SUPERCRITICAL FLUID EXTRACTION OF ANTIOXIDANTS FROM PEPPER (*Capsicum annuum* L.)

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Pepper (*Capsicum annuum* L.) is an excellent source of natural antioxidants, including carotenoids and phenolic compounds which role appears relevant to prevent some human diseases and to be used in food industry to prevent the decay by peroxidation of lipid bearing foods. The recovery of these compounds requires however the use of mild technologies, such as supercritical fluid extraction (SFE), in order to preserve their antioxidant power. On this basis, a research has been developed with the aim of testing the technical feasibility of a SFE process for the fractionated recovery of carotenoids and phenolic compounds from pepper. The paper reports the experimental protocol followed, together with the kinetics of the extractions, knowledge of which allows the optimisation of working parameters and the determination of the process yields. On the basis of the results obtained a 2-step SFE process is tentatively proposed. Such a process involves a preliminary $ScCO_2$ extraction of carotenoids, followed by the recovery of polyphenols by means of ethanol coupled with $ScCO_2$.

INTRODUCTION

Fresh pepper (*Capsicum annuum* L.) contains a large number of carotenoidic and phenolic antioxidants which role appears relevant to prevent some human diseases [1-3]. Among carotenoids, which level increases extensively during maturation, the most representative are α- and β-carotene, β-cryptoxanthin, capsanthin, lutein and zeaxanthin [4-6], while phenolic fraction includes mainly phenolic acids (cinnamic acid derivatives and hydroxy-substituted benzoic acids) and flavonoids (e.g. quercetin and luteolin)[5, 7, 8].

Due to their antioxidant power, these compounds can be profitably extracted and used in the food industry to prevent the decay by peroxidation of lipid bearing foods [9, 10]. The recovery of these compounds requires however the use of mild extraction technologies, in order to preserve their antioxidant power. In such a contest, the use of supercritical fluid extraction (SFE) is of interest, allowing the recovery of bioactive extracts, whose commercial value could remunerate the process.

While some experimental papers are available on the SFE of antioxidants from pepper, only limited information exists on their fractionated recovery and on the kinetics of the process [6, 11, 12]. In such a contest, a mathematical model able to describe the kinetics of a SFE process and allowing the optimisation of working parameters was applied.

MATERIALS AND METHODS

Fresh peppers from Italian cv Tolomeo (CRP 4993) were supplied by the Dipartimento di Agronomia Ambientale e Produzioni Vegetali of the University of Padova. Fruits were washed, cut, lyophilised, ground to a particle size of 0.37 mm and stored at –20 °C until use.

Extractions were performed using a commercial pilot plant apparatus (Sitec, Maur, Switzerland), which allows the recovery and the subsequent recycling of the solvent, with a minimal loss of CO_2. A supplementary pump provides the addition of a co-solvent to the CO_2 stream, when desired. The apparatus is detailed in a previous paper [13].

SFE of carotenoidic fraction was performed using 280 g of lyophilised matter per each run, with a working pressure (P) of 40 and 70 MPa and a temperature (T) of 40 and 60 °C. The extraction time was fixed in 180 min, while the rate of $ScCO_2$ was 10 $kg \cdot h^{-1}$. Extraction yields were determined gravimetrically, while the carotenoidic concentration in both extracts and lyophilised pepper was spectrophotometrically determined and expressed as β-carotene [14]. Extraction of carotenoids by percolation with n-hexane for 240 min was also performed using a Soxhlet apparatus.

The recovery of the phenolic fraction was carried out using the samples resulted from the SFE of carotenoids and left into the extractor. As a preliminary experimentation showed that pure supercritical $ScCO_2$ is a poor solvent for these polar compounds and that water is not suitable as co-solvent being unable to generate a homogeneous phase with $ScCO_2$, ethanol (EtOH) was used coupled to $ScCO_2$ (50 %, w/w) and pure. Extractions were carried out using a P of 30 and 50 MPa and a T of 50 and 80 °C. Because of different $EtOH/CO_2$ ratios used, variable solvent flow rates were employed, while the extraction time was fixed in a maximum of 120 min. Extraction yields were determined gravimetrically, while the polyphenolic concentration in both extracts and lyophilised pepper was spectrophotometrically determined and expressed as chlorogenic acid [15] Extraction of polyphenols by percolation with pure EtOH for 180 min was also performed using a Soxhlet apparatus.

RESULTS AND DISCUSSION

Extraction of carotenoids

To optimise the extraction parameters, a kinetic approach developed during a previous research was adopted [16]. This approach, based on the Fick's law, uses the following exponential equation to describe the evolution of extracted carotenoids over time (t):

$$Ce = H^* \cdot [Cs] \cdot (1 - e^{-k \cdot t}) \quad (1)$$

where: Ce = grams of carotenoids extracted at a random time t per gram of biomass submitted to the extraction (adimensional); H* = adimensional constant, ranging from 0 to 1, related to the equilibrium constant H (H* = H/(H+1)); [Cs] = carotenoidic concentration in starting lyophilised material (adimensional); k = kinetic constant (s^{-1}).

The extraction rate (R) calculated as first derivative of the equation 1:

$$R = dCe/dt = H^* \cdot [Cs] \cdot k \cdot e^{-k \cdot t} \quad (2)$$

reaches its maximum value (R_{max}) at the beginning of extraction, when t is close to 0:

$$R_{max} = k \cdot H^* \cdot [Cs] \quad (3)$$

According to Yu et al. [17], the value of R_{max} (s^{-1}) was assumed as an index to evaluate the efficiency of the extraction system *versus* the carotenoidic fraction. In particular, while the constant k (s^{-1}) gives information on the kinetics of the process, the adimensional product $H^* \cdot [Cs]$, representing the asymptotic value of the extraction curve when $t \rightarrow \infty$, measures the maximum amount of carotenoids extractable in the working conditions adopted. In presence of a highly efficient extraction process, H* tends to 1 and therefore the maximum amount of carotenoids extractable per unit of biomass is equal to their concentration in the starting material. The identification of the best values to be assigned to the equation parameters k and $H^* \cdot [Cs]$ was carried out by a commercially available statistical program (BURENL©) described in a previous paper [18].

Table 1: Amount (mg) of carotenoids extracted per kg of lyophilised pepper as a function of extraction time and working conditions (pressure and temperature) adopted.

Run time (min)	40 °C --- 40 MPa	40 °C --- 70 MPa	60 °C --- 40 MPa	60 °C --- 70 MPa	Soxhlet extraction (n-hexane)
5	304	506	463	565	--
10	525	721	665	814	401
20	748	856	804	949	598
30	834	941	904	981	744
40	879	951	914	997	793
60	833	1005	1007	1003	879
90	1013	1024	1016	1017	940
120	1011	1009	1010	1012	982
180	1017	1018	1021	1022	997
240	--	--	--		1017

Table 1 reports the amount of carotenoids extracted from lyophilised pepper as a function of run time and working conditions adopted, while Table 2 reports the values assumed by R_{max} and by the functional parameters k and $H^* \cdot [Cs]$. On the basis of the data tabled, the following remarks can be done: a) the increase of P and T, at the least in the experimented range, does not influence significantly the total amount of carotenoids extractable at equilibrium (extraction time = ∞), as testified by the values assumed by the equation parameter $H^* \cdot [Co]$. This product, which represents the mgs of carotenoids extracted from 1 g of lyophilised pepper when the equilibrium is reached, assumes in fact, for all SFE runs, values close to the concentration of carotenoids in starting material (1016 mg/kg) and to that obtained when percolation with n-hexane is used; b) P highly affects the kinetics of extraction, as confirmed by the values of constant k when working at the same T; c) also T affects the kinetics of SFE, as testified by the value that k assumes when working at the same P, but such influence is lower; d) the binomial pressure-temperature is however more crucial than T and P alone in determining the kinetics of the SFE process. In fact the value of k increases of 2.3 times passing from the mildest (40 °C and 40 MPa) to the hardest (60 °C

and 70 MPa) working conditions; e) in all conditions adopted, the extraction with $ScCO_2$ resulted faster than that carried out by percolation with n-hexane, as testified by the values assumed by k and/or R_{max}. For example, when working at the hardest SFE conditions, the value of R_{max} is about 3.5 times that related to the Soxhlet extraction; f) the values of k reported in Table 2 are relatively close to those determined in the SFE of carotenoids from tomato and reported in a previous paper [19]. This consideration, together with the high values assumed by the square of the correlation coefficient, testifies the suitability of the hypotheses introduced and gives a measure of the validity of the mathematical model proposed.

Table 2: Extraction of carotenoids from pepper. Values assumed by R_{max} and by the equation parameters k and H*·[Cs] as a function of working conditions adopted: T = temperature; P = pressure; r = correlation coefficient.

T (°C)	P (MPa)	$R_{max}\cdot10^6$ (s^{-1})	$k\cdot10^4$ (s^{-1})	$H^*\cdot[Cs]\cdot10^3$ (adimensional)	r^2
40	40	1.16	11.98	0.97	0.95
40	70	2.11	21.32	0.99	0.97
60	40	1.75	17.76	0.99	0.95
60	70	2.74	27.26	1.01	0.98
Soxhlet extraction		0.75	7.69	0.98	0.98

Note: for all runs, the confidence interval (p = 0.05) resulted ≤ 0.01.

Extraction of polyphenols

The same approach above reported to described the kinetics of SFE of carotenoids was adopted to optimise the working parameters related to the extraction of polyphenols from pepper by means of pure EtOH or $ScCO_2$ coupled with EtOH (1:1, w/w). In particular, the following equation was used to determine the maximum extraction rate (R'_{max}):

$$R'_{max} = k'\cdot H'^*\cdot[Ps] \quad (4)$$

where: k' = kinetic constant (s^{-1}); H'* = adimensional constant, ranging from 0 to 1, related to the equilibrium constant H'; [Ps] = polyphenolic concentration in starting lyophilised material (adimensional).

Table 3 reports the polyphenols extracted as a function of run time and working conditions adopted, while in Table 4 are reported the values assumed by R'_{max} and by the functional parameters k' and H'*·[Ps]. On the basis of the data tabled, the following remarks can be done: a) as previously observed working with potato and tomato [19, 20], pure $ScCO_2$ is confirmed to be a poor solvent for polyphenols, even when high values of density are adopted; b) EtOH is a suitable co-solvent to pilot the polarity of solvent phase, but a high percentage (≥ al 50 %) needs to obtain high extraction yields; c) when working at the same EtOH/$ScCO_2$ ratio (1:1) and P (30 MPa), T highly affects the extraction process, with particular reference to the total amount of extractable polyphenols. In fact, while the kinetic constant k' does not change markedly when T increases from 50 to 80 °C, the equation parameter H'*·[Ps] almost triplicates passing from 50 °C to 80 °C. This means that such temperature increase determines the solubilisation of phenolic compounds otherwise not

collectable; e) to obtain the extraction of the whole phenolic fraction, pure EtOH at 80 °C and 30 or 50 MPa needs. In such conditions the extraction process is two times faster than that performed using the Soxhlet apparatus, as testify by the values assumed by the kinetic constant k'; f) when working at 80 °C and 30 MPa, if EtOH decreases from 100 % to 50 %, only a little decrease in the extraction yield and kinetics is paid.

Table 3: Amount (mg) of polyphenols extracted per kg of lyophilised pepper as a function of extraction time and working conditions (EtOH/CO_2 ratio, pressure and temperature).

Run time (min)	EtOH 50 % - CO_2 50 %		EtOH 100 %		Soxhlet extraction (EtOH)
	30 MPa		30 MPa	50 MPa	
	50 °C	80 °C	80 °C	80 °C	
5	--	--	6098	9302	--
10	3111	9209	12258	15462	6314
20	6283	15369	21652	26334	15400
30	6252	22946	27812	29414	--
40	8655	24702	28428	30646	23131
60	9702	27751	30831	30923	26519
90	9856	27720	30738	30728	29291
120	9764	27689	--	--	30246
180	--	--	--	--	30430

Table 4: Extraction of polyphenols from pepper. Values assumed by R'_{max} and by the equation parameters k' and H'*·[Ps] as a function of working conditions adopted: T = temperature; P = pressure; r = correlation coefficient.

EtOH (%)	$ScCO_2$ (%)	T (°C)	P (MPa)	$R'_{max}\cdot 10^6$ (s^{-1})	$k'\cdot 10^4$ (s^{-1})	$H'^*\cdot[Ps]\cdot 10^3$ (adimensional)	r^2
50	50	50	30	7.00	6.92	10.12	0.95
50	50	80	30	21.48	7.48	28.73	0.97
100	--	80	30	29.28	9.16	31.96	0.98
100	--	80	50	40.48	12.78	31.68	0.98
Soxhlet extraction (EtOH)				16.63	5.38	30.90	0.99

Note: for all runs, the confidence interval ($p = 0.05$) resulted ≤ 0.01.

CONCLUSION

On the basis of the results obtained a 2-step SFE process could be tentatively proposed. Such a process involves a preliminary $ScCO_2$ extraction of carotenoids, followed by the recovery of polyphenols by means of EtOH coupled with $ScCO_2$ (1:1, w/w). The economical aspects of such process are under evaluation together with the analytical characterisation of the raw extracts.

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