



Thermal and light stabilities and antioxidant activity of carotenoids from tomatoes extracted using an ultrasound-assisted completely solvent-free method



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ABSTRACT

The use of petroleum-derived solvents, particularly volatile organic compounds (VOCs), in the chemical industry has increased the contamination and residual effects of these solvents. Ionic liquids (ILs) can potentially replace VOCs, thereby reducing the risks of environmental contamination and toxicity. In this context, the objectives of this study were as follows: 1 – to obtain an ionic liquid for use in extracting carotenoids from tomatoes with ultrasound assistance; and 2 – to determine the stability and antioxidant activity of tomato carotenoid extracts. Ultrasound can also efficiently extract carotenoid compounds with ionic liquids in comparison with conventional VOC solvents (obtained from an all-*trans*-lycopene 7.5–8.0 $\mu\text{g}\cdot\text{g}^{-1}$ tomato sample by IL and 6.2–7.7 $\mu\text{g}\cdot\text{g}^{-1}$ by acetone). Similarly, the activation energies (E_a) in aqueous medium were obtained for the IL carotenoid extract (10.8 $\text{kJ}\cdot\text{mol}^{-1}$) and acetone carotenoid extract (9.4 $\text{kJ}\cdot\text{mol}^{-1}$). The antioxidant activities of the tomato carotenoid extract were 7.4 and 12.4 relative to α -tocopherol for the ionic liquid extract and acetone extract, respectively. The combination of chromatographic analysis and degradation kinetics provided data for positive assessment similarity of thermal and light stabilities of tomato carotenoids extracted by IL and extracted by acetone.

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1. Introduction

Solvents play essential roles in chemical processes, not only for placing reactants in contact with each other *via* dissolution but also by affecting the rates, stability and kinetics of the final reaction products. Solvents are also used in purification and extraction steps of reactions, which substantiate their broad applications (Collom et al., 2013).

Currently, there are numerous substitutes for VOC solvents for synthesis purposes and a few for extractions. For example, supercritical fluid extraction (Da Porto, Lino, & Decorti, 2015) and synthesis methods that employ metal catalysis, solid supports, acid salts, eutectic solvents, ionic liquids (Hallett & Welton, 2011), and even water (Andrade & Alves, 2005) are available. By combining the use of these substituents with heating and homogenization techniques to promote a reaction, such as continuous-flow microreactors, ultrasound (US) and microwaves (MWs), it is possible to practice green chemistry that is free of volatile solvents (Boukroufa, Boutekedjiret, Petigny, Rakotomanomana, & Chemat, 2015).

Natural carotenoid compounds have been studied for decades, and research has highlighted that the appropriate consumption of such compounds is beneficial to human health (Islamian & Mehrali, 2015). Tomatoes are an important source of carotenoids, and disposal of this

abundant fruit and losses during transport and storage are lofty (Moretti & Sargent, 2000). The conventional method for extracting carotenoids from tomatoes uses volatile organic solvents, such as acetone and hexane (Saha, Walia, Kundu, Sharma, & Paul, 2015). However, as these solvents cannot be used in food products, a new method for extracting tomato carotenoids using an ionic liquid ultrasound-assisted method was developed (De Rosso & Martins, 2015). This technique uses organic salts with low toxicity, allowing for sustainable and eco-friendly reuse (Cassol, Ebeling, Ferrera, & Dupont, 2006).

During the various stages of the extraction process, the unsaturated structure of a carotenoid may be disrupted depending on the temperature, oxygen availability, light exposure, water activity, acidity, presence of metals and the structure itself (Gordon & Bauernfeind, 1982; Jiang et al., 2015; Mei et al., 2015).

Stability and antioxidant activity studies predict that for industrial processes, the use of ultrasound in the extraction process may have a significant impact on the stability of the extract (Anese, Bot, Panozzo, Mirolo, & Lippe, 2015). This is similar to the antioxidant activity because the antioxidant potential of the individual chemical compounds in an extract does not always correspond to the total antioxidant capacity of the crude extract (Kim, Oh, Yi, Kim, & Lee, 2015). We selected the most suitable method for evaluating the antioxidant activity of carotenoids (Rodrigues, Mariutti, Chiste, & Mercadante, 2012).

Accordingly, the objectives of this study were as follows: 1- to synthesize and purify an IL for use in the extraction of carotenoids from

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tomatoes with ultrasound assistance and without VOCs; and 2- to determine the stability and antioxidant activity of tomato carotenoid extracts.

2. Materials and methods

2.1. Chemicals and instrumentation

We used high-purity (99%) reagents from Sigma-Aldrich® (Darmstadt, Germany), including 1-methylimidazole, 1-chloro-butane, deuterated acetone, all-*trans*- β -carotene, all-*trans*-lycopene, α -tocopherol, azobisisobutyronitrile (AIBN) and the solvent dimethyl sulfoxide (DMSO). The fluorescent reagent used was C₁₁-BODIPY^{581/591} undecanoic acid (Invitrogen®, Carlsbad, CA, USA). The salt reagents included potassium hexafluorophosphate from Fluka® (Darmstadt, Germany) and sodium sulfate from Labsynth® (Diadema, SP, Brazil). Organic solvents from Labsynth® (Diadema, Brazil) were of analytical standard grade and included chloroform, acetone and ethanol. HPLC-grade methanol and *tert*-butyl methyl ether were obtained from Merck® (Darmstadt, Germany). The water was purified using a Pilsen Quimis® (São Paulo, SP, Brazil) model Q341 water distiller.

The melting point determination was performed using an Instrutherm® (São Paulo, Brazil) model DF-3600 with an analog thermometer. The ultrasound probe was a Branson Digital Sonifier® 450 (Danbury, CO USA). The ESI-MS/MS (electrospray ionization – mass spectra) were obtained using a LTQXL Thermo® Ion Trap (Waltham, MA, USA). The nuclear magnetic resonance (NMR) spectra were obtained using a 300 MHz Bruker® DRX300 instrument (Bremen, Germany). A Shimadzu® (Kyoto, Japan) model LC-20a was used for the HPLC chromatographic analysis, and it was equipped with loop injector to inject 20 μ L of Rheodyne, an online degasser. The system also included a diode array detector (DAD) (model SPDA-20a) and a data acquisition and processing system.

2.2. Tomato sample

Tomato samples discarded as not suitable for sale were donated by a local market (Santos, Brazil). Approximately 10 kg of sample with no selection criteria was homogenized and reduced to laboratory samples. Being a microbiologically contaminated sample (from disposal), a sterilization process (121 °C for 15 min) was performed prior to the onset experiments, according to Nunes and Mercadante (2004), and then refrigerated at –40 °C until use. In addition to ensuring microbiological safe work, the sterilization process contributes to more effective extraction of lycopene due to softening of the cell wall. The applied sterilization process did not contribute to a significant increase in *cis* isomers, as performed in a previous study of Nunes and Mercadante (2004).

2.3. Traditional synthesis and purification of ionic liquids

1-Butyl-3-methylimidazolium chloride ([BMIM][Cl]) was synthesized in a round-bottom flask by mixing 0.10 mol of 1-methylimidazole and 0.10 mol of 1-chloro-butane. The mixture was stirred and refluxed (70–80 °C) for 72 h (Fang et al., 2008). A viscous yellow liquid, which was washed twice with dichloromethane, was obtained. [BMIM][Cl] was dried under vacuum at 100 °C and crystallized at –40 °C.

An ion exchange reaction was performed according to Schrekker et al. (2008). A mixture containing 0.01 mol of [BMIM][Cl] and 0.01 mol of potassium hexafluorophosphate in distilled water was stirred vigorously for 45 min. The upper aqueous phase that formed was separated and discarded; the remaining liquid was added to distilled water and stirred for 15 min. Then, 40 mL of chloroform was added. The formed organic phase was separated, dried with sodium carbonate and filtered using Celite column chromatography (SPE cartridge 3 cm). The solvent was evaporated under vacuum to yield a viscous

liquid with a slightly yellow color hexafluorophosphate, called 1-butyl-3-methylimidazolium ([BMIM][PF₆]). Both of these synthesized products were characterized by melting point (MP) determination, mass spectrometry (ESI-MS/MS) and nuclear magnetic resonance spectroscopy (NMR).

2.4. Synthesis and purification of ionic liquids obtained with ultrasound assistance and solvent-free method

In a cylindrical glass tube (diameter of 6 cm) were mixed 0.10 mol of 1-methylimidazole and 0.10 mol of 1-chloro-butane. The mixture was maintained for 30 min, which was optimized experimentally, in the ultrasound (US) probe in the range of 65% (Zbancioc, Mangalagiu, & Moldoveanu, 2015). The temperature was monitored throughout the reaction period using LabVIEW® software (National Instruments Corporation, Austin, TX, USA). A viscous yellow liquid was obtained, dried under vacuum at 100 °C and crystallized at –40 °C to yield the final product, 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), which was chemically characterized using the same techniques applied to the product synthesized using the traditional method.

To prepare the 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆]) ionic liquid, the same reaction procedures were conducted as described in the traditional synthesis, except for the purification step. After the reaction, the organic phase was separated and then dried over anhydrous sodium sulfate (Na₂SO₄), followed by separation of the precipitated salt to obtain [BMIM][PF₆], a viscous slightly yellow liquid. It was dried under vacuum and characterized chemically using the same conditions as used for all of the other products.

2.5. Chemical characterization of the synthesized ionic liquids

The mass spectra of the synthesized ionic liquids were obtained using an ESI-MS/MS Ion Trap. The conditions for the mass spectrometric (MS) analysis were as follows: ionization in positive mode for 2 μ L of sample at a 10 mL/min N₂ (0.22 μ A) flow rate, a spray voltage of 5 kV, a capillary voltage of 14 V and a temperature of 280 °C. The experiment was performed by direct infusion of the reaction product dissolved in methanol.

For all of the NMR analyses, approximately 30 mg of the ionic liquids was added to a 5 mm NMR tube. A stem coaxial capillary tube that was loaded with 0.6 mL of deuterated acetone was inserted into the 5 mm NMR tube. Structural analysis of the produced ionic liquid was performed using a 300 MHz nuclear magnetic resonance instrument. The instrument was equipped with a 5 mm probe for direct detection (¹³C) or a 5 mm trinuclear probe and an inverse detection probe with a z-gradient (TXI) (¹H and ²D spectra). Temperature spectra were recorded at 7 and 25 °C. All chemical shifts are expressed in ppm relative to the solvent, deuterium, or to TMS and were acquired and processed using TopSpin 1.3 (Bruker BioSpin®).

2.6. Carotenoid extraction with ionic liquid and conventional solvent (acetone)

Conventional extraction was used for comparison in all analyses. Following the sample treatment described in the previous section, five grams of tomato sample was subjected to complete extraction with acetone. Pestle homogenization was performed, and the resulting carotenoids were concentrated in a rotary evaporator (38 °C under vacuum). The carotenoid extract was stored at –40 °C until use (De Rosso & Mercadante, 2007a).

For extraction with ionic liquids, each individual [BMIM][PF₆] and [BMIM][Cl] and a combination of the ILs were dissolved in 5 g of water-free tomato sample for extraction in the absence of light and homogenization using an ultrasound probe at 0 °C. In brief, the ILs were diluted in ethanol (1:1) mixed with tomato sample by US, and then the extracted carotenoids were vacuum filtered, ethanol rotary evaporated,

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