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Characterization of essential oil from the peel of three citrus species grown in Sikkim Himalaya

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Abstracts

The fruit peel of citrus is known to be rich in essential oil. Essential oil content and its characteristics vary amongst the species and climatic factors play a major role. Essential oil, to be suitable for the specific commercial purpose should have basic characteristics. In the present study, extracted oil of selected three citrus species *Citrus reticulata*, *C. maxima* and *C. jambhiri* grown at different altitude of Sikkim Himalaya were characterized. The oil yield was up to 2.4 % in mature fruits and 2.5 % in immature fruits at an altitude of > 1600 m in mandarin orange with significant variations concerning species and altitude. The various analysed oil parameters and their highest values in the study are refractive index (1.49), specific gravity (0.87), free fatty acid (7.83 %), acid value (8.37 %), saponification (163.37 mg KOH / g of oil), iodine value (116 g I_2/100 g), peroxide value (36.33 meq O_2/ kg), thiocyanogen (77.29 %), ester value (57.63 mg KOH/g), phenol (81.92 mg/g). These parameters also showed variations with altitude and the species. The *C. reticulata* grown at higher altitude (> 1600 m from MSL) was found to have better essential oils than *C. maxima* and *C. jambhiri* with some exceptions. The characteristics highly endorsed the use of these essential oils for food and cosmetic industries. The waste management with simultaneous production of valuable compound is the major perspective sought in the study.

Key words: Essential oil, hydro distillation, Sikkim mandarin (*Citrus reticulata*), pomelo (*C. maxima*), rough lemon (*C. jambhiri*)

Introduction

Citrus fruits, which are important horticultural crops, are not only used for human consumption as deserts or processed products but also have enormous scope for extraction of valuable oils from fruit peel. The extraction of essential oil from the waste (peel and pomace), is seen as the major proposition for strategic waste management in fruit processing units.

Essential oils are the volatile liquids with natural flavour and fragrances usually exploited commercially for food, cosmetics and pharmaceutical industry, nutrition supplements, health aspect and aromatherapy. The odour and flavour of essential oil are mainly due to presence of organic compounds. Plant parts like flowers, leaf, peel, seed, bark, roots and pomace are known to contain essential oil and can be extracted by various methods. The essential oil extracted from the fruit peel is known to be commercially exploited for edible as well as nonedible purposes.

Essential oil is synthesised by plants as defensive measure and are known to have antibacterial, antifungal and anti-parasitic properties for which its application has been increased tremendously. It can also be used as perfumes, cosmetics and bath products, for flavouring food and drink, incense, household cleaning products and medicinal use (Abdurahman *et al.*, 2013). Several species of citrus are organically grown in Sikkim Himalayas, of which Sikkim Mandarin is most commercial, though pomelo and rough lemon are also cultivated. After extraction of juice, the orange peels are treated as waste and lead to environmental pollution due to improper disposal. However this waste can be used for the extraction of citrus oil (Sikdar *et al.*, 2016). A plethora of studies are available on essential oil and its characterization. However, no such comparative study investigating the influence of altitude and maturity stages in the Citrus species being grown in this part of the country have ever been reported.

Because of growing application of essential oil derived from citrus peel, the present study was undertaken on Sikkim mandarin (*Citrus reticulata*), pomelo (*C. maxima*) and rough lemon (*C. jambhiri*) collected from five different altitude (800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m) of Sikkim in two stages *i.e.* mature and immature .

Materials and methods

Three species of *Citrus viz.,* Sikkim mandarin (*C. reticulata*), pomelo (*C. maxima*) and rough lemon (*C. jambhiri*) were collected at mature and immature stage from five different altitude range of Sikkim (800-1000 m, 1000-1200 m, 1200-1400 m, 1400-1600 m and >1600 m). The fruits were harvested at two stages of maturity: immature (green stage) and mature (green-yellow stage). The fruits were washed separately then peeled off carefully with the help of a sharp knife to avoid any damage to oil glands. Statistical analysis was performed using OPSTAT online software. *P* value less than 0.05 was considered statistically significant.

Extraction of the essential oil: The peel of fresh *Citrus* spp fruits were subjected to hydro distillation for 3 h using a Clevenger-type-apparatus. The distillates of the essential oils were dried in anhydrous sodium sulphate, filtered and stored at -4 °C until analyzed (Hussain *et al.*, 2008).

Methods of analysis: The physical and chemical characterization of essential oil comprising of solubility, refractive index, specific gravity, colour, acid value, peroxide value, iodine value, saponification value, thiocyanogen value, ester value, phenol and DPPH were determined based on the methods described by the

Association of Official Analytical Chemists (AOAC, 2005). Each experiment was carried out in triplicate to ensure reproducibility and the mean values are reported.

Oil yield: Oil yield was determined by dividing the weight of extracted oil with weight of the fresh peel and was expressed as percent.

The yield of the oil extracted was calculated using equation below

Yield(%) = (Weight of oil extracted/ weight of sample used) X 100

Solubility: A small amount of oil was mixed with water which was stirred. It resulted into two layer indicating that the oil was insoluble in water.

Specific gravity: Pycnometer was weighed (B) and filled with the oil (A). After that water was placed by removing oil (C). The equation was then used to determine specific gravity.

Specific gravity (at 30 ºC) = A- B/C-B

Where, A = weight in g of specific gravity bottle with oil at 30 °C; B = weight in g of specific gravity bottle at 30 °C; C = weight in g of specific gravity bottle with water at 30 °C

Refractive index: The refractive index of oil was determined by Butyro refractrometer. A drop of oil was poured around the prism of refractrometer and RI was noted down.

Acid value: 10 mL of oil was taken in conical flask along with 50 mL of ethanol. After that 2-3 drops of phenolphthalein indicator was added and titrated against 0.1N KOH till the pink colour was attained. The volume of KOH displaced for titration was noted down and by using the equation acid value was determined for the oil samples.

The acid value was calculated using equation:

Acid Value = (56.1 × Normality of NaOH)/ Weight of Sample (g)) × Titre value

Saponification value: In a 250 mL conical flask 2 mL of essential oil, 50 mL of 0.5M alcoholic KOH was added. It was stirred for about 1 hour and titration was done with 0.5 M HCl by applying phenolphthalein indicator till pink colour disappears.

Saponification value = ((S-B) × C × 56.1)/M) × 100

Where S is the sample titre value; B is the blank titre value, C is the concentration of the HCl, 56.1 is the molecular weight of KOH and M is the weight of the sample.

Peroxide value: 2 mL of oil and 30 mL of glacial acetic acid was taken in a flask. Then KI (0.5 mL) was added and finally 30 mL of distilled water. It was then titrated with 0.1 M sodium thiosulphate using, 0.5 mL starch indicator. Blank titration was also done (Saad, 2015).

Peroxide value = ((Sample titre - Blank titre)/ Weight of oil taken) × Normality of sodium thiosulphate solution × 1000

Free fatty acid: 1 g of the essential oil was poured in a beaker and warmed; 25 mL of methanol was added to the sample and stirred thoroughly followed by 2 drops of phenolphthalein indicator and a drop of 0.14 N NaOH. The mixture was titrated against NaOH solution until a light pink colour which persisted for about 1 minute was observed. The end-point was recorded which was used for calculation of free fatty acid from equation (Rapisararda, 1999; Saad, 2015).

Acid value= Free Fatty Acid (%) × 1.99

Iodine value: 1 g of the essential oil was added to10 mL of CCl_4. The entire content was dissolved in 10 mL of wiji's solution by swirling. It was kept in dark for 30 minutes. The solution was titrated against sodium thiosulphate with starch as an indicator. The same procedure was repeated for blank titration (Saad, 2015). Iodine value was calculated using following equation:

I.V = (Blank titre – Sample titre) / Weight of sample (g)) × N of $Na_2S_2O_3$ × 12.69

Thiocyanogen value (T.V): 1 mL of oil mixed with 25 mL of thiocyanogen solutions was stored in dark for 24 hours. Then KI (1.66 g) was added, mixed thoroughly and 30 mL of distilled water was added and titrated with 0.1 N $Na_2S_2O_3$ using starch indicator.

T.V= ((Blank titre – Sample titre) / Weight of sample (g)) × N of $Na_2S_2O_3$ × 12.69

Ester value: It was obtained as the difference between the saponification value and the acid value of the oil (Saad, 2015).

Ester value = Saponification value - Acid value

Phenol: Folin-Ciocalteu and Gallic acid reagent were used to determine the phenolic content in oil sample using spectrophotometer. It was expressed as mg g^{-1} GAE (Singleton *et al.*, 1999).

DPPH: 50 µL of essential oil dissolved in 1000 µL methanol. In a test tube 50, 100, 125, 150, 200 µL of methanolic solution was taken and adjusted to 300 µL with methanol and 2700 µL fresh methanolic solution of DPPH was added. The decrease in absorbance at 517 nm related to the colour decrease was determined for all samples.

Results and discussion

Physico chemical characterization

Solubility: Essential oil extracted from the peel of immature and mature fruits of mandarin, pomelo and rough lemon was found to be insoluble in water. It was in corroboration the finding of (Javed *et al.*, 2014) as reported for mandarin, grape fruit, tangerine, malta and mousami that the essential oil extracted from citrus fruit peels are insoluble in water.

Yield of citrus peel essential oils: Yield of essential oil obtained from hydro distillation at 60 ºC is shown in Table 1, 3 and 5. The highest oil yield was found in immature fruits of Sikkim manadarin (2.50 %) as compared to mature (2.40 %) at >1600 m altitude followed by 2.03 and 2.06 % at mature and immature stage at 1400-1600 m altitude in mandarin. Likewise, in pomelo and rough lemon, oil yield was found higher for the samples collected from >1600 m altitude at mature (1.73 % and 2.31 %) and immature stages (1.72 % and 1.65 %). Javed *et al.* (2014) reported that *C. paradisi*, *C. sinensis* var. Malta, *C. reticulata* var. Mandarin, *C. sinensis* var. Mousami, *C. reticulata* var. Tangerine oil yield was 0.45, 0.37, 0.33, 0.30 and 0.28 %, respectively which is comparatively lower than our finding. This shows that the citrus species taken for present study have high potential for essential oil production and yield almost five times of oil than reported earlier for other species of citrus.

Refractive index (RI): Refractive Index of essential oil extracted from immature and mature stages of fruits was 1.49 and 1.48 at

Table 1. Physical properties of the essential oil at mature and immature stage from mandarin peel

Altitude (m)	Parameter							
	Colour		Specific gravity		Refractive Index		Yield (%)	
	Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature
800-1000	0.81±0.02	0.69±0.02	0.84±0.01	0.85±0.01	1.46±0.01	1.46±0.01	1.48±0.08	1.49±0.07
1000-1200	0.90±0.04	0.87±0.06	0.85±0.01	0.84±0.01	1.47±0.01	1.48±0.01	1.83±0.07	1.95±0.24
1200-1400	0.95±0.02	1.04±0.01	0.86±0.01	0.82±0.01	1.47±0.01	1.47±0.01	1.84±0.062	1.97 ±0.11
1400-1600	0.75±0.03	0.97±0.05	0.85±0.01	0.86±0.01	1.47±0.01	1.48±0.01	2.03±0.061	2.06 ±0.06
>1600	0.73±0.03	1.06±0.14	0.86±0.01	0.82±0.01	1.48±0.01	1.49±0.01	2.40±0.115	2.50±0.11
LSD (P=0.05)	0.09	0.23	0.01	0.03	0.01	0.01	0.26	0.44

Table 2. Chemical properties of essential oil at mature and immature stage from mandarin peel

Altitude (m)	Stage	800-1000	1000-1200	1200-1400	1400-1600	>1600	LSD (P=0.05)
Free fatty acid (%)	Mature	1.50±0.29	1.06±0.13	5.04±0.13	5.38±0.19	5.50±0.27	0.67
	Immature	6.77±0.18	6.21±0.56	7.83±0.18	5.74±0.32	5.54±0.43	1.16
Acid value (mg KOH/g oil)	Mature	7.63±0.26	5.27±0.18	6.12±0.06	5.80±0.11	8.30±0.15	0.53
	Immature	8.17±0.13	5.87±0.13	5.74±0.13	8.37±0.32	8.33±0.33	0.73
Saponification value (mg KOH/g oil)	Mature	152.67±1.33	114.34±0.33	163.67±0.88	142.00±1.53	161.34±0.67	3.33
	Immature	66.38±1.90	56.70±1.76	41.05±1.95	45.67±0.67	30.03±0.98	4.93
Iodine value (g I_2/100g oil)	Mature	86.00±1.73	106.46±1.86	109.96±1.19	116.00±0.58	114.64±1.66	4.73
	Immature	102.00±1.16	116.00±0.98	91.34±1.95	84.50±1.05	112.67±1.76	1.44
Peroxide value (meq.O_2/kg oil)	Mature	7.70±0.35	15.40±0.60	18.50±0.25	23.67±0.88	27.33±0.33	1.71
	Immature	15.00±0.58	23.00±0.58	31.33±0.67	34.70±0.30	36.33±0.33	1.64
Thiocyanogen value (%)	Mature	71.67±0.33	73.63±1.17	75.29±0.65	74.96±0.55	75.40±0.40	2.18
	Immature	72.13±1.65	75.10±0.09	75.17±0.24	77.30±1.35	75.40±0.12	3.07
Ester value (mg KOH/g oil)	Mature	145.03±1.57	109.07±0.48	157.54±0.82	136.20±1.61	153.04±0.78	3.66
	Immature	57.63±1.23	50.83±1.66	35.31±2.08	37.30±0.85	21.70±0.65	4.45
Phenol(mg/g)	Mature	79.07±0.64	81.21±0.12	80.81±0.45	80.79±0.22	81.92±0.91	1.74
	Immature	79.67±0.33	80.83±0.44	80.87±0.13	80.41±0.30	81.63±0.19	0.96
DPPH(%)	Mature	25.67±1.20	42.67±1.20	63.33±1.86	71.75±1.18	81.04±0.58	4.05
	Immature	57.83±0.60	80.95±0.95	59.33±1.45	57.00±1.53	74.66±0.66	3.54

>1600 m altitude, respectively for mandarin. Pomelo recorded maximum RI (1.49) at mature and immature stages at >1600 m altitude. Likewise rough lemon recorded 1.48 (1200-1400 m, >1600 m) in mature stage and 1.48 (1400-1600 m and >1600 m) as maximum RI. Ali (2015) reported 1.48, 1.47 and 1.42 RI in lemon, orange and grapefruit. Njoku and Evbuomwan (2014) reported 1.47, 1.48 and 1.48 RI in orange, lemon and lime peel oil, respectively, which are in range with the present finding. In orange peel oil RI was 1.49 (Zaker, 2017).

Specific gravity: Mandarin showed the highest specific gravity (0.86) at >1600 m, 1200-1400 m altitude in mature stage and 0.86 at 1400-1600 m altitude in immature stage. 0.85 specific gravity was found in pomelo at mature stage at elevation range of 1000-1200 m, 1200-1400 m and >1600 m. In immature fruits highest specific gravity (0.87) was recorded for the fruits collected from 1400-1600 m altitude. On the other hand, Rough lemon showed 0.85 (>1600 m, 1000-1200 m altitude) as maximum specific gravity in mature and 0.86 (at 800-1000 m and >1600 m altitude) in immature fruits. The essential oil of mandarin, mousami, grape fruit, malta and tangerine was reported earlier up to 0.84, 0.85, 0.86, 0.85 and 0.84 specific gravity (Javed *et al.,* 2014).

In another research (Njoku and Evbuomwan, 2014), specific gravity was found to be 0.85 and 0.84 for grapefruit and tangerine peel oil. As mentioned by Sharma (2016), in Kinnow mandarin peel oil, the specific gravity was 0.81, 0.80, 0.86, 0.83 and 0.85 in various methods of oil extraction *viz.,* physical method and hydro distillation, physical method, hydro distillation, mechanical dried peel by hydro distillation and solar dried peel by hydro distillation. Likewise, sweet orange peel showed 0.84 as specific gravity (Ezejiofor *et al.*, 2011).

In orange peel oil specific gravity was 0.85 (Zaker, 2017). Citrus oil should have specific gravity between 0.85 and 0.86 (Codex Alimentarium Commission, 1981). The specific gravity in the present study is in corroboration with the finding of the study. The specific gravity so obtained for all the samples indicates that the extracted oil was pure.

Colour: Mandarin (1.06) showed the maximum value at >1600 m altitude at immature stage. However it was found to be at par with 0.87 (1000-1200 m), 1.04 (1200-1400 m), 0.97 (1400-1600 m altitude), respectively. In addition, mature stage showed 0.95 (1200-1400 m) altitude as the highest and 0.73 (>1600 m altitude) as the lowest.

Further pomelo was found with 0.80 and 1.09 as the highest and lowest value at 800-1000 m and >1600 m altitude in mature stage. Likewise in immature stage 0.57 (800-1000 m altitude) was accorded with the lowest value. However it was found to be at par with 0.68 (1000-1200 m) and 0.76 (1200-1400 m altitude). Moreover, >1600 m altitude showed the highest value (1.04).

Rough lemon, on the other hand, showed 1.40 (>1600 m) and 1.26 (1200-1400 m) as the highest value in terms of colour in mature and immature stages, respectively. As per Zaker (2017) Orange peel oil showed colour as 3.47, -2.39, 0.82, and 2.47 for light, green, yellow, chroma and hue value.

Free fatty acid (%): The extracted essential oil was analysed to determine its free fatty acid content. Highest free fatty acid (7.83 %) was estimated in immature samples collected from1200-1400 m altitude and it was observed to be 5.50 % at >1600 m altitude at mature stage in mandarin. As can be seen from the Table 6, the pomelo oil had highest free fatty acid (6.01 %) at altitude >1600 m which was at par with that of 1400-1600 m altitude (5.76 %). On the other hand, immature stage fruit showed 7.38 % as the highest at >1600 m altitude. In addition value of 2.57 % (800-1000 m altitude) was recorded as the lowest. Further, it was at par with 2.90 % at 1000-1200 m altitude.

The trend in rough lemon was different than pomelo and mandarin at immature stage. It showed highest value (8.26 %) at 1400-1600 m altitude which was at par with 7.60 % recorded for the samples from 1200-1400 m altitude. On the other hand, immature stage was found with 7.90 % at >1600 m altitude. The high free fatty acid during this study is in disagreement with the finding of Khan (2013) who mentioned sweet orange and sour orange with 5.8 % and 2.3 % of free fatty acid. In addition, Sharma (2016) reported free fatty acid to the tune of 1.74, 2.53, 2.05, 2.18 and 2.24 % in physical method, hydro distillation, mechanical dried peel by hydro-distillation and solar dried peel by hydro-distillation, respectively. The present finding showed free fatty acid up to the extent of 6 percent which showed it could be used for making soap. As illustrated by Olabanji *et al.* (2016) free fatty acid content in seed oil (11.86 %) was higher than peel oil (12.61 %) so it can be used to make soap as FFA were higher than 2.5 and 1.4 % (FAO/WHO,1993)

Acid value (mg KOH/g oil): Mandarin, pomelo and rough lemon oil were analysed for acid value content in immature and mature stages as depicted in Table 2, 4 and 6.

The acid value in mandarin was 8.37 mg KOH/g oil in the samples from 1400-1600 m altitude as highest which was at par with 8.33 mg KOH/g oil (>1600 m) and 8.17 mg KOH/g oil (800-1000 m)

Table 3. Physical properties of the essential oil at mature and immature stage from pomelo peel

Altitude (m)	Parameter							
	Colour		Specific gravity		Refractive Index			
	Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature
800-1000	0.80±0.03	0.57±0.01	0.84±0.01	0.84±0.01	1.46±0.01	1.46±0.04	0.88±0.04	1.19±0.03
1000-1200	0.87±0.05	0.68±0.08	0.85±0.01	0.86±0.01	1.47±0.01	1.47±0.01	1.48±0.20	1.60±0.05
1200-1400	0.89±0.01	0.76±0.15	0.85±0.01	0.86±0.01	1.48±0.03	1.47±0.01	1.57±0.09	1.81±0.04
1400-1600	1.01±0.06	0.92±0.12	0.82±0.01	0.87±0.01	1.49±0.01	1.48±0.02	1.60±0.06	1.83±0.07
>1600	1.09 ±0.05	1.04±0.04	0.85±0.01	0.86±0.01	1.49±0.01	1.49±0.03	2.00±0.10	2.40±0.15
LSD (*P*=0.05)	0.14	0.31	0.02	0.02	0.02	0.01	0.22	0.26

Table 4. Chemical properties of essential oil at mature and immature stage from pomelo peel

Altitude (m)	Stage	800-1000	1000-1200	1200-1400	1400-1600	>1600	LSD (*P*=0.05)
Free fatty acid(%)	Mature	2.26±0.08	2.61±0.28	3.14±0.39	5.76±0.12	6.01±0.05	0.71
	Immature	2.57±0.04	2.90±0.02	3.16±0.02	5.85±0.07	7.38±0.32	0.47
Acid value (mg KOH/g oil)	Mature	6.08±0.25	6.20±0.18	7.14±0.70	7.82±0.74	10.11±0.64	1.77
	Immature	6.20±0.41	8.51±0.05	10.23±0.72	12.74±0.81	13.35±0.68	1.92
Saponification value (mg KOH/g oil)	Mature	130.67±5.82	159.34±3.17	180.62±2.92	136.34±3.18	131.41±4.85	13.25
	Immature	43.03±0.98	49.09±4.05	43.72±1.64	58.37±1.17	83.43±3.44	8.23
Iodine value (g I_2/100g oil)	Mature	101.77±1.30	104.45±1.09	93.39±1.24	112.67±1.76	93.06±1.57	4.51
	Immature	104.00±0.58	104.29±1.81	78.38±1.16	92.00±1.53	105.62±1.16	4.19
Peroxide value (meq.O_2/kg oil)	Mature	8.84±0.42	10.8±0.42	12.98±0.38	15.67±0.18	17.04±0.23	1.07
	Immature	9.48±0.29	11.33±0.54	13.96±0.20	16.03±0.26	17.68±0.22	1.02
Thiocyanogen value (%)	Mature	74.91±0.40	75.55±0.24	75.86±0.57	77.08±0.51	77.87±0.93	1.84
	Immature	74.96±0.55	75.63±0.32	75.84±0.20	76.09±0.22	77.09±0.20	1.03
Ester value (mg KOH/g oil)	Mature	123.54±5.51	151.96±3.46	174.54±3.15	130.14±3.34	122.71±2.58	11.96
	Immature	31.88±1.06	36.75±3.48	33.34±2.62	50.49±0.29	71.74±5.51	10.15
Phenol (mg/g)	Mature	79.80±0.42	81.15±0.45	81.33±0.55	81.88±0.77	82.67±0.42	1.72
	Immature	79.13±0.69	80.81±0.18	80.87±0.10.13	80.91±0.10	81.17±0.44	1.22
DPPH (%)	Mature	47.68±0.87	65.75±9.86	77.78±7.78	81.37±2.44	82.00±0.58	7.28
	Immature	69.99±3.20	77.75±1.38	70.99±2.49	68.77±0.26	77.84±2.93	7.43

Table 5. Physical properties of the essential oil at mature and immature stage from rough lemon peel waste

Altitude (m)	Parameter							
	Colour		Specific gravity		Refractive Index		Yield (%)	
	Mature	Immature	Mature	Immature	Mature	Immature	Mature	Immature
800-1000	0.79±0.02	0.61±0.03	0.84±0.01	0.86±0.01	1.46±0.01	1.46±0.01	1.37±0.03	0.77±0.03
1000-1200	0.80±0.04	0.67±0.05	0.85±0.01	0.83±0.01	1.47±0.01	1.47±0.01	1.54±0.04	1.41±0.02
1200-1400	1.30±0.03	1.26±0.03	0.83±0.01	0.84±0.01	1.48±0.01	1.47±0.01	2.50±0.18	1.53±0.01
1400-1600	1.26±0.07	0.22±0.07	0.82±0.01	0.85±0.01	1.47±0.01	1.48±0.01	2.31±0.05	1.64±0.03
>1600	1.40±0.02	0.79±0.01	0.85±0.01	0.86±0.01	1.48±0.01	1.48±0.01	2.61±0.17	2.14±0.03
LSD (*P*=0.05)	0.13	0.13	0.02	0.02	0.01	0.01	0.37	0.08

in immature stage. Likewise mature stage was found with 8.30 mg KOH/g oil acid value at >1600 m altitude. In pomelo acid value was as high as 10.11 at >1600 m altitude and as low as 6.08 mg KOH/g oil in the samples from 800-1000 m. The acid value in the immature stage was higher than the mature one, maximum was recorded to be 13.35 mg KOH/g oil (>1600 m altitude) which was at par with 12.74 mg KOH/g oil (1400-1600 m altitude) and the lowest value was 6.20 mg KOH/g oil (800-1000 m altitude). In rough lemon, 8.97 mg KOH/g oil (1000-1200 m altitude) was recorded with maximum acid value which was at par with 7.96 mg KOH/g oil (1200-1400 m altitude) in mature stage. Further in immature stage, 8.43 mg KOH/g oil (1400-1600 m) showed the maximum value which was at par with 7.94 mg KOH/g oil (1000-1200 m), 8.24 mg KOH/g oil (1200-1400 m) and 8.34 mg KOH/g oil (>1600 m altitude) accordingly.

The present study showed comparatively higher acid value than that of the findings of Ali (2015) with 1.79, 1.99 and 2.23 mg/KOH/g oil in lemon, orange and grape fruit. Njoku and Evbuomwan (2014) have also reported acid value of 1.99, 1.77 and 2.20 mg KOH/g oil in orange, lemon and lime peel oil. Sharma (2016) mentioned 3.46, 5.02, 4.07, 4.34 and 4.46 mg KOH/g acid value in kinnow mandarin peel oil in different methods of oil extractions *viz.,* physical and hydro distillation, physical, hydro distillation, mechanical dried peel by hydro distillation and solar dried peel by hydro distillation methods which was lower than the present finding. In orange peel oil 0.28 mg KOH/g oil of acid value was found (Zaker, 2017). The acid value was comparatively higher in the present study which indicates that it is suitable for making oil paint for industry and the oil will have outstanding storage life.

Saponification value (mg KOH/g oil): The saponification value is shown in Table 2 and Table 4. It is observed that samples from 800-1000 m and 1200-1400 m altitude had the highest value of 66.38 mg KOH/g oil and 163.37 mg KOH/g oil, respectively, at immature and mature stages in mandarin peel oil. In pomelo, the maximum value was 180.62 mg KOH/g oil (1200-1400 m) in mature fruit oil and 83.43 mg KOH/g oil (>1600 m) in immature fruits. In case of rough lemon lowest and highest value were 134.41 mg KOH/g oil (>1600 m altitude) and 163.73 (1000-1200 m altitude), respectively, at mature stage. In immature fruit, maximum value was 39.74 (>1600 m altitude).

The present study estimated comparatively higher value than reported by Njoku and Evbuomwan (2014) for orange, lemon and lime (16.19, 13.76 and 13.23 mg KOH/g oil). Whereas Khan (2013) reported saponification value of 171 and 183 mg/KOH/g oil in sour and sweet orange peel oil. Saponification value reported by Ali (2015) in lemon, orange and grapefruit showed 16.75, 13.5 and 13.7 mg/KOH/g oil is almost 10 times less than that of present value. Saponification value of kinnow oil reported by Sharma (2016) was at par with the values of present studies. The high saponification value of the orange seed oil indicates the presence of high percentage of fatty acids in the oil (Omolara and Dosummu, 2009). The relatively high value recorded is indicative that it has potential for use in the industry (Akubugwo and Ugbogu, 2007).

Iodine value (g I_2/100g oil): Mandarin, pomelo, and rough lemon oil were analysed for iodine value, as presented in Table 2, 4, 6. Mandarin accorded the highest value of 116.00g I_2/100g oil at 1400-1600 m at immature stage, which was at par with 112.67 g I_2/100g oil (>1600 m), 102 (800-1000 m), respectively. On the other hand, samples of mature stage had iodine value of 116.00 (>1600 m) as the highest value, which was at par with 114.64 g I2/100g (>1600 m altitude). In pomelo, at 1400-1600 m altitude maximum value 112.67 g I_2/100g oil was estimated in mature stage and 105.62 g I_2/100g oil in immature fruits. In rough lemon, iodine value was highest (115.67 g I2/100g) at 1400-1600 m in mature stage and in immature stage it was 111.01 g I_2/100g oil (1000-1200 m).The present finding is in line with Khan (2013), who reported sour orange and sweet orange peel oil with 100 and 103 g/100g of iodine. In orange peel oil, it was reported to be 120 g I2/100g g/100 g (Zaker, 2017). Higher the iodine value, greater the number of C=C double bonds. It means that oil is unsaturated and hence can be used in the production of soap (Akinhanmi *et al.*, 2009).

Table 6. Chemical properties of essential oil at mature and immature stage from rough lemon peel

Parameters	Stages	Altitude (m)					
		800-1000	1000-1200	1200-1400	1400-1600	>1600	LSD (P=0.05)
Free fatty acid (%)	Mature	2.16±0.17	3.8±0.15	7.60±0.47	8.26±0.23	5.13±0.18	0.85
	Immature	5.80±0.42	6.40±0.21	7.77±0.41	7.60±0.06	7.90±0.40	1.05
Acid value (mg KOH/g oil)	Mature	6.77±0.15	8.97±0.12	7.96 ±0.51	7.31±0.64	7.25±0.03	1.19
	Immature	6.70±0.25	7.94±0.47	8.24±0.33	8.43±0.01	8.34±0.07	0.9
Saponification value (mg KOH/g oil)	Mature	143.34±2.02	163.73±2.37	154.01±3.46	156.68±1.76	134.41±3.59	8.76
	Immature	74.67±2.40	78.74±2.78	86.99±2.89	36.73±1.64	39.74±2.34	7.82
Iodine value (g I_2/100g oil)	Mature	94.76±1.53	92.73±1.26	94.00±1.15	115.67±1.20	86.41±1.21	4.08
	Immature	103.33±1.20	111.01±0.58	74.00±1.53	106.67±1.76	104.67±1.45	4.36
Peroxide value (meq.O_2/kg oil)	Mature	11.33±0.88	5.37±0.32	14.17±1.30	22.70±1.76	6.03±0.61	3.51
	Immature	22.67±1.20	12.00±1.16	5.50±0.27	12.67±1.45	12.33±1.45	3.80
Thiocyanogen value (%)	Mature	72.33±0.33	74.73±0.88	75.71±0.01	75.35±0.22	75.78±0.33	1.44
	Immature	73.73±0.37	75.21±0.13	75.61±0.20	75.46±0.29	75.63±0.46	0.99
Ester value (mg KOH/g oil)	Mature	132.66±2.92	156.16±4.19	147.00±2.61	149.37±2.06	127.50±3.37	9.93
	Immature	67.40±3.15	70.98±3.21	79.41±2.16	28.30±1.63	32.82±3.11	8.70
Phenol (mg/g)	Mature	79.81±0.42	80.40±0.20	80.43±0.07	80.54±0.33	81.40±0.31	0.92
	Immature	80.63±0.32	87.51±1.05	85.33±0.88	86.00±1.00	89.59±0.80	2.68
DPPH (%)	Mature	47.00±1.53	71.72±1.25	79.68±1.19	81.71±1.24	71.60±1.14	5.79
	Immature	44.05±0.53	66.00±0.58	80.95±0.95	70.82±0.43	72.43±0.97	2.29

Peroxide value (meq.O_2/kg oil): All the studied crops, mandarin, pomelo and rough lemon showed maximum peroxide value of 36.33, 17.68 and 22.67 meq O_2/kg oil at immature stages at >1600 m, >1600 m and 1400-1600 m altitude. In mature stage, mandarin had highest peroxide value of 27.33 meq O_2/kg oil followed by pomelo (17.04 meq O_2/kg oil) and rough lemon (22.70 meq O_2/kg oil) at >1600 m >1600 m and 800-1000 m altitude, respectively.

In earlier reports, peroxide values were 13.67, 18.68 and 9.59 meq O_2/kg oil in lemon, orange and grapefruit (Ali, 2015) and 68.75, 13.57 and 9.57 meq O_2/kg oil in orange, lemon and lime (Njoku and Evbuomwan, 2014), and 35.2 and 13.5 meq O_2/kg oil in sour orange and sweet orange peel oil (Khan, 2013). The result indicated that peroxide value was higher than the standard values for vegetable oil (below15 meq/kg), so it can be used in industry instead of cooking oil. Further, Olabanji *et al.* (2016) mentioned that the orange seed oil value more than 10 milli eq./kg of the oil as per FAO/WHO,1993 can be used for the cosmetics industry, which includes soap making, perfumes and not suitable for consumption.

Thiocyanogen value (%): The data of thiocyanogen value is shown in Tables 2, 4 and 6. Mandarin was found with 77.29 % (1400-1600 m) as the highest value, which was in turn at par with 75.39 % (>1600 m), 75.16 % (1400-1600 m), 75.10 % (1000-1200 m altitude) in immature stage. At mature stage it was 75.40 % (>1600 m altitude) as the maximum value followed by 75.29 % (1200-1400 m), 74.96 % (1400-1600 m altitude). Pomelo was noted with 77.87 % and 77.09 % at >1600 m altitude as the highest value in mature and immature stages, respectively.

In rough lemon, the highest content (75.78 %) of TV was in >1600 m altitude, which was at par with 74.73 % (1000-1200 m), 75.71 % (1200-1400 m) and 75.73 % (1400-1600 m altitude) in mature fruits. Likewise immature stages showed the maximum TV (75.63 %), which was found to be at par with 75.21 % (1000-1200 m), 75.61 % (1200-1400 m) and 75.46 % (1400-1600 m altitude).

Ester value (mg KOH/g oil): In mandarin, ester value was estimated up to 57.63 mg KOH/g oil at 800-1000 m altitude as the lowest and 157.54 mg KOH/g oil at 1200-1400 m altitude as the highest ester value. Likewise in pomelo of mature stage, ester value of 122.71 mg KOH/g oil was recorded, which was lowest value but significantly at par with 130.14 mg KOH/g oil (1400-1600 m altitude). Likewise in immature stage 800-1000 m altitude was found with 31.88 mg KOH/g oil as lowest value, which was at par with 36.75 mg KOH/g oil (1000-1200 m altitude) and 33.34 mg KOH/g oil (1200-1400 m altitude).

Rough lemon, on the other hand, showed 156.16 mg KOH/g oil for 1000-1200 m altitude as the highest value. At mature stage, it was at par with 147.00 mg KOH/g oil (1200-1400 m) and 149.37 mg KOH/g oil (1400-1600 m altitude). Whereas in immature stage, 79.41 mg KOH/g oil (1200-1400 m) had highest ester value. It was found to be at par with 70.98 mg KOH/g oil at 1000-1200 m altitude.

Phenol (mg/g): In mature stage samples, mandarin had phenol content up to 81.92 mg/g of fruits at >1600 m altitude followed by significantly at par value of 80.89 mg/g (1200-1400 m) and 80.79 (1400-1600 m). In immature stage, samples from >1600 m had 81.63 mg/g phenol followed by the significantly at par value of 80.83 mg/g (1000-1200 m) and 80.87 mg/g (1200-1400 m altitude).

Likewise in pomelo at mature stage, highest phenol content (82.67 mg/g) at >1600 m altitude showed the maximum value which was at par with 81.15 mg/g (1000-1200 m), 81.33 mg/g (1200-1400 m) and 81.88 mg/g (1400-1600 m altitude). On the other hand highest value of phenol content (81.17 mg/g) was observed at >1600 m altitude. It was found to be at par with 80.81 mg/g (1000-1200 m), 80.87 mg/g (1200-1400 m) and 80.91 mg/g (1400-1600 m altitude).

In case of rough lemon, fruits from >1600 m altitude showed the highest phenol content (81.40 mg/g). However, at immature stage it was found to be 89.59 mg/g (>1600 m altitude). Further, it was found to be at par with phenol content of 87.51 mg/g at (1000-1200 m altitude). Similar kind of result was reported in lemon peel oil (81.82 mg gallic acid equivalent/g of essential oil) by Moosavy *et al.* (2017).

Free radical scavenging activity using the DPPH method: As illustrated in Tables 2, 4 and 6, there was variation in altitude for mandarin, pomelo and rough lemon regarding free radical scavenging activity. Mandarin (80.95 %) at 1000-1200 m in immature and 81.04 % at >1600 m altitude in mature stage was observed. Likewise pomelo was noted with 82 % free radical scavenging activity at >1600 m altitude as the highest at mature stage. In addition, at immature stage maximum value (77.84 %) was found at >1600 m.

On the other hand, rough lemon had maximum value of 81.71 % (1400-1600 m) at mature stage which was at par with 79.68 % (1200-1400 m). In addition, immature stage showed 80.95 % (1200-1400 m) and 44.05 % (800-1000 m) as the highest and lowest free radical scavenging activity, respectively. As mentioned by Javed *et al.* (2014), free radical scavenging activity was 91.1 % in mandarin 88 % in tangerine, followed by pomelo (87.2 %), malta (86 %) and mousami (83.2 %). It also indicates that mandarin has high antioxidant activity compared to other *Citrus* spp which is similar to the present finding in immature fruits. Sharma (2016) mentioned 55.09 % inhibition from free radical scavenging activity in lemon peel oil, which was lower than the present research.

Present study revealed that the citrus fruit peel is rich in essential oils. Its content and characteristics are very much influenced by the altitude and species. The oil yield was up to 2.4 % in mature fruits and 2.5 % in immature fruits at an altitude > 1600 m in mandarin orange. *C. reticulata* grown at higher altitude (> 1600 m msl) had better essential oils than *C. maxima* and *C. jambhiri*.

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