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Integral valorization from industrial Persian lime processing wastes (*Citrus latifolia* Tanaka): simultaneous recovery of oils and antioxidants

Valorización integral de los residuos industriales del procesamiento de limón Persa (*Citrus latifolia* Tanaka): recuperación simultanea de aceites y antioxidantes

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Abstract

Mexico's Persian lime production in 2019 was close to 1.3 million of tons, inferring an approximately 390,000 tons of peel residues during its processing. This residues presents an interesting opportunity for obtaining value-added products, mainly due to its composition, formed principally by water, soluble sugars, fiber, organic acids, fatty acids, minerals, essential oils, flavonoids, and vitamins. Despite this, the valorization of this residual biomass has been almost completely ignored. In this paper, the residual peel was analyzed in order to determine its biotechnological potential, for this, different oil extraction methods were compared, the fatty acids profile was determined, also characterization and the antioxidant potential of the defatted residues were evaluated. The results showed that the principal fatty acids present in the oil extracted were Palmitic, Oleic and Linoleic, which allows its use for food and bioenergy purposes, moreover, the defatted residual biomass characterization presents a chemical composition which allows the use for as livestock, biogas production or agronomy. To our knowledge, this is the first report of antioxidant activity from defatted Persian lime residual biomass, wherein, the residues generated by steam distillation showed the bigger amount of phenolic compounds, but the obtained from hexane extraction presents a higher antioxidant activity.
Keywords: Persian lime, oil extraction, valorization, phenolic compounds, antioxidant activity.

Resumen

En 2019, la producción de limón Persa en México fue de 1.3 millones de toneladas, lo que infiere la generación de 390,000 toneladas de cáscara residual. Estos residuos presentan potencial para obtener productos de valor agregado, debido principalmente a su composición, que consta principalmente de agua, azúcares solubles, fibra, ácidos grasos, minerales, aceites esenciales, flavonoides y vitaminas. A pesar de esto, la valorización de este residuo ha sido casi ignorada; en este trabajo se caracterizó el potencial biotecnológico de este subproducto. Para ello se compararon metodologías diferentes para extracción de aceites y se determinó su perfil de ácidos grasos; además, la biomasa residual desgrasada (BRD) fue caracterizada, y se determinó su potencial antioxidante. Los resultados mostraron que los ácidos grasos mayoritarios fueron el Palmítico, Oleico y Linoleico, lo que permite su uso para fines alimenticios y bioenergéticos; adicionalmente, la BRD presentó una composición proximal con potencial uso para alimento de ganado, producción de biogás o fertilizante. Hasta donde sabemos, este es el primer reporte de actividad antioxidante de este material, donde los residuos generados por arrastre de vapor mostraron una mayor cantidad de compuestos fenólicos, sin embargo, los generados por extracción con hexano presentaron la mayor actividad antioxidante.
Palabras clave: Limón Persa, extracción de aceite, valorización, compuestos fenólicos, actividad antioxidante.

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1 Introduction

According to Food and Agriculture Organization (FAO, 2011) around 1.3 billion tons of food wastes are disposed annually worldwide, from these, it has observed that in industrialized countries, more that 40% of food wastes are of industrial origin which represents around 520 million tons. Agroindustrial wastes generate environmental, public health and economic problems, mainly due to its inadequate management and its scarce use, for that reason, results imperative to find alternatives for the use and recovery of these byproducts (Jiménez-Malpeque *et al*., 2020). About the citrus industry, 35-40% of fruits are destined to juice and 0.2-0.5% to essential oil production, while the remaining 60-65% represents wastes consisting in exhausted peels, pulp, and seeds. In these wastes, which normally are discarded or used as animal feed or fertilizers, remain useful and valuable substances such as polyphenols, limonene, carotenoids, vitamins, fibers (pectin), coumarins, among others, these substances are estimated at around 18mg·kg^{-1} (M'hiri *et al*., 2018; Costa *et al*., 2019).

Concerning lemon, during the marketing year 2018/2019, the worldwide production of lemons and limes reach more than 8.5 million metric tons, being Mexico the leading global producer, contributing with 2.6 million metric tons (USDA, 2020), the above would represent more than 5 million metric tons of lemon wastes around the world. The Persian lime (*Citrus latifolia* Tanaka) is mainly grown in the Gulf Coast of Mexico, in 2019 the production in Mexico was close to 1.3 million of tons, regarding residues of Persian lime peel, a production of up to approximately 390,000 tons can be inferred, if we consider that about 30% of the weight of the lemon is made up of peels (Fernández-Lambert *et al*., 2015; SIAP, 2020; Jiménez-Nempeque *et al*., 2020). Peels are composed mainly of water, soluble sugars, fiber, organic acids, amino acids, minerals, essential oils, flavonoids, and vitamins, which represents an area of opportunity for obtaining value-added products (Londoño *et al*., 2012).

Lemon oil and polyphenols, present in peels, are used in a wide variety of applications, mainly in the flavor, perfumery, and pharmaceutical industries, both are used for their nutraceutical properties, for example their antioxidant capacity (Sharma *et al*., 2017; Macías-Cortés *et al*., 2020). The amount and quality of the compounds recovered from peels are highly influenced by the extraction method used. For example, in case of oils, during hydrodistillation (which is one of the most used technology by industry due to its economic operation cost), the increased temperatures and extended extraction time used, can cause chemical modifications of the oil components and loss of the most volatile constituents (Gök *et al*., 2014). On the other hand, organic solvent methods showed that oil extraction at high temperatures enhanced solubility because of reduction in viscosity and increased mass transfer rate. The organic solvent extraction is dependent on the nature of the solvent, reaction time, process temperature and the solid/solvent ratio (Ali & Watson, 2013). Meanwhile, in case of polyphenols extraction, phenolics can occur as soluble fraction (free phenolics) or non-soluble fraction (bound phenolics), the last ones are attached to dietary fiber or protein, and they require an extra step of hydrolysis during extraction to make them soluble and profitable (Gutiérrez-Grijalva *et al*., 2016).

In this paper, the valorization of Persian lime peels is proposed, for this, a characterization of its main components was made; different oil extraction methods are reported and compared, which are steam distillation, hexane and chloroform/methanol Soxhlet extraction; also phenolic contents and the antioxidant potential of the defatted residues was evaluated. This work provides a basis for the exploration of the potential and use of the waste generated by the Persian lime industry.

2 Materials and methods

2.1 Plant material

Persian Lime (*C. latifolia Tanaka*) Peel samples were gently donated by Citrika Rancho Agricola S.A. de C.V., in Culiacán, Sinaloa, México, during the 2018-2019 harvest and processing season. The peels were cleaned repeatedly to remove impurities, subsequently the samples were chopped out for size reduction (3 x 3 cm), for solvents extractions the samples were dried in a forced recirculation oven previously heated to 70 °C for 12 hours, and finally the lemon peel were stored under refrigeration until its use (Badillo, 2011).

2.2 *Oil extraction from Persian lime peels*

The oil extraction from the Persian lime peels was carried out by three extraction methods, two with solvents by soxhlet and one by steam distillation. Extractions were performed by triplicates and the mean values was reported.

For oil extraction by soxhlet methods, dried lemon peel were used, besides two solvents combinations were performed: a) chloroform-methanol mixture (2:1) and b) hexane; also, the oil percentage extracted was obtained by the difference in weight. The defatted residual biomass (DRB) was dried at 60 °C for 12 hours to remove solvents excess and stored (4 °C) for later use (Bligh & Dyer, 1959; Santos-Ballardo *et al*., 2016).

On the other hand, fresh lime peels were submitted to steam distillation process using a Clevenger-type apparatus and extracted with 500 mL ultrapure water at 100 °C for 3 h (until no more oil was obtained). The oil was collected, and the extraction percentage was obtained by the difference in weight. The DRB was dried at 60 °C for 12 hours and stored (4 °C) for later use (Lopresto *et al*., 2014; Golmohammadi *et al*., 2018).

2.3 *Persian lime oil density calculations*

Density determination of the extracted oil from the lime peel was performed according to Dalm & Hogervost (2011), using the following formula:

$$\text{Density} = \frac{M}{v} \tag{1}$$

where the ratio of the sample was established, weighing 1 mL of heated oil to 20 °C.

2.4 *Fatty acids analysis*

Fatty acids (FAs) samples were analyzed by extraction, separation, methylation, purification and quantification by AOAC method number 969.33 (1995). The FAs detection and quantification were analyzed with a splitless injection technique using gas chromatography analysis performed with an instrument (Agilent Technologies 7820A GC) equipped with a Supelco omegawax 320 column (30 cm length, 0.32 mm internal diameter and 0.25 μm phase thickness). The oven temperature was programmed with an increment from 50 to 260 °C at 10 °C min^{-1}. Temperatures of 260 °C for 40 min, for the injector and detector were used. Methyl esters of FAs (FAME) were identified by comparing retention times with those of standard FAMEs (37 FAME compounds, SupelcoTM Mix C4-C24; trophic markers). FAs were quantified by integrating the areas under peaks in the gas chromatography traces (CHROMQUEST 4.1® software), with calibrations derived from standard FAs. The results are presented as % of saturated fatty acids (SFAs), mono-unsaturated fatty acids (MUFAs), poly-unsaturated fatty acids (PUFAs) and % of each marker.

2.5 *Defatted lime peel residual biomass characterization*

The biomass samples obtained after the oil extraction were collected and characterized in basis of the total Kjeldahl nitrogen (TKN) (Santos-Ballardo *et al*., 2015). The total protein content was calculated using the TKN results (Vratislav *et al*., 2015). Furthermore, the total organic carbon (TOC), was determined according to EPA (1999) and the organic matter content was calculated using TOC results (Iglesias-Jiménez & Pérez-Gárcía, 1992). Once the content of C and N was determined, the C/N of the organic material was calculated. Furthermore, the ash content was evaluated through standard methods (APHA, 1999).

2.6 *Phenolic compounds extraction*

2.6.1 *Free phenolic extract preparation*

A 1 g flour sample of Persian lime defatted peel was blended with 10 mL of chilled methanol/water (80:20, v/v), and agitated for 24 h. After centrifugation at 10,000 rpm, at 4 °C, for 15 min (ThermoFisher scientific sorvall TR), the supernatant was evaporated at 45 °C and reconstituted in 2 mL of methanol. The resulting extracts were frozen at -80 °C and stored until use (Rispail *et al*., 2005; Ambriz-Pérez *et al*., 2017).

2.7 *Bound phenolic extract preparation*

After extraction of free phenolic compounds, the residue was digested with 10 mL of 2 M sodium hydroxide in a water bath at 95 °C for 30 min. After, the sample was agitated for 1 additional hour at 25 °C. The mixture was acidified (pH<2.0) with 2 mL of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with 10 mL of ethyl acetate for each extraction. The ethyl acetate fraction was evaporated to dryness under vacuum at 35 °C. Bound phenolic compounds were reconstituted in 2 mL of methanol/water (80:20, v/v), were frozen at -80 °C and stored until use (Ambriz-Pérez *et al*., 2017).

2.8 Determination of total phenolic compounds

Phenolic content was determined by Folin-Ciocalteu (1927) assay, 20 μl of free and bound phenolic extracts of Persian lime defatted peel were placed in tubes, adding 1200 μL of distilled water, 100 μL of Folin-Ciocalteau reagent and 300 μL of 7% sodium carbonate; after 8 minutes 380 μL of water was added and incubate for 90 minutes. Finally, absorbance was measured at 765 nm using a spectrophotometer, and methanol 80% as blank. A calibration curve was prepared using gallic acid as standard and total phenolics were expressed as μM of gallic acid equivalent (GAE) per 1 g of dry weight (DW) of sample.

2.9 Antioxidant capacity

Antioxidant capacity was determined using the method described by Re *et al*. (1999). 20 μL of the samples were placed in vials adding 1980 μL of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$) (A_{734nm}=0.70±0.02); absorbance was measured 6 minutes after adding the radical, using a spectrophotometer at 734 nm. A calibration curve was prepared using Trolox as standard, and the $ABTS^{\bullet+}$ inhibition was considered as antioxidant capacity and expressed as μM of Trolox equivalent (TE) per 1 g of dry weight (DW) of Persian lime defatted peel.

2.10 Statistical analysis

The data was expressed as mean values ± standard deviation for triplicate measurements. Analysis of variance was performed using One-way or Two-way ANOVA depending the case. Significant difference of means among method of oil extraction and type of phenolic were considered at $p < 0.05$. Statistical analysis was done by using SigmaPlot 12.5 software.

3 Results and discussion

3.1 Oil extraction from Persian lime peel

Regarding to the oil extraction, the three methods used showed a large variation in the extraction percentage from the lime peel biomass, the oil yields oscillated from 0.46±0.03% to 13.23±0.23% (Table 1). These results are similar to reported for citrus peels by Palazzolo *et al*. (2013), which reported extraction percentages between 0.5 and 15.0% (w/v). Besides, the results obtained in the present work were higher than the range reported for lemon and orange peels (0.07 - 3.24%) by different authors (Ben Hsouna *et al*., 2017; Fakayode & Abobi, 2018; Matsuo *et al*., 2019; Özcan *et al*., 2020).

The difference between the oil extraction efficiency in different experiments could be caused by several factors. Regarding to this, Dugo *et al*. (2000) and Palazzolo *et al*. (2013), reported that different aspects, such as the genotype, soil type, climate, vegetative cycle, nature of the fruit, age and the extraction process used, could affect the quantity, composition and quality of citrus peel oil extraction.

On the other hand, regarding to the effect of the extraction technique used on the oil extraction efficiency; the most effective method was the soxhlet with chloroform/methanol (SOX-CM), followed extraction by soxhlet with hexane (SOX-H) and the less effective was the steam distillation (STM-D) (Table 1). This behavior was reported previously on vegetable materials, for example, Salomé-Abarca *et al*. (2015) reported oil extraction from *Calendula officianalis* by different methods, obtaining 0.9 ± 0.1 and 6.7 ± 0.2 for STM-D and organic solvent extraction, respectively.

Table 1. Oil extraction percentage and oil density according to the extraction method.

Extraction method	STM-D	SOX-H	SOX-CM
Oil yield (%)	0.468±0.03c	7.348±0.33b	13.236±0.23a
Oil density (g·mL^{-1})	0.857 ± 0.005c	0.9598 ± 0.0032b	1.5717 ± 0.0044a

Mean of triplicate ± standard deviation. One-way ANOVA, Tukey test $p < 0.05$, different letters in the same row indicate statistically significant differences. STM-D: Steam distillation; SOX-H: Soxhlet with Hexane; SOX-CM: Soxhlet with Chlorofom-Methanol.

Respecting to the soxhlet extraction (organic solvents), it was expected higher extractions efficiency compared with STM-D, due to the nature of the process, moreover, the difference between the solvents used could be caused by the use of organic solvents mixture (Chlor/MeOH), which allows to extract a major quantity of compounds compared with one solvent (Hexane). On the other hand, even though the soxhlet method it is considered as a useful technique for processing many samples with high efficiency, is limited by the large amount of pigments that could be dragged in their extracts. Such pigments can produce phytochemical interference and could also interfere with other potential applications (Salomé-Abarca *et al*., 2015). It is important to remark that the oil yield obtained by STM-D (0.468%±0.03) is lower than the oil extraction of 3% reported by Ben Hsouna *et al*., (2017) for *Citrus lemon* peel, using the same extraction technique, moreover, the results in the present work was similar to the range reported (0.07% - 1.02%) for lemon peel (Özcan *et al*., 2020); also, it is within the range of 0.33% - 0.47% reported for *Citrus natsudaidai* peel (Matsuo *et al*., 2019). Otherwise, the difference between the diverse experimental setups, could be explained because the effectiveness of STM-D techniques could be affected by many factors, like the size of the samples, time, temperature and the plant species (Golkamani & Moayyedi, 2016).

Concerning to the oil density, the values obtained ranged between 0.857 and 1.5717 (g·mL^{-1}) (Table 1), showing significate difference between all the results. The values in the present work are similar to the density of 0.86 g·mL^{-1} reported for orange peel oil (TsegayeFekadu *et al*., 2019), also, most of the results were within the range reported between 0.84-0.91 for oils and fatty acids of *Citrus latifolia* (Gobato *et al*., 2015; Goncalves *et al*., 2018). Moreover, the oil extracted with SOX-CM showed a higher density than the reported previously, this could be explained due to the extraction process with a solvent mixture might dragged out other compounds which could affect the density of the lipid material obtained. On the other hand, density is an important factor which influences the oil behavior in food industries (also in others such as cosmetics, and pharmaceuticals); due to this, it is important that the oil density obtained through the different extraction techniques were within the recommended range (Sahasrabudhe *et al*., 2017).

3.2 Fatty acids profiles for the oil extracted from Persian lime peel

The FAs composition Persian lime peel oil obtained after three different extraction methods are shown in Table 2. Palmitic, Oleic and Linoleic were the major FAs of Persian lime peel (presents on all the extraction techniques). The FAs contents in oils from Persian lime peel showed differences depending on the extraction methods used. Among them, the STM-D showed as principal FAs the Palmitic (26.77%) and Linoleic (22.47%); SOX-H presented the Linoleic (27.85%) and Palmitic (22.18%) as main FAs, and the SOX-CM showed the Oleic (35.58%) and Linoleic (23.35%) as principal FAs present. Also, the Linolenic (16.03%) and Myristic acids (10.15%) showed considerable presence only in STM-D. Moreover, the Caproic acid (10.97%) was only importantly detected in SOX-H. Furthermore, the contents of Oleic acid varied between 8.3 - 9.8% in SOX-H and SOX-CM, respectively and 35.58% for SOX-CM. Another important variation is in the presence of saturated (SFA) and unsaturated fatty acids (UFA) in the oils obtained. Concerning to these, the STM-D showed an equilibrated presence of fatty acids (SFA: 49.78% and UFA: 50.22%), meanwhile in SOX-H extraction the SFA showed majority presence (58.08%), and the SOX-CM accumulated the major UFA concentration (56.93%).

Özcan *et al*. (2020) reported the fatty acids profile of lemon peel oil, obtained through soxhlet extraction using Petroleum benzene as solvent, the major FAs present were Palmitic, Oleic, Linoleic and Linolenic acids, besides they reported an important presence of UFA (nearly to 70%). The authors also report variations in the FAs profiles, in response on the drying method prior to the oil extraction. On the other hand, Matsuo *et al*. (2019) presented the FAs profile of three cultivar of *Citrus natsudaidai* peel, wherein the major FAs acid present in all three cultivars was Linoleic acid, followed by Palmitic acid, α-Linolenic acid and Oleic acid. These results showed that UFA are predominant in all three cultivars, representing approximately 70% of total fatty acid content. They also reported difference in fatty acids profiles and total content among the cultivars.

However, Juhaimi *et al*. (2019) reported that variations in FAs compositions of vegetable oils were significantly affected by using different extraction solvents and methods, these differences can be probably due to extraction method, sample composition, nature of the oil in material, and solvent types.

Table 2. Fatty acids profile for the oil recovered according to extraction method.

Fatty acid	**Nomenclature**	**Fraction according to extraction method (%)**		
		Steam-distillation	**Soxhlet (Hexane)**	**Soxhlet (CHlor/MeOH)**
Caproic	C6:0	1.04	10.97	ND
Caprilic	C8:0	1.27	6.34	ND
Capric	C10:0	0.9	0.26	2.6
Undeanoic	C11:0	0.67	0.45	ND
Lauric	C12:0	2.79	8.42	14.2
Tridecanoic	C13:0	2.45	1.03	ND
Myristic	C14:0	10.15	0.06	5.18
Myristoleic	C14:1, cis-9	ND	0.26	ND
Pentadecanoic	C15:0	ND	0.32	ND
Cis-10-pentadecenoic	C15:1, cis-9	ND	0.9	ND
Palmitic	C16:0	26.77	22.18	8.87
Palmitoleic	C16:1, cis-9	2.82	3.22	ND
Heptadecanoic	C17:0	ND	1.14	ND
Cis-10-Heptadecenoic	C17:1	0.87	ND	ND
Stearic	C18:0	2.36	4.13	7.79
Oleic	C18:1, cis-9	8.03	9.8	35.58
Linoleic	C18:2, cis 9,12	22.47	27.85	23.35
Linolenic	C18:3, cis 9, 12, 15	16.03	0.14	ND
Gama-linolenic	C18:3, cis 6, 9, 12	ND	0.15	ND
Araquidic	C20:0	ND	0.12	ND
Araquidonic	C20:4, cis-5,8,11,14	ND	0.51	ND
Heneicosanoic	C21:0	1.38	1.74	ND
Behenic	C22:0	ND	ND	4.43
Total		100	100	100
Saturated		49.78	58.08	43.07
Unsaturated		50.22	41.92	56.93

ND: not detected.

3.3 Potential use of Fatty acids extracted from Persian lime peel

3.3.1 Potential uses in food

Regarding FAs extracted, the Palmitic, Oleic and Linoleic acids are found in the highest amounts, and thus presented valuable source of edible nutritive oils. The Palmitic and Oleic acids could be obtained directly from the dietary sources and also by endogenous synthesis in the body itself, whilst the Linoleic acid only could be obtained through the diet; besides, these FAs as phospholipids promote to maintain the structural integrity and the critical functioning of cellular membranes throughout the body. On the other hand, the Linolenic acid (with high presence in STM-D) only can be derived from dietary sources in the human body, and it has an important role in the pathways for desaturation and chain elongation of omega-6 and omega-3 fatty acids (Kaur *et al*., 2014; Zema *et al*., 2018). Based in the general FAs profile obtained from the extraction techniques, the oils obtained from Persian lime peel showed good potential for edible nutritional products.

3.3.2 Potential uses as biodiesel feedstock

Biodiesel is an alternative diesel fuel non-toxic, biodegradable, possesses inherent lubricity, and a high flash point, besides is considered free of sulfur and aromatic compounds, and usually is produced from vegetable oils and animal fats (Rashida *et al*., 2013).

The physical and chemical properties of a biodiesel are determined by its organic composition. The principal properties influencing the behavior and quality of the biodiesel are: the size distribution of the FA chains and the unsaturation degree of these FA chains. Many of the biodiesel variations produced from different feedstocks can be explained by these two properties. For example, an average degree of unsaturation was highly correlated with numerous biodiesel properties, including low temperature performance metrics, viscosity, and specific gravity, cetane number, and iodine value. On the other hand, increments in average unsaturation leads to lower cetane number and poorer oxidation stability, but improved low temperature performance (Fazal *et al*., 2011; Sánchez-Roque *et al*., 2020).

However, some authors proposed schemes respecting to two critical biodiesel properties such as: oxidative stability and low temperature performance. For an adequate low temperature performance, biodiesel feedstocks should content low concentrations of long-chain SFAs. At the same time, for better oxidative stability, biodiesel feedstocks should have low contents of PUFAs and elevated SFAs and MUFAs concentrations; it means that an equilibrate fatty acid profile is recommended in the feedstocks for obtaining biodiesel with an adequate performance (Kent-Hoekman *et al*., 2012). According to the FAs profiles (SFA: 43.07%-58.08%, and UFA: 41.92%-56.93%) reported in the present work, the oils obtained from Persian lime peels presents an adequate potential for being biodiesel feedstock.

3.3.3 Other uses

The FAs present in the Persian lime peels could be used as inactive ingredients (excipients) in drug preparations. According to Rustan & Devron (2005), the use of lipid formulations as active substances carriers is growing rapidly. Moreover, the largest amount of lipids used in pharmaceuticals are employed in the production of fat emulsions, mainly for clinical nutrition but also as drug vehicles.

Moreover, FAs derivatives such as fatty alcohols, fatty acid esters, and surfactants are used in a wide range of cosmetic and personal care products in an increasing number of functions. Furthermore, the increasing emphasis on sustainability will allow the importance of the use of agroindustrial residues (such as Persian lime peel) as FAs feedstocks for personal care products and cosmetics ingredients (Kelm & Wickett, 2017).

3.4 Persian lime peel defatted residual biomass (DRB) characterization

The protein, ash, organic matter, carbon and nitrogen contents (also C/N ratio) of the Persian lime peel DRB obtained from diverse oil extraction methods are shown in Table 3. Also, the complete Persian lime peel was characterized for comparative purposes. The highest protein content was 6.312±0.121% in SOX-H, followed by non-defatted biomass, STM-D and SOX-CM. Besides, ash content ranged from 2.272±0.14% and 5.063±0.07%. Furthermore, the organic matter content ranged from 52.755±0.999 to 53.823±1.155, showing no statistically significant difference between all the treatments. SOX-CM and SOX-H showed the highest carbon and nitrogen contents, respectively. However, the C/N ranged from 30.85 to 42.77, where SOX-CM showed the highest values.

No previous reports were found from Persian lime peel DRB. However, Olerumi *et al*. (2007) reported the proximate composition of *Citrus limonum* peel (dry matter: 89.35 %, crude protein: 9.30%, nitrogen free extract: 67.83%, ash: 5.18%); moreover, Egbuonu & Osuji (2016) analyzed the proximate content of *Citrus sinensis* peel (ash: 4.89±0.06%, protein: 11.0±0.10%, carbohydrate: 54.17±1.09%). Both works showed similar results compared with those obtained in the present study. On the other hand, Sztern & Pravia (2011) reported the C/N ratio and the organic matter content for citric residues, showing 22 and 33%, respectively. The organic matter content obtained in the present study was higher than the previous report, this could be explained because the oil extraction could affect the organic content in the DRB.

Table 3. Characterization of defatted residual biomass of lemon peel.

Lemon peel	**Ash content (%)**	**Nitrogen content (%)**	**Protein content (%)**	**Carbon content (%)**	**Organic matter content (%)**	**C/N**
Whole	$4.698{\pm}0.192^{b}$	$0.985{\pm}0.056^{a}$	$6.155{\pm}0.35^{a}$	$31.202{\pm}0.67^{a}$	$53.823{\pm}1.155^{a}$	31.67
Steam distillation	$2.272{\pm}0.14^{c}$	$0.98{\pm}0.0463^{a}$	$6.126{\pm}0.29^{a}$	$30.6{\pm}0.579^{a}$	$52.755{\pm}0.999^{a}$	31.22
Soxhlet (Hexane)	$4.899{\pm}0.128^{ab}$	$1.01{\pm}0.019^{a}$	$6.312{\pm}0.121^{a}$	$31.162{\pm}0.528^{a}$	$53.724{\pm}0.91^{a}$	30.85
Soxhlet (Chlor/MeOH)	$5.063{\pm}0.073^{a}$	$0.735{\pm}0.084^{b}$	$4.592{\pm}0.526^{b}$	$31.441{\pm}0.549^{a}$	$54.205{\pm}0.947^{a}$	42.77

Mean of triplicate ± standard deviation. One-way ANOVA, Tukey test $p < 0.005$, different letters in the same column indicate statistically significant differences.

Furthermore, to our knowledge this is the first report of C/N ratio for Persian lime peel DRB, however, the C/N ratio for citric residues was similar for most of the results in the present study, only the SOX-CM showed higher values of this parameters, this could be due to the higher oil extraction reached by this method.

3.5 Potential uses of Persian lime peel DRB

3.5.1 Potential uses for livestock

Contemporary animal feeding development is searching for inexpensive readily available feed resources which can partially substitute the scarce and expensive concentrate feeds. Concerning to the use of Persian lime residues represent an opportunity to contribute in this topic. However, the oil extraction of the Persian lime peel generates a nutritionally advantage since it is not desirable for animal feed to present high lipid contents, which could have some negative effects, such as mould development, and rancidity with adverse consequences on the animal health (Olerumi *et al*. 2007). Furthermore, the protein, ash and organic matter contents, and also the characteristic of being revalorized material from agroindustry, showed that the Persian lime peel DRB presents a good potential for use as feedingstuffs in livestock production.

3.5.2 Potential uses as biogas feedstock

The biogas is a gas mixture formed mainly by methane (CH_4) and carbon dioxide (CO_2). Furthermore, the production of biogas from agro-industrial waste has received attention as a promising option for a renewable energy source (Santos-Ballardo *et al*., 2016). On the other hand, the relationship between the amount of carbon and nitrogen present in the organic matter is represented as the C:N ratio. Moreover, it is well reported that the substrates C:N relation for an adequate anaerobic digestion (AD) presents an optimum range (some authors reports values from 20:1 to 30:1). Furthermore, insufficient amounts of carbon or nitrogen could limit AD performance in digestion of industrial food wastes (Leung & Wang, 2016; Serrano-Meza *et al*., 2020). The Persian lime DRB obtained by STM-D and SOX-H methods showed adequate C/N values for an optimum biogas production, meanwhile the obtained through SOX-DM presented an elevate C/N ratio (42.77) which means an elevated carbon content related with insufficient protein generation during the biogas production. Also, the high organic matter content present in all the treatments is a positive factor for biogas production because represents more material to be transformed in energy (Santos-Ballardo *et al*., 2016). In general, the Persian lime peel DRB showed a good potential for its use as feedstock in biogas production. It is important to remark that is necessary the determination of other parameters for determine the real potential for biogas production of this material, such as the total solids, volatile solids, pH, carbohydrates, etc.

3.5.3 Agronomic utilization

Agronomic utilization of organic residues by direct land spreading is a suitable practice to rise the soil organic matter content and improve its fertility. The addition of organic matter improves soil raindrop impacts, water infiltration capacity and the resistance, therefore the water overflow and soil erosion are reduced. The presence of organic matter also leads to a high-water retention capacity, greater porosity, air permeability and better efficiency in nutrient utilization in the soil. Due to the high organic matter content and the C/N ratio present in the Persian lime peel DRB (obtained from all the treatments), this material presents a high potential as being used by direct land spreading as fertilizer (Zema *et al*., 2018).

3.6 Phenolic composition of Persian lime peel DRB

The phenolic composition of free and bound phenolic extracts of Persian lime peel DRB is shown in Table 4. According to the statistical analysis realized, the oil extraction method and the type of phenolic extract influenced phenolic content in the extracts tested. Among the oil extraction methods, it was observed that STM-D significantly ($p < 0.05$) allowed to keep higher amount of free and bound phenolic compounds in peel extracts followed by SOX-H, it was observed even bigger phenolic content than non-defatted peel, which resulted unexpected, since Patrón-Vázquez *et al*. (2019) reported that phenolic compounds from *C. lemon* (L.) *Burn f* fluors suffered thermal degradation during drying processes and prolonged exposure to heat (40-110 °C/12-24 h). Regarding type of phenolics, all samples showed bigger amount of free phenolic, except for the peel after STM-D, which surprising presented more bound phenolics. The above mentioned is probably due not only to the temperature used during STM-D, but also due to the solubility of phenolics present in the lime peel.

Table 4. Comparison of Phenolic content and antioxidant capacity of free and bound phenolic extracts of Persian lime peel, according oil extraction method.

Lemon peel	**Total phenolic content (μM GAE/g DW)**			**Antioxidant capacity ABTS (μM TE/g DW)**	
	FPE	**BFE**	**Total**	**FPE**	**BFE**
Non-defatted	0.78±0.003^{cA}	0.35±0.002^{dB}	1.14±0.002^{c}	106.49±0.39^{cA}	21.13±0.24^{cB}
After Steam distillation	0.844±0.003^{aB}	1.82±0.02^{aA}	2.66±0.02^{a}	138.53±0.19^{bA}	23.00±0.03^{cB}
After Soxhlet (Hexane)	0.79±0.003^{bA}	0.45±0.005^{bB}	1.24±0.007b	182.29±0.19^{aA}	29.22±1.90^{bB}
After Soxhlet (Chlor/MeOH)	0.56±0.01^{dA}	0.40±0.003^{cB}	0.97±0.01d	138.30±1.96^{bA}	38.42±0.14^{aB}

FPE: Free phenolic extract; BPE: Bound phenolic extract; GAE: gallic acid equivalent; TE: trolox equivalent; DW: dry weight of sample. Mean of triplicate ± standard deviation. Two-way ANOVA, Tukey test $p < 0.05$, different lower-case letters indicate statistically significant differences in the same column (type of extraction method), different capital letters indicate statistically significant differences in the same row (type of phenolic extract).

The distillation method uses high temperatures for prolonged periods, which can cause chemical modifications of the peel components, allowing bigger exposition of phenolics, mostly those bound to other compounds, therefore, phenolics remain in the lime DRB available for their later extraction (Gök *et al*., 2014). Presumably, a portion of the phenolics present in Persian lime peel were solubilized in the organic solvents used for oil extraction due to its direct contact, mostly in Chlor/MeOH, since phenolics are more soluble in polar organic solvents due to the presence of a hydroxyl group, such as alcohol mixtures (Zahoor *et al*., 2016; Aryal *et al*., 2019); therefore, they could were dragged out from the DRB which was analyzed. It is important to mention, that according to several authors, the peels of citrus contain more phenolic compounds compare to other citrus wastes, such as pulp and seeds (Abeysinghe *et al*., 2007; Goulas & Manganaris, 2012; Sir Elkhatim *et al*., 2018).

3.7 Antioxidant capacity of Persian lime peel DRB

The antioxidant capacity (AoxC) of free and bound phenolic extracts of Persian lime peel DRB was determined by the free radical scavenging activity (Table 4). According to the statistical analysis performed, the method of oil extraction and the type of phenolic extract influenced antioxidant capacity in the tested extracts. Regarding type of phenolic, it was clearly observed that free phenolic extracts presented bigger AoxC than the bound ones. Among the extraction methods, it was observed that peel after SOX-H significantly ($p < 0.05$) showed higher AoxC, while the non-defatted peel presents the lowest; these results coincide with those reported by Patrón-Vázquez *et al*. (2019), who also observed an increase in antioxidant capacity in lemon flours after exposure to heat. Even though peel after STM-D had bigger amount of phenolics, did not had the biggest AoxC, this is due to the AoxC not only depends on quantity, but the specific combination of phenolics present in samples, since the chemical composition and chemical structures of active extract components are determinant; for example, it has already been reported that phenolic compounds with ortho- and para-dihydroxylations or hydroxy and methoxy group or both are more effective than simple phenolics (Amzad & Dawood, 2015). Variations among peel samples after different oil extraction methods could be due to the fact that depending upon the polarity of the solvent used, and the chemical nature of the extractable components, the amounts and type of phenolics removed from the material could change (González-Quijano *et al*., 2019; Ji *et al*., 2020). Due to this, desired phenolics might be individually extracted by using a specific extraction method each one (Zahoor *et al*., 2016; Rábago-Panduro *et al*., 2020). Citrus fruits, like Persian lime, have several bioactive components including vitamin C, carotenoids, and phenolic compounds, however, phenolics are considered as the major contributors to their antioxidant potential due to their notorious free radical scavenging activity (Zahoor *et al*., 2016). To our knowledge, although there are studies about lemon wastes, this is the first report of AoxC of Persian lime peel wastes; however, for better understanding of AoxC, further studies are needed, testing different Aox mechanisms, together with the isolation and identification of individual phenolic compounds as well as in vivo studies.

Conclusions

The present study demonstrates that the oils extracted from the Persian lime peels presents a fatty acid profile with a high potential for their use in different industries such as food, bioenergy and even for feedstock, health, or cosmetics products. Also, defatted Persian lime residual biomass presented protein, ash, organic matter, carbon and nitrogen contents (as well as C/N ratio), representing a high potential for its use in diverse economic activities as livestock production, biogas generation and agronomic purposes. On the other hand, oil extraction method and type of extract (free or bound phenolics) influence the phenolic content in the extracts tested, besides, defatted residual biomass obtained through steam distillation showed higher phenolic compounds content; furthermore, free phenolic extracts showed greater AoxC than bound ones. Additionally, defatted residual biomass obtained after hexane extraction presented higher AoxC. Finally, as far we know, this is considered the first report of AoxC of defatted Persian lime peel wastes; however, further analysis of other Aox mechanisms, isolation and identification of individual phenolic compounds as well as in vivo studies are necessary for better understanding of AoxC of this material.

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Nomenclature

$ABTS^{\bullet+}$: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical; AoxC: antioxidant capacity; BRD: biomasa residual desgrasada; C/N: carbon/nitrogen ratio; Chlor/MeOH: chloroform/methanol mixture; DRB: defatted residual biomass; DW: dry weight; FAMEs: methyl esters of fatty acids; FAs: fatty acids; AD: anaerobic digestion; GAE: gallic acid equivalent; M: weight; MUFAs: mono-unsaturated fatty acids; PUFAs: poly-unsaturated fatty acids; SFA: saturated fatty acids; SFAs: saturated fatty acids; SOX-CM soxhlet with Chlor/MeOH; SOX-H: soxhlet with hexane; STM-D: steam distillation; TE:Trolox equivalent; TKN: total Kjeldahl nitrogen; TOC: total organic carbon; UFA: unsaturated; v: volume.

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