Antioxidant and antibacterial capabilities of phenolic compounds and organic acids from *Camellia oleifera* cake

Duoduo Zhang¹ · Shaoping Nie¹ · Mingyong Xie¹ · Jielun Hu¹

Received: 10 December 2018 / Revised: 13 April 2019 / Accepted: 30 May 2019 / Published online: 18 July 2019
© The Korean Society of Food Science and Technology 2019

Abstract There is growing interest in the antioxidants and antibacterial activity from natural substances. The purpose of the research was to gain and distinguish phenolic substances and organic acids in the *Camellia oleifera* cake, and to study their antioxidant and antibacterial activities. The extraction and purification of them were achieved by solvent extraction and column separation, respectively. The conclusions displayed that purity of the phenolic substances was $94.1 \pm 0.5\%$ w/w and that of organic acid was $96.0 \pm 0.3\%$ w/w; Fifteen phenolic substances were certificated using HPLC–ESI–MS technology; oxalic, citric, acetic, malic, and succinic acids are discovered to be main organic acids. In addition, the phenolic substances and organic acids both have good antioxidant activity and obvious inhibition against six species of bacteria. These conclusions can be useful in the reuse of the waste of *Camellia oleifera* oil industry in the future.

Keywords *Camellia oleifera* cake · Phenolic substance · Organic acid · Antioxidant ability · Antibacterial ability

Introduction

Currently, more and more natural products have been found to have many benefits for the human body (Hu et al., 2018) and people are interested in seeking natural antioxidants to curb oxidative stress and reduce oxidative damage to viable cells. Oxidative harm is associated with development of many illnesses. Antioxidants have received extensive attention for the use of food preservatives and the treatment of diseases caused by oxidative stress (Masuda et al., 2003). Therefore, assessment of antioxidant capacity of natural substances has received great attention recently (Ester Hernández-Corroto et al., 2018). In addition, due to the current state of antibiotics, there is great interest in the natural products that control the microorganisms which produce toxins (Shen et al., 2008). Raising acquaintance of problems related to antibiotics has spurred research efforts to develop new methods for developing antimicrobial compounds.

The seeds of *Camellia oleifera* consist many compounds and nutritious oils which are good for human health. However, the defatted seeds, *C. oleifera* cake, are not taken seriously after refining. In our country, millions of tons of *C. oleifera* cakes are thrown annually (Shen et al., 2008). However, the *C. oleifera* cake contains a lot of active compounds. Hence, the extraction of phenolic compounds and organic acids from *C. oleifera* cake will be valuable in reuse of the waste in *Camellia oleifera* and energy conservation.

Polyphenols, a kind of secondary metabolite that plays an essential part in lightening oxidative stress, are reported to possess antioxidant abilities (Mattera et al., 2017). Kirakosyan et al. reported that phenolic compounds have antioxidant activities to help to protect cells against oxidative damage (Kirakosyan et al., 2003). What's more,

✉ Jielun Hu
hujielun@ncu.edu.cn
Duoduo Zhang
family581624@163.com
Shaoping Nie
spine@ncu.edu.cn
Mingyong Xie
myxie@ncu.edu.cn

¹ State Key Laboratory of Food Science and Technology, China-Canada Joint Lab of Food Science and Technology (Nanchang), Nanchang University, 235 Nanjing East Road, Nanchang 330047, Jiangxi Province, China

it can act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating agents (Hamilton et al., 1997). The phenol-rich substances extracted from natural products are increasingly attracting attention in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Difonzo et al., 2018). Phenolic substances are also believed to exhibit antibacterial properties (Zhang et al., 2013). In addition, organic acids are widely distributed in fruits, vegetables, herbs and plants. The role of organic acids in the scavenging of reactive oxygen species has been confirmed by other authors (Sentjurs et al., 2003). At the same time, the organic acids are very important antimicrobial compounds in plants (Mau et al., 2001). The characteristics of foods are closely related to the quantity of organic acids (Poyrazoglu et al., 2002).

According to what the author has learnt, there have limited reports on phenolic components of *C. oleifera* cake and their activities. Similarly, few researches have been carried out to study the organic acid compositions of *C. oleifera* cake. Considering deficiency of related data, the phenolic substances and organic acids of *C. oleifera* cake as well as their antioxidant along with antibacterial abilities were studied.

Materials and methods

Materials

The seeds of *C. oleifera* were collected in Yichun County and the cakes were made after expression in Meiling County both from the Jiangxi Province in China. The dried cakes were battered and crushed to small pieces by a pulverizer (GC-ZN-08, Xihua Corp. Beijing, China), and then grounded by a grinder (YD-313, Youji Corp. Shanghai, China) to fine powders. The powders were stored at room temperature (15 ± 3 °C) before use.

Escherichia coli (1.1369), *Staphylococcus aureus* (1.128), fungi *Mucor racemosus* (3.3446), *Bacillus subtilis* (1.1630), *Rhizopus stolonifer* (3.4110) and *Aspergillus oryzae* (3.5232), which were used to carry out antiseptic drugs sensitivity experiment, were gained from Institute of Microbiology Chinese Academy of Sciences. All reagents used in the study were analytically pure.

Preparation of phenolic substances and organic acids

The flour of *C. oleifera* cake (500 g) was degreased with petroleum ether for 3 h. After that, the organic solvent was volatilized using a rotary evaporator (RE-52A, Yarong

Bio-instrument Co., Shanghai, China), and then defatted powders were obtained.

Extraction and purification of phenolic component

Extraction and purification procedure of phenolic components was performed as follow: the defatted powders were extracted with 500 mL/L methanol [matters to solvent ratio (g/mL) = 1:15] in the 50 °C water bath for 2 h. Then mixtures were filtered and filtrates were concentrated at 55 °C. The concentrated filtrate was eluted through AB-8 macro-reticular resin column and eluted with 850, 700, 500, and 300 mL/L ethanol successively according to method of Singleton and Rossi (Singleton and Rossi, 1965). The 300 mL/L ethanol eluent, which was considered to embody the major phenolic substances, was considered as the phenolic component-rich fraction. Volatilization of fractions was done at 55 °C to dry state. The smallest volume of distilled water was added and the solution was freeze-dried. The procedures above were repeated a couple of times to obtain adequate sample.

Extraction and purification of organic acids

The defatted powders were extracted with deionized water [matters to solvent ratio (g/mL) = 1:10] in the 70 °C water bath for 2 h. Then the filter paper was used to filter mixture and the filtrate was volatilized at 55 °C. Then, concentrated filtrates were passed through HPDI00 macro-reticular resin column and cleared with distilled water and 950 mL/L ethanol in H₂O, successively. The ethanol eluent was evaporated to dryness by a rotary evaporator at 55 °C. The smallest volume of distilled water was added and then the obtained solution was frozen and dried. The procedure above was repeated a couple of times to obtain adequate sample.

The obtained phenolic components and organic acids were then redissolved in distilled water for subsequent operation.

Determination of phenolic compounds

Purity of phenols The purity of phenolic was determined with an improved method described by Singleton and Rossi (1965). 0.2 mL of the sample (1 g/L dissolved in distilled water) were mixed with 46 mL of distilled water and 1 mL of Folin–Ciocalteu reagent. After reacting for 3 min, 3 mL of an Na₂CO₃ aqueous solution (75 g of 1L) was added. Then mix them thoroughly. Incubation was carried out for 2 h at room temperature with intermittent oscillation, and then the absorbance of every mixture was determinated at 760 nm. The identical progress was also used for the standard solutions of gallic acid. Total phenolic was

described as gallic acid equivalents micrograms per kilogram of dry sample. The determination was repeated three times.

Identification of phenolic substances Agilent 1200 HPLC–MS system was used to analysis phenolic substances. HPLC was performed on a liquid chromatograph system. This system has a pump with four mobile phase paths, a controller of column temperature, and a photodiode array detector. The column temperature was maintained at 35 °C. RP-C18 column (4.6 mm × 250 mm, 5 µm) using 10 mL/L acetic acid aqueous solution (A) and 5 mL/L acetic acid in water and acetonitrile (50:50, v/v; B) under gradient conditions was used to separate sample [from 10 to 25% B (15 min), 25 to 35% B (15 min), 35 to 40% B (5 min), 40 to 50% B (10 min), 50 to 100% B (25 min), 100 to 10% B (10 min)]. The mobile phase was maintained at 0.3 mL/min for a period of 80 min and the detector was used at 280 nm. 20 µL of liquid was injected. The peak was assigned based on retention time. Relevant settings were used in ionization of phenolic substances. The gas pressure of the sprayer is 45 psi; the dry gas flow rate is 11 L/min; the ESR voltage of the ion source is 3000 V; the capillary temperature is 350 °C; the voltage of fragmentor is 120 V. Nitrogen (> 99.99%) was considered as sheath gas while He (> 99.99%) as damping gas.

Determination of organic acids

Analysis was obtained on a Waters liquid chromatograph system equipped with a Waters C18 column (4.6 mm × 250 mm, 5 µm, Waters, Milford, MA, USA), a guard column (10 × 4 mm, Waters, Milford, MA, USA) and a Waters 2996 PDA detector. Phosphate buffer solution (20 mmol/L KH₂PO₄, pH = 2.7) (A) and methanol (B) constituted an elution system. Separation was achieved through this program: 0–30 min, 5% B. The flow speed was 0.5 mL/min and 10 µL of sample was injected per run while the system was manipulated at room temperature. The PDA detector performs the detection at 214 nm.

Determination of antioxidant ability

Hydroxyl radical scavenging ability

An improved approach (Hu et al., 2013) was taken to determinate the scavenging capacity of hydroxyl radicals.

Superoxide radical-scavenging activity

Nitro blue tetrazolium (NBT) was used for determination of superoxide radical scavenging activity. In the presence of NBT, superoxide free radical O₂^{•−} was oxidized by

hydroxylamine hydrochloride and reduced to nitrite. Nitrite can produce color at 560 nm in the presence of EDTA. Specific determination methods referred to existing literature (Hu et al., 2013).

1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

Determination of scavenging activity of DPPH radical in samples was carried out by reported method (Chen et al., 2008; Hu et al., 2013).

Inhibition of lipid peroxidation

This experiment was carried out by the means of Zhang et al. (1996) with some improvement (Hu et al., 2013).

Antimicrobial activity assay

The activities of different samples were determined against bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and fungi *Mucor racemosus*, *Rhizopus stolonifera*, *Aspergillus oryzae*. The liquid mediums for bacteria and potato mediums for fungi were hot pressed at 121 °C for 20 min before inoculation. Then, the rings of each microorganism were inoculated into the cold liquid mediums. They were then stored in the condition of oxygen. Three strains of bacteria were cultured at 24 h for 37 °C, while the three fungi were cultivated at 48 h for 30 °C which was further adjusted to obtain a suitable turbidity for further use. After culture, average counts of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were 3.5×10^6 , 8.6×10^5 , 5.4×10^6 colony forming units (CFU/mL), and for fungi *Mucor racemosus*, *Aspergillus oryzae*, and *Rhizopus stolonifer* were 1.5×10^6 , 7.4×10^6 , and 2.9×10^6 (CFU/mL). The density of CFU/mL was allocated to determination board after the following steps. All the bacterial and fungal cultures were continuously diluted 10 times, and 100 µL were inoculated in the culture dish, then the colony count was carried out to calculate the density of the bacterial and fungal population.

Determination of microbial inhibitory effect

The antibacterial activity of the samples was determined by inhibition zone method for the first time. Twenty milliliters of medium were dumped to each culture dish. When medium became solid, 100 µL of suspension was inoculated on test bacteria. Wells were prepared and samples (100 or 200 mg/L) were added to the wells (20 µL), meanwhile, equal volume of gentamicin (1 g/L) was considered as a positive control. Deionized water was regarded as a negative control. After inoculation with bacterial

culture plate, after cultivated for 24 h culture at 37 °C and 48 h at 30 °C, the diameter of inhibition zone (mm) was the mean value of the largest diameter in three directions.

Determination of minimum microbial inhibitory concentration

Liquid medium dilution method was used to determine minimum inhibitory concentration (MIC). Two suitable antibiotics control samples were made in bacterial liquid medium and potato medium (Ibrahim and Osman, 1995). For liquid medium dilution test, each tube was added 100 µL of bacteria or fungus culture and cultured either at 37 °C for bacteria or at 30 °C for fungi for 24 h or 48 h. At the end of culture, turbidity was used as growth indicator. The minimum concentration of MIC was kept unchanged after microscopic evaluation, thus was recorded as mean concentration of triplicates.

Statistical analysis

All tests were done three times in parallel. Statistical analysis was carried out by SPSS. The results were conveyed on the basis of mean $\pm$ standard deviations and compared with 5% confidence level by Tukey test.

Results and discussion

Phenolic components and organic acids

Phenols, aromatic secondary plant metabolites, are widely distributed in nature. The purity of phenolic components obtained from *C. oleifera* cake is $94.1 \pm 0.5\%$ w/w. Figure 1 showed the HPLC chromatogram of the phenolic components. MS spectra of phenolic compounds in *C. oleifera* cake were obtained by positive ion mode under

LC–MS conditions and Table 1 presented their most possible identification. 15 phenolic compounds were detected in the phenolic compounds (Fig. 1, Table 1). The identification is supported by comparison of reference standards and related literatures. The detected phenolic components are pelargonidin-diglucoside, gallotannin, ellagitannin, cyanidin-glucoside, pelargonidin-glucoside, catechin, *p*-coumaroyl-glucoside, *p*-coumaroyl-ester, *p*-coumaroyl-glucoside, quercetin-rutinoside, ellagic acid, quercetin-glucoside, quercetin-glucuronide, methyl-ellagic acid-pentose, and kaempferol-glucuronide.

Organic acids are crucial for sensory attributes and nutritional value of fruits and vegetables. The purity of organic acids obtained from *C. oleifera* cake is $96.0 \pm 0.3\%$ w/w. Oxalic, acetic, citric, malic and succinic acids are main organic acids in the *C. oleifera* cake (Fig. 2).

Antioxidant activities

The role of antioxidant components in plant materials is arousing interest of scientists, food manufacturers and consumers as future trends are moving towards functional food (Akram and Kwon, 2010). Phenolic compounds have been reported to have much physiological function, including antioxidant activity. In this research, the antioxidant activities of phenolic compounds and organic acids were evaluated by four different methods. Table 2 sums up the IC₅₀ values of different samples.

Hydroxyl radical is a very active free radical shaped in a biological system. It is considered to be a very destructive species in the pathology of free radicals, which can destroy almost all the molecules in active cells (Hochstein and Atallah, 1988). As shown in Table 2, the scavenging force of phenolic compounds on hydroxyl radicals is greater than that of organic acids and ascorbic acid ($p < 0.05$).

Fig. 1 HPLC chromatogram of phenolic components obtained from *C. oleifera* cake

Table 1 Identification of phenolic compounds from *C. oleifera* cake using their HPLC–DAD, LC–MS and LC–MSⁿ data

Peak ^a	t _R (min)	Mw	Tentative ID
1	4.25	594	Plg-diglucoside
2	5.32	784	Gallotannin
3	11.89	640	Ellagitannin
4	13.64	448	Cyanidin-glucoside
5	14.58	432	Plg-glucoside
6	16.03	290	Catechin
7	27.76	325	<i>p</i> -coumaroyl-glucoside
8	30.29	356	<i>p</i> -coumaroyl-ester
9	31.82	326	<i>p</i> -coumaroyl-glucoside
10	31.74	610	Quercetin-rutinoside
11	34.20	302	Ellagic acid
12	37.41	464	Quercetin-glucoside
13	39.03	478	Quercetin-glucuronide
14	42.10	448	Methyl-ellagic acid -pentose
15	45.88	462	Kaempferol-glucuronide

^aPeak numbers refer to HPLC chromatograms in Fig. 1

Superoxide anion is important in the formation of active oxygen such as hydroxyl radical, singlet oxygen and hydrogen peroxide, which could induce oxidative damage in lipids, protein and DNA. According to Table 2, the IC₅₀ value of superoxide radical scavenging ability of phenolic was higher than that of organic acids and ascorbic acid ($p < 0.05$).

The model of scavenging the DPPH radical is widely applied to assess the ability of natural substances to scavenge free radicals. The scavenging effect of antioxidants on DPPH radical was owing to their hydrogen supply capacity (Milardovic et al., 2006). The DPPH scavenging activity of the phenols represent by IC₅₀ was 103 ± 8 mg/L (Table 2), which was higher than that of organic acids (184 ± 5 mg/L) and ascorbic acid (205 ± 3 mg/L) ($p < 0.05$).

Lipid peroxidation could produce many degradation outcomes, such as malondialdehyde, which can lead to cell destroy and cell membrane destroy, resulting in kidney damage, liver damage, aging, atherosclerosis. The IC₅₀ value (mg/L) displayed that the lipid peroxidation

Fig. 2 HPLC chromatograms of standard organic acids (A) and organic acids from *C. oleifera* cake (B). Peaks in the chromatograms followed by the order: 1, oxalic acid; 2, tartaric acid; 3, malic acid; 4, acetic acid; 5, maleic acid; 6, citric acid; 7, quinic acid; 8, succinic acid

Table 2 Half-inhibition (IC₅₀) values of antioxidant activities of phenolic components and organic acids measured using hydroxyl radical-scavenging, superoxide radical-scavenging, DPPH radical scavenging and inhibition of lipid peroxidation assays

Sample	IC ₅₀ (mg/L) ^a			
	Hydroxyl radical-scavenging	Superoxide radical-scavenging	DPPH radical scavenging	Lipid peroxidation inhibition
Phenolic components	184 ± 2 a ^b	221 ± 2 a	103 ± 8 a	203 ± 8 a
Organic acids	594 ± 3 b	671 ± 3 b	184 ± 5 b	376 ± 8 b
Ascorbic acid ^c	220 ± 3 c	304 ± 5 c	205 ± 3 c	440 ± 3 c

^aThe IC₅₀ value is expressed as mg/L and represents the concentration of sample that is required for 50% inhibition of hydroxyl radical, superoxide radical, DPPH radical, and lipid peroxidation. A lower IC₅₀ value indicates a higher antioxidant activity. Each value in the table was obtained by calculating the average of three determinations ± standard deviation

^bMean values in the same column with different letters are significantly different (Tukey test, $p < 0.05$)

^cAscorbic acid was used as a positive control

inhibition of phenolic components was higher than that of organic acids and ascorbic acid ($p < 0.05$).

15 phenolic substances have been found in *C. oleifera* cake (Fig. 1, Table 1). It can be concluded from above results that phenolic compounds in *C. oleifera* cake have good antioxidant capacity for superoxide radical scavenging, hydroxyl radical scavenging, DPPH scavenging and lipid peroxidation suppression. Some studies have shown that phenolic substances have a good ability to scavenge free radicals (Zhang et al., 2010) and are also effective metal chelators and lipid peroxidation inhibitors (Puupponen-Pimiä et al., 2001), which were the same as our conclusion. Moreover, different phenolic compounds may have different antioxidant abilities. Sanchez-Moreno et al. studied the inhibition of lipid oxidation of the phenolic substances and discovered that quercetin, catechin, and ellagic acid could have diverse antioxidant abilities in the system of linoleic acid. What's more, they have diverse free radical-scavenging activities. Thus, the phenolic compounds that we found in *C. oleifera* cake may also have different antioxidant capacities.

Other molecules, such as organic acids, are also present in a great amount in plants, fruits or vegetables. Previous studies have shown that scavenging of free radicals and other antioxidant capacities have direct effects on certain organic acids. (Chang and Chen, 2004). In addition, the effective antioxidant action of organic acids is known as their chelation, which can inactivate reduced cations. In this study, oxalic, citric, acetic, malic and succinic acids are found to be main organic acids in the *C. oleifera* cake (Fig. 2). It has been reported that these five organic acids all have certain antioxidant capacity. Oxalic acid is available as a natural antioxidant in some systems (Kayashima and Katayama, 2002). Citric acid and other hydroxycarboxylic acids such as malic, tartaric and isocitric acids also have antioxidant activity. For example, hydroxycarboxylic

acids can be used as metal scavengers and promote the degradation of hydroperoxides by not forming free radicals (Pokorný, 1991). So is succinic acid (Bespyatykh et al., 2011). In addition, some other works confirmed that organic acids have the bioactivity both in decreasing reactive oxygen species and in increasing phenolics biological availability (van den Berg et al., 2003). Thus, it could be supposed that the organic acids in *C. oleifera* cake might also improve the antioxidant activity of the phenolic substances in *C. oleifera* cake.

Antimicrobial activities

Plants can produce antibacterial substances to keep themselves away from microbial infection. The use of natural antibacterial compounds, especially those extracted from plants, is of increasing interest in preserving food. Besides, more consumers intend to suspect the safety of synthetic additives and prefer natural food. Scientists reported the antibacterial activity of some spices in roots, stems, leaves, flowers and fruits. The phenolic compounds and organic acids are very important antimicrobial compounds in plants.

In terms of microbial morphology, tolerance and identification, it is more significant to select *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* as the research objects of antimicrobial experiment. The diameters of bacteriostasis circle of the phenolic substances and organic acids from *C. oleifera* cake are shown in Fig. 3, and the MICs are shown in Table 3. As shown in Fig. 3, there is obvious difference in diameter of inhibition zone for phenolic components and organic acids (100 or 200 mg/L) compared with deionized water (negative control, $p < 0.05$). It was indicated from Fig. 3 that phenolic components and organic acids (100 or 200 mg/L) could inhibit these three bacteria, and their inhibiting capacity

Fig. 3 Inhibition zones of antimicrobial activities of phenolic components and organic acids from *C. oleifera* cake. Values were mean $\pm$ standard deviations of three independent experiments. Different letters mean significantly different ($p < 0.05$) from each other. (“high” and “low” represent the concentration of sample are 200 mg/L and 100 mg/L, respectively.)

Table 3 MICs^a of antimicrobial activity for phenolic components and organic acids from *C. oleifera* cake against bacteria and fungi

Microorganisms	MIC (mg L ⁻¹)	
	Phenolic components	Organic acids
<i>Escherichia coli</i>	125	250
<i>Staphylococcus aureus</i>	250	63
<i>Bacillus subtilis</i>	63	125
<i>Rhizopus stolonifer</i>	500	1000
<i>Aspergillus oryzae</i>	1000	500
<i>Mucor racemosus</i>	2000	1000

^aThe lowest concentration which the liquid medium remained clear after microscopic evaluation was taken as the minimum inhibitory concentration (MIC). It was recorded as the mean concentration of triplicates

was sometimes better than gentamicin (positive control, 1 g/L).

Inhibition zones of the phenolic components and organic acids for each experiment on test bacteria are opposite to MIC (Table 3). It means that higher inhibition zone will give lower MIC value which implies stronger antimicrobial activity. As shown in Table 3, MIC was at 125, 250 and 63 mg/L for phenolic components against bacteria *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*,

while it was 250, 63 and 125 mg/L for organic acids against the same bacteria. When against fungi, *Rhizopus stolonifer* was affected by the phenolic components and organic acids as the MICs were 500 and 1000 mg/L. In addition, phenolic components and organic acids could inhibit *Aspergillus oryzae* with MIC level of 1000 and 500 mg/L. Furthermore, MIC was 2000 and 1000 mg/L for phenolic components and organic acids, respectively against fungi *Mucor racemosus*. These results suggested that the phenolic substances and organic acids had lower inhibitory activity against fungi than bacterial test.

In our daily life, the crucial thing is to seek food additives and preservatives to inhibit food deterioration and prolong the storage life of ingredients. Many phenolic compounds are known to possess antimicrobial properties. In this work, there are 15 phenolic components found in *C. oleifera* cake (Fig. 1, Table 1). It was also reported that ellagitannins could be one of the main phenolic components leading to the inhibition against *Salmonella* (Puupponen-Pimiä et al., 2001), which was also one of our detected phenolic components in *C. oleifera* cake. In addition, different bacterial species have distinct sensitivities to phenolics, and the variations come from the surface structures of Gram-negative and Gram-positive bacteria. Specially, outer membrane of Gram-negative bacteria can be a preventive barrier to hydrophobic substances

(Helander et al., 1998). However, sometimes phenolics also existed activity to Gram-negative bacteria (Puupponen-Pimiä et al., 2001), which was consistent with our results. Helander et al. found some phenolic compounds could inhibit *Salmonella* and *E. coli*, and the inhibitory mechanism involved the diffusion of these substances to the outer membrane (Helander et al., 1998). The antimicrobial activity of phenolics may also be related to the glycosides in phenolics (Stammati et al., 1999). Thus, the antimicrobial activities of the phenolic substances from *C. oleifera* cake may also result from their glycosides.

It is also suggested that specific organic acids should be used to dominate microbial contamination and foodborne pathogen transmission in food production and processing. In this study, citric, oxalic, malic, acetic and succinic acids are considered to be main organic acids in *C. oleifera* cake (Fig. 2). The antibiosis mechanism of organic acids may rest with physiological state of the organism or the physical and chemical properties of the external environment (Ricke, 2003). Other toxicity mechanisms have also been raised that organic acids may possess membrane uncoupling capacity (Davidson and Taylor, 2013). Furthermore, undecomposed acids are deemed to be the active form, because they could freely diffuse through the cell membrane into the cytoplasm (Booth, 1985). However, the toxic mechanism of organic acids to foodborne pathogens has not been fully clarified. This is partly because of complexities involved in organic acids, and elements such as side chain composition, chain length, and hydrophobicity could influence antiseptic ability of organic acids (Hsiao and Siebert, 1999). This could be investigated in future study.

The study revealed that the purity of the phenolic substances was $94.1 \pm 0.5\%$ w/w and that of organic acid was $96.0 \pm 0.3\%$ w/w; Fifteen phenolic substances were certificated and oxalic, citric, acetic, malic, and succinic acids were discovered to be main organic acids in *Camellia oleifera* cake. Moreover, both phenolic substances and organic acids have good antioxidant and inhibitory activities against six species of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and fungi *Mucor racemosus*, *Rhizopus stolonifer*, *Aspergillus oryzae*). These results could provide theoretical basis for making full use *Camellia oleifera*. Moreover, their antioxidant and antibacterial activities can play an important role in food preservation and other aspects. The mechanism of antioxidant and antibacterial activities remain to be studied.

Acknowledgements This study was supported by National Natural Science Foundation of China (31770861); Young Elite Scientists Sponsorship Program by CA-ST (2018QNRC001); National Key Research and Development Program of China (2017YFC1600405); Jiangxi Provincial Major Program of Research and Development Foundation (Agriculture field, 20165ABC28004).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Akram K, Kwon JH. Food irradiation for mushrooms: A review. J. Korean. Olean. Soc. Appl. Biol. 53: 257–265 (2010)
- Bespyatykh OY, Kokorina AE, Domsik IA. State of antioxidant system of furbearers after injection of succinic acid. Russ. Agric. Sci. 37: 516–519 (2011)
- Booth IR. Regulation of cytoplasmic pH in bacteria. Microbiol. Rev. 49: 359–378 (1985)
- Chang C, Chen J. Study on the protective mechanism of organic acids in human umbilical vein endothelial cells. Acta Nutrimenta Sinica. 26: 280–283 (2004)
- Chen Y, Xie MY, Nie SP, Li C, Wang YX. Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. Food Chem. 107: 231–241 (2008)
- Davidson PM, Taylor TM. Chemical Preservatives and Natural Antimicrobial Compounds. Food Microbiology Fundamentals & Frontiers (2013)
- Difonzo G, Pasqualone A, Silletti R et al. Use of olive leaf extract to reduce lipid oxidation of baked snacks. Food Res. Int. 108: 48–56 (2018)
- Hernández-Coroto E, Marina ML, García MC. Multiple protective effect of peptides released from *Olea europaea* and *Prunus persica* seeds against oxidative damage and cancer cell proliferation. Food Res. Int. 106: 458–467 (2018)
- Hamilton RJ, Kalu C, Prisk E, Padley FB et al. Chemistry of free radicals in lipids. Food Chem. 60: 193–199 (1997)
- Helander LM, Alakomi HL, Latva-Kala K. Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agric. Food Chem. 46: 3590–3595 (1998)
- Hochstein P, Atallah AS. The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. Mut. Res. 202: 363–375 (1988)
- Hsiao C, Siebert KJ. Modeling the inhibitory effects of organic acids on bacteria. Int. J. Food Microbiol. 47: 189–201 (1999)
- Hu JL, Nie SP, Xie MY. Antidiabetic mechanism of dietary polysaccharides based on their gastrointestinal functions. J. Agric. Food Chem. 66: 4781–49849 (2018)
- Hu JL, Nie SP, Xie MY. High pressure homogenization increases antioxidant capacity and short-chain fatty acid yield of polysaccharide from seeds of *Plantago asiatica* L. Food Chem. 138: 2338–2345 (2013)
- Ibrahim D, Osman H. Antimicrobial activity of *Cassia alata* from Malaysia. J. Ethnopharmacol. 45: 151–156 (1995)
- Kayashima T, Katayama T. Oxalic acid is available as a natural antioxidant in some systems. Biochimica et Biophysica Acta. 1573(1): 1–3 (2002)
- Kirakosyan A, Seymour E, Kaufman OB, Warber S, Bolling S, Chang SC. Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (Hawthorn) subjected to drought and cold stress. J. Agric. Food Chem. 51: 3973–3976 (2003)
- Masuda T, Inaba Y, Maekawa T, Takeda Y, Yamaguchi H, Nakamoto K, Kuninaga H, Nishizato S, Nonaka A. Simple detection method of powerful antiradical compounds in the raw extract of plants and its application for the identification of antiradical plant constituents. J. Agric. Food Chem. 51: 1831–1838 (2003)
- Mattera R, Benvenuto M, Giganti MG, Tresoldi I, Pluchinotta FR, Bergante S, Tettamanti G, Masuelli L, Manzari V, Modesti A,

- Bei R. Effects of polyphenols on oxidative stress-mediated injury in cardiomyocytes. *Nutrients*. 9: 523 (2017)
- Mau JL, Chen CP, Hsieh PC. Antimicrobial effect of extracts from Chinese chive, cinnamon, and corni fructus. *J. Agric. Food Chem.* 49: 183–188 (2001)
- Milardovic S, Ivekovic D, Grabaric BS. A novel amperometric method for antioxidant activity determination using DPPH free radical. *Bioelectrochemistry*. 68: 175–180 (2006)
- Pokorný, J. Natural antioxidants for food use. *Trends. Food Sci. Tec.* 2: 223–227 (1991)
- Poyrazoglu E, Gokmen V, Artik N. Organic acids and phenolic compounds in pomegranates (*Punica grgranatum* L.) grown in Turkey. *J. Food Compos. Anal.* 15: 567–575 (2002)
- Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A, Oksman-Caldentey KM. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* 90: 494–507 (2001)
- Ricke SC. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry. Sci.* 82: 632–639 (2003)
- Sentjurc M, Nemec M, Connor HD, Abram V. Antioxidant activity of *Sempervivum tectorum* and its components. *J. Agric. Food Chem.* 51: 2766–2771 (2003)
- Shen J, Cao C, Su H, Yang X, Wei Z, Du L. Evidence of gastrointestinal system as an active and toxic target of sasanqua saponins extract. *Exp. Toxicol. Pathol.* 60: 43–49 (2008)
- Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16: 144–158 (1965)
- Stammati A, Bonsi P, Zucco F, Moezelaar R, Alakomi HL, von Wright A. Toxicity of selected plant volatiles in microbial and mammalian short-term assays. *Food Chem. Toxicol.* 37: 813–823 (1999)
- van den Berg AJ, Halkes SBA, van Ufford HC, Hoekstra MJ, Beukelman CJ. A novel formulation of metal ions and citric acid reduces reactive oxygen species in vitro. *J. Wound. Care.* 12: 413–418 (2003)
- Zhang EX, Yu LJ, Zhou YL, Xiao X. Studies on the peroxidation of polyunsaturated fatty acid from lipoprotein induced by iron and the evaluation of the anti-oxidative activity of some natural products. *Acta Biochimica Et Biophysica Sinica.* 28: 218–222 (1996)
- Zhang GW, Hu MM, He L, Fu P, Wang L, Zhou J. Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. *Food Bioprod. Process.* 91: 158–168 (2013)
- Zhang LL, Wang YM, Xu M, Chen JH. Comparisons of antioxidant activity and total phenolics of *Camellia oleifera* Abel fruit hull from different regions of China. *J. Med. Plants. Res.* 4: 1420–1426 (2010)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.