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Manipulation of fatty acid profile and nutritional quality of *Chlorella vulgaris* by supplementing with citrus peel fatty acid

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Microalgae could be an excellent resource of functional and essential fatty acids. To achieve viable microalgal biomass production, mass cultivation of microalgae is required; however, the high cost of nutrients is the obstacle. An inexpensive and nutritious material is required to feed *Chlorella vulgaris* in the pharmaceutical and food sectors. Citrus peel waste with a valuable nutritional quality could be one of the promising and inexpensive candidates. In this study, the fatty acid extract from different citrus peels was used as the organic nutrient source for the cultivation of *Chlorella*. The proximate composition of bitter orange, sweet orange, grapefruit, and mandarin peels were determined, and their nutritional quality was evaluated. Total fatty acids from the citrus peel were prepared by acidic methanol hydrolysis and hexane extraction. Fourier transforms infrared (FT-IR) and gas chromatography–mass spectrometry (GC–MS) was used to analyze the fatty acid composition and nutrient composition. Fatty acids from the citrus peels were added to the *Chlorella* culture medium to study their influences on biomass, lipid production, fatty acid profile, and nutritional quality of *Chlorella*. The most predominant citrus peel fatty acids were linoleic, palmitic, oleic, linolenic, and stearic acids. The citrus peels contain polyunsaturated, saturated, and monounsaturated fatty acids. The most unsaturated fatty acids were omega-6, omega-3, omega-9, and omega-7. The citrus peel had acceptable atherogenicity, thrombogenicity, omega-6/omega-3, peroxidizability, hypocholesterolemic, and nutritive value indices. The major fatty acids of *Chlorella* were palmitic, linoleic, oleic, alpha-linolenic, gamma-linolenic, 4,7,10,13-hexadecatetraenoic, palmitoleic, 7,10-hexadecadienoic, 7,10,13-hexadecatrienoic, lauric and 5,8,11,14,17-eicosapentaenoic acids. *Chlorella* contains polyunsaturated, saturated, and monounsaturated fatty acids. The most unsaturated fatty acids contain omega-6, omega-3, omega-9, and omega-7. *Chlorella* had acceptable atherogenicity, thrombogenicity, omega-6/omega-3, hypocholesterolemic, peroxidizability, and nutritive value indices. Supplementation of *Chlorella* with citrus peels fatty acid increases total biomass, lipid content, and nutritional quality of *Chlorella*. The present research shows that citrus peels have good nutritional quality and could be used for the inexpensive cultivation of *Chlorella* biomass with potential utility for food application.

Citrus is one of the most extensively grown horticultural fruits. The sweet orange, bitter orange, lemon, lime, grapefruit, and mandarin are the most extensively farmed and industrially important citrus fruits globally in the agro-industrial area. Citrus fruit may be used to make fresh juice, citrus-based drinks, and jelly, in addition to being consumed. Juice for beverages, jellies, marmalades, potpourris, jams, candied peel, flavoring mediator for beverages, oils and essences, fiber, and pectin for food components are the chief goods of the citrus processing industry¹. Citrus fruit is mostly made up of pulp (71%), peel (27%), and seeds (2.0%). Pectin (21%), citric acid (16%), pentosans (15%), fiber (11%), glucoside (10%), minerals (10%), proteins (9.0%), essential oils (5.0%), and lipids (3.0%) make up the dry matter². The derivative of the citrus processing industry is the citrus peel waste that accounts for 50–60% of the citrus processed, depending on the citrus cultivar. Peels (50%), internal tissue

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(30%), and seeds (10%) make up citrus peel waste with non-nutrient components like essential oil and nutrients like amino acids and fatty acids³. Citrus peel waste is now utilized primarily as animal feedstuff, an organic soil conditioner, a composting substrate, and bioethanol and biomethanol production substrate. Citrus peel waste has a high added value and may be used to make pectin, dietary fibers, protein, and fatty acids in the food sector. Citrus peel waste might potentially be used to extract flavonoids, favorable agents, and citric acid in the cosmetic and pharmaceutical sectors⁴. The macronutrient composition and profile of fatty acids, on the other hand, have been investigated to a lesser extent. Citrus peel waste offers enough nutrients for microalgae culture to enhance lipid and fatty acid content in the microalgae.

Microalgae are suitable for the food industry due to their quick growth rate and are used as a primary source of lipid and protein⁵. Proteins, lipids, carbohydrates, vitamins, pigments, and carotenoids are all in microalgae, the potential resource of biological constituents⁶. Microalgae lipid have also been shown to be harmless for digestion in numerous human and animal investigations, and it has been linked to favorable health outcomes such as decreased blood glucose, blood pressure, and cholesterol⁷. Microalgae are an excellent resource of proteins, and essential and non-essential amino acids, lipids, sugars, essential fatty acids, vitamin precursors, and mineral deposits, as well as organic acid, terpenoids, alkaloids, steroids, and phenolic compounds, all of which have been used as therapeutic foods to prevent hypertension, hypercholesterolemia, atherosclerosis, and diabetes mellitus⁸. Various studies reported that salinity and light intensity could improve lipid accumulation and manipulate fatty acid profiles in the microalgae⁹. Besides the light intensity, a nutritious and affordable substance for microalgae feeding is required to employ *Chlorella* in the food and pharmacological industries. These objectives can be met by feeding microalgae with nutritive ingredients and promoting fatty acids¹⁰. An inexpensive and nutritious material is required to feed *Chlorella* in the pharmaceutical and food sectors and manufacture biofuels to encourage the synthesis of lipids and fatty acids for this purpose¹¹. Citrus peel waste with a valuable nutritional quality could be one of the promising and inexpensive candidates. But the direct use of citrus peel has several limitations to microalgae culture due to antinutrient materials in the citrus peels. Partial extraction of fatty acid reduces antinutrients content and overcomes this problem¹².

Accordingly, in this research, the fatty acid extract from different citrus peels was used as the organic nutrient source for cultivating *Chlorella*. The nutritional quality of extracted fatty acids from bitter orange (*Citrus aurantium*), sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), and mandarin (*Citrus reticulata*) peel waste and the stimulatory effects of their fatty acids on the manipulation of fatty acid composition and nutritional quality of *Chlorella* are presented. The macronutrient composition, fatty acid profile, and lipid nutrition quality of citrus peel waste and *Chlorella* supplemented with fatty acid from citrus peel have been explored in this research for the first time so far.

Materials and methods

The bitter orange, sweet orange, grapefruit, and mandarin were grown at Fars Research Center for Agriculture and Natural Resources (Jahrom, Fars, Iran). Fresh peels from ripped, harvested fruits were got from randomly chosen, healthy trees in December. Jahrom Agricultural and Natural Resources Research Station are allocated at an altitude of 1070 m with the longitude of 53°, 37', 33", and a latitude of 28°, 30', 48". Jahrom Agricultural and Natural Resources Research Station confirmed all the source and batch number details. A voucher specimen has been deposited at the Herbarium of the Agricultural and Natural Resources Research Station. The characteristics of citrus trees in this research station were recorded in the book of Citrus cultivars of Jahrom Agricultural Research Station¹³. Experimental research and field studies on plants, including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation.

The peel was removed from the fruit and dried at room temperature before being crushed in a home grinder. The standardized protocols proposed in the literature were used to evaluate moisture content, ash content, total carbohydrate, total protein, total lipid, protein digestibility, and total energy of microalgae by following the Association of Official Analytical standard procedures Chemists method¹⁴. Fourier transforms infrared (FT-IR) spectroscopy in the range of 4000–400 cm⁻¹, done with a Bruker FTIR spectrophotometer (Germany), was used to characterize the chemical components of citrus peels and *Chlorella* supplemented with citrus peel.

Fatty acids preparation and profiling. Lipids from citrus peel powder (1.0 g) were hydrolyzed and esterified with 5.0 ml of acidic methanol (methanol: sulfuric acid 80:20, v/v) in a shaking water bath for 120 min at 80 °C. At that time, 4.0 mL of normal saline was added to the tubes and vortexed for 5.0 min. Then, 4.0 mL of hexane was added to the tubes and vortexed for 5.0 min, and centrifuged at 3000g for 20 min in the next step. The upper hexane phase was extracted and transferred to gas chromatography vials for fatty acid methyl ester analysis. GC–MS analysis was performed using Agilent (Agilent 7890B GC 7955A MSD) device equipped with a silica capillary column (HP-5MS (30 m × 0.25 mm id; thickness 0.25 μm)), coupled with a quadrupole mass spectrometer according to the standardized method that is described elsewhere in detail¹⁵. For the preparation of free fatty acid, we used acid hydrolysis (without methanol) with the same procedure, followed by the addition of normal saline for better extraction of fatty acid and then hexane extraction. After hexane evaporation, the free fatty acid extract was added to the microalgae culture medium.

Lipid nutritional quality index. Total saturated fatty acids (SFA), unsaturated fatty acids (UFA), polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), omega-6/omega-3 ratio, PUFA/SFA (P/S), hypocholesterolemic index (HI), atherogenicity index (AI), thrombogenicity index (TI), peroxidizability index (PI), and nutritive value index (NVI) were calculated according to the formula described in the literature¹⁶.

Microalgal culture and treatment. *Chlorella vulgaris* (IBRC: M50026) was obtained from the Iranian Biological Resources Center (IBRC) (Tehran, Iran). *Chlorella* was isolated using the agar plate procedure, then purified in Bold's Basal Media (BBM) before being transferred to the same liquid medium. The *Chlorella* cells were transferred to BBM liquid medium, bubbled, and maintained at 28 °C with an initial pH of 6.8 and 2500 E m⁻² s⁻¹ light intensity to raise biomass. A mechanical pump aerated the culture after it passed through a 0.22 m filter. The microalgal cells were supplied with fatty acid (5.0 g/1000 ml) at the logarithmic phase (after 12 days), and the cultures were incubated for 4 days¹¹. The biomasses from supplemented microalgal cultures were collected by centrifugation at the end of the stationary phase (after 17–18 days). It was washed with deionized water and lyophilized to remove the associated residues. The dry biomass was used to analysis of macronutrients and fatty acid composition¹⁴. The standardized protocols proposed in the literature were used to extract and profile fatty acids¹⁵.

Statistical analysis. The results are presented as averages with standard deviations from three replicates. Through SPSS software version 16, experimental data were evaluated as a completely randomized design using one-way analysis of variance (ANOVA) followed by Tukey post-hoc testing ($P \leq 5\%$). (SPSS Inc., Chicago, IL, USA). To investigate the apparent difference in the distribution of the components between the samples, the matrix of fatty acid composition and nutritional value data in the samples was evaluated for principal component analysis (PCA) using Minitab software (version 20.1.2).

Ethics approval. There were no human subjects or animal experiments in our study.

Results and discussions

Citrus peel proximate analysis. The typical FTIR spectrum of bitter orange, sweet orange, grapefruit, or mandarin peels is shown in Fig. 1. The 3700–3100 cm⁻¹ bands are associated with stretching vibrations of OH groups in water or hydrogen-bonded OH. Peaks in the 2900–2700 cm⁻¹ region are associated with the stretching vibrations of lipid and fatty acid CH, CH₂, and CH₃ groups. The stretching of C=O amides, C=C aromatics, N–H amines, or carboxyl groups in proteins is represented by the bands in the 1300–1100 cm⁻¹ region. Polysaccharide vibrations, including symmetric stretching of C–O–C and OH groups, cause the band at 1100–900 cm⁻¹. The bands at 900–500 cm⁻¹ are due to the vibration of terpenes and terpenoids¹⁷. According to FTIR analyses, citrus peels have a high concentration of carbohydrates (1100–900 cm⁻¹) and a lower concentration of protein, fatty acids, and terpenes (Fig. 1).

The macronutrient analysis also confirmed that the tested citrus peels have a large carbohydrate and lesser protein, lipid, and fatty acid (Table 1). The macronutrient composition of bitter orange, sweet orange, grapefruit, and mandarin is similar, although there are statistically significant differences ($P \leq 5\%$). Carbohydrates are the major macronutrient in citrus peels that make them to have high energy value, but the most abundant carbohydrates are fibers (pectin) that cannot be digested¹⁸. Bitter orange and grapefruit have the similar dry matter, carbohydrate, and fat content, while sweet orange and mandarin consider protein, moisture, ash contents, and digestibility (Table 1 and Fig. S1 in the supplemental file).

*Citrus maxima*¹⁹ and *citrus natsudaidai*²⁰ peels have chemical compositions similar to our findings. Citrus peel waste contains polysaccharides (cellulose and pectin), monosaccharides (sucrose, fructose, glucose, galactose), organic acid (citric, succinic, malic, tartaric, oxalic), fatty acid (palmitic, oleic, linoleic, linolenic, and stearic), and minerals (nitrogen, calcium, magnesium, potassium)²¹. Citrus peel waste also contains volatile constituents (alcohols, ester, aldehydes, ketone, hydrocarbon), flavonoids (flavanones, anthocyanins, flavones), essential oil (limonene and linalool), limonoids (limonin), enzymes (pectin esterase, peroxidase, phosphatase), carotenoids (carotene, lutein), polyphenolic constituents, nitrogen compounds (nitrate, ammonia), and vitamin (B groups vitamins, ascorbic acid)²². All of them make citrus peels, the raw and inexpensive materials for food-stuff, ingredients, and flavoring.

The fatty acid profile of the citrus peel. The typical FTIR patterns of fatty acid extracts of bitter orange, sweet orange, grapefruit, and mandarin peels are shown in Fig. 1. The strong peaks in the 2900–2700 cm⁻¹ region are associated with the stretching vibrations of lipid and fatty acid CH, CH₂, and CH₃ groups. The C=C stretching of unsaturated fatty acids and C=O of carboxyl groups can be related to the variations in FTIR patterns observed in the 1900–1500 cm⁻¹ region. The weak bands in the 1200–1000 cm⁻¹ might be related to lipophilic volatile hydrocarbon and terpenes¹⁷. There were no proteins and carbohydrates in prepared extracts of fatty acids.

The fatty acid composition of bitter orange, sweet orange, grapefruit, and mandarin peels are stated in Table 2. The most predominant components in the citrus oil were linoleic acid, palmitic acid, oleic acid, alpha-linolenic acid, stearic acid, palmitoleic acid, and myristic acid. The fatty acid composition of bitter orange, sweet orange, grapefruit, and mandarin remains similar, as there are some statistically significant differences, especially in the oleic acid and alpha-linolenic acid content ($P \leq 5\%$). Grapefruit had the highest oleic acid and alpha-linolenic acid and sweet orange the lowest, while mandarin had significantly the lowest oleic acid compared to all other citrus peels ($P \leq 5\%$). The contents of arachidic acid, heptadecanoic acid, docosadienoic acid, palmitic acid, tridecanoic acid, stearic acid, myristic acid, decanoic acid, and pentadecanoic acid were similar in sweet orange and mandarin ($P \leq 5\%$). At the same time, bitter orange and grapefruit peel fatty acid compositions were similar considering percentages of oleic acid, hexadecadienoic acid (C16:2n-6), gamma-linolenic acid, palmitoleic acid, and dodecanoic acid (Table 2 and Fig. S2 in the supplemental file).

Generally, the major fatty acids in bitter orange, sweet orange, grapefruit, and mandarin peels were linoleic, palmitic, oleic, linolenic, stearic, palmitoleic, and myristic acids. Fatty acid composition from *Citrus maxima*¹⁹

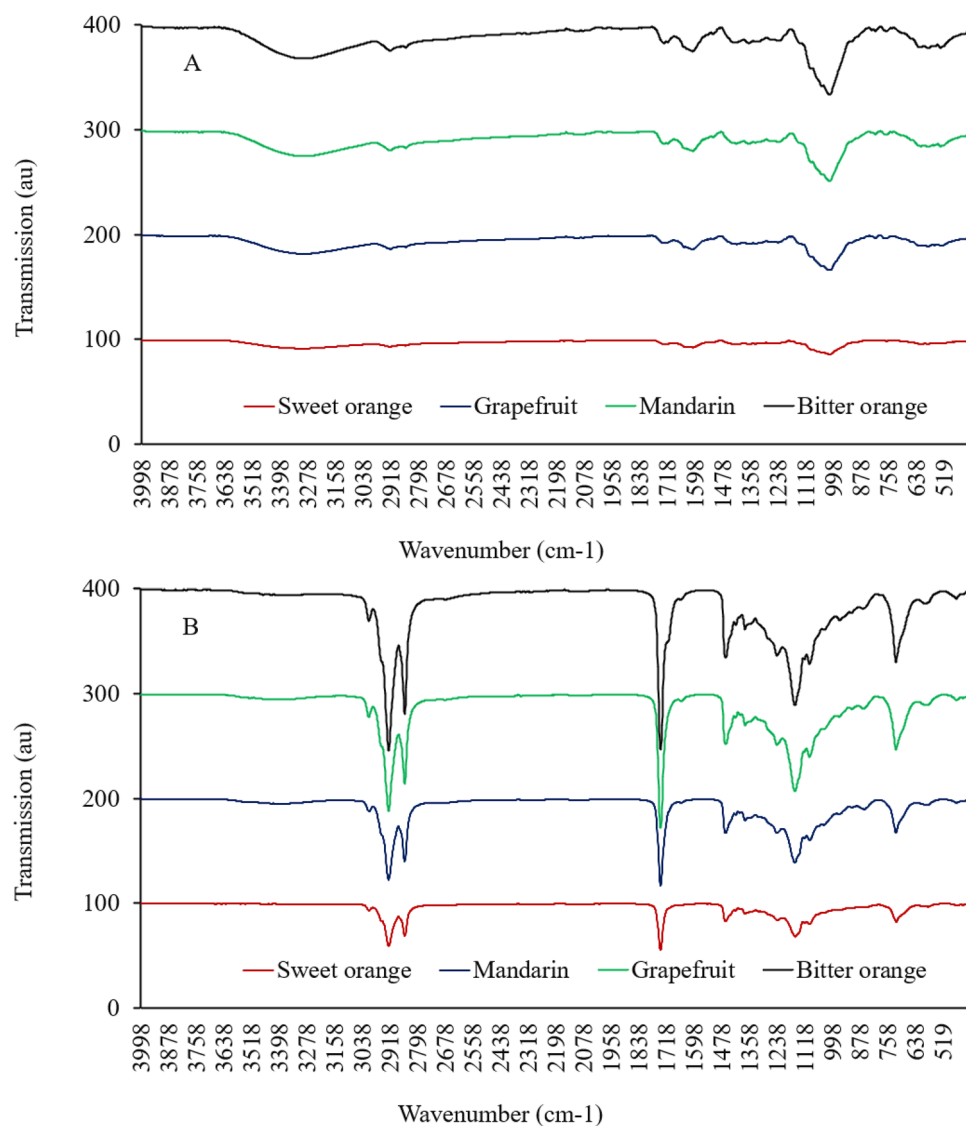


Figure 1. Fourier transform infrared spectrum of peel powder (A) and fatty acid (B) from bitter orange, sweet orange, grapefruit, and mandarin peels.

Parameters	Bitter orange	Grapefruit	Sweet orange	Mandarin
Moisture (g/100 g fw)	77.50 ± 3.01 ^a	75.40 ± 2.93 ^a	78.52 ± 3.05 ^a	80.32 ± 3.12 ^a
Dry matter (g/100 g fw)	22.50 ± 0.87 ^b	24.60 ± 0.96 ^a	21.48 ± 0.84 ^b	19.68 ± 0.77 ^c
Ash (g/100 g dm)	8.54 ± 0.33 ^a	9.56 ± 0.37 ^a	10.85 ± 0.42 ^a	9.63 ± 0.37 ^a
Carbohydrate (g/100 g dm)	57.42 ± 2.23 ^a	58.83 ± 2.29 ^a	56.10 ± 2.18 ^a	57.85 ± 2.25 ^a
Protein (g/100 g dm)	18.50 ± 0.72 ^a	17.61 ± 0.68 ^a	18.99 ± 0.74 ^a	19.20 ± 0.75 ^a
Fat (g/100 g dm)	13.83 ± 0.54 ^a	12.89 ± 0.50 ^a	12.67 ± 0.49 ^a	11.56 ± 0.45 ^a
Energy (kcal/100 g)	428.15 ± 16.65 ^a	421.78 ± 16.40 ^a	414.39 ± 16.11 ^a	412.24 ± 16.03 ^a
Digestibility (g/100 g)	53.70 ± 2.09 ^a	52.60 ± 2.05 ^a	54.33 ± 2.11 ^a	54.25 ± 2.11 ^a

Table 1. Biochemical composition of bitter orange, sweet orange, grapefruit, and mandarin peels. The values are expressed as means ± SD for three replicates experiments. Mean values with similar letters within a row are statistically similar, while mean values with different letters within a row are significantly different by the Tukey test ($P < 0.05$). *fw* fresh weight, *Dm* dry matter.

Fatty acid	Bitter orange	Grapefruit	Sweet orange	Mandarin
Decanoic acid (C10:0)	0.44 ± 0.02 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	1.59 ± 0.09 ^a
Dodecanoic acid (C12:0)	0.00 ± 0.00 ^b	0.85 ± 0.05 ^a	1.03 ± 0.06 ^a	0.00 ± 0.00 ^b
Tridecanoic acid (C13:0)	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.60 ± 0.03 ^a	0.56 ± 0.03 ^a
Tetradecanoic acid (C14:0)	0.75 ± 0.04 ^b	1.04 ± 0.06 ^b	1.65 ± 0.09 ^a	2.26 ± 0.13 ^a
Pentadecanoic acid (C15:0)	0.00 ± 0.00 ^c	0.35 ± 0.02 ^b	0.50 ± 0.03 ^a	0.51 ± 0.03 ^a
Hexadecanoic acid (C16:0)	27.97 ± 1.59 ^a	22.52 ± 1.28 ^b	29.38 ± 1.67 ^a	28.97 ± 1.64 ^a
9-Hexadecenoic acid (C16:1n7)	2.50 ± 0.14 ^a	2.12 ± 0.12 ^a	1.62 ± 0.09 ^b	1.97 ± 0.11 ^a
7,10-Hexadecadienoic acid (C16:2n6)	0.00 ± 0.00 ^b	0.73 ± 0.04 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b
Heptadecanoic acid (C17:0)	0.65 ± 0.04 ^b	0.43 ± 0.02 ^c	0.72 ± 0.04 ^b	1.06 ± 0.06 ^a
Octadecanoic acid (C18:0)	4.46 ± 0.25 ^a	2.95 ± 0.17 ^c	3.82 ± 0.22 ^b	4.70 ± 0.27 ^a
9-Octadecenoic acid (C18:1n9)	19.86 ± 1.13 ^a	20.80 ± 1.18 ^a	17.13 ± 1.01 ^a	9.76 ± 0.55 ^b
9,12-Octadecadienoic acid (C18:2n6)	33.20 ± 1.88 ^a	30.30 ± 1.72 ^a	33.90 ± 1.92 ^a	30.08 ± 1.71 ^a
9,12,15-Octadecatrienoic acid (C18:3n3)	8.20 ± 0.46 ^b	16.10 ± 0.91 ^a	6.35 ± 0.36 ^c	15.40 ± 0.87 ^a
6,9,12-Octadecatrienoic acid (C18:3n6)	0.00 ± 0.00 ^b	0.36 ± 0.02 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b
6,9,12,15-Octadecatetraenoic acid (C18:4n3)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Eicosanoic acid (C20:0)	1.32 ± 0.07 ^a	0.75 ± 0.04 ^b	1.57 ± 0.09 ^a	1.77 ± 0.10 ^a
Arachidonic acid (C20:0)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
13,16-Docosadienoic acid (C22:2n6)	0.54 ± 0.03 ^b	0.36 ± 0.02 ^b	0.90 ± 0.05 ^a	0.92 ± 0.05 ^a
Docosahexaenoic acid (C22:6n3)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Total	99.89 ± 5.66 ^a	99.66 ± 5.65 ^a	99.77 ± 5.66 ^a	99.55 ± 5.64 ^a
Saturated fatty acid (SFA)	35.59 ± 2.02 ^b	28.89 ± 1.64 ^c	39.27 ± 2.23 ^a	41.42 ± 2.35 ^a
Unsaturated fatty acid (UFA)	64.30 ± 3.65 ^{ab}	70.77 ± 4.01 ^a	60.50 ± 3.43 ^b	58.13 ± 3.30 ^b
UFA/SFA	1.81 ± 0.10 ^a	2.45 ± 0.14 ^a	1.54 ± 0.09 ^a	1.40 ± 0.08 ^a
Monounsaturated fatty acid (MUFA)	22.36 ± 1.27 ^a	22.92 ± 1.30 ^a	19.35 ± 1.10 ^b	11.73 ± 0.67 ^c
Polyunsaturated fatty acid (PUFA)	41.94 ± 2.38 ^a	47.85 ± 2.71 ^a	41.15 ± 2.33 ^a	46.40 ± 2.63 ^a
PUFA/MUFA	1.88 ± 0.11 ^b	2.09 ± 0.12 ^b	2.13 ± 0.12 ^b	3.96 ± 0.22 ^a
Omega-3 (ω-3)	8.2 ± 0.46 ^b	16.1 ± 0.91 ^a	6.4 ± 0.36 ^c	15.4 ± 0.87 ^a
Omega-6 (ω-6)	33.74 ± 1.91 ^a	31.75 ± 1.80 ^a	34.80 ± 1.97 ^a	31.00 ± 1.76 ^a
Omega-6/Omega-3	4.11 ± 0.23 ^a	1.97 ± 0.11 ^b	5.48 ± 0.31 ^a	2.01 ± 0.11 ^b
Omega-7 (ω-7)	2.5 ± 0.14 ^a	2.1 ± 0.12 ^a	1.6 ± 0.09 ^b	2.0 ± 0.11 ^a
Omega-9 (ω-9)	19.86 ± 1.13 ^a	20.80 ± 1.18 ^a	17.73 ± 1.01 ^a	9.76 ± 0.55 ^b
PUFA/SFA (P/S)	1.18 ± 0.07 ^a	1.66 ± 0.09 ^a	1.05 ± 0.06 ^a	1.12 ± 0.06 ^a
MUFA/SFA (M/S)	0.63 ± 0.04 ^a	0.79 ± 0.04 ^a	0.49 ± 0.03 ^b	0.28 ± 0.02 ^c
Atherogenicity index (AI)	0.48 ± 0.03 ^a	0.39 ± 0.02 ^a	0.61 ± 0.03 ^a	0.65 ± 0.04 ^a
Thrombogenicity index (TI)	0.63 ± 0.04 ^a	0.35 ± 0.02 ^c	0.75 ± 0.04 ^a	0.53 ± 0.03 ^b
Hypocholesterolemic index (HI)	2.13 ± 0.12 ^a	2.85 ± 0.16 ^a	1.87 ± 0.11 ^a	1.77 ± 0.10 ^a
Peroxidizability index (PI)	50.70 ± 2.85 ^b	64.88 ± 2.65 ^a	47.98 ± 2.70 ^b	62.09 ± 3.45 ^a
Nutritive value index (NVI)	0.87 ± 0.05 ^b	1.05 ± 0.06 ^a	0.73 ± 0.04 ^c	0.50 ± 0.03 ^d

Table 2. Fatty acid composition (percent) and lipid nutritional quality of bitter orange, sweet orange, grapefruit, and mandarin peels. The values are expressed as means plus standard deviation of three replicates. Mean values with similar letters within a row are statistically similar, while mean values with different letters within a row are significantly different by the Tukey test ($P < 0.05$).

and *C. natsudaidai* peel²⁰ display comparable results to those in our study. Citrus peel oils have a low omega-6/omega-3 ratio, high omega-9 fatty acid (mainly oleic acid), high omega-6 fatty acids, and high omega-3 fatty acids (especial grapefruit and mandarin peels) could be a better dietary source of fatty acids than most of the vegetable oils by authors opinion. Animals and humans cannot synthesize linoleic and linolenic acids, which are essential and provided exclusively from dietary sources. Citrus peel fatty acids could be a promising candidate for food supplements and ingredients to provide linoleic and linolenic acids²³.

The medium-chain SFA (8–12 carbon atoms) is linked to lower blood pressure, better cardiac function, lower cancer risk, lower atherosclerosis risk, and lower low-density lipoprotein (LDL) cholesterol^{24,25}. Moderate myristic acid consumption improves long-chain omega-3 fatty acid levels in plasma phospholipids, positively impacts cardiovascular health, and regulates key metabolic processes²⁶. Palmitic acid (16:0, PA) is the most common saturated fatty acid found in the human body and can be provided in the diet or synthesized endogenously from other fatty acids. PA is a precursor for palmitoleic acid (16:1n-7, POA) synthesis by delta 9 desaturase. The disruption of PA homeostatic balance, implicated in different physiopathological conditions such as atherosclerosis, neurodegenerative diseases, and cancer, is often related to an uncontrolled PA endogenous biosynthesis,

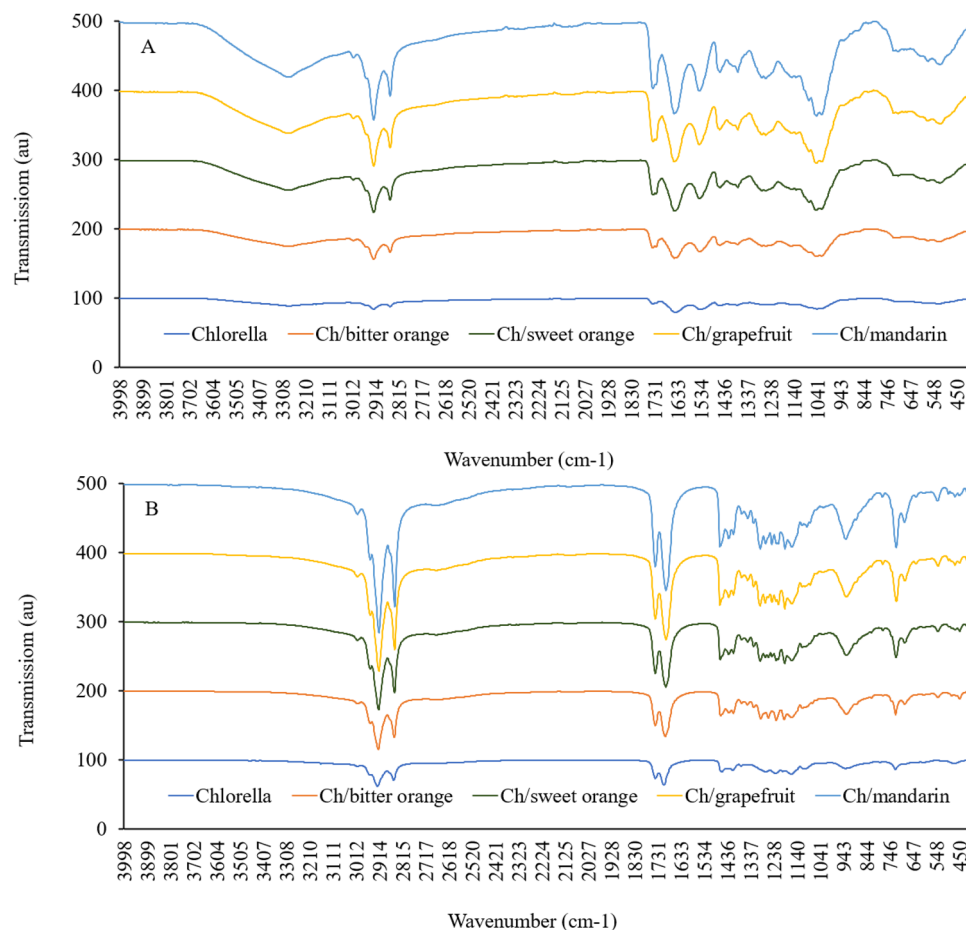


Figure 2. Fourier transform infrared spectrum of whole *Chlorella* (A) and *Chlorella* fatty acid (B) supplemented with fatty acid extract from bitter orange, sweet orange, grapefruit, and mandarin peels.

irrespective of its dietary contribution²⁷. On the other hand, although it belongs to saturated fatty acids, stearic acid is not atherogenic and reduces cardiovascular and cancer risk²⁸.

Nutritional quality of citrus peel fatty acid. The results for the nutritional quality of fatty acids from bitter orange, sweet orange, grapefruit, and mandarin peels are presented in Table 2. The citrus peel contains PUFA, SFA, and MUFA, respectively. PUFA mainly contains omega-6 and omega-3, respectively. MUFA mainly contains omega-9 and omega-7, respectively. Considering the fatty acid nutritional quality, bitter orange, sweet orange, grapefruit, and mandarin are similar, although there are statistically significant differences in the total MUFA and omega-9 and omega-3 fatty acid content ($P \leq 5\%$). Grapefruit had the highest MUFA, omega-9, and omega-3 fatty acid. Mandarin had the lowest MUFA and omega-9 fatty acids ($P \leq 5\%$). Bitter orange and grapefruit are similar according to UFA/SFA, HI, UFA, PUFA/SFA, NVI, MUFA/SFA, MUFA, omega-9, PUFA, omega-3, PI, and omega-7. Sweet orange and mandarin are similar according to SFA, AI, TI, PUFA/MUFA, and omega-6/omega-3 (Table 2 and Fig. S3 in the supplemental file). Dietary variables are closely linked to the development of diet-related disease, and the consumption of fatty acids is thought to play a unique role. Because of their relevance to health, an adequate supply of UFA plays an important role in disease treatment. The ratio of SFA to UFA in biological membranes is an important feature. Reduced levels of UFA in membranes have been linked to various pathophysiological conditions, including cardiovascular disease, diabetes, and cancer²⁹. MUFA are beneficial to biological membranes because they are liquid at body temperature but not quickly oxidized, allowing them to retain proper membrane fluidity. UFA also functions as a lipid hormone or lipokine, coordinating metabolic responses between tissues³⁰. Accordingly, citrus peels fatty acids with a good balance of PUFA, SFA, MUFA, omega-6, omega-3, omega-9, and omega-7 are beneficial for human health and microalgae culture and biomass production.

Biochemical of *Chlorella* supplemented with citrus peel. The FTIR patterns of *Chlorella* supplemented with fatty acid from bitter orange, sweet orange, grapefruit, and mandarin peels differ, indicating the various components in these products (Fig. 2). The 3700–3100 cm^{-1} bands are associated with the stretching vibrations of OH groups in water or aromatic molecules. Peaks in the 2900–2700 cm^{-1} region could be linked to the stretching vibration of lipid and fatty acid CH, CH₂, and CH₃ groups. The majority of the variations in FTIR

Parameters	Chlorella	Chlorella + bitter orange	Chlorella + grapefruit	Chlorella + sweet orange	Chlorella + mandarin
Moisture (g/100 g fw)	6.75 ± 0.25 ^a	7.37 ± 0.25 ^a	6.86 ± 0.26 ^a	7.57 ± 0.29 ^a	7.45 ± 0.28 ^a
Dry matter (g/100 g fw)	93.25 ± 3.51 ^a	92.63 ± 3.51 ^a	93.14 ± 3.51 ^a	92.43 ± 3.48 ^a	92.55 ± 3.49 ^a
Ash (g/100 g dm)	7.85 ± 0.30 ^a	6.54 ± 0.30 ^a	6.65 ± 0.25 ^a	6.85 ± 0.26 ^a	6.63 ± 0.25 ^a
Carbohydrate (g/100 g dm)	28.35 ± 1.07 ^a	26.32 ± 1.07 ^a	25.75 ± 0.97 ^a	26.81 ± 1.01 ^a	25.55 ± 0.96 ^a
Protein (g/100 g dm)	38.85 ± 1.46 ^a	33.50 ± 1.46 ^b	32.75 ± 1.23 ^b	34.29 ± 1.29 ^b	34.22 ± 1.29 ^b
Fat (g/100 g dm)	24.50 ± 0.92 ^b	33.23 ± 0.92 ^a	34.72 ± 1.31 ^a	31.67 ± 1.19 ^a	33.56 ± 1.26 ^a
Energy (kcal/100 g)	489.30 ± 18.43 ^b	538.35 ± 18.43 ^a	546.49 ± 20.58 ^a	529.43 ± 19.94 ^a	541.12 ± 20.38 ^a
Digestibility (g/100 g)	55.50 ± 2.09 ^a	57.73 ± 2.09 ^a	55.37 ± 2.09 ^a	56.33 ± 2.12 ^a	56.25 ± 2.12 ^a

Table 3. Biochemical composition of *Chlorella vulgaris* supplemented with of bitter orange, sweet orange, grapefruit, and mandarin peel fatty acid. The values are expressed as means ± SD for three replicates experiments. Mean values with similar letters within a row are statistically similar, while mean values with different letters within a row are significantly different by the Tukey test ($P < 0.05$). *fw* fresh weight, *Dm* dry matter.

patterns observed in the 2900–2700 cm^{-1} region can be attributed to a variation in fatty acid composition caused by citrus peel fatty acids. The C=C stretching of unsaturated fatty acids and the formation of MUFA and PUFA in *Chlorella* after supplementing with citrus peel can be related to the variations in FTIR patterns observed in the 1900–1500 cm^{-1} region. The stretching of C=O of amides, C=C of aromatics, N–H of amines, or carboxyl groups in proteins is represented by the bands in the region 1300–1000 cm^{-1} . The bands at 1100–900 cm^{-1} are created by polysaccharide vibrations, including symmetric stretching of the C–O–C and OH groups. The vibration of terpenes and terpenoids causes the bands at 900–500 cm^{-1} ³¹.

The macronutrient composition of *Chlorella* and *Chlorella* supplemented with citrus peel fatty acids is stated in Table 3. Protein, carbohydrates, and lipids are the major components of *Chlorella*, respectively. *Chlorella* supplementation with citrus peel fatty acids increases total biomass and fat content while reducing carbohydrates and protein to some extent ($P \leq 5\%$). In this research, microalgae were separated by centrifugation, washed with distilled water, and then dried by lyophilization after culture. Wet biomass washing and lyophilization reduce the moisture content and salt (ash) of the dry matter of microalgae. *Chlorella*/bitter orange, *Chlorella*/grapefruit, *Chlorella*/sweet orange, and *Chlorella*/mandarin are similar according to energy, fat, moisture, and digestibility. *Chlorella* is different from other samples based on the ash, protein, dry matter, and carbohydrate (Table 3, Fig. S4 in supplemental file). Accordingly, supplementation with fatty acids from tested citrus peels modulates the macronutrient composition of *Chlorella*, especially fatty acids. Previous reports suggested that *Chlorella* biomass included an average of 40 g of protein, 18 g of carbohydrates, 12 g of fiber, and 10 g of lipids per 100 g of dry biomass^{32–34}.

The fatty acid content of *Chlorella* supplemented with citrus peel. The FTIR patterns of fatty acid from *Chlorella* supplemented with bitter orange, sweet orange, grapefruit, and mandarin peel fatty acids are stated in Fig. 2. Peaks in the 2900–2700 cm^{-1} region could be linked to the stretching vibration of lipid and fatty acid CH, CH₂, and CH₃ groups. The majority of the variations in FTIR patterns observed in the 2900–2700 cm^{-1} region can be attributed to a variation in fatty acid composition caused by citrus peel fatty acids. The C=C stretching of unsaturated fatty acids and the formation of MUFA and PUFA in *Chlorella* after supplementing with citrus peel can be related to the variations in FTIR patterns observed in the 1900–1500 cm^{-1} region. The vibration of terpenes and terpenoids causes the bands at 1000–500 cm^{-1} ³¹. According to FTIR findings, *Chlorella* includes mostly proteins, carbohydrates, fatty acids, and lower hydrocarbons and terpenes. Because of the high level of essential fatty acid, high level of omega-3, and high levels of omega-6, microalgae such as *Chlorella* are preferred over bacteria and fungi as the single-cell oil for human eating. Carbohydrates (starch) and dietary fibers (cellulose) in microalgae biomass vary on average 20% and 12%, respectively. Because of the lack of lignin and hemicellulose, microalgae carbohydrates have been potential raw materials for bioethanol production. If the characteristics of *Chlorella* fatty acids are nutritionally attractive, *Chlorella* with citrus peel fatty acids can be marketed as a single-celled oil³³.

Fatty acid composition of *Chlorella* and *Chlorella* supplemented with bitter orange, sweet orange, grapefruit, and mandarin peel fatty acids stated in Table 4. The primary fatty acids of *Chlorella* are palmitic, linoleic, oleic, alpha-linolenic, gamma-linolenic, hexadecatetraenoic, palmitoleic, hexadecadienoic, hexadecatrienoic, lauric and eicosapentaenoic acids (Table 4). To some extent, the fatty acid composition reported in *Chlorella* is similar to the previous results^{32,34}. The fatty acid composition of *Chlorella* remains almost unchanged after supplementation with the fatty acids, as there are some statistically significant differences (Table 4). *Chlorella*/sweet orange, and *Chlorella*/mandarin according to docosahexaenoic acid, eicosanoic acid, heptadecanoic acid, myristic acid, tridecanoic acid, 4,7,10-hexadecatrienoic acid, pentadecanoic acid, 7,10,13-hexadecatrienoic acid, linoleic acid, palmitic acid, eicosapentaenoic acid, and lauric acid are correlated to each other ($P \leq 5\%$). *Chlorella*, *Chlorella*/bitter orange, and *Chlorella*/grapefruit samples are correlated according to 4,7,10,13-hexadecatetraenoic acid,

Fatty acid	Chlorella	Chlorella + bitter orange	Chlorella + grapefruit	Chlorella + sweet orange	Chlorella + mandarin
Decanoic acid (C10:0)	0.49 ± 0.03 ^b	0.31 ± 0.02 ^b	0.16 ± 0.01 ^c	0.24 ± 0.01 ^c	0.77 ± 0.05 ^a
Dodecanoic acid (C12:0)	2.42 ± 0.14 ^b	2.33 ± 0.14 ^b	2.56 ± 0.15 ^b	2.22 ± 0.13 ^b	3.35 ± 0.20 ^a
Tridecanoic acid (C13:0)	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.20 ± 0.01 ^b	0.19 ± 0.01 ^a
Tetradecanoic acid (C14:0)	0.44 ± 0.03 ^b	0.52 ± 0.03 ^b	0.56 ± 0.03 ^b	0.88 ± 0.05 ^a	1.02 ± 0.06 ^a
Pentadecanoic acid (C15:0)	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.12 ± 0.01 ^a	0.17 ± 0.01 ^a	0.17 ± 0.01 ^a
Hexadecanoic acid (C16:0)	23.16 ± 1.36 ^a	23.96 ± 1.41 ^a	22.08 ± 1.30 ^a	24.13 ± 1.42 ^a	24.94 ± 1.47 ^a
9-Hexadecenoic acid (C16:1n7)	4.18 ± 0.25 ^a	3.01 ± 0.18 ^a	2.99 ± 0.18 ^a	3.40 ± 0.20 ^a	3.20 ± 0.19 ^a
7,10-Hexadecadienoic acid (C16:2n6)	3.30 ± 0.19 ^a	1.56 ± 0.09 ^b	1.71 ± 0.10 ^b	3.96 ± 0.23 ^a	2.33 ± 0.14 ^b
7,10,13-Hexadecatrienoic acid (C16:3n3)	2.45 ± 0.14 ^b	1.23 ± 0.07 ^c	1.40 ± 0.08 ^c	6.54 ± 0.38 ^a	6.57 ± 0.39 ^a
4,7,10-Hexadecatrienoic acid (C16:3n6)	0.11 ± 0.01 ^b	0.07 ± 0.00 ^b	0.10 ± 0.01 ^b	0.36 ± 0.02 ^a	0.39 ± 0.02 ^a
4,7,10,13-Hexadecatetraenoic acid (C16:4n3)	4.41 ± 0.26 ^a	2.39 ± 0.14 ^b	2.73 ± 0.16 ^b	1.84 ± 0.11 ^b	2.14 ± 0.13 ^b
Heptadecanoic acid (C17:0)	0.00 ± 0.00 ^c	0.22 ± 0.01 ^a	0.14 ± 0.01 ^b	0.24 ± 0.01 ^a	0.35 ± 0.02 ^a
Octadecanoic acid (C18:0)	1.69 ± 0.10 ^b	2.78 ± 0.16 ^a	2.15 ± 0.13 ^a	1.93 ± 0.11 ^b	2.18 ± 0.13 ^a
9-Octadecenoic acid (C18:1n9)	17.80 ± 1.05 ^a	19.14 ± 1.12 ^a	18.52 ± 1.09 ^a	12.53 ± 0.74 ^a	10.20 ± 0.60 ^b
9,12-Octadecadienoic acid (C18:2n6)	17.93 ± 1.05 ^b	23.68 ± 1.39 ^a	22.57 ± 1.33 ^a	24.38 ± 1.43 ^a	21.99 ± 1.29 ^a
9,12,15-Octadecatrienoic acid (C18:3n3)	12.42 ± 0.73 ^b	11.86 ± 0.70 ^b	15.06 ± 0.89 ^a	9.91 ± 0.58 ^c	13.00 ± 0.76 ^b
6,9,12-Octadecatrienoic acid (C18:3n6)	4.63 ± 0.27 ^a	2.48 ± 0.15 ^b	2.61 ± 0.15 ^b	2.36 ± 0.14 ^b	2.39 ± 0.14 ^b
6,9,12,15-Octadecatetraenoic acid (C18:4n3)	1.95 ± 0.11 ^a	0.98 ± 0.06 ^b	1.20 ± 0.07 ^a	0.99 ± 0.06 ^b	1.06 ± 0.06 ^b
Eicosanoic acid (C20:0)	0.00 ± 0.00 ^c	0.44 ± 0.03 ^a	0.25 ± 0.01 ^b	0.52 ± 0.03 ^a	0.59 ± 0.03 ^a
Arachidonic acid (C20:4n6)	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b
5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	2.00 ± 0.12 ^a	2.41 ± 0.14 ^a	2.40 ± 0.14 ^a	2.47 ± 0.15 ^a	2.27 ± 0.13 ^a
13,16-Docosadienoic acid (C22:2n6)	0.00 ± 0.00 ^c	0.18 ± 0.01 ^b	0.12 ± 0.01 ^b	0.30 ± 0.02 ^a	0.31 ± 0.02 ^a
Docosahexaenoic acid (C22:2n6)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
Total	99.37 ± 5.84 ^a	99.55 ± 5.85 ^a	99.45 ± 5.84 ^a	99.55 ± 5.85 ^a	99.42 ± 5.84 ^a
Saturated fatty acid (SFA)	28.21 ± 1.66 ^b	30.56 ± 1.80 ^a	28.04 ± 1.65 ^b	30.52 ± 1.79 ^a	33.57 ± 1.97 ^a
Unsaturated fatty acid (UFA)	71.16 ± 4.18 ^a	68.99 ± 4.05 ^a	71.41 ± 4.20 ^a	69.03 ± 4.06 ^a	65.85 ± 3.87 ^{ab}
UFA/SFA	2.52 ± 0.15 ^a	2.26 ± 0.13 ^a	2.55 ± 0.15 ^a	2.26 ± 0.13 ^a	1.96 ± 0.12 ^a
Monounsaturated fatty acid (MUFA)	21.98 ± 1.29 ^a	22.15 ± 1.30 ^a	21.51 ± 1.26 ^a	15.92 ± 0.94 ^b	13.40 ± 0.79 ^b
Polyunsaturated fatty acid (PUFA)	49.19 ± 2.89 ^a	46.84 ± 2.75 ^a	49.90 ± 2.93 ^a	53.10 ± 3.12 ^a	52.45 ± 3.08 ^a
PUFA/MUFA	2.24 ± 0.13 ^a	2.11 ± 0.12 ^a	2.32 ± 0.14 ^a	3.33 ± 0.20 ^a	3.91 ± 0.23 ^a
Omega-3 (ω-3)	23.2 ± 1.36 ^a	18.9 ± 1.11 ^c	22.8 ± 1.34 ^{ab}	21.7 ± 1.28 ^{ab}	25.0 ± 1.47 ^a
Omega-6 (ω-6)	25.97 ± 1.53 ^a	27.97 ± 1.64 ^a	27.11 ± 1.59 ^a	31.36 ± 1.84 ^a	27.41 ± 1.61 ^a
Omega-6/Omega-3 (ω-6/ω-3)	1.12 ± 0.07 ^a	1.48 ± 0.09 ^a	1.19 ± 0.07 ^a	1.44 ± 0.08 ^a	1.09 ± 0.06 ^a
Omega-7 (ω-7)	4.2 ± 0.25 ^a	3.0 ± 0.18 ^a	3.0 ± 0.18 ^a	3.4 ± 0.20 ^a	3.2 ± 0.19 ^a
Omega-9 (ω-9)	17.80 ± 1.05 ^a	19.14 ± 1.12 ^a	18.52 ± 1.09 ^a	12.53 ± 0.74 ^b	10.20 ± 0.60 ^b
PUFA/SFA (P/S)	1.74 ± 0.10 ^a	1.53 ± 0.09 ^a	1.78 ± 0.10 ^a	1.74 ± 0.10 ^a	1.56 ± 0.09 ^a
MUFA/SFA (M/S)	0.78 ± 0.05 ^a	0.72 ± 0.04 ^a	0.77 ± 0.05 ^a	0.52 ± 0.03 ^a	0.40 ± 0.02 ^a
Continued					

Fatty acid	Chlorella	Chlorella + bitter orange	Chlorella + grapefruit	Chlorella + sweet orange	Chlorella + mandarin
Atherogenicity index (AI)	0.38 ± 0.02 ^a	0.41 ± 0.02 ^a	0.38 ± 0.02 ^a	0.43 ± 0.03 ^a	0.49 ± 0.03 ^a
Thrombogenicity index (TI)	0.27 ± 0.02 ^a	0.33 ± 0.02 ^a	0.27 ± 0.02 ^a	0.30 ± 0.02 ^a	0.29 ± 0.02 ^a
Hypocholesterolemic index (HI)	2.04 ± 0.12 ^a	2.23 ± 0.13 ^a	2.48 ± 0.15 ^a	1.87 ± 0.11 ^a	1.74 ± 0.10 ^a
Peroxidizability index (PI)	98.40 ± 5.78 ^a	85.20 ± 5.01 ^a	93.40 ± 5.49 ^a	93.51 ± 5.50 ^a	96.09 ± 5.65 ^a
Nutritive value index (NVI)	0.84 ± 0.05 ^a	0.92 ± 0.05 ^a	0.94 ± 0.06 ^a	0.60 ± 0.04 ^a	0.50 ± 0.03 ^a

Table 4. Fatty acid composition (percent) and lipid nutritional quality of *Chlorella vulgaris* supplemented with bitter orange, sweet orange, grapefruit, and mandarin peel fatty acid. The values are expressed as means plus standard deviation of three replicates. Mean values with similar letters within a row are statistically similar, while mean values with different letters within a row are significantly different by the Tukey test ($P < 0.05$).

gamma-linolenic acid, 6,9,12,15-octadecatetraenoic acid, oleic acid, palmitoleic acid, and alpha-linolenic acid (Table 4, Fig. S5 in supplemental file).

Fatty acid nutritional quality of *Chlorella* supplemented with citrus peel. Fatty acid nutritional quality of *Chlorella* and *Chlorella* supplemented with bitter orange, sweet orange, grapefruit, and mandarin peel fatty acids stated in Table 4. *Chlorella* mainly contains PUFA, SFA, and MUFA, respectively. PUFA mainly contains omega-6 and omega-3, respectively. MUFA contains omega-9 and omega-7, respectively (Table 4). *Chlorella* had nutritionally acceptable AI, TI, omega-6/omega-3, HI, PI, and NVI (Table 4). Supplementation of *Chlorella* with citrus peel fatty acids increases the total biomass and lipid content, while carbohydrate and protein, to some extent, decrease ($P \leq 5\%$). *Chlorella* and supplemented *Chlorella*'s nutritional quality and fatty acid composition are similar, but total fatty acid increased. *Chlorella*, *Chlorella*/bitter orange, and *Chlorella*/grapefruit are correlated according to MUFA/SFA, MUFA, omega-9, NVI, UFA, UFA/SFA, HI, and PUFA/SFA. *Chlorella*/sweet orange and *Chlorella*/mandarin are similar according to PUFA/MUFA, AI, SFA, PUFA, omega-3, and omega-6 (Table 4, Fig. S6 in supplemental file).

Balanced fatty acid in the *Chlorella* leads to an acceptable AI, TI, HI, PUFA/SFA, omega-6/omega-3 ratio, PI, and NVI, making *Chlorella* suitable for food supplements. Because the ratio of UFA/SFA in the *Chlorella* is above 1.5, it can recommend a promising candidate for raising high-density lipoprotein (HDL) cholesterol and depressing LDL cholesterol. Lowering triglycerides, preventing inflammation, increasing mitochondrial biogenesis, restoring insulin sensitivity, reducing central body fat, and suppressing thrombosis and inflammation are benefits of an omega-3-rich diet³⁵. Increased blood viscosity, vasoconstriction, and promoting prothrombotic, proinflammatory, and proaggregatory factors are linked to a diet with high omega-6 fatty acids. Maintaining good health necessitates a sensible omega-6/omega-3 ratio³⁶. *Chlorella* supplemented with citrus peel is beneficial as a rich source of essential fatty acids and the excellent omega-6/omega-3 ratio, which is crucial in dietary supplements to prevent and manage chronic illnesses and obesity problems³⁴.

A PUFA/SFA ratio of over 0.45 is advised to avoid cardiovascular disease and several chronic illnesses in human diets, including cancer. The recommended PUFA/SFA ratio, which indicates the quality of lipid nutrition in a given diet, is 1–2, decreasing blood cholesterol and lowering the risk of heart disease³⁴. Low AI and TI values indicate that foodstuffs have a greater preventive impact in avoiding heart and coronary illnesses, decreasing overall and abdominal obesity, and reducing diabetes in pregnant women. High PUFA levels are closely connected to high HI fatty acid ratios, which are more favorable for human health since they are regarded as the optimal quantity of cholesterol. The PI is a measure of PUFA sensitivity to oxidation. The range of 70–90 is a favorable PI ratio representing the lipid nutritional quality of a certain meal and lowers blood cholesterol and cardiovascular disease. A higher amount of fatty acid oxidation is associated with higher PI levels. However, high PI levels due to high omega-3 PUFA and omega-6 PUFA lead to greater antioxidant and anti-inflammatory actions³⁶.

Citrus peel oil produces a rise in the nutritional quality of *Chlorella* due to the findings, which is a valuable gain for *Chlorella* applications such as food supplements, medicinal benefits, and biodiesel generation. Citrus peel oil can be converted to acetyl-CoA, then converted to palmitic acid, and subsequently to an unsaturated fatty acid by elongases and desaturases. (1) Fatty acids may be incorporated into the phospholipids and glycolipids of the plasma membrane. (2) Fatty acids may be incorporated into the triglyceride and used as a storage resource. (3) Fatty acids may be incorporated into the metabolic pathway of fatty acids and converted to omega-3 and omega-6 fatty acids. Citrus peel oil enters directly into the synthesis pathway of unsaturated fatty acids like linoleic acid and linolenic acid³⁵. In the omega-3 pathway, 15-desaturase converts linoleic acid to alpha-linolenic acid. In the omega-6 pathway, 6-desaturase transforms linoleic acid into gamma-linolenic acid²⁴. When the diet contains high linoleic acid (citrus peel oil) levels, placed in the *Chlorella* culture medium, $\Delta 15$ -desaturase is involved in the biosynthesis of alpha-linolenic acid from linoleic acid¹¹. In contrast, $\Delta 6$ -desaturase is involved in the biosynthesis of gamma-linolenic acid from linoleic acid¹¹. Adding citrus peel oil to the culture medium of *Chlorella* leads to an acceptable production of omega-9, omega-6, omega-3, and nutritionally suitable omega-6/omega-3 ratio²⁶. Accordingly, humans whose diet is rich in these fatty acids have lower inflammation and better insulin

sensitivity than those with saturated fatty acids in their diet. So, these diets are beneficial for human health²⁶. The use of fatty acids by microalgae is complex, and explanation in this case is very difficult and need more studies.

Conclusion

Citrus peel oil is rich in carbohydrates, proteins, and lipids. Given fatty acid content, citrus peels are rich sources of linoleic acid, palmitic acid, oleic acid, linolenic acid, stearic acid, polyunsaturated fatty acid, saturated fatty acids, and monounsaturated fatty acids with good nutritional quality. With this nutritional quality, citrus peels could be used as an inexpensive nutrient for microalgae growth to improve microalgae biomass. But the direct use of citrus peel has several limitations due to antinutrient materials in the citrus peels. Partial extraction of fatty acids and removing antinutrients could reduce these limitations. Supplementing *Chlorella* with fatty acids in citrus peel increases total biomass and lipid content. Although further research is needed to increase the lipid and fatty acid content of *Chlorella*, recent findings suggest that citrus peel may be utilized to grow microalgae and provide microalgal biomass for nutritional supplements at a low cost.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Received: 16 December 2021; Accepted: 10 May 2022

Published online: 17 May 2022

References

- Montero-Calderon, A., Cortes, C., Zulueta, A., Frigola, A. & Esteve, M. J. Green solvents and ultrasound-assisted extraction of bioactive orange (*Citrus sinensis*) peel compounds. *Sci. Rep.* **9**, 1–8 (2019).
- Sharma, K., Mahato, N. & Cho, M. H. Converting citrus wastes into value-added products: Economic and environmentally friendly approaches. *Nutrition* **34**, 29–46 (2017).
- Mahato, N., Sharma, K., Sinha, M. & Cho, M. H. Citrus waste-derived nutra-pharmaceuticals for health benefits: Current trends and future perspectives. *J. Funct. Foods* **40**, 307–316 (2018).
- Zema, D. A. *et al.* Valorisation of citrus processing waste: A review. *Waste Manage.* **80**, 252–273 (2018).
- Markou, G., Wang, L., Ye, J. & Unc, A. Using agro-industrial wastes for the cultivation of microalgae and duckweeds: Contamination risks and biomass safety concerns. *Biotechnol. Adv.* **36**, 1238–1254 (2018).
- Chia, S. R. *et al.* Analysis of economic and environmental aspects of microalgae biorefinery for biofuels production: A review. *Biotechnol. J.* **13**, 1700618 (2018).
- Zhao, C., Wu, Y., Yang, C., Liu, B. & Huang, Y. Hypotensive, hypoglycaemic and hypolipidaemic effects of bioactive compounds from microalgae and marine micro-organisms. *Int. J. Food Sci. Technol.* **50**, 1705–1717 (2015).
- Katihar, R. & Arora, A. Health-promoting functional lipids from microalgae pool. *Algal Res.* **46**, 101800 (2020).
- Teh, K. Y. *et al.* Lipid accumulation patterns and role of different fatty acid types towards mitigating salinity fluctuations in *Chlorella vulgaris*. *Sci. Rep.* **11**, 1–12 (2021).
- Park, W. K. *et al.* Use of orange peel extract for mixotrophic cultivation of *Chlorella vulgaris*: Increased production of biomass and FAMES. *Biore. Technol.* **171**, 343–349 (2014).
- Nateghpour, B., Kavooosi, G. & Mirakhorli, N. Amino acid profile of the peel of three citrus species and its effect on the combination of amino acids and fatty acids *Chlorella vulgaris*. *J. Food Compos. Anal.* **98**, 103808 (2021).
- Kolawole, S. E., Obueh, H. O. & Emokpae, B. A. Nutritional and antinutritional evaluation of grapefruit (*Citrus paradisi*) juice using different extraction methods. *J. Adv. Food Sci. Technol.* **4**, 84–90 (2017).
- Khorram, F. Citrus cultivars of Jahrom Agricultural Research Station. Publisher, Ministry of Agriculture-Jahad. Agricultural Research, Education and Extension Organization of Fars. Pages, 10–140 (2009).
- AOAC. *Official Methods of Analysis of AOAC International* 20th edn. (Rockville, 2016).
- Loh, S. H., Chen, M. K., Fauzi, N. S., Aziz, A. & Cha, T. S. Enhanced fatty acid methyl esters recovery through a simple and rapid direct transesterification of freshly harvested biomass of *Chlorella vulgaris* and *Messastrum gracile*. *Sci. Rep.* **11**, 1–9 (2021).
- Chen, J. & Liu, H. Nutritional indices for assessing fatty acids: A mini-review. *Int. J. Mol. Sci.* **21**, 5695 (2020).
- Tayyab, M. *et al.* UHPLC, ATR-FTIR profiling and determination of 15 LOX, α -glucosidase, ages inhibition and antibacterial properties of citrus peel extracts. *Pharm. Chem.* **155**, 176–186 (2021).
- Satari, B. *et al.* Process optimization for citrus waste biorefinery via simultaneous pectin extraction and pretreatment. *BioResources* **12**, 1706–1722 (2017).
- Ani, P. N. & Abel, H. C. Nutrient, phytochemical, and antinutrient composition of *Citrus maxima* fruit juice and peel extract. *Food Sci. Nutr.* **6**, 653–658 (2018).
- Matsuo, Y., Miura, L. A., Araki, T. & Yoshie-Stark, Y. Proximate composition and profiles of free amino acids, fatty acids, minerals, and aroma compounds in *Citrus natsudaidai* peel. *Food Chem.* **279**, 356–363 (2019).
- Sharma, K., Mahato, N. & Lee, Y. R. Extraction, characterization, and biological activity of citrus flavonoids. *Rev. Chem. Eng.* **35**, 265–284 (2019).
- Satari, B. & Karimi, K. Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization. *Resour. Conserv. Recycl.* **129**, 153–167 (2018).
- Assefa, A. D., Saini, R. K. & Keum, Y. S. Fatty acids, tocopherols, phenolic and antioxidant properties of six citrus fruit species: a comparative study. *J. Food Meas. Charact.* **11**, 1665–1675 (2017).
- Astrup, A. *et al.* Saturated fats and health: A reassessment and proposal for food-based recommendations: JACC state-of-the-art review. *J. Am. Coll. Cardiol.* **76**, 844–857 (2020).
- Dayrit, F. M. The properties of lauric acid and their significance in coconut oil. *J. Am. Oil. Chem. Soc.* **92**, 1–15 (2015).
- Verruck, S. *et al.* Dairy foods and positive impact on the consumer's health. *Adv. Food Nutr. Res.* **89**, 95–164 (2019).
- Carta, G., Murru, E., Banni, S. & Manca, C. Palmitic acid: Physiological role, metabolism, and nutritional implications. *Front. Physiol.* **8**, 902–915 (2017).
- Senyilmaz-Tiebe, D. *et al.* Dietary stearic acid regulates mitochondria in vivo in humans. *Nat. Commun.* **9**, 1–10 (2018).
- Shramko, V. S., Polonskaya, Y. V., Kashtanova, E. V., Stakhneva, E. M. & Ragino, Y. I. The short overview on the relevance of fatty acids for human cardiovascular disorders. *Biomolecules* **10**, 1127 (2020).
- Das, U. N. Essential fatty acids and their metabolites could function as endogenous HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules. *Lipid Health Disease* **7**, 1–18 (2008).

31. Arif, M. *et al.* A complete characterization of microalgal biomass through FTIR/TGA/CHNS analysis: An approach for biofuel generation and nutrients removal. *Renew. Energy* **163**, 1973–1982 (2021).
32. Matos, A. P. *et al.* Chemical characterization of six microalgae with potential utility for food application. *J. Am. Oil Chem. Soc.* **93**, 963–972 (2016).
33. Nagappan, S. *et al.* Potential of microalgae as a sustainable feed ingredient for aquaculture. *J. Biotechnol.* **341**, 1–20 (2021).
34. García, J. L., De Vicente, M. & Galán, B. Microalgae, old sustainable food, and fashion nutraceuticals. *Microb. Biotechnol.* **10**, 1017–1024 (2017).
35. Liu, R. *et al.* High ratio of ω -3/ ω -6 polyunsaturated fatty acids targets mTORC1 to prevent high-fat diet-induced metabolic syndrome and mitochondrial dysfunction in mice. *J. Nutr. Biochem.* **79**, 108330 (2020).
36. Dawczynski, C. *et al.* Incorporation of n-3 PUFA and γ -linolenic acid in blood lipids and red blood cell lipids together with their influence on disease activity in patients with chronic inflammatory arthritis—a randomized controlled human intervention trial. *Lipid Health Disease* **10**, 1–13 (2011).

Acknowledgements

The authors are very grateful to researchers in the central lab of Shiraz University for performing gas chromatography-mass spectrometry and Fourier transforms infrared analysis.

Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by G.K., K.G.J., Z.H.K. K.G.J. wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors were involved in the review and editing of the draft manuscript. G.K. was involved in funding acquisition, validation, supervision, review of the final manuscript, and submission. G.K. and A.S. have read and approved the final manuscript. All authors reviewed the manuscript.

Funding

The fund for this research was provided by Shiraz University (Grant No. 88-GR-AGRST-108).

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-022-12309-y>.

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