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Optimizing the supercritical fluid extraction process of bioactive compounds from processed tomato skin by-products

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Abstract

A supercritical fluid extraction (SC-CO_2) was used to extract high-quality oil from tomato skin by-products. The effects of pressure and extraction time on oil yield was investigated in the study. Lycopene and β-carotene content as well as *p*-coumaric acid, ferulic acid, caffeic acid, chlorogenic acid, vanillic acid, epicatechin, naringenin, catechin, quercetin and luteolin were estimated. The highest oil yield of 79.00% was obtained after 80 min with a pressure of 550 bar. The resulting oleoresin in carotenoids with lycopene and β-carotene content respectively of 0.86 and 1.5 mg/100 g, this oleoresin was found to be the richest. Naringenin was the most abundant flavonoid identified with a maximum content in oleoresin extracted at 550 bar (84.04 mg/kg DW) followed by caffeic acid (26.60 mg/kg DW). A moderate radical scavenging potential was further observed. Overall, results highlight that pressure is a key parameter for the extraction bioactive oleoresin from tomato skin by-products.

Keywords: supercritical carbon dioxide extraction; tomato by-products; bioactive compounds.

Practical Application: Tomato processing waste as source of functional ingredients.

1 Introduction

Non-negligible amounts of various kinds of waste are generated in the vegetable processing industry. In particular, waste represents 2-3% of the raw material in the tomato processing industry, consisting mainly of peels (about 60%) and seeds (around 40%). Production companies generating this kind of waste due to disposal processes (Ćetković et al., 2012) are faced with the burden of additional costs. Several research studies have pointed out that tomato peels contain a high level of bioactive compounds including phenols, lycopene, ascorbic acid compared to their pulp and seeds (Vinha et al., 2014). Peels and seeds contribute particularly to 53% of the total amount of phenols, 52% of flavonoids, 48% of lycopene, 43% of ascorbic acid and 52% of the antioxidant activity occurring in tomatoes. These results demonstrate that the removal of peels and seeds during cooking or industrial processing leads to a significant loss of all the major antioxidants (Toor & Savage, 2005). Lycopene represents the most promising compound among tomato antioxidants (Story et al., 2010; Kaliora et al., 2006; Visioli et al., 2003). The request for lycopene as a functional ingredient for nutraceutical products or for functional food formulations has increased dramatically over the past decade due to its acknowledged healthy properties. Since there is still no favourable synthetic process allowing for its production, lycopene extraction from natural plant matrices continues to be necessary. Currently, the most common methods for extracting lycopene from tomatoes or tomato processing residues are based on the use of organic solvent solvents (Machmudah et al., 2012). After the extraction procedure, lycopene is subjected to a long and expensive purification process that does not lead, however, to a high degree of purity. To overcome these drawbacks, new solvent-free and environmental friendly compatible extractions support the use of supercritical fluids such as carbon dioxide (CO_2) in order to obtain toxic-free solvent products (Amaral et al., 2018a, b). This technology is configured as an interesting alternative for the food industry due to the increased nutrient retention (Amaral et al., 2017).

Extraction by means of supercritical fluids is an alternative method to the traditional processes of fractional distillation, steam distillation, solvent extraction. Supercritical fluid extraction is based on the principle of a direct correlation between solvent power and density. In addition, the critical temperature of CO_2 is close to the ambient temperature, so it is also capable of treating thermolabile substances (Nahar & Sarker, 2012).

The aim of the present study was to optimize an extraction process of carotenoids and, in particular, of lycopene and β-carotene from tomato skins through the use of carbon dioxide as an extraction fluid in a supercritical state. Antioxidant activity and bioactive compound content were further determined.

2 Materials and methods

2.1 Chemicals

Standards of lycopene, β-carotene, *p*-coumaric acid, ferulic acid, caffeic acid, chlorogenic acid, vanillic acid, epicatechin, naringenin, catechin, quercetin, and luteolin were obtained from Extrasynthese (Genay-France). ABTS [2,2'-azinobis

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(3-ethylbenzothiazoline-6-sulfonate)], 2,2-diphenyl-1-picryl hydrazyl (DPPH), 6-Hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid, 97% (Trolox) were purchased from Sigma-Aldrich Chem. Co. (Milan, Italy). All solvents and other reagents used were of HPLC grade and were purchased from Carlo Erba Reagents (Milan, Italy).

2.2 Materials

The tomato cultivars used in the current research were of the San Marzano type, named "Docet", "Ercole", "Fuzzer", "Herdon", "Komolix", "Player" and "Ulisse" due to their oblong shape, which are grown as field crops in the Southern Italian Regions of Campania and Apulia. These cultivars were chosen as they are commonly used in the tomato processing industry, where their separation is extremely difficult since they continuously reach the technological plant from different tomato plantations. Although the different cultivars are crushed together, this is not an influential factor for the present research which focuses on the processing conditions. Tomato lots were, in fact, processed with a treatment at 65-80 °C. Tomatoes were chopped beforehand to separate the pulp from seeds and peels and then floatation was conducted to separate the latter two. Peels were exposed to sunlight (12 h) to evaporate the majority of the water and oven-heating was applied at 40-50 °C (24 h) to evaporate residual humidity. Tomato skins were dehydrated to constant weight using a Christ ALPHA 2-4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Dried skins were homogenized in a laboratory ultra-centrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through 35 mesh (350 μm sieves).

2.3 Supercritical-CO_2 extraction (SC-CO_2)

Super Critical-CO_2 extraction (SC-CO_2) was carried out using a laboratory apparatus (Speed SFE system, Applied Separations, Allentown, PA, USA) fitted with a 25 mL stainless-steel extraction vessel (ø = 1 cm; h = 25 cm). An aliquot of 18 g of raw material, packed into the vessel, was used for extraction which lasted between 20-80 min. Temperature, carbon dioxide flow rate and extraction time were kept constant respectively at 60 °C and 2 mL/min, while pressure was varied between 350, 450 and 550 bar. The oil yield percentage was calculated. The extracted oils were stored under an enriched CO_2 atmosphere and protected from light at -20 °C until further analyses. Each extraction was repeated at least three times.

2.4 Spectrophotometric determination of carotenoids in tomato by-products oil

As Fish et al. (2002). report, lycopene and β-carotene contents in tomato by-products were determined using an UV-Vis spectrophotometer (Agilent 8453 Technologies, Italy). To minimize the interference from other carotenoids, the concentration of lycopene was calculated at λ=503 nm using the molar extinction coefficient ε= 17.2 x 10^4 M^{-1} cm^{-1}. β-carotene absorbance was measured at λ= 450 nm and quantification was carried out using a standard curve. All analyses were made in triplicate and results were expressed as mean ± standard deviation (SD).

2.5 RP-HPLC/DAD determination of bioactive compounds

p-Coumaric acid, ferulic acid, caffeic acid, chlorogenic acid, vanillic acid, luteolin, epicatechin, naringenin, catechin, and quercetin were selected as maker and quantified by HPLC in oleoresins extracted from tomato peels by supercritical fluid at a different pressure. A Knauer (Asi Advanced Scientific Instruments, Berlin, Germany) HPLC system, equipped with two pumps Smartiline Pump 1000, a Rheodyne injection valve (20 μL) and a photodiode array detector UV/VIS with a semi-microcell, was used. Compounds were separated on a TSK gel ODS-100 V (TOSOH Bioscience, Germany) column (250 mm ×3.0 I.D.; 3 μm) at 30 °C and the flow rate used was 0.5 mL min^{-1}.

The mobile phase consisted of water/formic acid (99.9:0.1, v/v; solvent A) and acetonitrile/formic acid (99.9:0.1, v/v; solvent B) and the gradient profile was as follows: 0.01-20.00 min 5% B isocratic; 20.01-50.00 min, 5-40% B; 50.01-55.00 min, 40-95% B; 55.01-60.00 min 95% B isocratic.

2.6 Evaluation of radical scavenging activity of SC-CO_2 oils

The radical scavenging potential of SC-CO_2 was investigated by means of DPPH and ABTS radical assays. The DPPH test was assessed following the method previously reported (Brand-Williams et al., 1995). An aliquot of 2.5 mL of 0.06 mM DPPH· solution was added to 20 μL of oleoresin. Absorbances at t_0 and t_5 were measured at λ= 515 nm using a UV-Vis Agilent 8453 spectrophotometer (Agilent Technologies, Italy). Trolox was used as a standard antioxidant and tomato skin oil activity was expressed in mM of Trolox equivalents (TE).

The ABTS (2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical test was carried out as described by Re et al. (1999). The radical was generated by mixing 7 mM of ABTS and $K_2S_2O_8$ 140 mM and followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted (1:80) with ethanol to give an absorbance of 0.70 at λ = 734 nm. An aliquot of 50 μL of sample extract was added to 950 mL of ABTS solution. Trolox was used as a control and tomato skin oil activity was expressed in mM of TE.

2.7 Statistical analysis

Results were expressed as mean ± SD of three replicates. All data were analyzed using one-way analysis of variance (ANOVA) with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) statistical software. Significant differences were calculated according to Duncan's multiple range tests. Differences at $P<0.05$ were considered to be statistically significant while at $P<0.01$ were considered to be highly significant. Studies of the *Pearson's* correlation coefficient (*r*) and linear regression, assessment of repeatability, calculation of average and relative standard deviation were performed using Microsoft Excel 2010 software. Principal Component Analysis (PCA) were applied through the use of SPSS software for Windows, version 15.0 (Chicago, IL, USA).

3 Results and discussion

3.1 Effect of extraction parameters on carotenoids

This research investigated the effect of pressure and extraction time on the recovery of lycopene, β-carotene and phenolic compounds from tomato skin by-products. In the case of extraction with supercritical CO_2, temperature appears to be one of the most important parameters for yielding the target compound. In the specific case of lycopene extraction, different research studies indicate temperature as a parameter of fundamental importance in order to increase the yield of the extract (Reverchon & De Marco, 2006; Egydio et al., 2010; Konar et al., 2012; Yi et al., 2009). At the temperature of 80 °C, lycopene solubility in supercritical CO_2 showed a decrease, probably due to its thermal degradation. For this reason, the decision to operate at 60 °C was made. Lycopene recovery is reported to have increased slightly when the temperature rose from 40 to 60 °C and persisted almost the same before reaching 80 °C. Pressure effects on lycopene extraction are similar to temperature ones. Literature data show that an increase in supercritical carbon dioxide pressure causes an increase in the amount of extracted lycopene (Reverchon & De Marco, 2006; Yi et al., 2009; Nobre et al., 2012). The level of lycopene at a higher pressure is due to the increased density of SC-CO_2 as a result of a greater interaction between the lycopene molecule and the supercritical carbon dioxide (Yi et al., 2009; Baysal et al., 2000). The use of different pressure values did not result in a difference in extraction yield with values of 3.60, 3.68 and 4.21% after 60 minutes for pressure of 350, 450 and 550 bar respectively (Table 1, Figure 1).

After 80 minutes, the achievement of a plateau with the maximum yield (0.79 g) at 550 bar was observed.

Table 1. Effect of extraction time on extraction yield at different pressure.

Extraction time	Extraction Yield (g)		
	A	B	C
	350 Bar	450 Bar	550 Bar
0	0.00	0.00	0.00
20	0.37	0.57	0.67
40	0.60	0.64	0.74
60	0.65	0.66	0.76
80	0.67	0.68	0.79

Data are reported as mean ± standard deviation (n = 3).

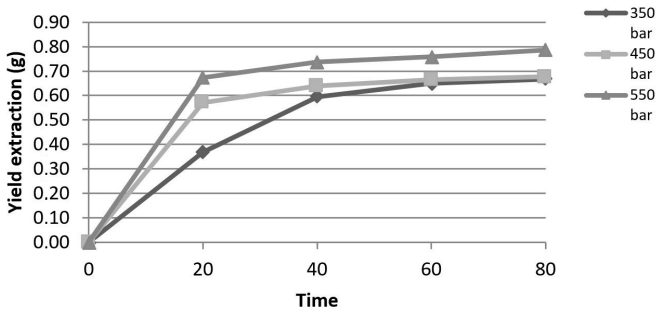

Figure 1. Extraction rate curves for tomato skin oil at 60 °C as a function of extraction pressure.

According to our data, Kehili et al. (2017) reported that the recovery of oleoresin seems to increase with pressure values respectively of 4.86 and 5.56% at 400 and 500 bar and a temperature value of 60 °C with an extraction time of 105 minutes. Evolution of the pressure effect on β-carotene and lycopene extraction (Table 2) showed that no significant differences occurred at 350 and 450 bar. Nevertheless, the application of 550 bar resulted in an increase of carotenoid content with values respectively of 0.86 and 1.5 mg/100 g FW.

These data diverge from those reported by Kehili et al. (2017), who pointed out a correlation between pressure and β-carotene without any effect on lycopene. Nobre et al. (2012) investigated the pressure and temperature effects on lycopene extraction. Carotenoid recovery increases with pressure and rises drastically when pressure increases from 200 to 300 bar, which is expected due to the increase in the solvent density with pressure (Machmudah et al., 2012). According to some authors, the rise in temperature at constant pressure determines an increase in the vapour pressure of lycopene (Reverchon & De Marco, 2006; Egydio et al., 2010; Sabio et al., 2003; Saldana et al., 2002; Rozzi et al., 2002) which, in turn, determines greater solubility in SC-CO_2 (Reverchon & De Marco, 2006; Egydio et al., 2010; Sabio et al., 2003). Ultimately, we can claim that lycopene increase or decrease is mainly dependent on the interaction between pressure and temperature. In fact, the higher density of the SC-CO2 that occurs with the increase in pressure and consequently the greater extraction of lycopene, determines a defence of the solvent power following an increase in temperature. The density of SC-CO2 can decrease with an increase in temperature while keeping the pressure constant, but the density decrease becomes smaller at high pressures (Saldana et al., 2002; Rozzi et al., 2002). Genival et al. (2008) found that an increase in temperature at high pressures led to an increase in lycopene yield, while the lycopene yield decreased with increasing temperature at lower pressures. Lycopene solubility in SC-CO_2 and consequently its retention increase when temperature and pressure parameters are both increased (De la Fuente et al., 2006; Topal et al., 2006).

3.2 The effect of extraction parameters on phenolics

Analysis of the influence of applying a different pressure on phenolic acids generally revealed that their extraction is strictly connected to the pressure applied. An increase in pressure from 350 to 550 bar resulted, in fact, in a 1.56-time high extraction yield for all except for caffeic acid. A 1.94-time high extraction performance was obtained by using the application of 550 bar in comparison to lower pressure on ferulic acid. A similar trend was observed also for *p*-coumaric acid. No significant differences were recorded for pressure on chlorogenic acid content (Table 3).

Table 2. Comparative β-carotene and lycopene content in oils obtained at different pressure. Data are expressed as mg/100 g DW.

	Lycopen	β-Caroten
A	0.50 ± 0.05	1.3 ± 0.1
B	0.62 ± 0.04	1.3 ± 0.4
C	0.86 ± 0.06	1.5 ± 0.4

Data are reported as mean ± standard deviation (n = 3).

Table 3. Comparative phenolic profile in oils obtained at different pressure. Data are expressed as mg/kg DW.

	Vanillic acid	*Caffeic acid*	*Ferulic acid*	*p-Coumaric acid*	*Chlorogenic acid*	*Quercetin*	*Epicatechin*	*Catechin*	*Naringenin*	*Luteolin*
A	1.74 ± 0.02	16.32 ± 0.08	4.06 ± 0.02	5.52 ± 0.24	4.02 ± 0.08	6.71 ± 0.13	1.08 ± 0.05	3.12 ± 0.04	62.33 ± 0.15	0.24 ± 0.05
B	1.61 ± 0.09	26.8 ± 0.04	5.05 ± 0.07	6.55 ± 0.06	3.49 ± 0.57	6.58 ± 0.05	2.37 ± 0.06	3.57 ± 0.03	77.62 ± 0.08	0.16 ± 0.03
C	2.71 ± 0.05	26.60 ± 0.05	7.88 ± 0.04	10.05 ± 0.06	4.05 ± 0.07	6.85 ± 0.06	2.83 ± 0.03	4.02 ± 0.05	84.04 ± 0.10	0.27 ± 0.06

Data are reported as mean ± standard deviation (n = 3).

High content of chlorogenic acid has been also found in tomato processing waste by Nour et al. (2018).The effect of applying pressure on phenolic extraction was monitored using quercetin, epicatechin, catechin, naringenin and luteolin s markers. Data analysis highlighted how the increase in pressure led to a rise in the extraction performance of both epicatechin (respectively 1.08 mg/kg DW *vs* 2.83 mg/kg DW at 350 and 550 bar) and catechin (respectively 3.12 mg/kg DW *vs* 4.22 mg/kg DW at 350 and 550 bar). Among the flavonoids selected, only naringenin extraction seems to be conditioned by pressure (1.33-time higher at 550 bar). Tomato phenolic content varies considerably between skin and pulp; tomato skin contains a higher concentration of phenolic compounds. George et al. (2004) studied the antioxidant fraction in 12 tomato genotypes, and reported that tomato skin contains significant amounts of polyphenols and ascorbic acid with a phenolic content ranging from 10.4 to 40.0 catechin mg/100 g FW. A similar observation was made by Toor & Savage (2005) who reported a total phenolic content of 29.1 mg GAE/100 g FW. Kalogeropoulos et al. (2012) investigated the HPLC profile of the whole tomato and its by-products (pomace constituted by skin and seeds) and underlined how hydroxycinnamic acids predominated in the whole fruit, whereas flavonoids prevail in the pomace rather than glycosylated compounds. According to our results, naringenin was the main abundant compound (328.6 mg/kg DW). The high flavonoid content (98%) of tomato skin has been previously reported also by Stewart et al. (2000) who analysed 20 different tomato varieties.

3.3 Radical scavenging potential of tomato oleoresin

The antioxidant potential was measured by using the two different radical scavenging tests DPPH and ABTS. Samples did not show any difference due to the method applied and an increase in the TEAC value depending on the pressure with a high value at 550 bar (respectively 0.08 and 0.11 mM Trolox for DPPH and ABTS tests) (Table 4).

It is worth noting that no correlations were found between the radical scavenging potential and the content of phenols or carotenoids. The activity is, in fact, no longer high in the extract obtained at 550 bar and characterized by the highest content of bioactive compounds. This evidence is consistent with that reported by Kehili et al. (2017) who observed the highest DPPH radical scavenging activity in oleoresin samples extracted under relatively mild conditions of pressure and temperature (300 bar and 50 °C). This evidence is likely the consequence of a greater conservation of bioactive compounds when mild operating conditions are used as in our study. The radical scavenging potential of tomato skin product after a different drying process was demonstrated. Samples showed a percentage of DPPH radical ranging from 0.19 to 7.63 for 1 g of DW extract (Albanese et al., 2014).

Table 4. Radical scavenging potential of oils obtained at different pressure.

	DPPH	ABTS
	(mM Trolox)	(mM Trolox)
A	0.06 ± 0.006	0.09 ± 0.04
B	0.07 ± 0.002	0.10 ± 0.02
C	0.08 ± 0.006	0.11 ± 0.01

Data are reported as mean ± standard deviation (n = 3).

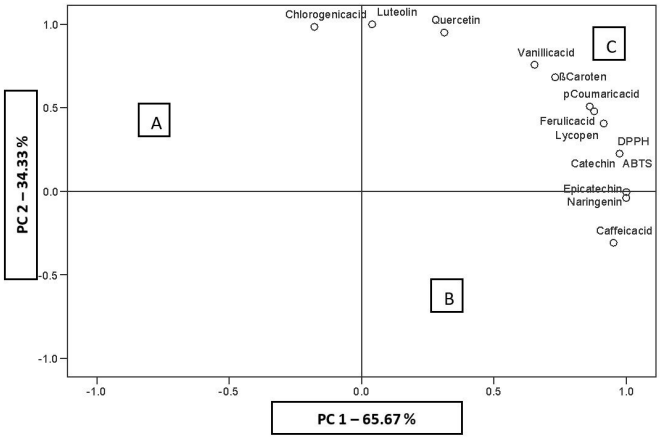

Figure 2. Two-dimensional map representation of PCA distribution of variables and distribution of samples. (A) 350 bar; (B) 450 bar; (C) 550 bar.

3.4 Multivariate analysis

PCA was performed with all parameters obtained from the determination of antioxidant activity through DPPH, ABTS and individual phenolic compounds quantified by HPLC-DAD. After the statistical analysis of data, the PCA model retained two principal components (PC), which explained 100% of the total variability (PC1 explains about 65,67% and PC2 about 34.33%). The loading plots of the first two principal components are shown in Figure 2.

PC1 correlated positively with lycopen and β-carotene, vanillic acid, caffeic acid, ferulic acid,p-coumaric acid, epicatechin, catechin, naringenin, DPPH and ABTS. Principal Component 1 (PC1) was inversely correlated with chlorogenic acid. PCA showed that tomato skin samples extracted by SC-CO_2 at 550 bar and 80 min were characterized by a higher content of phenolic compounds with great antioxidant activity.

4 Conclusion

The effect of different pressures on carotenoids and phenols SC-CO_2 extraction from tomato skin by-products was investigated. If the extraction conditions exceed optimal operating conditions, this may result in a decrease in the recovery of phytochemicals or in the bioactivity of the extract. It is interesting to note that in applying maximum pressure, there is a double effect, namely a major phytochemicals recovery was observed together with a negative impact on antioxidant activity. These results prove that pressure can be considered a key parameter for the SC-CO_2 extraction of bioactive oleoresin from tomato skin by-products.

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