

## Structural Features and Biological Activities of Bioactive Compounds from *Fortunella margarita* (Lour.) Swingle: A Review Postprint

**Authors:** ZENG Hong-Liang, CHEN Pei-Lin, HUANG Can-Can, SHEN Jin-Ye, CHANG Qing, ZHENG Bao-Dong, ZHANG Yi

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### Abstract

*Fortunella margarita* (Lour.) Swingle, commonly known as kumquat, is the smallest citrus fruit. It thrives in southeastern China and is widely cultivated and consumed worldwide due to its multiple health benefits. It has been used as an important herbal medicine in traditional Chinese medicine and also as one of the most popular fruits. Various bioactive compounds are present in *F. margarita*, such as polysaccharides, limonoids, essential oils, flavonoids, phenolic acids, vitamins, dietary fiber, etc. In addition, many studies have reported that these bioactive compounds can be utilized as antioxidants, antimicrobials, hypolipidemic agents, and drosophila lure components in functional foods, pharmaceuticals, and daily chemical products due to their biological activities. This review focuses on the structural features and biological activities of polysaccharides, limonoids, essential oils, flavonoids, and other bioactive substances from *F. margarita*, as well as their potential applications in the food, daily chemical, and pharmaceutical industries.

### Full Text

## Structural Features and Biological Activities of Bioactive Compounds from *Fortunella margarita* (Lour.) Swingle: A Review

**Hong-Liang Zeng**, Pei-Lin Chen, Can-Can Huang, Jin-Ye Shen, Qing Chang, Bao-Dong Zheng, Yi Zhang

- (1) College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China

- (2) Fujian Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, Fujian Agriculture and Forestry University, Fuzhou 350002, China
- (3) China-Ireland International Cooperation Centre for Food Material Science and Structure Design, Fujian Agriculture and Forestry University, Fuzhou 350002, China
- (4) College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China

## ABSTRACT

*Fortunella margarita* (Lour.) Swingle, commonly known as kumquat, is the smallest citrus fruit. It thrives in southeastern China and is widely cultivated and consumed worldwide due to its multiple health benefits. It has been used as an important herbal medicine in traditional Chinese medicine and also as one of the most popular fruits. Various kinds of bioactive compounds exist in *F. margarita*, such as polysaccharides, limonoids, essential oils, flavonoids, phenolic acids, vitamins, and dietary fiber. Many studies have reported that these bioactive compounds can serve as antioxidant, antimicrobial, and hypolipidemic agents, as well as drosophila lure components in functional foods, pharmaceuticals, and daily chemical products due to their biological activities. This review focuses on the structural features and biological activities of polysaccharides, limonoids, essential oils, flavonoids, and other bioactive substances from *F. margarita* and their potential applications in food, daily chemical, and pharmaceutical industries.

**Keywords:** *Fortunella margarita* (Lour.) Swingle; bioactive compounds; structural features; biological activities; application

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## 1 INTRODUCTION

*Fortunella margarita* (Lour.) Swingle, known as kumquat or cumquat, originates in southeastern China and is grown for its delicious fruit in many parts of the world, including Europe, Japan, USA, Puerto Rico, Guatemala, Suriname, Colombia, Brazil, Australia, South Africa, and India [1]. It is the smallest citrus fruit and is distinguished by the fact that it can be eaten completely, including the peel [2]. *F. margarita* is a characteristic fruit resource in China and is widely cultivated in Fujian, Zhejiang, Jiangxi, Hunan, and Guangxi provinces [3]. In 2015, its production exceeded 500,000 tons in China, with an output value reaching up to 10 billion [4]. The fruit is highly nutritious and contains numerous bioactive compounds, such as polysaccharides, limonoids, essential oils, flavonoids, phenolic acids, vitamins, dietary fiber, and amino acids [5, 6]. *F. margarita* has been used to prevent blood vessel rupture, reduce capillary

fragility and permeability, and slow arterial hardening [7]. Furthermore, it is used in traditional herbal medicines, especially for treating coughs and colds [8].

The biological activities of *F. margarita* are closely related to its abundant bioactive compounds. Zeng et al. [9] reported that the antimicrobial activities of *F. margarita* were associated with its polysaccharides. Its polysaccharide fractions also displayed antioxidant activities, pancreatic lipase inhibitory effects, and bile acid-binding activities [10]. Different polysaccharide fractions had various structural features, which resulted in differences in biological activities. Limonin and nomilin were the main components of the limonoids from *F. margarita*, and their antioxidant activities were investigated by Meng [11]. Essential oils from *F. margarita* peel and seed were characterized in previous studies [12, 13]. These oils displayed different chemical components and biological activities. Zheng et al. [14] and Li et al. [15] investigated the chemical components and functional properties of flavonoids from *F. margarita*. Additionally, the structural characteristics and functional activities of other bioactive compounds from *F. margarita*, such as phenolic acids, vitamins, dietary fiber, amino acids, and minerals, have also been studied [16]. All of these bioactivities were related to the chemical components and molecular structural features of the bioactive compounds. These basic scientific studies make it possible to utilize the bioactive compounds from *F. margarita* as active ingredients in food, pharmaceutical, and daily chemical industries.

The objective of this review was to generalize and summarize information on bioactive compounds from *F. margarita*. To generate summary tables and figures, the bioactivities and chemical structures of bioactive compounds were compiled from original papers. Moreover, the potential applications of bioactive compounds as ingredients in food, daily chemical, and pharmaceutical industries were discussed.

## 2 CHEMICAL COMPOSITION

*F. margarita* is highly nutritious, containing a variety of bioactive compounds, such as non-starch polysaccharides, essential oils, limonoids, and flavonoids. Table 1 exhibits the chemical composition of *F. margarita*. Some data are provided by USDA Food Composition Databases. The fruit contains 80.85 g of water, 1.88 g of protein, 0.86 g of total lipid, 0.52 g of ash, 15.9 g of carbohydrate, 6.5 g of fiber, and 9.36 g of sugars per 100 g of edible portion [17]. It is also rich in minerals, vitamins, carotene, cryptoxanthin, lutein, zeaxanthin, and other nutrients. *F. margarita* displays multiple health benefits due to its various bioactive compounds. Several studies have reported that the hypocholesteremic and hypolipidemic effects of citrus fruits were attributed to their polysaccharides [18]. Pectic polysaccharides from citrus fruits inhibited lipase activity [19]. Additionally, the glycemic index was regulated by feeding kumquat juice in mice, which was related to its flavonoid compounds.

### 3 POLYSACCHARIDES

#### 3.1 Structural Properties

Polysaccharides, as the main bioactive compounds from *F. margarita*, account for approximately 12% of dried *F. margarita* [4]. The yield of polysaccharides from *F. margarita* (FMPS) reached up to  $9.15 \pm 0.13\%$  using ultrasonic-microwave synergistic extraction [20], which increased by 405.52%, 128.18%, and 76.64% compared to hot water extraction [21], ultrasonic-assisted extraction [22], and microwave-assisted extraction [9] methods, respectively. FMPS is a macromolecular heteropolysaccharide containing four polysaccharide fractions with different concentrations and molecular weights [10]. Size exclusion chromatography, ultrafiltration, and antisolvent precipitation are the primary methods for fractionating macromolecular polymers. Chromatography is a more accurate method for purifying and isolating polysaccharides compared to ultrafiltration and antisolvent precipitation methods. As shown in Fig. 1 [Figure 1: see original paper], four polysaccharide fractions, named FMPS1, FMPS2, FMPS3, and FMPS4, were isolated sequentially by DEAE Sepharose CL-6B column and Sephadex G-100 gel column [10].

Different polysaccharides had various structural properties. There are also structural differences between crude and purified polysaccharides [23]. The aggregation of purified polysaccharide molecules in solution was observed by confocal laser scanning microscopy (CLSM) (Fig. 2 [Figure 2: see original paper]). The polysaccharide network of purified FMPS was observed to be unevenly distributed in the medium, with compact and smooth aggregation shapes, while the molecules of crude FMPS were dispersed in the solution system without network structure. Structural features were also affected by extraction method. Zeng et al. [24] investigated the effects of different extraction methods on the molar mass distribution and chain conformation of polysaccharides from *F. margarita*. They found that ultrasonic-assisted and microwave-assisted extraction methods had significant degradation effects on the molar mass of polysaccharides, while ultrasonic-microwave synergistic extraction had no influence on the polysaccharides.

Detailed structural features of various polysaccharides are shown in Table 2. All polysaccharides had monosaccharide compositions of galactose, galacturonic acid, and mannose. FMPS1 and FMPS2 contained glucose, FMPS2 and FMPS3 contained arabinose, while FMPS1, FMPS3, and FMPS4 contained rhamnose [10]. Moreover, the relative molar percentages of their monosaccharide compositions differed. The molecular weights of FMPS, FMPS1, FMPS2, FMPS3, and FMPS4 were  $6.192 \times 10^4$  ( $\pm 2.59\%$ ),  $2.572 \times 10^4$  ( $\pm 0.517\%$ ),  $1.755 \times 10^4$  ( $\pm 2.009\%$ ),  $2.563 \times 10^4$  ( $\pm 1.784\%$ ), and  $2.411 \times 10^4$  ( $\pm 1.808\%$ ), respectively [24]. FMPS3 and FMPS4 had similar molecular weights, indicating these fractions were not easily isolated by ultrafiltration and antisolvent precipitation [24]. The glycosidic linkages of FMPS and FMPS3 were mainly  $\alpha$ -glycosidic with a small amount of  $\beta$ -glycosidic bonds, while FMPS1 and FMPS2 were mainly  $\beta$ -glycosidic

with a small amount of  $\alpha$ -glycosidic bonds, and FMPS4 had only  $\beta$ -glycosidic linkages. The chain conformation of the polysaccharides in aqueous solution varied: FMPS1 had a tight uniform spherical conformation, FMPS2 had a random coil conformation, whereas FMPS, FMPS3, and FMPS4 displayed highly branched structures.

### 3.2 Biological Activities

Polysaccharides from many plant species play important roles in cell-cell communication, cell adhesion, and molecular recognition in the immune system [25]. They exhibit various biological activities, including antioxidant, antitumor, hypoglycemic, antilipidemic, anticancer, and radioprotective effects [4]. The biological activities of polysaccharides from *F. margarita* are shown in Table 2. FMPS displayed antioxidant and antibacterial activities. Zeng et al. [9] reported that FMPS showed good antibacterial effects against *Staphylococcus aureus* Rosenbach. The minimal inhibitory concentrations of polysaccharide against *Staphylococcus aureus* Rosenbach, *Salmonella*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas* were 3.13, 50.00, 12.50, 12.50, and 12.50 mg/mL, respectively. FMPS had capacity to scavenge hydroxyl, superoxide, and DPPH radicals, and the antioxidant activities increased with concentration [24]. Among the four fractions, FMPS1 and FMPS3 had stronger inhibitory effects on pancreatic lipase, FMPS1 and FMPS2 had stronger bile acid-binding abilities, while FMPS3 and FMPS4 exhibited greater scavenging activities against hydroxyl, superoxide, and DPPH radicals [10].

Moreover, the effects of FMPS on serum lipid levels and antioxidant indices of plasma and tissues were investigated in hyperlipidemic rats [4]. The results showed that feeding FMPS to hyperlipidemic rats reduced TG, TC, LDL-C, and NEFA contents while significantly increasing HDL-C and LIPA. Meanwhile, feeding FMPS enhanced SOD, GSH-Px, GST, and T-AOC abilities and reduced MDA content in hyperlipidemic rats. There was a concentration-response relationship. Furthermore, the body weight and liver and spleen indices of hyperlipidemic rats were significantly reduced, which was related to FMPS concentration. Histopathological micrographs of hepatic tissue and blood vessel morphology showed that feeding FMPS to hyperlipidemic rats reduced fat deposition in liver cells and protected vascular endothelial cells. These results indicated that FMPS displayed significant regulatory roles in lipid metabolism disorders of hyperlipidemic rats. Combined with the hypolipidemic effect in vitro, the hypolipidemic mechanism of polysaccharides from *F. margarita* in hyperlipidemic rats was achieved by increasing lipase activity, reducing lipid content, and enhancing antioxidant enzyme activity.

### 3.3 Structure-Bioactivity Relationship

The hypolipidemic mechanism, including inhibiting pancreatic lipase activity, binding bile acid, and antioxidant activity, was affected by the preliminary structural characteristics of polysaccharide fractions from *F. margarita* (Fig. 1). The

inhibitory effects on pancreatic lipase activity were influenced by the monosaccharide composition of the polysaccharide fractions, especially pectic polysaccharides [26]. The ability of polysaccharides to bind bile acid might be related to their anionic and cationic properties, physical characteristics, monosaccharide composition, and molecular weight [27]. Glucan could effectively bind bile acids through molecular interactions with bile salts, and the high viscosity of polysaccharides had hydrodynamic restrictions on bile acid-binding [28]. Several factors affected the antioxidant activities of polysaccharides, including their monosaccharide composition, glycosidic linkage, molecular weight, and chain conformation [29]. FMPS3 were mainly pectic polysaccharides with appropriate molecular weight, -glycosidic linkages, and highly branched chain conformation in aqueous solution.

## 4 LIMONOIDS

### 4.1 Structural Features

Limonoids are a class of highly oxidized triterpenes of secondary metabolites. They exist in the form of free ligands and sugar ligands in citrus fruits, especially in fruit peel and seed. Currently, more than 300 limonoids have been identified, with limonin and nomilin as the representative compounds. Acidic limonoids are water-soluble, whereas neutral ones are not easily dissolved [14]. Limonoids are the main substances responsible for the bitter taste of citrus fruit juice. The extraction, purification, isolation, and structural properties of limonoids from *F. margarita* were studied by Meng [11]. Extracts obtained by supercritical carbon dioxide method were isolated and purified by recrystallization. Two crystals were obtained, and FT-IR and NMR analysis revealed molecular structures containing olefin, lactone, vinyl ether, epoxy compounds, and -CH - and -CH groups. The chemical structures of the crystals were characterized by 1D NMR, 2D NMR, and LC-MS/MS [11]. The limonoids from *F. margarita* were composed of limonin and nomilin. The fragment analysis of limonin and nomilin from *F. margarita* by secondary mass spectrometry is shown in Tables 3 and 4. These chemical structures were consistent with standard substances. The content of limonin from *F. margarita* was higher than that of nomilin, consistent with results reported by Zheng et al. [14].

### 4.2 Biological Activities

Limonoids from *F. margarita* are the main active ingredients with anti-cancer properties. Moreover, they have anti-inflammatory, anti-anxiety, and sedative activities, and can regulate cholesterol and prevent atherosclerosis (Fig. 3 [Figure 3: see original paper]) [30]. They have been used as ingredients in food and pharmaceutical industries due to their health benefits. Li et al. [31] reported that limonoids from *F. margarita* had inhibitory effects on DNA oxidation and could reduce the oxidation reaction of lard, affecting the oxidation rate [11]. These limonoids had strong antibacterial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Shigella*, *Salmonella*,

and *Saccharomyces cerevisiae*, with minimum inhibition concentrations of 1.25, 1.25, 1.25, 2.50, 2.50, 2.50, and 5.00 mg/mL, respectively. Acidic conditions could promote their antibacterial activities [11]. Murthy et al. [32] reported that limonin and limonin glycosides inhibited the proliferation of human colon cancer cells. Patil et al. [33] studied the anticancer activities of five purified limonoids. Studies showed significant inhibition of pancreatic cancer cells, consistent with Zhang's research [34]. Hafeez et al. [35] reported that limonoids had insecticidal effects and resistance capacity against *Aedes*.

## 5 ESSENTIAL OILS

### 5.1 Chemical Components

Essential oils are aromatic and volatile liquids extracted from various plants as secondary metabolites. *F. margarita* peel and seed contain numerous oil cells with pigment and aromatic oil. When ripe, the essential oil content from *F. margarita* peel is generally 22%-28% [36]. The aromatic oil has excellent quality and serves as an important and popular natural chemical raw material and edible flavor. Peel essential oil can be extracted by squeezing, hydro-distillation, oil separation, carbon dioxide extraction, and continuous subcritical water extraction. Essential oils from *F. margarita* peel were extracted by microwave, ultrasonic, and supercritical CO<sub>2</sub> fluid methods, with the highest yield reaching 5.08%. The physicochemical properties of the peel essential oil were as follows: acid value of 0.4668, ester value of 4.2456, density of 0.8380 g/mL, dioptr of 1.4707, and optical rotation of 1.8920 [37].

The component analysis of peel essential oil was studied by Wang et al. [38]. There are 88 components in peel essential oil, including alkanes, alkenes, alcohols, acids, ketones, aldehydes, and esters. Essential oils from *F. margarita* peel were mainly composed of D-limonene, myrcene, and  $\alpha$ -pinene with relative amounts of 72.90%, 6.88%, and 3.60% [13]. Moreover, the physicochemical properties and chemical components of seed oil from *F. margarita* were investigated by Xie et al. [13]. The physicochemical properties were as follows: saponification value of  $200.00 \pm 3.45$ , iodine value of  $130.12 \pm 2.67$ , refractive index of 1.47, proportion of 0.92, acid value of  $1.58 \pm 0.06$ , and peroxide value of  $5.02 \pm 0.14$ . The chemical components were detected by GC-MS, with the main components being linoleic acid (47.82%), oleic acid (17.06%), and methyl palmitate (15.88%) [39]. The unsaturated fatty acid content reached up to 64.88%. Its linoleic acid content was generally higher compared to rapeseed oil, linseed oil, peanut oil, and sesame oil.

### 5.2 Biological Activities

The biological activities of essential oils from *F. margarita* peel and seed are shown in Fig. 4 [Figure 4: see original paper]. The antimicrobial activities of peel essential oil demonstrated inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Aspergillus niger*, and yeast. The inhibitory ef-

fect was promoted by adding acid solution, but the antimicrobial activities had thermal instability [12]. The peel essential oil displayed effective antioxidant activity in edible fats, especially in olive and lard oils. The peroxide values of olive and lard oils decreased with the addition of peel essential oil [38]. Interestingly, Wang et al. [38] found that peel essential oil had significant drosophila lure effect. When the test time was 40 min, 10 L/mL of peel essential oil displayed the strongest drosophila lure effect, with a luring rate up to 81.0%. It can be used as a potential attractive substance in agriculture. In addition, its seed oil displayed strong scavenging abilities against hydroxyl and superoxide radicals and had great total antioxidant activity. The scavenging ability against hydroxyl radical was stronger compared to vitamin C [12].

## 6 FLAVONOIDS

### 6.1 Chemical Components

Flavonoids are plant secondary metabolites present in higher plants, usually existing in the form of aglycones and glycosides (with one or more glycosyl groups on the ring). Flavonoids are widely found in the peel and pulp of *F. margarita*. Zheng [14] used UPLC-PDA-MS to determine the content of nine flavonoid compounds, including apigenin 8-C-rutinoside, hesperidin, neohesperidin, rhoifolin, poncirin, acacetin 7-O-rutinoside, phloretin, phloretin 3,5-di-C-glycopyranoside, apigenin, acacetin 8-C-neohesperidoside, and acacetin 6-C-neohesperidoside. Phloretin 3,5-di-C-glycopyranoside was found to be the main flavonoid component from *F. margarita*. Acacetin 3,6-di-C-glucoside, vicenin-2, lucenin-2,4-methyl ether, narirutin 4-O-glucoside, and apigenin 8-C-neohesperidoside were identified for the first time in kumquat juice by Barreca et al. [40]. According to literature, the flavonoids from *F. margarita* were mainly composed of rhoifolin, phloretin 3,5-di-C-glucoside, vicenin-2, narirutin 4-O-glucoside, acacetin 8-C-neohesperidoside, and didymin (Fig. 5 [Figure 5: see original paper]) [41]. These main components affected the biological activities of flavonoids from *F. margarita*.

### 6.2 Biological Activities

Flavonoids have important health effects on the human body. Studies have shown that flavonoids have good antioxidant activity, cardiovascular disease prevention, anti-cancer activity, anti-inflammatory and antibacterial properties, antiviral activity, free anticoagulant, and antithrombotic effects [42]. Li et al. [43-45] reported that flavonoids from *F. margarita* had significant antioxidant effects, promoted immune function in mice, and significantly relieved diabetes symptoms in mice. Chen et al. [46] reported that total flavonoids could protect the liver from acute alcoholism in mice. Antioxidant tests of flavonoids from kumquat showed that the inhibition rates against DPPH· and ABTS· were up to 79% and 93%, respectively [40].

## 7 OTHER BIOACTIVE COMPOUNDS

In addition to polysaccharides, limonoids, essential oils, and flavonoids, *F. margarita* is also rich in phenolic acids, vitamins, dietary fiber, and other bioactive substances. Phenolic acids are compounds with a benzene ring containing multiple phenolic hydroxyl groups. They are important secondary metabolites in plants, ranking second only to flavonoids. Most phenolic acids in *F. margarita* are hydroxylated derivatives of cinnamic and benzoic acids [47]. Wang et al. [48] determined coumaric, chlorogenic, caffeic, erucic, and ferulic acids from *F. margarita* in Taiwan using HPLC. They found that ferulic and erucic acids were the most important soluble phenolic acids in *F. margarita* peel. Studies have shown that *F. margarita* phenolic acids have various biological activities, such as anti-virus, anti-inflammatory, anti-allergy, immune function enhancement, antibacterial activity, and pest control effects. *F. margarita* peels contained high total amounts of lutein, zeaxanthin, -cryptoxanthin, and -carotene, which exhibited antioxidant activity, anti-cancer activity, immune function enhancement, osteoporosis prevention, night blindness prevention, cardiovascular disease prevention, anti-aging activity, and tumor cell growth inhibition [48]. *F. margarita* also contained a certain amount of dietary fiber, which could increase metabolic granule activity to prevent constipation [16].

## 8 APPLICATIONS

Thus far, *F. margarita* is mainly used as an edible fresh fruit and is rarely processed into products. Its products only include fruit juice, concentrated juice, jam, wine, vinegar, and dried fruit. The comprehensive development and utilization of bioactive compounds from *F. margarita* have become an important direction for the future development of the *F. margarita* industry, especially regarding polysaccharides, limonoids, essential oils, and flavonoids. FMPS and FMPS3 can be used as novel natural hypolipidemic and antioxidant agents in the food industry, respectively. The limonoids and flavonoids can be utilized as natural antibacterial and antioxidant agents in functional food and pharmaceutical industries, respectively. The essential oils have been developed as raw materials in the Chinese daily chemical industry, such as in sunscreen, skin cream, hand cream, and soap products.

## 9 CONCLUSION

In this review, literature analysis revealed that *F. margarita* contains abundant bioactive compounds. The structural features of these bioactive compounds contributed to their biological activities. The polysaccharide was a macromolecular heteropolysaccharide containing four polysaccharide fractions with different molecular weights. Different polysaccharide fractions displayed antibacterial and antioxidant activities and hypolipidemic effects. The limonoids were composed of limonin and nomilin, which had anti-cancer, anti-inflammatory, anti-anxiety, and sedative activities, and could regulate cholesterol and prevent

atherosclerosis. Essential oils from *F. margarita* peel were mainly composed of D-limonene, myrcene, and  $\alpha$ -pinene, while linoleic acid, methyl palmitate, and oleic acid were the major components of *F. margarita* seed oils. Essential oils displayed antimicrobial and antioxidant activities and drosophila lure effects. The flavonoids were mainly composed of vicenin-2, narirutin 4-O-glucoside, phloretin 3,5-di-C-glucoside, rhoifolin, acacetin 8-C-neohesperidoside, and didymin, which had strong capacity to eliminate free radicals. Therefore, evidence suggests that bioactive compounds from *F. margarita* have potential as active ingredients for preparing various functional foods, pharmaceutical, and daily chemical products due to their valuable biological functions and beneficial health effects.

## REFERENCES

- (1) Agócs, A.; Nagy, V.; Szabó, Z.; Márk, L.; Ohmacht, R.; Deli, J. Comparative study on the carotenoid composition of the peel and the pulp of different citrus species. *Innov. Food Sci. Emerg.* 2007, 8, 390-404.
- (2) Peng, L.; Sheu, M.; Lin, L.; Wu, C.; Chiang, H.; Lin, W.; Lee, M.; Chen, H. Effect of heat treatments on the essential oils of kumquat (*Fortunella margarita* Swingle). *Food Chem.* 2013, 136, 532-537.
- (3) Zheng, Y. N. Study on the Technologies of Kumquat Juice Concentrating Process. Fujian Agriculture And Forestry University 2012.
- (4) Zeng, H. L. Structural Characterization and Hypolipidemic Mechanism of Polysaccharides from *Fortunella Margarita* (Lour.) Swingle. Fujian Agriculture And Forestry University 2015.
- (5) Ramful, D.; Tarnus, E.; Aruoma, O. I.; Bourdon, E.; Bahorun, T. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Res. Int.* 2011, 44, 2088-2099.
- (6) Wu, H. Q. Study on Kumquat Juice Processing Technology with Two Kinds of Enzymatic Method. Fujian Agriculture And Forestry University 2011.
- (7) Abirami, A.; Nagarani, G.; Siddhuraju, P. In vitro antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits. *Food Sci. Human Well* 2014, 3, 16-25.
- (8) Zeng, H. L. Extraction, Isolation, Purification, Antibacterial and Antioxidative Activity of Polysaccharides from *Fortunella margarita*. Fujian Agriculture And Forestry University 2012.

- (9) Zeng, H. L.; Huang, C. C.; Chang, Q.; Xu, W. Y.; Zhang, Y.; Zheng, B. D. Optimization of microwave-assisted extraction of polysaccharide from *Fortunella margarita* (Lour.) Swingle and its antibacterial effect. *Food Machin.* 2016, 32, 154-160.
- (10) Zeng, H. L.; Miao, S.; Zhang, Y.; Lin, S.; Jian, Y.; Tian, Y. T.; Zheng, B. D. Isolation, preliminary structural characterization and hypolipidemic effect of polysaccharide fractions from *Fortunella margarita* (Lour.) Swingle. *Food Hydrocolloid.* 2016, 52, 126-136.
- (11) Meng, P. Study on Extraction, Purification, Structural Identification and Biological Activities of Limonoids from Kumquat. Fujian Agriculture And Forestry University 2013.
- (12) Wang, S. Y. Studies on the Extraction and Function of Essential Oil from Kumquat Peel. Fujian Agriculture And Forestry University
- (13) Xie, J. F. Studies on the Extraction Technology and Components of *Fortunella margarita* Seed Oil. Fujian Agriculture And Forestry University 2011.
- (14) Zheng, J. Nutritional and Functional Compounds of Major Varieties of Kumquat (*Fortunella* Swingle) in China. Southwestern University 2015.
- (15) Li, J. L. The Research on Extraction and Bioactivities of Active Components in Kumquat. Central South University of Forestry and Technology 2007.
- (16) Wang, Y. C.; Chuang, Y. C.; Ku, Y. H. Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. *Food Chem.* 2007, 102, 1163-1171.
- (17) Meng, P. Study actuality of kumquat and its exploitive foreground. *Academ. Period. Farm Prod. Proc.* 2009, 35-37.
- (18) Chau, C. F.; Huang, Y. L.; Lin, C. Y. Investigation of the cholesterol-lowering action of insoluble fibre derived from the peel of *Citrus sinensis* L. cv. Liucheng. *Food Chem.* 2004, 87, 361-366.
- (19) Espinal, R. M.; Parada, A. F.; Restrepo, S. L. P.; Narváez, C. C. E. Inhibition of digestive enzyme activities by pectic polysaccharides in model solutions. *Bioact. Carboh. Diet. Fibre* 2014, 4, 27-38.
- (20) Zeng, H. L.; Zhang, Y.; Lin, S.; Jian, Y. Y.; Miao, S.; Zheng, B. D. Ultrasonic-microwave synergistic extraction (UMSE) and molecular weight distribution of polysaccharides from *Fortunella margarita* (Lour.)

Swingle. *Sep. Purif. Technol.* 2015, 144, 97-106.

- (21) Zeng, H. L.; Lu, X.; Bian, Z. Y.; Lin, Y. F.; Zhang, Y. Optimization of the extraction technique of *Fortunella margarita* polysaccharides via response surface analysis. *J. Fujian Agr. For. Univ.* (Nat. Sci. Edit.) 2012, 41, 315-319.
- (22) Zeng, H. L.; Zhang, Y.; Zhao, Y. T.; Tian, Y. T.; Miao, S.; Zheng, B. D. Extraction optimization, structure and antioxidant activities of *Fortunella margarita* Swingle polysaccharides. *Int. J. Biol. Macromol.* 2015, 74, 232-242.
- (23) Zeng, H. L.; Zhang, Y.; Jian, Y. Y.; Tian, Y. T.; Miao, M.; Zheng, B. D. Rheological properties, molecular distribution, and microstructure of *Fortunella margarita* (Lour.) Swingle polysaccharides. *J. Food Sci.* 2015, 80, 742-749.
- (24) Zeng, H. L.; Zhang, Y.; Liu, J.; Zheng, B. D. Molar mass distribution and chain conformation of polysaccharides from *Fortunella margarita* (Lour.) Swingle. *Chin. J. Struct. Chem.* 2014, 33, 1245-1252.
- (25) Mu, R. J.; Pang, J.; Yuan, Y.; Tan, X. D.; Wang, M. Chen, H.; Chiang, W. T. Progress on the structures and functions of aerogels. *Chin. J. Struct. Chem.* 2016, 35, 487-497.
- (26) Huang, Y. L.; Chow, C. J.; Tsai, Y. H. Composition, characteristics, and in-vitro physiological effects of the water-soluble polysaccharides from Cassia seed. *Food Chem.* 2012, 134, 1967-1972.
- (27) Fijan, R.; Basile, M.; Šostar, T. S.; Žagar, E.; Žigon, M.; Lapasin, R. A study of rheological and molecular weight properties of recycled polysaccharides used as thickeners in textile printing. *Carbohydr. Polym.* 2009, 76, 8-16.
- (28) Gunness, P.; Flanagan, B. M.; Gidley, M. J. Molecular interactions between cereal soluble dietary fibre polymers and a model bile salt deduced from <sup>13</sup>C NMR titration. *J. Cereal Sci.* 2010, 52, 444-449.
- (29) Jahanbin, K.; Gohari, A. R.; Moini, S.; Emam-Djomeh, Z.; Masi, P. Isolation, structural characterization and antioxidant activity of a new water-soluble polysaccharide from *Acanthophyllum bracteatum* roots. *Int. J. Biol. Macromol.* 2011, 49, 567-572.
- (30) Khalaf, A.; Moore, G. A.; Jones, J. B.; Gmitter Jr, F. G. New insights into the resistance of Nagami kumquat to canker disease. *Physiol. Mol. Plant P* 2007, 71, 240-250.

- (31) Li, J. L.; Zhang, H.; Zeng, C. Z.; Li, Z. H. Study on ultrasonic extraction technology of limonin from the kumquat. *J. Chin. Inst. Food Sci. Technol.* 2009, 9, 96-102.
- (32) Murthy, K. N. C.; Jayaprakasha, G. K.; Kumar, V.; Rathore, K. S.; Patil, B. S. Citrus limonin and its glucoside inhibit colon adenocarcinoma cell proliferation through apoptosis. *J. Agr. Food Chem.* 2011, 59, 2314-2323.
- (33) Patil, J. R.; Jayaprakasha, G. K.; Murthy, K. N. C.; Chetti, M. B.; Patil, B. S. Characterization of *Citrus aurantifolia* bioactive compounds and their inhibition of human pancreatic cancer cells through apoptosis. *Microchem. J.* 2010, 94, 108-117.
- (34) Zhang, J. J.; Luo, G.; He, L.; Zhou, L. M. Inhibiting effects of limonin on human hepatocarcinoma cells SMMC-7721 in vitro. *Sichuan J. Physiol. Sci.* 2007, 29, 157-160.
- (35) Hafeez, F.; Akram, W.; Shaalan, E. A. Mosquito larvicidal activity of citrus limonoids against *Aedes albopictus*. *Parasitol. Res.* 2011, 109, 221-229.
- (36) Su, D. L.; Shan, Y. Review of physiologically-active compounds in citrus peels. *Mod. Food Sci. Technol.* 2006, 22, 260-262.
- (37) Fu, W. Q.; Wang, S. Y.; Zheng, B. D.; Zeng, S. X.; Zhang, Y. Optimization of supercritical CO<sub>2</sub> fluid extraction technology of essential oil from kumquat peel and its physicochemical properties. *Chin. J. Trop. Agr.* 2015, 35, 55-59.
- (38) Wang, S. Y.; Zhang, Y.; Zheng, B. D. The extraction process of essential oil from *Fortunella* peel via steam distillation combined with microwave and its influence on the attracted activity of the fruit fly. *J. Chin. Inst. Food Sci. Technol.* 2014, 14, 37-44.
- (39) Zhang, Y.; Xie, J. F.; Zeng, S. X.; Zheng, B. D. Study on the extraction technology and components of *Fortunella Margarita* seed oil by ultrasonic. *J. Chin. Inst. Food Sci. Technol.* 2013, 13, 35-42.
- (40) Barreca, D.; Bellocco, E.; Caristi, C.; Leuzzi, U.; Gattuso, G. Kumquat (*Fortunella japonica* Swingle) juice: flavonoid distribution and antioxidant properties. *Food Res. Int.* 2011, 44, 2190-2197.
- (41) Kumamoto, H.; Matsubara, Y.; Iizuka, Y.; Okamoto, K.; Yokoi, K. Structure and hypotensive effect of flavonoid glycosides in orange (*Citrus sinensis* OSBECK) peelings. *Agr. Biol. Chem.* 1986, 50, 781-783.

- (42) Lou, S. N.; Lai, Y. C.; Huang, J. D.; Ho, C. T.; Ferng, L. H.; Chang, Y. C. Drying effect on flavonoid composition and antioxidant activity of immature kumquat. *Food Chem.* 2015, 171, 356-363.
- (43) Li, J. L.; Cui, P. W.; Wu, Y. H.; Zeng, C. Z.; Liu, Z. M. Effect of kumquat flavonoids on the anti-oxidation in mice. *Lishizhen Med. Mat. Med. Res.* 2009, 20, 1031-1032.
- (44) Li, J. L.; Zhang, H.; Zeng, C. Z.; Li, Z. H. Study on extraction, purification, structural identification and biological activities of limonoids from kumquat. *J. Chin. Inst. Food Sci. Technol.* 2009, 9, 96-102.
- (45) Li, J. L.; Li, Z. H.; Zhong, H. Y.; Liu, Z. M. Effect of kumquat flavone on blood glucose in mice. *Pharmacol. Clin. Chin. Mat. Med.* 2007, 23, 42-44.
- (46) Chen, Z. Y. Study on the anti-Alcoholism and Protecting Liver Effects of Kumquat Component. Hunan Agricultural University
- (47) Hayat, K.; Zhang, X.; Farooq, U.; Abbas, S.; Xia, S.; Jia, C.; Zhong, F.; Zhang, J. Effect of microwave treatment on phenolic content and antioxidant activity of citrus mandarin pomace. *Food Chem.* 2010, 123, 423-429.
- (48) Wang, Y. C.; Chuang, Y. C.; Hsu, H. W. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chem.* 2008, 106, 277-284.

**Fig. 1.** Structural characterization and hypolipidemic effect of polysaccharide fractions from *F. margarita* [10]. Reprinted with permission.

**Fig. 2.** CLSM images of FMPS solutions: (a) Crude FMPS; (b) Purified FMPS [23]. Reprinted with permission.

**Fig. 3.** Biological activities of limonoids from *F. margarita*.

**Fig. 4.** Biological activities of essential oils from *F. margarita*.

**Fig. 5.** Chemical components of flavonoids from *F. margarita*.

**Table 1.** Chemical Composition of *F. margarita*

Nutrient	Units	Value per 100 g of edible portion
Water	g	80.85
Protein	g	1.88
Total lipid (fat)	g	0.86
Carbohydrate	g	15.9
Fiber, total dietary	g	6.5

Nutrient	Units	Value per 100 g of edible portion
Sugars, total	g	9.36
Minerals		
Calcium, Ca	mg	62
Iron, Fe	mg	0.86
Magnesium, Mg	mg	20
Phosphorus, P	mg	19
Potassium, K	mg	186
Sodium, Na	mg	10
Zinc, Zn	mg	0.17
Copper, Cu	mg	0.095
Manganese, Mn	mg	0.135
Vitamins		
Vitamin C	mg	43.9
Thiamin	mg	0.037
Riboflavin	mg	0.09
Niacin	mg	0.429
Pantothenic acid	mg	0.208
Vitamin B-6	mg	0.036
Folate, total	mcg	17
Folate, DFE	mcg_DFE	17
Vitamin A, IU	IU	290
Vitamin A, RAE	mcg_RAE	15
Vitamin E	mg	0.34
Lipids		
Fatty acids, total saturated	g	0.102
Fatty acids, total monounsaturated	g	0.154
16:1 undifferentiated	g	0.02
18:1 undifferentiated	g	0.134
Fatty acids, total polyunsaturated	g	0.163
18:2 undifferentiated	g	0.132
18:3 undifferentiated	g	0.031
Other		
Carotene, alpha	mcg	155
Cryptoxanthin, beta	mcg	193
Lutein + zeaxanthin	mcg	129

**Table 2.** Structural Features and Biological Activities of Polysaccharides

Component name	Monosaccharide composition	Molecular weight (Da)	Glycosidic linkage	Chain conformation	Biological activities
FMPS	Galactose, galacturonic acid, mannose	$6.192 \times 10$ ( $\pm 2.59\%$ )	- glycosidic with a small amount of - glycosidic bonds	Highly dispersive large polymer	Antioxidant activities [4, 8, 9, 23]
FMPS1	Galactose, glucose, galacturonic acid, rhamnose, mannose	$2.572 \times 10$ ( $\pm 0.517\%$ )	- glycosidic with a small amount of - glycosidic bonds	Tight uniform spherical conformation	Pancreatic lipase active inhibition, bile acid-binding abilities [4, 10]
FMPS2	Galactose, glucose, galacturonic acid, arabinose, mannose	$1.755 \times 10$ ( $\pm 2.009\%$ )	- glycosidic with a small amount of - glycosidic bonds	Random coil conformation	Bile acid-binding abilities [4, 10]
FMPS3	Galactose, galacturonic acid, arabinose, rhamnose, mannose	$2.563 \times 10$ ( $\pm 1.784\%$ )	- glycosidic with a small amount of - glycosidic bonds	Highly branched polymers	Pancreatic lipase active inhibition, antioxidant activities [4, 10]
FMPS4	Galactose, galacturonic acid, rhamnose, mannose	$2.411 \times 10$ ( $\pm 1.808\%$ )	- glycosidic linkage	Highly branched polymers	Antioxidant activities [4, 10]

**Table 3.** Fragment Analysis of Limonin from *F. margarita* by Secondary Mass Spectrometry [11]. Reprinted with Permission

Order	Mass charge ratio MS/MS (m/z)	Fragment ions Fragment affiliation	Structural formula
1	515.23	C H O [M+H]	
2	471.22	C H O [M+H-CH O ]	
3	455.23	C H O [M+H-C H O ]	
4	441.21	C H O [M+H-C H O ]	
5	411.22	C H O [M+H-C H O ]	
6	205.05	C H O [M+H-C H O ]	

**Table 4.** Fragment Analysis of Nomilin from *F. margarita* by Secondary Mass Spectrometry [11]. Reprinted with Permission

Order	Mass charge ratio MS/MS (m/z)	Fragment ions Fragment affiliation	Structural formula
1	515.19	C H O [M+H]	
2	497.18	C H O [M+H-H O]	
3	455.17	C H O [M+H-C H O ]	

*Note: Figure translations are in progress. See original paper for figures.*

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