., Salinas, M. R., & Alonso, G. L. (2006). Generation of saffron volatiles by thermal carotenoid degradation. *Journal of Agricultural and Food Chemistry, 54*, 3834–6825.

Cremer, D. R., & Eichner, K. (2000). Formation of volatile compounds during heating of spice paprika (*Capsicum annuum*) powder. *Journal of Agricultural and Food Chemistry, 48*, 2454–2460.

Crouzet, J., & Kanasawud, P. (1997). Formation of volatile compounds by thermal degradation of carotenoids. In L. Packer, *Methods in Enzymology, Vol. 213. Carotenoids, Part A. Chemistry, separation, quantitation, and antioxidation*. San Diego: Academic Press, Inc.

Edmunds, F. S., & Johnstone, R. A. W. (1965). Constituents of cigarette smoke part IX. The pyrolysis of polyenes and the formation of aromatic hydrocarbons. *Journal of Chemical Society, 46*, 2892–2897.

Environmental protection agency. (1981). *Exposure and risk assessment for toluene*. Final draft report. Washington DC: US Environmental protection agency. Office of Water Regulations and Standards.

Felicissimo, M. P., Bittencourt, C., Houssiau, L., & Pireaux, J. J. (2004). Time-of-flight secondary ion mass spectrometry and X-ray photoelectron spectroscopy analyses of bixa orellana seeds. *Journal of Agricultural and Food Chemistry, 52*, 1810–1814.

Foote, C. S., & Brenner, M. (1968). Chemistry of singlet oxygen.VIII. An unusual allenic oxygenation product. *Tetrahedron Letters, 9*, 6041–6044.

Frankel, N. (1993). Formation of headspace volatiles by thermal decomposition of oxidized fish oils vs.oxidized vegetable oils. *JAOCS, 70*, 767–772.

Hallagan, J. B., Allen, D. C., & Borzelleca, J. F. (1995). The safety and regulatory status and food, drug and cosmetics colour additives exempt from certification. *Food Chemistry and Toxicology, 33*, 515–528.

Jia, M., Zhang, Q. H., & Min, D. B. (1998). Optimization of solid phase micro extraction analysis for headspace ?avor compounds of orange juice. *Journal of Agricultural and Food Chemistry, 46*, 2744–2747.

Jun, H. R., & Kim, Y. S. (2002). Comparison of volatile compounds in red pepper (*Capsicum annuum* L) powders from different origins. *Food Science and Biotechnology, 11*, 293–302.

Kanasawud, P., & Crouzet, J. C. (1990). Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium.1. β-Carotene degradation. *Journal of Agricultural and Food Chemistry, 38*, 237–243.

Klok, J., Baas, M., Cox, H. C., De Leeuw, J. W., Rijpstra, W. I. C., & Schenck, P. A. (1984). Loliolides and dihydroactinidiolides in a recent marine sediment probably indicate a major transformation pathway of carotenoids. *Tetrahedron Letters, 25*, 5577–5580.

Kubow, S. (1990). Toxicity of dietary lipid peroxidation products. *Trends in Food Science and Technology, 1*, 67–71.

McKeown, G. G. (1963). Composition of oil-soluble annatto food colours. II. Thermal degradation of bixin. *Journal of the Association of Official Analytical Chemists, 46*, 790–796.

Mousseron-Canet, M., Dalle, J. P., & Mani, J. C. (1968). Photooxydation sensibilisee de composes apparentes aux carotenoides. Analogie avec la structure des carotenoides oxygenes, alleniques et autres. *Tetrahedron Letters, 9*, 6037–6040.

Pérez-Gálvez, A., Rios, J. J., & Mínguez-Mosquera, M. I. (2005). Thermal degradation products formed from carotenoids during a heat-induced degradation process of paprika oleoresins (*Capsicum annuum* L.). *Journal of Agricultural and Food Chemistry, 53*, 4820–4826.

Repeta, D. J. (1989). Carotenoid diagenesis in recent marine sediments: II. Degradation of fucoxanthin to loliolide. *Geochimica et Cosmochimica Acta, 53*, 699–707.

Scotter, M. J. (1995). Characterisation of the coloured thermal degradation products of bixin from annatto and a revised mechanism for their formation. *Food Chemistry, 53*, 177–185.

Scotter, M. J., Wilson, L. A., Appleton, G. P., & Castle, L. (1998). Analysis of annatto (Bixa orellana) food colouring formulations. 1. Determination of colouring components and coloured thermal degradation products by high-performance liquid chromatography with photodiode array detection. *Journal of Agricultural and Food Chemistry, 46*, 1031–1038.

Scotter, M. J., Wilson, L. A., Appleton, G. P., & Castle, L. (2000). Analysis of annatto (Bixa orellana) food colouring formulations. 2. Determination of aromatic hydrocarbon thermal degradation products by gas chromatography. *Journal of Agricultural and Food Chemistry, 48*, 484–488.

Teranishi, R., Keller, U., Flath, R. A., & Mon, T. R. (1980). Comparison of batchwise and continuous steam distillation-solvent extraction recovery of volatiles from oleoresin capsicum, african type (Capsicum frutescens). *Journal of Agricultural and Food Chemistry, 28*, 156–157.

Whitfield, F. B., & Last, J. H. (1991). Vegetables. In H. Maarse (Ed.), *Volatile compounds in foods and beverages*. New York: Marcel Dekker Inc.

Wissgott, U., & Bortlik, K. (1996). Prospects for new natural food colourants. *Trends in Food Science and Technology, 7*, 298–302.

Yamauchi, R., Miyake, N., Inoue, H., & Kato, K. (1993). Products formed by peroxyl radical oxidation of β-carotene. *Journal of Agricultural and Food Chemistry, 41*, 708–713.