



MASS TRANSFER AND KINETIC MODELLING OF SUPERCRITICAL CO₂ EXTRACTION OF FRESH TEA LEAVES (*Camellia sinensis* L.)

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Abstract – Supercritical carbon dioxide extraction was employed to extract solids from fresh tea leaves (*Camellia sinensis* L.) at various pressures (15 to 35 MPa) and temperatures (313 to 333K) with addition of ethanol as a polarity modifier. The diffusion model and Langmuir model fit well to experimental data and the correlation coefficients were greater than 0.94. Caffeine solubility was determined in supercritical CO₂ and the Gordillo model was employed to correlate the experimental solubility values. The Gordillo model fit well to the experimental values with a correlation coefficient 0.91 and 8.91% average absolute relative deviation. Total phenol content of spent materials varied from 57 to 85.2 mg of gallic acid equivalent per g spent material, total flavonoid content varied from 50.4 to 58.2 mg of rutin equivalent per g spent material and the IC₅₀ value (antioxidant content) varied from 27.20 to 38.11 µg of extract per mL. There was significant reduction in polyphenol, flavonoid and antioxidant content in the extract when supercritical CO₂ extraction was carried out at a higher pressure of 35 MPa.

Keywords: Supercritical CO₂; Fresh tea leaves; Mass transfer; Caffeine; Polyphenols; Antioxidants

INTRODUCTION

Green tea is one of the highly preferable raw materials for the food and pharmaceutical industry due to the presence of many biological active molecules such as polyphenols, caffeine, theanine and specially catechins which impart the flavor, taste and health benefits to human beings (Park et al., 2012). The catechins possess anti-cancer, anti-inflammatory, anti-microbial and anti-obesity properties. Green tea contains caffeine (3 to 4% w/w), which is a kind of alkaloid and some clinical studies show that it possess health benefits to cure Alzheimer's disease and cancer treatment (Eskelinen and Kivipelto, 2010; Gadkari and Balaraman, 2013; Kang et al., 2010).

There are several techniques used to extract the bioactives from the tea matrix such as conventional solvent extraction, pressure assisted solvent extraction, ultrasound assisted extraction, microwave assisted extraction and supercritical fluid extraction (Gadkari et al., 2014; Ghoreishi and Heidari, 2012, 2013; Pan et al., 2003; Park et al., 2012; Xia et al., 2006). Several studies reported that supercritical CO₂ could be employed for selective extraction of bioactives from herbaceous materials (Lang and Wai, 2001). Due to the higher initial capital investment, supercritical fluid extraction (SFE) is less preferred than other techniques of extraction, but it has a unique quality of selective separation of bioactive compounds with slight manipulation of pressure and temperature (Gadkari et

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al., 2015). Green tea has been extracted using different solvent systems and different methods for improving the extract quality (Gadkari and Balaraman, 2015; Gadkari et al., 2014). Green tea contains polar compounds but much less non-polar components and hence polar solvents such as water, ethanol, methanol, etc. are more preferred for extraction. The process of extraction of components from the matrix involves dissolution of a solid component within the matrix into the fluid and then diffusion of the solid from the matrix controlled by external mass transfer processes (Roy et al., 1996).

The main advantages of supercritical CO₂ extraction compared to other methods are: residue-free extracts, low-temperature of processing, less number of unit operations, less thermal degradation, higher mass transfer rates and many more. A supercritical fluid (SCF) has liquid-like densities, gas-like viscosities and diffusivity, and zero surface tension which cause superior mass transfer characteristics and solvent effectiveness with density control (Ghoreishi and Heidari, 2012). In the 21st century, SCF extraction and encapsulation processes have been hailed by researchers as a solution for separation of important biomolecules and developing effective delivery systems for them (Gadkari and Balaraman, 2015). Supercritical fluids have high potential in downstream processing, but reliable and versatile mathematical models are needed to understand mass transfer for their use in process design and economic feasibility studies (Brennecke and Eckert, 1989). There are many applications of SCF reported in the literature (Brunner, 2005; Herrero et al., 2006; King, 2014; Knez et al., 2014). Hacer and Gurub (2010) studied supercritical CO₂ extraction of caffeine from Turkish tea stalk and fiber and found that with addition of ethanol during the extraction process, the yield of caffeine could be increased from 62.5 to 63.1% (w/w) and the extraction time reduced from 7 to 2 h. Caffeine was extracted from guaraná seeds and mate tea leaves with solubility values of caffeine ranging from 6.01×10^{-4} to 1.11×10^{-5} mole fraction at a CO₂ pressure 10 MPa and temperature from 313 to 343 K (Saldana et al., 2002). To date, there are no reports on the kinetics of extraction of fresh tea leaves using supercritical CO₂ and analysis of spent material.

The main objective of study was to understand the mass transfer processing during supercritical CO₂ extraction of fresh tea leaves with application of diffusion models and a solubility model to identify the dominant mass transfer mechanism. Also, the spent material was extracted and analyzed for its polyphenol, flavonoid content and in vitro antioxidant activity.

Diffusion and Kinetic models for extraction

There are many models available for understanding the mass transfer process during extraction of solute from a

solid matrix and these models are based on mass transfer integration with the following assumptions;

1. Particles are considered as spherical with radius (R).
2. Solute free solvent is entering the system.
3. Extraction of solute (extractable solids) happens in a single step.
4. At the interface, thermodynamic equilibrium is established.

During the extraction solute diffuses to the surface and internal diffusion is modelled using either an effective diffusion coefficient or solid-phase mass transfer coefficient (Campos et al., 2005). If several components exist in the sample matrix, the fitting has been made by taking into account just a single one, called the solute. The extraction system is considered as a fixed bed comprised of two phases:

- (i) Solid (static): tea leaf matrix which holds the solute.
- (ii) Fluid (mobile): supercritical CO₂ + polar solvent.

The solvent flow rate and physical properties are constant during the extraction process. Pressure losses, temperature gradients and heat of dissolution are neglected in the bed. Superficial velocity was calculated from the supercritical fluid flow rate by neglecting the extracted solute flow rate. To understand the mass transfer process during extraction, the following models are employed.

Fick's Diffusion model

Supercritical CO₂ extraction is a diffusion-based process in which the solute is leached out from the leaf matrix into the solvent phase. The law states that the flux is proportional to concentration gradient and diffusion of a solute occurs in the direction of decreasing concentration. The general form of Fick's diffusion equation is given as follows (Aguerre et al., 1985):

$$D_e \left[\frac{\partial^2 C}{\partial r^2} \right] = \frac{\partial C}{\partial t} \quad (1)$$

where, C is the concentration of solute at the time t and at the radial position r within the (planar) leaf matrix; the initial and boundary conditions are as below:

at the center : $F_0 > 0, r = 0, \frac{\partial c}{\partial t} = 0;$

at the surface : $F_0 > 0, r = 1, c = 0;$

at the start; $F_0 = 0, 0 \leq r \leq 1, c = 1.$

The solution of Eq. 1 can be given as follows (Aguerre et al., 1985; Gadkari and Balaraman, 2015).

$$C^* = \sum_{n=1}^{\infty} \frac{6}{(n\pi)^2} \exp\left(- (n\pi)^2 \frac{D_e t}{R^2}\right) \quad (2)$$

where $C^* = \frac{(\bar{C} - C_{\infty})}{(C_0 - C_{\infty})}$ and C_{∞} is the concentration of

solute at infinity, \bar{C} is the average concentration of solute in the solvent phase, C_0 is the concentration of solute at time ($t=0$), and D_e is the effective diffusivity (m²/s). When the Fourier number (F_0), defined as $\left(\frac{D_e t}{r^2}\right)$, is greater than 0.1, all terms other than $n = 1$ can be neglected and Eq. 2 is simplified as follows:

$$C^* = \frac{6}{\pi^2} \exp\left(- (\pi)^2 \frac{D_e t}{R^2}\right) \quad (3)$$

From the slope of the plot of $\ln C^*$ versus time, one can determine the value of the effective diffusivity D_e .

Langmuir model

To evaluate the mass transfer process, two simple models, i.e., the exponential and Langmuir model have been repeatedly used by researchers (Manohar and Kadimi, 2012; Murthy and Manohar, 2014). The Langmuir model is one of the well-known models used to explain the extraction kinetics. Though the adsorption model is usually employed for studying the extraction process of oil seed materials, it can also be used for the extraction process of a leafy matrix. The material is soaked in fluid (supercritical CO₂ + EtOH) in an extraction vessel and after some time solute diffuses from the internal matrix and gets adsorbed on the surface, which further travels to the separator vessel in the solvent. The Langmuir extraction model is presented in the following form,

$$Y = \frac{Y_f \cdot t}{(K_L + t)} \quad (4)$$

where Y is % extraction yield (w/w), Y_f and K_L are constants (Y_f is the yield at infinite time).

The temperature dependence of the adsorption

coefficient is governed by an Arrhenius equation as follows (Al-Jabari, 2003),

$$K_L = K_L^0 \exp\left(\frac{-E}{RT}\right) \quad (5)$$

where E is the activation energy (kJ/mol), K_L^0 is the pre-exponential coefficient, and R is the universal gas constant.

The Gordillo model for caffeine solubility

The Gordillo model is an empirical model used for correlating the solubility of a solute in supercritical CO₂. The model gives the relationship between pressure, temperature of extraction and their influence on the solubility of the solute. Gordillo et al. (1999) proposed a modification of the original equation presented by Yu et al. (1994) in order to correlate experimental solubility data of Penicillin G. The Gordillo model is represented as follows:

$$\ln y_2 = D_0 + D_1 P + D_2 P^2 + D_3 P T + D_4 T + D_5 T^2 \quad (6)$$

where D_0 to D_5 are model coefficients, y_2 is the mole fraction caffeine solubility, P is the pressure and T is the temperature.

In order to provide a reliable criterion to compare the accuracy of the model, the average absolute relative deviation (AARD) was calculated from:

$$\text{AARD, \%} = \frac{100}{N} \sum_{i=1}^N \frac{|y_{\text{exp}} - y_{\text{pred}}|}{y_{\text{exp}}} \quad (7)$$

where y_{exp} is the experimental solubility, y_{pred} is the predicted solubility and N is the total number of experiments.

MATERIALS AND METHODS

Fresh tea leaves (*Camellia sinensis*) were supplied by M/s. Dollar tea estate, Ooty, India. After picking, the leaves were transferred to a freezer (-253 K) within 5 h. Food grade carbon dioxide (99 % pure) was procured from M/s Kiran Corporation, Mysore, India. Aluminum chloride and folin-ciocalteu reagent were procured from SRL Chemicals (Mumbai, India). DPPH* (2,2-diphenyl-1-picrylhydrazyl), gallic acid, rutin, and trolox were procured from Sigma-Aldrich Company Ltd., Germany. Absolute ethanol, HPLC grade methanol, acetonitrile and acetic acid

were purchased from Merck Chemicals, Mumbai, India.

Extraction of Fresh leaves under Supercritical CO₂

Supercritical CO₂ is a non-polar solvent and reported by many researchers to be a powerful tool for extraction of non-volatiles in their natural form (Brunner, 2005; Campos et al., 2005; Manohar and Kadimi, 2012). Ethanol was added (1.2 % w/w) as a polarity modifier during supercritical CO₂ extraction to enhance the solubility of polar compounds into the solvent (Gadkari et al., 2015). The polarity modifier was pumped into the extractor vessel using a high pressure pump (Milton Roy™ duplex pump, USA). The leaves were extracted in a pilot scale supercritical fluid extraction unit (NOVA Swiss WERKE AG, Switzerland) designed for working pressure up to 100 MPa, temperature up to 373 K. The frozen tea leaves were crushed in the presence of dry ice to an average particle size less than 1.5 mm in an analytical mill (model A10, IKA, Germany) prior to supercritical extraction. 100 g of crushed tea leaves were loaded in the extractor vessel with injection of a polarity modifier into the extractor vessel where CO₂ is continuously circulated through a closed loop system. Each fraction was collected separately at various time intervals up to 9 h extraction and weighed on an analytical balance (AT-201, Mettler, USA).

Caffeine solubility measurement

There are different methods for measuring solubility of the solute (caffeine) in supercritical CO₂, i.e., static, dynamic and recirculation methods. Most researchers use a dynamic method for determination of solute solubility in supercritical CO₂ due to the simplicity of the method (Ismadji and Bhatia, 2003). It was assumed that the saturation of solute in the CO₂ was attained at lower superficial velocities (2.9×10^{-5} and 4.6×10^{-5} m/s). The solubility of caffeine was determined from slope values obtained by fitting a second-order polynomial equation to the curve where the X-axis represents the kg of caffeine and the Y-axis represents the kg of CO₂ used (Campos et al., 2005). The solubility values were further converted to molefractions prior to the fit of the Gordillo model.

Extraction of spent material

After supercritical CO₂ extraction, the spent material obtained after each experiment was extracted using water as a solvent (material: solvent, 1:50) at 353 K for 40 min in a hot water bath (Labbe et al., 2006). The extracts were brought to room temperature (300 K) under running water and then filtered through a 0.22µm syringe filter. To prevent the oxidation of extracts due to light and temperature, the extracts were stored in amber coloured glass vials in refrigerated condition (277 K) until analysis.

Total polyphenol content

Total polyphenol content (TPC) was determined using a well established spectroscopic method with slight modification, the ISO14502-1 method as described in earlier studies (Gadkari et al., 2014). It is a colorimetric assay in which the polyphenols present in the extract react with Folin–Ciocalteu reagent to produce a blue coloured complex. The absorbance of the complex formed was determined at 765 nm for further calculation purposes. Gallic acid was used as the polyphenol standard and the standard calibration curve was obtained in the range 0 to 40 µg of gallic acid. The results were expressed as mg of gallic acid equivalent per g of spent material.

Total Flavonoid content

The total flavonoid content of the extract was quantified using the method described in earlier studies (Gadkari et al., 2015). It is a colour-producing spectrophotometric assay where aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols to form coloured complexes. Rutin was used as a standard and the calibration curve was plotted with different concentrations from 0 to 1000 µg. Finally, the total flavonoid content was expressed as mg rutin equivalent per g of spent material.

DPPH assay (IC₅₀ value)

The antioxidant activity was determined using the DPPH assay with a slight modification in methodology and the results were presented in terms of the IC₅₀ value (amount of extract required to achieve 50 % of inhibition against DPPH radical) (Kutti Gounder and Lingamallu, 2012). 1 mL of extract or trolox or ethanol as blank (0-100 µg/mL) was mixed with 1 mL of 0.4 mM of DPPH solution (prepared in ethanol). The mixture was vortexed for a minute and allowed to stand in the dark for 30 min. Finally, the absorbance of the mixture was observed at 517 nm using a UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan). The DPPH scavenging activity was calculated using Eq. 8,

$$\text{DPPH inhibition, \%} = \left(1 - \frac{A_S}{A_B}\right) \times 100 \quad (8)$$

where A_S is the absorbance of the sample and A_B is the absorbance of the blank.

HPLC analysis of extractable solids

The samples were dissolved in HPLC grade methanol

and then filtered through a 0.22 µm syringe filter. The separation of caffeine and individual compounds was carried out on a Shimadzu LC-10A system (Tokyo, Japan.) equipped with a reverse phase C18 (15 µ-Diamonsil) column (250 mm × 4.6 mm) and a PDA detector set to range from 200 to 600 nm. The peak integration and data collection was carried out with Class 10 software (Shimadzu, Tokyo, Japan). The mobile phase was prepared and degassed under vacuum as per our earlier studies (Gadkari et al., 2014). The identification and quantification of individual compounds were done using authenticated analytical standards.

Statistical analysis

All experiments were carried out in duplicate and values were expressed with their means. The regression analysis for each model was carried out using the Excel program (MS Office® 2013).

RESULTS AND DISCUSSION

Influence of Extraction pressure and temperature on extraction yield

In order to study the effect of extraction pressure and temperature, the experiments were carried out at pressures from 15 MPa to 35 MPa and temperatures from 313 K to 333 K (Table 1). Figure 1a shows that, when extraction was carried out at a pressure 15 MPa with varying temperature (313 K to 323 K), the % extract yield increases slightly from 2.59 % to 2.66%. But when the temperature changed to 333 K, the % extract yield increased to 3.76 %. The sudden increase in % extract yield may be attributed to temperature dominancy where the temperature effect

dominates over the solvent density effect, which can lead to an increase in % extract yield at higher temperatures (Park et al., 2012). Also, the same effect was observed at the pressures of 25 MPa and 35 MPa where a similar trend was observed from Fig. 1b and Fig. 1c.

Diffusion and Langmuir models for extraction

The Fick's 2nd law model has been employed for several decades for understanding the mass transfer process during extraction of herbaceous material. Eq. 1, which is the basic form, was further resolved into Eq. 3 for $F_0 > 0.1$. The F_0 number calculated after obtaining the diffusion coefficient for each experiment is presented in Table 2. The diffusion coefficients obtained from the slope of the curve

$$C^* = \frac{(\bar{C} - C_\infty)}{(C_0 - C_\infty)}$$

versus time are presented in Figure 2 and the model regressed well with correlation coefficients >0.94. Diffusion coefficients varied from 3.50x10⁻¹¹ to 6.71x10⁻¹¹ m²/s depending on the extraction pressure and temperature. At lower pressure and temperature, i.e., 15 MPa, 313K, a higher diffusion rate was found due to the higher density of solvent at lower temperature, which increases the yield of extractable solids. The matrix of the leaf is not very strong, so the extraction completed within 9 h is comparatively less than that of conventional solvent extraction (Gadkari et al., 2015). These extracts when subjected to HPLC showed a major peak of caffeine; more than 85% (w/w) of the caffeine was extracted with very little amount of chlorophyll (not quantified). The effective diffusivity of mango ginger (*Curcuma amada* Roxb.) extract varied from 0.669×10⁻¹² to 18.50×10⁻¹² m²/s with extraction

Table 1. Experimental conditions for the supercritical CO₂ extraction of fresh tea leaves

Pressure (MPa)	Temperature (K)	Density (kg/m ³)	superficial velocity (m/s)	Viscosity (kg/m.s)
15	313	780.3	7.45x10 ⁻⁵	6.78x10 ⁻⁵
15	323	699.8	8.30x10 ⁻⁵	5.65x10 ⁻⁵
15	333	603.9	9.62x10 ⁻⁵	4.61x10 ⁻⁵
25	313	879.6	6.61x10 ⁻⁵	8.71x10 ⁻⁵
25	323	834.4	6.96x10 ⁻⁵	7.80x10 ⁻⁵
25	333	786.8	7.38x10 ⁻⁵	6.98x10 ⁻⁵
35	313	934.9	6.21x10 ⁻⁵	1.01x10 ⁻⁵
35	323	899.4	6.46x10 ⁻⁵	9.22x10 ⁻⁵
35	333	863.2	6.73x10 ⁻⁵	8.43x10 ⁻⁵

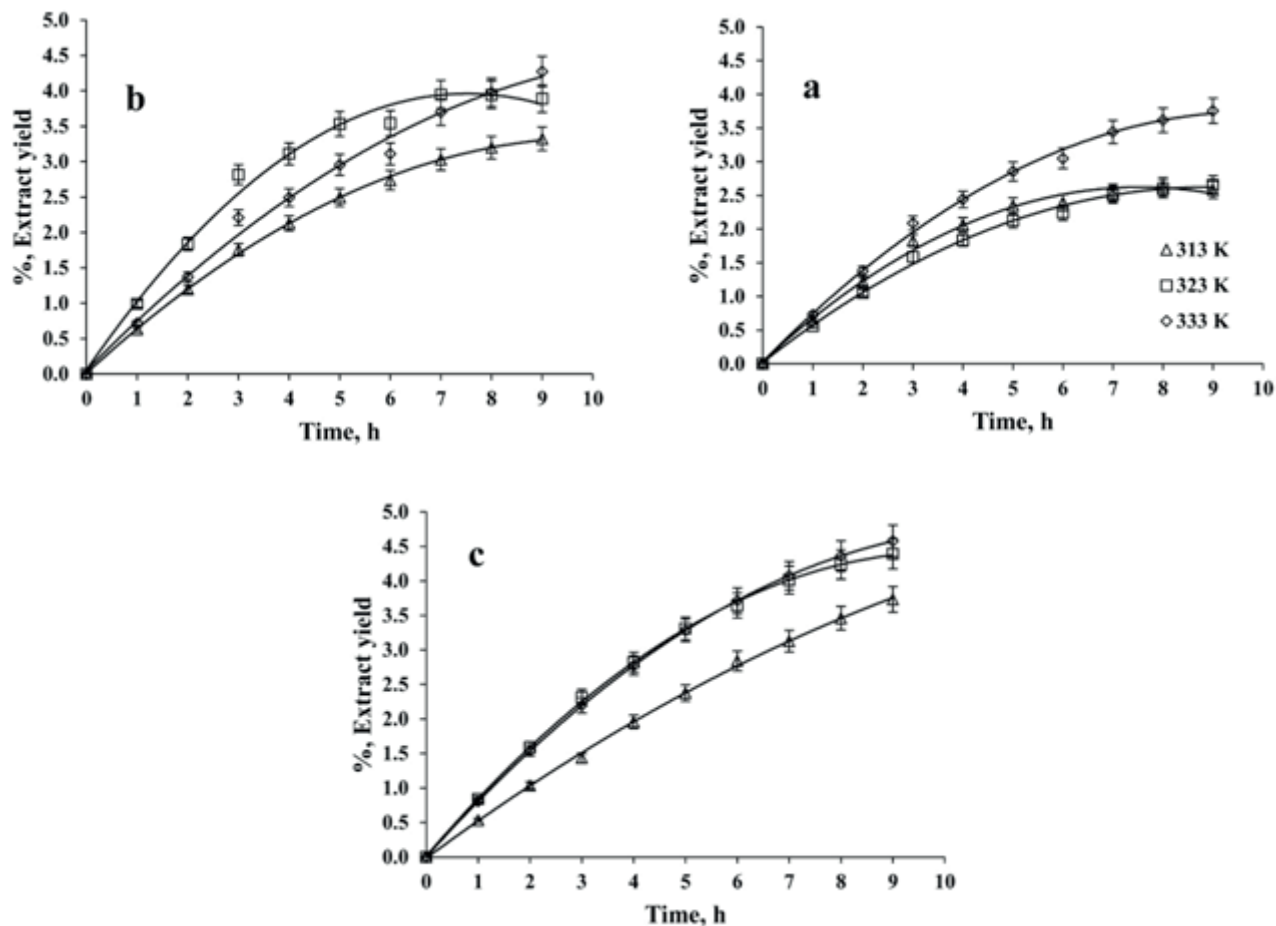


Figure 1. Extraction curves obtained at different pressures (15 to 35 MPa) and temperatures (313 to 333 K). (a) 15 MPa, (b) 25 MPa, (c) 35 MPa.

pressure (10 to 35 MPa) and temperature (313 to 333 K) in supercritical CO₂ (Murthy and Manohar, 2014). The effective diffusivity (D_e) in the present study was found to be about 9 times greater than D_e for certain seeds like coffee beans and guaraná seeds reported in the literature (Table 3). This can be attributed to the loose structure of

the tea matrix compared to the seeds. But, diffusivities in Korean tea leaves are observed to be about 10 times larger than those of the present study. However, it should be noted that diffusivities in supercritical CO₂ are much less than those found in water extraction (Table 3).

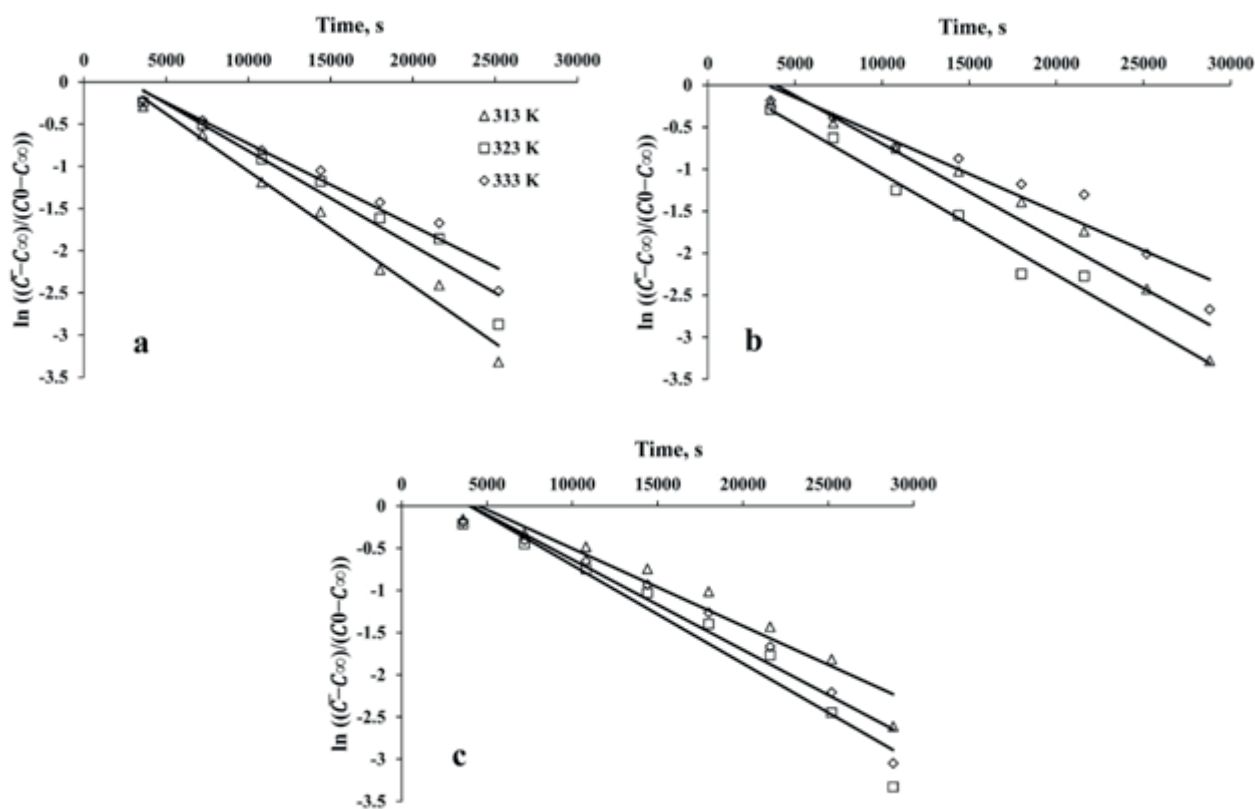
Table 2. Parameters of the Langmuir (K_L), Fick's (D_e) and Arrhenius models for overall extraction

Pressure (MPa)	Temperature (K)	Langmuir model rate constant K_L (s/ wt. %)	Effective Diffusivity D_e (m ² /s)	Fourier number F_0	Activation energy E_a (kJ/mol)
15	313	2.49×10^4	6.71×10^{-11}	1.39	20.57
	323	3.54×10^4	4.80×10^{-11}	0.99	
	333	4.21×10^4	4.29×10^{-11}	0.89	
25	313	4.44×10^4	4.67×10^{-11}	0.97	11.94
	323	2.50×10^4	6.25×10^{-11}	1.30	
	333	5.94×10^4	3.50×10^{-11}	0.73	
35	313	1.06×10^4	3.53×10^{-11}	0.73	29.91
	323	4.42×10^4	4.72×10^{-11}	0.98	
	333	5.38×10^4	4.30×10^{-11}	0.89	

Correlation coefficients for all models were greater than 0.94.

Table 3. Comparison of effective diffusivity (D_e) of present study to literature available

Raw material	Solvent	Pressure (MPa)	Temperature (K)	Effective diffusivity D_e (m ² /s)	Reference
Green tea dried	Water	-	323 - 363	$(0.03 - 1.38) \times 10^{-9}$	Ziaedini et al. (2010)
Coffee beans	SC-CO ₂ + Water	15 - 25	313 - 333	$(3 - 7) \times 10^{-12}$	Machmudah et al. (2012)
Guaraná seeds	SC-CO ₂	20	328	0.46×10^{-14}	Mehr et al. (1996)
Korean tea leaves	SC-CO ₂ + 20% Water	10 - 40	313 - 353	$(2.64 - 27.5) \times 10^{-10}$	Kim et al. (2007)
Indian Fresh tea leaves	SC-CO ₂ + 1.2 % Ethanol	15 - 35	313 - 333	$(3.5 - 6.71) \times 10^{-11}$	Present study

**Figure 2.** Regression of the Diffusion model to the extraction yield at various pressures. (a) 15 MPa, (b) 25 MPa, (c) 35 MPa.

The Langmuir model correlates well to the experimental data with correlation coefficients greater than 0.94. It was observed that the rate of extraction increases with an increase in extraction pressure and temperature (Table 2).

At higher pressure and temperature of extraction, the vapor pressure of the solute increases which directly matches the increased rate of extraction with increase in yield of extractable solids (Figure 3). Also, the Langmuir model

was used to predict temperature dependent supercritical CO₂ extraction curves, where the rate constant was found to follow the Arrhenius type of equation. The rate constants have been further used to calculate the activation energy

(E_a, kJ/mol); at an extraction pressure of 25 MPa, the lowest activation energy is 11.94 kJ/mol. The Arrhenius model explained well the temperature dependence of extraction, with good correlation (Al-Jabari, 2003).

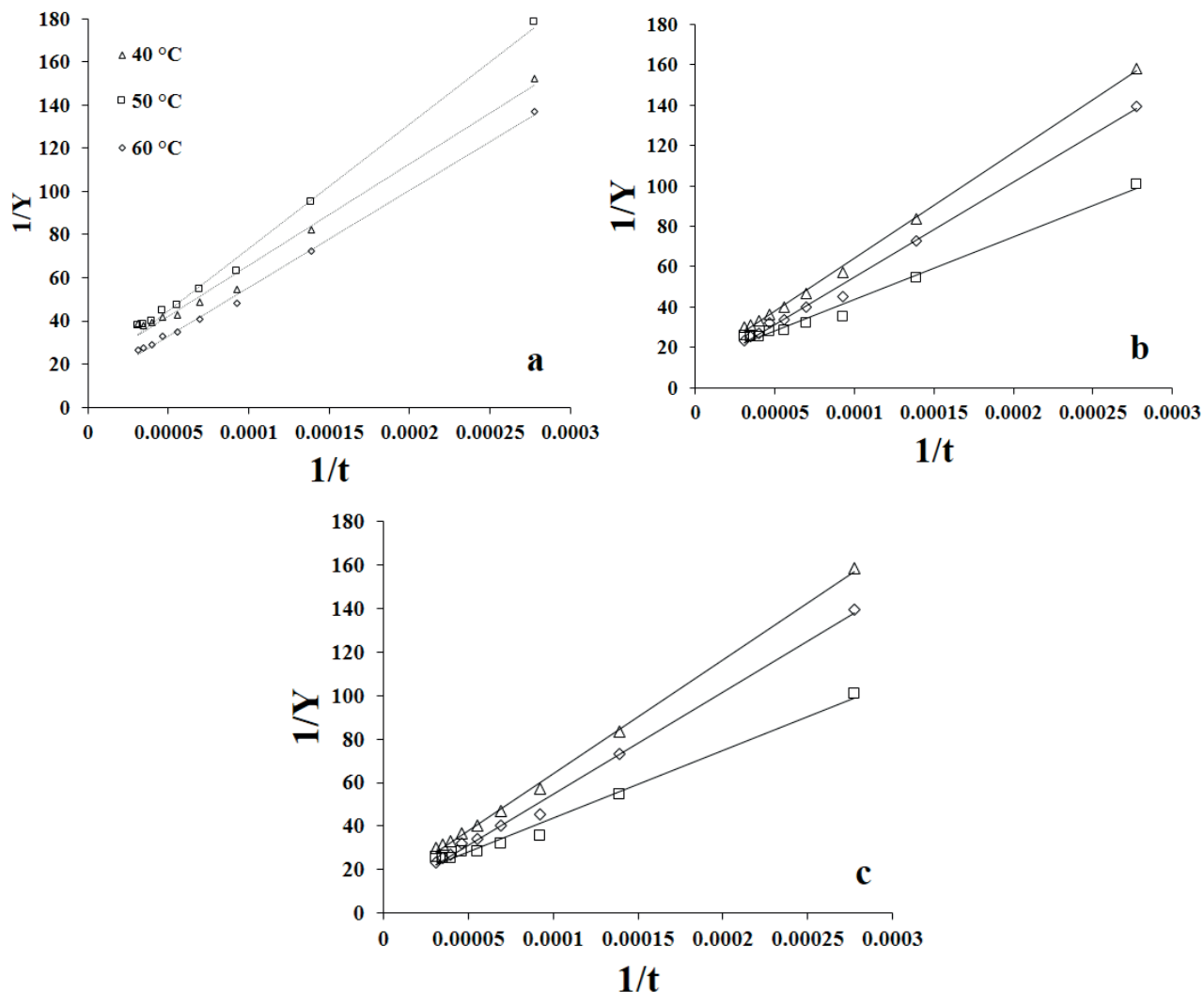


Figure 3. Regression of the Langmuir model for the extraction yield at various pressures. (a) 15 MPa, (b) 25 MPa, (c) 35 MPa.

Solubility of caffeine in supercritical CO₂+EtOH

The extractable solids were rich in caffeine and quantified using HPLC analysis (Figure. 4). Caffeine solubility in weight fraction (kg/kg) was converted to mole fraction as represented in Table 4. The Gordillo model gave the best fit to experimental data with a correlation

coefficient of 0.91 and AARD of 8.91% (Figure 5). The solubility of caffeine was found to be maximum (149.55×10^{-6}) at a pressure of 25 MPa and temperature of 323 K in supercritical CO₂. The phenomena may be due to the dominant effect of the increased vapor pressure of the solute at higher temperature, although it leads to decreased solvent density and reduced dissolving power of the solvent (Park et al., 2012; Kim et al., 2008).

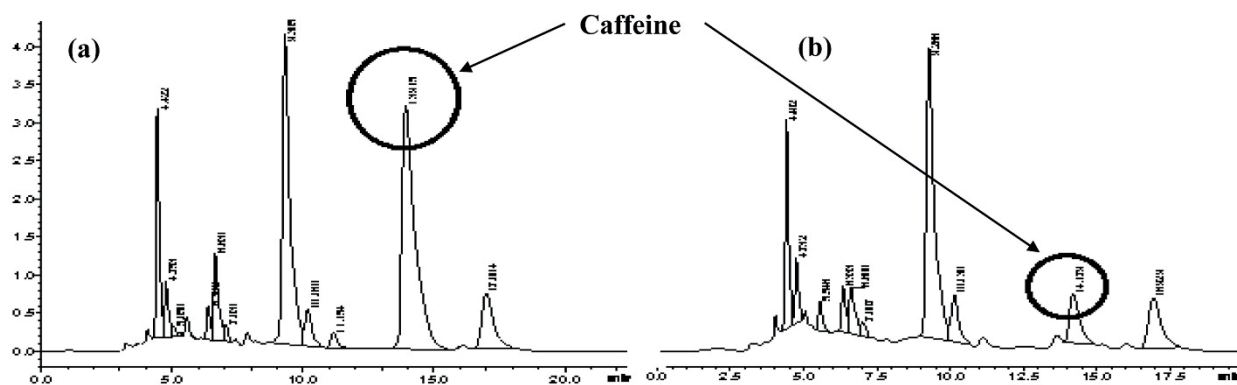


Figure 4. HPLC chromatogram of green tea; (a) Fresh tea, (b) After Supercritical CO₂ extraction.

Table 4. Experimental solubility of caffeine and its prediction by using the Gordillo model

P (MPa)	T (K)	Solubility (mole fraction, x10 ⁻⁶)	
		Experimental ^a	Gordillo model
15	313	60.96±0.20	53.55
	323	83.62±0.38	102.19
	333	106.28±1.23	99.00
25	313	64.81±0.49	72.68
	323	149.55±1.14	133.75
	333	125.31±0.78	124.95
35	313	44.19±1.03	44.86
	323	87.01±0.56	79.62
	333	66.62±0.45	71.73
Model coefficients			
D0	-376.4197		
D1	0.30181		
D2	-0.00393936		
D3	-0.000363221		
D4	2.22605		
D5	-0.0034		
AARD (%)	8.91		
R2	0.96		
F value	65.4		

^a Experimental solubility of caffeine in mole fraction with its mean (n=2) and standard deviation

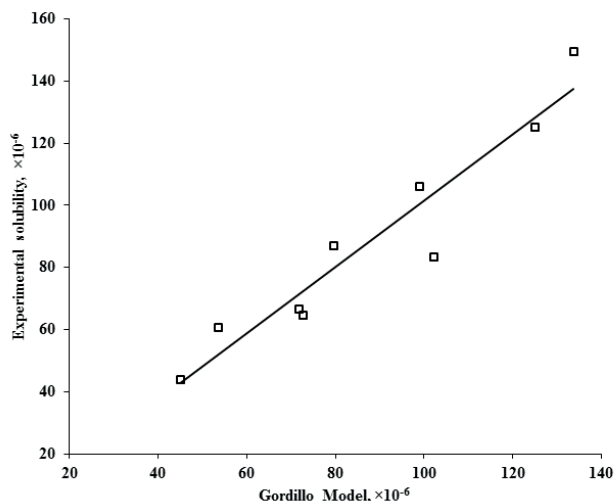


Figure 5. Gordillo Model values vs. Experimental values of caffeine solubility in Supercritical CO₂.

Total polyphenol, total flavonoid content and antioxidant activity of spent material

The spent material (decaffeinated tea) was analyzed for its polyphenol and flavonoid content and antioxidant activity (Figure 6). There was maximum loss of 33.1 % and 30.9 % polyphenols and flavonoids respectively in spent tea extracted at 35 MPa as compared to fresh tea. As the extraction pressure increases the solubility of important polyphenols and flavonoids increases, which results in loss of these components through extractable solids during extraction. Figure. 6 shows that there was a decrease in the content of polyphenol from 85.2 to 57 mg gallic acid equivalent per g of spent tea. Extraction

at lower pressures (15, 25 MPa) resulted in reduced loss of polyphenols (Bhattacharya et al., 2014). Flavonoids are also an important active biological group present in fresh tea leaves. We observed that there was a decrease in flavonoid content as the extraction pressure increased from 15 to 35 MPa. The IC₅₀ value is an indication of the amount of sample required to achieve 50 % antioxidant activity against free radicals such as DPPH. When extraction was done at 15 MPa from a fresh sample, the antioxidant content of fresh tea leaves was good, but as the extraction pressure increased to 35 MPa, the IC₅₀ value of the extract increased from 27.20 to 38.11 $\mu\text{g}/\text{mL}$, which shows that there was a decrease in antioxidants at higher pressure of extraction.

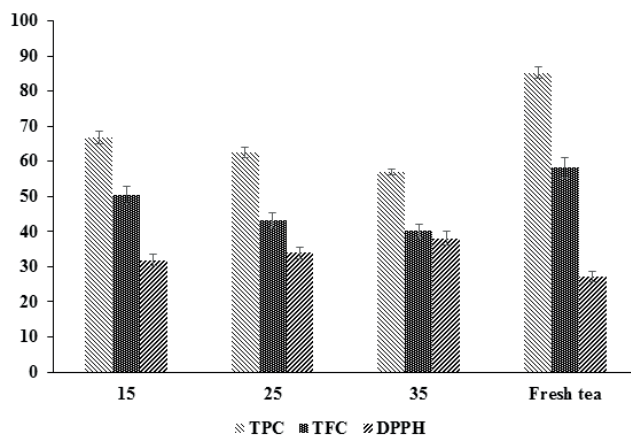


Figure 6. Composition of spent material obtained after extraction at various pressures (15 to 35 MPa), Total polyphenol content (mg gallic acid equivalent per g of spent material), total flavonoid content (mg rutin equivalent per g of spent material), DPPH IC₅₀ value (μg of extract/ mL).

CONCLUSIONS

The supercritical CO₂ extraction of fresh tea leaves was carried out successfully for a range of conditions of pressure (15 to 35 MPa) and temperature (313 to 333 K) with 1.2% (w/w) ethanol as polarity modifier. The mass transfer process was studied with application of the diffusion model, Langmuir model and Gordillo model. The diffusion and Langmuir model fit well to the experimental results with correlation coefficients greater than 0.94. These models were found to be useful for discussing the mass transfer process. When the extracts were subjected to HPLC analysis, caffeine was the major component identified and quantified. It was concluded that supercritical CO₂ + EtOH could be an effective technique for selective removal of caffeine from fresh tea leaves. Further, using a dynamic method, we measured the solubility of caffeine in supercritical CO₂ and the Gordillo model was used to correlate the solubility of caffeine. The spent material (decaffeinated tea) was further extracted and analyzed for its total polyphenol, flavonoid content and antioxidant activity. At an extraction pressure of 35 MPa there was a significant loss in polyphenol, flavonoid content and antioxidant activity from the fresh tea leaves. To prevent the losses of important polyphenols, flavonoids and antioxidants, it is recommended that the extraction be carried out at the lower pressure range from 15 to 25 MPa.

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NOMENCLATURE

P	Pressure, MPa
R	Universal gas constant, J gmol ⁻¹ K ⁻¹
T	Temperature, K
t	Time, s
y, y ₂ phase	Mole fraction of solute in the supercritical phase
r	space coordinate, dimensionless
R	Radius of spherical particle, m
D _e	Effective diffusivity, m ² s ⁻¹
F ₀	Fourier number
E	Activation energy, kJ mol ⁻¹
KL	Langmuir model constant
K _L ⁰	Pre-exponential coefficient

Y _f	Constant in equation 4
D ₀ - D ₅	Empirical model coefficients
AARD	Average absolute relative deviation
SFE	Supercritical fluid extraction
CO ₂	Carbon dioxide

Greek characters

ρ	Density, kg m ⁻³
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Subscripts

exp	Experimental value
pred	Predicted value

REFERENCES

- Aguerre, R., Gabitto, J., Chirife, J., Utilization of Fick's second law for the evaluation of diffusion coefficients in food processes controlled by internal diffusion. *International Journal of Food Science & Technology*, 20, 623-629 (1985).
- Al-Jabari, M., Modeling analytical tests of supercritical fluid extraction from solids with Langmuir kinetics. *Chemical Engineering Communications*, 190, 1620-1640 (2003).
- Bhattacharya, M., Srivastav, P., Mishra, H., Optimization of process variables for supercritical fluid extraction of ergothioneine and polyphenols from *Pleurotus ostreatus* and correlation to free-radical scavenging activity. *The Journal of Supercritical Fluids*, 95, 51-59 (2014).
- Brennecke, J.F., Eckert, C.A., Phase equilibria for supercritical fluid process design. *AIChE Journal*, 35, 1409-1427 (1989).
- Brunner, G., Supercritical fluids: technology and application to food processing. *Journal of Food Engineering*, 67, 21-33 (2005).
- Campos, L. M., Michielin, E. M., Danielski, L., Ferreira, S. R., Experimental data and modeling the supercritical fluid extraction of marigold (*Calendula officinalis*) oleoresin. *The Journal of Supercritical Fluids*, 34, 163-170 (2005).
- Eskelinen, M.H., Kivipelto, M., Caffeine as a protective factor in dementia and Alzheimer's disease. *Journal of Alzheimer's Disease*, 20, 167-174 (2010).
- Gadkari, P.V., Balaraman, M., Catechins: Sources, extraction and encapsulation: A review. *Food & Bioproducts Processing*, 93, 122-138 (2015).
- Gadkari, P.V., Balaraman, M., Extraction of catechins from decaffeinated green tea for development of nanoemulsion using palm oil and sunflower oil based lipid carrier systems. *Journal of Food Engineering*, 147, 14-23 (2015).
- Gadkari, P.V., Balaraman, M., Kadimi, U.S., Polyphenols from fresh frozen tea leaves (*Camellia assamica* L.) by supercritical carbon dioxide extraction with ethanol entrainer-application of response surface methodology. *Journal of Food Science & Technology*, 52, 720-730 (2015).
- Gadkari, P.V., Kadimi, U.S., Balaraman, M., Catechin concentrates of garden tea leaves (*Camellia sinensis* L.): extraction/isolation and evaluation of chemical composition. *Journal of the Science of Food & Agriculture*, 94, 2921-2928 (2014).

- Ghoreishi, S., Heidari, E., Extraction of epigallocatechin gallate from green tea via modified supercritical CO₂: Experimental, modeling and optimization. *The Journal of Supercritical Fluids*, 72, 36-45 (2012).
- Ghoreishi, S., Heidari, E., Extraction of Epigallocatechin-3-gallate from green tea via supercritical fluid technology: Neural network modeling and response surface optimization. *The Journal of Supercritical Fluids*, 74, 128-136 (2013).
- Gordillo, M., Blanco, M., Molero, A., Martinez De La Ossa, E., Solubility of the antibiotic penicillin G in supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 15, 183-190 (1999).
- Hacer, I., Gurub, M., Effect of ethanol content on supercritical carbon dioxide extraction of caffeine from tea stalk and fiber wastes. *The Journal of Supercritical Fluids*, 55, 156-160 (2010).
- Herrero, M., Cifuentes, A., Ibanez, E., Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. *Food Chemistry*, 98, 136-148 (2006).
- Ismadji, S., Bhatia, S., Solubility of selected esters in supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 27, 1-11 (2003).
- Kang, S.S., Han, K.S., Ku, B.M., Lee, Y.K., Hong, J., Shin, H.Y., Almonte, A.G., Woo, D.H., Brat, D.J., Hwang, E.M., Yoo, S.H., Chung, C.K., Park, S.H., Paek, S.H., Roh, E.J., Lee, S.J., Park, J.Y., Traynelis, S.F., Lee, C.J., Caffeine-mediated inhibition of calcium release channel inositol 1, 4, 5-trisphosphate receptor subtype 3 blocks glioblastoma invasion and extends survival. *Cancer Research*, 70, 1173-1183 (2010).
- Kim, W. J., Kim, J. D., Kim, J., Oh, S. G., Lee, Y. W., Selective caffeine removal from green tea using supercritical carbon dioxide extraction. *Journal of Food Engineering*, 89, 303-309 (2008).
- Kim, W. J., Kim, J. D., Oh, S. G., Supercritical carbon dioxide extraction of caffeine from Korean green tea. *Separation Science & Technology*, 42, 3229-3242 (2007).
- King, J.W., Modern Supercritical Fluid Technology for Food Applications. *Annual Reviews in Food Science Technology*, 5, 215-238 (2014).
- Knez, Z., Markocic, E., Leitgeb, M., Primožic, M., Knez Hrncic, M., Skerget, M., Industrial applications of supercritical fluids: A review. *Energy*, 77, 235-243 (2014).
- Kutti Gounder, D., Lingamallu, J., Comparison of chemical composition and antioxidant potential of volatile oil from fresh, dried and cured turmeric (*Curcuma longa*) rhizomes. *Industrial Crops & Products*, 38, 124-131 (2012).
- Labbe, D., Tremblay, A., Bazinet, L., Effect of brewing temperature and duration on green tea catechin solubilization: Basis for production of EGC and EGCG-enriched fractions. *Separation & Purification Technology*, 49, 1-9 (2006).
- Lang, Q., Wai, C.M., Supercritical fluid extraction in herbal and natural product studies-a practical review. *Talanta*, 53, 771-782 (2001).
- Machmudah, S., Martin, A., Sasaki, M., Goto, M., Mathematical modeling for simultaneous extraction and fractionation process of coffee beans with supercritical CO₂ and water. *The Journal of Supercritical Fluids*, 66, 111-119 (2012).
- Manohar, B., Kadimi, U.S., Extraction Modelling and characterization of bioactive components from *Psoralea corylifolia* L. Obtained by supercritical carbon dioxide. *Journal of Food Processing & Technology*, 3, 144 (2012).
- Mehr, C., Biswal, R., Collins, J., Cochran, H., Supercritical carbon dioxide extraction of caffeine from guarana. *The Journal of Supercritical Fluids*, 9, 185-191 (1996).
- Murthy, T.P.K., Manohar, B., Mathematical Modeling of Supercritical Carbon Dioxide Extraction Kinetics of Bioactive Compounds from Mango Ginger (*Curcuma amada* Roxb). *Open Journal of Organic Chemistry*, 2, 36-40 (2014).
- Pan, X., Niu, G., Liu, H., Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing: Process Intensification*, 42, 129-133 (2003).
- Park, H.S., Im, N.G., Kim, K.H., Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves. *LWT-Food Science & Technology*, 45, 73-78 (2012).
- Roy, B.C., Goto, M., Hirose, T., Extraction of ginger oil with supercritical carbon dioxide: experiments and modeling. *Industrial Engineering and Chemistry Research*, 35, 607-612 (1996).
- Saldana, M. D. A., Carsten, Z., Rahoma, S. M., Brunner, G., Extraction of methylxanthines from guarana seeds, mate leaves, and cocoa beans using supercritical carbon dioxide and ethanol. *Journal of Agricultural & Food Chemistry*, 50, 4820-4826 (2002).
- Xia, T., Shi, S., Wan, X., Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion. *Journal of Food Engineering*, 74, 557-560 (2006).
- Yu, Z. R., Singh, B., Rizvi, S. S., Zollweg, J. A., Solubilities of fatty acids, fatty acid esters, triglycerides, and fats and oils in supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 7, 51-59 (1994).
- Ziaedini, A., Jafari, A., Zakeri, A., Extraction of antioxidants and caffeine from green tea (*Camelia sinensis*) leaves: kinetics and modeling. *Food Science & Technology International*, 16, 505-510 (2010).