DYNAMIC MEASUREMENT OF THE EQUILIBRIUM PARTITION OF TOMATO EXTRACT BETWEEN SUPERCRITICAL CO_2 AND PRETREATED TOMATO

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Abstract. A function (isotherm/isobar) relating the partition of a vegetable extract (solute) between supercritical CO_2 ($scCO_2$) and a vegetable substrate commonly is a best-fit parameter in the modeling of supercritical fluid extraction processes, and inaccuracies of scale-up procedures might be related to adjusted parameters misrepresenting the physical reality. We developed a dynamic experimental methodology based in chromatographic principles to experimentally measure oleoresin (lycopene) partition between pelletized and milled carotenoid-rich tomato and $scCO_2$ at 40 °C and 29 MPa. The methodology proposes the injection of tomato oleoresin dissolved in $scCO_2$ into a column packed with pretreated (pelletized, milled, sieved, and $scCO_2$-extracted) tomato. Constant and stable oleoresin concentrations in $scCO_2$ at the inlet of the tomato column were achieved by injecting pulses to a column packed with glass beads. Elution profiles were monitored measuring $scCO_2$ absorbance at 486 nm. A linear relationship was found between the average absorbance and the average injected carotene concentration in $scCO_2$ for eight points in the range of 0-1 Absorbance Units (AU). Five curves of dissolved oleoresin injected to a completely $scCO_2$-extracted tomato column were measured.

Keywords : sorption isotherm/isobar, tomato extract, supercritical extraction, pretreated substrate, pelletization

1. Introduction

Carotenoids function in foods as pigments, antioxidants, and essential nutrients. Because of these functional properties, the food industry is interested in isolating carotenoids from biological substrates to use them as ingredients in nutraceuticals and functional foods. Tomatoes and tomato-derived products are an important source of carotenoids, particularly lycopene, and its extraction with supercritical CO_2 ($scCO_2$), represents an alternative to their extraction with organic solvents [1].

Mathematical simulation of the process makes different assumptions about kinetic and equilibrium factors controlling mass transfer during $scCO_2$ extraction of vegetable substrates [2]. Thus, these factors should be experimentally measured instead of best-fitted to integral extraction curves measured at laboratory and pilot plant scales. The viability of industrial $scCO_2$ extraction processes depends critically on scale-up analysis of experimental data gathered at laboratory and pilot-plant scales. In our opinion, a reliable scale-up procedure should be based on robust mathematical models to simulate the extraction process, to account for scale-

specific issues that are less relevant in one-pass laboratory units and pilot plants with CO_2 recycling capabilities, such as residual oleoresin in the $scCO_2$ stream entering the extraction vessel, a variable oleoresin content in the $scCO_2$ stream entering the vessel in industrial plants equipped with three or more [3], and non ideal flow conditions (e.g., channelling) resulting in radial gradients of residual oleoresin concentration within large vessels [4].

Extraction rate depends on the partition of the extract between the substrate and $scCO_2$ (equation of sorption isotherm/isobar relating solute concentrations in the $scCO_2$ phase, C_f, and the substrate, C_s), an internal mass transfer parameter (effective diffusivity of extract in the substrate), and external mass transfer parameters (axial dispersion coefficient and film coefficient) [2]. In the case of sheared substrates devoid of inner mass transfer barriers (such as those obtained by pelletization [5]), del Valle and Urrego [6] proposed using dimensionless correlations for packed beds operating with SC Fluids (SCFs) to estimate axial dispersion and film coefficients, and an independent measurement of the sorption isotherm/isobar to characterize the equilibrium (solute partition between $scCO_2$ and the substrate), so as to limit the model to best-fit the effective diffusivity of the extract in the substrate.

Urrego et al. [7] proposed and standardized a methodology to measure the sorption isotherm/isobar of vegetable extracts between $scCO_2$ and a pretreated (pelletized and milled) substrate. This methodology intersperses extraction (to reduce the extract content) and equilibration (by recirculation of the $scCO_2$ phase) steps, the vegetable extract is sampled on each step, the extract concentration in the $scCO_2$ is obtained by direct measurement, and the extract concentration in the substrate is calculated with mass balances. This methodology, however, requires long periods of time to obtain an isotherm/isobar curve (due to the equilibration steps) and alternative methodologies might be considered.

In the field of adsorption from supercritical phases, Brunner and Johannsen [8] stated that static methods to measure sorption isotherm/isobar have the advantage that no kinetic effects disturb the results, but since they are time consuming, alternative dynamic methods are of practical interest. Three dynamic methods proposed for the experimental determination of adsorption isotherm/isobar curves in supercritical fluid-solid systems are: (1) breakthrough curves, (2) perturbation experiments, and (3) the peak fitting method. In the analysis of breakthrough curves a solute-free substrate is contacted with a $scCO_2$ phase containing a certain concentration of the solute, the contact continues until the concentration of the solute at the outlet is the same as in the inlet, then the substrate is assumed to be in equilibrium with the concentration of the solute and the equilibrium loading can be determined by a mass balance. The method has some complications for mixtures because different compounds might adsorb differently and do not reach equilibrium at the same concentration level at the outlet [8]. For the perturbation method, the substrate is brought in equilibrium as in the previous case and from a small change of the concentration of the solute the slope of the sorption isotherm/isobar can be determined. For the peak fitting method (or inverse method) [9], a mathematical model accounting an isotherm/isobar equation is used to minimize discrepancies between an experimental chromatogram and the model prediction.

The objective of this work was to measure oleoresin (lycopene) partition between pretreated (pelletized, dried, and milled) tomato and pure $scCO_2$, using a modified methodology derived from chromatographic principles and methods (tomato extract diluted in $scCO_2$ injected into a column packed with completely $scCO_2$-extracted tomato).

2. Materials and Methods

2.1. Materials

Commercial tomato flakes were acquired from Invertec Foods (Santiago, Chile). Flakes were pelletized in a Pellet Pros PP85 device (Dubuque, IA) into cylindrical particles of 4-mm diameter and ~10-mm length. Then pellets were milled in a Moulinex chopper (Barcelona, Spain), milled particles were sieved so as to separate a fraction of 355-425 μm diameter, milled tomato was partly dried in a oven at 60 °C overnight. Pretreated tomato contained ~1.8% w/w oleoresin (total extractable oleoresin by soxhlet extraction with hexane), and ~12% w/w moisture (dried in a oven at 105 °C). Samples were packed in kraft paper into polethylene bags and stored in a refrigerator (5 °C) up to analysis.

Around 400 g of pretreated tomato were extracted using the method of Germain et. al. [10], by using a Thar Technologies' (Pittsburgh, PA) SFE-1L process development unit equipped with a computer-controlled system to adjust the extraction pressure and solvent flow rate. Extraction experiments were performed using food-grade CO_2 from Indura (Santiago, Chile). A 500-cm^3 extraction vessel (model 500-mL-ph) was

employed. Temperature (60 °C) inside the extraction vessel was controlled by passing through an external jacket hot water from a PolyScience (Niles, IL) 8205 thermostated bath. The system was equipped with a P-200A-220V pump and a Micro Motion (Boulder, CO) CMF010M324NU mass flow sensor to maintain the desired solvent flow rate. The extraction pressure (50 MPa) was maintained by a back-pressure regulator (BPR) BPR-A-200B1 placed at the outlet of the extractor. The outlet line of the BPR was connected to a 200-cm^3 cyclone separator (model CS-200-mL). The high-pressure pump was operated at 150 g/min (superficial solvent velocity of 1.10 mm/s) for 12 h.

Tomato extract was collected from the cyclone into a 10-cm^3 amber vial, the extracted water was removed with a Pasteur Pipette from the bottom of the vial, the extract (~ 4 g) was dried with a nitrogen stream for ~10 min, the vial was closed with nitrogen atmosphere and stored in a freezer (-20 °C) up to analysis. Residual tomato oleoresin in the pretreated tomato was re-extracted (up to additional 6 h) until residual oleoresin was not gravimetrically detected. The $scCO_2$-extracted tomato was packed in kraft paper into polethylene bags and stored in a refrigerator (5 °C) up to analysis.

2.2. Isotherm/isobar equipment and procedure

The experimental device consists of two systems as depicted in Figure (1). Area (A) delimits equipment and accessories used to measure the isotherm/isobar with chromatographic methods and procedures (dynamic system), whereas Area (B) delimits equipment to dilute vegetable extract in $scCO_2$ (to be injected to the dynamic system, Area A). Food-grade CO_2 from Indura (Santiago, Chile) is used for both systems, and cooled in a 3-m (1/8" high-pressure tubing) coil placed into a PolyScience (Niles, IL) cooling bath operated at -10 °C. The operation temperature is achieved within a thermostated air bath equipped with a Hillesheim HT42-10P (Waghäusel, Germany) controller, and a Leister Hotwind S (Sarnen, Switzerland) hot-air blower.

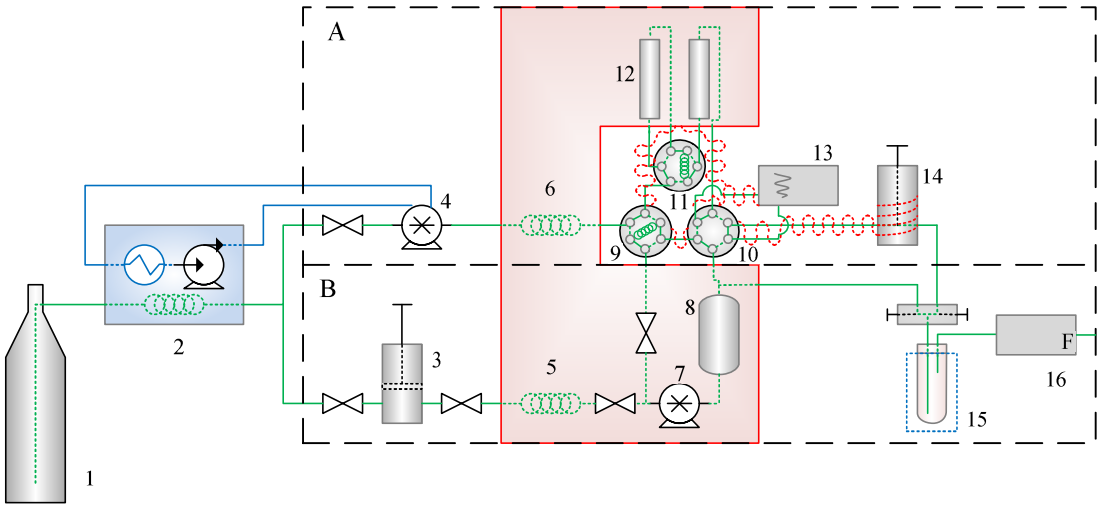

Figure 1. Configuration of the experimental device: (1) CO_2 cylinder, (2) cooling bath, (3) Teledyne Isco 260D (Lincoln, NE) syringe pump, (4) Jasco PU-2086 Plus (Tokyo, Japan) HPLC pump, (5-6) 6-m heating coils, (7) high-pressure gear pump Micropump GAH-T23 (Vancouver, WA) powered by a Siemens Micromaster 411 (Congleton, UK) motor, (8) 40-cm^3 equilibrium cell, (9) Jasco HV-2080-01 (Tokyo, Japan) two column selection valve with a Siemens LOGO 230RC (Munich, Germany) timing relay, (10,11) Rheodyne 7000 (Rohnert Park, CA) manual switching valves, (12) 12-cm^3 cells, (13) Agilent G1314A (Santa Clara, CA) UV/Vis detector equipped with a high-pressure cell (40 MPa), (14) Swagelok KHB-6000 (London, UK) BPR, (15) 50-cm^3 sampling tube and dewar flask, and (16) drum-type Ritter TG-05/5 (Bochum, Germany) volumetric gas meter. The system has a Jasco LC-NET II/ADC (Tokyo, Japan) interface to digitalize the analog output signal of the UV/Vis detector as well as to allow the operation of the HPLC pump with the Jasco ChromPass Chromatography Data System ver. 1.7.403.1 (Tokyo, Japan) software.

Dissolution of tomato extract in $scCO_2$ (Figure 1B): the vegetable extract (amount below 40% of the cell volume) was loaded in the equilibrium cell and placed horizontally in the system, the air bath was heated up to the desired temperature and then the system was pressurized with $scCO_2$ using the syringe pump. Equilibration was done recirculating $scCO_2$ through the equilibrium cell with the gear pump while monitoring

carotenoid concentration in the $scCO_2$ stream with the UV/Vis detector at 486 nm (wavelength of maximum absorbance for tomato oleoresin in $scCO_2$). During equilibration, the gear pump operates at 30 Hz (~40 cm^3/min, considering the performance of the pump using distilled water). Equilibration was assumed after absorbance remained unchanged for 1 h. The system then remained in standby to subsequently inject portions (defined by the volume of the loop) of $scCO_2$ saturated with the vegetable extract to the dynamic system.

Setting of the system (Figure 1A): one of the 12-cm^3 cell was packed with glass beds (500 μm diameter), the other was carefully packed with fully $scCO_2$-extracted tomato so as to attempt a chromatographic-like column packing, both cells were connected to the system, the air bath was heated up to the desired temperature and then the system was pressurized with $scCO_2$ with the HPLC pump set at 3 cm^3/min, pressure was controlled with the manual BPR, the actual CO_2 flow was measured with the gas meter.

Dynamic measurement of isotherm/isobar curves: Once temperature, pressure and flow conditions were stable the automatic switching valve and timing relay were switched on, and the UV/Vis detector and the chromatographic software were set to record the change in absorbance of the $scCO_2$ at the outlet of the tomato packed bed. With each pair of switches of the automatic valve (2-ways), portions of $scCO_2$ saturated with tomato oleoresin were injected to the dynamic system and pure $scCO_2$ were carried out to the static system, thus, pulses (pure and saturated $scCO_2$) are pumped to the inlet of the column of glass beads and a stable oleoresin concentration in $scCO_2$ was achieved at the outlet of the column. In this way the system allows the measurement of sorption isotherm/isobar with frontal analysis and the peak fitting method. For the perturbation method the manual switching valve (11 in Figure 1) allows bypassing the column packed with glass beads (for a short period of time) and therefore to pump pure $scCO_2$ to the tomato packed column causing the perturbation.

Total carotenoid content in oleoresin samples was determined using the spectrophotometric method of Estrella et. al. [11]. Extracts were dissolved in HPLC-grade hexane (J.T. Baker, NJ) and absorbance measurements were performed at 472 nm (wavelength of maximum absorbance for tomato oleoresin in hexane) in a Shimadzu UVmini-1240 (Kyoto, Japan) UV/Vis spectrophotometer. Carotenoid concentration in the extracts was estimated with the extinction coefficient in hexane of lycopene ($E^{1\%}$ = 3450), the most important carotenoid pigment in tomato, and the results were expressed in mg carotenoid/g oleoresin.

2.3. Experimental design

To obtain a stable oleoresin concentration in $scCO_2$ at the inlet of the column packed with completely $scCO_2$-extracted tomato, which also contains a carotenoid composition enough to adsorb in the range of 0-1 absorbance unit (AU), three loop sizes (5.0, 20 and 50 μL), switching times between 3 and 21 s (based on the $scCO_2$ flows times were calculated to ensure that the loop volume was completely carried out while the automatic valve was in the position connecting the loop with the dynamic system), and HPLC pump flows of 2.5 and 3.0 cm^3/min were tested. In order to record the extract concentration at the outlet of the column packed with glass beads, the system was modified to bypass one column and packaging glass beads to the connected column. Seven different concentrations that adsorbed in the range of 0-1 absorbance units were recorded.

Six grams of completely $scCO_2$-extracted tomato were used for the dynamic isotherm/isobar curve. From the analysis of a stable oleoresin concentration in $scCO_2$ five combinations of switching times (leaving loop and pump flow constant) were selected.

3. Results and Discussion

The injection methodology proposed in this work differs from the commonly applied methodologies in chromatographic procedures where there is a constant solute concentration diluted in the solvent pumped into the column [8], in this work, the injected "solute" is a vegetable extract with interest in its carotenoids content. Initial and unreported results showed that the carotene concentration in oleoresin-saturated $scCO_2$ was over the UV/Vis measuring range (>3 AU), therefore extract dilution in $scCO_2$ was required. Authors explored the use of pulses and a column packed with glass beads as an alternative to achieve lower carotenoid concentrations. Figure (2) shows the actual steps of carotenoids concentration in $scCO_2$ being injected into the glass beads column for three total switching times (4, 9 and 21 s, each including 1.5 s to carry out the loop content) in a one-minute lapse, the carotenoid concentration represents the case when HPLC pump operates at 3.0 mL/min (2.3 g CO_2/min for a solvent superficial velocity of 1.1 mm/s) and the automatic switching valve has a 50-μL loop with oleoresin and carotene concentrations in $scCO_2$ of 6.68 g/kg and 2.32 mg/g,

respectively (concentrations were measured by sampling 7.29 g of $scCO_2$ from the static system once equilibration was achieved).

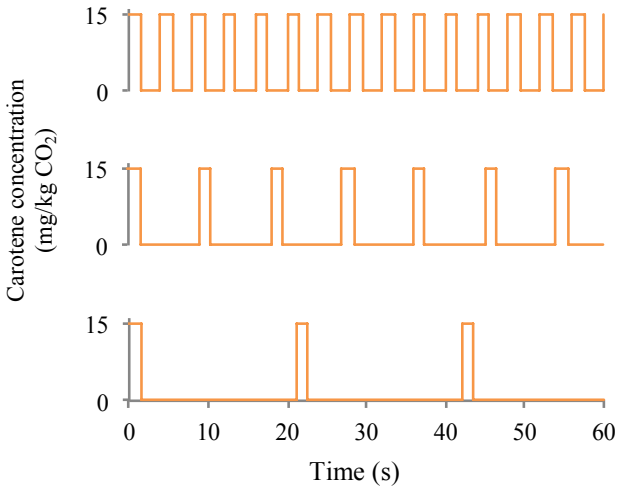

Figure 2. Pulses (1.5 s) of carotenoid concentration injected into the glass beads column for 4-, 9- and 21-s switching times.

Figure (3) shows the response signal recorded at the outlet of the glass beads column for switching times varying from 4 to 21 s. By comparing Figures (2 and 3) one might conclude that the column packed with glass beads allowed stabilization of the oleoresin concentration thanks to mixing and dispersion effects. Nevertheless, it must be noted that at the lowest switching time the horizontal asymptote shows a high dispersion and switching values over 4 s should be preferred. At higher switching time values, the dispersion might represent noise effects. The combinations of loop volume (50 μL), switching times (between 4 and 21 s), and flow (3 cm^3/min) used in Figure (3) were the only ones giving horizontal asymptotes in the full range of 0-1 AU, and were applied to measure the sorption isotherm/isobar dynamically. Results from other combinations are not reported. Differences in the width of the runs are only due to differences in the total time of injections and are not related to any adsorption or desorption effects, and this is represented by the compressed (shock) fronts and rear of the curves [9].

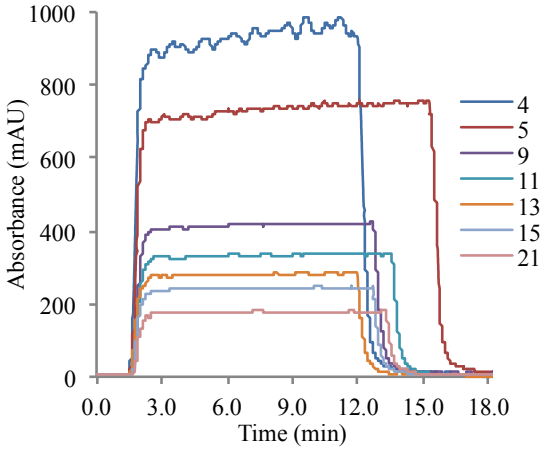

Figure 3. Response signal for different switching times (in s), of tomato oleoresin diluted in $scCO_2$, in a glass beads column.

Figure (4) shows correlations between the average absorbance (with error bars representing the standard deviation) observed in Figure (2) and the average carotene and oleoresin concentrations in $scCO_2$. In the assumption that the $scCO_2$+oleoresin in the loop of the automatic switching valve is completely carried out by the HPLC pump (1.17 s are required by the pump to displace 50 μL, and timing relay was set to stay at this

position for 1.50 s), and that the loop is completely refilled with oleoresin-saturated $scCO_2$ by the recirculating-gear pump, average oleoresin and carotene concentrations on each set of switching times were calculated based on the total switching time and the CO_2 flow. Lines in Figure (4) represents linear regressions of the data and the fit might suggest that the assumptions of completely carry out of the loop in 1.5 s as well as the assumption that the carotenoids concentration in the oleoresin during the experiments remains constant were correct. The linear regression of carotene was applied to convert the recorded absorbance into carotene concentration in further analysis.

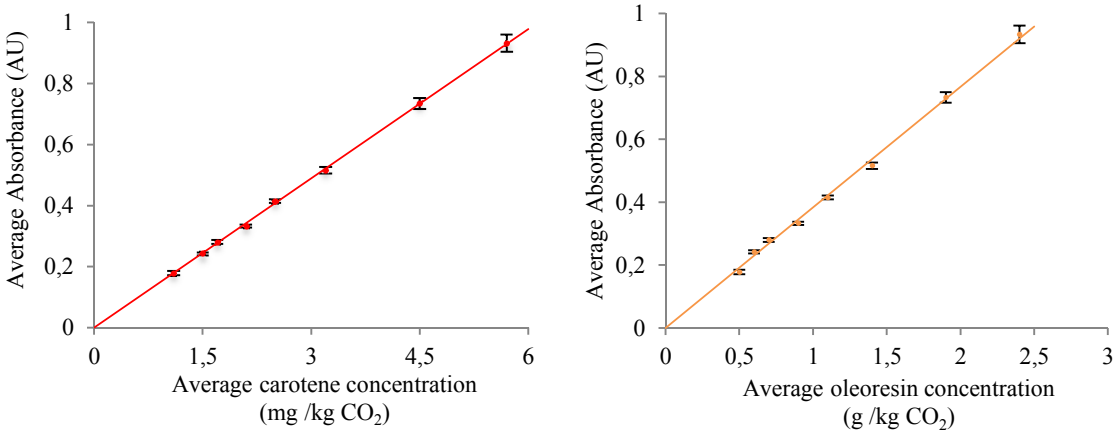

Figure 4. Correlation between the average absorbance and the average carotene and oleoresin concentrations.

The assumption of negligible adsorption/desorption on glass beads was also evaluated by comparing curves from Figure (3) under a same basis, by converting absorbance into carotene concentration in $scCO_2$ and dividing it by the theoretical-average concentration (Figure 5). The shared frontal shape implies that glass beads do not adsorb the carotene since different carotene concentrations in $scCO_2$ reach the outlet of the column at the same time, and that any eventual difference gets confounded within the noise of the methodology.

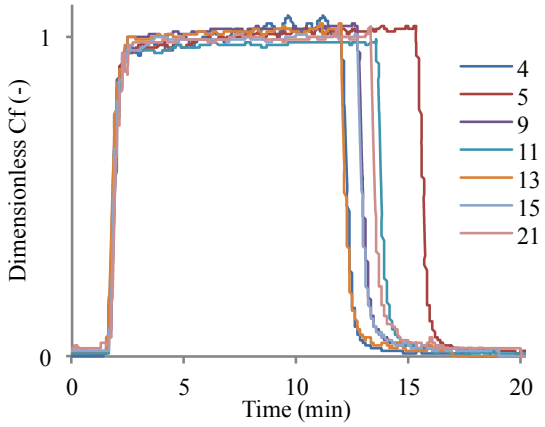

Figure 5. Elution response comparison of dimensionless carotene concentrations in $scCO_2$, for different switching times (in s).

Based in the correlation observed in Figure (4) seven average carotene concentrations in $scCO_2$ (1.10, 1.90, 2.60, 3.50, 4.10, 5.00 and 5.60 mg carotene/kg CO_2) were selected, and the required switching times to achieve those concentration were calculated (20.5, 12.0, 8.50, 6.50, 5.50 4.50 and 4.00 s, respectively) to measure the isotherm/isobar with the pretreated and completely $scCO_2$-extracted tomato. Figure (6) shows the experimental runs obtained with the dynamic methodology, with perturbations made once equilibration was assumed to be set and the elution profiles produced by turning off the injection valve and allowing pure CO_2 to desorb the carotenes from the equilibrated column. Elution profiles in Figure (6) represent the case where an equilibrated pretreated tomato is desorbed with pure $scCO_2$ (analysis of the desorption front, time was recalculated to zero at the moment when switching valve was turned off). This method states that only one

eluted profile contains the whole information of the adsorption isotherm/isobar if sufficient high quantities of the solute are injected [8], hence, it is expected that every elution profile fall into a same line as it was the case observed in Figure (6).

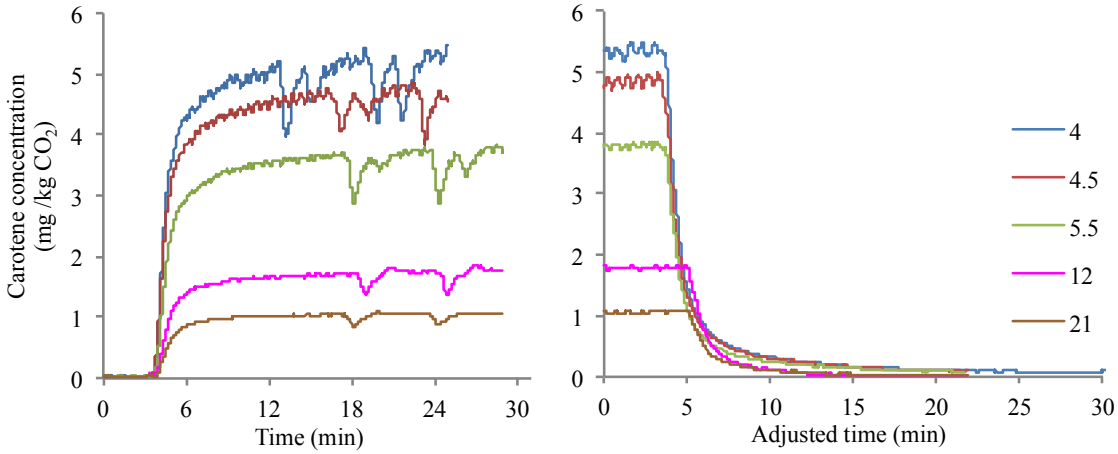

Figure 6. Elution profiles of different concentration of tomato extract injected to a completely $scCO_2$-extracted tomato column at 40 °C and 29 MPa.

Ongoing work is aimed at evaluating the different methods to calculate the isotherm/isobar curve from data in Figure (6), as well as to compare such results with an isotherm/isobar curve measured with a static and standardized methodology proposed by del Valle and Urrego [7].

Acknowledgements

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