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Supercritical and subcritical carbon dioxide extraction of Indian orange peel oil

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ABSTRACT

The viscosity of SC-CO_2 increases rapidly in the critical region, its order of magnitude is an order less than those of liquid organic solvents even at very high pressures. Hence an attempt was made to extract the oil using SC-CO_2 to study the quality, quantity and compositions of the oil. Pressures ranging from 80-150 bars with temperatures, ranging from 28-60°C were employed. SC-CO_2 yielded 2.22 wt% (2 h) of orange oil and hydro distillation gave 1.14wt% (9 h) of orange oil. The constituents of SC-CO_2 orange oil was α-pinene 0.14, β-pinene 0.99, myrecene 2.65, limonene 88.68, terpinolene 0.55, C_8 aldehyde 0.33, citronellol 0.11, and linalool 0.75 respectively. Hydro distillation yielded α-pinene 1.79, β-pinene 2.20, d-limonene 76.93, myrecene 5.18 and unidentified 14.18 respectively. Supercritical carbon dioxide extraction technology facilitates the uniform bed that allows reasonable heat and mass transfer as the surface area of the grounded orange peels either wet and/or dried increase the surface area for better penetration of the fluid under sub/supercritical conditions of set parameters. That gave the enhanced yield and extractability of the Orange peel oil constituents. Small changes in pressure or temperature have shown large changes in density and yield.

KEYWORDS

SC-CO_2;
Orange peels;
Hydro distillation;
Pre treatments;
Compositions.

INTRODUCTION

Supercritical fluid (SCF) extraction is an extraction process utilizing a fluid as an extract-ant at temperatures and pressures exceeding its critical temperature and pressure. It is possible to separate a multi component mixture when a supercritical fluid is used as an extractive solvent by capitalizing on both the differences in component volatilities (i.e., salient features of distillation) and the differences in the specific interaction between the mixture components and the SCF solvent. The application of SCF solvents is based on the experimental observations that many gases exhibit enhanced solvating power when compressed to conditions above the critical point[1].

Supercritical fluid extraction (SFE), which has received much interest in its use and further development for industrial applications, is a method that offers some advantages over conventional methods, especially for the palm oil industry. SC-CO_2 refers to supercritical fluid extraction (SFE) that uses carbon dioxide (CO_2) as a solvent which is nontoxic, inexpensive, non flammable and non polluting supercritical fluid solvent for the extraction of natural products. Almost 100% oil can

be extracted and it is regarded as safe, with organic solvent-free extracts having superior organoleptic profiles[2].

Desorption of bigarade peel oil from a polar adsorbent was performed by supercritical CO_2 to improve the oil quality by selectively eliminating hydrocarbon terpenes and coumarins. The oil fractions obtained at 40 °C, at pressures between 7.7 and 12 MPa, and at different desorption times were analyzed by GC"MS. (Gonzalo A. N. et al. 2010) studied the solubility in supercritical carbon dioxide (CO_2) of farnesol. The differences in solubility between farnesol, naringenin, and other sesquisterpenes or flavonoids reported in the literature were partially explained by differences in molecular weight and polarity between solutes[3].

Researchers have studied the extraction of citrus peel oil from Japanese citrus fruits employing supercritical CO_2 at 333K and 20 MPa in order to compare the compositions and efficiency of extraction from the slurries. Oxygenated compounds (contain an aldehyde and ester group) in the oil represented 8.84, 5.5 and 4.49% in lemon and vice versa[4].

The supercritical fluid extraction of orange essential oil was studied employing dehydrated orange peel, (0.0538 kg H_2O kg^{-1} dm) from nave line cultivars as raw material and CO_2 as solvent. The effect of operation conditions was analyzed in a series of experiments at 313, 323 K, and pressures between 1 and 25 MPa. Furthermore, the effect of CO_2 flow rate and particle size of orange peel was studied in the range of 0.5 to 3.5 kg h^{-1} and 0.1 to 10 mm. The authors have reported the yield of the oil as 1.3% by simultaneous distillation extraction and 0.3% by steam distillation and 0.3% by lyophilisation[5].

Our[23] objective of the research was to develop the feasible process of extracting Orange peel oil and to best manage the demand of orange oil. The study was carried by varying pressure, temperature, and batch time. Flow rate of supercritical carbon dioxide was not of great importance as the higher flow rates did not contributed in the yield enhancement. Hence the study was investigated with the flow rate, 5 kg h^{-1}. Study conducted by various authors using SC-CO_2 had shown the interesting results.

Supercritical fluid extraction has become very important in the field of extraction of foods materials. It has advantages such as excellent mass transfer rates, ease of control of solubility and solvents free extracts. CO_2 is one of the most commonly used supercritical fluids (SCF). This is because it has low critical temperature (31.1°C) and critical pressure (73.8 bars). It is abundantly available at low cost and odourless, colourless, non toxic, non inflammable, non corrosive in nature[6].

The processes like Cold-press method, hydro distillation have been mentioned in the literatures and until the SC-CO_2 technique came into the existence the commercial exploration of Orange oil (bitter or sweet) were being carried out by the said methods. As a matter of facts these two techniques have been found to suffering from the low yield as the oil emulsifies while processed by Cold-Press method and hydro distillation that yield the oil with low oxygenated composition and the oil suffer with burning note. The conventional techniques found to lose the important oxygenated compounds along with D-limonene being the major constituents of the oil. As supercritical conditions, have the low density as compare to subcritical conditions and hence the extraction was also conducted under the subcritical conditions to study and compare the compositional statutes. The various treatments were found to be lacking in retaining the some of the oxygenated compounds like C_8 aldehyde, citronellol and lialool whereas these were extracted in better yield under subcritical conditions than supercritical conditions due to better mass and heat transfer. Two components are necessary for a citrus odour, that is, d-limonene and citral. It is responsible for the base sensory character of the citrus oils. The individual odours of the different citrus fruits and their various cultivars are not due to various different chemicals but rather to the proportions of the various chemical components. Very recently researchers elaborated the effects of modified atmosphere on physico-chemical characteristics and sensory evaluation of Indian bitter orange oil that supports our claim for the present study with supercritical/subcritical carbon dioxide extraction conditions[7,8].

EXPERIMENTAL

Orange peels fresh as such, sun dried, alkaline treated, freeze stored at 15°C for over 15 days were

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employed for extracting oil using SFE and hydro distillation. In case of SFE the fresh peels were ground with moderate mixing and were charged into the extractor of the pilot plant. Pressure and temperatures were varied along with batch time and flow rate of SC-CO_2.

Material and methods

SC-CO_2 extraction

Carbon dioxide gas (99.9%) was purchased from Indian Oxygen Limited. Orange peels were collected freshly from fruit merchant, local area of Matunga, Mumbai suburbs. The standards orange oil constituents were purchased from Sigma-Aldrich Co. (St. Louis, MO). The SC-CO_2 pilot plant (Figure 1), with extractor and separator capacities of 1 L each, was imported from UHDE GmbH (Dortmund, Germany).

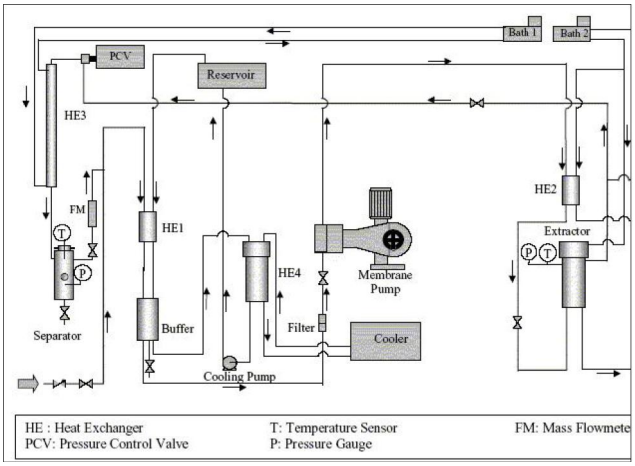

Figure 1 : SC-CO2 Pilot Plant schematic flow

The SC-CO_2 pilot plant was procured with a grant from DST (Government of India).

Orange peels (*citrus aurantium)*, bitter oranges, were collected from the fruit processing merchant procured from the Nagpur region of India and covers huge area for cultivation of Oranges. The merchant was requested to place the peels in the refrigerator so that an entire study may use the peels from the same lot of oranges.

Operation of the supercritical plant

The CO_2 from the cylinder was allowed to pass through the pre heat exchanger to bring to the fluid state and later was stored in the 20 litre reservoir. This process takes 45-50 minutes and was utilized until the extraction parameters complete. In the mean time the grounded orange peels were weighed and charged into the extractor of 1 litre capacity. The extraction temperature was set by switching on the thermostat. Once the reservoir was full with fluid sate CO_2, then the CO_2 feed was stop. The flow rate was set as per the calibration graph of pneumatic pump and the pneumatic pump was put on to start the process. Accordingly the pressure of the pilot plant was also set every time of the cycles. The extraction was carried out under the set parameters of temperatures and pressures. The subcritical state of CO_2 fluid was brought about by adding ice into the thermostat, as such so that the temperature be maintained at 28°C (subcritical temperature). Once the set pressure of extraction was achieved then the solenoid valve automatically cut of the pressure and the extracts of orange peel oil were collected into the sample tube. The oil was then analyzed for assessing its constituents by GC and capillary GC each time of the set parameters.

Sun dried Orange peels

The peels were kept in the shade exposed to sunlight and were dried for about 8-10 hrs. The entire yield calculations in every technique studied is based on the dry basis.

Analysis of orange oil

Analysis of Orange oil was done by using gas chromatography (Perkin-Elmer 8500), column specification and temperature programme are described as follows: column SE30 (10%) on chromosorb W, column material S.S, column length 4 meter, internal diameter 1/8 mm, injector temperature 300°C, FID temperature 300°C, flow rate of N_2 30 ml/minute and temperature programming 100-250°C with 5°C/minute rise of temperature, final hold time 5 minute[9,10].

Supercritical carbon dioxide extracted orange oil was analyzed by capillary gas chromatography (Hewlett Packard 5890). The analysis conditions and column specification of capillary column is as follows: column BP5 (equivalent to SE54), column material Vitreous silica, column length 50 meter, type bonded phase, internal diameter 0.22 mm, orifice diameter 0.33 mm, injector temperature 250°C, FID temperature 280°C, carrier pressure 10 psi, split ratio 1:10 and temperature programme 70-240°C with 10°C/minute rise of tem-

perature, final hold time 20 minute[11].

RESULTS

Effect of temperature on the extraction of Orange oil

The extraction was carried under subcritical condition (28°C,) pressure 150 bars, batch time of 2 hour and flow rate of 5 kg h^{-1} (Figure 2). It was indicated that D-limonene was the major constituent of the orange oil. It was found that at constant pressure with increase in temperature the yield of the orange peel oil was found to increase till 55°C. At 60°C the extraction of orange oil was found to decrease slightly. (TABLE 1) Major constituents of (TABLE 2) the oil, D-limonene was found to increase up to 89.28% at 60°C. The components α-pinene, β-pinene, terpinolene, C_8-aldehyde, linalool and citronellol were found to be less than 1% while myrecene was less than 3% in the extracted oil.

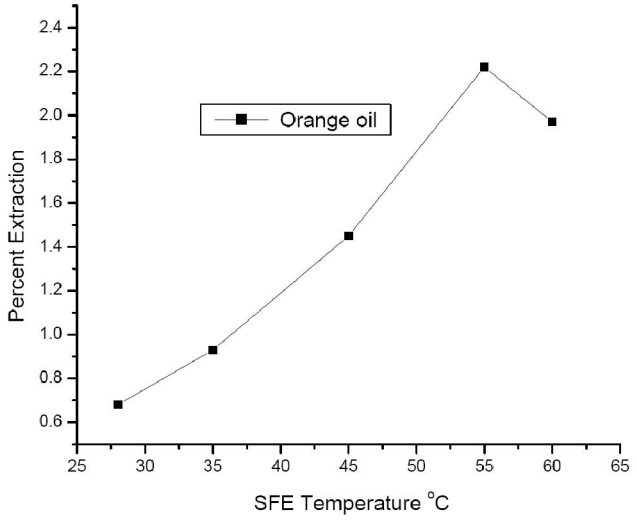

Figure 2 : Effect of SFE temperatures at 150 bars on extraction of Orange oil

TABLE 1 : Effect of temperature on the extraction of orange peel oil

Extraction Temperature (°C)	Yield of Oil (wt % of peels)
28	0.58
35	0.93
45	1.45
55	2.22
60	1.97

The compositions of the constituents at these conditions were α-pinene (0.14%), β-pinene (0.99%), myrecene (2.65%), D-limonene (88.68%), terpinolene (0.55%), C8-aldehyde (0.33%), citronellol (0.11%) and linalool (0.13%). The decrease in the extraction of orange oil may be attributed to the fact that above 55°C some degradation products start forming, thus reducing the yield[12,13].

TABLE 2 : GC analysis of SC-CO_2 extracted orange peel oil

T °C	P Bars	Composition							
		1	2	3	4	5	6	7	8
28	150	0.08	0.58	1.97	88.25	0.58	0.16	0.45	0.24
35	150	0.10	0.76	2.30	76.72	0.58	0.27	0.16	0.12
45	150	0.10	0.75	2.09	86.62	0.16	0.38	0.10	0.46
55	150	0.14	0.99	2.65	88.68	0.55	0.34	0.11	0.75
60	150	0.12	0.79	2.27	89.28	0.54	0.14	0.41	0.13

1) α-pinene 2) β-pinene 3) myrecene 4) d-limonene 5) terpinolene 6) C_8 aldehyde 7) citronellol 8) linalool

Effect of temperature had shown the progress of extraction of various constituents in appreciable amount specifically the oxygenated compounds (bears aldehyde and ester functional group) C_8-aldehyde, citronellol and linalool. Unlike SC-CO_2 was found to extract the oil in lesser process time and the presence of the moisture within the Orange peels have found to play a role of an entrainer to facilitate the non-polar nature of the carbon dioxide to polar and enhances the extractability in short batch time[12,13].

Effect of pressure on the extraction of orange oil

In this part of study pressure was varied in between 80-250 bars, at 55°C, 5kg h^{-1} flow rate of SC-CO_2 and 2 hour batch extraction time. With an increase in pressure at constant temperature the yield of the oil was found to increase till 150 bars. 150 bars onwards decrease in the degree of extraction was noticed specifically at 200 and 250 bars. Most of the volatile oil components get extracted at pressures in the range of 80-150 bars. This pressure onwards no increase in the yield of the oil was found. Anomalous behaviour of decrease in yield at high pressures at 55°C may be due to the obstruction for the flow of SC-CO_2 because of decrease in void space of the packed bed. (Figure 3) may be referred[12,13].

The compositions of the extracted orange oil have shown that d-limonene was the major constituents (about 90%) of the oil. The other components were α-

pinene, β-pinene, myrecene, terpinolene, C_8-aldehyde, citronellol and linalool. Out of these except myrecene that is 2.5-3%, the rest of the constituents were less than 1% each. Percent composition of the orange peel oil at 150 bars and 55°C are α-pinene (0.14%), β-pinene (0, 99%), myrecene (2.65%), D-limonene (88.68%), terpinolene (0.55%), C_8-aldehyde (0.33%), citronellol (0.11%) and linalool (0.75%)[14,15].

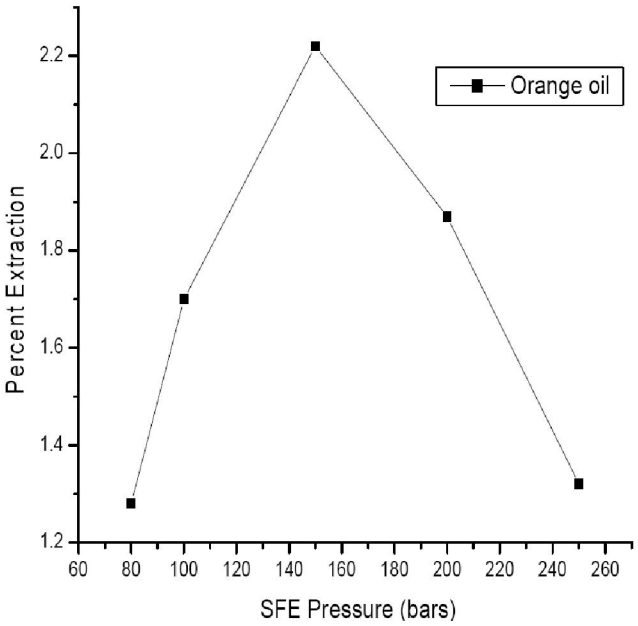

Figure 3 : Effect of pressure on extraction of Orange oil at 55°C

Hydro distillation of orange peel oil

The ungrounded fresh peels (Figure 4) were extracted using hydro distillation process. The data for batch time of 3-12 hours for hydro distillation and their compositions is presented in (TABLE 3&4). It was seen that even employing 12 hours of batch time, the yield of oil was only 1.27 wt%. The poor yield may be explained on the basis of increased thickness through which the oil must diffuse out of peels to available for distillation. The lack of pre-treatment such as grinding may have not exposed the oil bearing sacks for obtaining the oil. This further reduces the yield. Though the oil yield was slightly found to increase at the 12th hour but keeping in mind the optimum yield and the optimum batch time the distillation was not carried farther. Fractionation study was conducted employing hydro distillation and each result was analyzed by gas chromatography[16,17].

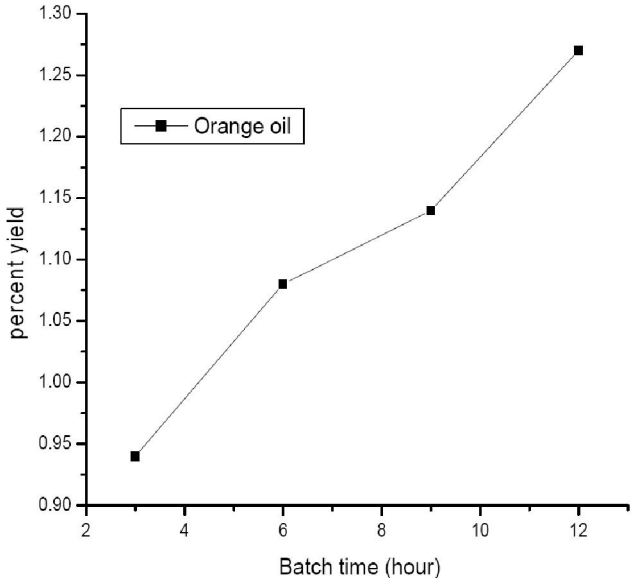

Figure 4 : Effect of batch time using hydro distillation on yield of Orange peel oil (un-ground fresh peels)

TABLE 3 : Chemical composition of orange peel oil sun dried stored in refrigerator at 0°C in polythene bag

Batch time (h)	Chemical composition (%area)		
	1	2	3
1	0.6564	2.0620	93.565
2	0.8564	2.0666	93.590
3	0.8197	2.2227	92.907
4	1.0951	2.9984	90.320
5	1.1725	2.5204	93.205
6	1.0266	2.9505	95.420

1) α-pinene 2) β-pinene 3) D-limonene

TABLE 4 : Effect of fractionation on yield and composition of sun dried Orange peel oil

Time (h)	%Yield	Percent composition				
		1	2	3	4	5
1	1.25	1.79	2.02	76.93	9.18	14.18
2	0.80	1.09	1.23	76.77	9.68	11.23
3	0.65	0.83	0.94	77.62	7.82	12.79
4	0.60	0.68	0.77	79.01	6.40	13.14
5	0.45	0.60	0.67	75.55	5.63	17.55
6	0.20	0.57	0.64	73.31	5.39	20.09

1) α-pinene 2) β-pinene 3) D-limonene 4) myrecene 5) unidentified

Effect of batch time on sun-dried ground peels stored in cooling chamber at 0°C

The pre-treatment of orange peels involve sun-drying, grinding and then storing the peels in cooling cham-

ber at 0°C. The orange peel powder had a moisture content of 10% (Karl-Fischer method). This was used for hydro distillation with different batch times from 1-6 hours. The data have shown gradual increase in the yield of oil and d-limonene with batch time of hydro distillation[16,17].

The d-limonene in the oil was more than 93%. The α-pinene and β-pinene were also present more than 1 and 2.5% respectively at batch times of 4-6 hours. It was observed that pH of water post distillation was acidic. This may be due to the degradation of thermally labile constituents that on oxidation resulted conversion to acids. In view of this observation, alkaline treatments of orange peels were thought to be promising[16,17].

Hydro distillation of sundried orange peel yielded oil, in which geranyl acetate was found to be about 0.1 %. Linalool and C_8-aldehyde was found to be absent. Highly volatile fraction of the orange oil responsible for characteristic odour did not get distilled during the process.

Effect of batch time on fresh ground peels with 0.4% alkaline treatment

The fresh peels were first soaked in 0.45% alkaline (Na_2CO_3) solution for 4-5 hours. This was then subjected to grinding and was used for hydro distillation. Prior to distillation the water used for the processing was heated first and then the ground peels were charged. The recovery of the oil was not much better in comparison to sundried peels. The pH of process water was found to be neutral (post distillation). This was due to the pre alkalinity, but yield of major constituent d-limonene was found to be lower. Analysis of the oil shown the presence of C_8-aldehyde (0.05%) and linalool (0.37%), but geranyl acetate was absent in the distilled orange oil[17,18].

Effect of batch time on sundried ground peels stored more than 15 days in the refrigerator at 15°C

In these sets of experiment the ground sundried peels stored more than 15 days in the refrigerator at 15°C were employed for hydro distillation with cold water circulation through the condenser to trap the constituents. It was found that the yield of the oil was appreciably decreased and more volatile constituents responsible for orange fragrance were trapped. As a result the fragrance of the oil recovered was excellent. The yield of the oil, β-pinene, and d-limonene was found to be less. Linalool was found to be lost as the ground peels were stored in the refrigerator for more than 15 days. Geranyl acetate (0.06%) and C_8-aldehyde (0.22%) were found to be present in the oil[17,18].

When the refrigerated stored orange peels were hydro distilled over the batch time of 1-6 hours then the yield of the orange oil was found to be reduced drastically not only that but the yield of the d-limonene also reduced. The unidentified constituent was found to recover in good yield. The study was quite important to reveal the increasing yield of the unidentified constituent. (Figure 5) shows the comparison of the constituents yielded by the hydro distillation and SC-CO_2, it has clearly indicated that the hydro distillation of Orange peel oil was not efficient to recover the major terpenes and oxygenated constituents as compare to SC-CO_2 extraction technology in lesser batch time[17,18].

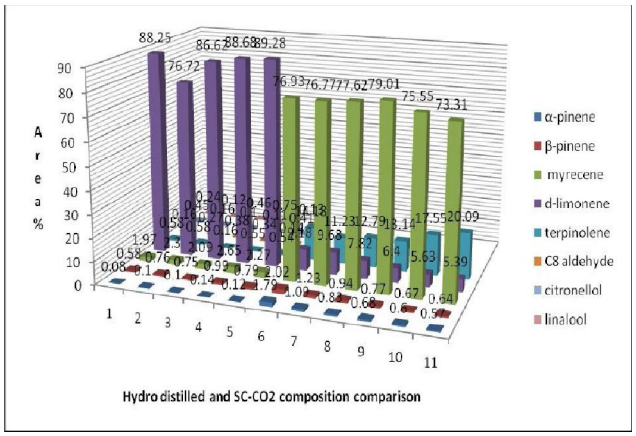

Figure 5 : Comparison of Orange peel oil's constituents by hydro distillation and SC-CO2

DISCUSSION

Interpretation of data (figure 5) gave great understanding about the extractability of compositions by supercritical carbon dioxide and conventional methods. The extracts obtained by SC-CO_2 gave the oxygenated compounds in high percentage as compare to conventional techniques. The extracts by SC-CO_2 sustained the better shelf life as a fact that the SC-CO_2 acts as germicide that kills all the pathogens responsible for deteriorating the quality of the oil or natural products. Hydro distilled orange oil had the oxygenated compounds

in very less percentage with burnt odour. The orange oil constitutes only 5% of the oxygenated components and hence difficult to be recovered by any conventional techniques but SC-CO_2 efficiently does that. Now days the demand of SC-CO_2 extracted oil is very high since it is free of any contamination. The oil with high oxygenated contents fetches the high price in the commerce. The physical properties evaluated by various analytical instruments and met the specification of SC-CO_2 extracted orange oil by the international standards[17-20].

The SC-CO_2 was operated with ease with regards to low flow rate, short batch time and moderate extraction pressure and the CO_2 was quite recoverable and reusable. The extracted oil was recovered by depressurization during the processing. Moisture content of the peels was determined using Clevenger apparatus and Karl-Fischer method. SFE was utilized by varying the pressures and temperatures to study the effect on yield and quality of orange oil. Comparative studies using conventional technology did have some disadvantages on the yield and quality of oil. Physical properties were evaluated to approve the conformation of the extracted oil and were found to conform to the values reported[17-20].

Hydro distilled orange oil shown that α-pinene and β-pinene were distilled in good yield as compare to SC-CO_2 but the oil was more prominent in its terpenic contents than its oxygenated constituents' content and was colourless oil. The contents in sundried ground peels were found to be less than that of the oxygenated compound as 14.18-20.09%. It was quite interesting to know this but could not be established as what the constituent was. Surprisingly the myrecene content was also higher as compare to SC-CO_2 results[17-23].

It was reported that the yield of the oil as 1.3% by simultaneous distillation extraction, 0.3% by steam distillation and 0.3% by lyophilisation. Sulphated orange peels stored at 0.1°C for 2 months the level of extracted essential oil increased from 0.44 to 1.06% where as for cold stored untreated peels the yield it increased only from 0.44 to 0.66%. They reported the yield of the oil as 1.1-1.4% by using hexanol, hot methanol, and 1% citric acid by maintaining the pH at 2.6[17-23].

The process of hydro distillation yielded the Orange peel oil with the 1.27% (pH 4.27) in 12 hours but revealed to lose all the oxygenated compounds. The yield of the terpenes was good as compare to SC-CO_2. Whereas the sun dried ground peels when stored for the 15 days under refrigeration and hydro distillation have yielded the Orange peel oil that found to contain an unidentified constituent with the good yield varying from 14.18-20.09%. Predictably the bed size of the orange peels whether sun dried and wet had shown to play an important role on the surface area and diffusivity by carbon dioxide under sub/supercritical conditions on the yield of the oil and their constituents[17-23].

Properties of hydro distilled oil

Physical properties of orange oil were determined. The values were found to be conforming to the specified values having mentioned in the literatures. These physical values are of utmost importance from the quality point of view and decide the shelf life of the oil. It also helps in evaluating the progress of the distillation of the various constituents and also helps in indentifying the rancidity of the oil with regards to its oxidation. The manual adulteration (TABLE 5) could also be examined with the help of these values[17-23].

TABLE 5 : Physical properties of the subcritical carbon dioxide extracted orange peel oil

Physical property	Values
Colour of the oil	light pale yellow
Refractive index	1.47175
Acid value	7.14
Optical rotation $[\alpha]^{D}_{20}$	+93.58[b]

[b] **determined as a 1% solution of oil in absolute alcohol (w/v)**

TABLE 6 : Refractive index of physically treated orange peel oil

Treatments	Values
Sun dried peels	1.4701
Treated peels	1.4712
Stored peels	1.4791

Significant difference was found in the values of refractive index of oil (TABLE 5) of pre treated peels extracted from each batch. Refractive index values were found to be varying. Thus RI as a major physical value of the oil was found to vary due to pre treatment and different batch time conditions carried out for the study. Refractive index values were determined employing Bausch-Lomb refractometer. Marked variations in the values of RI (TABLE 6) were observed in case of

stored peels at 2, 5, and 6 hours of batch time.

CONCLUSIONS

The optimum conditions for the volatile oil extraction of orange peels using SC-CO_2 were 55°C, 150 bars pressure, 5kg h^{-1} of flow rate and 2 hour batch time. The yield of the oil was 2.22 wt%. Quality of oil was absolutely fragrant and resembling as that of fresh orange peel oil. The colour of the oil was light pale yellow.

Various treatments were given to orange peels. The best results were obtained when sun dried ground peels stored in cooling chambers at 0°C processed by hydro distillation. The yield of the oil varied from 3.42 wt%-3.99 wt%. The pH of water was found to be acidic during hydro distillation. Pre treatments have found to shown remarkable results on yield and quality of the orange oil.

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