Physicochemical Characteristics of Calamondin (*Citrus microcarpa*) from Hainan

Yi ZOU[1], Hongjian ZHANG[2], Lianhe ZHENG[1*]

1. Hainan Institute of Grain and Oil Science, Qionghai 571400, China; 2. Hainan Green Kumquat Food Technology Co., Ltd., Qionghai 571400, China

Abstract Some physicochemical characteristics of calamondin (*Citrus microcarpa*) from Hainan were determined in this study. The mean length, width, and thickness of calamondin were (2.763 ±0.262), (2.721 ±0.189), and (2.684 ±0.201) cm, respectively. The average values for geometric mean diameter, sphericity, fruit shape index, mass, surface area, volume, true density, bulk density and porosity were (2.721 ±0.196) cm, (98.79 ±5.149)%, (101.64 ±7.609)%, (11.18 ±2.149) g, (23.36 ±3.362) cm^2, (11.16 ±2.115) cm^3, (1.00 ±0.060) g/cm^3, 0.44 g/cm^3, and (56.40 ± 0.027)%, respectively. The mean edible part, juice rate, and soluble solids were (85.77 ±3.53), (48.36 ±4.20), and (7.86 ±0.09)%, respectively. The total flavonoids, pectin, limonin, reducing sugar and volatile oil contents of the peel were 1.00, 7.14, 0.51, 5.98, and 4.25%, respectively. The total flavonoids, pectin, limonin and reducing sugar contents of the pulp were 0.02, 5.04, 0.03 and 4.56%, respectively. The total flavonoids, total acid, reducing sugar and vitamin E contents of the juice were 0.11, 6.74, 0.37%, and 1.68 mg/kg, respectively, while the content of vitamins A, B_1, B_2, B_3, B_6, and D in the juice was very low. The seeds had limonin, reducing sugar and oil contents of 0.46, 1.39, and 30.46%, respectively. The vitamin B_2, B_3, B_6 and E contents of the fruit residue were 44.83, 19.31, 17.55, and 15.72 mg/kg, respectively. The amino acid profile of the residue included aspartic acid, glutamate, cystine, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine, with respective contents of 4.42, 1.81, 2.10, 0.78, 0.59, 0.20, 0.72, 0.16 0.67, 3.65 0.50, 0.67, 0.59, 1.00, 0.76, 0.57 and 0.91 g/kg.

Key words Hainan calamondin, Physical characteristic, Chemical characteristic, Nutrient content

1 Introduction

Calamondin (*Citrus microcarpa*) (Rutaceae: *Citrus*) is an economically important small fruit-bearing citrus tree, and a kumquat hybrid. It has traditionally been consumed in Hainan, China. Calamondin is commonly found in China (known locally as Qingjinju), Malaysia (kasturi lime), Indonesia (jeruk kasturi), Thailand (ma-nao-wan), Philippines (kalamondin, kalamunding, kalamansi, calamansi, limonsito or agridulce), Taiwan, China (gold lime)[1] and other Southeast Asian countries. The trees are typically 2 – 7.5 m in height and characterized by a spiny or thorny stem, leathery green leaves, and clusters of small aromatic white flowers. The fruit is round and small, with a typical diameter of 2 – 4 cm, and has a smooth and shiny skin. The unripe fruit is green and becomes yellowish orange upon ripening. The pulp is juicy and orange with a very sour taste, and usually contains numerous seeds[1]. The harvest yield is very high; and according to statistics, the annual output of one calamondin tree is typically 50 – 100 kg and the yield is 75 – 120 t/ha. The fruit is predominantly used for juicing and the fruit pulp is discarded as waste after processing, which has led to environmental pollution and wastage of resources.

Calamondin pomace contains an abundance of nutrients, including flavonoids, pectin, and limonin. Flavonoids from various citrus species have known nutritional benefits, particularly antioxidant and anti-inflammatory properties. The flavonoids in the calamondin samples were diosmin (5.99 μg/g), hesperetin (3.31 μg/g), hesperidin (0.42 μg/g), naringin (1.66 μg/g), and quercetin (0.53 μg/g)[2]. Phytosterols are generally dominated by the structure of cholesterol with one or two extra carbon atoms in the side chain. They can inhibit the absorption of endogenous cholesterol, leading to the cholesterol-lowering effect[3]. The calamondin samples contained sitosterol (1.57 μg/g), stigmasterol (4.52 μg/g), campesterol (4.43 μg/g), and amyrin (2.07 μg/g)[2]. Limonins have various nutraceutical properties, including anticancer, antioxidant, antibacterial, and antifungal effects. The calamondin samples were rich in limonins, with a content of 1.85 μg/g[2].

The appealing flavor and high nutritional value of calamondin has led to its use not only as a seasoning, but in the production of processing fruits powders, fruit slices, juice, jam and fruit wine. However, little research into the basic physical properties of calamondin has been previously conducted. Furthermore, there is no specialized mechanical equipment for the harvest and screening of calamondin, and the use of unspecialized equipment greatly reduces production efficiency and hinders the development of the calamondin industry. Therefore, the physical properties of the cal-

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Yi ZOU, master, engineer, engaged in research on food science and engineering.

* Corresponding author. Lianhe ZHENG, doctor, researcher, engaged in research on food science and engineering.

Editorial Office E-mail: asiaar@163.com

amondin need to be studied in detail.

Hainan is a province in South China with a tropical climate suitable for the production of citrus. The only area planted with more than 700 ha of calamondin is Haikou City, which reports an annual output of over 50 000 t. However, the lack of effective mechanical equipment has led to delayed picking and processing, which results in a large amount of waste.

This study aimed to present the first report on the physicochemical characteristic of Hainan calamondin. These findings are expected to contribute to the design of appropriate machinery and promote subsequent in-depth research and product development.

2 Materials and methods

2.1 Plant materials Fresh calamondin fruits were obtained directly from a vegetable market in Jiaji in Qionghai, Hainan. Samples were washed three times with water to remove dust, leaves, immature fruit and broken fruit, and blow dried at room temperature.

Fruit powder was obtained by separating the processed fresh fruit into peels, seeds, pulp and juice. The seeds, peels and pulp were dried at 50 ℃ and milled by multifunctional food processing machine (js80d-200).

2.2 Physical properties

2.2.1 Geometric mean diameter[4]. The length (L), width (W) and thickness (T) of the fruits were measured using a vernier caliper with a resolution of 0.02 mm. The geometric mean diameter (D) was calculated using formula (1):

$$D = (LWT)^{1/3} \quad (1)$$

2.2.2 Sphericity[4]: Sphericity was calculated using formula (2):

$$\text{Sphericity} = \frac{D}{L} \times 100\% \quad (2)$$

2.2.3 Fruit shape index[5]: Fruit shape index was calculated using formula (3):

$$\text{Fruit shape index} = \frac{L}{W} \times 100\% \quad (3)$$

2.2.4 Surface area[4]: Surface area of the fruit was calculated using formula (4):

$$\text{Surface area} = \pi D^2 \quad (4)$$

2.2.5 True density[4]: The mass (m) of fruit was measured using an electronic balance (±0.01 g) and the volume (v) was measured using the water displacement method. The true density (ρ_t) was calculated using formula (5):

$$\rho t = \frac{m}{v} \quad (5)$$

2.2.6 Bulk density[4]: The bulk density was determined by filling a 250 mL cylindrical container with calamondins and determining the mass (m_b). The bulk density (ρ_b) was calculated using formula (6):

$$\rho_b = \frac{m_b}{250} \quad (6)$$

2.2.7 Porosity[4]: Porosity of the fruit was calculated using formula (7):

$$\text{Porosity} = \frac{\rho_t - \rho_b}{\rho_t} \quad (7)$$

2.2.8 Edible percentage[5]: All fruit except seeds are edible. A sample of 10 – 20 fruits was weighed (±0.1 g). The peel (including the white cortex), stalk, seeds and other parts of each fruit were separated and weighed (±0.1 g). The edible percentage was calculated using formula (8):

$$\text{Percentage of edible part} = \frac{w' - w_{12}}{w'} \times 100\% \quad (8)$$

where w' is the total mass of the whole fruit (g) and w_{12} is the mass of the seeds (g).

2.2.9 Juice rate[5]: A sample of 10 – 20 fruits was weighed (±0.1 g). Each fruit was sliced in two parts, juiced using a juicer and pressed to remove all remaining juice, which was collected in a beaker. The peel, seeds and pomace (including the wall of the sputum and the wall of the juice) were weighed (±0.1 g). The juice rate was calculated using formula (9):

$$\text{Juice rate} = \frac{w' - (w_{11} + w_{12} + w_{14})}{w'} \quad (9)$$

where w' is the total mass of the sample fruit (g), w_{11} is the mass of the peel (g), w_{12} is the mass of the seeds (g) and w_{14} is the mass of the fruit residue (g).

2.2.10 Soluble solids[5]: The juice was filtered through two layers of gauze into a beaker and stirred well. A pipette was used to sample 1 – 2 drops of the juice, which was quickly transferred to the prism of an Abbe Refractometer and rested for a few seconds to reach 20 ℃. The light source was aligned, and the eyepiece was used while rotating the compensator spiral to achieve a clear light and dark boundary line. The scale pointer spiral was rotated to focus the light and dark boundary line on the "×" mark of the objective lens and the ruler. The sugar reading and temperature were recorded, and testing was conducted in duplicate or triplicate to determine a mean value.

2.3 Chemical properties

2.3.1 Determination of total flavonoids[6]. The total flavonoid content of the peel, pulp and juice was determined according to the China Industry Standard for Agriculture: *Determination of Total Flavonoids in Citrus Fruits and Derived products* (NY/T2010-2011).

2.3.2 Determination of total acid[7]. The total acid of fruit juice was determined according to the National Food Safety Standard: *Determination of Total Acid in Foods* (GB/T 12456-2008).

2.3.3 Determination of pectin[8]. The pectin content of peel, pulp and whole fruit was determined according to the China Industry Standard for Agriculture: *Determination of Pectin Content in Fruit and Derived Products* (*Spectrophotometry Method*) (NY/T 2016-2011).

2.3.4 Determination of limonin[9]: A limonin standard was formulated in anhydrous ethanol to a standard working solution of 130 μg/mL. Different volumes of the standard solution (0, 0.5, 0.8, 1.2, 1.5, and 2.0 mL) were added to 6 tubes, along with 2.0 mL of absolute ethanol and 5.0 mL of the reaction solution. The reaction solution was obtained by dissolving 125 mg p-dimethylaminobenzaldehyde and 0.5 mL of 0.9% ferric chloride solution in 100 mL of sulfuric acid-anhydrous ethanol mixed solution (Vsulfuric acid: Vanhydrous ethanol = 35:65). The mixed solution was shaken and rested for 30 min before taking an absorbance reading at 500 nm. Linear regression was performed to obtain standard curve. 10.0 g of peel and seed powder (accurate to 0.001 g) was placed into petroleum ether as a solvent to completely degrease the samples over 24 h using Soxhlet extraction. Samples were dried, wrapped in filter paper and placed in the Soxhlet extractor. Extraction with acetone solution was conducted for 6 h. The extracted solution was concentrated under low pressure, dissolved in dichloromethane, filtered, concentrated under low pressure second time, dissolved in absolute ethanol and diluted to 25 mL.

1.5 mL of sample preparation solution was diluted to 2.0 mL with absolute ethanol, the absorbance of the sample preparation solution was measured according to the standard curve method, and limonin concentration in the sample preparation solution was determined from the standard curve by the absorbance. The limonin concentration was used to calculate the limonin content according to formula (10):

$$\text{Content of analog in the raw material} = \frac{175\rho}{LM} \times 100\% \quad (10)$$

where ρ is limonin concentration determined using the standard curve (ug/mL), L is the volume of the sample solution(mL) and M is the sample quality (g).

2.3.5 Determination of reducing sugars[10]. The reducing sugar content of the peel, pulp, seeds and juice was determined according to the National Food Safety Standard: *Determination of Reducing Sugars in Foods*.

2.3.6 Determination of vitamin content[11-15]. The vitamin content of the fruit residue and juice was determined according to the National Food Safety Standard: Determination of vitamins A, D and E in food, vitamin B_1 in food, vitamin B_2 in food, vitamin B_3 (nicotinic acid and nicotinamide) in cosmetics (HPLC and HPLC-MS/MS) and vitamin B_6 in food.

2.3.7 Determination of amino acid content[16]. The amino acid content of the fruit residue was determined according to the National Food Safety Standard: *Determination of Amino Acids in Foods*.

3 Results and analysis

3.1 Physical properties of calamondin fruit The mass of calamondin varied widely from 7.78 to 15.19 g[mean = (11.18 ± 2.149) g], the volume ranged from 7.50 to 15.85 cm^3 [mean = (11.16 ± 2.115) cm^3] and the fruit ranged from 2.182 to 3.220 cm in length[mean = (2.763 ± 0.262) cm], from 2.400 to 3.054 cm in width[mean = (2.721 ± 0.189) cm], and from 2.100 to 2.980 cm in thickness (Table 1). The calamondin was bigger than the musk lime from Malaysia[1] but smaller than bergamot[17], Valencia oranges, interdonate lemons, red blush grapefruit and satsuma mandarins[18], indicating that the calamondin fruit is one of the smallest members of the citrus family. This insight is very useful when designing systems for the transportation, blanking and processing of the fruit.

Table 1 Physical properties of calamondin

Physical property	Minimum	Maximum	Mean value
Length//cm	2.182	3.220	2.763 ±0.262
Width//cm	2.400	3.054	2.721 ±0.189
Thickness //cm	2.100	2.980	2.684 ±0.201
Geometric mean diameter//cm	2.377	3.059	2.721 ±0.196
Sphericity//%	92.36	117.24	98.79 ±5.149
Fruit shape index//%	75.24	112.21	101.64 ±7.609
Surface area//cm^2	17.74	29.38	23.36 ±3.362
Volume//cm^3	7.50	15.85	11.16 ±2.115
Mass//g	7.78	15.19	11.18 ±2.149
True density//g/cm^3	0.83	1.10	1.00 ±0.060
Bulk density//g/cm^3	–	–	0.435 76
Porosity//%	47.44	60.34	56.40 ±0.027
Seed weight//g	0.92	3.3	1.82 ±0.451
Peel weight (plus fruit residue)//g	3.33	7.43	4.83 ±0.814
Edible percentage//%	74.16	91.89	85.77 ±3.530
Juice rate//%	38.40	55.20	48.36 ±4.200
Soluble solids//%	7.66	7.96	7.86 ±0.090

The geometric mean diameter varied from 2.377 to 3.059 cm [mean = (2.721 ± 0.196) cm], while the sphericity and fruit shape index varied from 92.36% to 117.24% and from 75.24% to 112.21%, respectively[mean sphericity = (98.79 ± 5.149)%; mean fruit shape index = (101.64 ± 7.609)%]. This indicated that the shape has a strong tendency toward a sphere and that the fruit has a high probability of rolling during processing.

The mean bulk density was 0.44 g/cm^3, while the true density and porosity ranged from 0.83 to 1.10 g/cm^3 and from 47.44% to 60.34%, respectively[mean true density = (1.00 ± 0.060) g/cm^3; mean porosity = (56.40 ± 0.027)%]. Density, true density, bulk density and porosity of calamondin were higher than bergamot[17] but lower than the musk lime from Malaysia[1]. This parameter is important in determining the storage capacity of the fruits, where the small size of the fruit likely contributes to the high bulk density[1].

The edible percentage varied from 74.16% to 91.89 % [mean = (85.77 ± 3.53)%], higher than the navel orange, ponkan and pomelo[19]. The proportion of the fruit's machinable section was large, which shows promise for reducing processing waste and environmental pollution with further experimental research and comprehensive implementation of specialized strategies.

The juice rate and juice soluble solids content were lower than the navel orange, ponkan and pomelo[19]. As the yield and edible percentage of calamondin is high and the price is low, giving the fruit a high research and processing value.

3.2 Chemical properties of calamondin peel The contents of total flavonoids, pectin, limonin, reducing sugars and volatile oils in the peel were 1.00%, 7.14%, 0.51%, 5.98% and 4.25%, respectively (Table 2). The total flavonoid content of the calamondin peel was higher than 'Miyagawa Wase' satsuma mandarin, Quzhou ponkan, 'Hamlin' orange, Changshanhuyou tangelo and tonkan[20] but lower than mandarin, lemon and bitter orange[21]. The limonin content of the peel was 2 – 4 times higher than many citrus fruits, such as lemon, grapefruits, orange and pomelo[22]. While the pectin content was lower than those of other citrus fruits, such as pomelo and mandarin[23].

Table 2 Chemical properties of calamondin peel

Chemical component	Content // %
Total flavonoids	1.00
Pectin	7.14
Limonin	0.51
Reducing sugars	5.98
Volatile oils	4.25

3.3 Chemical properties of calamondin pulp The contents of total flavonoids, pectin, limonin and reducing sugars in the pulp were 1.02%, 5.04%, 0.03% and 4.56%, respectively (Table 3). The limonin content was higher than many citrus fruits such as lemon, grapefruits, pomelo and orange[22].

Table 3 Chemical properties of calamondin pulp

Chemical component	Content // %
Total flavonoids	1.02
Pectin	5.04
Limonin	0.03
Reducing sugars	4.56

3.4 Chemical properties of calamondin juice The contents of total flavonoids, total acid, reducing sugars and vitamin E in the juice were 0.11%, 6.74%, 0.37% and 1.68 mg/kg, respectively. The total flavonoid content was lower than the kasturi lime[24]. The total acid of the juice was higher than that in calamansi juice from Malaysia, Philippines and Vietnam, while the reducing sugar content was lower[25].

Table 4 Chemical properties of calamondin juice

Chemical component	Content
Total flavonoids // %	0.11
Total acid // %	6.74
Reducing sugar // %	0.37
Vitamin A // mg/kg	Not detected (0.3)
Vitamin B_1 // mg/kg	Not detected (0.12)
Vitamin B_2 // mg/kg	Not detected (6.15)
Vitamin B_3 // mg/kg	Not detected (10.87)
Vitamin B_6 // mg/kg	Not detected (0.57)
Vitamin D // mg/kg	Not detected (0.02)
Vitamin E // mg/kg	1.68

3.5 Chemical properties of calamondin seed The contents of limonin, reducing sugars and volatile oils in the seed were 0.46%, 1.39% and 30.46%, respectively. The limonin content was 0.46% and lower than the chrysanthemum core, south orange, Xupu Ordinary Sweet Orange[22]. The oil content of 30.46% was lower than musk lime, tangelo, lime and bitter orange, but higher than grapefruit and tangerine[1].

Table 5 Chemical properties of calamondin seed

Chemical component	Content // %
Limonin	0.46
Reducing sugars	1.39
Volatile oils	30.46

3.6 Chemical properties of calamondin fruit residue The contents of vitamins B_2, B_3, B_6 and E in the fruit residue were 44.83, 19.31, 17.55, and 15.72 mg/kg, respectively. The amino acid profile included aspartic acid, glutamate, cystine, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine contents of 4.42, 1.81, 2.10, 0.78, 0.59, 0.20, 0.72, 0.16, 0.67, 3.65, 0.50, 0.67, 0.59, 1.00, 0.76, 0.57 and 0.91 g/kg, respectively.

Table 6 Some chemical properties of calamondin fruit residue

Chemical component	Content
Vitamin A // μg/100g	Not detected (30)
Vitamin B_1 // mg/kg	Not detected (0.22)
Vitamin B_2 // mg/kg	44.83
Vitamin B_3 // mg/kg	19.31
Vitamin B_6 // mg/kg	17.55
Vitamin D // mg/kg	Not detected (2)
Vitamin E // mg/kg	15.72
Aspartic acid // g/kg	4.42
Glutamate // g/kg	1.81
Cystine // g/kg	2.10
Serine // g/kg	0.78
Glycine // g/kg	0.59
Histidine // g/kg	0.20
Arginine // g/kg	0.72
Threonine // g/kg	0.16
Alanine // g/kg	0.67
Proline // g/kg	3.65
Tyrosine // g/kg	0.50
Valine // g/kg	0.67
Methionine // g/kg	0.59
Isoleucine // g/kg	1.00
Leucine // g/kg	0.76
Phenylalanine // g/kg	0.57
Lysine // g/kg	0.91

4 Conclusions

The means of length, width and thickness of the calamondin fruits were (2.763 ± 0.262), (2.721 ± 0.189) and (2.684 ± 0.201) cm, respectively. The mean geometric mean diameter, sphericity, fruit shape index, mass, surface area, volume, true density, bulk density and porosity were (2.721 ± 0.196) cm, (98.79 ± 5.149)%, (101.64 ± 7.609)%, (11.18 ± 2.149) g, (23.36 ± 3.362) cm^2, (11.16 ± 2.115) cm^3, (1.00 ± 0.060) g/cm^3, 0.44 g/cm^3 and (56.40 ± 0.027)%, respectively. The mean edible percentage, juice rate and soluble solids contents were $(85.77 \pm 3.53)\%$, $(48.36 \pm 4.20)\%$ and $(7.86 \pm 0.09)\%$, respectively.

The total flavonoid, pectin, limonin, reducing sugar and volatile oil contents of the peel were 1.00%, 7.14%, 0.51%, 5.98% and 4.25%, respectively. The total flavonoid, pectin, limonin and reducing sugar contents of the pulp were 1.02%, 5.04%, 0.03% and 4.56%, respectively. The total flavonoid, total acid, reducing sugar and vitamin E contents of the juice were 0.11%, 6.74%, 0.37% and 1.68 mg/kg, respectively, while the content of vitamins A, B_1, B_2, B_3, B_6 and D was very low. The limonin, reducing sugar and volatile oil contents of the seed were 0.46%, 1.39% and 30.46%, respectively. The vitamin B_2, B_3, B_6, and E contents of the fruit residue were 44.83, 19.31, 17.55, and 15.72 mg/kg, respectively. The amino acid profile of the residue included aspartic acid, glutamate, cystine, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine and lysine contents of 4.42, 1.81, 2.10, 0.78, 0.59, 0.20, 0.72, 0.16, 0.67, 3.65, 0.50, 0.67, 0.59, 1.00, 0.76, 0.57 and 0.91 g/kg, respectively.

The technical data obtained in this study can be used to further improve the study and processing of calamondins, and to aid the development of specialized harvesting and processing equipment. The chemical properties of the peel, pulp, juice and seeds further research and value-added product development focused on the calamondin fruit.

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