Date: 29.09.2024

Analysis report – SFE extracts

Description of sample(s)

Sample name: Extracts of tomato pomace and lyophilised pulp

Sample type: Supercritical CO_2 extract

Scientific name: *Solanum lycopersicum*

Manufacturing date: -

Volume/mass of sample(s): A total of 80 samples were taken for analysis (10 ± 0.1 mg)

Customer: -

Applied methods of sample preparation and analysis

Analysis date: 15.09.2024

Analyst: Dr. Deniz Can Köseoğlu

Sample preparation:

Carotenoids: Dilution in 40:60 chloroform:acetone at a concentration of 1.0 ± 0.1 mg mL^{-1}

Analyses and additional services:

Test supercritical fluid extractions (CO_2) and determination of extract yield (%)

Construction of the Overall Extraction Curve (OEC) for SFE

Gravimetric analysis of moisture content (%)

HPLC-DAD analysis of carotenoids (lycopene and β-carotene)

Checked and approved by:

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Professor Abdullah Akdoğan

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Description of laboratory sample preparation and analysis protocols

Supercritical CO_2 extraction (SFE)

Sample preparation of tomato pomace consisted of grinding on a Robot Coupe cutter with a blade speed of up to 28000 rpm to a particle diameter of ≤1 mm. Grinding of two types of tomato pulp (homegrown and store-purchased) to a powder was carried out in a similar manner after lyophilization of thin slices (Deep Dryer equipment) to a moisture content of ≤10%. For comparison purposes, sea buckthorn pomace was also prepared as an alternative source of carotenoids.

SFE extractions were carried out on a SuperEx SC-1000 system with an extraction vessel of 1 L able to withstand maximum operating pressure and temperature of 500 bar and 200 °C, respectively. The raw material was placed in the vessel using a steel loading basket equipped with 10 µm mesh filters. After sealing, the vessel was heated to the set temperature and pressurized. Upon reaching the specified temperature and pressure, extraction was carried out in a dynamic (flow-through) mode with a constant flow of CO_2. The outflow pressure after exiting the vessel was released by an automatic backpressure regulator, settling the extract in the separator. After the specified extraction time or the required solvent to raw material ratio was reached, the extract was collected using a built-in needle valve. Any residues stuck to the walls of the separator were washed off with food-grade ethanol, which was subsequently evaporated off in a rotary evaporator.

In total, 5 SFE extractions were performed with the parameters summarized in the table below.

#	Raw material	Extraction parameters				
		Pressure (bar)	Temperature (°C)	CO_2 flow (mL min^{-1}*)	Time (min)	S/M**
1	Pomace (tomato)	450	65	80	300	78
2	Pomace (tomato)	450	65	80	300	78
3	Pomace (sea buckthorn)	450	65	100	240	69
4	Pulp (homegrown)	450	65	100	300	98
5	Pulp (store-bought)	450	65	100	300	98

*Assuming density of approximately 0.890 g mL^{-1}; **The solvent/material mass ratio (*w/w*)

Traditional Soxhlet extraction

To compare the yield and concentration of lycopene and β-carotene with the SFE method, Soxhlet extractions were carried out using chloroform at an S/M ratio = 25 for 8 hours (or until the solution became colourless to the naked eye). The flask with the solvent was covered with foil to prevent photodegradation of carotenoids.

Sample preparation of carotenoids and analysis by liquid chromatography (HPLC-DAD)

A sample of the extract (10.0 ± 0.1 mg) was diluted in 40:60 chloroform:acetone at a concentration of 1.0 ± 0.1 mg ml^{-1} in an amber glass vial, shaken by hand (1 min) and vortexed (1 min). Then, an aliquot of 1 ml was passed through a membrane filter (PTFE, 0.22 µm) into a vial for HPLC-DAD analysis (injection volume 10 µl).

The following equipment and parameters were used to analyze the obtained solution: Shimadzu i-Series LC-2050-3D liquid chromatograph with diode array detector, C_{18} column (250 × 4.6 mm, 5 µm) at 30 °C, isocratic elution with isopropanol : acetonitrile : methanol : water (52 : 39 : 5 : 4 *v/v/v/v*, 1.2 mL min^{-1}) for 25 min. Peak area measurements were performed at wavelengths of 450 nm (β-carotene) and 472 nm (lycopene). Calibration was performed using a series of lycopene (20–120 ppm) and beta-carotene (20–500 ppm) standard solutions.

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General information – Moisture content in samples

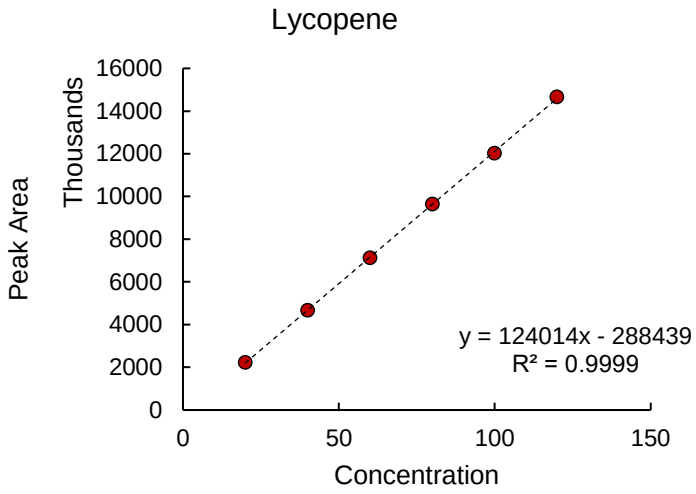
Sample type	Replicate measurements			Mean ± SD
	1	2	3	
Pomace (tomato)	5.77	6.12	5.76	5.88 ± 0.21
Pulp (homegrown)	10.73	10.68	10.12	10.51 ± 0.34
Pulp (store-bought)	10.51	9.37	10.10	9.99 ± 0.58
Pomato (sea buckthorn)	6.78	6.32	6.56	6.55 ± 0.23

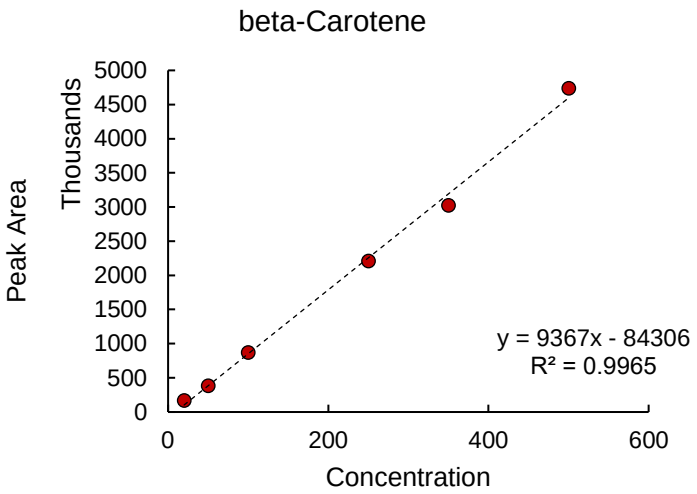
General information – Calibration data for carotenoids

Analyte name	CAS	Concentration (ppm)	RT* (min)	Peak area
Lycopene	502-65-8	20	12.685	2222882
		40	12.698	4665838
		60	12.774	7124012
		80	12.825	9653756
		100	12.873	12025681
		120	12.401	14662943
β-Carotene	7235-40-7	20	19.077	168168
		50	19.185	384063
		100	19.266	869748
		250	19.345	2207743
		350	19.422	3022197
		500	18.923	4738317

*Retention time

General information – Calibration graphs of carotenoids





Extracts of tomato pomace and lyophilised pulp *(Solanum lycopersicum)*, supercritical CO_2
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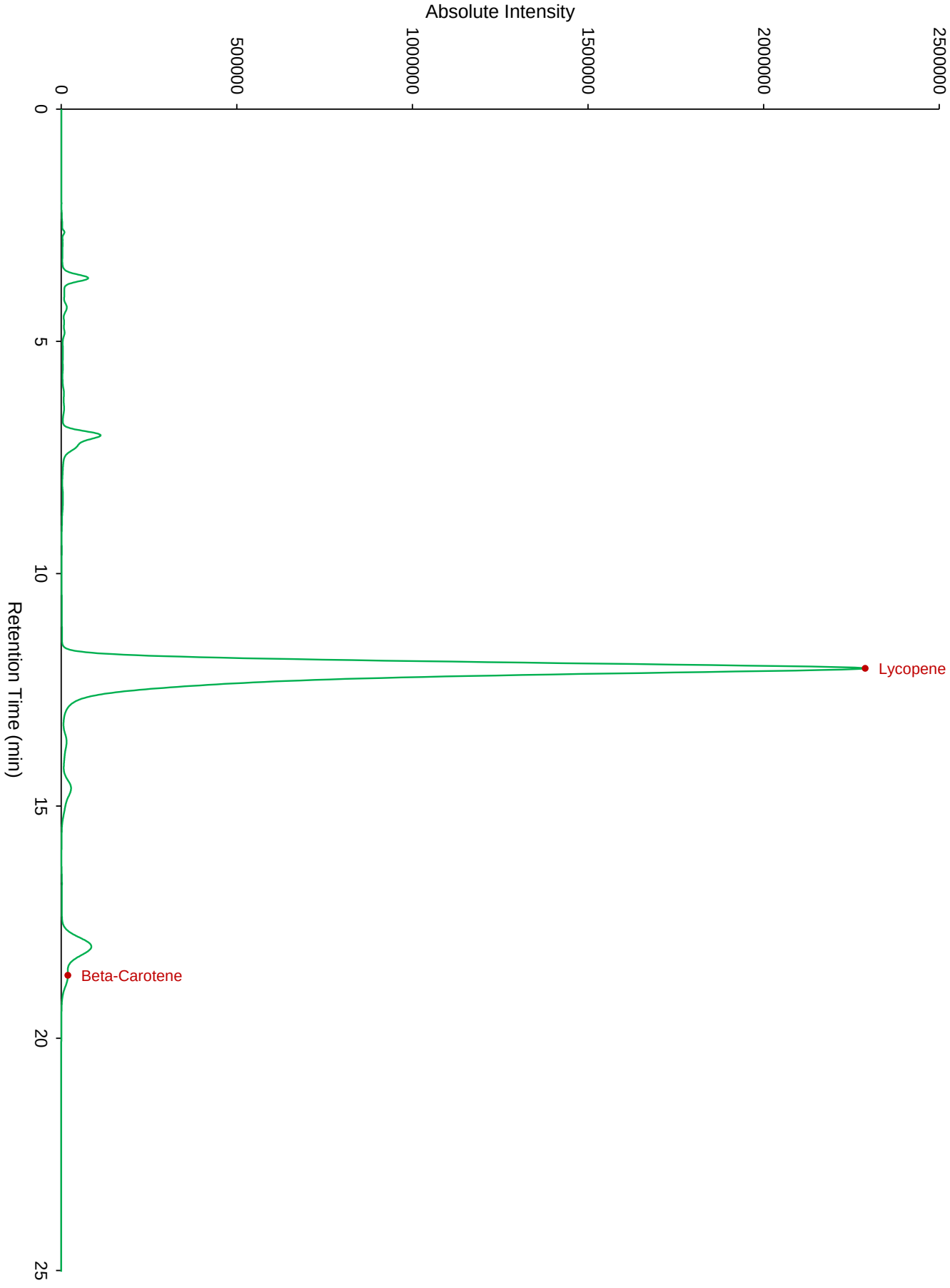
Results – Example chromatogram of carotenoids (HPLC-DAD)



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Results – Overall extraction curves (OECs) of yield and carotenoid content

In order to construct extraction curves and determine the optimal extraction time, samples of tomato and (for comparison purposes) sea buckthorn pomace extracts were collected from the separator every 30 minutes **during extractions 1 and 3**.

Results for tomato pomace

Time (min)	S/M	Overall yield*		Concentration (ppm)		Recovery (mg g^{-1} d.w.)	
		Grams	% dry weight	Lycopene	β-Carotene	Lycopene	β-Carotene
30	8	8.893	3.45	1365	1817	0.047	0.063
60	16	2.644	1.03	1760	3236	0.018	0.033
90	23	1.069	0.42	2062	4383	0.009	0.018
120	31	1.606	0.62	645	1641	0.004	0.010
150	39	2.087	0.81	1143	2259	0.009	0.018
180	47	0.347	0.13	1168	2725	0.002	0.004
210	55	0.361	0.14	1576	4570	0.002	0.006
240	62	0.294	0.11	889	2304	0.001	0.003
300	78	0.968	0.38	1673	4335	0.006	0.016
Residue**	-	5.789	2.25	-	-	0.031	0.041
TOTAL	**-**	**24.058**	**9.34**	-	-	**0.13**	**0.21**

*Based on a 273.560 g **wet** material loading; **Residue adhered to separator walls after extraction

Results for sea buckthorn pomace

Time (min)	S/M	Overall yield*		Concentration (ppm)		Recovery (mg g^{-1} d.w.)	
		Grams	% dry weight	Lycopene	β-Carotene	Lycopene	β-Carotene
30	9	10.88	3.78	513	2321	0.019	0.088
60	17	3.02	1.05	613	3496	0.006	0.037
90	26	1.22	0.43	663	4509	0.003	0.019
120	35	1.10	0.38	486	3288	0.002	0.013
150	43	1.86	0.65	697	5080	0.005	0.033
180	52	1.88	0.65	566	4022	0.004	0.026
240	69	1.43	0.50	323	2023	0.002	0.010
Residue**	-	2.74	0.95	-	-	-	-
TOTAL	**-**	**24.14**	**8.39**	-	-	**0.04**	**0.23**

*Based on a 307.860 g **wet** material loading

The results indicate an overall yield of approximately **9%** from tomato pulp at a S/M ratio of **70–80**, with a lycopene content of approximately **0.15%** (**5–6 times higher** than that of sea buckthorn pulp). Both pulps show **comparable beta-carotene** yields (2–3 mg g^{-1} dry weight), which exceed the lycopene yield by 2–5 times, respectively. The extraction rate slows down as the readily available fraction of oleoresin is extracted. For tomato pulp, more than **89.5% of the extract is recovered at S/M = 40** (approximately 150 min at 80 ml min^{-1} CO_2 flow), while for sea buckthorn the extraction does not slow down until S/M = 60 (probably due to the higher oil content in the raw material).

The extraction curve data are also summarized in the figure below.

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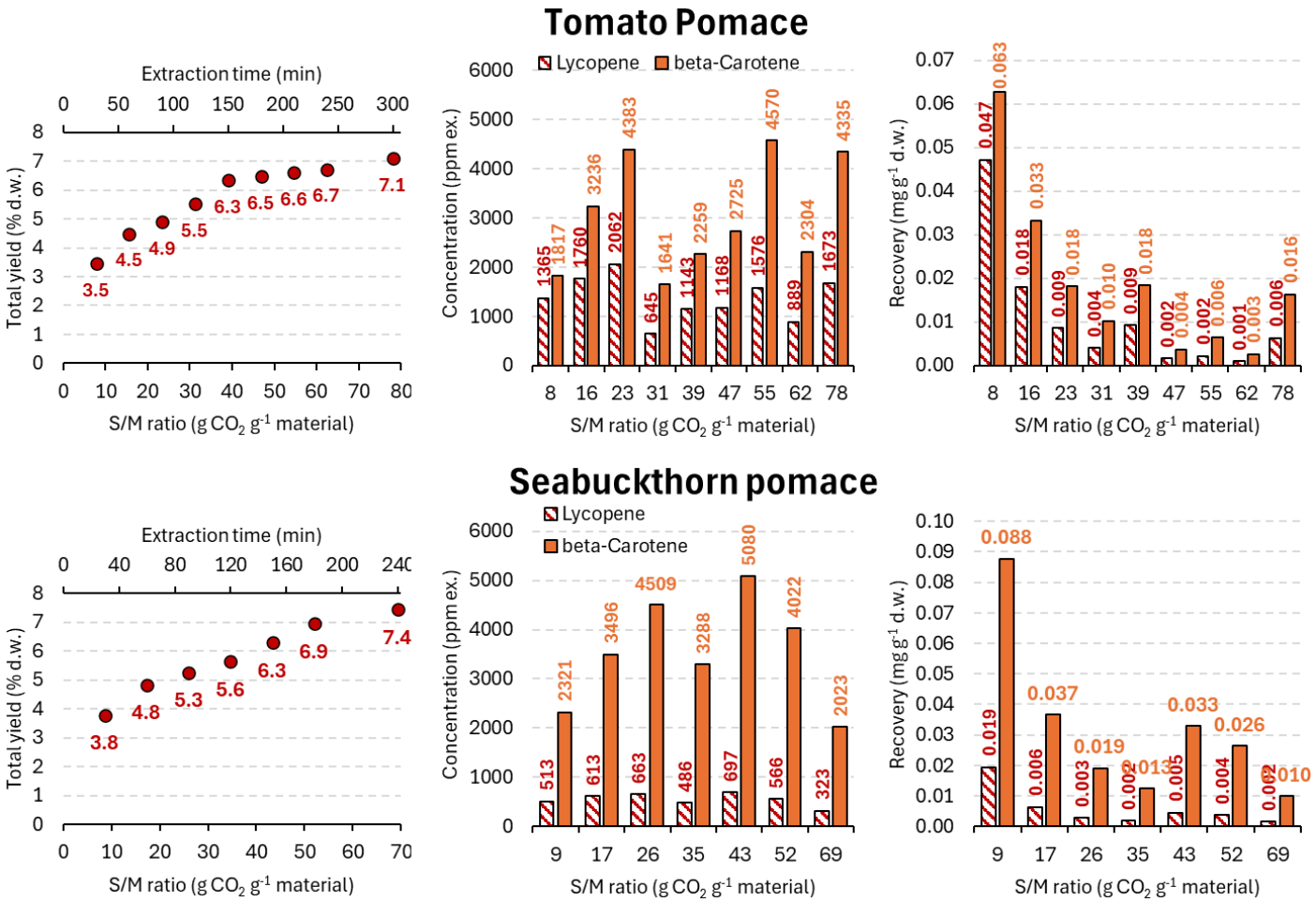
Results – Carotenoid content in tomato pulp via SFE and traditional Soxhlet extraction



Below are the results of SFE and (for comparison) Soxhlet extractions for **three types** of raw materials: tomato pomace and two types of lyophilized pulp (store-bought and organic garden-grown tomatoes).

General Information			Concentration (% extract)		Recovery (mg g^{-1} d.w.)	
Method	**Sample type**	**Yield (%)**	**Lycopene**	**β-Carotene**	**Lycopene**	**β-Carotene**
SFE	Pomace	7.78	0.16	0.25	0.13	0.20
	Pulp (garden grown)	7.20	5.61	0.39	4.04	0.28
	Pulp (store)	6.55	7.40	1.05	4.84	0.69
Soxhlet	Pomace	11.00	0.27	0.32	0.51	0.61
	Pomace (**post-SFE***)	7.64	0.24	0.14	0.19	0.10
	Pulp (garden grown)	8.17	7.69	1.06	6.29	0.87
	Pulp (store)	10.29	6.68	2.35	6.87	2.42

*Exhausted material from **extraction number 2**

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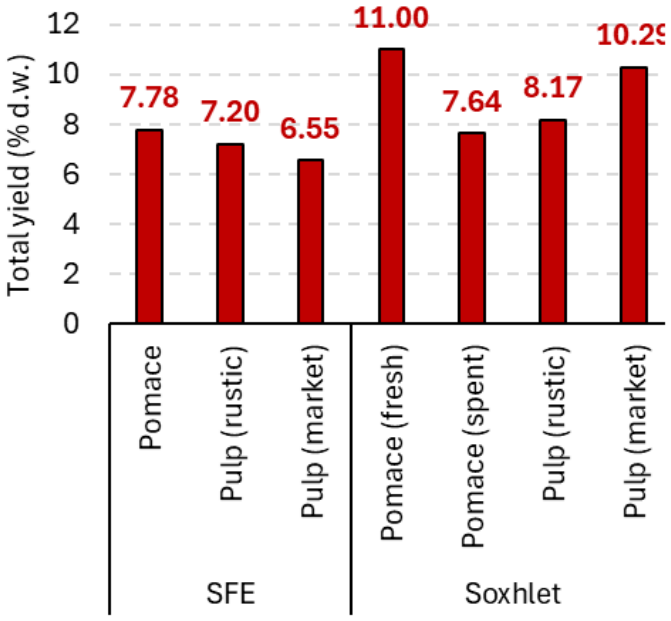

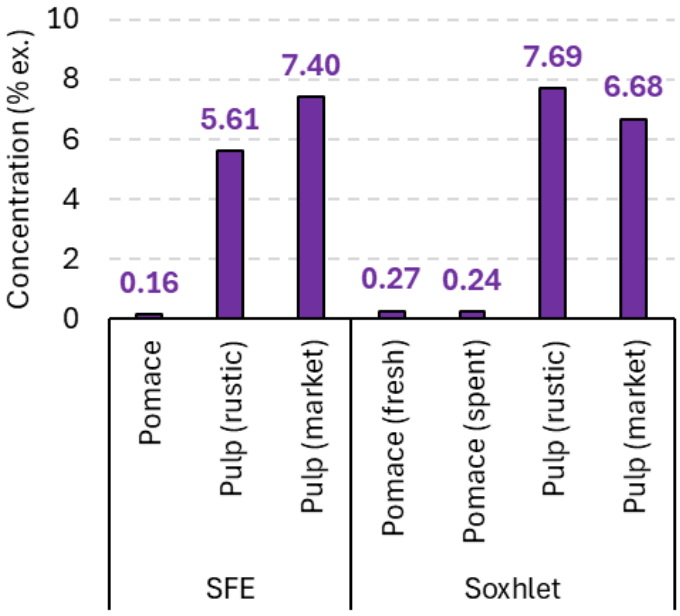

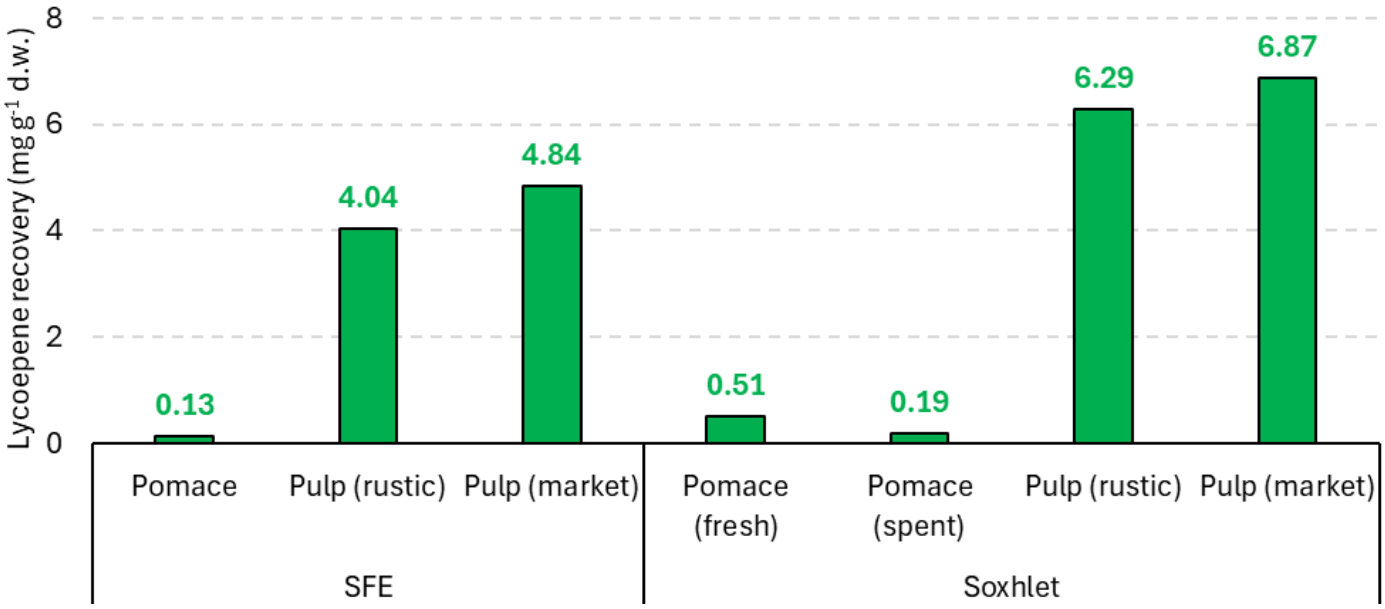

Main outcomes

- SFE of pomace allows to extract less than 1 mg of lycopene per gram of raw material, while the concentration in the extract **rarely exceeds 0.15%**. The remainder of the total yield (**7-9%** of dry weight) probably consists of **fatty acids** and other constituents of tomato seeds.
- The yield and concentration of lycopene in pulp extracts exceeds the values in the cake extract by 26–32 times. Thus, with SFE it is possible to achieve a concentration of **7–8% lycopene** (yield of **5–8 mg g^{-1}** of raw material).
- The yield of lycopene using a 5-hour SCE process is approximately 65–70% of the yield of exhaustive Soxhlet extraction (8 hours), regardless of the raw material. **This yield can be increased by process optimization.**

Freeze-dried tomato pulp can be recommended as a source of "biological" lycopene with the possibility of standardizing the concentration within 4–5% (the concentration most commonly encountered on the world market).

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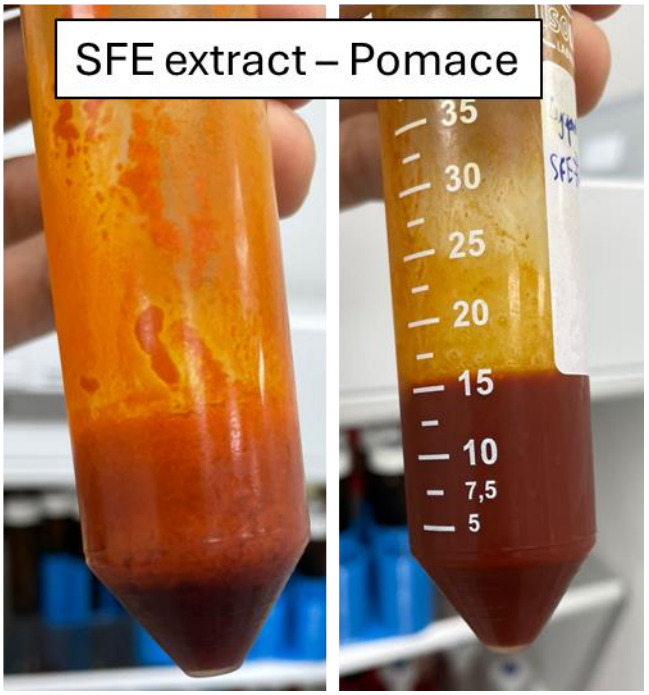
Extract and raw material photographs



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