

RESEARCH PAPER

Effect of supercritical carbon dioxide conditions on extraction of food phytochemical constituents from *Moringa oleifera*. Lam seed kernels

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ABSTRACT

Supercritical fluid extraction (SFE) has shown a great potential for the extraction and isolation of phytochemicals from various food samples as it minimizes sample handling, provides fairly clean Process extracts, SC-CO₂ is the fluid most commonly used in SFE with several advantages. Supercritical fluid extraction was carried out at selected SC-CO₂ pressures (100, 150 and 200 bar) and temperatures (40, 50 and 60 °C). The concentrations of food phyto-chemicals viz., total phenols, total flavonoids, total carotenoids, total sterols and total tocopherols from *Moringa oleifera*. Lam seed kernels were found to be 41.82 to 44.71 mg GAE/g, 14.82 to 18.25 mg RE/g, 15.45 to 17.06 ppm, 892.05 to 984.17 ppm, 80.27 to 92.26 ppm were highest at 200 bar pressure and 50 °C temperature. All of these parameters were significantly (p<0.01) affected by SC-CO₂ conditions. With increasing in pressure from 100 to 200 bar, the extraction of phyto-chemicals increased, in case of temperature up to 50 °C phyto-chemicals extraction increased, further increasing in temperature from 50 to 60 °C there will be a decrease in phyto-chemical concentration. Extraction yield of phyto-chemical constituents found 35.26 % in a SFE, which is more than the conventional soxhlet extraction technique (17.12 %). Therefore, phyto-chemical constituents present in the *Moringa oleifera*. Lam seed kernels could be efficiently extracted by using SC-CO₂ with standardized process conditions.

Keywords: Supercritical carbon dioxide, phenols, flavonoids, carotenoids, sterols, tocopherols

Moringa oleifera. Lam (Common name: Drum stick) is well known to the ancient world, but only recently it has been rediscovered as a multipurpose tree with a tremendous variety of potential uses. Its seeds are round with a brownish semi-permeable seed hull, with three papery wings. Seed hulls are generally brown to black, but can be white if kernels are of low viability (Paliwal *et al.*, 2011). It has been reported by Bureau of Plant Industry that, moringa is an outstanding source of nutritional components.

Almost every part of the plant (leaves, flowers, seeds, roots and bark) can be used as the food or medicinal and therapeutic purposes, especially in developing countries (Anwar *et al.*, 2006). The seeds can be eaten green, roasted, powdered and steeped for tea or used in curries. The seeds have been reported to possess strong coagulative and anti-microbial properties on pathogenic strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Salmonella typhi* and *Shigella dysenteriae* (Oluduro *et al.*, 2010).

The extraction of active food phyto-chemicals of plants require the technique that does not damage these biochemically active compounds. Supercritical fluid extraction (SFE) complies with these requirements. Carbon dioxide (CO₂) is the most widely used as Su solvent. Its critical temperature (31 °C) makes it an ideal solvent for extracting thermally labile materials and it eliminates from the extract after extraction. CO₂ is also non-toxic, non-flammable, environmentally acceptable and inexpensive. These properties of SFE make the products more advantageous in the field of food, pharmaceuticals and cosmetics (Machmudah *et al.*, 2007). SC-CO₂ therefore, has been proposed as an alternative of light petroleum fractions for vegetable oil extraction and biological active compounds. Previous works on optimization of supercritical fluid extraction process for moringa (PKM-1) seed kernel oil have been reported recently (Dinesha *et al.*, 2015; Zhao and Zhang, 2013; Nguyen *et al.*, 2011), but the influence of extraction conditions on food phytochemicals have not been reported so far. Further although, adoption of moringa seed essential oil extraction with SC-CO₂ implies the necessity for studying the effect of SC-CO₂ extraction parameters, *i.e.* temperature and pressure on extraction. So the study was conducted and reported here.

MATERIALS AND METHODS

Raw material

The experiment was conducted in the Department of Processing and Food Engineering, College of Agricultural Engineering, Raichur, Karnataka. Clean and dried seeds of moringa (PKM-1) were procured from M/s. Bharath seeds, Raichur (Karnataka). The shells were removed manually and the kernels were ground in a laboratory hammer mill to obtain fine powder (Nguyen *et al.*, 2011). The solvents, chemicals and reagents (analytical grade) used throughout the experiment were procured from M/s. Sigma Aldrich Chemicals, Bangalore (Karnataka).

Experiment

The supercritical carbon dioxide (SC-CO₂) extraction system (Thar; SFE 500) was used for extraction of food phytochemicals from *Moringa oleifera*. Lam seed kernels. Experimental setup for SC-CO₂ extraction of Moringa seed kernels is shown in Plate. 1. Deionized water (at 5 °C) was used for cooling different zones in the SC-CO₂ extraction system. The independent variables selected for the study were SC-CO₂ pressures of 100, 150 and 200 bar and temperatures of 40, 50 and 60 °C at constant dynamic extraction time of 90 min (Liza, 2010).

Fifty grams of Moringa seed kernel powder was placed into the extracting vessel. The flow rates of supercritical CO₂ and co-solvent (ethanol) were maintained at 20 and 2 g/min, respectively (Pradhan *et al.*, 2010) and static extraction process was performed for 30 min (Palafox *et al.*, 2012). After attaining desired pressure and temperature, dynamic extraction time (90 min) was started by opening the exit valve of the SC-CO₂ extraction system.

Soxhlet extraction method used as a control (SOCS-PLUS apparatus; Make: Pelican Equipments; Model: SCS-08) with hexane as solvent. The extraction was carried out at 85 °C temperature and extraction time of 90 min (Malapit, 2010).

The static extraction time allowed the sample to soak in the CO₂ and co-solvent in order to equilibrate the mixture at desired pressure and temperature. During the dynamic extraction time, CO₂ carrying the crude extract flowed out of the extraction vessel and then into a collection vessel, where the CO₂ was separated through the vent connected to the fume hood. Moringa seed kernel extract obtained from SC-CO₂ extraction was collected and the residual content of co-solvent (ethanol) was removed by using a rotary flash vacuum evaporator (Superfit, Rotavap; PBU-6D) under vacuum at 40 °C (Mani *et al.*, 2007).

Analysis of phytochemicals

The concentration of total phenols in the *Moringa oleifera*. Lam seed kernel extract (FCR) was estimated

with the Folin Ciocalteu reagent method. Moringa seed kernel extract of 100 μ l was added to 900 μ l of distilled water and 0.5 ml of FCR was added, mixed and kept for 3 min. After 3 min, one ml of 15% Na_2CO_3 solution was added, the contents were mixed and the volume was made upto 10 ml with distilled water. After 45 min of incubation at room temperature, the absorbance was measured at 750 nm against the reagent blank. Gallic acid was used as the standard for preparing the calibration curve (0.05-0.4 mg/ml). The sample absorbance was interpolated on the standard graph and the total phenolic compound (such as gallic acid) was calculated and expressed as mg GAE/g (Bhatnagar and Krishna, 2013).



Plate 1: Experimental setup for SC- CO_2 extraction of *Moringa oleifera*. Lam seed kernels

Total flavonoids in *Moringa oleifera*. Lam seed kernel extract was determined by aluminum trichloride colourimetric method by using rutin as standard. This method was based on formation of flavonoid-aluminum complex. The concentrated seed kernel extract (0.1 ml) in methanol (100 μ g/ml) was mixed with 0.2 ml of 5% sodium nitrate, then allowed to react for 5 min, thereafter 0.2 ml aluminum trichloride in methanol (10%) and one ml of sodium hydroxide (1M) were added, then allowed to stand at room temperature for 15 min. The absorbance

was read at 510 nm against reagent blank. The amount of total flavonoids was calculated from rutin calibration curve. The results were expressed in mg of rutin equivalent per gram of sample (mg RE/g) (Ogbunugafor *et al.*, 2011).

The total carotenoid was determined according to the concentration method given by Dauqan *et al.* (2011). 100 mg of moringa seed kernel extract was weighed and diluted with 10 ml of acetone, mixed well by vortexing and read at 446 nm (UV-1601, UV visible spectrophotometer) against a blank of pure acetone. One mg/ml standard β -carotene in acetone solution was prepared and its five aliquots (0.5, 1.0, 1.5, 2.0 and 2.5 ml) were read at 446 nm standard curve was generated by plotting the absorbance

of β -carotene against the concentrations of aliquots. The sample absorbance was interpolated on the standard graph and the total carotenoids content (β -carotene) was calculated and expressed as ppm (Bhatnagar and Krishna, 2013).

The total sterols content in the *Moringa oleifera*. Lam seed kernel extract was determined by Liberman-Burchard method (Sabir *et al.* 2003). One gram

of concentrated moringa seed kernel extract was weighed and diluted with 10 ml chloroform and mixed well by vortexing. Aliquot of three ml was taken and two ml of the Liberman-Burchard reagent (0.5 ml of sulphuric acid dissolved in 10 ml of acetic anhydride) added and the final volume was made upto 7 ml with chloroform. Liberman-Burchard reagent reacted with the sterol and produced a characteristic green colour whose absorbance was read at 640 nm. A standard cholesterol solution (1 mg/ml) was prepared and its five aliquots of 0.5, 1, 1.5, 2, and 2.5 ml were taken. A quality of two ml of Liberman-Burchard reagent was then, added and the final volume was made up to 7

ml with chloroform. This mixture was incubated in the dark for 15 min and read at 640 nm. A standard cholesterol curve was generated by plotting the absorbance of cholesterol against the concentrations of cholesterol. The *Moringa oleifera*. Lam seed kernel extract absorbance was interpolated on the standard graph and total sterol concentration was calculated and expressed as ppm (Bhatnagar and Krishna, 2013).

The total tocopherols content of moringa seed kernel extract was determined according to method described by Wong *et al.* (1988). Concentrated extract about 0.1 to 0.2 mg, 5 ml of toluene and 3.5 ml of 2, 2- bipyridine (0.07% w/v in 95% aqueous ethanol) were added into 10 ml volumetric flask. This solution was made up to 10 ml with 95% aqueous ethanol. After standing for one minute the absorption at 520 nm was determined using as a reference solution, prepared as above but omitting the sample. Solution was protected from strong light during colour development (Dauqan *et al.*, 2011).

The method was calibrated by preparing standard

containing 0.24 µg of pure α-tocopherol in 10 ml of toluene and then analyzed as above. The concentration of tocopherol in the moringa seed kernel extract was calculated by using equation given below:

$$\text{Total tocopherols (ppm)} = \frac{(A - B)}{M \times W} \quad \dots (1)$$

where, A= Absorption of sample, B= Absorption of blank, M= Gradient of absorbance Vs weight graph for α-tocopherol calibration (8.0×10^{-3} absorbance / µg α-tocopherol) and W= Weight of sample, g.

Statistical Design

The experiments were conducted with factorial design (3^2), which referred to two independent variables and three levels selected for each independent variable. The analyses were performed using the software, Design Expert Version 7.7.0 (State-Ease, Minneapolis, MN). SEM and CD @ 1% were calculate by using Simple CRD design.

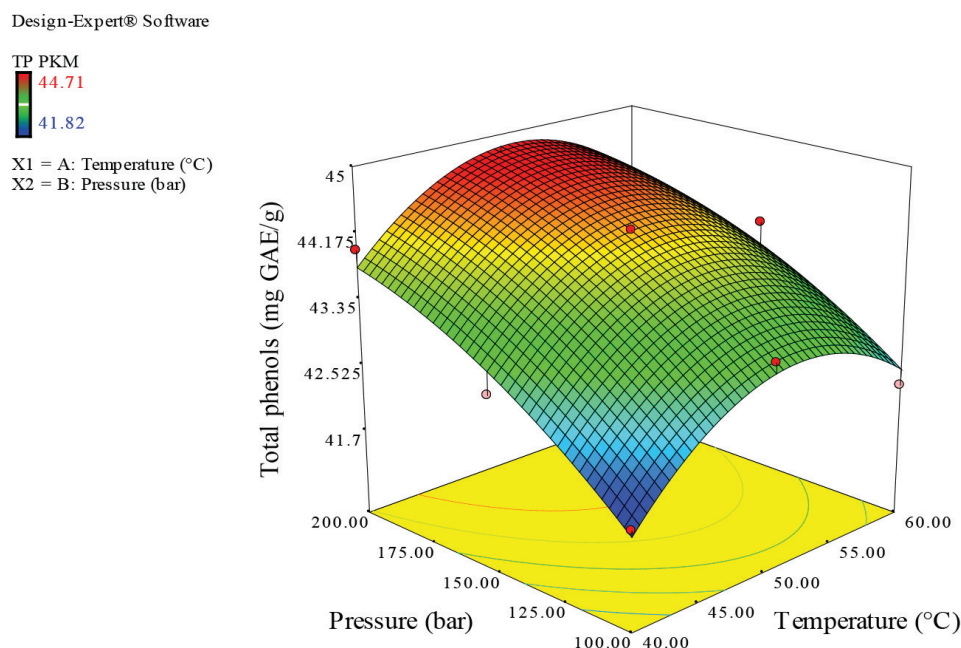


Fig. 1: Effect of temperature and pressure of SC-CO₂ on total phenols

RESULTS AND DISCUSSION

Effect of SC-CO₂ temperature and pressure on total phenols

The effect of SC-CO₂ temperature and pressure on total phenols of *Moringa oleifera* seed kernels presented in Fig. 1, shows that, as the pressure increased from 100 to 200 bar, the total phenols in the extract also increased. This might be due to the fact that, the increased extraction pressure led to higher fluid density thereby, increased the solvent strength and solubility of the phenols in CO₂. It is also evident from the figure that the total phenols increased as temperature was increase from 40 to 50 °C.

However, a temperature increased from 50 to 60 °C caused a decreased in the total phenolic content which might be due to reduction in the density of CO₂. Similar effect of pressure and temperature on extraction of bioactive compounds from *Ampelopsis grossedentata* stems had been reported by Wang *et al.* (2010). Soxhelt extraction (control) found total phenol concentration of 40.18 (mg GAE/g), it is lower than the SFE.

From the results, it can also be observed that the temperature used for the extraction of phenolic

compounds varies greatly. Some phenolic compounds are thermosensitive and therefore higher extraction temperatures need to be carefully used. The density of CO₂ at constant pressure is reduced with increased temperature that leads to the reduction of fluid solvent power. The effect of temperature on solute solubility is different at pressures in the critical range. Near the system critical pressure, the fluid density is very sensitive to temperature. A moderate increase in temperature can lead to a large decrease in fluid density, with a consequent reduction in solute solubility (Roop *et al.*, 1989). However, the increase in temperature will also accelerate mass transfer and improves the extraction yield (Wang *et al.*, 2008). The increase in temperature can increase the vapor pressure of the extractable compounds. Thus, the tendency of the compounds to be extracted is increased as they can pass to the supercritical fluid phase. For a volatile solute, there is competition between its solubility in supercritical carbon dioxide and its volatility (Chen *et al.*, 2009). However, it is always difficult to predict the effect of temperature on the extraction yield of phenolic compounds.

Kwon *et al.* (2010) reported supercritical carbon dioxide extraction of phenolics and tocopherols enriched oil from wheat bran. At constant pressure of

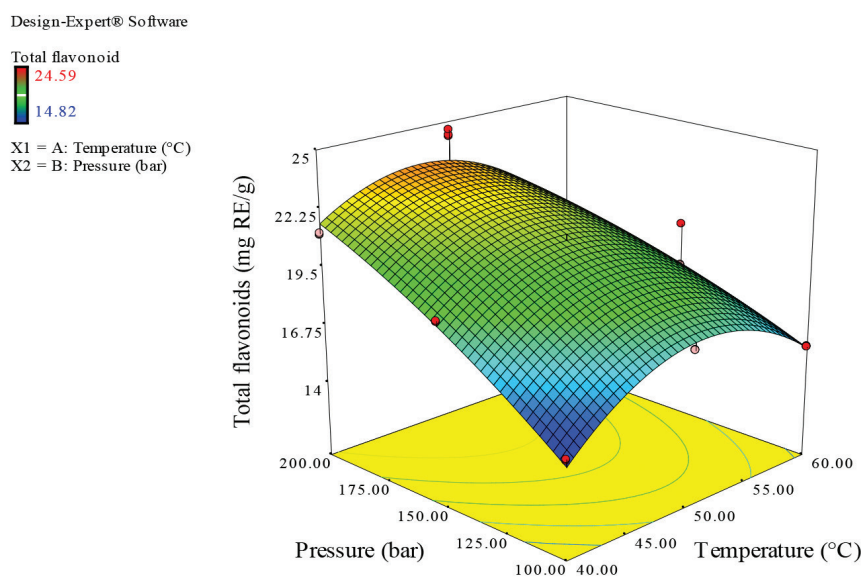


Fig. 2: Effect of temperature and pressure of SC-CO₂ on total flavonoids

15, 20, 25 and 30 MPa, the solubility of total phenolic content (TPC) increased with increase in temperature. Since, increase in temperature decreased the viscosity and increased the diffusivity resulting in an increased extraction rate. But at 15 MPa, the solubility of total phenolic content (TPC) decreased with increasing temperature.

Effect of SC-CO₂ temperature and pressure on total flavonoids

The effect of SC-CO₂ temperature and pressure on total flavonoids of *Moringa oleifera* seed kernels (Fig. 2) revealed that the variation in total flavonoids ranging from 14.82 to 18.25 mg RE/g of extract. Among the different treatment combinations, the total flavonoids of 18.25 mg RE/g was recorded to be the highest at SC-CO₂ pressure of 200 bar and temperature of 50 °C, whereas the lowest value of 14.82 mg RE/g was recorded at SC-CO₂ pressure of 100 bar and temperature of 40 °C. The effect SC-CO₂ temperature and pressure on total flavonoids was significantly different (p<0.01) at one per cent level. Total flavonoids concentration increased with the increase of pressure from 100 to 200 bar. This might be due to the increased SC-CO₂ density which caused the increase in solvent strength and solubility of the flavonoids in CO₂ (Ogbunugafor *et al.*, 2011). The control extraction technique showed lower values of total flavonoids concentration (13.90 mg RE/g) compared to the SC-CO₂ extraction.

It is also observed from the figure that the total flavonoids content increased as temperature was increased from 40 to 50 °C. However, the temperature increased from 50 to 60 °C caused a decrease in the flavonoids content which might be due to decreased fluid density and thus reduced the total flavonoids in the *Moringa* seed extract. The effect of pressure and temperature on extraction of total flavonoids confirmed with the results reported by Liza *et al.* (2010) and Cao *et al.* (2007).

Effect of SC-CO₂ temperature and pressure on total carotenoids, sterols and tocopherols

It is evident from the Table 1 that, as the pressure

increased from 100 to 200 bar, the carotenoids content in the extract also increased. Further the total carotenoids content also increased as temperature increased from 40 to 50 °C. It might be due to the fact that increased temperature contributes to decompositions of cell walls, and as the results carotenoids availability for extraction was increased. However, a temperature increase from 50 to 60 °C caused a decline in the carotenoids content which might be due to decreased fluid density and thus, reduced the total carotenoids in the extract (Machmudah *et al.*, 2007; Uquiche *et al.*, 2012).

Table 1: Effect of SC-CO₂ temperature and pressure on total carotenoids, sterols and tocopherols

Temperature °C	Pressure (bar)	TC** (ppm)	TS*** (ppm)	TT*** (ppm)
40	100	15.45	892.05	80.27
	150	15.75	901.57	84.77
	200	16.62	930.31	92.26
50	100	16.87	917.86	78.94
	150	16.88	922.62	83.69
	200	17.06	964.54	84.72
60	100	16.75	920.35	76.75
	150	16.77	930.42	81.45
	200	16.81	984.17	85.15
	Mean	17.78	929.49	83.45
	SEM	0.18	9.62	1.50
	CD@ 1%	0.12	0.35	0.36
	CV	0.29	0.01	0.2
	Soxhlet extraction (Control)	15.12	880.57	75.12

Number of replications=3. TC, total carotenoids; TS, total sterols; TT, total tocopherols.

Significance level p<0.01 *Significance level p<0.001

Sterols are minor components of all the vegetable oils comprising major portion of the unsaponifiable fraction of the oil (Dunford and King, 2000). The total sterols of supercritical fluid extracted *Moringa oleifera* seed kernels varied from 892.05 to 930.31 ppm at

40°C temperature and at different extraction pressures, whereas at 50 and 60 °C temperatures, the total sterol content varied from 917.86 to 964.54 ppm and from 920.35 to 984.17 ppm, respectively. The effect of SC-CO₂ conditions on extraction of total sterols was significantly found different ($p < 0.01$) at one per cent level. The total sterols of 984.17 ppm was recorded to be the highest at SC-CO₂ pressure of 200 bar and temperature of 60 °C, whereas the lowest value of 892.05 ppm were recorded at SC-CO₂ pressure of 100 bar and temperature of 40 °C. It is evident from the table that, as the pressure increased from 100 to 200 bar, the total sterols content in the extract was also increased. This might be due to increased fluid density which caused the increase in solvent strength and solubility of the total sterols in CO₂. The density of supercritical CO₂ increased at constant pressures, but magnitude of such density changes becomes smaller at elevated pressures (Ixtain, *et al.*, 2010). The effect of pressure and temperature in the present investigation is similar with the results obtained by Dunford and King, (2000).

The effect of SC-CO₂ temperature and pressure on total tocopherols of *Moringa oleifera* seed kernels are also included in Table 1. The pressure increased from 100 to 200 bar, the total tocopherols content in the extract also increased. This might be due to increased fluid density which caused the increase in solvent strength and solubility of the total tocopherols in CO₂. The total tocopherols content decreased as temperature was increased from 40 to 60 °C. This might be due to increase the temperature increased the viscosity and decreased the diffusivity resulting a decreased solubility of salute (Kwon *et al.*, 2010; Machmudah *et al.*, 2007). The effect of pressure and temperature in the present investigation is similar to the results reported by Uquiche *et al.* (2012).

The soxhelt extraction was found total carotenoids, sterols and tocopherols concentration of 15.12 (ppm), 880.57 (ppm) and 75.12 (ppm), these were lower than the SC-CO₂ extraction.

CONCLUSION

Moringa seed kernel phyto-chemicals were extracted in a high pressure apparatus using SC-CO₂ at different temperatures and pressures. In the conditions performed in this study, the highest total phenols, total flavonoids and total carotenoids content of extract was found at 50°C and 200 bar of SC-CO₂. Moringa seed kernel extract had maximum amount of total sterols at pressure of 200 bar and temperature of 60 °C. Total tocopherols content of 92.26 ppm was found maximum at SC-CO₂ pressure of 200 bar, temperature of 40 °C. Therefore, phytochemical constituents in moringa seed kernels could be efficiently extracted by using SC-CO₂ with standardized process conditions.

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