Lipid Composition in Tomato Skin Supercritical Fluid Extracts with High Lycopene Content

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ABSTRACT: The use of supercritical fluid extraction is proposed for obtaining stable extracts with high added value from natural and economical sources. Lipid composition, namely, of FFA, TAG, and FAME, in tomato skin extracts with high lycopene content was determined. Separation of different classes of lipids was achieved from tomato extracts using TLC followed by transesterification and GC, and lycopene and other carotenoids were analyzed by HPLC with a photodiode array detector. In lycopene extracts obtained using supercritical fluids, no FFA were found and polyunsaturated TAG represented only 9.2% of the total TAG content.

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KEY WORDS: Lipids, lycopene, supercritical fluid extraction, tomato.

The increasing demand for natural products and the need for environmentally friendly technologies that meet safety regulations have contributed to the development of new strategies for extraction processes that minimize, or ideally avoid, the use of organic solvents. In this respect, supercritical fluids (1–3) have already proved their usefulness for performing extractions of high value-added compounds from organic matrices (4–8). In these cases, adequate optimization of experimental variables (e.g., pressure and temperature) at which both the extraction and the subsequent depressurization are carried out results in a wide range of solvent strengths, enabling the selectivity of the extraction to be increased. Further characteristics of supercritical fluids, such as their low viscosities and high diffusivities, also contribute to an efficient mass transfer, which allows the use of mild experimental conditions especially suited to the extraction of thermally unstable and reactive compounds.

Lycopene, an acyclic unsaturated carotenoid whose major source is tomatoes and tomato foods, is in high demand by different industries (e.g., cosmetic, pharmaceutical, food and feed industries) because its bioactivity is associated with several health benefits. In fact, in the last few years the cancer prevention activity of lycopene has been demonstrated, and previous work by various authors has established that the antioxidant properties of lycopene inhibit the oxidation of LDL and, consequently, lower the risk for atherosclerosis (9–12).

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Besides lycopene and other carotenoids, the presence in tomatoes of certain kinds of lipids, e.g., palmitic (16:0), stearic (18:0), and oleic (18:1n-9) acids, also has been described previously (13). Generally speaking, the presence of lipids in the supercritical fluid-extract could be of special significance because of their nutritional properties; they act as an energy reserve and play an important role as a vitamin source. Lipids also contribute to the proper functioning of many vital organs (14). For instance, linoleic acid (18:2n-6) and the biosynthetically related α-linolenic acid (18:3n-3) are important EFA whose deficiency may result in growth disturbances. Likewise, α-linolenic acid is metabolized to eicosapentaenoic acid, a precursor of eicosanoids, which have a wide range of biological effects (15). However, degradation of lycopene might be observed during lipid peroxidation (16); consequently, the antioxidant effectiveness and stability of lycopene can eventually be altered in those extracts also containing lipids.

On the other hand, the use of natural, economical sources provides an excellent opportunity for new food development in the global market. An interesting approach to this concept is the use of nutritional supplements to supply carotenoids by way of foods and beverages; these are known as nutraceutical, or functional foods. Specifically, the potential benefits that suggest the use of supercritical fluid extraction (SFE) to obtain lycopene from surplus tomato production and by-products of tomato processing (e.g., tomato juice or tomato paste production) have recently been reported (17–22).

However, the stability of the extracted material, an important point concerning lycopene production and its subsequent incorporation into commercial products, has not been considered in depth. For that reason, optimizing lycopene yield for specific large-scale applications of SFE demands studies to optimize not only the selectivity of the fractionation procedure but also the potential for oxidation of the extracts and, consequently, their stability. In this respect, the use of adequate analytical techniques, typically TLC, GC, or HPLC (23–25), to determine the occurrence of fatty components is strongly required.

The objective of this work was to evaluate the usefulness of SFE to obtain stable, high value-added extracts from tomato skin. To this aim, the occurrence in the extracts of compounds other than lycopene present in tomatoes was investigated to estimate the potential risk of lycopene oxidative degradation or, on the contrary, the possibility of synergistic antioxidative effects.

EXPERIMENTAL PROCEDURES

Materials. Different compounds (19:0 TAG, 21:0, 23:0 methyl ester) were purchased from Sigma Chemical (St. Louis, MO) (99% purity in all cases) and were used as reference compounds to optimize the thin-layer separation procedure. A lycopene standard used for identification in the supercritical fluid extract was also obtained from Sigma Chemical. Tomatoes (*pear* type) were acquired in the commercial market, and the skins were dried in a freeze-dryer, ground, and put into a Teflon screw-capped tube, which was wrapped in aluminum foil and swept with nitrogen. Until SFE, samples were kept at –18°C. Carbon dioxide (99% purity) was obtained from Carburos Metálicos (Madrid, Spain) and filtered through active charcoal (activated carbon microcolumn).

SFE instrumentation and procedure. The SFE unit was designed and manufactured by Iberfluid (Barcelona, Spain). It consisted of a pump (450 bar), a flow meter, and a 300-mL extraction vessel connected to two 100-mL separation vessels (referred to as separation vessel 1 and separation vessel 2). A number of metering valves and thermocouples connected by electronic relays enabled the pressure and temperature of the extraction vessel and both separation vessels to be controlled.

A 20-g sample of freeze-dried tomato skins was placed into the extraction vessel and a stream of carbon dioxide was passed through while the pressure and the temperature were maintained at 32 MPa and 40°C, respectively, as described elsewhere (22). The decrease in solvating power resulting from lowering the pressure (up to 15 MPa) and increasing the temperature up to 50°C in separation vessel 1 (and, consequently, from lowering the carbon dioxide density) allowed the most insoluble and less volatile compounds to be collected as a deep-red solid. The extract was recovered by washing the vessel with dichloromethane; the extract was then flushed with nitrogen to dryness and analyzed as described below. The carbon dioxide was then passed through separation vessel 2, in which experimental conditions (6 MPa and 25°C) were established to retain medium-volatile compounds. The fraction collected in separation vessel 2 was also recovered with dichloromethane as described for separation vessel 1. Because no recirculation of carbon dioxide was done, the pressure and temperature of the stream leaving the second separation vessel were decreased by means of a valve until the carbon dioxide became a gas that was released to the atmosphere. The overall extraction time was 2 h, and each experiment was run in duplicate.

TLC separation of lipids followed by formation of FAME. The dry supercritical fluid extracts from separation vessels 1 and 2 were dissolved in 0.5 mL of chloroform/methanol (2:1). A solution (200 µL) containing 19:0 TAG (196 mg/L) and 21:0 (40.8 mg/L) was used as internal standard to establish the efficiency of TLC in separating the different groups of lipids and to quantify the compounds of interest. A 40-µL volume of the tomato supercritical fluid extracts was deposited on the TLC plate to carry out lipid separation. A mixture of isohexane/diethyl ether/acetic acid (40:10:1) was used as the eluent. Also, 19:0 TAG, 21:0, and 23:0 methyl ester were used to identify the bands on the plate. Four main bands corresponding to four fractions of lipids were obtained from the TLC plate, namely, polar lipids (band 1), which included phospholipids and glycolipids; FFA (band 2); TAG (band 3); and FAME fractions (band 4). To recover the lipid compounds, the plate was scraped and materials sorbed onto the silica were dissolved in 1.0 mL of methanol for the polar fraction and 1.0 mL of toluene for the other three fractions. After adding a 100-µL volume of 23:0 methyl ester (256 mg/L), which was used as an internal standard for quantification purposes, and 2.0 mL of methanol/sulfuric acid (1%), the samples were heated at 50°C on a heating block for 15 h. After making up the FAME, all fractions were treated as follows. A 5-mL volume of 5% sodium chloride together with 3 mL of isohexane containing BHT (50 mg/L) was added to every sample. After shaking and centrifuging the content of the tubes, the upper isohexane layer was removed and put through an anhydrous sodium sulfate column. The extraction was repeated once more with 3 mL of isohexane with BHT, and the column was then washed through with 1 mL of isohexane with BHT. The solvent was evaporated under a stream of nitrogen at 30°C. The samples were then dissolved in 1.0 mL of isohexane with BHT and put through a Florisil column, which was finally washed through by using 8.0 mL of isohexane with BHT/acetone (96:4). Subsequently, the solvent was evaporated under nitrogen gas at 30°C and the solids were redissolved in 50 and 200 µL of isohexane for the FFA fraction and the remaining fractions, respectively. The samples were then ready for analysis by GC.

GC of FAME. GC of FAME was performed using a Hewlett-Packard (Palo Alto, CA) model 5890, series II, gas chromatograph equipped with a split/splitless injector, autosampler, and FID. A fused-silica capillary column coated with CP-Wax 52CB^{TM} (0.25 mm i.d. × 25 m in length, 0.2-µm film thickness; Chrompack UK Ltd., London, United Kingdom) was used, and hydrogen was the carrier gas at an initial flow rate of 1 mL/min. After holding the temperature at 170°C for 3 min, the column was temperature-programmed at 4°C/min to 220°C, then held at this point for 15 min. In all analyses, the detector was set at 300°C, the injector at 230°C, and a split ratio of 50:1 was used. An HP 3365 Chemstation (Hewlett-Packard Ltd., Stockport, United Kingdom) was used for data acquisition.

Calculations. Lipid composition, expressed as weight percentage of total lipids, was calculated using area counts obtained from the gas chromatogram of the FAME. Total lipid contents, as weight percent of the tomato extract, were measured using Equation 1

$$\%\ \text{FAME} = \sum \frac{(A_{\text{XA}} * \text{CF}_{\text{X}}) * W_{\text{IS}}}{A_{\text{IS}}} \times 100 \qquad [1]$$

where A_{XA} = area counts of individual FAME, A_{IS} = area count of 23:0 methyl ester internal standard, W_{IS} = mass internal standard added to the sample, CF_{X} = theoretical correction factor relative to 23:0 methyl ester (IS) (23).

HPLC analysis of lycopene. The lycopene content of the extracts obtained was established using an HPLC system (model 126; Beckman, Fullerton, CA) and a 168 Beckman photodiode array detector (wavelength range of 200–600 nm). Analyses were performed on a 250 × 4.6 mm Develosil UG column (Nomura Chemical, Sojo, Japan) packed with a polymeric C_{30} phase operated at 22ºC, and a mixture of methanol/water (96:4, vol/vol) and methyl *t*-butyl ether was used as the mobile phase (21).

RESULTS AND DISCUSSION

For tomato extracts collected in both separation vessels, the lipid content corresponding to every band from the TLC plate was determined by using the procedure described above for at least two replicates. Relative SD values obtained for all lipid groups were lower than 16% and, in most cases, no higher than 10%. Quantification of polar lipids such as phospholipids and glycolipids could not be carried out because they occurred at trace levels in both separation vessels, probably owing to their lack of solubility in the supercritical fluid under the experimental conditions used to perform the extraction.

FFA were not found in the extract collected from separation vessel 1, but they represented 42.8% of the total lipid content in separation vessel 2. This is probably due to the relatively low vapor pressures of the FFA compared to the TAG. Table 1 depicts the FFA composition in tomato extract collected in separation vessel 2 (wt% of total FA). As can be seen, stearic acid (18:0) was the major component followed by 16:4, gondoic (20:1n-9), palmitoleic (16:1n-7), and palmitic (16:0) acids. The latter FA is probably the most common saturated FA and is found in all animal and plant fats and oils. Stearic acid is also relatively common and may occasionally be more abundant than palmitic acid (24). The presence of gondoic, palmitoleic, and 16:4 acids was, however, more unexpected: They are common components of seed oils, but their occurrence in other tissues is rare. This fact was particularly surprising for 16:4, which is not frequent in nature.

TAG were mainly present in separation vessel 1 in which 73.8% of the total lipid content was found. However, a lower percentage was also found in separation vessel 2 (39.8%). Similarly, FAME appeared in both separation vessels, although in lower proportions (26.2 and 17.3% of the total lipid content obtained in separation vessels 1 and 2, respectively).

Generally speaking, it is clear that SFE lipid fractionation was not complete, because some TAG and FAME were found in both separation vessels. Nevertheless, it is interesting to note that FFA were not detected in separation vessel 1. It can therefore be concluded that different types of lipids can be separated from tomato skins into two different fractions using SFE.

Table 2 shows the TAG composition of supercritical fluid extracts collected in separation vessels 1 and 2. As can be seen when comparing TAG fractions found in both separation vessels, saturated TAG represented 71.5% of total TAG in separation vessel 1, whereas only 21.5% was estimated in separation vessel 2. In contrast, polyunsaturated TAG made up only 9.2% of total TAG in separation vessel 1, whereas 52.7% was found in separation vessel 2. This is because longer, saturated-chain FA are less soluble in carbon dioxide, and were thus more likely to occur in separation vessel 1. Furthermore, the higher solubility of polyunsaturated TAG in supercritical carbon dioxide is also due to their lower M.W. with respect to those of saturated TAG of the same chain length; consequently, the former group was preferentially found in separation vessel 2. As can be seen in Table 2, stearic acid TAG was by far the major component in separation vessel 1, whereas 16:2, which is shorter and more unsaturated and therefore of higher solubility in carbon dioxide, occurred as the main TAG component in separation vessel 2. In any case, stearic acid TAG also occurred at a significant concentration in separation vessel 2, thus becoming the most important FA found in the tomato extract. As already mentioned, the presence of this acid was not unexpected because it is usually quite frequent in foods and plants (24).

Likewise, the ratio of saturated/unsaturated TAG appeared to be considerably higher in separation vessel 1 (2.5) than in separation vessel 2 (0.3). The same trend was also observed with respect to the ratio of monounsaturated/polyunsaturated TAG, which resulted in 2.1 and 0.5 in separation vessels 1 and 2, respectively.

TABLE 1
FFA Composition in Tomato Extract Collected in Separation Vessel 2 (wt% of total lipids)

FA	Percentage	FA	Percentage
14:0	4.9	18:0	14.9
15:0	8.7	18:1n-9	4.6
16:0	9.5	18:2n-6	5.5
16:1n-7	9.7	α-18:3n-3	4.6
16:2	3.7	18:4	7.3
16:3	1.8	20:1n-9	11.1
16:4	11.7	22:6n-3	1.7

TABLE 2
TAG Composition in Tomato Extract Collected in Separation Vessels 1 and 2 (wt% of total lipids)

TAG	Percentage (vessel 1)	Percentage (vessel 2)
14:0	0.97	1.2
16:0	1.50	3.8
16:1n-7	0.64	3.5
16:2	—	20.4
16:4	4.21	9.3
18:0	69.04	18.6
18:1n-9	5.84	4.4
18:1n-11	1.71	3.9
18:2n-6	1.27	3.4
18:4	2.31	5.7
20:1n-9	11.04	18.9
20:4n-3	—	2.8
20:4n-6	—	4.0
22:5n-3	1.46	—

With regard to the FAME group, in spite of its lower presence in comparison with TAG and FFA components, it also clearly showed the same trend. Actually, polyunsaturated FAME represented 63.8% of the total content of FAME in separation vessel 2 compared to 38.4% in separation vessel 1, and ratios of saturated/unsaturated FAME and monounsaturated/polyunsaturated FAME equal to 0.6 and 0.6, respectively, were found in separation vessel 1 compared to 0.1 and 0.2 in separation vessel 2.

Interestingly, the low concentration of polyunsaturated compounds found in the same separation vessel in which lycopene was collected (i.e., in separation vessel 1) seems to be advantageous regarding the stability of the extract obtained. This is because polyunsaturated compounds are particularly opportune targets for free radicals and bring about chain reactions generating a cascade of reactive intermediates that eventually may contribute to lycopene oxidation.

On the other hand, the low percentage at which lipids were found in separation vessel 1 is worth noting. Here lycopene represented approximately 85–90% of the total supercritical fluid extract. Moreover, other carotenoids identified in this extract (e.g., β-carotene) may also act synergistically with lycopene as natural antioxidants, thus contributing to the effectiveness of the supercritical fluid extract.

The present study shows the possibility of using SFE to obtain lycopene extracts in which FFA are not found and polyunsaturated TAG occur at low percentages. Consequently, the risk of lycopene degradation caused by lipid peroxidation can be lowered, thereby preserving the antioxidant effectiveness and stability of lycopene.

On the basis of our experience concerning SFE of lycopene, careful control of experimental conditions affecting extraction, storage, handling, and analysis are strongly recommended to minimize oxidative degradation and isomerization.

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