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Effect of Supercritical Carbon dioxide (SC-CO_2) Temperature and Pressure on Physico-chemical Properties of Moringa (*Moringa oleifera Lam.*) Seed Kernel Oil

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Oil was extracted from moringa (PKM-1) seed kernel by using supercritical fluid (SC-CO_2) extraction process. Supercritical fluid pressures of 100, 150 and 200 bar and temperatures of 40, 50 and 60 °C were considered for SC-CO_2 extraction process. Maximum extraction yield of 37.76 g/100g and extraction efficiency of 98.43% were obtained at supercritical fluid pressure of 200 bar and temperature of 40 °C. The SC-CO_2 temperature had significant effect on most physico-chemical properties than the pressure. Physico-chemical properties *viz*., density, specific gravity, color value (*b**), acid value and saponification value decreased with increase in extraction temperature. Colour value *L**, color value *a**, iodine value and peroxide value increased with increasing temperature. The physico-chemical properties of SC-CO_2 extracted moringa seed kernel oil were found to be superior over solvent extracted oil. The production cost of oil from moringa (PKM-1) seed kernel using supercritical fluid extraction equipment was estimated and benefit cost ratio was found to be 1.83:1.

Introduction

Moringa (*Moringa oleifera Lam.*) belongs to the family "*Moringaceae*" with genus "*Moringa Adans*" and species "*M. oleifera Lam*". It 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. India is the largest producer of moringa, with an annual production of 1.10 to 1.30 million tonnes of tender fruits from an area of 380 km^2. Among the states, Andhra Pradesh leads in both area and production (156.65 km^2) followed by Karnataka (102.8 km^2) and Tamil Nadu (74.08 km^2) (Lalas and Tsaknis, 2002).

It has been reported by Bureau of Plant Industry that moringa is an outstanding source of nutritional components. Moringa seeds were reported to have 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). Moringa seed oil is considered equivalent to olive oil in terms of its chemical properties and contains a large quantity of tocopherols (Middleton *et al.,* 2000). The refined moringa seed oil is clear, odorless and resists rancidity. The oil contains 70% of oleic acid, an 18-carbon long mono-unsaturated fatty acid (MUFA). Since the oleic acid has good oxidative stability when compared with poly-unsaturated fatty acids (PUFAs), it has found use in the food industry, as it allows longer storage and high temperature frying of foods (Ojiako and Okeke, 2013).

Furthermore, *M. oleifera* seed has been found to be a potential new source of oil especially with the advent of the need for oleo-chemicals and oils/ fats derived fuels (Biodiesel) all over the world (Anwar and Rashid, 2007). However, the plant has been identified as one of the under explored plant and there is dearth of information on physico-chemical properties of the moringa seed kernel oil which has limited its applications (Dinesha *et al.,* 2015).

There are a number of conventional extraction methods for essential oil extraction from plant materials. Some methods have been used for many years such as Soxhlet Extraction (SE), Heat Reflux Extraction (HRE) and Steam Distillation (SD). The main disadvantage of conventional extraction methods include long extraction time, usage of a large amount of solvent and the possibility of thermal decomposition of the target compounds (Qun, 2011).

Solvent extraction is being practiced for extraction of oil from moringa seed kernels. However, this method has the major disadvantage of solvent residue in the extracts. Recently, supercritical fluid extraction has gained increasing attention over the traditional techniques in the recovery of edible and essential oils. In the field of natural products, the new technique of supercritical fluid extraction (SFE) utilises smaller amount of organic solvents. Supercritical carbon dioxide is an alternative that does not have any of the negative effects related to traditional organic solvents, at optimal conditions (Casas *et al.,* 2009).

To date, any research article on supercritical fluid extraction of moringa (PKM-1), a world's most successful high productivity variety of *Moringa oleifera* seed kernel oil has not been reported. Keeping in view of these facts, the investigation on "Effect of Supercritical Carbon Dioxide (SC-CO_2) Temperature and Pressure on Physico-chemical Properties of Moringa (*Moringa oleifera Lam*) Seed Kernel Oil" was undertaken in the Department of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka (India).

Materials and Methods

Raw materials

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).

Soxhlet extraction of moringa seed kernel oil

Moringa seed kernel oil extraction was carried out by soxhlet extraction method using SOCS- PLUS apparatus (Make: Pelican Equipments; Model: SCS-08) with hexane as solvent. Accurately, 50 g of the moringa seed kernel powder was taken into the thimble and placed it in the sample compartment of the extractor. Sample compartment was attached to a 500 ml round bottom flask containing 300-350 ml hexane. SOCS- PLUS set-up was assembled and heated in a mantle. The SOCS-PLUS apparatus was run at 85 ^oC for 90 min. Hexane in the oil extract was distilled out by using a rotary flash vacuum evaporator (Superfit, Rotavap; PBU-6D) (Malapit, 2010).

Supercritical fluid ($SC\text{-}CO_2$) extraction of moringa seed kernel oil

The supercritical carbon dioxide extraction system (Thar; SFE 500 system) was used for extraction of moringa (PKM-1) seed kernel oil. Deionized water (at 5 °C) was used for cooling different zones in the $SC\text{-}CO_2$ extraction system. The independent variables selected for the study were supercritical fluid ($SC\text{-}CO_2$) pressures of 100, 150 and 200 bar and temperatures of 40, 50 and 60 °C at constant dynamic extraction time of 90 min (Liza *et al.*, 2010). The details of experiments are given in Table 1 and 2.

Fifty grams of moringa seed kernel powder was placed into the extractor vessel. The flow rates of supercritical CO_2 and co-solvent (ethanol) were maintained at 20 and 2 g/min, respectively (Pradhan *et al.*, 2010). 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 $SC\text{-}CO_2$ extraction system. The static extraction time allowed the sample to soak in the CO_2 and co-solvent in order to equilibrate the mixture at desired pressure and temperature. During the dynamic extraction time, CO_2 carrying the crude extract flowed out of the extraction vessel and then into a collection vessel, where the CO_2 was separated through the vent connected to the fume hood (Dinesha *et al.*, 2016).

Extraction yield

The moringa (PKM-1) seed kernel oil from the $SC\text{-}CO_2$ extraction was collected and the residual content of co-solvent was removed by using a rotary flash vacuum evaporator (Superfit, Rotavap; PBU-6D) under vacuum at 40 °C. The oil was then placed in the oven at
40 °C for 30 min for further removal of solvent traces. The extraction yield was computed by using the following equation (Liza *et al.*, 2010).

$$\text{Extraction yield (g/100g)} = \frac{M_{extract}}{m_{feed}} \times 100 \qquad (1)$$

where,

$M_{extract}$ = Mass crude extract, g

mfeed = Feed mass, g

Extraction efficiency

The extraction efficiency was calculated as per the method described by the Olawale (2012). It is the ratio of the quantity of oil extracted during the process to the actual amount of oil present in 100 g of moringa seed kernel.

$$\text{Extraction efficiency (\%)} = \frac{\text{Oil extracted, g/100 g of sample}}{\text{Actual oil present, g/100 g of sample}} \times 100 \quad \ldots (2)$$

Physico-chemical properties

The physico-chemical properties of moringa (PKM-1) seed kernel oil extracted from $SC\text{-}CO_2$ process were determined and compared with solvent extracted oil. Density and specific gravity of the oil samples were determined using the method described by Adejumo *et al.,* (2013). Hunter lab colourimeter (Model: Colour Flex EZ) was used for the measurement of colour values (*L**, *a**, *b** and Chroma) (Cho *et al.,* 2010). Acid value, saponification value, iodine value and peroxide value of oil samples were determined by using respective AOAC (1990) methods.

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. Experimental data were subjected to analyses of variance (ANOVA) and multiple comparison tests were performed using a least significant difference (LSD), suitable for factorial design (Cho *et al.,* 2010). The analyses were performed using the software, Design Expert Version 7.7.0 trial version (State-Ease, Minneapolis, MN).

Cost of production of moringa seed kernel oil using SC-CO_2 extraction process

The cost of production of moringa seed kernel oil using supercritical fluid extraction equipment was estimated by considering the fixed and variable costs as well as other related costs. The standard procedure in accounting and cost calculation given by Ababa *et al.,* (2004) was followed.

Results and Discussion

Effect of SC-CO_2 temperature and pressure on extraction yield and extraction efficiency moringa seed kernel oil

The extraction yield and extraction efficiency of oil obtained from moringa (PKM-1) seed kernel powder at different SC-CO_2 temperature and pressure combinations are depicted in the Figure 1 and 2. The extraction yield and extraction efficiency varied in the range of 31.87 to 37.76 g/100 g and 83.32 to 98.43%, respectively. The interaction effect between different treatment combinations are significant ($p < 0.01$) at one per cent level.

From the Figure 1 and 2, it can be noticed that, as the supercritical fluid (CO_2) pressure increased from 100 to 200 bar, the extraction yield and efficiency increased. This might be due to the fact that the increase in pressure increased the density of the CO_2 thereby increasing the solvent strength and solubility of the oil in CO_2 (Liza *et al.,* 2010). Results are in agreement with the earlier findings of Nguyen *et al.,* (2011) who reported optimal oil yield and extraction efficiency of 37.84 g/100 g and 90.90% at a pressure of 28.97 MPa and temperature of 44.30 °C for *Moringa oleifera* seed kernels. The increase in pressure might have also accelerated the mass transfer of oil and enhanced the extraction yield. Similar findings were reported by Zhao and Zhang (2013), who reported oil yield of 37.12% for *Moringa oleifera* seeds.

Increasing the temperature of SC-CO_2 reduced the solvent density and thus its salvation power at constant pressure (Couto *et al.,* 2009). It can be observed that, the extraction yield and extraction efficiency decreased with the rise of temperature at low pressures, due to the reduced density of CO_2 with increased temperature. However, in the present experiment at lower pressures 200 bar, the extraction yield and extraction efficiency decreased with the rise of temperature.

From the table, it is also seen that the moringa (PKM-1) seed kernel oil yield from solvent extraction (control) found to be lower (29.12 g/100g) with extraction efficiency of 76.29% compared to SC-CO_2 extraction. This might be due to lower solvation of oil by the solvent and higher temperature applied in soxhlet extraction compared to SC-CO_2 extraction. Higher temperature might have led to the thermal degradation of fatty acids, especially unsaturated fatty acids (Uddin and Chun, 2010).

Effect of SC-CO_2 temperature and pressure on physical properties of moringa seed kernel oil

Density

The densities of the SC-CO_2 extracted moringa (PKM-1) seed kernel oil varied between 0.915 to 0.998 g/cc. According to Table 3, the highest oil density of 0.998 g/cc was recorded at SC-CO_2 pressure of 200 bar and temperature of 40 °C, whereas the lowest of 0.915 g/cc was recorded at 100 bar and 60 °C. Density of oil decreased as temperature increased. This shows that the higher the temperature, lower the density of oil. This might be due to the fact that the oil bearing seeds tend to lose some of the properties when heated to higher temperatures, resulting in reduction in its density (Adejumo *et al.*, 2013). The soxhlet extracted (control) moringa (PKM-1) seed kernel oil was found to be lower with density value of 0.913 g/cc. Results found in good agreement with the findings of Rahaman *et al.*, (2012).

Specific gravity

Specific gravity of SC-CO_2 extracted moringa (PKM-1) seed kernel oil varied from 0.93 to 1.05, which shows that, it is less dense than water. The values decreased with increasing extraction temperatures (Table 3). Among the different treatment combinations, the specific gravity value of 1.05 was recorded highest at SC-CO_2 pressure of 200 bar and temperature of 40 °C, whereas the lowest value of 0.93 was recorded at 100 bar and 60 °C. However, the values are significantly different at $p<0.01$. The values of specific gravity of the samples were within the range of 0.9-1.16 for edible oil given by FAO/WHO (2009). This trend is similar to that of observed by Adejumo *et al.*, (2013) for solvent extraction of moringa seed oil. These values are in good agreement with the results reported by Belewu *et al.*, (2010); Tint and Mya (2009) for *Jatropha curcas* seed oil.

The specific gravity of Soxhlet extracted (control) moringa (PKM-1) seed kernel oil was found to be 0.92. This value was found to be less compared to SC-CO_2 extracted moringa seed kernel oil. This might be due to higher temperature of 85 ^{o}C applied in soxhlet extraction that lowered the specific gravity of the oil. This value is in agreement with the earlier findings (0.86) of Orhevba *et al.*, (2013) for soxhlet extracted moringa seed oil.

Colour

The maximum colour value (L^*) of the sample was found to be 19.34 at SC-CO_2 temperature of 60 °C and 200 bar pressure. The colour values of a^*, b^* and Chroma were observed to be -11.22, 6.12 and 9.50 respectively at 40 °C and 200 bar (Table 3). As pressure increased from 100 to 200 bar, the L^* value of the oil increased significantly ($p<0.01$).

This might be due to the fact that the increased extraction pressure led to higher fluid density thereby increased the solvent strength (Liza *et al.*, 2010). It is also evident from the table that, the colour value L^* increased as temperature increased from 40 to 60 °C. This might be due to temperature increases the vapor pressure of the solutes, therefore increasing their solubility in the supercritical solvent (Couto *et al.*, 2009). Pressure increased from 100 to 200 bar, a^*, b^* and chroma value of the oil also increased significantly ($p<0.01$). The colour value *a**, *b** and Chroma were decreased with the rise of temperature at low pressures, due to the reduced density of CO_2 with increased temperature. Similar effects have been reported by Lee *et al.*, (2013) for SC-CO_2 extracted ginseng seed oil.

The colour values *L**, *a**, *b** and Chroma of soxhlet extracted (control) moringa (PKM-1) seed oil were found to be 2.06, -0.93, 1.11 and 0.60. These values were less compared to SC-CO_2 extracted oil. This might be due to the residual solvent in the oil. The data were comparable with the previous reports of Anwar *et al.*, (2003) for *Moringa oleifera* seed oil.

Table.1 Supercritical Fluid Extraction Process Parameters

Extraction time	**: 90 min**
Co-solvent flow rate	: 20 g/min
CO_2 flow rate	: 2 g/min
Sample size	: 50 g

Independent Parameters	**Levels**		
	1	**2**	**3**
Extraction temperature (°C)	40	50	60
Extraction pressure (bar)	100	150	200

Table.2 Treatment combinations for supercritical fluid extraction of moringa (PKM-1) seed kernel oil

Treatment	Temperature °C	Pressure(bar)
T_0	85	Normal
T_1	40	100
T_2	40	150
T_3	40	200
T_4	50	100
T_5	50	150
T_6	50	200
T_7	60	100
T_8	60	150
T_9	60	200

To = Control - Soxhlet extraction carried out at 85 ^{o}C for 90 minutes

Table.3 Effect of SC-CO_2 temperature and pressure on physical properties for moringa (PKM-1) seed kernel oil

Tr	Temperature (°C)	Pressure (bar)	Density (g/cc)	Specific gravity	Color value *L**	Color value *a**	Color value *b**	Chroma (C*)
T_0	-	-	0.913	0.92	2.06	-0.93	1.11	0.60
T1	40	100	0.948 [a]	0.95 [a]	4.82 [a]	-7.05 [a]	2.11 [a]	6.72 [a]
T2	40	150	0.974 [a]	0.98 [a]	6.84 [a]	-8.85 [a]	3.27 [a]	9.43 [a]
T3	40	200	0.998 [a]	1.05 [a]	6.94 [a]	-11.22 [a]	6.12 [a]	9.50 [a]
T4	50	100	0.935 [a]	0.94 [a]	7.79 [a]	-5.39 [a]	1.09 [a]	5.27 [a]
T5	50	150	0.972 [a]	0.97 [a]	10.87 [a]	-6.05 [a]	1.68 [a]	5.81 [a]
T6	50	200	0.984 [a]	1.01 [a]	12.40 [a]	-6.24 [a]	2.08 [a]	5.88 [a]
T7	60	100	0.915 [a]	0.93 [a]	14.00 [a]	-1.89 [a]	0.09 [a]	2.12 [a]
T8	60	150	0.969 [a]	0.96 [a]	14.26 [a]	-3.09 [a]	0.53 [a]	3.36 [a]
T9	60	200	0.978 [a]	0.99 [a]	19.34 [a]	-3.52 [a]	1.03 [a]	9.26 [a]
		Mean	0.96	0.97	10.78	-3.93	1.99	5.82
		CD @ 1%	0.01	0.01	10.91	0.59	0.27	0.01
		CV	0.35	0.52	3.72	-3.91	5.70	0.47

Tr= Treatment; T_0 = Control - Soxhlet extraction carried out at 85 °C for 90 minutes; a= Significant at $p<0.01$; *L** = Lightness to darkness; *a**= Redness to greenness
*b**= Yellowness to blueness

Table.4 Effect of SC-CO_2 temperature and pressure on chemical properties for moringa (PKM-1) seed kernel oil

Tr	Temperature (°C)	Pressure (bar)	AV	SV	IV	PV
T_0	-	-	3.71	188.25	61.15	0.55
T1	40	100	2.88[a]	169.66 [a]	65.59 [a]	0.59 [a]
T2	40	150	2.91 [a]	184.00 [a]	66.28 [a]	0.62 [a]
T3	40	200	3.04 [a]	185.66 [a]	66.71 [a]	0.65 [a]
T4	50	100	2.17 [a]	162.66 [a]	66.76 [a]	0.64 [a]
T5	50	150	2.70 [a]	172.00 [a]	67.15 [a]	0.65 [a]
T6	50	200	2.80 [a]	184.00 [a]	67.55 [a]	0.68 [a]
T7	60	100	2.02 [a]	147.33 [a]	67.88 [a]	0.70 [a]
T8	60	150	2.61 [a]	171.00 [a]	68.03 [a]	0.72 [a]
T9	60	200	3.65 [a]	178.00 [a]	68.06 [a]	0.77 [a]
		Mean	2.63	172.82	57.81	0.55
		CD @ 1%	0.03	0.39	0.45	0.05
		CV	0.13	0.09	0.36	4.2

AV= Acid value (mg of KOH/g of oil); SV= Saponification value (mg of KOH/g of oil)
IV=Iodine value (g/mol); PV=Peroxide value (meq. of O_2/ kg of oil)

Fig.1 Effect of SC-CO_2 temperature and pressure on extraction yield of moringa (PKM-1) seed kernel oil

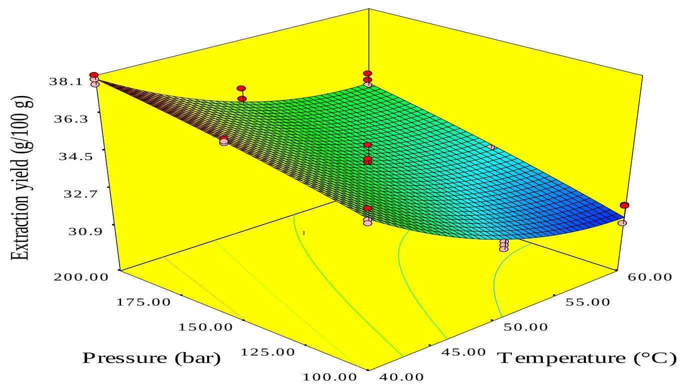
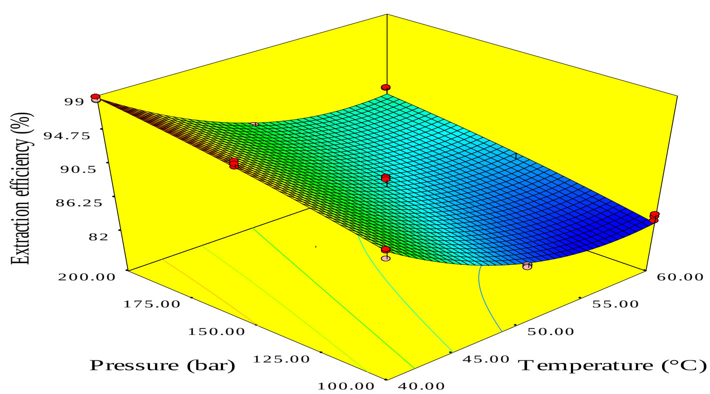


Fig.2 Effect of SC-CO_2 temperature and pressure on extraction efficiency of moringa (PKM-1) seed kernel oil



Effect of SC-CO_2 temperature and pressure on chemical properties of moringa seed kernel oil

Acid value

Table 4 shows the acid values of SC-CO_2 extracted moringa (PKM-1) seed kernel oil. Acid value depends on the degree of rancidity which is used as an index of freshness. The acid values obtained are in the range of 2.02 to 3.65mg KOH/g of moringa seed kernel oil and are significantly different at ($p<0.01$) one per cent level. The acid value decreased in a linear manner with the increase in temperature of SC-CO_2 and the trend is similar to that of reported by Adejumo *et al.,* (2013) for moringa seed oil. The values are within the range of 2.89±0.01 specified for edible oil as given by FAO/WHO (2013). The acid value of soxhlet extracted (control) moringa (PKM-1) seed oil was found to be 3.71 mg of KOH/g of oil which is significantly higher compared to the acid value of SC-CO_2 extracted oil. This might be due to higher oxidative rancidity of oil at higher temperature employed in soxhlet extraction process (Dinesha *et al.,* 2018).

Saponification value

It is also seen from the Table 4 that the saponification values of the SC-CO_2 extracted moringa (PKM-1) seed kernel oil samples varied from 147.33 to 185.66 mg of KOH/g. These values are within the range (188 to 265 mg KOH/g) recommended for edible oil by FAO/WHO (2013). The saponification value decreased with the increase in temperature and the samples differed significantly at ($p<0.01$) one per cent level. This trend is similar to the results reported by Adejumo *et al* (2013) for moringa seeds and also compared favorably with the values of 199.50 mg/KOHg obtained by Anderson *et al.*, (2012) for blighasapida seeds and 189 to 190 mg KOH/g reported by Mohammed and Hamza (2008) for sesame seeds. The saponification value of soxhlet extracted (control) moringa (PKM-1) seed kernel oil was found to be 188.25 mg of KOH/g.

Iodine value

The iodine value of SC-CO_2 extracted moringa seed kernel oil samples ranged from 65.59 to 68.06 g/mol as shown in Table 4. These are lower than the range (80 to 106 g/mol) specified by FAO/WHO (2013) for edible oil. The iodine value increased with the increase in temperature and pressure of SC-CO_2 and the values are significantly different at one per cent level. The iodine value of moringa seed kernel oil is in close agreement with the value 31.06 ± 0.80 mg/100g of African star apple seed oil reported by Akubugwo and Ugbogu (2007). The low iodine value indicates that the oil has a low content of unsaturated fatty acids (Dosumu and Ochu. 1995). Its suitability for the manufacture of soaps, lubricating oil and candles makes it as an attractive option because this oil, being not known yet commercially for consumption, can help to minimize dependence on use of known edible oils for making such products (Ochigbo and Paiko, 2011). The iodine value of soxhlet extracted (control) moringa (PKM-1) seed oil was found to be 61.15 g/mol.

Peroxide value

Peroxide value of SC-CO_2 extracted moringa (PKM-1) seed kernel oil samples ranged from 0.59 to 0.77 meq of O_2/ kg of oil as shown in Table 4. These values will below the standard (<10 meq of O_2/kg of oil) specified by FAO/WHO (2013) for fresh edible oil. Though peroxide value increased with the increase in temperature, the samples showed significant difference at one per cent level. The peroxide value of soxhlet extracted (control) moringa (PKM-1) seed kernel oil was found to be 0.55 meq of O_2/kg of oil.

Cost of production of oil from moringa seed kernel powder using SFE equipment

Estimation of cost of production of moringa (PKM-1) seed kernel oil was obtained as Rs. 13,652 /- per kg of oil with benefit cost ratio of 1.83:1.

In conclusion the SC-CO_2 extracted moringa seed kernel oil yield and extraction efficiency decreased with increase in extraction temperature and increased with increase in pressure. The SC-CO_2 temperature had significant effect on most physico-chemical properties than the pressure. Physico-chemical properties *viz*., density, specific gravity, color value (b^*), acid value and saponification value decreased with increase in extraction temperature. Colour value L^*, color value a^*, iodine value and peroxide value increased with increasing temperature.

The production cost of oil from moringa (PKM-1) seed kernel powder using supercritical fluid extraction equipment was found to be Rs. 13,652 /- per kg of oil with benefit cost ratio of 1.83:1. Hence, the developed technology could be adopted for commercial extraction of moringa (PKM-1) seed kernel oil.

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