Comparative Evaluation of Physiochemical and GC-MS Analysis of Sour Oranges and Sweet Oranges Peels Oil

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Abstract: The studied were carried out for the comparative evaluation of physiochemical and GCMS analysis of sour and sweet oranges peel oil of Pakistan. The physiochemical analysis of sour oranges were shown that moisture (%), density, iodine value (g/100g), saponification value (mg/g), peroxide value (mg eq/kg), free fatty acid value (%) 0.34 ±0.22, 0.92 ± 0.16, 100 ± 0.44 , 171 ± 0.32 , 35.2 ± 0.12 and 5.8 ± 0.31 respectively, while similarly the sweet oranges peels physiochemical analysis value were 0.42 ± 0.15 moisture (%), 0.94 ± 0.13 density, 103 ± 0.54 iodine value (g/100g), saponification value (mg/g), peroxide value (mg eq/kg) and free fatty acid value (%) were 183 ± 0.11 , 13.5 ± 0.17 and 2.3 ± 0.16 respectively. GC-MS analysis of sour orange and sweet orange peels oil were also calculated. The results of the present study demonstrated that the seeds of citrus species investigated are a potential source of valuable oil which might be utilized for edible and other industrial applications.
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Key words: Sour Oranges Peel Oil, Physiochemical Analysis, Saturated Fatty Acid and Un-Saturated Fatty Acid.

1. Introduction

Pakistan is one of the most important countries amongst citrus fruits producer. Citrus cultivation is probably one of the most important commercial and industrial agricultural activities of the world. The peel of *Citrus* fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants

(1). It is not surprising that the taxonomic family to which *Citrus* belongs, the Rutaceae, which include approximately 160 genera and 1700 species, has been used in herbal medicine

(2). In addition to various food products from pulp, *Citrus* peels are candied, fed to livestock, used to scent perfumes and soap products. Also it has been shown that limonene oil from peel has an insecticidal property. *Citrus* seeds are used to drive a cooking oil, and oils for plastic and soaps. Their flowers and foliage are used in perfume manufacturing. *Citrus* species essential oil contains: terpens, aliphatic sesquiterpene, oxygenated derivatives and aromatic hydrocarbons. The composition of the terpenic mix varies depending on the typology of examined *Citrus* fruit of the species to which it owns. Anyway, the mix of each typology is in different proportion made of: limonene, α–pinene, β-pinene, myrcene, linalol, and terpinen. Monoterpenes are important constituents of essential oil of *Citrus* fruits and other plants. A number of these monoterpenes have an antitumor activity. For example, d-limonene which comprises >90% of the orange peel oil has chemopreventive activity against rodents mammary, skin, liver, lung and forestomach cancer

(3).Many natural substances may play a fundamental role in the host plant/pathogen relationship: the essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobic, allelopathic, antioxidant and bio-regulatory properties. The antimicrobial abilities of essential oils, among which citrus oils are also shown to be a particularly interesting field for applications within the food and cosmetic industries

(4). Preparation from peel, flowers and leaves of bitter orange (*citrus aurantium* L.) are popularly used in order to minimize central nervous system disorders

(5). Some essential oils were used in skincare products and for acne control

(6). The chemical composition of the oil extract consequently gives a qualitative identification of oils, and is a very important area in the selective application guide in the commercialization and utility of oil products. Such analysis as the iodine value, gives an index of the drying and polymerizing properties of oil, while flash points indicate a substantial removal of solvent from a solvent-extracted oil like the one being discussed where a standard of not below 250 ^{o}F (121.1 ^{o}C) is required, for example by the open cup method of analysis. Apart from establishing the identity of oils, proximate analytical data expresses the particular type or grade of oil, despite the varying properties and processing characteristics of oils, due to geographical origin and the method of extraction from the oil-bearing material. It is then very important to obtain the specific data for samples of oil from a particular area, because there exist a range of free fatty acid content of these oils due to geographical origin, eg, Maloyan, Congo, Lagos, Niger palm oils or Ceylon, cochin coconut oils, etc (7). Finally, the aim of this work is to find use for the peel of the Pakistani sour orange which was being wasted, by extracting the oil and assessing the quality of the oil so extracted, in order to know in what industry it can be best utilized.

2. Materials and Methods

2.1 Procurement of Raw materials

The sour oranges and sweet oranges peels were obtained from the Food Pilot Plant of PCSIR Laboratories Complex Peshawar. The peels were dried in a dehydrator at 50°C for 24 hrs. A grinder mill was used to obtain a powder particle size of less than 0.2 mm.

2.2 Extraction of Oil from Peels

A Soxhlet extractor was used for solvent extraction of the oil. The solvent (*n*-Hexane) was removed from the extract by distillation and the residual oil component collected and used for the analytical work. A 500 ml capacity Soxhlet extractor was used in the extraction of the oil from the peel powder. 20 g of the ground peel powder were packed in a whatman filter paper and inserted into the Soxhlet extractor and Hexane was used as the extracting solvent. The period of continuous extraction was 16 hours. At the end of this period, the solvent was recovered by simple distillation and the residual oil was oven-dried at 65°C ± 2°C for one hour. The oil was then transferred to a desiccator and allowed to cool, before being weighed. The drying, cooling and weighing was repeated until a constant dry weight was obtained (three cycles of treatment), to within 0.01 g. The extracted oil sample was in a well sealed dark brown colored glass bottle and kept for analytical tests (8).

2.3 Physiochemical analysis of Peels Oil

Moisture (%), density, iodine value (g/100g), saponification value (mg/g), peroxide value (mg eq/kg) and free fatty acid (%) value were determined according to the standard method of analysis (8).

2.4 GC-MS analysis

2.4.1 Preparation of standard

2.4.2 Internal standard

13.7 mg of tridecanoic acid methyl ester was dissolved in 1 ml hexane.

2.4.3 External standard

10 mg of 37 component FAMEs mix standard was diluted to 10 ml with dichloromethane.

2.4.4 Preparation of FAMEs

Fatty acids present in the sample were derivatized according to the AOAC standard reference method. To a known amount of sample (equivalent to 25 mg fat) was added 0.1 ml internal standard (1.37 mg) and 1.5 ml of sodium hydroxide solution in methanol (0.5 N), sealed and heated in boiling water bath for 5 minutes. The hydrolyzed sample was cooled and added 2.5 ml of boron triflouride solution in methanol (10%). The solution was then sealed and heated in boiling water bath for 30 minutes and cooled. To the esterified solution was added 5 ml saturated sodium chloride solution and extracted twice with 1 ml hexane. The hexane extract was filtered through 0.45 µm membrane filter and injected 1 µl to GCMS using auto injector system.

2.4.5 Chromatographic separation of FAMEs

A gas chromatograph from Shimadzu hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) equipped with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used. Helium was used as carrier gas. All chromatographic separations were performed on a capillary column (TRB-FFAP; Technokroma) having specifications: length; 30 m, i.d.; 0.35 mm, thickness; 0.250 µm, treated with polyethylene glycol. Other GC-MS conditions are: ion source temperature (EI); 250 ℃, interface temperature; 240 ℃, pressure; 100 KPa, solvent cut time; 1.8 min. 1 µL of sample and standard were injected into the GC column. Injector was operated in a split mode with a split ratio 1:50. Injection temperature was 240 °C. The column temperature program started at 50 °C for 1 min and changed to 150 °C at the rate of 15 °C/min. The temperature was raised to 175 °C at the rate of 2.5

°C/min and hold for 5 minutes. Then the temperature was increased to 220 °C at the rate of 2.5 °C/min and kept constant for 3 minutes. Total elution time was 43 minutes. MS scanning was performed from m/z 85 to m/z 380. GC-MS solutions software provided by the supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of standard mass spectra from the NIST library (NIST 05).

3.0 Results and Discussion

The physiochemical analysis of sour orange and sweet orange peel oils were shown in table.1. The moisture (%) content of sweet orange peel oil was high (0.42 ± 0.15) than sour orange peel oil **(**0.34 ± 0.22). Similarly the density value of sweet orange peel oil was more than sour orange peel oil. The Iodine value (g/100g) of sweet orange and sour orange peel oil were103 ± 0.54 and 100 ± 0.44 respectively. The sweet orange Saponification value (mg/g) value was high (183 ± 0.11) as compared with sour orange (171 ± 0.32). The Peroxide value (mg eq/kg) of sour orange was high 35.2 ± 0.12 than sweet orage oil (13.5 ± 0.17 0. The sweet orange and sour orange peel oil Free Fatty Acid value (%) values were 2.3 ± 0.16 and 5.8 ± 0.31 respectively. The value of the saponification number, projects the oil in good light in such areas as soap making and in the detection of adulteration of the oil. Saponification value is used in checking adulteration. The high saponification value of the orange seed oil indicates the presence of high percentage of fatty acids in the oil (9). The relatively high value recorded is indicative that it has potential for use in the industry (10).

Saturated fatty acid of sour oranges peel oil were shown in Table.2. The highest concentration (%) was calculated Palmitic acid 49.75, while the lowest concentration was calculated to be Caprylic acid 0.03%. The other high concentration were calculated Stearic acid 8.06%, Myristic acid 2.14%, Tetracosanoic acid 1.53%, Lauric acid 1.03%, while the other lowest value Undecanoic acid 0.06, Tricosanoic acid 0.40, Capric acid 0.33, Behenic acid 0.81, Arachidic acid 0.64, Pentadecanoic acid 0.48, Margaric acid 0.82. The unsaturated fatty acid of sour orange peel oil (Table 3) revealed that the highest value were Linoleic acid 18.98%, followed by Oleic acid 8.22%, Linolenic acid 5.37%. While the lowest unsaturated value were Palmitoleic acid 0.44, Elaidic acid 0.94. Free faty Acid (FFA) concentration of the sweet orange and sour orange peel oil was for the above maximum limit of 2.0% reported for high grade Codex Alimentarius (11), even though the peroxide value was as low as 0.3. Oil having high percentages of peroxide is unstable and grows rancid easily (an unpleasant odor) (12). Iodine value is a chemical parameter that characterized oil based on the degree of unsaturation (13). The African sweet orange seed oil has saponification value 192, iodine value 108 and peroxide value 92.84 (14).

The saturated fatty acid of sweet oranges peel oil were shown in Table.4, palm tic acid was found the in highest concentration 48.16%, while the lowest concentration was found Undecanoic acid 0.07%. The other moderate value of the saturated fatty acid were 0.36, 0.55, 0.77, 4.76, 6.85, 0.41, 0.81, 4.57, 0.50, 1.16, 0.52 and 2.71 Hexanoic acid, Caprylic acid, Capric acid, Lauric acid, Lauric acid, Myristic acid, Pentadecanoic acid, Palmitic acid, Margaric acid, Stearic acid, Arachidic acid, Behenic acid, Tricosanoic acid and Tetracosanoic acid respectively. The unsaturated fatty acid of sweet oranges peel was presented in table.5. The linoleic acid was found in highest concentration 20.02%, while the lowest concentration was found elaidic acid 0.84%. The Linolenic acid and Oleic acid were found 3.54% and 3.42% respectively.

Table.1. Physiochemical Analysis of Sour Oranges and Sweet Oranges Peel Oil

S#	Parameters	Sour oranges	Sweet oranges
1	Moisture (%)	**0.34 ±*0.22	0.42 ± 0.15
2	Density	0.92 ± 0.16	0.94 ± 0.13
3	Iodine value (g/100g)	100 ± 0.44	103 ± 0.54
4	Saponification value (mg/g)	171 ± 0.32	183 ± 0.11
5	Peroxide value (mg eq/kg)	35.2 ± 0.12	13.5 ± 0.17
6	Free Fatty Acid value (%)	5.8 ± 0.31	2.3 ± 0.16

*Standards deviation values for the three measurements results; ** Average of three measurement

Table. 2. Quantitative Analysis of Saturated Fatty Acid of Sour Oranges Peel Oil.

S #	Saturated fatty acid	Retention Time	Concentration %	Area
1	Caprylic acid C 8:0	4.962	0.03	953
2	Capric acid C 10:0	6.798	0.33	11525
3	Undecanoic acid C 11:0	7.656	0.06	1953
4	Lauric acid C 12:0	8.551	**1.03**	36202
5	Myristic acid C 14:0	10.992	**2.14**	75654
6	Pentadecanoic acid C 15:0	12.656	0.48	16866
7	Palmitic acid C 16:0	14.678	**49.75**	1756728
8	Margaric acid C 17:0	16.964	0.82	28980
9	Stearic acid C 18:0	19.681	**8.06**	284631
10	Arachidic acid C 20:0	27.259	0.64	22471
11	Behenic acid C 22:0	34.390	0.81	28442
12	Tricosanoic acid C 23:0	37.646	0.40	14157
13	Tetracosanoic acid C 24:0	40.738	**1.53**	53851

Table. 3. Quantitative Analysis of Unsaturated Fatty Acid of Sour Oranges Peel Oil.

S #	Unsaturated fatty acid	Retention Time	Concentration %	Area
1	Palmitoleic acid C 16:1	15.199	0.44	15462
2	Oleic acid C 18:1	20.236	8.22	290184
3	Elaidic acid C 18:1	20.469	0.94	33152
4	Linoleic acid C 18:2	21.857	18.98	670331
5	Linolenic acid C 18:3	24.400	5.37	189602

Table 4. Quantitative Analysis of Saturated Fatty Acid of Sweat Oranges Peel Oil.

S #	Saturated fatty acid	Retention Time	Concentration %	Area
1	Hexanoic acid C 6:0	3.069	0.36	5576
2	Caprylic acid C 8:0	4.963	0.55	8363
3	Capric acid C 10:0	6.799	0.77	11803
4	Undecanoic acid C 11:0	7.657	0.07	1019
5	Lauric acid C 12:0	8.551	4.76	72828
6	Myristic acid C 14:0	10.993	6.85	108555
7	Pentadecanoic acid C 15:0	12.657	0.41	6211
8	Palmitic acid C 16:0	14.667	48.16	737072
9	Margaric acid C 17:0	16.965	0.81	12378
10	Stearic acid C 18:0	19.675	4.57	69873
11	Arachidic acid C 20:0	27.266	0.50	7716
12	Behenic acid C 22:0	34.390	1.16	17798
13	Tricosanoic acid C 23:0	37.649	0.52	7920
14	Tetracosanoic acid C 24:0	40.736	2.71	41492

Table 5.Quantitative Analysis of Unsaturated Fatty Acid of Sweat Oranges Peel Oil.

S #	Unsaturated fatty acid	Retention Time	Concentration %	Area
1	Oleic acid C 18:1	20.220	3.42	52305
2	Elaidic acid C 18:1	20.461	0.84	12782
3	Linoleic acid C 18:2	21.840	20.02	306339
4	Linolenic acid C 18:3	24.400	3.54	54113

4.0 Conclusion

The results of the present study demonstrated that the seeds of citrus species investigated are a potential source of valuable oil which might be utilized for edible and other industrial applications.

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