Aqueous extraction kinetics of soluble solids, phenolics and flavonoids from sage (*Salvia fruticosa* Miller) leaves

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Abstract In the present study, aqueous extraction kinetics of total soluble solids (TSS), total phenolic content (TPC) and total flavonoid content (TFC) from *Salvia fruticosa* leaves were investigated throughout 150 min. of extraction period against temperature (60–80 °C), particle size (2–8 mm) and loading percentage (1–4 %). The extract yielded 25 g/100 g TSS which contained 30 g/100 g TPC and 25 g/100 g TFC. The extraction data in time course fit with reversible first order kinetic model. All tested variables showed significant effect on the estimated kinetic parameters except equilibrium concentration. Increasing the extraction temperature resulted high extraction rate constants and equilibrium concentrations of the tested variables notably above 70 °C. By using the Arrhenius relationship, activation energy of the TSS, TPC and TFC were determined as 46.11 ± 5.61 , 36.80 ± 3.12 and 33.52 ± 2.23 kJ/mol, respectively. By decreasing the particle size, the extraction rate constants and diffusion coefficients exponentially increased whereas equilibrium concentrations did not change significantly. The equilibrium concentrations of the tested parameters showed linear behavior with increasing the loading percentage of the sage, however; the change in extraction rates did not show linear behavior due to submerging effect of 4 % loading.

Keywords *Salvia fruticosa* · Sage · Extraction kinetics · Phenolics · Flavonoids

Introduction

Salvia species, commonly known as sage, have been used since ancient times for more than sixty different ailments of aches, epilepsy, colds, bronchitis, tuberculosis, hemorrhage, and menstrual disorders (Topcu 2006). *Salvia fruticosa* Miller (synonym, *Salvia triloba* L.) is one of the important sage species which is native to Mediterranean countries and Turkey is one of the leading countries with high production and export capacity (1,544 t in 2009) of *S. fruticosa* along with *S. officinalis* (Çakıroğlu 2010). *S. fruticosa* contains considerable amounts of bioactive compounds such as phenolics and terpenoids which have anti-inflammatory (El-Sayed et al. 2006), antimicrobial (Delamare et al. 2007) and antioxidant (Tepe et al. 2006; Dincer et al. 2012) properties.

Traditionally, the leaves of the sage are used as herbal tea by infusing it into hot boiled water for 3–5 min. Recently, it has been marketed in tea bags and has also potential for processing into instant herbal tea but the extraction process of the sage is the main issue for the potential herbal tea products. From an engineering point of view, understanding of mass transfer phenomenon at the solid–liquid interface in sage extraction is important for optimizing process, scaling up to pilot, consequently development of industrial application.

Unsteady diffusion, film theory, empirical equation of Ponomaryov, Peleg model and Fick's law of diffusion are the typical kinetic models of solid–liquid extractions (Qu et al. 2010). Generally kinetics of tea extraction fits reversible first order kinetic model as has been reported by previous researchers (Spiro and Jago 1982; Jaganyi and Price 1999; Stapley 2002).

Type of solvent, temperature, solid–liquid ratio, particle size and agitation are important parameters for extraction of plant metabolites. There are several studies on extraction behavior of different plant materials such as coffee bean (Spiro and Selwood 1984), soy bean

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(Jokic et al. 2010), feijoa fruit (Tuncel and Yilmaz 2013), grape seeds (Bucic-Kojic et al. 2007), turmeric (Sogi et al. 2010), fumitory (Rakotondramasy-Rabesiaka et al. 2008), cassia seeds (Medoua and Mbofung 2007) and olive leaves (Carcel et al. 2010). These studies were conducted in aqueous form or solvent extraction based on the above mentioned parameters. It is generally reported that extraction rate increases with increasing in temperature and solid/liquid ratio, and decreasing the particle size. Earlier works reported extraction efficiency in different sage species by using different extraction methods, such as; conventional (Schneider et al. 2011), pressurized liquid (Hossain et al. 2010), ultrasonic assisted, (Velickovic et al. 2006; Velickovic et al. 2008), supercritical CO₂ (Aleksovski and Sovova 2007; Glisic et al. 2010) and ultrasonic assisted–supercritical CO₂ combination (Glisic et al. 2011).

The leaf based material extraction kinetics for soluble solids were modeled for yerba mate tea (Linares et al. 2010), green tea (Ziaedini et al. 2011) and olive (Carcel et al. 2010). However, there is no reported study on water extraction behavior of total soluble solids, total phenolics and flavonoids of *S. fruticosa* leaves.

Therefore, the present study was undertaken to determine water extraction behavior of soluble solids, total phenolics and flavonoids from sage leaves depending on the temperature, particle size and sage loading percentage in order to establish useful kinetic parameters for sage processing.

Materials and methods

Material

The sage (*Salvia fruticosa* Miller) leaves were harvested in flowering season (May–June) from its natural habitat located in West Mediterranean Region of Turkey. They were dried to equilibrium moisture level (~8 g/100 g moisture content) in shade and kept stored in polyethylene bags at room temperature till the treatments. The leaves were thick, fluffy with an oval form having striking nerve structure at the bottom. The mean thickness of the leaves (from 10 leaves) was measured as 0.99 ± 0.1 mm by using digital caliper.

Extraction

The sage samples were crushed into small particles with a blender (Beko BKK–2155 Maxi Hand Blender, Turkey) and sieved into 3 different fractions (2–4 mm; 4–6 mm and 6–8 mm) by a laboratory test sieve (Retsch, Germany) before the extraction treatments. Extraction was accomplished according to the procedure of Jaganyi and Price (1999) with minor

modifications. Four grams of the sage sample was immediately added to 196 mL of deionized water and conditioned at extraction temperature in a round bottom flask plugged with a stopper. The mixture was extracted by steady shaking in orbital shaking water bath (GFL, Germany) at 150 rpm to submerge all particles into water and to perform forced convective diffusion. The mixtures were uniformly maintained at five different constant temperatures (60, 65, 70, 75 and 80 °C) and these temperatures were validated with a thermocouple throughout each extraction process. The extraction process was performed by time dependent manner at different time intervals (0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10, 15, 20, 30, 50, 70, 90, 120, and 150 min) to equilibrium soluble solids concentration (150 min). The same procedure was applied to three different particle sizes (2–4 mm, 4–6 mm and 6–8 mm) and sage–water ratios (1, 2 and 4 %) at 80 °C.

Measurement of total soluble solids (TSS)

The total soluble solids (TSS) of sage extract were measured by its specific absorbance ($\lambda_{\max}$ 330 nm) with spectrophotometer (Shimadzu UV–Vis 160A, Japan) in a certain volume (250 µL for sage extract to measurable concentration) of dilution. The absorbance value was expressed as total soluble solids by using absorbance (A) versus TSS concentration curve of the sage extract ($\text{TSS} = 0.0075(A) - 0.002$, $R^2 = 0.999$). The stability of the absorbance was tested at 80 °C by time dependent manner.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) analyses were performed using the method developed by Skerget et al. (2005). For this purpose, 0.5 mL of the extract was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent and 2 mL of Na₂CO₃ solution (75 g/L). This mixture was incubated at 50 °C for 5 min followed by immediate cooling. The absorbance of the final solution was recorded with a spectrophotometer (Shimadzu UV–Vis 160A, Japan) at 760 nm. The results were expressed as gallic acid equivalent (g GAE/L).

Determination of total flavonoid content (TFC)

Total flavonoid content analyses of the samples were determined according to Chang et al. (2006). 2.5 mL distilled water and 150 µL of 5 % NaNO₂ solution were added into 0.5 mL extract of the samples and were allowed to stand for 5 min. after vortex. Then 300 µL of 10 % AlCl₃ solution was added into this solution and allowed to stand for further 5 min. One milliliter of 1 M NaOH solution was added and the final volume was made up to 5 mL with distilled water. Sample absorbance was measured at 510 nm by using a

spectrophotometer (Shimadzu UV–Vis 160A, Japan). The results were expressed as (+)-catechin equivalent (g CE/L).

Kinetic modeling

The majority of kinetic studies of leafy material extraction have been studied based upon the kinetic expression (Eq. 1) developed by Spiro and Siddique (1981).

$$\ln\left(\frac{C_{\infty}}{C_{\infty}-C}\right) = k_{\text{obs}}t \quad (1)$$

where C_{∞} is the equilibrium concentration of extractable solids i.e. TSS, TPC or TFC (g/L) in aqueous phase, C is the concentration of extractable solids (g/L) in aqueous phase at time t , and k_{obs} is the rate constant (s^{-1}) of the extraction process.

The data followed the straight line equation when the t was plotted versus $\ln[C_{\infty}/(C_{\infty}-C)]$. The straight line must pass through the origin; however, the experimental data did not well fit this linear model and missed the origin by a significant degree. This can be associated with a quantity of solute present on the surface of the solid which is mostly washed off into the solvent at the beginning of the experiment. As a matter of fact, the first order kinetic model (Eq. 1) is revised as reversible first order kinetic model (Eq. 2) for modeling tea infusion (Stapley 2002).

$$\ln\left(\frac{C_{\infty}}{C_{\infty}-C}\right) = k_{\text{obs}}t + a \quad (2)$$

where a is a constant for washing step of leafy material extraction.

Another useful kinetic parameter of an extraction process used for leaf based materials is half-life ($t_{1/2}$) which is the time required for half of equilibrium of the extractable solids concentration ($C_{\infty}/2$) in aqueous phase (Eq. 3).

$$t_{1/2} = \frac{(\ln 2 - a)}{k_{\text{obs}}} \quad (3)$$

Considering that the extraction process was carried out in shaking flask, the mass transfer was mostly controlled by diffusion in leaf particles. When it is considered that the shape of sage leaves is flat slab, diffusion coefficient of the extractable solids in the sage leaf can be estimated by using following equation (Eq. 4).

$$D_{\text{leaf}} = \frac{L^2 \cdot k_{\text{obs}}}{2} \quad (4)$$

where D_{leaf} is the diffusion coefficient of extractable solid in the sage leaf (m^2/s) and L is the half thickness of the swollen sage leaf (m).

The extraction rate constant depends on temperature according to the Arrhenius law (Eq. 5).

$$\ln(k_{\text{obs}}) = \ln A - \left(\frac{E_a}{RT}\right) \quad (5)$$

where A is a pre-exponential constant, E_a is the activation energy of extraction (J/mol), R is the universal gas constant (8.314 J/mol K), and T is the absolute temperature (K).

Statistical analyses

The extractions were conducted using a factorial design (3 particle size $\times$ 5 temperatures $\times$ 3 loading percentage) in two replicates. The data obtained for two parallel measurements of the replicate samples in time course was evaluated. The kinetic parameters were estimated by Sigma Plot (Version 12, Systat Software, Inc., Chicago, IL) using the reversible first order kinetic model (Eq. 2). The results of the estimated parameters were statistically evaluated by Variance Analysis and Duncan Multiple Range Test using the Statistical Analytical Systems software (SAS Institute, Cary, NC, USA).

Results and discussion

Extraction kinetics and modeling

Aqueous extraction kinetics of the total soluble solids (TSS), total phenolic content (TPC) and total flavonoid content (TFC) of sage were studied with respect to the operating parameters of temperature, particle size and loading percentage. The extraction processes were achieved by orbital shaking through force convective diffusion to prevent high concentration film forming at the surface of each particle and to provide diffusion controlled mass transfer process. The extractions were performed at different time intervals within the range of 0–150 min. accounting for the operating parameters. Almost 50 % of the extractable solids were extracted in first 30 min. followed by slow extraction until the equilibrium concentration was achieved. Similar results were published by terming the extraction stages washing, swelling and diffusion mechanism for yerba mate tea by Linares et al. (2010). In the current aqueous extraction study, the time (t) versus concentration (C) plots of TSS, TPC and TFC of the sage samples well fit with reversible first order model ($\text{Adj-R}^2 > 0.99$; $\text{RMSD} < 0.11$) in the same manner as reported previously for green tea (Price and Spitzer 1994), black tea (Jaganyi and Price 1999) and yerba mate tea (Linares et al. 2010). The model parameters for the present data were

estimated by nonlinear regression analyses. The diffusion coefficient of the extractable solids in the leaf and half-life time of the extraction process were also calculated (Tables 1, 2, and 3).

Effect of temperature

Extraction behavior of TSS, TPC and TFC of sage samples at different temperatures is illustrated in Fig. 1. The extraction was performed at five different temperatures in the range of 60–80 °C as reflecting over all temperature range of the traditional sage tea serving. However, the extractable solids must be verified for stability at high temperature extraction processes. Therefore, stability of the extractable solids was also checked at 80 °C in the present study and the stability was verified.

The extraction rate constant (k_{obs}) and equilibrium concentration (C_e) of TSS, TPC and TFC for the examined temperatures were found in range of $0.250\text{--}0.583 \times 10^{-3} \text{ s}^{-1}$, $0.333\text{--}0.683 \times 10^{-3} \text{ s}^{-1}$, $0.317\text{--}0.617 \times 10^{-3} \text{ s}^{-1}$ and $3.546\text{--}4.997 \text{ g/L}$, $1.207\text{--}1.661 \text{ g GAE/L}$, $0.826\text{--}1.352 \text{ g CE/L}$, respectively. When extraction process was performed in 2 % sage loading at different temperatures, the concentration of TSS corresponded with the extract yield of 17.73 and 24.99 g/100 g contained 6.04–8.31 g/100 g TPC and 4.13–6.77 g/100 g TFC. Velickovic et al. (2006) studied two different *Salvia* species with different solvents and they reported 28.0 % aqueous extract yield from *Salvia officinalis* and 34.4 % from *S. glutinosa* at 80 °C. Durling et al. (2007)

extracted *S. officinalis* with 81 % methanol and they found that extract yield and recovery of bioactive components of *S. officinalis* increased by increasing temperature due to higher solubility and diffusion coefficient.

Generally, the rate of extraction processes and equilibrium concentration of TSS, TPC and TFC were significantly ($p < 0.05$) increased by increasing temperature. This can be explained by disruption of the cell wall by heating or by the breakdown of insoluble phenolic compounds into soluble phenolics which leads to better extractability (Boateng et al. 2008).

Slight differences between the courses of extractions at different temperatures were observed and plotted (Fig. 1). Especially, there is explicit difference in extraction efficiency between 65 °C and 70 °C. It means that considerable amounts of soluble solids of the sage samples need at least 70 °C to dissolve and release from the leaf particles. It may also be related with microstructure of the sage which may radically change after a certain temperature. Linares et al. (2010) reported that changing in microstructure of yerba mate tea during the extraction process altered mass transfer rate and mechanism which can be associated with temperature.

The change in extraction rate constant as a function of absolute temperature obeyed the Arrhenius relationship (Eq. 5). Temperature dependence of the extraction processes was observed by plotting straight line of $1/T$ versus $\ln(k_{\text{obs}})$ (Fig. 2). E_a values for the extraction of TSS, TPC and TFC were determined as 46.11 ± 5.61 , 36.80 ± 3.12 and $33.52 \pm 2.23 \text{ kJ/mol}$, respectively. The results (14–59 kJ/mol) are in

Table 1 Kinetic parameters for the extraction of TSS, TPC, TFC from sage leaf particles (2–4 mm) in shaking water at 2 % (Mean $\pm$ SE, $n=2$)

	Temperature (°C)	C_e (g/L)	$k_{\text{obs}} \times 10^3$ (s^{-1})	a	$D_{\text{leaf}} \times 10^9$ (m^2/s)	$t_{1/2}$ (min)	Adj-R ²	RMSD
TSS	60	$3.546^c \pm 0.234$	$0.250^c \pm 0.033$	$0.042^a \pm 0.001$	$0.117^c \pm 0.017$	$45.39^a \pm 5.36$	0.995	0.076
	65	$3.968^c \pm 0.102$	$0.283^c \pm 0.033$	$0.033^b \pm 0.000$	$0.133^c \pm 0.017$	$39.30^{ab} \pm 3.84$	0.998	0.065
	70	$4.552^b \pm 0.044$	$0.367^b \pm 0.000$	$0.033^b \pm 0.001$	$0.183^b \pm 0.000$	$29.56^{bc} \pm 0.14$	0.996	0.111
	75	$4.780^{ab} \pm 0.019$	$0.550^a \pm 0.000$	$0.023^c \pm 0.000$	$0.267^a \pm 0.000$	$20.11^c \pm 0.15$	0.999	0.074
	80	$4.997^a \pm 0.015$	$0.583^a \pm 0.000$	$0.022^c \pm 0.001$	$0.283^a \pm 0.000$	$19.41^c \pm 0.15$	0.997	0.101
TPC	60	$1.207^c \pm 0.061$	$0.333^c \pm 0.033$	$0.065^a \pm 0.002$	$0.167^c \pm 0.017$	$31.19^a \pm 2.66$	0.992	0.036
	65	$1.309^c \pm 0.036$	$0.417^b \pm 0.000$	$0.050^b \pm 0.003$	$0.200^b \pm 0.000$	$25.84^b \pm 0.39$	0.995	0.036
	70	$1.541^b \pm 0.004$	$0.450^b \pm 0.000$	$0.048^b \pm 0.002$	$0.217^b \pm 0.000$	$23.89^b \pm 0.01$	0.998	0.026
	75	$1.593^{ab} \pm 0.009$	$0.650^a \pm 0.017$	$0.035^c \pm 0.002$	$0.317^a \pm 0.000$	$16.72^c \pm 0.15$	0.998	0.027
	80	$1.661^a \pm 0.003$	$0.683^a \pm 0.000$	$0.037^c \pm 0.001$	$0.333^a \pm 0.000$	$15.90^c \pm 0.16$	0.997	0.034
TFC	60	$0.826^c \pm 0.007$	$0.317^d \pm 0.017$	0.045 ± 0.001	$0.150^d \pm 0.000$	$33.92^a \pm 1.61$	0.995	0.020
	65	$0.921^d \pm 0.000$	$0.400^c \pm 0.000$	0.034 ± 0.000	$0.200^c \pm 0.000$	$27.43^b \pm 0.06$	0.997	0.018
	70	$1.163^c \pm 0.009$	$0.450^b \pm 0.017$	0.033 ± 0.006	$0.217^b \pm 0.017$	$24.07^c \pm 0.70$	0.998	0.019
	75	$1.277^b \pm 0.000$	$0.617^a \pm 0.000$	0.025 ± 0.006	$0.300^a \pm 0.000$	$18.28^d \pm 0.09$	0.999	0.019
	80	$1.352^a \pm 0.014$	$0.600^a \pm 0.017$	0.031 ± 0.001	$0.300^a \pm 0.000$	$18.22^d \pm 0.29$	0.999	0.019

Values in a column followed by different superscript letters are significantly ($p < 0.05$) different

Table 2 Kinetic parameters for the extraction of TSS, TPC, TFC from different size of sage leaf particles in shaking water at 80 °C (Mean $\pm$ SE, $n=2$)

	Particle size (mm)	C_e (g/L)	$k_{obs} \times 10^3$ (s ⁻¹)	a	$D_{leaf} \times 10^9$ (m ² /s)	$t_{1/2}$ (min)	Adj-R ²	RMSD
TSS	2–4	4.997 $\pm$ 0.015	0.583 ^a $\pm$ 0.000	0.022 ^a $\pm$ 0.001	0.283 ^a $\pm$ 0.000	19.41 ^c $\pm$ 0.15	0.999	0.019
	4–6	5.003 $\pm$ 0.015	0.400 ^b $\pm$ 0.000	-0.013 ^b $\pm$ 0.000	0.200 ^b $\pm$ 0.000	29.61 ^b $\pm$ 0.43	0.995	0.035
	6–8	5.178 $\pm$ 0.091	0.317 ^c $\pm$ 0.000	-0.015 ^c $\pm$ 0.000	0.150 ^c $\pm$ 0.000	38.31 ^a $\pm$ 0.60	0.996	0.033
TPC	2–4	1.661 $\pm$ 0.003	0.683 ^a $\pm$ 0.000	0.037 ^a $\pm$ 0.001	0.333 ^a $\pm$ 0.000	15.90 ^b $\pm$ 0.16	0.997	0.034
	4–6	1.641 $\pm$ 0.035	0.467 ^b $\pm$ 0.017	-0.013 ^b $\pm$ 0.001	0.233 ^b $\pm$ 0.017	25.02 ^a $\pm$ 0.89	0.996	0.041
	6–8	1.558 $\pm$ 0.004	0.467 ^b $\pm$ 0.000	-0.015 ^b $\pm$ 0.000	0.233 ^b $\pm$ 0.000	25.70 ^a $\pm$ 0.34	0.997	0.035
TFC	2–4	1.352 $\pm$ 0.014	0.600 ^a $\pm$ 0.017	0.031 ^a $\pm$ 0.001	0.300 ^a $\pm$ 0.000	18.22 ^b $\pm$ 0.29	0.999	0.019
	4–6	1.297 $\pm$ 0.047	0.433 ^b $\pm$ 0.000	-0.019 ^b $\pm$ 0.003	0.217 ^b $\pm$ 0.000	27.35 ^a $\pm$ 0.58	0.995	0.035
	6–8	1.258 $\pm$ 0.021	0.400 ^b $\pm$ 0.033	-0.020 ^b $\pm$ 0.004	0.200 ^b $\pm$ 0.017	30.16 ^a $\pm$ 2.25	0.996	0.033

Values in a column followed by different superscript letters are significantly ($p<0.05$) different

agreement with findings of previous studies on different leafy materials (Price and Spitzer 1994; Jaganyi and Price 1999; Spigno and De Faveri 2009; Linares et al. 2010). The differences in activation energy are either related to material utilized or extraction procedure used. To the best of our knowledge, no comparable kinetic study is reported for soluble solids and phenolic extraction of *S. fruticosa*.

The extraction rate of TSS and TFC in sage particles were found to be comparatively more temperature dependent than TPC as shown by Fig. 2. This may be related to their location in leaf structure e.g., the TPC fraction may be placed near to the surface of the particles and it is easy to be washed into solvent at any temperature. However, other fractions which were more temperature dependent can be extracted from the leaf particles through diffusion and the diffusion rate increased by increasing temperature. This can also be explained in the sense of molecular size of the fractions and their solubility in water. As the TPC contains both phenolic acid and flavonoids,

TFC represents only flavonoids which are mostly bounded as esters or glycosides with bigger molecular size (Lee 2004; Skerget et al. 2005). Likewise, Medoua and Mbofung (2007) propound molecular size hypothesis for extraction rate of water soluble solids in *Cassia* seeds.

Effect of particle size

Extraction of the TSS, TPC and TFC of sage was also investigated as a function of particle size. Extractions were performed on three different sizes (2–4, 4–6 and 6–8 mm) by loading 2 % sage in shaking water at 80 °C. The particle size had significant ($p<0.05$) effect on the extraction of the TSS, TPC and TFC of sage (Fig. 3). The kinetic parameters of the extractions were determined by using the reversible first order kinetic model and the results were statistically evaluated depending on the particle size of sage samples (Table 2). The particle size showed significant ($p<0.05$) effects on the rate

Table 3 Kinetic parameters for the extraction of TSS, TPC, TFC from different amount of sage leaf particles (2–4 mm) in shaking water at 80 °C (Mean $\pm$ SE, $n=2$)

	Sage loading (%)	C_e (g/L)	$k_{obs} \times 10^3$ (s ⁻¹)	a	$D_{leaf} \times 10^9$ (m ² /s)	$t_{1/2}$ (min)	Adj-R ²	RMSD
TSS	1	2.544 ^c $\pm$ 0.053	0.600 ^b $\pm$ 0.017	0.027 $\pm$ 0.006	0.283 ^b $\pm$ 0.000	18.71 ^a $\pm$ 0.44	0.999	0.034
	2	4.997 ^b $\pm$ 0.015	0.583 ^b $\pm$ 0.000	0.022 $\pm$ 0.001	0.283 ^b $\pm$ 0.000	19.41 ^a $\pm$ 0.15	0.997	0.101
	4	11.385 ^a $\pm$ 0.150	0.667 ^a $\pm$ 0.017	0.029 $\pm$ 0.003	0.333 ^a $\pm$ 0.017	16.52 ^b $\pm$ 0.43	0.998	0.194
TPC	1	0.911 ^c $\pm$ 0.007	0.700 ^b $\pm$ 0.017	0.019 ^b $\pm$ 0.006	0.350 ^b $\pm$ 0.000	15.90 ^a $\pm$ 0.50	0.998	0.016
	2	1.661 ^b $\pm$ 0.003	0.683 ^b $\pm$ 0.000	0.037 ^a $\pm$ 0.001	0.333 ^b $\pm$ 0.000	15.90 ^a $\pm$ 0.16	0.997	0.034
	4	3.444 ^a $\pm$ 0.027	0.833 ^a $\pm$ 0.000	0.043 ^a $\pm$ 0.000	0.400 ^a $\pm$ 0.000	13.13 ^b $\pm$ 0.00	0.998	0.059
TFC	1	0.688 ^c $\pm$ 0.008	0.700 $\pm$ 0.067	-0.004 ^b $\pm$ 0.002	0.350 $\pm$ 0.033	16.75 $\pm$ 1.45	0.988	0.031
	2	1.352 ^b $\pm$ 0.014	0.600 $\pm$ 0.017	0.031 ^a $\pm$ 0.001	0.300 $\pm$ 0.000	18.22 $\pm$ 0.29	0.999	0.019
	4	2.920 ^a $\pm$ 0.000	0.717 $\pm$ 0.017	0.038 ^a $\pm$ 0.005	0.350 $\pm$ 0.000	15.38 $\pm$ 0.13	0.997	0.062

Values in a column followed by different superscript letters are significantly ($p<0.05$) different

Fig. 1 Influence of the temperature on extraction of TSS (a), TPC (b) and TFC (c) from sage

constant, diffusion coefficient and half-life of the extraction time of TSS, TPC and TFC. However, the equilibrium concentration of the extractable solids were not significantly

Fig. 2 Arrhenius plot for the extraction of TSS, TPC and TFC from sage

($p > 0.05$) affected by particle size. Generally, the particle size greater than 4 mm gave almost same results in the kinetic parameters, however; the particles smaller than 4 mm caused the highest extraction rates thereby the shortest half-life (Table 2). Considering that the leaf particles are flat slap and the thicknesses are almost same for each class, the extraction rates which are mostly controlled by diffusion of the extractable solids through the broader side of the particles would not change with increasing particle size.

The experimental data of the particle size versus diffusion coefficient (D_{leaf}) of the TSS, TPC and TFC can be expressed in terms of exponential function (Eq. 6) as stated by Bucic-Kojic et al. (2007),

$$D_{leaf} = a \ln(S_p) + b \quad (6)$$

where S_p is average particle size, a and b are equation constants. The equation parameters are estimated for TSS, TPC and TFC by nonlinear regression analyses (Table 4).

The change in diffusion coefficient of the TSS depending on average particle size can be represented in a better way by exponential function than the other parameters as shown in Table 4. This may also be associated with location of the extractable solids in the leaf particles.

Effect of sage loading percentage

During conventional solvent extraction process, driving force is the gradient concentration and increasing the loading percentage (at both constant temperature and time) enhances the solute recovery. Owing to this fact, the extraction of TSS, TPC and TFC from sage was also investigated at different loading concentration of the sage.

Extraction behavior of the tested parameters for 1 %, 2 % and 4 % loading concentration of the sage are illustrated in Fig. 4. The higher loading amount of the sage was not possible due to its soaking and a

Fig. 3 Influence of particle size on extraction of TSS (a), TPC (b) and TFC (c) from sage

homogenous extraction was troublesome. The equilibration time of the tested parameters was found around 80 min (Fig. 4). The time courses of the extraction

Table 4 Results of non-linear regression analyses for D_{leaf} dependence of average particle size

Parameters	$a \times 10^9$	$b \times 10^9$	R^2
TSS	-0.2	0.5	0.994
TPC	-0.1	0.5	0.869
TFC	-0.1	0.4	0.917

data, depending on the loading amount, were fitted to reversible first order kinetic model (Eq. 2) by nonlinear regression analyses. The estimated equilibrium concentration, extraction rate constant and diffusion coefficient are presented in Table 3. It is inferred from the results that the equilibrium concentration of the mixtures loaded in 1 %, 2 % and 4 % sage samples were determined in the range of 2.44–11.39 g/L for TSS, 0.91–3.44 g/L for TPC and 0.69–2.92 g/L for TFC. The equilibrium concentrations were found according to the expectation as they were consistent with the loading percentages. The TSS yield was about 25–28 % with the loading amounts of 1–4 %. The equilibrium concentrations of the tested parameters increased linearly by increased the loading percentage of the sage sample. The similar linear relationship of loading amount in extraction treatments are reported previously with both water and water–ethanol mixture (Cacace and Mazza 2003; Durling et al. 2007; Rakotondramasy–Rabesiaka et al. 2008).

Extraction rate constant thereby diffusion coefficient and half-life of the extraction time of TSS and TPC showed significant ($p < 0.05$) changes by the loading percentages, however, no significant ($p > 0.05$) changes were observed for the TFC. Especially, 4 % of loading amount resulted in noticeable differences in the kinetic parameters for the TSS and TPC fractions. The differences in the kinetic parameters for TFC were not significant. Normally, mass transfer phenomenon in a solid–solvent extraction is related to concentration differences between solid and solvent fractions. The increasing amount of sage loading is supposed to decrease the mass transfer rate due to higher concentration of soluble solids in solvent fractions. Interestingly, in case of 4 % loading for the present study led to increase the extraction rate constant compared to 1 and 2 % loading. Spigno and De Faveri (2009) reported that increasing the liquid/solid ratio (loading percentage) at constant temperature of extraction process enhances mass transfer due to the driving force of gradient concentration. The differences in 4 % sage loading for the present study may be associated with enhancement of solid–solvent interaction due to submerging effect of the higher load amount.

Fig. 4 Influence of sage loading percentage on extraction of TSS (a), TPC (b) and TFC (c) from sage

Conclusion

In the present study, aqueous extraction behavior of TSS, TPC and TFC of *S. fruticosa* as time course was studied depending

on temperature, particle size and loading amount. Almost 25 % of TSS, which contained at least 30 % TPC and 25 % TFC, was yielded from the extractions at 80 °C. The stability of the results obtained from tested variables was verified throughout the extraction period at the highest extraction temperature of 80 °C. The data obtained was found to be well fit with the reversible first order kinetic model which was also used in previous studies on extraction of similar leafy materials. Based on the estimated kinetic parameters, all of the studied variables were evaluated and they showed significant effects on the kinetic parameters. Among the variables, only particle size did not show any significant effect on the equilibrium concentration. From a practical point of view, the dried *S. fruticosa* leaves should be loaded up % 4, extracted at least at a critical temperature of 70 °C for 80 min and after crushing into 4 mm particle size to get higher extraction yield.

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