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ORANGE PEEL FIXED OIL (*CITRUS SINENSIS* "VALENCIA"), PHYSIOCHEMICAL PROPERTIES, FATTY ACIDS PROFILE, POTENTIAL USES AND THE EFFECT OF ENVIRONMENTAL FACTORS ON IT

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Abstract

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Studying chemical and physical properties of oils plays a role in determining its importance and the fields that can be used in, orange fruits contain oils and many valuable materials due to that, physical and chemical properties of orange peel fixed oil (OPFO) have been determined, which extracted from two samples collected from two areas which are different in environmental factors. Several devices and methods used to calculate Density which was 0.95 g/ml, Kinematic Viscosity nearly 100 cSt, Refractive Index 1.499 nD, Surface Tension was lower than many vegetable oils, Pour Point 31.7-32.35°C, Acid Value nearly 24 mg KOH/g, Saponification Value 141-134 mg KOH/g, Ester Value 110 mg KOH/g, Glycerol Content 5%, Iodine Value about 75.68-77.85 I_2g/100g and Saponification Equivalent nearly 400 g oil/mol KOH. The fatty acids content was determined by GC-MS, where Palmitic, Oleic, Linoleic and Stearic acids were the most compounds are present in OPFO. The result shows the effect of environmental factors (climate and soil composition) on oil properties. Some of fixed oil properties were similar to some vegetable oils properties and differ to others. This oil was semi-solid at room temperature. Orange peel is a potential source of valuable oil and suitable for numerous industrial applications such as cosmetic, soap and candles industries.

Key words: orange peel; fixed oil; physical and chemical properties; fatty acids; environmental factors

Introduction

Many fruits containing pulp are rich in oil and seeds contain different ratios of it, sometimes oil is extracted from the fruit or pulp, followed by the extraction of oil from seeds, that depends mainly on the type of fruits. Physical and chemical properties of vegetable oils are related with the chemical structure of the fatty acids, as well as the way they linked with glycerol, and how the glycerides distributed in oil. It's important to study the fatty acids which reflect the oil properties, and the fatty acids ratios of each local oils, because they represent biochemical diversity. The vegetable oils are one of the most important plant components, mostly are used in food, and as a source of energy and renewable alternative of fossil diesel in the early last century, that's because of the increasing in global production at the same century (Mielke, 2002).

Agricultural is one of the most important productive activities in the world, especially after the global interesting in agriculture, infrastructure development and attention to water resources. The agricultural sector plays an important role in the national economy of many countries in the world; where agricultural exports contribute to foreign trade, and the provision of foreign currency, as the agricultural sector

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provides a lot of raw materials for the various other economic and industrial sectors. In addition it contributes to the operation of labor and provides a living for large numbers of individuals, so the work in production of vegetable oils provides new jobs; that is ranging from plant cultivation, harvesting, transferring, squeezing, treatment of their waste, production and marketing at the end.

Citrus are well known as one of the world's major fruit crops that are produced in many tropical or subtropical climate countries, Brazil, USA, Japan, China, Mexico, Pakistan, countries of the Mediterranean region (i.e., Syria) and South Asia are the major citrus producers (Kamal et al., 2011; Rashid et al., 2013), global production reach 48 million metric tons (Foreign Agricultural Service/USDA- Office of Global Analysis, 2017) which confirms the importance of this crop.

Orange tree is often cultivated, rarely founds in the forests, and was planted for the first time in southern China, northeast India and Burma, it's in China since 2500 BC (Spiegel-Roy and Goldschmidt**,** 1996; Nicolosi et al., 2000).

Orange fruit was considered as one of the most important food, medical and valuable materials sources, due to the presence of many chemicals, elements, and secondary waste that obtained from the citrus industry, this materials can be affected by climatic factors such as temperature, precipitation, and humidity, and also affected by soil properties, and all this factors can be named the environmental factors. In general, peels contain Essential oil which is one of the active substances against fungi and bacteria (Al-Saadi et al., 2009; Velázquez-Nuñez et al., 2013; Hasija et al., 2015). It is important to mention that limonene and some of its derivatives possess effective against *Leishmania* and *Trypanosoma* (Graebin et al., 2010). Phenolic compounds which found in a high rate in citrus peel, recently acquired importance especially flavonoids due to the free radicals scavenging (anti-oxidants activity); which give it a practical importance in human health (Gorinstein et al., 2001; Imeh and Khokhar, 2002; Ross and Kasum, 2002; M'hiri et al., 2014). Fixed oil has been characterized with its fatty acids content (Khan et al., 2013), this oil was converted to biodiesel by transesterification, density, viscosity, Mg, Na, Ca, K, P content of biodiesel have been determined (Bull and Obunwo, 2014), and characteristics of single cylinder diesel engine working by diesel with 10-20% orange peel oil methyl ester (biodiesel) have been determined by experimental investigation (Deep et al., 2013). Glycerol is a by-product of this process, promising and abundant carbon source for industrial microbiology (Da Silva et al., 2009). Pigments percentage has been determined (especially carotenoid which represent the majority of pigments in orange peel) (Agócs et al., 2007). Regarding using orange peel fixed oil, fatty acid profile of this oil indicated that it will be more useful in cosmetic, soap and lubricant industries due to its high content of palmitic acid (Islam et al., 2011; Khan et al., 2013), and any oil have a high content of this acid will not be preferred in medicine field (Mancini et al., 2015).

Orange peel can also be used as a carbon source additive for agricultural medium in microbial growth, or a source for beverage clouding agents, or can be used in animal feeding (Gohl, 1970; Sreenath et al., 1995; Mamma and Christakopoulos, 2008).

Plant parts contain oil, especially fruits and seeds, the oil is extracted by physical or chemical (solvents) methods, that basically depends on fruit type. Commonly *n*Hexane is used as nonpolar solvent (relative 0.009) for the extraction of vegetable oils that contain 90% triglycerids at least which have nonpolar feature due to the long chains of fatty acids esterified with glycerol, and the presence of acids in a free form (Gunstone, 2002; Reichardt, 2003), The main aim of this study is determine the properties of orange peel fixed oil to determine the fields which can this oil used in, and the effect of environmental factors on its properties to determine the suitable climate and soil properties for getting higher yield and the best oil properties.

Materials and Methods

Plant material and Environmental factors

200 fruits of orange (*Citrus sinensis* "Valencia" is cultivated variety planted in open farms) have been collected at the same time period from each two areas {Al-ssisanyah (S), Al-jma'ashyah (G)} in Tartous city (Syria), which are different in environmental factors due to the different topographic site, Al-ssisanyah is located in the southern-east of Tartous city, with 30 km distance from the Mediterranean sea, about 200 meters above sea level (34°46′46″N 36°8'31″E). The second area Al-jma'ashyah is located in the northern-east of Tartous city, with 10 km distance from the Mediterranean sea, about 300 meters above sea level (34°56′48″N 35°59′51″E). The climatic factors were collected from the nearest monitor climatic factors station of the two sampling areas, the two stations belong to The Water Resources Directorate – Syria, the averages monthly temperature and precipitation rate were calculated from 2005 to 2015 and showed as an average to determinate the main climate of the two areas.

Soil properties of the two sampling areas (Organic carbon %, $CaCO_3$%, Total nitrogen, electrical conductivity and percentages of Sand, Silt and Clay), were determined in The General Commission for Scientific Agricultural Research – Syria, and trypan blue assay (Phillips and Haymann, 1970) was used to studying mycorrhizas in roots.

Extraction process

Fruits were squeezed and the peel was cut into 1-2 mm^2 pieces, dried in hot air incubator (JSGI-250DT –JSR) at 50°C for 3 days, then peel was dried in an oven at 50°C for 2 hour till a constant dry weight was obtained.

Dried orange peel was made into fine powder by grinder, (50 g) of peel powder was extracted with (230 ml) *n*Hexane at 60°C for 6 hours in Soxhlet apparatus (Agarry et al., 2013), and to reduce *n*Hexane consumption in each extraction, each 5 extracts (a mixture of oil and *n*Hexane) were collected together, then filtrated to get rid of micro particles in the extract by Büchner funnel with filter 0.2 micrometers (11407-47-ACN Cellulose Nitrate Filter – Sartorius stedim).

After filtration, rotary evaporator (RV 10 digital IKA) was used for solvent evaporating at 60°C under pressure, then the oil was transferred to a glass container (100 ml) and placed in the shaking incubator (JSSI-100C –JSR) at 60°C for 4 hours or weight constant was obtained.

After yield calculation, 50 ml of *n*Hexane were added to the glass container to prevent oil oxidation relatively (Christie, 1982), and then stored in refrigerator at 2°C for three days till using it.

Physical and chemical properties determination

The solvent was evaporated before starting studying the crude oil properties.

The volumetric mass (Density) was measured by weighting a known volume of oil using milimitric pipette at 15°C.

Kinematic viscosity was measured by (Ubbelohde – Size 2 – NC 0.1 – Comecta), within the thermostat (VB-1423 Selecta) for thermal equilibration during the measurement process at 50°C, then required time for oil movement was measured.

The surface tension was measured in a direct way using (DST 30 series – S.E.O. Co., Ltd) by ring of Palladium 1.5 cm diameter.

The refractive index was measured using Abbe's refractometer (Digital Refractometers – DR6000 Series – A. Kruss Optronic) at 20°C.

Pour Point was measured by pipette (10 ml), the temperature of the oil was raised to become liquid, the oil was absorbed in the pipette and left to become in a solid state, the pipette was placed in a hot air incubator, with the sensitive heating probe (C-MAG HS7 digital – IKA), then the temperature was raised slowly by 0.1°C/10 min (started from 30°C) until the oil becomes liquid, at this point the temperature was determined.

Acid value was measured by alkaline titration, 0.5 g of oil was placed in a glass vial and dissolved in 5 ml of ethanol (99%), the solution was titrated with NaOH (0.05N) with 3 drops of phenolphthalein indicator, till pink color appearance which indicates the end of titration, the acid value was calculated by the formula: $V \times N \times 56.1 / W$, so Free Fatty Acid % = Acid Value × 0.502.

Saponification value was measured by adding 20 ml ethanolic potassium hydroxide (0.5N) to 1 g of oil, the mixture was boiled until saponification (about an hour), the mixture was cooled, several drops of phenolphthalein were added and titrated by hydrochloric acid (0.5N), till pink color disappearance (colorless) which indicates the end of titration, Saponification value was calculated by the formula: $V \times N \times 56.1 / W$

Ester value = Saponification value – acid value, Glycerol % = Ester Value × 0.054664

Saponification Equivalent (g oil/mol KOH) = 56108/ Saponification value

Iodine value was measured by Wij's method, 0.1 g of oil was placed in erlenmeyer flask and dissolved with 10 ml CCl_4, 10 ml of Wij's solution were added, erlenmeyer was stoppered and left for 30 min in dark, then 15 ml of potassium iodine (10%) and 100 ml distilled water were added, and the solution was titrated with sodium thiosulphate (0.1N), no indicator was used, so the dark-red color of the mixture was disappeared (colorless) which indicates the end of titration, iodine value was calculated by the formula: $V \times 1.269 / W$.

All experiments were performed by triplicate and results are presented as the mean ± standard deviation.

Determination of fatty acids content by GC-MS

Fatty acids in OPFO for two samples (S and G) were converted to their methyl ester and analyzed by Gas Chromatography (Agilent Technologies 7890A), 1 microgram of methyl ester fatty acids of oil and *n*Hexane (1:9 v/v) injection, thermal program: 150°C for 1 min, then 4°C/min to 200°C for 0 min, then 5 °C/min to 270°C for 22.5 min, processing time 50 min, carrier gas (helium) at a flow rate 1 ml/min, pressure 13.332 psi, column (HP-5MS 5% Phenyl Methyl Silox: 1785.43335, 325 °C: 30 m × 250 µm × 0.25 µm), and mass spectrometry (Agilent Technologies 5975C – Ei), fatty acids were identified using mass spectrum and matching with mass spectral library (NIST).

Statistical analysis

SPSS V20 software was used to calculate the significant differences (sig < 0.05) between mean values, One-way ANOVA procedure was used to perform the analysis of variance.

Results and Discussion

The study of environmental factors shows that Al-ssisanyah area is higher than Al-jma'ashyah in temperature and precipitation, and the soil properties show that Al-ssisanyah area have more nutrition than Al-jma'ashyah, in addition it has more clay and less sand percentage which make this soil more ability for materials storage, and there are significant differences between the two areas in climatic factors and soil properties except percentage of nitrogen and silt, as shown in Table 1.

Table 1
Environmental factors in two sampling areas

		S	G
Temperature, °C		19.68 a	18.46 b
Precipitation, ml		950.73 a	906.52 b
Organic Carbon, %		6.59 a	6.18 b
$CaCO_3$, %		10.5 a	9.6 b
Total Nitrogen, %		0.336 a	0.330 a
Electrical Conductivity mmohs cm^{-1}		2.9 a	1 b
Percentages % of	Sand	24 a	36 b
	Silt	16 a	16 a
	Clay	60 a	48 b

S: Climatic factors and soil properties of the Al-ssisanyah region; G: Climatic factors and soil properties of the Al-jma'ashyah region

Table 2 shows that moist peel composes 40% of fruit weight, it didn't reach referential percentage (50%) that's because the third layer of fruit (pulp) wasn't used. The statistical analysis shows that there are significant differences between two samples regions in peel and moisture percentage and fruit weight average, without significant difference in oil yield. Moisture percentage wasn't in accordance with (Khan et al., 2013) which indicated that moisture percentage 42%. Regarding the difference in fixed oil yield between the two areas peel is due to the different environmental factors in the cultivation samples; so higher temperature and yield of oil have a positive relation as shown in previous study (Sadeghi and Talaii, 2002) which stated that warmer climate increases the oil content. Highest oil rate was observed in Al-ssisanyah which has the lowest altitude; this result was confirmed by (Turhan et al., 2010). Regarding the effect of soil properties, the results show that Al-ssisanyah is more nutritious also that increases the yield of oil, as agreed with (Shukry, 2001), and the yield of orange peel fixed oil wasn't in accordance with (Bull and Obunwo, 2014).

Vascular arbuscular mycorrhizal fungi (VAM) was the mycorrhizal type which found in two areas samples roots, that's in accordance with (Ishii and Kadoya, 1996), it's play an important role in support, protection and increase the yield of plant components especially oils, in addition to further benefits (Nadeem et al., 2013; Heidari and Karami, 2014; Yadava et al., 2015).

Table 2
Orange peel properties

		S	G
Fruit weight Average		132.1 a	142.3 b
Peel percentage, %		a36.75	b42.86
Moisture, %		a76.86	b78.81
Fixed oil yield	g oil/ 250 g peel	3.62 ±0.15 a	3.56 ±0.18 a
	%	1.450 a	1.425 a

S: *Citrus sinensis* collected from the Al-ssisanyah region; G: *Citrus sinensis* collected from the Al-jma'ashyah region

The relative yield of the extraction; total weight of fruits 27000 g, contains 11170 g moist peels, give 3574 g dried peels, which have 51.46 g crude oil, equal 54.17 ml, that will shows the viability of this oil for industrial uses.

Table 3 shows the physical and chemical properties of orange peel fixed oil for two areas, density is mass of the unit of volume, was approximately 0.95 g/ml at 15°C, that met with (Khan et al., 2013) which indicated 0.94 g/ml for sweet orange fixed oil. Viscosity is a measure of resistance between the adjacent layers of a fluid, which hinders the sliding of one over the other; it was high for the two samples (nearly 100 cSt) at 50°C comparing with other vegetable oils (Fasina and Colley, 2008). Refractive index is the ratio of the velocity of light in a vacuum to its velocity in a specified medium, was also high at 20°C comparing with other vegetable oils (about 1.45-1.47 nD) (Kanthack, 1995; Fakhri et al., 2011). Surface tension is a measure of the energy required to increasing the surface area of a liquid by a unit of area caused by the attraction of the particles in the surface layer by the bulk of the liquid which tends to minimize surface area, for OPFO it was lesser than many vegetable oils (30-32) n/m (Esteban et al., 2012). Pour point is the temperature at which a fluid begins to flow or becomes semi solid and loses its flow characteristics, for OPFO it was 31.7-32.35°C; close to Palm oil 30.8-37.6°C and nearly to Coconut oil 23-26°C, and higher than other vegetable oils which were under 0 C in many degrees (Ajithkumar, 2009).

Acid Value is the number of milligrams of potassium hydroxide required to neutralize the free fatty acid in one gram of a fat, FFA% is the grams of free fatty acids in 100g of oil or fat, in this work it was 23.4-26.4 mg KOH/g, and FFA% 11.7-13.2, the FFA% wasn't agreed with sweet OPFO which was 2.3% (Khan et al., 2013), and it's high comparing with other vegetable oils (Neagu et al., 2013). Saponification value is defined as the weight of potassium hydroxide in milligrams needed to saponify one gram of fat, it was 141.3-134.6 mg/g, so lower than sweet orange fixed oil (183 mg/g) and lower than many vegetable oils [15,34]. Ester value is mil-

Table 3
Physical and chemical properties of OPFO

		S	G
Volumetric mass (Density), g/ml		0.965 ±0.0184 a	0.945 ±0.0120 a
Kinematic viscosity, cSt-mm^2/s		111.64 ±2.57 a	93.64 ±2.55 b
Refractive index, nD		1.4999 ±0.0001 a	1.4993 ±0.000351 a
Surface tension (n/m) (dyn/cm)	40°C	26.084 a	23.736 b
	46°C	25.840 a	18.542 b
	52°C	25.515 a	15.715 b
Pour point, °C		31.7 ±1.6 a	32.35 ±0.77 a
Acid value, mgKOH/g		23.4 ±0.86 a	26.449 ± 0.87 b
FFA%		11.74 a	13.27 b
Saponification value mg KOH/g oil		141.35 ±3.68 a	134.63 ±7.30 a
Ester value mgKOH/g		117.955 a	108.186 b
Glycerol%		5.926 a	5.399 b
Saponification Equivalent g oil/mol KOH		396.94 a	416.75 b
Iodine value, I_2g/100g		75.68 ±0.57 a	77.85 ±0.58 a

OPFO: Orange peel fixed oil; S: *Citrus sinensis* collected from the Al-ssisanyah region; G: *Citrus sinensis* collected from the Al-jma'ashyah region

ligram of KOH required to react with glycerol, it was 117.9-108.1 mgKOH/g, so lower than sweet orange fixed oil (178.4 mg/g) and lower than many vegetable oils, glycerol content was about 5%, also lower than many vegetable oils (Dileesh, 2012; Khan et al., 2013). Saponification Equivalent is the amount in grams of an ester that consumes one gram-equivalent of alkali in saponification; it was approximately 400 g oil/mol KOH in this work. Iodine value is the number of iodine grams consumed by 100g of fat, higher iodine value indicates higher degree of fatty acids unsaturation, it was 75.68-77.85 I_2 g/100 g in this work, and wasn't agreed with sweet OPFO (103 I_2 g/100 g) in referential study (Khan et al., 2013), and comparing to other vegetable oils; it was nearly to Olive oil (80-88 I_2 g/100 g) and higher than Palm oil (44-51 I_2 g/100 g) and lower than many vegetable oils such as Corn, Sunflower and Soybean oils (109-133, 125-144, 120-136 I_2 g/100 g) respectively (Thomas, 2012).

The slight difference between oils characteristics for both samples is due to the differences in environmental factors (climate and soil) in cultivation samples area. There are significant differences between two oils in some properties such as viscosity, surface tension, acid value, FFA%, Ester value, glycerol content and saponification equivalent, on the other hand there aren't significant differences in density, refractive index, pour point, saponification value and iodine value; but the absence of statistical differences in some properties doesn't mean absence of actual differences. There is a positive relation between density and viscosity. The iodine value is important property which affect on viscosity and pour point, increasing in iodine value will decreases viscosity (Dutt and Prasad, 1989). The coolest area (Al-jma'ashyah region) have higher iodine value; that's for more resistance to the coldness, because increasing iodine value will make the oil more fluidity that's make the performance of oil is more better (Becker et al., 2006), so the viscosity of Al-jma'ashyah area have lesser than another area. Regarding the relation between iodine value and pour point, the increasing in iodine value will decreases pour point, but pour point also depends on the polymorphic form of the glycerides i.e., the crystalline structure (Thomas, 2012) which may play an importance role in pour point of orange peel fixed oil more the iodine value.

Due to the difference between oil characteristics of both samples; fatty acids profile has significant differences as a statistical analysis shows (Table 4); especially in oleic and linoleic acids, were fatty acids profile shows orange peel fixed oils contain only palmitic, oleic, linoleic and stearic acids, other fatty acids weren't observed; due to the absence of it or presence in undetectable percent. Al-jma'ashyah oil has higher number of mono and polyunsaturated fatty acids collectively, that's due to the decreasing in temperature (Becker et al., 2006).

Fatty acids profile shows difference between OPFO and fixed oil from another orange peels which were studied referentially (Islam et al., 2011; Khan et al., 2013); due to the differences in varieties and orange cultivation area. Regarding the difference between OPFO and other vegetable oils (Orthoefer, 1996; Anonymous, 2011c); that is due to many

Table 4
Fatty acids profile of OPFO for two samples

Fatty Acids %	OPFO-S	OPFO-G	1	2	3-Soybean	4-Sunflower	5-Cottonseed	6-Corn
Palmitic acid $C_{16:0}$	47.8709 b	46.6404 a	48.16	38.6	11	6.8	24.7	10.9
Stearic acid $C_{18:0}$	3.4207 b	3.2262 a	4.57	4.1	4	4.7	2.3	1.8
Oleic acid $C_{18:1}$	38.5917 b	36.5242 a	3.42	57.2	23.4	18.6	17.6	24.2
Linoleic acid $C_{18:2}$	10.1167 b	13.6092 a	20.02	-	53.2	68.2	53.3	58

OPFO-S: Orange peel fixed oil of *Citrus sinensis* collected from the Al-ssisanyah region; OPFO-G: Orange peel fixed oil of *Citrus sinensis* collected from the Al-jma'ashyah region; 1: Sweet Orange Peels Oil [15]; 2: Orange Peels Oil [20]; 3,4,5: Other vegetable oil [42]; 6: Corn oil [43]; (-): not existing or not determined factors especially genetics factors, planting and harvesting time (Gupta et al., 1994; Flagella et al., 2002; Baydar and Erbas, 2005). The increasing in oleic acid synthesis (depending on genetic trait and environmental conditions) causes a reduction of linoleic acid percentage that confirmed referentially (Onemli, 2014). The highest content of unsaturated fatty acids was in Al-jma'ashyah is related with higher content of iodine value and that's make this oil more fluidity than Al-ssisanyah oil, and the percent of saturated fatty acids was higher in Al-ssisanyah oil which make this oil less in fluidity.

The nutrient of soil play an important role in fatty acids compositions, so Al-ssisanyah oil must has higher unsaturated fatty acids, but the increasing in soil nutrition (Over-Fertilization) cause a decreasing in soil material efficiency, and this increasing considering as obstacle factor in plant nutrient utilization (Peng et al., 2006), on other hand Al-jma'ashyah soil has higher sand content which increasing the water movement in the soil; that facilitate soil nutrient movement and plant utilization (Crouse, 2016).

The uses of oil in any field depends on its characteristics, so physical and chemical properties play a main role in uses of oil as a biodiesel, viscosity and pour point are very important for any diesel; the oil in this paper will not be useful as a biodiesel, because it has high viscosity and pour point which may cause many problems in the system of fuel injection will lead to severe engine deposits, injector coking and piston ring sticking (Pestes and Stanislao, 1984; Perkins et al., 1991; Xiao and Gao, 2011) unless high percentage of diesel will be added to this biodiesel to make its properties good for diesel engine (Agarry et al., 2013), it's good to use this oil as a crude motor oil (viscopedia.com, 2017). Regarding the uses in cosmetic and medical fields; due to high content of unsaturated fatty acids, OPFO can be useful for cosmetic and medicine (Zielinska and Nowak, 2014), but any oil have a high percent of palmitic acid will be not preferred for medical uses because it will causes many diseases except using it as suppository drug (Noordin and Chung, 2007; Pugunes and Ugandar, 2013), and that's will make it non edible oil (Mancini et al., 2015); so the uses of this oil in cosmetic, soap and candles industries will be more suitable (Henson, 2012).

Conclusion

Orange peel is a potential source of valuable oil, the increasing in temperature gives higher yield and more unsaturated fatty acids, and the increasing of soil nutrition in large amount is not good for oil properties. Some of fixed oil properties were similar to some vegetable oils properties and differ to others, orange peel fixed oil was semi-solid at room temperature; it does not require hydrogenation. It has unique fatty acids content which makes it suitable for numerous industrial applications such as biodiesel (blending with diesel), bio-lubricant and cosmetic due to high content of palmitic acid which make any oil unsuitable for edible and medicine uses.

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