Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 327–345

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Natural bioactive compounds of *Citrus limon* for food and health

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ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 19 June 2009
Accepted 22 July 2009
Available online 29 July 2009

Keywords:
Lemon
Phytochemical
By-products
Analytical techniques
Diet
Food industry

ABSTRACT

Citrus genus is the most important fruit tree crop in the world and lemon is the third most important *Citrus* species. Several studies highlighted lemon as an important health-promoting fruit rich in phenolic compounds as well as vitamins, minerals, dietary fiber, essential oils and carotenoids. Lemon fruit has a strong commercial value for the fresh products market and food industry. Moreover, lemon productive networks generate high amounts of wastes and by-products that constitute an important source of bioactive compounds with potential for animal feed, manufactured foods, and health care. This review focuses on the phytochemistry and the analytical aspects of lemon compounds as well as on the importance for food industry and the relevance of *Citrus limon* for nutrition and health, bringing an overview of what is published on the bioactive compounds of this fruit.

Contents

Abbreviations: AAS, atomic absorption spectrometer; AOAC, Association of Official Analytical Chemists; APCI, atmospheric pressure chemical ionization; BGE, background electrolyte; BHT, butylated hydroxytoluene; β-cry, β-cryptoxanthin; β-CD, β-cyclodextrin; CC, coupled column or multicolumn; CE, capillary electrophoresis; CE-ED, capillary electrophoresis with electrochemical detection; CEC, capillary electrochromatography; CHD, coronary heart disease; CID, collision induced dissociation; CVD, cardiovascular disease; CZE, capillary zone electrophoresis; DCIP, 2,6-dichlorophenol-iodophenol; DEPT, distortionless enhancement by polarization transfer; DHAA, dehydroascorbic acid; 3,4-DHCA, 3,4-dihydroxycinnamic acid; DMF, dimethylformamide; ECD, electrochemical detection; EI, electron ionization; EIC, extracted ion chromatogram; EOF, electroosmotic flow; ESI, electrospray ionization; FAB, fast atom bombardment; FAs, flavanone aglycones; FGs, flavanone glycosides; FID, flame ionization detector; HT–HR GC–MS, high-temperature–high-resolution GC coupled to mass spectrometry; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum coherence; HPCE, high-performance capillary electrophoresis; HRGC high-resolution, gas chromatography; HSCCC, high-speed counter-current chromatography; HT–HR, high-temperature–high-resolution; IDF, insoluble dietary fiber; IS, internal standard; LDL, low density lipoproteins; LOD, limit of detection; MAE, microwave-assisted extraction; MALDI, matrix-assisted laser desorption ionization; MECC or MEKC, micellar electrokinetic capillary chromatography; MS, mass spectrometry; NI, negative ion; NMR, nuclear magnetic resonance; NSP, non-starch polysaccharide; ODS, octadecyl silane; PDA, photodiode-array detector; PE, pectinesterase; PEG, polyethylene glycol; PI, positive ion; PMF, polymethoxyflavone; PPO, polyphenoloxidase; PTFE, polytetrafluoroethylene; RDA, recommended dietary allowance; RIA, radioimmunoassay; RP, reverse-phase; SDF, soluble dietary fiber; SPE, solid phase extraction; TA, titratable acidity; TBS, turbo-ionspray; TLC, thin-layer-chromatography; TMS, trimethylsilane; TOF, time-of-flight; TSS, total soluble solids.

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doi:10.1016/j.jpba.2009.07.027

1. Introduction

Citrus is the most important fruit tree crop in the world, with an annual production of approximately 102 million tonnes. According to the morphological system established by Tanaka [1], lemon is classified as *Citrus limon* (L.) Burm. f. Lemon is the third most important *Citrus* species after orange and mandarin, with its production totalling over 4 200 000 tonnes in 2007, with 750 000 tonnes for 2007/2008 Spain being the third main lemon producing country in the world [2], as it is summarized in Table 1. Spain as a leading representative of the Mediterranean lemon producing countries, concentrates its production in orchards in the South-East of Valencia and Murcia, representing about 95% of the *Citrus*-growing areas of Spain [3].

Citrus trees are obtained by rootstock propagation systems using different scions budded onto different rootstocks. *Citrus* rootstocks affect many external and internal fruit characteristics including size, shape, peel thickness, juice content, total soluble solids and phytonutrient composition [3,4]. However, the differences between clones and cultivars are small at industrial level purposes, since the major differences are hidden on industrial procedures [5–7]. In general, *Citrus* cultivars have undergone numerous genetic modifications, due to frequent spontaneous mutations, sporadic hybridizations, and natural selection [3], rendering cultivars (Table 2) with economic impact worldwide [8].

2. Bioactive composition of lemon fruit

Lemon fruit [*C. limon* (L.) Burm. f.] contains many important natural chemical components, including phenolic compounds (mainly flavonoids) and other nutrients and non-nutrients (vitamins, minerals, dietary fiber, essential oils and carotenoids) (Tables 3 and 4). Their health-promoting effects and properties have been associated with their contents, namely vitamin C and flavonoids, due to their natural antioxidant characteristics [9–11]. Overall, lemon fruits, rich in flavonoids, are a very important part of a balanced diet, particularly for their role in prevention of diseases, such as obesity, diabetes, blood lipid lowering, cardiovascular diseases, and certain types of cancer [12–18].

2.1. Phenolic compounds

Flavonoids are one of the most widespread groups of secondary plant metabolites [19], present in a wide variety of edible fruits and vegetables. Flavonoid skeleton is composed of two aromatic rings (namely, A and B), which are connected through a pyrone or hydropyrone ring (C), the flavones or flavanones, respectively (Fig. 1) [20]. More than sixty individual flavonoids have been identified in *Citrus* sp. and most of them can be classified into three groups: flavanones, flavones and flavonols [21]. In addition, other phenolic compounds (phenolic acids, etc.) are also present in *Citrus* species.

Citrus flavonoids are present in the glycoside or aglycone forms. Nevertheless, and thus, flavonoids in juices are mainly present as their glycosyl derivatives (flavonoid glycosides, FGs) [22]. Among the glycoside forms, two types of di-glycosides, L-rhamnosyl-D-glucosyl derivatives, are classified as neohesperidosides and rutinosides, linked via an α-1,2 or α-1,6 interglycosidic bond, respectively. Moreover, *Citrus* juices also contain smaller amounts of flavones *C*-glycosides, in which the substitution is generally on either the *C*-6 or the *C*-8, or in both positions [20,23]. Summarizing, flavonoids can exist as free aglycones but most of them commonly occur as *C*- or *O*-glycosides. These sugars affect the taste of *Citrus* fruits and juices. For example, neohesperidosides, present in grapefruits, are intensively bitter [24], while rutinosides, present in lemons, are tasteless [25,26].

2.1.1. Flavanones

Flavanones are the most abundant *Citrus* flavonoids (e.g. 98% in grapefruit, 96% in limes and 90% in lemons) [25]. Flavanones are weak acids and can be easily converted to isomeric chalcones in alkaline or acid media [26]. Their chemical structures are almost specific for every species, which renders them as markers of adulteration in commercial juices [27,28].

Flavanones exist as a pair of diastereomers because of the presence of a chiral centre in the aglycone (C-2) and the optically active sugar residue (Fig. 1). Naturally occurring flavanones usually have the 2*S* configuration, but racemization can take place during extraction [26,29]. For example, lemon juices contain eriocitrin stereoisomers in equal amount (50%), but hesperidin is almost exclusively found as the 2*S* isomer [29–31].

These compounds are not equally distributed in the lemon fruit. Hesperidin and eriocitrin occur mainly in lemon juice [5,15,32–35] (Fig. 1). Two isomers of hesperidin, neohesperidin and homoeriodictyol 7-*O*-rutinoside have also been identified in lemon juices (Table 3) [4,33].

The peel is richer in flavonoids than are the seeds [23]. Lemon seeds contain eriocitrin and hesperidin more abundantly and very low amounts of naringin. On the contrary, the peel is rich in neoeriocitrin, neohesperidin and naringin and has minor amounts of narirutin [36–38].

Table 1
Listing of countries ranking their lemon market trade (in 1000 tonnes of fresh fruit).

Rank country	Production	Imports	Total supply	Exports	Fresh domestic consumption	For processing	Total distribution
Mexico	1850	1	1851	397	1158	296	1851
Argentine	1400	0	1400	350	50	1000	1400
Spain	750	60	810	430	80	300	810
United States	638	60	698	120	348	230	698
Italy*	576	93	669	35	330	304	669
Turkey	500	0	500	200	290	10	500
South Africa	200	10	210	110	20	80	210
Israel	50	0	50	3	41	6	50
Greece	42	43	85	2	80	3	85
Morocco	25	0	25	0	25	0	25
Cyprus*	14	0	14	8	3	3	14
Japan	4	70	74	0	74	0	74

http://indexmundi.com/agriculture/?commodity=lemons&graph=production (year of estimate: 2007).
* Italy and Cyprus, year of estimate 2006.

In addition, the flavonoid concentrations in lemon fruits depend on the cultivar, maturity stage, etc. [5,39] (Fig. 1).

2.1.2. Flavones and polymethoxylated flavones (PMFs)

Miyake et al. [40] isolated two *C*-glucosylflavones from lemon fruit: diosmetin 6,8-di-*C*-glucoside and diosmetin 6-*C*-D-glucoside. These flavones are also present in limes, but not in other *Citrus* fruits [23,41]. Lower amounts of vicenin-2, and diosmin were determined in lemon juices [4,32,34,35,42]. In addition, chrysoeriol 6,8-di-*C*-glucoside, apingenin 7 (malonylapiosyl)-glucoside [4,33,42], and diosmetin 8-*C*-D-glucoside have been also identified [42] (Fig. 1). There are no studies on flavone composition in seeds. On the other hand, lemon peels contain the three most abundant flavones: diosmetin 6,8-di-*C*-glucoside [40], vicenin-2, and diosmin [36] (Table 3).

Regarding the PMFs on lemon fruit the literature is very scarce. As far as we are aware, studies are only available either on peel or on the edible portion of the lemon fruit, identifying sinensetin, nobiletin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, natsudaidain and tangeretin [34,35,38,43] (Table 3) (Fig. 1).

2.1.3. Flavonols and other phenolic compounds

Rutin and myreIcetin are the most abundant flavonols identified in lemon juice [33,44], while quercetin and kaempferol are in both peel and juice [33–35,38]. Iso/limocitrol 3-β-glucoside, limocitrin 3-β-D-glucoside and limocitrol were identified, as polymethoxylated flavonols, in peel [33,36,45] (Table 3) (Fig. 1).

Table 2
Listing of lemon cultivars of commercial importance worldwide[a].

Lemon cultivars	Other names	Location(s)
Bears		Florida
Berna	Verna, Bernia, Vernia	Spain, Algeria, Morocco
Eureka		S. Africa, Argentina, Australia, California, Greece, Israel, Mexico, Pakistan, China, Arizona, Florida
Femminello ovale or Feminello comune	Commune, Ruvittaru	Sicily
Interdonato	Speciale	Sicily
Kusner		Russia
Lisbon		California, Algeria, China, Greece, Mexico, Morocco, Argentina, Portugal
Mesero	Fino, Primifiori	Italy, Spain
Monachello	Moscatello	Italy
Monroe		California
Sicilian lemon		Brazil
Villafranca		Sicily, California

Extracted and modified from Refs. [3,8].
[a] Lemon cultivars [*Citrus limon* (L.) Burm. f.]. Some of them are listed in [297].

Other phenolic compounds such as hydroxycinnamic acids are also known to be present in very low concentrations (caffeic, cholorogenic, ferulic, sinapic and *p*-coumaric acids) [34,35,37,46], in addition to benzoic acids (protocatechuic, *p*-hydroxybenzoic and vanillic acids) [47] (Table 3) (Fig. 2).

Recently, Miyake et al. [48] identified 1-feruloyl-β-D-glucopyranoside and 1-sinapoyl-β-D-glucopyranoside in lemon juice samples (Table 3) (Fig. 2).

2.2. Other nutrients and non-nutrients of lemon fruit

Lemon has been valued as a fundamental part for a healthy diet. It is well established that lemon fruit and its by-products constitute an interesting source of phenolic compounds (mainly flavonoids) and other nutrients and non-nutrient compounds (vitamins, minerals, dietary fiber, essential oils, organic acids and carotenoids), which are essential for the normal growth and the correct functioning of the human physiological systems [49,50].

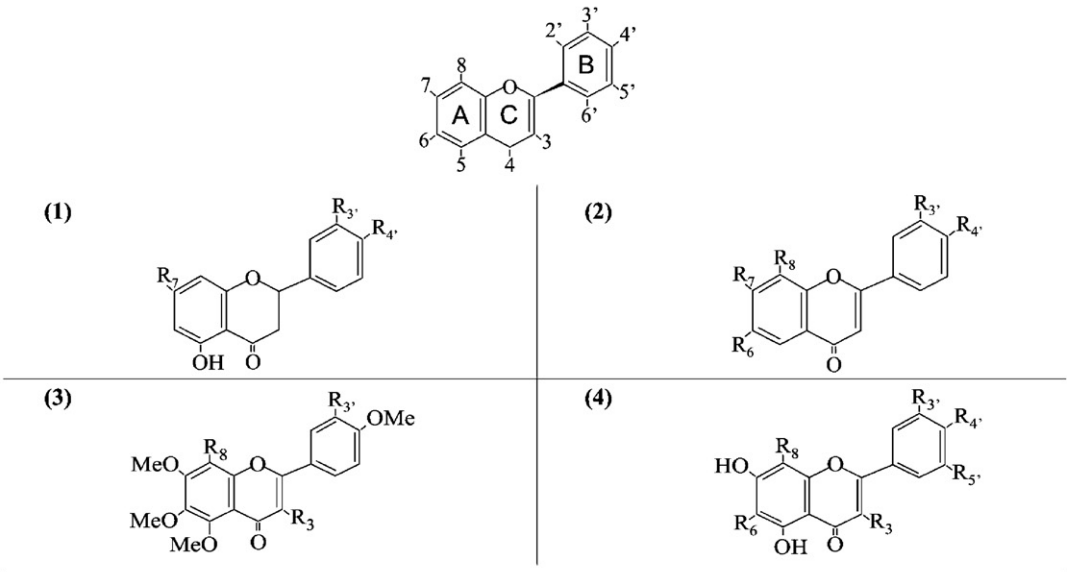
Lemon is a rich source of vitamins for human diet, vitamin C being the main one present in this *Citrus*. Other vitamins present in minor quantities are A and B-group (B1, B2, B3, B6 and B9) [51] (Table 4).

Vitamin C is highly bioavailable and is the most important water-soluble antioxidant in cells as well as an efficient scavenger of reactive oxygen species with two biologically active forms: ascorbic acid (L-AA) and dehydroascorbic acid (L-DHAA) [52,53]. The antioxidant function of vitamin C is based on its ability as hydrogen donor that lets it inactivate free radicals preventing proteins, lipid and DNA damages [54–56]. Vitamin C has also been related with the formation of collagen as part of the connective tissue [57].

The main mineral present in lemon is potassium, although other minerals like calcium, magnesium and phosphorus are also present in minor levels. Moreover, lemon contains trace levels of copper, iron, manganese, selenium, sodium and zinc (Table 4). Potassium constitutes an essential mineral for human health since it is essential to maintain the water–acid balance and it participates in the transmission of nerve impulse to muscle [58].

Lemon constitutes an interesting source of dietary fiber (Table 4), also called non-starch polysaccharides (NSP), that may be classified as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) [59]. The SDF/IDF ratio is fundamental for dietary and functional properties. From this point of view, it is generally accepted that those fibers suitable for use as food ingredients should have an SDF/IDF ratio close to 1:2 [60]. Lemon peel is the structure that presents the major content of dietary fiber [61] and pectin is the major component of fiber present in lemon. A reasonable dietary fiber intake is considered 25–30 g/day and *Citrus* lemon may constitute a valuable contribution to meeting the daily fiber requirements [58].

Flavonoids



Flavanones (1)	R_7	$R_{3'}$	$R_{4'}$
Eriocitrin (Eriodictyol 7-*O*-rut)	*O*-rut	OH	OH
Hesperidin (Hesperitin 7-*O*-rut)	*O*-rut	OH	OMe
Homoeriodictyol 7-*O*-rut	*O*-rut	OMe	OH
Naringin (Naringenin 7-*O*-nh)	*O*-nh	H	OH
Neohesperidin (Hesperetin 7-*O*-nh)	*O*-nh	OH	OMe
Neoeriocitrin (Eriodictyol 7-*O*-nh)	*O*-nh	OH	OH
Narirutin (Naringenin 7-*O*-rut)	*O*-rut	H	OH

Flavones (2)	R_6	R_7	R_8	$R_{3'}$	$R_{4'}$
Diosmetin 6,8-di-*C*-β-gluc (Lucenin-2 4'-methyl ether)	Gluc	OH	Gluc	OH	OMe
Diosmin (Diosmetin 7-*O*-rut)	H	*O*-rut	H	OH	OMe
Vicenin-2 (Apigenin 6,8-di-*C*-gluc)	Gluc	OH	Gluc	H	OH
Chrysoeriol 6,8-di-*C*-gluc (Stellarin-2)	Gluc	OH	Gluc	OMe	OH
Lucenin-2 (Luteolin 6,8-di-*C*-gluc)	Gluc	OH	Gluc	OH	OH
Luteolin 7-*O*-rut	H	*O*-rut	H	OH	OH
Apigenin 7-(malonylapiosyl)-gluc	H	*O*-Api-Gluc	H	H	OH
Diosmetin 6-*C*-β-D-gluc	Gluc	OH	H	OH	OMe
Diosmetin 8-*C*-β-D-gluc (Orientin 4'-methyl ether)	H	OH	Gluc	OH	OMe

Flavone Aglycon (2)	R_6	R_7	R_8	$R_{3'}$	$R_{4'}$
Luteolin	H	OH	H	OH	OH

PMFs (3)	R_3	R_8	$R_{3'}$
Sinensetin	H	H	OMe
Nobiletin	H	OMe	OMe
3,5,6,7,8,3′,4′-Heptamethoxyflavone	OMe	OMe	OMe
Natsudaidain	OH	OMe	OMe
Tangeretin	H	OMe	H

Flavonols (4)	R_3	R_6	R_8	$R_{3'}$	$R_{4'}$	$R_{5'}$
Rutin (Quercetin 3-*O*-rut)	*O*-rut	H	H	OH	OH	H
Quercetin	OH	H	H	OH	OH	H
Kaempferol	OH	H	H	H	OH	H
Myricetin	OH	H	H	OH	OH	OH
Iso-/limocitrol 3-β-D-gluc	*O*-Gluc	OMe	OMe	OH	OMe	H
Limocitrin 3-β-D-gluc	*O*-Gluc	OMe	OMe	OMe	OH	H

Gluc: glucoside; rut: rutinoside; nh: neohesperidoside; PMFs: polymethoxyflavones

Fig. 1. Listing of flavonoids identified in lemon fruit.

Essential oils are aromatic and volatile compounds present in several plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). Of the essential oils group from lemon, we can find about sixty individual components [62]. The major component is D-limonene (45–75%). The aldehyde-citral has been also shown to be present in lemon, in the form of stereoisomers neral and geranial [63]. Linalool shows concentrations of 0.015%. Citral and linalool are thought to be the most potent aroma compounds in *C. limon* [64].

Citric acid is the most representative organic acid in lemon [65], comprising as much as 8% in dry weight, that represents 5–6 g/100 mL [51] (Table 4).

Table 3
Listing of phenolic compounds identified in lemon fruit and related references.

Phenolic compounds	Handly juice	Commercial juice	Peels	Seeds	Edible portions
Flavanones					
Eriocitrin (Eriodictyol 7-*O*-rut)	[4,5,7,22,32,33] and [29–31,118] *	[13,28,134]	[15,36,73]	[37]	[38]
Hesperidin (Hesperetin 7-*O*-rut)	[4,5,7,22,32,33,47,271] and [29–31] *	[13,28,47,80,134]	[34,36]	[37]	[35,38]
Homoeriodictyol 7-*O*-rut	[4]				
Naringin (Naringenin 7-*O*-nh)		[80]	[34,37]	[37]	[35]
Neohesperidin (Hesperetin 7-*O*-nh)	[33,271]	[298]	[34,37]		[35]
Neoeriocitrin (Eriodictyol 7-*O*-nh)		[28]	[37]		[38]
Narirutin (Naringenin 7-*O*-rut)		[28]	[36]		[38]
Flavones					
Diosmetin 6,8-di-*C*-β-gluc	[4,5,32,33,41]		[40]		
Diosmin (Diosmetin 7-*O*-rut)	[4,5,7,13,32]	[80]	[34,36]		[35,38]
Vicenin-2 (Apigenin 6,8-di-*C*-gluc)	[4,5,32,33,41,42]		[36]		
Chrysoeriol 6,8-di-*C*-gluc (Stellarin-2)	[4]				
Luteolin 6,8-di-*C*-gluc (Lucenin-2)			[36]		
Luteolin 7-*O*-rut	[7]				
Apigenin 7-(malonylapiosyl)-gluc	[33]				
Diosmetin 6-*C*-β-D-gluc	[33,42]		[40]		
Diosmetin 8-*C*-β-D-gluc	[42]				
Aglycons					
Luteolin			[34]		[35,38]
PMFs					
Sinensetin			[34]		[35]
Nobiletin					[38,43]
3,5,6,7,8,3′,4′-Heptamethoxyflavone					[38,43]
Natsudaidain					[38,43]
Tangeretin					[43]
Flavonols					
Rutin (Quercetin 3-*O*-rut)	[33,44]		[34]		[35,38]
Quercetin	[44]		[34]		[35]
Kaempferol			[34]		[35]
Myricetin	[44]				
Iso-/limocitrol 3-β-D-gluc	[33]		[36,45]		
Limocitrin 3-β D-gluc	[33]		[36,45]		
Limocitrol			[36,45]		
Hydroxycinnamic acids					
Caffeic acid			[34,47]	[37]	[35]
Cholorogenic acid			[34,47]		[35]
Ferulic acid			[34,47]	[37]	[35]
Sinapic acid (or sinapinic acid)			[34,47]	[37]	[35]
p-Coumaric acid			[34,47]	[37]	[35]
Benzoic acids					
Protocatechuic acid	[47]				
p-Hydroxybenzoic acid	[47]				
Vanillic acid	[47]				
Others					
1-feruloyl-β-D-glucopyranoside	[48]				
1-sinapoyl-β-D-glucopyranoside	[48]				

gluc: Glucoside; rut: rutinoside; nh: neohesperidoside; PMFs: polymethoxyflavones.
* Diastereomers.

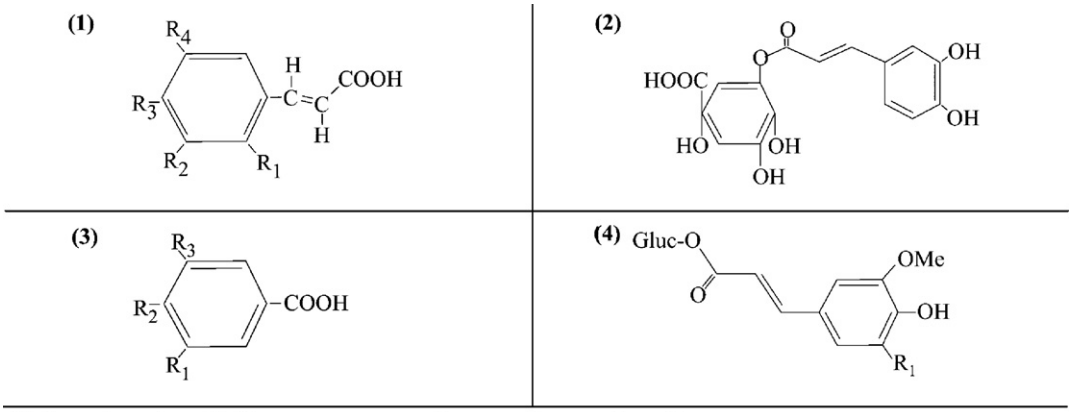
Lemon is a rich source of carotenoids, constituents that protect against photo-oxi damage [66]. However, the concentration of carotenoids is strongly dependent on *Citrus* variety and growing conditions [67]. In recent years, many researches have focused their work on lemon in relation to the major carotenoid components of several orange species and orange juices showing that lemon contains reasonable quantities of carotenoids for a daily nutrition [68,69]. Mature lemon accumulates β-cryptoxanthin (β-cry) as a principal carotenoid [70] and it accumulates predominantly in the flavedo and juice sacs in mature fruits [71].

3. Analysis of lemon phenolic compounds

3.1. Extraction, purification and isolation

The early isolations of lemon phenolic compounds were carried out by Rusznyák and Szent-Györgyi in 1936 [72] extracting 'Citrin' from lemon peel, a mixture of two flavonoids, namely, eriodictyol and hesperidin. Then, in 1960 the isolation of eriocitrin in the peel of lemon fruit was carried out by chromatography on silicic acid by Horowitz and Gentili [73]. More recently, the method fundamental in reverse-phase (RP) high-performance liquid chromatography is employed for isolate eriocitrin from lemon peel [15,48]. Nogata et al. [74], isolated eriocitrin from lemon peel, after freeze-drying n-hexane extraction, filtering under vacuum, and re-extracting again with dichloromethane and hot ethanol, which was concentrated under vacuum and partitioned between n-hexane and 80% aqueous methanol. The aqueous methanol layer was evaporated to dryness, redissolved to a small amount of 40% methanol and subjected to Amberlite XAD-2 column chromatography (750 mm × 80 mm) for purification.

Abad-García et al. [75] showed that polar solvents as methanol are suitable to extract *Citrus* polyphenols, and reducing polyphenol oxidase (PPO) activity. This approach showed that solvents combinations with low percentages of methanol do not inactivate



Hydroxycinnamic acids (1)	R_1	R_2	R_3	R_4
Caffeic acid	H	H	OH	OH
Ferulic acid	H	H	OH	OMe
Sinapic acid (or sinapinic acid)	H	OMe	OH	OMe
p-Coumaric acid	H	H	OH	H
Chlorogenic acid (2)				

Benzoic acids (3)	R_1	R_2	R_3
Protocatechuic acid	H	OH	OH
p-Hydroxybenzoic acid	H	OH	H
Vanillic acid	H	OH	OMe

Others (4)	R_1
1-Feruloyl-β-D-glucopyranoside	H
1-Sinapoyl-β-D-glucopyranoside	OMe

Gluc: glucoside.

Fig. 2. Listing of phenolic compounds identified in lemon fruit.

Table 4
Listing of essential nutrients and non-nutrients in *Citrus limon*.

Major components	g/100 g	
Energy	29 kcal	
Carbohydrates	6.52	
Proteins	1.1	
Saturated fat	0.039	
Citric acid	5–6 g/100 mL	
Total fat	0.3	
Dietary fiber	2.8	
Vitamins	**mg/100 g**	**Recommended daily intake (mg)**
Vitamin A (retinol)	0.003	0.6
Vitamin B_1 (thiamin)	0.04	1.4
Vitamin B_2 (riboflavin)	0.02	1.6
Vitamin B_3 (niacin)	0.1	18
Vitamin B_6 (piridoxina)	0.08	2
Vitamin B_9 (folic acid)	53	400
Vitamin C (ascorbic acid)	0.0106	75
Minerals		
Calcium	26	1000
Copper	Trace amounts	2
Iron	0.6	15
Magnesium	8	350
Manganese	Trace amounts	5
Phosphorus	16	1000
Potassium	138	350
Selenium	Trace amounts	35
Sodium	2	2400
Zinc	0.06	15

http://www.lenntech.com/fruit-vegetable-vitamin-content.htm (year of estimate 2008).

this enzyme completely, resulting in low extraction efficiencies. However, since high amounts of methanol decreased significantly the resolution of the chromatographic peaks, a moderate mixture of methanol–water–acetic acid, has been proposed as the best selection as extractant. Sometimes, antioxidants, such as butylated hydroxytoluene (BHT), L-AA or sulfites are added to the extraction solvent to protect analytes from oxidation [75].

FGs could also be analysed as aglycones, since the hydrolysis of glycosides is a useful method to obtain structural elucidation and characterization information. The rate of acid or basic hydrolysis of glycosides is also a good method to distinguish different structures. For example, acid hydrolysis does not affect *C*-glycosides, while *O*-glycosidic bounds are quickly hydrolysed [41,76]; studies of Ferreres et al. [41,76] reported alkaline hydrolysis of *O*-glycosyl-*C*-glycosyl flavones extracts to remove exclusively acylated residues.

One disadvantage of using strong alkalis (e.g. NaOH) for extraction is the possible conversion of flavanones (e.g. hesperidin) to their corresponding chalcone derivatives. This effect may be reversed by acids [77]. Flavanones, less soluble than chalcones, tend to separate first in fractional crystallization and are easily precipitated at low pH, specially if solutions are chilled or frozen [26]. In these cases, heating or preferably the use of strong solvents such as dimethylsulfoxide (DMSO), dimethylsulfoxide/methanol mixtures (1:1), dimethylformamide (DMF), as well as ethanol, acetone or mixturing them with water is essential to ensure efficient recovery [4,18,20,26,28,41].

Other pretreatment technique involves microwave-assisted extraction (MAE) for phenolics [78] but this method is very time-consuming and not so generalized.

Regarding extraction of phenolic acids, this is very similar to the aforementioned methods. Methanol and DMSO are usually used as extractants [34,35,37] followed by filtration, solvent is washed and

Table 5
Listing of rutinary available methods for identifying common phenolic compounds in lemon fruit.

Techniques	Bioactives			Reference
	Flavanone	Flavones	Other	
UV–Vis	Eriocitrin, hesperidin	Diosmetin 6,8-di-*C*-β-gluc, vicenin-2, diosmin		[32,41]
IR		Diosmetin 6,8-di-*C*-β-gluc, diosmetin 6-*C*-β-D-gluc		[40]
CE SBE-β-CD	Eriocitrin, hesperidin			[30]
RP-CEC	Eriocitrin, hesperidin			[134]
CC–LC-ESI-MS	Eriocitrin, hesperidin*			[29]
Quiral CE	Eriocitrin, hesperidin*			[31]
HPLC (UV, Vis, DAD)	Eriocitrin, hesperidin, homoeriodictyol 7-*O*-rut, naringin, neohesperidin neoeriocitrin, narirutin	Diosmin, luteolin, luteolin 7-*O*-rut, diosmetin 6,8-di-*C*-β-gluc, vicenin-2, chrysoeriol 6,8-di-*C*-gluc, diosmetin 6-*C*-β-D-gluc	Flavonols (rutin, quercetin and kaempferol) Hydroxycinnamic acids PMFs (Sinensetin, nobiletin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, natsudaidain) Other (1-feruloyl-β-D-glucopyranoside, 1-sinapoyl-β-D-glucopyranoside)	[4,5,7,28,34,35,38,40,44,47,48,80,118,298]
HPLC-PDA-MS	Eriocitrin, hesperidin, homoeriodictyol 7-*O*-rut	Diosmetin 6,8-di-*C*-β-gluc, vicenin-2, diosmin, chrysoeriol 6,8-di-*C*-gluc		[4,22]
HPLC-DAD-TSP-MS	Eriocitrin, hesperidin, narirutin	Diosmin, vicenin-2, luteolin 6,8-di-*C*-gluc	Iso-/limocitrol 3-β-D-gluc, limocitrin 3-β D-gluc, limocitrol	[36]
HPLC-DAD-ESI-MS	Eriocitrin, hesperidin, neohesperidin, neoeriocitrin, naringin	Diosmin, luteolin 7-*O*-rut	Hydroxycinnamic acids	[7,37]
FAB-MS and HR-FAB-MS		Diosmetin 6,8-di-*C*-β-gluc, diosmetin 6-*C*-β-D-gluc	Other (1-feruloyl-β-D-glucopyranoside, 1-sinapoyl-β-D-glucopyranoside)	[40,48]
HPLC-PDA-APCI-MS-MS	Eriocitrin, hesperidin	Diosmetin 6,8-di-*C*-β-gluc, vicenin-2, diosmin		[32,41]
Micro-HPLC-ESI-MS	Eriocitrin, hesperidin, neohesperidin	Diosmetin 6,8-di-*C*-β-gluc, diosmetin 6-*C*-β-D-gluc, vicenin-2, apigenin 7-(malonylapiosyl)-gluc	Iso-/limocitrol 3-β-D-gluc, limocitrin 3-β D-gluc	[33]
Fast microparticle HPLC			PMFs (Nobiletin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, natsudaidain, tangeretin)	[43]
1H RMN and 13C NMR	Eriocitrin, hesperidin, diosmin, diosmetin 6-*C*-β-D-gluc	Diosmetin 6,8-di-*C*-β-gluc, vicenin-2	Other (1-feruloyl-β-D-glucopyranoside, 1-sinapoyl-β-D-glucopyranoside)	[32,40,48]

gluc: Glucoside; rut: rutinoside; PMFs: polymethoxyflavones.
* Diastereomers

evaporated to dryness under vacuum and redissoluted [37]. Other option is dilution in distilled water followed by alkaline hydrolysis (NaOH) and acidification (HCl). Finally, a centrifugation lets to extract the phenolic acids from the hydrolysate with organic solvents [47].

The pretreatment of samples by hydrolysis can produce loss of phenolics, due to decomposition and polymerization. Sakakibara et al. [79] developed a direct determination of the FGs in foods without hydrolysis and also El-Shafae et al. [77] proposed methods for the determination of hesperidin and diosmin without involving alkali or acid extraction.

For the preparation of standard stock solutions (hesperidin, diosmin or naringin), researchers usually use DMSO:methanol in different proportions, e.g. 1:9, 1:1 (v:v) [77,80], whereas the standard solutions of the aglycones (e.g. hesperetin and naringenin) are more suitable to be prepared on acetonitrile [81].

The lemon samples can be partially purified by solid phase extraction (SPE) procedures with C18 Sep-Pak cartridges [82–87], and the collected fractions can be eluted in solution containing internal standard (IS) using different filters [32,41].

Among *C*-glucosylflavones, the diosmetin 6,8-di-*C*-glucoside was extracted, at first, from lemons with boiling methanol by Gentili et al. [45]. After that, this diosmetin 6,8-di-*C*-glucoside and the diosmetin 6-*C*-glucoside were isolated from the lemon peel with methanol at room temperature for 3 days, filtered through a cloth and concentrated under vacuum. The peel extract was then dissolved in water and chromatographed on a Cosmosil 75C 18-OPN ODS column (column size Ø 37 mm × 500 mm). The column was successively eluted with increasing proportions of methanol/water (20%, 40% and 100%). The 40% fraction was further concentrated under vacuum, dissolved in methanol, and applied to a preparative HPLC using a YMC-Pack ODS column [40].

There are a few scientific papers related to the extraction and purification of the phenolic compounds of lemon destined to pharmaceutical and food industry. Lemons fruits are processed by the food industry, mainly to obtain juice and for canning. This activity generates a large quantity of lemon waste useful by the chemical industry to extract flavonoids and others bioactives [88]. Chemical industry extracts and purifies the phenolic compounds of the lemon

peel by heat drying (60 °C) until stable weight, grinding phenolics in DMSO [13].

3.2. Analytical techniques

3.2.1. Classical techniques for lemon fruit quality

Titratable acidity (TA), total soluble solids (TSS) as well as pH are considered quality indexes for *Citrus* juices. Thus, TA is determined elsewhere by titrating with 0.1N NaOH in accordance with AOAC [89]. Total soluble solids (TSS) are recorded in a refractometer at 20 °C with values expressed as °Brix and the pH values are normally measured at 20 °C by using a pH-meter [5,47].

On the other hand, several classical techniques for the determination of phenolic compounds have been described. Initially, the 'Davis method' was used, a quick semiquantitative spectrophotometric method for measuring total flavanones [90]. Then, Rowell [91] developed a quantitative and also spectrophotometric determination of flavanones based on the development of red color in the reducing reaction of flavanones. Later, other methods developed for the spectrophotometric determination of hesperidin by forming complexes with certain metals were described: Copper (II)—[92], uranil (II)—[93], zirconium (IV)—[94] and aluminium (III)–hesperidin complexes [95] in water–methanol systems [24]. Moreover, for hesperidin determination spectrofluorimetry based on the reaction between rutin and DMF to form a fluorescent chelate has been used [96], and radioimmunoassay (RIA) utilizing antibodies raised against a hesperidin-4-*O*-carboxymethyl-oxime hapten and a tritiated radiotracer prepared by direct reduction of hesperidin with $NaB[^3H]_4$ have been developed [97]. Finally, there were other methods, not widely used in the analysis of hesperidin and diosmin that included spectrodensitometry [98] and voltammetry techniques [99].

Nowadays, the total phenolic content (TPC) of lemon fruit extracts and juices is determined spectrophotometrically by the Folin–Ciocalteau's phenol reagent procedure according to Singleton [100] with small modifications [5,35].

3.2.2. Thin-layer-chromatography and gas chromatography

Early in the 1960s, chromatography method such as thin-layer-chromatography (TLC) has been used in flavonoid analysis. For example, Höerhammer and Wagner [101] separated and purified *Citrus* flavonoids (hesperidin, hesperetin, eriodictyol, naringin and naringenin) using preparative TLC on silica gel with butanol, acetic acid and water (4:1:5). Greenham [102] determined different flavones (apigenin and luteolin, also present in lemon) by TLC in plant extracts and more recently Baranowski [103] studied the effect of several mobile phases in RP-18 TLC plates, on the separation of flavanones, flavones and flavonols.

On the other hand, gas chromatography (GC) techniques are not so generalized for determining FGs, due to the analytical difficulty, even after derivatization, besides the long time required of these reactions and the low limits of detection (LOD) [104]. Nevertheless, in some reports a high-temperature–high-resolution GC coupled to mass spectrometry (HT–HR GC–MS) has been used with capillary columns that can support temperatures up to 400 °C for analysing hesperidin in plant extracts [104].

3.2.3. HPLC techniques

The most widely used methods, for the determination of the phenolic structures present in food samples, are based on reversed-phase high-performance liquid chromatography (RP-HPLC) using hydrophilic endcapped C18- or C8-bonded silicagel columns as the stationary phase coupled to ultraviolet–visible (UV–Vis) or diode-array or photodiode-array detection (DAD and PDA, respectively) [27,47,74,75,77,80,105–111]. Based on their UV spectra flavonones show a strong absorption peak in the range 270–295 nm (Band II) and a shoulder at 320 nm (Band I), while flavones show two strong absorbance peaks at 280 (Band II) and 320–360 (Band I) [20]. Techniques used to characterize the content of phenolic compounds in lemon fruit are listed in Table 5.

There are several packing materials for stationary phases in the design of the columns, such as NovaPak RP-C18 (300 mm × 3.9 mm; i.d. 4 μm), HydroBond PS18 (100 mm × 3 mm or 25 mm × 3.2 mm with i.d. 5 μm) [112], Lichrosorb RP-C18 [77], Lichrospher [5], Octadecyl silane-3 (ODS-3) columns [82], RP narrow-bore column [113], Hypersil ODS-1 [114]. HPLC mobile phases generally consist of acetonitrile/water as well as methanol/water mixtures for aglycones and glycosides [22,27,83]. The addition of a weak acid (up to 5%), acetic [27,115], formic [22], or citric acid [80], to the solvent system suppresses the ionization of phenol groups leading to better separation of aglycones (e.g. naringenin, hesperetin) as well as FGs. Moreover, the separation is further improved when the column temperature is increased from 34 °C to 45 °C giving symmetrical and sharp peaks [47,80,83,107].

Preparative HPLC using C18 column has been used as techniques of purification [20,32,41,116].

PMFs (nobiletin, sinensetin and 3,5,6,7,8,3′,4′-heptamethoxyflavone) have been identified by this method at 330 nm in *Citrus* juices, but not all of them specifically in lemon [43,86,106,111,117]. Phenolic acids (hydroxycinnamic and benzoic acids) have been isolated from lemon juice samples [47] and in *Citrus* by-products, such as lemon peel molasses (ferulic, *p*-coumaric and sinapic acids) [46] (Table 5).

3.2.3.1. Stereospecific separation by HPLC. Yáñez et al. [118] developed a stereospecific separation method for eriodictyol in raw lemon juice using RP-HPLC using a Chiralpak OJ-RH column and UV detection. Later, the stereospecific content of the flavanone glycosides (hesperidin, naringin and eriocitrin) and their aglycones (hesperetin, naringenin and eriodictyol) in fruit juices was quantified and verified by HPLC and LC/MS, respectively by Yañez et al. [119].

3.2.3.2. Bidimensional chromatography system (LC–LC) and CC–LC. There are many TANDEM methods of LC coupled to other techniques including LC (2D, LC–LC) [29], or mass spectrometry (LC–MS) [4,7,22,32,33,36,37,40,41,48,80,106,120] for determining the phenolic compounds in *Citrus* juices, including lemon, biological fluids or pharmaceutical formulations (Table 5). In all cases, HPLC is coupled to DAD, electrochemical detection (ECD) [82,112].

One advantage of bidimensional chromatography systems (LC–LC) in comparison to single-dimension separations is the increase of the peak capacity and the resolution of rutin, naringenin, hesperetin and hesperidin [121]. Determining phenolic acids, ferulic and *p*-coumaric acids, also in lemon fruit, by 2D LC-HPLC using a Purospher Star RP-18e column, and two parallel Zirconia Carbon columns, for the first and second dimension, respectively [122].

On the other hand, the coupled column LC (CC–LC) combined with cyclodextrines (CDs) allows to resolve flavanone stereoisomers [29]. These studies report the use of a RP-C18 column for the separation of FGs, and a carboxymethylated β-cyclodextrin-based (β-CD) column for the stereoisomers separation, combined with MS in negative ion electrospray ionization (ESI-MS), and determining the diastereomeric composition of hesperidin and eriocitrin besides neohesperidin, naringin, and narirutin in lemon juice [29].

3.2.3.3. HPLC coupled to mass spectrometry. The use of MS implies tandem techniques (LC–MS), involving distinct types of ionization sources as the interface between LC and the mass detector. Among them, electrospray ionization (ESI-MS) and atmospheric pressure chemical ionization (APCI-MS) [22,85] are currently the most popular ionization sources used for small molecule metabolites

such as bioflavonoids. Also a turbo-ionspray (TBS) interface [120] and the matrix-assisted laser desorption ionization (MALDI) could be used in combination with 'time-of-flight' techniques offering new possibilities for compound identification, as the elucidation of the molecular formula of eriocitrin analysed by ESI-TOF-MS [74]. The compound elucidation using these techniques is based on the analysis of the fragments produced in the collision cell, originating ions by dehydration and cleavage of the sugar pyrano ring [42]. Abad-Garcia et al. [42], reported the guidelines that allow the easy differentiation of *C*-6- and *C*-8-monoglycoside isomers using ESI-MS in positive ion mode, while Zhou et al. [81], developed and validated an MS technique in full-scan-mode for the simultaneous detection and quantification of two flavanone aglycones, naringenin and hesperetin. In these studies, the protonated flavanone aglycones produced specific fragmentation patterns using a positive APCI-MS/MS technique which formed a characteristic collision induced dissociation (CID) CID-MS fingerprint.

Analysis of flavanones, flavones, as well as flavonols in lemon peel and juice was carried out by HPLC-DAD-ESI-MS techniques [4,36,123–126]. Recently, Ding et al. [78], used perfluorinated carboxylic acids as ion-pairing reagent to improve the separation and retention of flavonoids in LC-ESI-MS for the simultaneous determination of hesperidin, and naringin in samples containing alkaloids [78]. On the other hand, the use of HPLC-PDA-ESI/MS/MS has been used by many researches to determine characteristic *C*-glycosyl flavones of lemon, i.e. vicenin-2, diosmetin 6,8-di-*C*-glc, etc. in juice [41], in *Citrus Aurantifolia* leaves [127], and in bergamot (*Citrus* Bergamia Risso) juice [126], as well as phenolic acids (ferulic and *p*-coumaric) in lemon juice [37], and nobiletin, sinensetin, and 3,5,6,7,8,3′,4′-heptamethoxy, in tangerine peels [128,129] (Table 5).

3.2.3.4. μHPLC. Nowadays, the use of microcolumns (μHPLC) is increasing due to the ability to work with small sample size (e.g. V_{inj}—0.65 μL) and small volumetric flow-rates (e.g. 40 μL/min). The coupling of this powerful and sensitive technique with ESI/MS (micro-HPLC-ESI/MS) is of special interest. Nevertheless, in order to achieve efficiency in the ionization process a splitter is commonly incorporated when conventional HPLC columns are used. Using a micro-bore HPLC column (C18) obviates the need for a splitter between HPLC system and ESI interface, otherwise, a C18 narrow-bore column may be used (V_{inj}—2 μL, and flow—200 μL/min) [33] (Table 5). By this method most of the phenolic compounds present in lemon juice have been analysed, but hesperidin and myricetin have been detected by a simple extraction in methanol and semi-micro-HPLC with electrochemical detection (μHPLC-ECD) using a micro-bore ODS C18 column (150 mm × 1.0 mm, i.d. 3 μm), and applying a potential at +0.9 V versus an Ag/AgCl reference electrode [130].

3.2.4. Capillary electrophoresis (CE)

CE is based on the different electrophoretic mobilities of substances in solution under the action of an electric field. The main properties of capillary electrophoresis (CE) are the high speed of analysis, the high separation efficiency and the great variety of applications using reduced sample volume and solvent consumption. Several reviews have been published on the application of CE methods in food analysis and the good demonstrative data of the possibilities of this technique in this field [131]. CE coupled with electrochemical detection (CE-ECD) has been used for flavonoid determination (hesperidin, naringin, hesperetin, narigenin and rutin) in different *Citrus* samples prepared in borate buffer (pH 9.0) [132–134].

CE is then a useful technique for the separation of stereoisomers. In this aspect, flavanones can be ring-opened in alkaline media and converted to isomeric 2′,6′-dihydroxy-substituted chalcones which are unstable and with a strong tendency to rapidly cyclise back to flavanones. Since this reaction is not enzymatical, it results in a racemization of the corresponding flavanone. Flavanones with a free hydroxy group at position 4′ (e.g. glycosides of naringenin and eriodictyol) racemize easier than with a methoxy group (e.g. glycoside of hesperetin) [31] (Table 5).

Separation on CE depends on the amount of the CDs, the pH of the buffer and the concentration of the background electrolyte (BGE). Thus, the chiral separation of flavanone glycoside diastereomers, mainly the flavanone-7-*O*-glycosides eriocitrin and hesperidin, could be achieved by ionization at wish pH using a borate buffer (0.2 M, at pH 10.0) and with 5 mM γ-CD (a native CD) as chiral selector [135]. Aturki and Sinibaldi [30] proposed β-cyclodextrin sulfobutyl ether (SBE-β-CD) as a chiral buffer additive of CE for the separation of the epimers of naringin, narirutin, hesperidin, neohesperidin, and also eriocitrin in *Citrus* fruit juices.

The use of micellar electrokinetic capillary chromatography (MECC or MEKC) separates the samples by differential partitioning between a pseudo-stationary micellar phase and an aqueous mobile phase. In 1994, Ferreres [136] identified hesperidin, by MECC; nevertheless it was recently demonstrated that most of the FG standards can be fully separated by this technique [133,134,137].

3.2.5. Capillary electrochromatography (CEC)

Capillary electrochromatography (CEC) is a hybrid analytical technique combining the best features of CE and HPLC, i.e. capillaries packed with a stationary phase and the use of an electroosmotic flow (EOF) generated under an electric field as the mobile phase [134]. RP-CEC was initially developed and then extensively applied to the separation of non-polar neutral compounds, such as the FGs. Thus, the separation of most flavanone-7-*O*-glycoside constituents of *Citrus* was carried out by isocratic RP-CEC using a 75 μm i.d. silica fused column packed with 5 μm ODS silica gel [134]. One advantage of this technique is the short time in the analysis versus the time consuming of gradient elution that is necessary in the FG analysis by liquid chromatography (Table 5).

3.2.6. Nuclear magnetic resonance (NMR)

NMR technique, requires prior isolation of the compounds and possibly a high degree of structural information. NMR and two-dimensional NMR techniques (COSY, NOESY, HMQC and HMBC) have been used to achieve the structural elucidation and the complete H and C assignments. These methods usually use trimethylsilane (TMS) as internal standard (IS) [32,41,138]. As examples, flavanones and *C*-glycosyl-flavones (vicenin-2 and diosmetin 6,8-di-*C*-glucoside) in lemon juice [32,40,48] (Table 5), China *Citrus* [139] and in a hydroalcoholic extract of *C. Aurantifolia* leaves [127] have been analysed. Diosmin and luteolin were fully characterized by Park et al. [140]. Narirutin and hesperidin have also been detected in by-products by means of spectral analyses using heteronuclear multiple bond correlation (HMBC), ^{1}H NMR, ^{13}C NMR and 2D NMR [141]. Also PMFs, nobiletin, sinensetin and 3,5,6,7,8,3′,4′-heptamethoxy were determined by ^{1}H NMR, ^{13}C NMR and 2D NMR spectral studies in tangerine peel [128,129] and its metabolites in murine urine and faeces samples in rats as well [142]. Also ^{1}H NMR spectra of *C*-glucoside flavonoids are particularly interesting on diastereomers elucidation because of the presence of rotamers resulting from hindered rotation around the *C*-glycosidic linkage [20].

4. Analysis of other nutrients and non-nutrients of lemon fruit

4.1. Extraction, purification and isolation

Lemon fruit and its by-products from lemon manufacturer process are fundamentally composed of flavonoids, vitamins, minerals,

soluble and insoluble dietary fiber, essential oils, organic acids and carotenoids [49,50,143]. However, the proportion of all these components in the lemon juice and its residues depends, mainly, on the lemon juice extraction system employed [7].

Both lemon and lemon juice, constitute an important source of vitamin C. To extract vitamin C from lemon, it must be peeled and the edible portion must be chopped in small portions and homogenized at room temperature. This slurry is diluted in double distilled water, sonicated and spun. The supernatant after centrifugation is used as vitamin C source [144]. Vitamin C extracted from lemon may suffer a reduction in its concentration during storage. Concretely, oxygen, heat light, time, storage temperature and storage time affects the ascorbic acid retention in *Citrus* juices [145,146].

To extract the mineral contained in lemons, they must be chopped in small portions. The minerals from lemon are extracted using 0.3N HCl by shaking the samples at 37 °C for several hours [147].

Lemon juice industrial by-products (mainly lemon's peel) can be used to obtain high dietary fiber powder. Washing by-products prior to drying prevents fiber browning during drying procedure [148]. Lemon SDF and IDF are obtained from lemon peel by cutting the peel residue, washing it with warm water, drying and grinding to a particle size of 500–600 μm. The material is washed under mild conditions to avoid losses of some soluble fiber components as well as bioactive components. Grinding to a large particle size must be performed to not affect the hydration characteristics on the textures of the fiber concentrates [149]. Different components from both SDF and IDF in lemon have been recently characterized by means of several techniques following sequential extraction in a Soxhlet extractor and acid hydrolysis [61].

Pectin is extracted from lemon peel using two different methods: acid extraction and dried pectin preparation. Acid extraction from lemon peel is followed by filtration and precipitation using an alcohol as 2-propanol [150]. The extraction of pectin from *Citrus* peel is about 25% [151]. These extraction conditions induce the proteolysis that is not adequate for the quality or quantity of pectin extracted. On the other hand, dried *Citrus* pectin extracts, containing 70% pectin are produced at a yield of 20–26% [152]. Nevertheless, the extraction of pectin at high temperatures caused the dissolved pectin to degradation while it slightly improved the yield of pectin [153]. Moreover, longer extraction time generally decreased the degree of methylation, because extraction condition must take into accounts both recovery yield and characteristics of extracted pectin [49].

In order to increase the yield of pectin from fruits, Fishman et al. [154] described a treatment by microwave heating. This method has also been reported as favourable on yield and quality of pectin due to the partial disintegration of the tissue structure and the hydrolysis of propectin together with the fast deactivation of hydrolytic enzymes [155]. The use of the microwave over the extraction was optimized recently in apple pomace by Wang et al. [35], showing a critical reduction of the extraction time that could help to improve pectin extraction from lemon and its by-products the juice industry. Ralet et al. [156] obtained water-soluble pectins after extrusion cooking from lemons.

Several procedures can be used to extract essential oils from different *C. limon* plant parts, including water or still distillation, solvent extraction, extraction under pressure, supercritical fluid, subcritical water-extractions and cold-pressing [157,158]. Distillation is the major method to produce essential oils with commercial objective [159]. However, hexane based extraction of essential oils lets to obtain essential oils with alternative properties than those observed when distillation procedure is used [160]. The presence of essential oils in lemon extract decreases over time due to the evaporation of the volatile compounds [161]. Microencapsulation of essential oils constitutes an interesting technology that is still being used in food industry, to prevent the volatilization of essential oils and extend the useful-life of these biological compounds [162].

To extract citric acid, lemons are chopped in small pieces and seeds are picked out. The material obtained from lemon fruit and seeds are heated at 50 °C for 12 h. Resultant material is finally heated at 120 °C for 2 h. The final product is dried at 105 °C for 24 h and cooled in a desiccator [163]. When citric acid is preformed from lemon juice, calcium citrate is precipitated by addition of calcium hydroxide in the juice at temperature of 60 °C. The citric acid is then reconstituted by addition of sulfuric acid [164].

Carotenoids are extracted from lemon peel using n-hexane–acetone–ethanol (v/v/v; 50:25:25) and then analysing the absorbance of the dilution [34,35].

4.2. Analytical techniques

Concerning the analysis of L-AA from lemon, it is essential to inactivate degradative enzymes and to fix the L-AA /L-DHAA redox equilibrium. In order to avoid L-AA oxidation an acidic extraction solvent must be employed, to suppress metabolic activity upon disruption of the cell and to precipitate proteins [165]. In 1980 and through our days, L-AA determinations can be performed by spectrophotometry. This method is based on the reduction of 2,6-dichloroiodophenol (DCIP) by L-AA, and the reduction of iron from its ferric form to ferrous by L-AA, followed by the formation of a chromogenic ferrous iron complex, using chelators as 2,2′-dipiridyl and ferrizone. After treatment with sulfuric acid, a colored product that absorbs at 520 nm is formed. Fluorometric determination is also possible to analyse vitamin C, and it is based on the condensation of L-DHAA of *o*-phenylenediamine to form quinexaline derivative. This method suffers from a weak sensitivity and specificity [166]. Moreover, this procedure is unable to determine L-DHAA directly [167]. Determination of vitamin C by means of titration method using DCIP led to report a rapid decrease of vitamin C levels [145].

HPLC constitutes an interesting alternative to correct the lack of selectivity and sensitivity of classical methods. HPLC can be performed coupled to electromechanical detection [165,166,168], fluorescence detection [169], refractive index detection [170] or UV detection [171]. Moreover, high-performance capillary electrophoresis (HPCE) is a technique increasing in the recent years to analyse L-AA as faster than HPLC, although HPCE shows a limit of detection higher than that observed in HPLC [167]. The Association of Official Analytical Chemists (AOAC) recommends the volumetric titration using DCIP as titrant for the determination of vitamin C in lemon preparations. Microfluorometry and fluorometry is suggested for quantification of total vitamin C in food including lemon and its preparations [172]. After the vitamins have been released by enzymatic and hydrolytic digestion, a substance-specific cleanup followed by HPLC–MS analysis provides interference-free determination at the lowest concentration levels [165]. Vitamin C from lemon can be also analysed by means of LC on a RP with UV detection [173] (Table 6).

To analyse vitamin C from lemon, which also shows a functional role as antioxidant compound, it is necessary to perform the analysis of the antioxidant capacity. In order to test the antioxidant ability of vitamin C, the authors suggest that a general testing protocol should properly test various oxidant conditions, measure both initial and secondary oxidation products, compare antioxidants at the same molar concentrations of active components and quantify in basis of induction period. Major antioxidant capacity assays can be divided into two categories (1) hydrogen atom transfer reaction based assays and (2) single electron transfer reaction based assays [174,175].

Several methods have been used to determine the mineral content in lemon tissues and manufactured products. The most widely used method for the analysis of mineral content is the atomic absorption spectrometer (AAS). The minerals and trace elements are determined by AAS using the instrumental conditions recommended for each mineral [173]. Instrumental Neutron Activation Analysis constitutes a technique that can be considered an alternative tool not only for its high sensitivity and accuracy [176], but also because it allows multielement analysis for each single sampling of a complex matrix such as lemon juice [177] (Table 6).

Dietary fiber composition of lemon is determined by enzymatic–gravimetric methods as described by Lee et al. [178]. Figuerola et al. [149] analysed the content of total dietary fiber in Fino49 and Eureka lemon finding a higher amount of total dietary fiber in the former, in which the major content is represented by insoluble fiber [149]. Pectin content of the samples is determined by colorimetric method using m-hydroxydiphenyl [179]. Several components from both SDF and IDF in lemon have been characterized using a number of techniques including alkaline hydrolysis and colorimetric determination with carbazole for pectin, acetic nitric acid digestion for cellulose and titration with thiosulfate for hemicellulose [61] (Table 6).

Detailed analysis of essential oils composition has been achieved by high-resolution gas chromatography (HRGC) and high-resolution gas chromatography coupled to mass spectrometry (HR GC–MS) [64], being performed with two columns with different stationary phase polarities [43,180,181]. Gas chromatography with flame ionization detector (GC-FID) has also been employed in order to characterize the essential oils present in lemon fruit [182] (Table 7).

Citric acid is usually measured by HPLC, NMR and ion chromatography in lemon juice [51,183].

To identify and quantify the carotenoids present in lemon, three different gradient elution schedules of HPLC and RP-HPLC are used. Moreover, lemon carotenoid contents are easily determined by UV–Vis methods [34,35,70,184] (Table 6).

Table 6
Listing of rutinary available methods for identifying other nutrients and non-nutrients in *Citrus limon*.

Techniques	Other nutrients and non-nutrients					Reference
	Vitamins	Minerals	Dietary fiber	Organic acids	Carotenoids	
Alkaline hydrolysis			Pectin			[61,149,178,179,272]
Colorimetric determination			Pectin			[149,178,179,272]
Enzymatic gravimetric method			Pectin, cellulose, hemicellulose, lignin			[149,178,179,272]
Acetic nitric acid digestion			Cellulose			[149,168,178,179,272]
Titration	Vitamin C		Hemicellulose			[145,149,165–172,178,179,272,299]
Sequential extraction in a Soxhlet extractor			Lignin			[149,178,179,272]
Acid hydrolysis			Lignin			[149,178,179,272]
GLC	Vitamin A					[51]
HPLC	Vitamin C and vitamins B_1, B_2, B_3, B_6, B_9			Citric acid	Neochrome, Violaxantine, furanoid, Phytoene, (-Carotene, (-Carotene, (-Carotene, (*Z*)-(-Cry, (-Cry, (-Cry, Lutein, (*13Z*)-violaxantine, (*9Z*)-Violaxantine, Lutheoxantin, (*Z*)-(-citraurin, (-Citraurin	[34,35,51,70,145,152,165–172,183,184,299]
Spectrometry	Vitamin C					[145,165–172,299]
Fluorometric and microfluorometry	Vitamin C					[145,165–172,299]
UV detection	Vitamin C				Neochrome, Violaxantine, furanoid, Phytoene, (-Carotene, (-Carotene, (-Carotene, (*Z*)-(-Cry, (-Cry, (-Cry, Lutein, (*13Z*)-violaxantine, (*9Z*)-Violaxantine, Lutheoxantin, (*Z*)-(-citraurin, (-Citraurin	[34,35,70,145,165–172,184,299]
Refractive index detection	Vitamin C					[145,165–172,299]
HPCE	Vitamin C					[145,165–172,299]
Electrochemical detection	Vitamin C					[145,165–172,299]
Atomic absorption spectrometry		Ca, Cu, Fe, Mg, Mn, P, K, Se, Na, Zn				[173]
Instrumental Neutron Activation Analysis		Na, Zn, Fe				[176,177]
NMR				Citric acid		[178]
Ion chromatography				Citric acid		[178]

Table 7
Listing of rutinary available methods for identifying essential oils in *Citrus limon*.

Techniques	Essential oils					Reference
	Monoterpenes	Oxygenated monoterpenes	Sesquiterpenes	Oxygenated sesquiterpenes	Other oxygenated compounds	
GC and GC–MS	(-Thujene (-Pinene Camphene (-Pinene (-Myrcene (-Phellandrene (-Terpinene Limonene (-3-Carene (-Terpinene Terpinolene Ocimene	*cis*-Sabinene hydrate Linalool *endo*-Fenchol *Cis*-*p*-Ment-2-en-1-ol *Trans*-Limonene oxide Camphor Citronellal Borneol Terpin-4-ol (-Terpineol Nerol Neral Carbone Geraniol Geranial Perilla alcohol	(-Elemene (*E*)-Caryophelene (-*trans*-Bergamotene (-Humulene (*E*)-(-Farnesene (-Curcumene Valencene Bicyclogermacrene (-Muurolene (*Z*)-(-Bisabolene (-Bisabolene (*Z*)-(-Bisabolene (-Cadinene (*E*)-(-Bisabolene	Caryophelene alcohol Germacrene D-4-ol (-Muurolol (-Cadinol (-Bisabolol *Epi*-(-Bisabolol (*E*,*E*)-Farnesol Nootkatone	n-Nonanal Decanal Undecanal Methyl geranate Citronellyl acetate Neryl acetate Geranyl acetate Geranil *n*-propanoate Butyl acetate Dill apiole Citroptene Palmitic acid Linoleic acid *p*-Cymene Citral	[64]
GC-FID	(-Pinene (-Pinene (-Terpinene Limonene Ocimene	Linalool Terpin-4-ol (-Terpineol	β-Lonone	Valencene Nonanool 3-Heptanone	*p*-Cymene Citral	[182]

Summarizing the treated aspects cited here, in relation to the different compounds described in lemon and the analytical techniques for determining them, is given in Tables 6 and 7.

5. *C. limon* and food industry

Lemons are consumed fresh and processed, as juices, jam, jellies, molasses, etc. Chemical industry extracts from lemon bioactive compounds like flavonoids, vitamins, minerals, dietary fiber, essential oils, etc. are used in the food, cosmetic and pharmaceutical industry [185]. A number of agro-industrial by-products or wastes like *Citrus* pulp, meals, seed meals, molasses and peels are generated from fresh *Citrus*, after the main products of interest have been removed or extracted, during processing or peeling [186]. Most of lemon juice industry by-products can be used as functional ingredients, in the development of healthy foods (functional foods). Specially, non-digestible carbohydrates, dietary fiber and bioactive compounds (flavonoids and ascorbic acid) are employed with this objective [7,187].

Lemon fruit can be kept for several months maintaining its levels of juice, vitamins, minerals, fibers and carbohydrates. Both lemon and lemon juices suffer a reduction on the vitamin C content during storage or industrial processes such as pasteurization. Specifically, oxygen, heat light, time, storage temperature and storage time affect the ascorbic acid retention in *Citrus* juices [145,146]. Moreover, since ascorbic acid decomposes easily in acid solutions, lemon juice concentrate (pH 1.82) may show high ascorbic acid destruction [188]. To prevent the loss of ascorbic acid levels and its antioxidant capacity of both lemon and lemon juice, they should be kept at 0–5 °C and protected from water loss by proper packaging, with high relative humidity during distribution. Under these conditions lemon products show a good retention of vitamin C and antioxidant capacity [189].

On the other hand, phenolic compounds are also known to be influenced by processing and storage [68]. Comparing manually or industrially squeezed juices, the compositions of the pressed ones are therefore enriched by the release of components from the albedo and flavedo (e.g. PMFs mainly present in the fruit peels) [7,20,29,111,134]. Indeed, Marín et al. [7] showed that the effect of the system extraction is practically irrelevant regarding acidity, soluble solids or ascorbic acid, although the flavonoid content is strongly affected. In addition, significant differences of the diastereomeric ratios for flavanones are observed between freshly squeezed juices and those from commercial sources [29,31].

Regarding the concentration techniques, it seems that it is not deleterious for the major bioactive flavonoids in *Citrus* fruit [18]. Likewise, pasteurization may produce thermal degradation of different compounds. Nevertheless, Dhuique-Mayer et al. [124] showed that pasteurization does not modify hesperidin content. Therefore, the importance of both sample preparation and processing in analytical methods should not be undervalued.

Vitamins are molecules that have been reported as fundamental to health and well-being, although they are present in a nutritional diet at very small concentrations. An increasing number of nutrition and dietary supplements are enriched with vitamins, many of them, extracted from lemon by the pharmaceutical industry [165].

Nowadays, the beverage market is turning towards hybridization of traditional beverage categories. These hybrids include traditional drinks combined with fruit juices and nutrients that improve their health-promoting characteristics. This tendency has let the development of new products with functional properties. Lemon, by means of its bioactive compounds like those derived from the processed of its by-products, is showing an increasing interest in the food industry. Vitamin C obtained from lemon by-products led to improving the antioxidant activity of new beverages, measured according to the methodologies mentioned above [190].

Lemon peel represents between 50 and 65% of the whole fruit weight, constituting the principal by-product and the main source of environmental pollution. The use of these by-products is conditioned by the lack of a specific industry and the high cost of all industrial procedures involved [191,192]. However, the growing use of this by-product is due to the benefits that can be obtained from lemon's peels after fruit processing. This fact is being kept in mind in order to gain a further insight in the economic profit of lemon's peels, as source of health-promoting compound (oils, pectin, flavonoids, etc.) [37].

Albedo is the part of lemon peel that shows the major content in dietary fiber, and it is used in two forms: raw albedo and dehydrated raw albedo (dried at 50 °C during 48 h). However, dietary fiber is not only desirable for its nutritional properties, but also

for its functional and technological properties, that led to improve the pharmaceutical uses and the cooking yield, reducing formulation cost and enhancing texture of several manufacturer products [193,194]. The most rational use of lemon industry by-products may be considered the production of pectin [195]. Pectin extracted from *Citrus* peel is used in a wide range of food industrial process as gelling agent, including the manufacturing of jam, jellies and as thickener, texturizer, emulsifier and stabilizer in diary products. Moreover, pectin is used in pharmaceutical, dental and cosmetic industries for their jellifying properties [196]. The finding of dietary fiber utility has let to upgrade agricultural by-products as food ingredients [195,197].

The well-known capacity of lemon dietary fiber to increase texture, observed after heating, may be caused by the formation of a solid matrix and constitutes a useful property in enrichment of some foods where viscosity in mouth is desired. Dietary fiber from lemon increases emulsion capacity and is appropriate for several food products, in which shows water and fat binding properties and improve texture [143,198]. The criterion for incorporation of dietary fiber in the manufacture of food products should be related to the desired physical, chemical or sensory characteristics of the particular food [149]. The final powder presents a high water holding capacity, which favours its use as a functional ingredient to modify viscosity and texture of foods [199].

This development in food industry has led to obtaining non-vegetable foods that could help to incorporate the diary intake requirement of fiber by means of the use of lemon by-products in food industry [199]. The presence of bioactive compounds (flavonoids and vitamin C) with antioxidant properties in albedo from lemon peel, converts it in the most interesting candidate as fiber source. These compounds exert higher health-promoting effects than the dietary fiber from other vegetable foods [7,195]. For instance, one application of dietary fiber from lemon has been its addition to cooked and dry-cured sausages. The incorporation of raw and cooked albedo to both cooked and dry-cured sausages produces lower residual nitrite levels, due to the interaction between the nitrites and the bioactive compounds presents in the albedo. The healthy effects derivated of this reduction must be due to a reduction of the possibility of nitrosamines formation [143].

Currently, the main use of the essential oils from lemon is as flavoring in foods, perfumes and pharmaceutical formulations due to their functional properties (antimicrobial, antifungal, etc.) [158,200–202]. Since essential oils show antibiotic and flavoring properties, they have also been used in the elaboration of shampoos, toothpaste, disinfectants, topical ointments and cosmetics [203]. Linalool and citral are the main essential oils from lemon with antimicrobial effects both in direct oil and vapour form [204]. However, the antimicrobial effect induced *in vitro* by essential oils, should be considered with caution in an eventual extrapolation to food products since the presence of fat, carbohydrates, proteins, salt, pH reaction and water activity alter the effectiveness of essential oils as antimicrobial agents in foods compared with that observed *in vitro* [162,200,205]. High concentrations required in food systems to achieve equivalent results than those observed *in vitro*, may induce undesirable organoleptic properties which must be kept in mind when assessing the potential bioactivity of lemon essential oils [206]. The use of lemon essential oils, linalool and citral, in seafood is especially interesting due to the short shelf-life of this kind of food. Furthermore, lemon essential oils show utility in the production of some types of cheese because they reduce significantly the population of microorganisms, especially *Enterobacteriaceae* [207].

Lemon juice industry also constitutes an important source of citric acid that can be used effectively in the treatment of heavy metal ions from aqueous solutions [163]. Moreover, it is a natural flavoring and preservative and it is also used to add an acidic, or sour, taste to foods and soft drinks [51]. Even more, citric acid solution added to samples of olive oil during its production increases the oxidant stability of oils significantly. This effect has been shown from bergamots, however, the addition of lemon fruits does not exert the same effect [208].

Another industrial application of lemon is the liquor's elaboration ("limoncello") by means of maceration of lemon peels in ethanol, water and sugar, lemons being the most suitable for making limoncello free from pesticide residues. This beverage has raised an evident interest in order to make further use of lemon [209].

In an attempt to look for a further use for lemon cultures, leaves, peel and pulp derived from *C. limon* used directly for consumption in human diet have been found as useful post-harvest waste products that can be used to feed animals, although the growing use of pesticides, food additives and veterinary drugs as residual agricultural chemical in *Citrus*, may constitute a handicap in their use in animal feed [168]. The treatment with pesticides may cause not only toxicity for potential consumers (humans or animals), but also induce changes in the essential oils composition as an effect on plant metabolism. The criteria for the use of lemon fruits and its by-products for fresh commercialization are different to those for processing. Whereas fruit size and color is critical for its fresh commercialization, for processing, soluble solids, juice, pectin and essential oils content are the most relevant characteristics [50].

6. *C. limon* and health

The beneficial effects of the dietary *Citrus* fruits can be attributed, not only to the vitamin C, minerals, dietary fiber, essential oils, organic acids and carotenoids, but also to the antioxidant activity of their flavonoids. This property is linked particularly to the chemical structures of these flavonoids [9–11]. Three structural groups are important for the evaluation of their antioxidant capacity: the ortho-dihydroxy (catechol) structure in the B-ring, the 2,3-double bond in conjugation with a 4-oxo function and the presence of both 3-(a)- and 5-(b)-hydroxyl groups. Indeed, vitamin C and flavonoids are, in part, synergistic regarding its biological activities (Fig. 1) [24]. A diet rich in grain, legumes, vegetables and fresh fruit, such as *Citrus* fruits and juices, has beneficial effects on human health [13,16,17,21,23].

6.1. Bioavailability of lemon compounds

A much greater number of bioactive natural products are ingested by human consumption as foods or dietary supplements (functional foods and nutraceuticals). Understanding the bioavailability, transport and metabolism of *Citrus* flavonoids after consumption of *Citrus* fruits is a prerequisite for understanding the mechanisms of their protective effects in humans. Nevertheless, it is noteworthy that the metabolic fate could be different depending on the individual human, thereby their health-related properties would be affected by the bioavailability of flavonoids as was seen by Silberberg et al. [210].

Most of the polyphenol *O*-glucosides undergo intestinal hydrolysis to release the respective aglycons by media of UDP-glucuronosyltransferase found in the intestine mucous membrane intestinal [24,211]. Because the aglycons and their metabolites are more hydrophobic, they are more efficiently transported across the wall of the gastrointestinal tract than their respective glucosides. They are both converted in the gut wall and the liver, as well as at peripheral tissue sites into phase I and phase II metabolites [212]. Then, a glucuronidation/sulfation (to form β-glucuronides) as well as methylation reactions is precise to flavonoid metabolism and absorption [24,119]. In addition, studies of Serra et al. [213], on the ionization constants and the bidirectional permeability

Table 8
Listing of health-promoting activity of minerals and vitamins present in *Citrus limon*.

Minerals	Health-promoting activity
Calcium	It is the main constituent of bones and teeth and it has key metabolic functions.
Copper	It is a trace element that is essential for human health.
Iron	It is an essential part of haemoglobin.
Magnesium	It is essential in muscle contraction
Manganese	It activates metalo-enzymatic complex in fat metabolism
Phosphorus	It is a part of DNA materials and they take part in energy distribution.
Potassium	It plays an important role in the physical fluid system of humans and it assists nerve functions.
Selenium	Selenium shows an antioxidant role
Sodium	It is necessary for humans to maintain the balance of the physical fluids system. It is also required for nerve and muscle functioning.
Zinc	It is essential for human health.
Vitamins	
Vitamin A (retinol)	Development of bone, skin and eyesight. Regulation of the expression of growth hormone.
Vitamin B_1 (thiamin)	Nervous system, heart and brain maintenance. Cofactor in gastrointestinal and muscular functions.
Vitamin B_2 (riboflavin)	Coenzyme in oxidation and reduction reactions.
Vitamin B_3 (niacin)	Systemic metabolism
Vitamin B_6 (piridoxina)	Component of haemoglobin. It regulates metabolism, digestion and fluid balance.
Vitamin B_9 (folic acid)	It plays a role in the metabolism of nucleic acid and amino acid. It is essential for development and nervous system functioning.
Vitamin C (ascorbic acid)	Formation of collagen and connective tissue. Absorption of inorganic iron. Antioxidant properties.

using the cellular model Caco-2 of FG (diosmin, hesperidin and naringin) and their aglycones (FA), showed that aglycones are ionized at physiological pH whereas glycosides are in the neutral form, and permeation was only detected for the aglycones confirming the need for metabolism before absorption through the intestinal microvilli [213].

Previous studies on oral flavonoids glycosides and their aglycones support that hesperidin and hesperetin may be metabolized into hesperetin glucuronides/sulfatoes after oral administration [125,214]. Eriocitrin is metabolized to eriodictyol (the aglycone) in the intestinal flora and it is metabolized to homoeriodictyol and hesperetin through methoxylation and finally to 3,4-dihydroxycinnamic acid (3,4-DHCA) and phloroglucinol by human intestinal bacteria [118,119,211,215,216].

After ingestion of FGs and flavonone aglycosides (FAs) in humans, hesperetin and eriodictyol, the flavanone aglycones, as well as its metabolites were detected in both human urine and plasma as the glucuro- and/or sulfo-conjugates, also suggesting that eriodictyol undergoes extensive phase II metabolism [217] and in minor extent in the unconjugated form (e.g. diosmetin) [83]. Most of the absorbed *Citrus* flavanones undergo glucuronidation before urinary excretion [83,84]. Nevertheless, different kinetics have been reported depending on the phenolic compound [218].

Analysis of biological fluids, mainly flavanone and flavone and their metabolites in both plasma and urine samples, have been carried out similarly to the analysis described above varying only the sample preparation: the same ODS columns with HPLC and UV [217] or electrochemical detection (750 mV) [211], etc.

Vitamin C, which is also apported by lemon fruit in large quantities, is part of glucose metabolism. This vitamin is not accessible to humans since they lack L-gulonolactone oxides, the last enzyme in the biosynthetic pathway [219]. The intake of vitamin C reduces the mortality, fundamentally by cardiovascular disease prevention [220]. The antioxidant action from vitamin C that is greater than that of vitamin E or β-carotene has been considered the mechanism responsible for the vascular protection [221,222] (Table 9). Furthermore, at the present date, the interest in vitamin C is focused on its ability to prevent the cell damage done by "free radical" molecule as they oxidize protein, fatty acids and DNA [55,56]. Vitamin C, by means of its antioxidant activity, may help to prevent cardiovascular diseases and arteriosclerosis [221]. Plasma levels of L-AA in large sections of the population are suboptimal for the protective health effects of this vitamin. For adults, the dietary intake is constituted of 60 mg per day, and the recommended dietary allowance (RDA) of vitamin C is 75–90 mg per day (smokers need a supplement of 50% of this vitamin) [168].

Lemon fruit constitutes, in addition, a rich source of minerals necessary for health care and maintenance. Concretely, in lemon composition, two groups of minerals are clearly separated, the main elements (K, Ca, Mg and P) and those present in trace levels (Na, Fe, Zn, Cu, Mn and Se). Minerals belonging to the first group, which are essential for human health must be consumed in amounts of >20 mg/day. The second group is formed by elements which are essential in lower daily intake to perform a wide spectrum of physiological functions [223]. Availability of trace elements to the body depends on their chemical form and absorption [176]. A relation of the minerals present in lemon and its health-related properties is showed in Table 8.

Regarding the determination of phenolic metabolites in biological fluids in bioavailability studies with human subjects, the samples of plasma, biliary, and urinary excretions, are analysed either directly or after enzymatic hydrolysis in order to release the flavonoid aglycones and its metabolites from the glucuronic acid and sulfate conjugates, by using glucuronidases and sulfatases generally at 37 °C (during 1 h, at a low pH, e.g. pH 4.5 with an acetate buffer), and sometimes in the presence of ascorbic acid [214], and adding oxalic acid at the end [116,211].

6.2. Lemon and health benefits

Health effects of polyphenols depend on the amount consumed and on their bioavailability. Research on polyphenol bioavailability allows us to establish correlations between polyphenol intakes with one or several accurate measures of bioavailability (such as concentrations of key bioactive metabolites in plasma, urine and tissues) and with potential health effects in epidemiological studies.

6.2.1. Cancer

Recently, the influence of *Citrus* flavonoids on cancer has been revised [12]. *Citrus* flavonoids are considered quite safe and non-toxic drugs. At least 35% of all cancers worldwide are caused by an incorrect diet, and in the case of colon cancer, diet may account for 80% of the cases [16]. *Citrus* flavonoids act as modulators of tyrosine kinases, are particularly important today because of the implications in the treatment of cancer. Eriocitrin (from lemon juices and its metabolites) was tested in different *Citrus* juices by Ogata et al. [215]. This work showed that eriocitrin induced apoptosis in HL-60 cells [215]. Moreover, hesperidin in different *Citrus* juices showed antiproliferative activity [108,224] (Table 9).

Miller et al. [225], studied the inhibition of oral carcinogenesis by *Citrus* flavonoids in hamsters and the antineoplastic activity, concluding that hesperetin, neohesperetin, tangeretin and nobiletin

Table 9
Listing references of experimental data support of the health-promoting activity of lemon and its bioactive compounds.

Therapeutic activity	Experimental model		
	In vitro models	*In vivo* models	Clinical trials
Health-promoting activity	[16,17,21]	[16]	[35,239,268]
Cancer	[16,110,215,227,229,231,236]	[12,224–226,228,230,232–234,237,265]	[221,238,239,247,264]
Cardiovascular, coronary heart disease and oxidative damage	[48,77,283,287–290]	[291]	[14,56,220–222,244,284,285,300]
Lipid metabolism and obesity	[248,250]	[15,246,247]	
Gastrointestinal diseases		[257–263]	[245,251]
Diabetes			[255,256]
Antimicrobial activity	[270,278–281]	[203]	
Urinary diseases			[51]
Psychiatric diseases			[274–277]
Bone protection	[273]		

were ineffective, while naringin and naringenin gave good results. Synthetic hesperidin and diosmin were effective as chemopreventive agents in urinary-bladder carcinogenesis [226]. Lemon showed relatively potent antiproliferative activities on HepG2 human liver-cancer cell growth in a dose-dependent manner [227] (Table 9).

Other studies showed eriocitrin as an inhibitory compound acting against both rat platelet 5- and 12-lipoxygenases, and its aglycone, eriodictyol, was a much more potent inhibitor of such lipoxygenases, which are involved in the biosynthesis of various bioregulators that are closely related to the pathogenesis of several diseases such as allergy and atherosclerosis and cancer [74]. Indeed, the type of glycosilation also exhibits a certain bioactive activity proving that rutinosides group attenuates the apoptosis-inducing activity of flavonoids by the activation of caspase-3 [110]. On the other hand, quercetin and rutin appear to inhibit colonic neoplasia induced by azoxymethanol [228].

Strong antioxidant power to the polymethoxylated flavones which inhibit the transport of talinolol in a Caco-2 cell monolayer, and have other health-related properties (e.g. anti-inflammatory, anti-bacterial activities, etc.) mainly due to their chemical structure has been reported [229,230]. Nevertheless, since its content is negligible in the lemon fruit we consider them in low attention (Table 9).

It is currently accepted that essential oils could be used to protect body organs against carcinogenesis. Exploration of citral as a cancer chemopreventive agent targeted towards inflammation-related carcinogenesis such as skin cancer and colon cancer has been reported [231]. Citral also increased the hepatic glutathione-S-transferase (GST) and aminopyrine demethylase activities, and reduced glucuronyl transferase activity [232]. Although citral is generally recognized as safe for human consumption as a food additive, it is classified as a potential teratogen and primary irritant [233]. Other essential oils from lemon elicit hepatoprotective activity due to their phenolic and/or monoterpene [234]. In addition, D-limonene exhibits chemopreventive efficacy in preclinical hepatocellular carcinoma models [235,236]. The mode of action originates, in part, from the induction of the specific cytochrome P450 isozymes [237]. D-limonene is also a candidate for the chemoprevention of skin cancer, although it can cause contact dermatitis, especially when oxidized [238] (Table 9).

Moreover, this cancer suppressive activity of essential oils observed *in vivo* is supported by a number of *in vitro* assays in which all their components have been tested on several human cancer cell lines. D-limonene showed antiangiogenic and proapoptotic effects on human gastric cancer implanted in nude mice, thus inhibiting tumor growth and metastasis and can also induce the formation of apoptotic bodies in gastric cancer cells. This compound may contribute in the chemoprevention and/or therapy of chemically induced human solid tumor cells across its activity as enzyme inhibitor [236].

Beneficial effect of the dietary fiber to the colon cancer has also been reported [239–241]. Vitamin C, by means of its antioxidant activity, may also help to prevent cancer [221] (Table 9).

6.2.2. Cardiovascular and coronary heart diseases (CVD and CHD) and oxidative damage

There are clinical studies about the effect of lemon juice on blood pressure in treatment of hypertension [242]. El-Shafae et al. [77], reported that hesperidin and diosmin could be effective for the treatment of chronic venous insufficiency, chronic haemorrhoids and venous leg ulcer. Vicenin-2 and 6,8-di-*C*-glucosyldiosmetin showed a suppressive effect on the expression of blood adhesion molecules [48].

Defects in coagulation, as thrombosis are closely related with atherosclerosis in the development of cardiovascular diseases by which their prevention becomes as critical to reduce cardiovascular episodes with dramatic ending [243]. The use of lemon extracts to prevent these clinical entities constitutes a response to the side effects from agents traditionally used with this purpose, including gastric erosion and systemic haemorrhages [244]. However, essential oils from lemon have not been related with this activity yet.

Epidemiological research has demonstrated the relationship between the absence of dietary fiber intake and the development of cardiovascular diseases and several other disorders [239].

On the other hand, soluble dietary fiber is related with a reduction in cholesterol absorption, due to increased excretion of cholesterol and bile acids [245].

6.2.3. Lipid metabolism and obesity

Both eriocitrin and hesperetin metabolites played an important role in plasma, serum and hepatic lipids, with lipid-lowering activities *in vivo* in high-cholesterol fed rats [15,246]. Recently, other studies reveal that polymethoxylated flavones (PMF, i.e. nobiletin) reduced plasma cholesterol levels at lower doses than required for flavanones (i.e. hesperetin), explaining the hepatic mechanisms that underlie this differential potency [247,248].

Recent studies showed an interaction between nutritional elements and receptional sites, such that led to the birth of nutritional biotherapy that selects the foods for the treatment of several pathologies [249]. In order to gain a further insight in the activity of lemon compounds, in lipidic metabolism, several assays have been performed. The oral administration of lemon pectin to rats reduces and delays the peak plasma triacylglycerol concentration. Pectin inhibits the hydrolysis of trileoyglycerol emulsified with soybean phosphatidylcholine by pancreatic, carboxylester, and lingual lipases in a concentration-dependent manner. Results showed in previous reports indicated that pectin might interact with emulsified substrates and inhibit the adsorption of lipase to the surface of substrate emulsion [250]. Citric acid and related compounds from

lemon are functional foods driving thermogenesis and reducing obesity risk [249].

6.2.4. Activity against other diseases

Both dietary soluble fiber and insoluble fiber show several beneficial effects on human health. Insoluble dietary fiber has a beneficial effect related to intestinal motility. Consumption of this fiber type delays gastric emptying slowing the absorption process in the small intestine. The above mentioned delay in gastric emptying, reduces the energy absorption, promotes satiety, prevents a surge in blood glucose levels and interferes with the reabsorption of bile acids that reduce plasma cholesterol levels [239,251–254]. Moreover, soluble dietary fiber could delay the absorption of macronutrients, including fat and carbohydrates, which could lead to increase insulin sensitivity [255] triacylglycerol concentration [256] (Table 9).

From the histological point of view, pectin is a soluble fiber that has been reported to have a trophic effect to the intestine, increasing villus weight and crypt depth in the small intestine [257,258]. Pectin stimulates intestinal cell proliferation, the activity of brush border membrane enzymes and the short-chain fatty acid production in the cecum [259,260]. Pectin feeding has been found to increase the levels of plasma enteroglucagon [259], which constitutes a humoral factor for ileal mucosal growth [261], and of plasma glucagon-like peptide-2 [260] that promotes cell proliferation in the gastrointestinal tract [262]. All these changes are closely related with modification in protein metabolism in this tissue, increasing energy and protein requirements and reducing peripheral nutrient availability [263] (Table 9).

By-products that derivate from the lemon industry attract an increasing interest as a source of dietary fiber, which play an essential role in the prevention of digestive diseases, such as constipation, haemorrhoids, hypercholesterolemia and colorectal cancer [143,264–266].

C. limon, is very rich in citric acid and efficient to prevent the metabolic pathologies [249]. Knowledge of citric acid content of beverages may be useful in nutrition therapy for calcium urolithiasis, especially for patients with hypocitraturia. The intake of beverages containing citric acid, like lemon juice, increase the total volume of urine, reducing the saturation of calcium and other crystals, and may enhance urinary citrate excretion [51]. Moreover, beverages containing lemon citric acid are useful for people who frequently feel fatigue, since its administration attenuates the fatigue feeling [267,268]. Finally, citric acid is useful in therapy for calcium urolithiasis, since citrate is a naturally occurring inhibitor of urinary crystallization [51] (Table 9).

Li et al. [269], reported that hesperidin may have a therapeutic value for the clinical treatment of rheumatoid arthritis (studies in rats). Also lemon juice possesses anti-bacterial activity [270]. In addition, naringenin and hesperidin [83] and *C. limon* [271] have also been reported as inhibitors of selected cytochrome P450, as well as P450 3A4 (CYP3A4), enzymes resulting in drug interactions to obtain increased oral bioavailability of drugs [126,272].

Hesperidin also shows bone-sparing effects in ovariectomised (OVX) animals inducing bone protection [273] (Table 9).

Aromatherapy constitutes a "nice" line or treatment of behaviour and psychological symptoms in dementia, as an alternative to the traditional use of antipsychotics. The former constitutes a safer alternative to treat this clinical presentation, compared with traditional alternatives. The most common mode of application is by massage onto skin, which reduces pain and tension, increases circulation and aids relaxation [274]. In dementia, essential oils promote sleep [275], increasing motivational behaviour and improving disturbed behaviour [276]. Although exact mechanism of essential oils action remains unknown, it is considered that the pleasant odour and the volatile constituents exert both physiological and psychological effects respectively. Physiological effect is due to the absorption of the essential oils components through the skin and/or respiratory system [277] (Table 9).

Several components of lemon are responsible for the different targets in the infectious entities [203]. Essential oils can be used as antimicrobial and antifungal agents [158,200–202]. The antimicrobial effect of essential oils may be considered with care, because although, essential oils may constitute a good alternative to combat the increasing resistance pathogens [278], they could also produce an imbalance in gut microflora [279]. Essential oils also show antiviral activity which constitutes an interesting alternative to the antiviral drugs because of their weaker toxicity [280,281]. The main lemon essential oil with antiviral activity is citral [282].

Increased concentrations of oxidative modified low density lipoproteins (LDLs) in cholesterol play a substantial role in atherosclerosis disease initiation [283–286]. The increase of the antioxidant intake in diet daily could help to prevent atherosclerosis, by a reduction in the LDLs oxidation. Essential oils from lemon prevent the LDL oxidation and are able to reduce the plasmatic cholesterol and triglycerides. The mechanism by which essential oils reduce LDL oxidation is by avoiding the oxidation of intrinsic carotenoids of LDL [287,288]. Compounds present in lemon peel have been described to prevent LDL oxidation and to reduce plaque formation, preventing the risk of cardiovascular disease (CVD) [289,290] (Table 9).

Recent reports also show a hepatoprotective activity of essential oils from lemon. Their protective effect on experimental liver damage induced by carbon tetrachloride, and the ethyl acetate soluble fraction of the extract was evaluated on HepG2 cell line. This experimental approach led to demonstrate that ethanol extract from lemon normalized the levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total and direct bilirubin, which were altered due to carbon tetrachloride intoxication in rats. In the liver tissue, treatment significantly reduced the levels of malondialdehyde, hence the lipid peroxidation, and raised the levels of the antioxidant enzymes superoxide dismutase and catalase [291] (Table 9).

6.3. Nutrigenomics

Dietary phytochemicals may alter gene expression in a different way in different subject. This is the principal guiding nutrigenomic approaches to understand the influence of diet on health on an individual basis. Genes and nutritional genomics offer a futuristic way to optimize human health and well-being through designed foods. Bioactive components of the diet may regulate gene expression at the transcriptome, protein abundance and the protein turnover levels [292]. Current publications are growing evidences that diet flavonoids have a role in the activation state of target molecules and/or the modulating of gene expression [293]. Concretely, the regulation of the expression of the gene codifying for the low density lipoprotein receptor (LDLR) expression by *Citrus* flavonoids has been reported [248]. Hesperetin, hesperidin and naringenin have been described as modulators of the expression of several genes (NFkB, microsomal cytochrome P450 A1 and COX 2) [294–296]. However, although no further evidences exist on the implication of lemon in nutrigenomic therapies, the presence of bioactive compounds in its composition (i.e. hesperetin, hesperidin, and naringenin), actively related to the modulation of the gene expression, may point to this characteristic biological effect.

Acknowledgements

EGM wishes to thank the CSIC-I3P Fellowship Programme, partially funded by the European Social Funds (ESF). RDP was sup-

ported by a contract from the Spanish Ministry of Science and Innovation (MICINN) under a PETRI Research Project (PET2006-0305) and a CSIC-Project (200870I070). The authors also wish to thank the C.I.C.Y.T. for the financial support (AGL2007–61694/ALI).

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