

Novel Cascade Process for Recovery of Valuable Compounds from Tomato Processing Waste, using Supercritical CO₂ and Subcritical Water as Sequential Solvents

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Italy stands as the largest producer of tomatoes in Europe, with a total area of approximately 75 thousand hectares dedicated to this crop. The environmental impact of tomato production is a growing concern, with many studies devoted to the carbon footprint associated with different cultivation practices. Pressure on the environment could be alleviated by turning waste from tomato processing into useful products, according to circular economy principles. Among many different substances, tomatoes contain significant amounts of carotenoids (e.g., lycopene) and proteins/peptides. This work proposes and evaluates a novel valorisation route for tomato waste, consisting of supercritical CO₂ extraction followed by subcritical water extraction. Such an approach can enhance the recovery of lycopene, with its concentration in the recovered oil reaching 0.66 mg/g, due to its high solubility in lipid-like phases which are efficiently extracted by supercritical CO₂. Subcritical water extraction of the residual solids allows the recovery of proteins and peptides.

1. Introduction

Tomato is one of the most commonly grown fruits worldwide (Méndez-Carmona *et al.*, 2022). Among many different substances important for human well-being (Abbasi-Parizad *et al.*, 2020), tomatoes contain significant amounts of carotenoids (Nguyen, Francis and Schwartz, 2001; Liu *et al.*, 2021) including β -carotene and lycopene (Schweiggert *et al.*, 2012; Méndez-Carmona *et al.*, 2022). Carotenoids present in tomatoes are stored in chromoplasts, with lycopene stored mostly in crystalline chromoplasts (Camara *et al.*, 1995; Nguyen, Francis and Schwartz, 2001; Schweiggert *et al.*, 2012). Food processing can increase the bioavailability of lycopene by disrupting cellular membranes and helping to release lycopene from the tissue matrix (Story *et al.*, 2010). Lycopene is soluble in lipids, therefore using 10%_{w/w} of hazelnut oil as co-solvent has been reported to be optimum for supercritical extraction of lycopene from tomatoes (Margotta and De Simone, 2020). High carotenoid recovery, including 98.5% recovery of lycopene from tomato flesh, has been reported for supercritical CO₂ (SC-CO₂) extraction using ethanol as co-solvent (de Andrade Lima *et al.*, 2019); however, the subsequent recovery of co-solvent is a downside of such a process. Moreover, breaking the biomass matrix could be beneficial: the application of hydrolytic enzymes has been successfully attempted for the recovery of valuable products from tomato pomace, including lycopene (Scaglia *et al.*, 2023). Processes based on SC-CO₂, such as extraction, impregnation, and grafting (Nowak *et al.*, 2023), attracted some attention recently, due to the fact that SC-CO₂ is an excellent solvent for non-polar solutes (Duba and Fiori, 2015). Moreover, SC-CO₂ extraction allows relatively easy recovery of the solvent by simple depressurisation and subsequent condensation, so no elaborate distillation is needed to close the CO₂ loop. Thus, the economics of such a process is in many cases favourable with high return on investment and short payback

periods (Duba and Fiori, 2019). Therefore, an effective process for lycopene recovery from tomato waste should consider unit operations facilitating breaking down the solid matrix, where lycopene is stored (chromoplasts), selective dissolving of lycopene in the extraction solvent, energy-efficient recovery of the solvent, and dissolving of the recovered lycopene in oil for increased bioavailability. The cascade biorefinery approach seems to be capable of providing viable solutions for such issues. One such concept (Kehili *et al.*, 2016) proposed SC-CO₂ extraction of lycopene and β -carotene, followed by hydrolysis in NaOH solution (0.05 M) for the recovery of proteins, and subsequent hydrothermal treatment (120 and 200 °C) for the recovery of sugars and carboxylic acids. Additionally, a method based on acetic acid extraction and subsequent solid phase extraction has been proposed (Abbasi-Parizad *et al.*, 2023), aiming at the recovery of glycoalkaloids (all-tomatine) used as biopesticides. Overall, breaking the structure of the waste biomass along with a selective extraction process aiming at the recovery of different bio-compounds, e.g. through SC-CO₂ and subcritical water extraction (SWE), could be considered a viable solution for modern biorefineries. Processing methods should be evaluated for the recovery of valuable compounds, their influence on further steps and their consumption of energy and auxiliary substrates/catalysts. Such a cascade approach would be well in line with the principles of circular economy and the modern zero-waste approach.

2. Materials and methods

The sample of tomato waste, peels and seeds (PS) with a small amount of woody material coming from pedicels, was provided by a company producing tomato sauce. The sample was pre-dried by vacuum drying at 40 °C, sealed in air-tight plastic bags and stored at 4 °C. The supercritical CO₂ extraction (SCO₂E) of the PS was performed using the rig shown in Figure 1 (left). During these experiments, the water route (with HPLC-type pump) was cut off by a valve, and only CO₂ was supplied to the extraction basket. The CO₂ flow was measured using a Coriolis digital mass flow meter (mini Cori-Flow, Bronkhorst, Ruurlo, The Netherlands). The mass of the collected oil was recorded manually every 10 minutes. Process conditions were selected based on previous works on this type of feedstock (Scaglia *et al.*, 2020; Squillace, Adani and Scaglia, 2020). Subcritical water extraction (SWE) in a continuous mode was performed using the same setup as for SCO₂E, whereas, for the batch SWE experiments a small autoclave was used, as shown in Figure 1 (right). Batch SWE experiments were performed with a dry biomass-to-water ratio of 0.1. Pressure in the batch SWE experiments depended on the water saturation pressure (at process temperature) and the production of gases. A summary of the process conditions for the performed experiments is shown in Table 1. The baseline oil content of PS was determined using submersion method (Thiex *et al.*, 2003), with SER 148/3 Randall extractor (SER 148, Velp Scientifica). Extraction using *n*-hexane (purity 95%, Honeywell Riedel-de Haën), which exhibits one of the highest efficiencies in soxhlet extraction of lipids (Ramola *et al.*, 2019), took place at the boiling temperature of the solvent (69 °C), with a 60 min immersion step in the hot solvent followed by 110 minutes of reflux washing similar to the standard Soxhlet technique. The cellulose thimbles with the solid samples residual from extraction were subsequently placed in a ventilated oven at 105 °C overnight for drying, evaporating any remaining hexane. The mass of both beakers with oil and thimbles with extracted solids was determined on the next day, using Mettler Toledo ML204/01 balance (max. weight 220 g; permissible error 0.1 mg). Residual moisture content of PS was determined by a gravimetric method, drying PS at 105 °C for approximately 20 hours, in compliance with EN ISO 18134-2:2015. Average PS particle density was determined using pycnometry, according to the standard EN ISO 17892-3, with ethanol used instead of water, due to the fact that some particles within the sample batch did not sink completely into the water. SCO₂E-p410T70 and SCO₂E-p390T70 oil extracts were analysed for lycopene content and the quantification was performed by using Agilent 1260 Infinity HPLC system equipped with a C30 Develosil column (5 μ m, 250 x 4.6 mm) (Scaglia *et al.*, 2020). The protein fraction was solubilized in 50 mM sodium phosphate buffer, pH 9 with a dry biomass-to-water ratio of 0.1. The suspension was stirred for 1 hour at 4 °C and the solubilized protein fraction was separated by centrifugation at 10000 \times g for 20 min. The protein profiling was carried out on the extracts by SDS-PAGE in reducing condition according to (Laemmli, 1970) and the densitometric analysis was performed using ImageLab software (BioRad). Proteins were loaded on a 17% acrylamide gel and run in a Miniprotean II apparatus (Bio-Rad Laboratories, Hercules, CA, USA). The gel was Coomassie Blue-stained after fixing with 10% Tricolori Acetic Acid (TCA). Primary amines were quantified by the O-Pthalaldehyde (OPA) assay (Church *et al.*, 1985) with slight modifications. The OPA working solution was prepared by mixing 0.5 mL of OPA reagent (40 g/L in ethanol), 2.5 mL of Sodium Dodecil Sulphate (SDS) 10% w/v, 12.5 mL of 0.1 M borate buffer, 0.5 mL of 2-mercapto-ethansulfonic acid solution (200g/L), 1.25 mL of Triton X-100 (100 g/L), and 7.75 mL of water. Samples (8 μ L) and standards were added to a 96-well plate, followed by 232 μ L of the OPA working solution. After incubation at 30 °C for 10 min in the dark, absorbance was measured at 335 nm by a microplate reader (Tecan Infinite® M Nano+). Results were expressed as mmol of primary amine L⁻¹ determined through a calibration curve by using L-glutamic acid in 0.5 M perchloric acid as a standard.

Table 1: Process conditions for each extraction experiment (A – autogenic pressure; n.a. – not available).

Solvent	Process type	T (°C)	p (bar)	Flow rate (g/min)	t (min)	V _{extr.} (mL)	Code
Supercritical CO ₂	Continuous	60	380	22.2	30	200	SCO2E-p380T60
Supercritical CO ₂	Continuous	70	380	40.5	40	200	SCO2E-p380T70
Supercritical CO ₂	Continuous	70	410	9.5	130	200	SCO2E-p410T70
Supercritical CO ₂	Continuous	70	390	5.7	460	500	SCO2E-p390T70
Water	Continuous	120	90	3.0	30	200	SWEC-p90T120
Water	Batch	100	A	n.a.	30	50	SWEB-T100
Water	Batch	120	A	n.a.	30	50	SWEB-T120
Water	Batch	170	A	n.a.	30	50	SWEB-T170
Water	Batch	220	A	n.a.	30	50	SWEB-T220

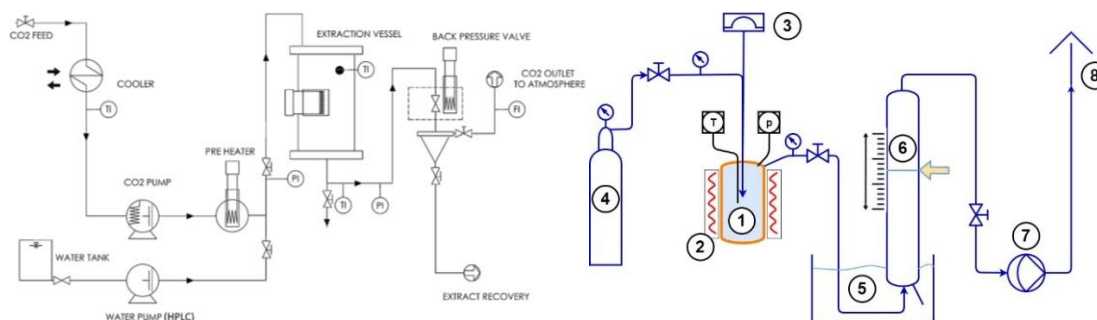


Figure 1: Left: Diagram of the rig used for supercritical CO₂ extraction and subcritical water extraction in continuous mode (from (Duba et al., 2015)). Right: subcritical water extraction batch rig (1 – extractor vessel; 2 – band heater; 3 – rupture disc; 4 – N₂ cylinder; 5 – open tank with water; 6 – cylinder for gas volume measurement; 7 – vacuum pump; 8 – vent of the fume hood cabinet; T – thermocouple; p – pressure sensor)

3. Results and discussion

The residual moisture content of the pre-dried tomato peels and seeds (PS) was 6.8 % (wet basis) and its density was 1.22 g/cm³. The oil yield resulting from Randall extraction was 0.108 g_{oil}/g_{dry-feedstock}. During SCO₂E, extraction efficiencies higher than 50% were achieved (Table 2). Performed research clearly shows that, apart from temperature and pressure, also flow rate is an important parameter since SCO₂E-p380T60 and SCO₂E-p380T70 experiments were performed with much higher flow rates than SCO₂E-p410T70 and SCO₂E-p390T70, the two latter achieving much higher efficiencies. Since the solubility of any solute in SC-CO₂ depends on the temperature and pressure of extraction (Duba and Fiori, 2015), it seems plausible to hypothesize that the observed dependence of the oil extraction efficiency on the CO₂ flow rate is not caused by differences in solubility, but rather by losses during depressurisation and oil collection stage. For the same geometry and temperatures, a higher flow rate is always associated with higher velocities. Since the depressurised solution (CO₂ + oil) was split in a glass separation funnel, it seems sensible to suspect that higher velocities caused a higher loss of droplets of condensing oil, entrained by the CO₂ flowing out of the separator. Additionally, during SCO₂E-p380T60 and SCO₂E-p380T70 experiments, the formation of dry ice was observed, which could be attributed to the high flow rates exacerbating the Joule-Thomson cooling effect. Extraction time influenced the SCO₂E efficiency only for the case of short extraction times, i.e., 30 min for SCO₂E-p380T60 and 40 min for SCO₂E-p380T70, which resulted in relative oil extraction efficiencies of 34.9 and 41.9%, respectively. For SCO₂E-p410T70 and SCO₂E-p390T70, differences in extraction time did not matter in terms of their respective relative oil extraction efficiencies, since both experiments were conducted until no more oil was extracted. Much longer extraction time in the case of SCO₂E-p390T70 was related to the use of a bigger volume of the extraction basket (500 vs 200 mL), which was filled with more PS, thus requiring a longer time to exhaust the matrix. Nonetheless, from the point of view of SCO₂E kinetics, basket size did not matter, as similar kinetics could be observed for both SCO₂E-p410T70 and SCO₂E-p390T70 (Figure 2). Especially, for the part of the extraction process limited by the solubility of the oil in SC-CO₂, a similar linear trend was found (see trend lines for the linear part of the curve – Figure 2).

Table 2: Yields for performed SC-CO₂ extraction and SWE (n.d. – not determined; n.a. – not available).

Sample	Oil Yield (g _{oil} /g _{dry-feedstock})	Solid yield (%)	Liquid yield (%)	Gas yield (%)	Rel. Oil extr. effic. (%)	Tot. lycopene (mg/g oil)
SCO ₂ E-p380T60	0.038	n.a.	n.a.	n.a.	34.9	n.d.
SCO ₂ E-p380T70	0.045	n.a.	n.a.	n.a.	41.9	n.d.
SCO ₂ E-p410T70	0.063	n.a.	n.a.	n.a.	58.6	0.66
SCO ₂ E-p390T70	0.059	n.a.	n.a.	n.a.	54.7	0.63
SWEC-p90T120	n.d.	76.6	n.d.	n.d.	n.d.	n.a.
SWEB-T100	n.d.	84.7	15.1	0.3	n.d.	n.a.
SWEB-T120	n.d.	83.8	15.6	0.6	n.d.	n.a.
SWEB-T170	n.d.	71.0	27.6	1.4	n.d.	n.a.
SWEB-T220	n.d.	71.9	24.7	3.3	n.d.	n.a.

Obtained solid yields for batch SWE show that mass loss due to hydrolysis significantly increases when increasing the temperature, in particular from 120 to 170 °C (Table 2). Further increase in temperature did not lead to significant variation in solid yield, which could be explained by the concurrent and counterbalancing densification of the original PS undergoing solid to solid carbonization reactions and the formation of secondary chars resulting from liquid to solid reactions, which is known to occur already at 220 °C (Ischia *et al.*, 2024). For the same temperature (120 °C) continuous SWE resulted in comparably lower solid yield (continuous: 76.6% vs batch: 83.8%), which could be explained by the fact that during continuous SWE treatment, the solution of water and extractives was gradually being replaced by the flow of fresh solvent, thus preventing saturation of the solution. There was only a slight numerical difference in amount between lycopene content of SCO₂E-p410T70 and SCO₂E-p390T70 oil extracts, with concentrations reaching 0.66 and 0.63 mg of lycopene per g of oil, respectively. This is less than achievable lycopene concentration reported by (Squillace, Adani and Scaglia, 2020) as 3.75 and 1.59 mg of lycopene per g of oil for tomato peels and raw tomato fruits, respectively. This indicates that increasing the oil content, either by improving SCO₂E efficiency or adding a co-feedstock rich in oil, could improve the overall performance of lycopene extraction (Vasapollo *et al.*, 2004). The protein profile in solid sample after SCO₂E is comparable to that of the untreated sample (Figure 3A) suggesting that SCO₂E did not affect the overall properties of proteins. On the contrary, peptides represent the only species present in both the SWE extract and in the solid materials remaining after the SWE treatment. The SDS-PAGE tracings indicate that the peptide profile in the liquid phase from SWE treatments at various temperature is similar to that of the corresponding residual solids. These results suggest an effect of SWE on the overall protein organization, with complete disappearance of the large-size proteins present in peels and seeds sample as well as in solids from SCO₂E. Even SWE treatments at the lowest temperature (100 °C) resulted in the formation of protein polymers that are insoluble in the non-denaturing conditions used for preparing samples to be analysed by SDS-PAGE. The densitometric analysis confirms the similarity between liquid and solid SWE samples also from a quantitative point of view and highlights that, among the liquid samples, SWEB-T120 has a peptide content 30% higher than SWEB-T100 and 20% higher than SWEB-T170. Data from the OPA assay (Figure 3B) shows that the amount of free primary amines in SWE liquid samples increases, especially when the treatment is carried out at 170 and 220 °C, compared to untreated sample. Given that the OPA assay detects all free amines (including free ammonia) and considering the apparent absence of stainable peptides in the SDS-PAGE profiles shown in Figure 3A, it is likely that SWE samples from high-temperature treatments are rich in very small-sized peptides or in free amino acids, or even in ammonia from the deamidation of glutamine and asparagine. All these species are not visible in the SDS-PAGE tracings, either because they run out of the electrophoretic gel or because they do not have enough hydrophobic surfaces able to bind the Coomassie Blue stain. Observed influence of the temperature is in good qualitative agreement with the model proposed for behaviour of proteins in hydrothermal conditions by (Kosińska *et al.*, 2023), which suggests decomposition of proteins to single polypeptides and amino acids at temperatures of 150 -180 °C.

The recovery of valuable compounds, using non-toxic solvents, represents a practical application of the circular economy in the tomato processing sector. Main economic benefits of such approach include avoiding waste generation and creation of additional revenue streams of high added value products. Such products can have a profound effect on the economic performance of biorefinery, e.g., recovery of lycopene from tomato waste using SCO₂ has been reported to have a payback time of 0.32 years (Hatami and Ciftci, 2023). Techno-economic analysis is advised to confirm similar effects for the cascade approach. Life cycle assessment is also recommended as a mean of quantifying the environmental benefits of the cascade approach.

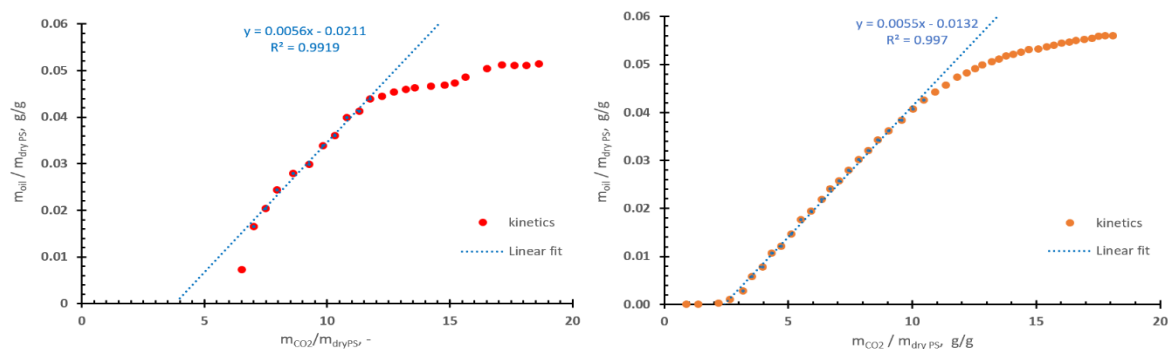


Figure 2: Kinetics of SCO₂E. Left: $p=410$ bar, $T=70$ °C, CO_2 flow rate=9.5 g/min, extraction basket volume=200 mL (SCO₂E-p410T70). Right: $p=390$ bar, $T=70$ °C, CO_2 flow rate=5.7 g/min, extraction basket volume 500=mL (SCO₂E-p390T70).

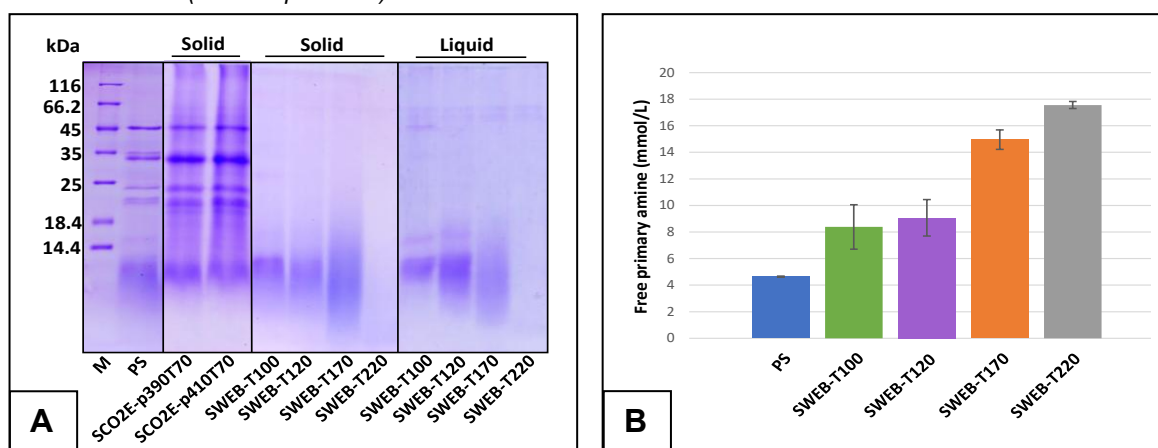


Figure 3. A: SDS-PAGE profiles - under reducing conditions - of proteins in samples from: tomato waste (PS); SCO₂E solid samples; batch SWE solid and liquid samples; B: O-Pthalaldehyde (OPA) assay of tomato waste (PS) and batch SWE liquid samples (M - Unstained Protein Molecular Weight Marker (Thermoscientific): 116=b-galactosidase, 66.2=Bovine serum albumin, 45=Ovalbumin, 35=Lactate dehydrogenase, 25=REase Bsp98l, 18.4=b-lactoglobulin, 14.4=Lysozyme);

4. Conclusions

Recovery of oil during SCO₂E was not limited by the solubility of oil in CO₂ but more likely by losses during depressurisation and oil collection stage. Thus, effective depressurisation and oil collection system seems to be as much important as correct selection of SCO₂E parameters. Moreover, increasing the oil content of the feedstock, either by improving SCO₂E efficiency or adding a co-feedstock rich in oil, could improve the overall performance of lycopene extraction, which in current settings reached 0.66 mg/g of recovered oil.

The overall properties of the proteins in solids after SCO₂E are non-modified. On the contrary, SWE deeply changes the overall properties of proteins, leaving small-sized peptides (and free amino acids) as the only soluble fraction. The amount of small-sized products is highest when treatment is performed at 170 and 220 °C, although the true nature of possible chemical modifications occurring at these temperatures (such as deamidation or breakdown of peptide bonds) remains to be assessed.

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