8 Recent Advances in Longan Polysaccharides and Polyphenols Extraction, Purification, Physicochemical Properties and Bioactivities

Yuzhu Mao and Hongshun Yang National University of Singapore National University of Singapore (Suzhou) Research Institute

Caili Fu National University of Singapore (Suzhou) Research Institute

Siyong You Fuzhou University

Hui Cao University of Shanghai for Science and Technology

Shaojuan Lai Guizhou University of Traditional Chinese Medicine

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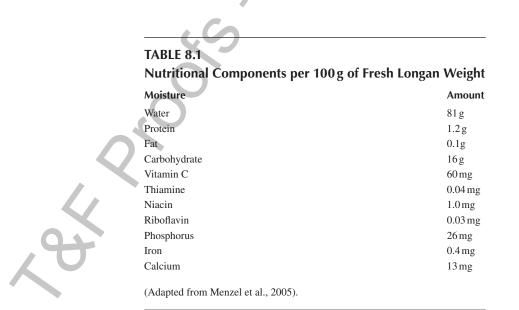
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8.1 INTRODUCTION

Longan (*Dimocarpus longan* Lour.) is a subtropical plant belonging to the *Sapindaceae* family and widely planted in China and southeast Asia countries (Jiang et al., 2002). Due to their desired taste and health benefits, longan fruits have gained popularity over the world (Jiang et al., 2002; Yi et al., 2012). With the growing market demand and the improvement of plant management, longan production has risen significantly (Jiang et al., 2002). In 2010, the total longan production in China reached 1.283 million metric tons, accounting for 60% of the yield over the world (Qiu, 2012). The increase in the production brings prospects for further utilization in the longan fruits.

Longans are nutritious fruits (Table 8.1) which are commonly eaten in fresh form; the edible section is white and sweet. Except for the succulent white aril, there is a thin leathery pericarp surrounding the edible part and the dark brown seed. The seed accounts for approximately 17% of the whole fruit, and it takes a significantly higher proportion when compared with longan pericarps (Jiang et al., 2013). Due to their positive effects on human health, the edible arils, seeds, and pericarps of longans have also been used as a traditional medicine for treating neural pain and swelling in Chinese medicinal formulations (Yang et al., 2011). However, seeds and pericarps of longans, which occupy about 15%–20% of the whole weight, are often directly discharged into estuaries, resulting in environmental pollution and waste of resources (Fu et al., 2015). Therefore, it



is worthwhile to extract and purify the bioactive components from the longan seeds and pericarps and then evaluate their contribution to human health.

Recently, some studies have focused on extracting bioactivity compounds from longan byproducts to add their commercial value. The results demonstrated that longan by-products contain high amounts of bioactive compounds, i.e., polysaccharides and polyphenols. These bioactive compounds found in longan by-products revealed that they possess a variety of health-functional effects, such as antitumor, immunomodulatory, and antioxidant activities and promoting healing of burn. Although longan bioactive compounds possess prominent therapeutic potential, and many studies have been carried out, the systematic review of their extraction, purification, properties, and bioactivities is very limited. Therefore, this chapter aims to summarize the existing extraction and purification methods of bioactive compounds in longan by-products, as well as their physicochemical properties, functional biological activities, and processing.

8.2 EXTRACTION, PURIFICATION, AND PHYSICOCHEMICAL PROPERTIES OF POLYSACCHARIDES AND POLYPHENOLS IN LONGANS

8.2.1 EXTRACTION, PURIFICATION, AND PHYSICOCHEMICAL PROPERTIES OF POLYSACCHARIDES IN LONGANS

Polysaccharides are a kind of vital macromolecule carbohydrate in the growth of organisms (Yang et al., 2009a). Recently, polysaccharides from longan by-products have attracted great attention in the field of functional food and pharmacology because of their high content, specific structure, ease of extraction, mild side effects, and excellent therapeutic potential. Usually, the biological activities of polysaccharides are closely correlated with their chemical characterization such as types, molecular sizes, features of glycosidic linkages, and ratios of constituent monosaccharides (Yang et al., 2008c). Therefore, a basic understanding of chemicophysical properties and biological activities of longan polysaccharides (LPs) will be helpful for their multifunctional applications.

8.2.1.1 Extraction of Longan Polysaccharides

The extraction methods of LPs based on different mechanisms have been widely investigated in recent years, mainly including hot water extraction, microwave-assisted extraction, ultrasonic-assisted extraction, high pressure-assisted extraction, and ultrahigh pressure-assisted enzymatic extraction. The different extraction methods and their schematic diagram are shown in Figure 8.1. The longan fruits (pulp, seeds, and pericarps) are ground into powder and extracted further. Then, the crude polysaccharide solution is preliminarily purified with a series of combined techniques, such as protein removal by Savage reagent and precipitation with alcohol.

The yields of LPs with different extraction methods are summarized in Table 8.2. It was found that each one has its superiority, and therefore, selecting the appropriate method is very important for the extraction of different LPs.

8.2.1.1.1 Hot Water Extraction

The hot water extraction is a simple, easy-to-control, and lower-cost method for obtaining crude polysaccharides from longan by-products. The primary process for hot water extraction includes pulp weighing, hot water extraction, filtration, evaporation and concentration, protein removal, alcohol precipitation, washing, and drying (Figure 8.1). As a conventional and widely used method, hot water extraction plays an essential role in the extraction of LPs. Yang et al. (2009b) successfully adopted hot water to extract the crude water–soluble polysaccharides from longan dry pericarps, and the yield of polysaccharides was 10.6 ± 0.7 mg galactose equivalents (GE)/g dry pericarps which accounted for $93.3\%\pm2.7\%$ of the dry extract. However, the hot water treatment cannot destruct the cell membrane thoroughly and thus might only extract extracellular polysaccharides from longan

Asian Berries: Health Benefits

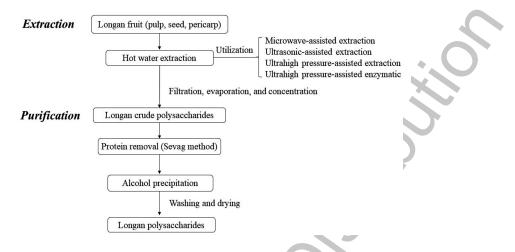


FIGURE 8.1 Extraction and purification of longan polysaccharides.

by-products. Also, the diffusion rate of polysaccharides in hot water is relatively slow and therefore leads to long processing time and low extraction efficiency.

8.2.1.1.2 Microwave-Assisted Extraction

Microwave-assisted extraction is an excellent extraction method for LPs, which combines the advantages of microwave and traditional solvent extraction methods. To increase the extraction efficiency and reduce the extraction time and the consumption of the solvent, many researchers have adopted the microwave-assisted extraction method to isolate the LPs. Yang et al. (2007) adopted the microwave pretreatment to prompt the cell rupture by destroying cytoderm. As a result, the extraction efficiency of LPs was significantly improved with a shorter time microwave of 60 s. Meanwhile, the direct heating of water molecules in solvent by microwave energy led to an increase in the solubility of polysaccharides due to the accelerated thermal movement of polysaccharide molecules. They also found that microwave time and power were essential because the long time (over 60 s) resulted in high solvent temperature and degradation of LPs.

8.2.1.1.3 Ultrasonic-Assisted Extraction

Recently, ultrasonic-assisted extraction method has received wide attention due to its easy operation, less energy input, and improved efficiency under mild reaction conditions. For example, Yang et al. (2008a) optimized the ultrasonic extraction condition of polysaccharides from longan pulp with the highest yield of $4.455\% \pm 0.093\%$ within 4.5 min. They found that the mechanism of ultrasonic extraction mainly involves two physical processes: the dissolution of the polysaccharides near the particle surface (rinsing) and the diffusion of polysaccharides to the liquid extract (slow extraction). Operational parameters also affected the yield of polysaccharide extraction directly (Yi et al., 2012). The long ultrasonic time could enhance the diffusion rate of polysaccharides to the liquid extract, leading to a high extraction recovery rate. However, the relatively high ultrasonic power may relate to the lower recovery rate of crude polysaccharides (polysaccharides from longan fruit pericarp [PLFP]) because ultrasonic wave may destroy glycosidic bonds and cause polysaccharides degradation.

8.2.1.1.4 Other Extraction Methods

Ultrahigh pressure–assisted extraction also has been investigated for the extraction of polysaccharides from longan pericarps, pulp, and seeds. However, Yang et al. (2009a) found that ultrahigh-pressure treatment decreased the yields of water-soluble polysaccharides, and no significant differences were observed compared with that of alkali-soluble treatment. The lowest yield

| TABLE 8.2 | | | | | |
|---|-----------|---|------------------------------------|---|--------------------------|
| Extraction and | Purificat | ion Methods of Longan | Polysaccharides | | |
| Extraction | Source | Condition | Yield | Purification | References |
| Hot water | Pericarp | Solid/liquid ratio 1:25g/mL; 55°C; 120min | 10.6±0.7 mg GE/g dry pericarp | Sephadex G-100 gel filtration column | Yang et al. (2009b) |
| | Pulp | Solid/liquid ratio 1:40 g/mL; 50°C; 90 min | 2.23% | Savage reagent; ethanol | Huang et al. (2019c) |
| Superfine grinding | Pulp | Superfine grinding for 2 min | 3.16% | precipitation | |
| Superfine grinding- assisted enzymatic | Pulp | Hydrolyzed with cellulase (140 U/g; pH 5.0) | 5.07% | | |
| Microwave | Pulp | Solid/liquid ratio 1:15 g/mL; 700 W, 60 s; 100°C, 7 h | 9.0 mg/g dry pulp | TCA method; ethanol precipitation | Yang et al. (2007) |
| Ultrasonic | Pericarp | Solid/liquid ratio 1:25 g/mL; 120 W; 20 min; 70°C | 5.49 mg GE/g dw | Savage reagent | Yang et al. (2008a) |
| | Pericarp | Solid/liquid ratio 1:25g/mL; 120 W, 22 min, 60°C; 241 W, 18 min, 51°C | 5.47±0.16 mg/g dw | Savage reagent | Yang et al. (2008b) |
| | Pulp | Solid/liquid ratio 1:25 mL/g; 680 W; 4.5 min; 50°C | 4.455%±0.093% | N/A | Zhong and Wang (2010) |
| | Pulp | Solid/liquid ratio 1:40 mL/g; 250 W; 55°C–80°C; pH 5.0; 2h (at 80°C) | LP1 2.14%; LP2 6.85%; LP3 9.77% | Anion exchange resin D301-F | Yi et al. (2012) |
| | Seed | Solid/liquid ratio 14.3 mL/g; 268.7 W; 35 min | 5.51 mg GE/g dw | Sephadex G-100 gel column | Jiang et al. (2013) |
| Ultrahigh pressure | Pericarp | Solid/liquid ratio 1:15 mL/g; 25°C; 500 MPa; 30 min | 6.4±0.6 mg/g | Savage reagent; ethanol precipitation | Yang et al. (2009a) |
| Ultrahigh pressure– assisted enzymatic | Pulp | Solid/liquid ratio 1:10 g/mL, 6 min, 407 MPa; hydrolyzed with cellulase (42 mL/g, pH 5.0, 1.7 h) | 8.55%±0.42% | Savage reagent; ethanol precipitation | Bai et al. (2017) |

GE, galactose equivalents; LP, longan polysaccharide; TCA, trichloroacetic acid.

 $(6.4\pm0.6 \text{ mg/g})$ was obtained at 500 MPa. One possible reason is that the high pressure destroyed the glycosidic bonds of LPs and then led to the decrease of polysaccharides yield. It is clear that the extraction conditions of LPs by high-pressure treatment need to be further optimized.

Enzyme-assisted extraction is an effective method to improve the extraction rate of the polysaccharides because the enzyme could specifically hydrolyze the components of the cell membrane. Bai et al. (2017) compared the effects of three different extraction methods on the extraction efficiency of LPs. They found that the yield of ultrahigh pressure-assisted enzymatic extraction was $8.55\% \pm 0.42\%$, which was higher than yields of the hot water method ($4.81\% \pm 0.23\%$) and ultrahigh-pressure method ($6.62\% \pm 0.14\%$). It is evident that the enzymatic treatment increased the yield of LPs. Huang et al. (2019c) investigated the effect of superfine grinding and superfine grinding-assisted enzymatic extraction on the yield of LPs with the comparison of hot water extraction. They found that the LPs from the superfine grinding-assisted enzymatic method (LP-SE) contained the most sugar among LPs from other methods and lower content of uronic acid than that of superfine grinding (LP-S).

8.2.1.2 Purification of Longan Polysaccharides

The polysaccharides from longan by-products usually do not exist singly but are conjugated with other components, such as protein, pigment, and other chemicals. To obtain purified LPs, a series of combined techniques have been used to remove these nonpolysaccharide impurities, such as precipitation with alcohol, protein removal by Savage reagent, phenol removal through macroporous resin, and decolorization by activated carbon and resin.

At the first step, Sevag method is usually adopted to remove the protein from longan crude polysaccharides (Navarini et al., 1999; Yang et al., 2009b), and then the polysaccharides were precipitated by anhydrate ethanol as the common purification process. In the Sevag method, a mixture of chloroform and butanol (5:1, v/v) was added to crude polysaccharide solution and then mixed and centrifuged to remove the denatured proteins (Okuda et al., 1999). The advantage of this method is that the treatment condition is mild and will not cause the degeneration of polysaccharides. However, this method needs to repeat the extraction over four times, which may result in high sample loss.

To analyze the structures of LPs, the column chromatography method has been widely used to further remove impurities. Yi et al. (2010) selected anion exchange resin (D301-F) to purify the longan crude polysaccharides. The results showed that the retention rate of polysaccharide was 85.75% with the decolorization rate of 90.21% and protein removal rate of 73.12% under optimal conditions. Yi et al. (2012) adopted an anion-exchange DEAE52-cellulose column (50 cm×2.6 cm) to purify the crude polysaccharide. Sephadex G-100 gel column chromatography was adopted to collect the polysaccharide (Jiang et al., 2013; Yang et al., 2009b). Although column chromatography has the advantage of high purity of polysaccharides obtained, this methods has several limitations, such as relatively long extraction time, low extraction capacity, and separation rate. Therefore, a feasible, efficient, economic, and easy method for LP purification is still greatly demanded.

8.2.1.3 Physicochemical Properties of Longan Polysaccharides

To clarify the physicochemical basis of LPs activity, the following information is needed: (1) molecular weight and monosaccharide composition of LPs, (2) glycosidic linkage and sequence of sugar residues, (3) sugar ring configuration, and so on. Therefore, we summarized the molecular weight, monosaccharides composition, and glycosidic linkages of LPs in Table 8.2. Because of differences in sources and isolation protocols, a wide range of LPs have been obtained with different physicochemical properties and bioactivities.

Molecular weight is one of the most important physical characteristics of LPs. As shown in Table 8.3, the polysaccharides found in longan pulp had a wide range of molecular weight (13.8 kDa -9.64×10^3 kDa). The molecular weight of crude polysaccharides (PLFP) from longan pericarps was about 420 kDa (Yang et al., 2009b).

The monosaccharide compositions of polysaccharides from longan pulp, seeds, and pericarps have been analyzed by gas chromatography (GC) and GC–mass spectroscopy (GC-MS). The results showed that LPs are mainly heterogeneous polysaccharides, and the most extensively found monosaccharide compositions involve glucose, ribose, and arabinose. Less frequently found components of LPs were xylose, mannose, and rhamnose. The polysaccharides from different materials have different monosaccharide compositions. Yang et al. (2009b) obtained PLFP by hot water method and found that PLFP was consisted of L-arabinofuranose with α -form and D-glucopyranose, D-galactopyranose, and D-galacturonic acid with β -form. Yang et al. (2008c) isolated a neutral polysaccharide (LPS-N) consisting of xylose and glucose residues, an acidic polysaccharide (LPS-A1) consisting of rhamnose, xylose, arabinose, galactose, and an acidic monosaccharide (LPS-A2) including rhamnose from the longan pulp using high-performance liquid chromatography (HPLC). Further analysis indicated that the LPS belongs to β -type heteropolysaccharide with pyran group. Jiang et al. (2013) analyzed the monosaccharide compositions of LSP1, LSP2, and LSP3 polysaccharides from longan seeds and found that LSP1 was consisted of Fuc, Fru, Xyl, Man, Ara, Gal, and Glc in the ratio of 1:1.19:1.61:3.51:6.16:9.95:41.99, LSP2 was consisted of Rha, GalA, Man, Ara, Fru,

| TABLE 8.3 Physicoch | .3 hemical P | TABLE 8.3 Physicochemical Properties of Longan | of Longan Polysaccharides | | | |
|------------------------|-----------------|---|---|--|-----------------------------------|--------------|
| Compound | | < | | | | |
| Name | Source | Mw (kDa) | Monosaccharides Composition | Glycosidic Linkages | Bioactivities | References |
| LPS-N | Pulp | 13.8 | Xylose and glucose (1:1.9) | N/A | N/A | Yang et al. |
| LPS-A1 | Pulp | 1382 | Rhamnose, xylose, arabinose, galactose (1:1.64:4.33:2.28) | | | (2008c) |
| LPS-A2 | Pulp | 571 | Rhamnose | | | |
| LPI | Pulp | 14.59 | Glucose, mannose, and arabinose | $(1 \rightarrow 6)$ - α -D-Glcp, $(1 \rightarrow 4)$ - β -D-Manp, | Immunomodulatory | Yi et al. |
| | | | | $(1 \rightarrow 5) - \alpha - L - Araf$ | activities | (2012) |
| LPII | Pulp | 68.34 | Glucose, mannose, arabinose, galactose, and ribose | $(1 \rightarrow 6)$ - α -D-Glcp, $(1 \rightarrow 5)$ - α -D-Araf, and β -D-Galp | | |
| LPII | Pulp | 107.4 | Arabinose, rhamnose, galactose, and ribose | $(1 \rightarrow 4)$ - β -D-Rhap, and $(1 \rightarrow 5)$ - α -L-Draf | | |
| LPIV | Pulp | 5,282 | Xylose, ribose, mannose, and glucose | N/A | | |
| LPI | Pulp | 110 | Glc, GalA, Ara, Gal (5.39:1.04:0.74:0.21) | \rightarrow 4)- α -D-Glcp-(1 \rightarrow 4)- α -D-GalpA-(1 \rightarrow 4)- α -D- | Antitumor and | Meng et al. |
| | | | | Glcp-(1→4)-β-D-Glcp-(1→ and →2)-β-D-Fruf-(1→2)-L-sorbose-(1→ | immunomodulatory activities | (2014) |
| H-H- | Pulp | 238.243 | Arabinose and glucose | \rightarrow 3)- α -l-Araf-(1 \rightarrow , \rightarrow 3,6)- β -d-Galp-(1 \rightarrow , | Prebiotic activities | Huang et al. |
| | | | | α -l-Rhap(l \rightarrow , \rightarrow 4)- β -D-Glcp(l \rightarrow | | (2019c) |
| LP-S | Pulp | 227.770 | Arabinose and galactose | $\rightarrow 3)-\alpha \text{-l-Araf-}(1 \rightarrow 3, 6)-\beta \text{-d-Galp-}(1 \rightarrow ,$ | | |
| | | | | α-1-πιαρ(1→, →4)-p-D-Gaip-(1→ | | |
| LP-SE | Pulp | 189.943 | Arabinose and galactose | $ \rightarrow 3)-\alpha-l-Araf-(1 \rightarrow, \rightarrow 3,6)-\beta-d-Galp-(1 \rightarrow, \alpha-l-Rhap(1 \rightarrow, \rightarrow 5)-\alpha-L-Araf-(1 \rightarrow) $ | | |
| LPD2 | Pulp | 9.64×10^{3} | Arabinose, mannitus, glucose, and galactose | $(1 \rightarrow 4)$ - β -Glc and $(1 \rightarrow 6)$ - β -Man | Immunological | Rong et al. |
| | | | (0.25:0.49:1:0.5) | * | activity | (2019) |
| LSP1 | Seed | N/A | Fuc, Fru, Xyl, Man, Ara, Gal, and Glc (1:1.19:1.61:3.51:6 | \rightarrow 4)-Ara-(\rightarrow 1, \rightarrow 6)-Gal-(\rightarrow 1, Glc-(\rightarrow 1, | Radical scavenging | Jiang et al. |
| | | | .16:9.95:41.99) | \rightarrow 6)-Glc-(1 \rightarrow | activity | (2013) |
| LSP2 | Seed | N/A | Rha, GalA, Man, Ara, Fru, Fuc, Glc, and Gal (1.00:1.07:6 .53:8.30:8.99:9.62:29.25:34.86) | \rightarrow 6)-Gal-(\rightarrow 1, Glc-(\rightarrow 1 and 6)-Glc-(1) | | |
| LSP3 | Seed | N/A | Man, Ara, GalA, Glc, and Gal (1:1.40:1.90: 6.65:7.70) | \rightarrow 3)-Gal-(\rightarrow 1, Glc-(\rightarrow 1, 6)-Glc-(1 \rightarrow | | |
| PLFP | Pericarp | 420 | L-arabinofuranose, D-glucopyranose, D-galactopyranose, | \rightarrow 5)-L-Araf-(1 \rightarrow , \rightarrow 6)-D-Glcp-(1 \rightarrow , \rightarrow 3)-D- | Antiglycated activity Yang et al. | Yang et al. |
| | | | D-galacturonic acid (32.8%, 17.6%, 33.7%, 15.9%) | GalpA-(1→, →6)-D-Galp-(1→, 2:1:1:1:1 | ドー | (2009b) |
| PLFP, polys | accharides fro | PLFP, polysaccharides from longan fruit pericarp. | t pericarp. | | | (|
| | | | | | | 5 |
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Fuc, Glc and Gal in the ratio of 1.00:1.07:6.53:8.30:8.99:9.62:29.25:34.86, and LSP3 was consisted of Man, Ara, GalA, Glc, and Gal in the ratio of 1:1.40:1.90: 6.65:7.70.

The chemical structure of polysaccharides from longan pulp, seeds, and pericarps was also reported, mainly including sequence of monosaccharides, configuration of glycosidic linkages, and type of glycosidic linkages. Meng et al. (2014) investigated the chemical structure of longan pulp polysaccharides (LP1) and found that LP1 consisted of a backbone of \rightarrow 4)- α -D-Glcp-(1 \rightarrow 4)- α -D-Glcp-(

The different extraction processes also have different effects on the physicochemical properties of polysaccharides. Huang et al. (2019c) studied the polysaccharide extracted from longan pulp by using hot water (LP-H), ultrafine crushing (LP-S), and ultrafine enzymatic crushing (LP-SE). Three LPs contained similar glycosidic linkage of \rightarrow 3)- α -l-Araf-(1 \rightarrow , \rightarrow 3,6)- β -d-Galp-(1 \rightarrow and α -l-Rhap(l \rightarrow , while they each contained specific glycosidic linkage of \rightarrow 4)- β -d-Glcp(l \rightarrow , \rightarrow 4)- β -d-Galp-(1 \rightarrow and \rightarrow 5)- α -l-Araf-(1 \rightarrow in LP-H, LP-S, and LP-SE. LP-SE exhibited the prebiotic activities. The structural features and activities of LP provide the insights into LP application in functional food and medical industry.

8.2.2 STRUCTURAL MODIFICATION OF THE LONGAN BIOACTIVE POLYSACCHARIDES

The bioactivities of polysaccharides are directly or indirectly restricted by their molecular structure, such as types of glycosidic bond, ways of connections, and monosaccharide compositions. Therefore, it is difficult to compare their activities with those of synthetic drugs. Some methods have been employed to modify the properties of LPs in gaining the desired bioactivities, e.g., chemical modification, physical modification, and biological modification (Figure 8.2).

The chemical modification is the most common method which can change the structures of longan polysaccharides by introducing substituent groups. Chemical modification methods include methylation, sulfidation, Maillard reactions (MRs), and others. Yang et al. (2010) modified the PLFP by attaching a sulfate group to a hydroxyl group of monosaccharides. They found that the

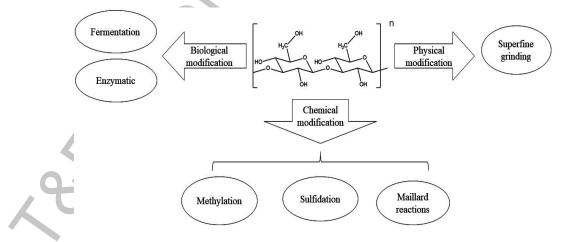


FIGURE 8.2 The scheme of longan polysaccharides modification.

methylation led to a decrease in the scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity of PLFP, while the scavenging superoxide anion free radical activity of PLFP decreased with the increase of methylation degree. The results also illustrated that the hydroxyl groups of monosaccharide units were vital for the radical scavenging activity of PLFP. Jiang et al. (2014) sulfated polysaccharides (LP1-S) using the sulfuric acid (sulfuric acid and butanol complex in the ratio of 3:1). The Fourier transform infrared results showed a symmetrical C-O-S vibration associated with a SO_4 group and the presence of the bonds of S=O, indicating that the sulfation reaction had occurred. Compared with LP1, the LP1-S showed higher antiproliferative activity against human nasopharyngeal carcinoma HONE1 cells, which may result from the sulfate group. These results may be useful for developing antitumor medical and functional foods. Yi et al. (2019) selected Lys to modify LPs by MRs and then investigated the properties of MR-modified LPs (MLPs). They found that particle-like MLPs contained 94% fractions with an Mw less than 7.07 kDa; by contrast, network-like MLPs contained 45% fractions with an Mw larger than 264.1kDa. In addition, MLPs have strong free radical scavenging ability and immune stimulation effect to macrophages, but weak inhibition ability to the growth of cancer cells. The results showed that MR was a good polysaccharide modification method with excellent biological properties.

Fermentation modification is a common biological modification method and has gained wider acceptability as it proceeds in milder conditions. Huang et al. (2019a) modified the LPs by using lactic acid bacteria fermentation method. With the comparison of LP, the modified longan polysaccharides showed a good promotion effect on the proliferation of *Leuconostoc mesenteroides* and *Lactobacillus casei*.

Physical modifications always rely on extra energy. Huang et al. (2019c) obtained polysaccharide (LP-SE) from longan pulp through ultrafine enzymatic crushing. Compared with LP-H and LP-S, the yield, sugar content, solubility, arabinose, and mannose content of LP-SE increased, whereas the apparent viscosity, particle size, molecular weight, and glucose content decreased.

8.2.3 EXTRACTION, PURIFICATION, AND PHYSICOCHEMICAL PROPERTIES OF LONGAN POLYPHENOLS

Phenolic compounds have the ability to remove reactive oxygen species and regulate cellular signaling processes such as cell growth, differentiation, and many other cellular characteristics (Rangkadilok et al., 2005). In the past two decades, around 30 phenolic compounds have been identified from longan seeds and pericarps. The total polyphenol content extracted from longan seeds was up to approximately 80.90 mg/g dry weight, and the content in pericarps was about 52.9 mg/g dry matter (Chen et al., 2015; He et al., 2009; Sudjaroen et al., 2012). These phenolic compounds found in longan by-products have been confirmed to have potentially effective for human health and prevention of various chronic diseases. Therefore, the agriculture waste of longans such as seeds and pericarps is considered as a rich source of polyphenols, which may be used as functional ingredients or natural antioxidants (Chen et al., 2015; Zheng et al., 2009).

Longan polyphenols can be divided into three different categories according to the chemical structures of the aglycones (Tsao, 2010), namely, phenolic acids, flavonoids, and other polyphenols (Figure 8.3). The phenolic acids are nonflavonoid constituents and can be further divided into two principal types, namely, benzoic acid and cinnamic acid derivatives, according to their C1–C6 and C3–C6 backbones. The gallic acid (GA), ethyl gallate, and 1- β -O-galloyl-D-glucopyranose from longan seeds (Zheng et al., 2009) and protocatechuic acid from pericarps (Bai et al., 2019) all belong to benzoic acids. Flavonoids in longan by-products can be further divided into a number of subgroups and mainly including flavonols, flavanols and proanthocyanidins. Quercetin in longan pericarps belongs to flavonols, and quercetin-3-O-rhamnoside belongs 3-hydroxy derivative flavonols (Bai et al., 2019; Soong & Barlow, 2005). The other polyphenols in longan seeds and pericarps include methyl brevifolin carboxylate, brevifolin, corilagin, isoscopoletin, monogalloyl-glucose, monogalloyl-diglucose, and so on.

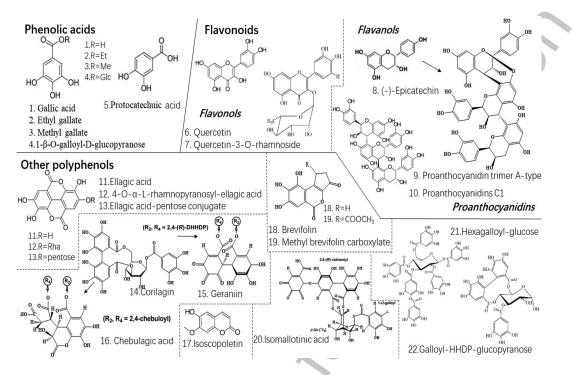


FIGURE 8.3 The structure of longan polyphenols. (Adapted from Zheng et al., 2009; Bai et al., 2019; Soong & Barlow, 2005; Sudjaroen et al., 2012.)

The total phenols in longan by-products were usually extracted by methanol (Sudjaroen et al., 2012). Recently, the ultrasonic-, microwave-, and high pressure–assisted extraction methods have been adopted for a more efficient extraction of polyphenols from longan seeds and pericarps. Prasad et al. (2009b) optimized the condition of high-pressure extraction (HPE) for longan polyphenols, and the optimum conditions were 50% ethanol, the solid-to-liquid ratio of 1:50 (w/v), 500 MPa, and holding time 2.5 min. To further analyze the molecular structure of longan polyphenols, the column chromatograph method is often used to purify the crude polyphenols, such as Toyopearl HW-40 and Sephadex LH-20 (Sudjaroen et al., 2012).

8.2.3.1 Proanthocyanidins

Proanthocyanidins are a kind of major bioflavonoid compounds in longan by-products, which are the biomacromolecules formed by catechin and epicatechin because an acid-catalyzed cleavage of the polymeric chains produces anthocyanidins (Tsao, 2010). Due to their prominent antioxidant activity, the structure and physicochemical properties of longan pericarp proanthocyanidins (LPPs) have been investigated by ¹H and ¹³C nuclear magnetic resonance spectra. As illustrated in Figure 8.4, the LPPs are composed of dominant procyanidin units (catechlin/epicatechin) and a small amount oligomer gallates containing one galloyl group. The successive procyanidin units are coupled each other by A-type and B-type linkages: A-type structure in which monomers are linked through C2–O–C7 or C2–O–C5 bonding and B-type structure in which monomers are linked mainly through C4–C6 or C4–C8 (Tsao, 2010).

The structure of LPPs was further characterized by electron spray ionization-mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry (MALDI-TOF-MS) (Fu et al. 2015). For LPPs, A-type PC trimer (m/z 863) was the most abundant followed by B-type PC trimer (m/z 865) and dimmer (m/z 577) (Figure 8.5a). After m/z 2300, no obvious proanthocyanidin signal was observed, suggesting that LPPs were almost

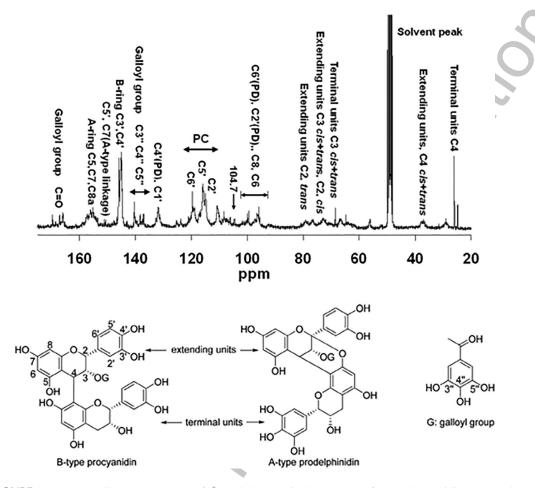


FIGURE 8.4 Upper, C-NMR spectrum of LPPs; below, typical structures of proanthocyanidins (Fu et al., 2015). LPPs, longan pericarp proanthocyanidins; NMR, nuclear magnetic resonance.

composed of oligomeric proanthocyanidins (Figure 8.5b). ESI-MS and MALDI-TOF-MS results clearly showed that A-type PC trimer and B-type PC dimer and trimer in LPPs were the major individual proanthocyanidins.

The proanthocyanidins extracted from longan seeds and pericarps showed strong antioxidant activity, oxygen radical scavenging capacity (ORAC), and α -amylase inhibitory activity. The anti-oxidant activity of proanthocyanidins in longans was as high as 1.23×10^4 mol TE/g (Fu et al., 2015). In addition to the proanthocyanidins found in longan seeds and pericarps, the proanthocyanidins derived from longan flower (LFP) have also shown ability to inhibit the AGAR proliferation and implant-independent growth of two colorectal cancer cells in vitro. The levels of epidermal growth factor receptor (EGFR) and Akt phosphorylation were reduced with 200 µg/mL LFP treatment (Chung et al., 2018). It is clear that proanthocyanidins in longan are effective natural antioxidants that can remove free radicals.

The proanthocyanidins in longan by-products have been isolated using solvent extraction (Fu et al., 2015) or ultrasonic-assisted extraction methods (Yang et al., 2011) due to their prominent antioxidant activity. Currently, the solvent extraction method is still widely used because it has been well established and is easy to perform (Figure 8.6). Fu et al. (2015) adopt 70% acetone-extracted proanthocyanidins from longan pericarps with yield of 269 mg/kg. Ho et al. (2007) obtained proanthocyanidins from longan flowers with twice extraction, and the yield was 37.42%.

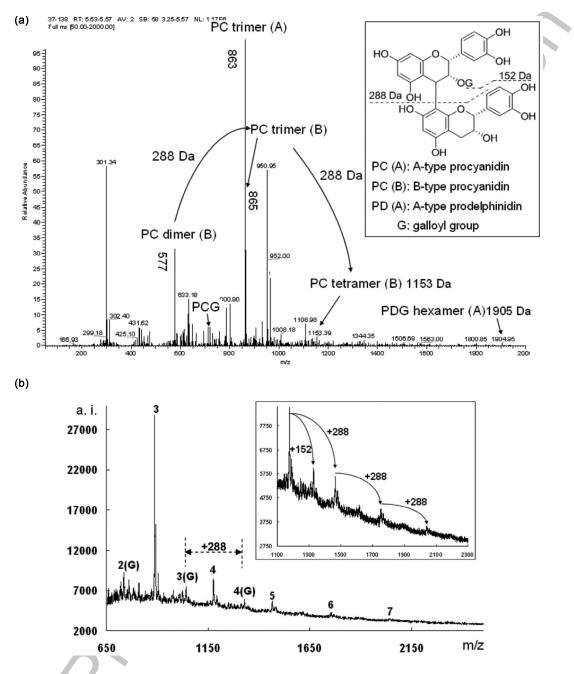


FIGURE 8.5 ESI-MS recorded in the negative ion mode of LPPs (A) ESI-MS with negative ion mode; (B) MALDI-TOF-MS recorded in the $[M+Na]^+$ mode (Fu et al., 2015). ESI-MS, electron spray ionization-mass spectrometry; LPPs, longan pericarp proanthocyanidins.

There are few studies on the purification of proanthocyanidins in longan by-products. Huang et al. (2014) reported the purification process of proanthocyanidins in longans by Sephadex LH-20 gel column. The impurities are the obstacles to the purification of proanthocyanidins and the identification of their structures. Therefore, efficient and more affordable methods to purify high-quality proanthocyanidins on a large scale are needed.

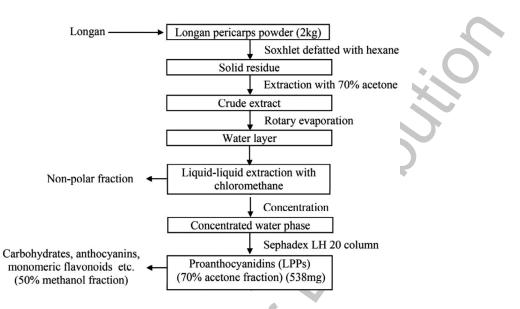


FIGURE 8.6 The process of extracting and purifying longan proanthocyanidins (Fu et al., 2015). LPPs, longan pericarp proanthocyanidins.

8.2.3.2 Corilagin

Corilagin (β -1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-D-glucose) is a main polyphenolic compound in longans with a basic structure of geraniin and chebulagic (Sudjaroen et al., 2012), which is a water-soluble inverse gallic tannin (Prasad et al., 2009a). Longan peel and seed contain a high level of corilagin (3.7–8.6 mg/g dw), whereas the dried pulp only contains a low level of corilagin (0.08–0.15 mg/g dw) (Rangkadilok et al., 2005).

The extraction methods of corilagin including hot water extraction, ultrasonic extraction, and HPE. Prasad et al. (2009a) compared three extraction methods and found that the corilagin content extracted by using HPE (9.65 mg/g dry matter) was higher than that extracted by using ultrasonic extraction (7.91 mg/g dry matter) and conventional hot water extraction (2.35 mg/g dry matter). The optimal conditions of HPE for corilagin extraction were 2.5 min, 30°C, and under 500 MPa.

Column chromatography is the most commonly used purification method for corilagin. Rangkadilok et al. (2005) adopt a reversed-phase HPLC (RP-HPLC) to purify the corilagin in longan fruits. The fixed phase was Phenomenex Luna C_{18} reversed-phase column (250 mm×4.6 mm, 5 µm). The mobile phase was water/tetrahydrofuran/TFA (98:2:0.1) (solvent A) and acetonitrile (solvent B) in a linear gradient. The column temperature was set at 25°C with a detection wavelength of 270 nm. He et al. (2009) purified the polyphenolic compounds using Sephadex LH-20 column chromatography and found the phenolic compounds separated fast and efficient on this gel column. Li et al. (2018b) isolated and purified corilagin using a combination of macroporous resin column chromatography, reversed-phase column chromatography, and gel filtration chromatography. 0.60 g of corilagin was obtained from 1 kg of longan seed powder with the yield of 0.06% and purity of 96.2%.

As one of the main phenolic constituents in longan seeds and pericarps, corilagin exhibits prominent antioxidant, anti-inflammatory, hypoglycemic, anticancer, and antitumor activities. Corilagin has also good effects on nerve cells, liver, and cardiovascular system against atherosclerosis and other aspects (Li et al., 2018b). Rangkadilok et al. (2007) investigated the antioxidant activities of corilagin in longan seed and pulp and found that the corilagin in longan seed showed the scavenging radical and the tyrosinase inhibitory activities. Chen et al. (2015) analyzed the structure– activity relationships of phenolic compounds extracted from longan seeds. They found that the

2-hydroxy-3-methoxycaffeic acid 5-O- β -D-glucopyranoside and 3'-O-methyl-4'-O-(4-O-galloyl- α -L-rhamnopyranosyl) ellagic acid (EA) are the main active components, not corilagin. Li et al. (2018b) found that longan seeds had the highest corilagin content (542.15±10.30 µg/g) by HPLC analysis. Also, the corilagin+ginsenoside Rh2 (Rh2) and corilagin+5-fluorouracil (5-FU) showed effects on ovarian cancer cells and cancer-preventing activities. Bai et al. (2019) found that the scavenging rate of DPPH free radicals by corilagin was 71.8%±0.5% and the inhibition rate of •OH was 75.9%±0.3%. In addition, corilagin also showed strong cytotoxicity with IC₅₀ value of 28.8±1.2µM.

8.2.3.3 Flavonoids

Flavonoids are important secondary metabolites naturally occurring in longans, which are found not only in pulps (Zhang et al., 2018), seeds (Huang et al., 2012), leaves (Zheng et al., 2018), and flowers (Ho et al., 2007) but also in commercial products such as honey (Chaikham & Prangthip, 2015). The flavan nucleus is the basic skeletal structure of flavonoids which consists of two aromatic rings linked by a three-carbon chain (C6–C3–C6), which are labeled A, B, and C (Figure 8.7). Flavonoids can be grouped into different classes. Major classes of flavonoids in longan are anthocyanins, flavan-3-ols, flavonos, flavanones, and flavanols (Tsao, 2010).

Extraction is the main step for the isolation and recovery of flavonoids from longan by-product before analysis. The extraction methods of longan flavonoids are summarized in Table 8.4. It is clear that solvent extraction is the most common extraction method for longan flavonoids due to its simple and wide applicability. Ethanol, methanol, and acetone with different proportions of water are often used for extracting flavonoids. Ho et al. (2007) and Yang et al. (2010) obtained flavonoids from longan flowers with solvent extraction, and the total yield was approximately 156.0 mg/g and 68.32 mg

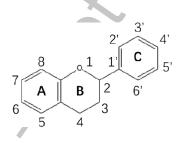


FIGURE 8.7 The basic skeletal structure of flavonoids.

TABLE 8.4

Extraction Method of Flavonoids in Longans

| Method | Materials | Solvent | Condition | Yield | References |
|-----------------------|---------------|--------------|---|----------------------------------|---------------------------|
| Solvent extraction | Dried flowers | 95% ethanol | Liquid/solid ratio, 20 mL/g (twice); room temperature; 24 h | 156.0 mg/g | Ho et al. (2007) |
| Solvent extraction | Dried flowers | Water | Liquid/solid ratio, 2.5% (w/v); temperature, 100°C; 30 min | 68.32 mg CE/ 100 mL | Yang et al. (2010) |
| Ultrasonic extraction | Seeds | 50% methanol | Temperature, 71°C; 70 min | 163.88 mg/g | Huang et al. (2012) |
| Solvent extraction | Seeds | 59% acetone | Temperature, 71°C; 2.3 h | 43.67 mg/g | Guan et al. (2014) |
| Ultrasonic extraction | Leaf powder | 95% ethanol | Liquid/solid ratio, 40:3; 2.5 h | 0.775 µg/mL (after purification) | Zheng et al. (2018) |

CE/100 mL, respectively. Guan et al. (2014) optimized the solvent extraction method of longan seed flavonoids and found that the yield was dependent on the extraction conditions, e.g., extraction time, solvent concentration, and temperature. In recent years, sonication-assisted extraction method has been developed for the extraction of longan flavonoids. Huang et al. (2012) extracted flavonoids from longan seeds using sonication-assisted extraction method with Box–Behnken response surface experiment design. The ultrasonic extraction rate of flavonoids was high (1.6388%). Zheng et al. (2018) extracted the flavonoids from longan leaves by using ultrasonic method at the condition of 40:3 mL/g of liquid ratio, the volume fraction of 95% ethanol, and extracting time of 2.5 h. After purification, the extraction yield of total flavonoids was up to 0.775 μ g/mL.

Longan by-products contain a series of similar flavonoids, with a slight change in functional groups or substitution patterns. Therefore, chromatography is the most powerful technology for the purification of longan flavonoids. Huang et al. (2011) separated the flavonoids in longan seeds using macroporous adsorption resin and then optimized their isolation condition. Zheng et al. (2018) applied macroporous resin AB-8 to purify total flavonoids in longan leaves with 70% ethanol eluent. The results showed that the recovery of flavonoids was $0.775 \,\mu$ g/mL, and the total flavonoids content of longan leaves reached 77.49%.

Flavonoids have showed a wide application potential in medicinal, nutraceutical, and cosmetic products as an indispensable ingredient due to their antimutagenic, antiinflammatory, antioxidative, and anticancer activities (Panche et al., 2016). Bioflavonoid-derived natural sources have relatively low molecular weight, can be quickly absorbed by the human body, and then easily pass the blood-brain barrier, which has unique advantages in the treatment of cerebrovascular diseases. Zhang et al. (2018) investigated total flavonoid contents of 24 longan cultivars in southern China. They found that the cellular antioxidant activity (CAA) of longan pulp had a positive correlation with phenolic and flavonoid contents. Zheng et al. (2018) analyzed the antitumor effects of crude flavonoids from longan leaves. The results indicated that extracts of flavonoids from longan leaves can effectively inhibit the growth of tumor cells and have the potential to be a new type of natural antitumor drugs.

8.2.3.4 Other Polyphenolic Fractions

In addition to proanthocyanidins, corilagins, and flavonoids, longan seeds and preicarps have other polyphenols, e.g., GA, EA, brevifolin, methyl brevifolin carboxylate, ethyl gallate, and unknown polyphenol compounds. Several different types of phenolic constituents in longans might form a multilevel system, which can protect against oxidations. Solvent extraction, ultrasonic-assisted extraction, and high pressure–assisted extraction are common methods to obtain these phenolic substances, and the purification was usually performed by the column chromatography (Prasad et al., 2009); He et al., 2009; Zheng et al., 2009).

GA and EA were common polyphenols in longans and have been widely studied for their pharmacological activities such as antiplasmodial, antimicrobial, antioxidant, and anticancer activities (Soong & Barlow, 2005; Sudjaroen et al., 2012). He et al. (2009) investigated 12 Chinese longan cultivars and found GA and EA were not the main substances that lead to the antioxidation effect of longan pericarps. Zheng et al. (2009) found eight polyphenols in longan seeds; especially, EA and GA exhibited scavenging activity toward DPPH radicals with SC₅₀ value of 0.80–5.91 µg/mL and toward superoxide radicals with SC₅₀ value of $1.04-7.03 \mu g/mL$. Li et al. (2016) found that EA and GA in longan fruit shell can reduce the concentration of postpranational hyperglycemia by inhibiting the activity of α -glycosidase and β -galactosidase. Therefore, extracts of longan nut shell may be a potential strategy for early treatment of postprandial hyperglycemia.

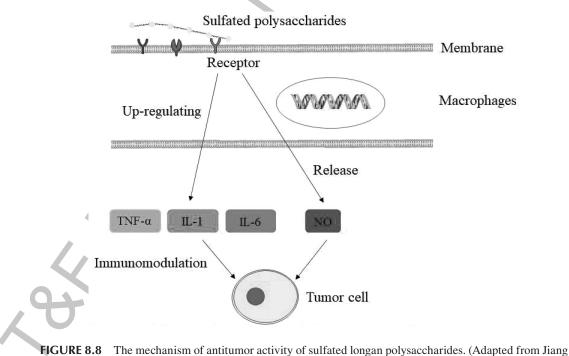
8.3 BIOACTIVITIES OF LONGAN EXTRACTIONS, POLYSACCHARIDES, AND POLYPHENOLS

Longans and their by-products contain a significant number of bioactive compounds, such as polysaccharides and polyphenols. Due to their positive effects for human health, the bioactivity of polysaccharides and polyphenols in longan has attracted great attention. Therefore, the multiple bioactivities and health benefits of longan extractions are summarized and compared in detail below.

8.3.1 ANTITUMOR ACTIVITY

Some studies have demonstrated that LPs exert strong antitumor activity with different mechanisms. The currently accepted antitumor mechanism for LP can be summarized as follows: (1) inducing the apoptosis of tumor cells; (2) improving the immune response to tumors; and (3) preventing the oncogenesis by the oral administration of polysaccharides. Zhong et al. (2010) investigated the inhibitory effect of longan pulp polysaccharide (UELP) on S180 tumor mice models. The result indicated that UELP with medium dose (200 mg/kg) and low dose (100 mg/kg) had significant inhibitory effect on S180 cancer cells when compared with control group and maximum-dose group (400 mg/kg). Meng et al. (2014) obtained a new water-soluble polysaccharide (LP1) from longan pulp and found that LP1 inhibited 40% of SKOV3 cells and 50% of HO8910 tumor cells. LP1 also significantly stimulated the activity of the murine macrophages and strengthened the B and T cell proliferation, which has the potential to be a natural antitumor agent. Jiang et al. (2014) demonstrated that the LP1 and its sulfated derivative (LP1-S) exerted significantly antiproliferative activity against human nasopharyngeal carcinoma HONE1 cells in vitro. This may be because the LP and LP-S enhance murine macrophages activity and upregulate the release of nitric oxide (NO), interleukin 6 (IL-6), IL-1 β , and tumor necrosis factor-alpha (TNF- α) in macrophages (Figure 8.8). Meanwhile, LP1-S exhibited higher antitumor activity than LP1, possibly due to the existence of the sulfate group.

The longan polyphenols have also significant influence on ovarian carcinoma. Li et al. (2018b) extracted the corilagin from longan seeds and demonstrated that this polyphenol could effectively inhibit the proliferation of ovarian cancer SKOv3ip cells and Hey cells when synergized with ginsenoside Rh2 and 5-fluorouracil. The phenolic substances from longan also indicated other antitumor activities. Zheng et al. (2018) isolated flavonoids from longan leaves and found that the



et al,. 2014.)

| Antitumor Compounds in Longans and Bioactivities | | | | | | |
|--|------------|-------------------------------|---|------------------------|--|--|
| Compound Name | Source | Tumor Models | Effects | References | | |
| UELP | Dried pulp | S180 tumor mice models | DTH response; macrophage phagocytosis; ConA-stimulated splenocyte proliferation | Zhong et al. (2010) | | |
| LP1 | Dried pulp | HONE1 tumor cells | Lymphocytes proliferation; macrophages activity; NO, IL-1β, IL-6, TNF-α ↑ | Jiang et al. (2014) | | |
| LP1-S | Pulp | SKOV3 & HO8910 tumor cells | Macrophages activity; B and T cell proliferation | Meng et al. (2014) | | |
| Corilagin | Seed | SKOv3ip & Hey cells | Tyrosinase ↓; scavenging nitrite; antioxidation; blocking nitrosamine synthesis | Li et al. (2018b) | | |
| Flavonoids | Leaf | A549 cell | Cell shrinkage; nuclear fragmentation; apoptosis | Zheng et al. (2018) | | |
| | | | | | | |

TABLE 8.5 Antitumor Compounds in Longans and Bioactivities

flavonoids induced shrinkage, nuclear fragmentation, and apoptosis of the A549 cells. These results showed that the longan polyphenols had certain immunomodulatory effects and antitumor effects (Table 8.5).

8.3.2 IMMUNOLOGICAL ACTIVITIES

Immunomodulation is considered an important function of natural bioactive substances, which can act as immunomodulators. The polysaccharides from longans have exhibited prominent immunological activities via participating in cell differentiation, adhesion, and proliferation. Yi et al. (2011) investigated immunoregulatory activity of polysaccharide-protein complex (LP3) from longan pulp by establishing a mouse immune hypoxia model. The results showed that LP3 could significantly improve the splenocyte proliferation and natural killer cell cytotoxicity. The LP3 also promoted the antibody secretion against chicken red blood cell (CRBC), interferon- γ (INF- γ), and IL-2 in serum. It is clear that the polysaccharide and protein complex from longan pulp exhibited a potential as immunotherapeutic adjuvants.

The immunological activities of LPs were also assessed on T cells, B cells, and macrophagedependent immune system responses. Yi et al. (2012) investigated the effect of polysaccharides (LPI–IV) from longan pulp on immunoregulatory activity in vitro. They found that the LPII–IV can enhance lymphocytes and B cells proliferation, but not T cells. Rong et al. (2019) analyzed the polysaccharide (LPD2) from longan pulp and found that it induced macrophage activation partly in mice. LPD2 also increased NO and IL-6 production of macrophage via the TLR2- and TLR4-mediated MyD88/IRAK4-TRAF6 signaling pathways. These results showed that the LP could improve humoral immunity and cellular immunity in mice. However, the mechanism of immunological activities in human still needs further studies.

8.3.2 RANGING OF BURN

There are few studies on the treatment of burns using longan extraction. Zhao et al. (2019) studied the ability of longan seed polyphenolic extracts as deep-burn wound-healing material. The results showed that the high-dose polyphenolic extracts could induce the formation of new blood vessels and capillaries and then regenerate new dermal tissues and reshape the newly formed tissues during the proliferation period. The utilization of longan polyphenolic extracts in treatment of burns needs to be further investigated.

8.3.3 INHIBITORY ACTIVITIES AGAINST AGLUCOSIDASE, A-AMYLASE, AND ACETYLCHOLINESTERASE

Diabetes mellitus (DM) is a kind of chronic diseases which are characterized by hyperglycemia due to deficiencies in insulin action. The α -amylase is related to the dietary starch hydrolysis that can adjust the glucose in the blood. The α -glucosidase can affect the starch breakdown and intestinal absorption (Lordan et al., 2013).

Polyphenols have been proved having inhibitory effects on α -amylase. Fu et al. (2015) found that LPPs can strongly inhibit the α -amylase activity in a dose-dependent manner (Figure 8.9a) and act as a noncompetitive inhibitor of the enzyme (Figures 8.9b and c). Moreover, LPPs exhibited a strong antioxidant activity of $1.23 \times 10^4 \mu$ M TE/g as measured by oxygen radical scavenging capacity and α -amylase inhibitory activity with IC₅₀ of 0.075 mg/mL. Li et al. (2016) found that the inhibiting effect of phenolic compounds in longan shells on α -glucosidase and β -galactosidase activities was the result of the interaction of various components. The 50% ethanol and water extracts had strong inhibitory activities on α -glucosidase. The GA and EA were identified as the major phenolic compounds from longan shells against α -glucosidase and β -galactosidase activities with the dried extraction from longan pulp can inhibit the α -amylase and α -glucosidase activities with the values of 97.56% and 88.58%, respectively. Acetylcholine is vital in brain memory function, and the acetylcholinesterase (AChE) inhibitor, which elevates acetylcholine levels, is one of the most efficient components used in the treatment for Alzheimer's disease (Schliebs & Arendt, 2006). Bai et al. (2017) extracted polysaccharides from longan pulp from different methods including hot

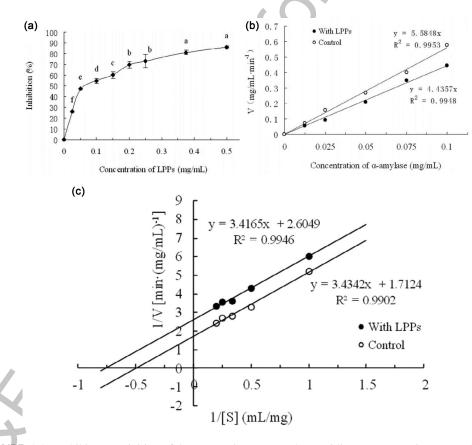


FIGURE 8.9 Inhibitory activities of longan pericarp proanthocyanidins (LPPs) against α -amylase. (a) Relationship between inhibition ratio and concentration of LPPs; (b) inhibitory kinetics curves of LPPs against α -amylase; (c) Lineweaver–Burk plot for the mode of inhibition of α -amylase by LPPs. (Adapted from Fu et al., 2015.)

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water (LP-H), ultrahigh pressure (LP-U) or enzymatic treatment (LP-E), and ultrahigh pressureassisted enzymatic method (LP-UE). LP-UE was found to have the highest inhibitory activity on the AChE.

8.3.4 ANTIOXIDANT ACTIVITIES

Oxidation phenomena have been implicated in many illnesses, and therefore, the antioxidant capacity of longan polyphenols has been evaluated through DPPH and hydroxyl radical scavenging assay. The polyphenols extracted from longan seeds (Chen et al., 2015) and pericarps (Sun et al., 2007) exhibited higher antioxidant properties, mainly including proanthocyanidins, corilagins, EA, GA, and unknown compounds. Chen et al. (2015) found the main phenolic compounds that contribute to the potent antioxidant activity of longan seeds were 2-hydroxy-3-methoxycaffeic acid 5-O- β -D-glucopyranoside and 3'-O-methyl-4'-O-(4-O-galloyl- α -L-rhamnopyranosyl) EA. Sun et al. (2007) compared the antioxidant activities of two phenolic compounds from longan pericarps and found that the 4-O-methylgallic acid had higher reducing power and DPPH-, hydroxyl radical-, and superoxide radical scavenging activities than (–)-epicatechin.

The extraction method had significant effects on the antioxidant activities of longan polyphenols. Pan et al. (2008) compared the effect of different extraction methods on the antioxidant activities of longan polyphenols. Compared with the conventional extraction and ultrasonic-assisted extraction methods, high pressure–assisted extraction had higher phenolic content and exhibited prominent scavenging activity toward DPPH and superoxide anion radicals (Prasad et al., 2009b, 2010). Yang et al. (2008b) isolated PLFP by ultrasonic treatment extraction method and then investigated their DPPH radical scavenging activity. The results showed that the optimal conditions to obtain the strongest antioxidant activity of PLFP were 120 W, 22 min, and 60°C, as well as 241 W, 18 min, and 51°C, respectively.

8.3.5 OTHER HEALTH BENEFITS

Several studies focused on other health benefits of longan bioactive substances, such as memoryenhancing, antiobesity, and hypolipidemic activities. Park et al. (2010) observed and compared the aqueous extract of longan fruit (ELE) and piracetam on learning and memory abilities in mice by the passive avoidance method. ELE exhibited beneficial effects on learning and memory by brain-derived neurotrophic factor expression and immature neuronal survival. Zhu et al. (2016) investigated the effects of LP on articular chondrocytes in rabbits by observing the effects on the proliferation, morphology, survival rate, glycosaminoglycan synthesis, and cartilage-specific gene expression. The results showed that LP could effectively promote the growth of chondrocytes and the secretion and synthesis of chondrocytes and extracellular matrix. Yang et al. (2010) found that the polyphenol-rich longan flower water extract (LFWE) exhibited antiobesity and hypolipidemic activities. The total flavonoids and phenolic acids from longans reduced the pancreatic lipase activity and fatty acid synthase (FAS) gene expressions in rats and inhibited the peroxisome proliferator– activated receptor-alpha (PPAR- α) gene expressions (Figure 8.10). Therefore, flavonoids from longans worked with phenolic acids to protect rats from obesity and hyperlipidemia.

8.4 EFFECTS OF PROCESSING ON BIOACTIVITIES AND QUALITY OF LONGAN PRODUCTS

Different processing techniques have been applied in longan products to obtain desired goals, such as extending shelf life, increasing bioactivity, rich nutrition, and flavor aroma. Li et al. (2017) investigated the effects of ultraviolet irradiation and refrigeration on shelf life and antioxidant levels of longans. Three forms (whole fruit, peeling, and nucleating) of fresh longans were treated by



Asian Berries: Health Benefits

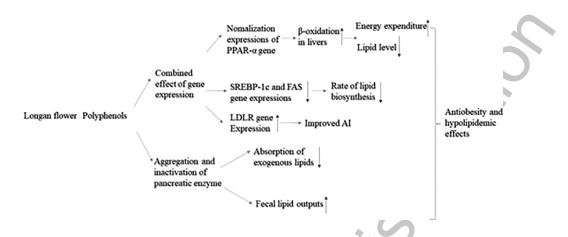


FIGURE 8.10 The mechanism of antiobesity and hypolipidemic effects of longan flower water extract in hypercaloric dietary rats. (Adapted from Yang et al., 2010.)

freezing and UV-C irradiation, respectively. After processing, the deterioration rate, phenolic compounds, and antioxidant capacity were quantified. The results showed that the shelf life of fruits was prolonged by refrigeration and UV-C irradiation treatment. However, UV-C caused the nutritional degradation of refrigerated longans and reduced the antioxidant capacity of longans. Li et al. (2018a) explored the effects of different lights on flavonoid metabolism through structural genes and transcription factors analysis. The blue light promoted the accumulation of flavonoids and the genes expression, while the synthesis of rutin was inhibited.

Coating is another method for extending the shelf life. Chitosan is a common compound adopted as a coating material. Lin et al. (2020) analyzed the effect of chitosan treatment on the quality and storage time of longans. They found that the chitosan treatment could keep the quality of longans and extend their shelf life due to the lower respiration rate, lower pericarp cell membrane permeability, pericarp browning index, pulp breakdown index, fruit disease index, and weight loss. The content of bioactive substances was also increased after chitosan coating, such as anthocyanins, flavonoids, and total phenols.

Fermentation is also a common processing method for longan products because this treatment method improved the bioactive compounds in longans. Huang et al. (2019a) investigated the effects of lactic acid bacteria fermentation in different fermentation time on polysaccharide substances and antioxidant activities in longan pulp. The different fermentation time significantly changed the yield, molecular weight, and monosaccharide composition of longan pulp. The fermentation also affects the physicochemical properties of longan pulp with increased prebiotic activities. Huang et al. (2019b) found that compared with longan pulp (LP), fermented longan pulp (LP-F) exhibited stronger stimulation on macrophages secretion of IL-6 and NO, as well as better proliferation of *Leuconostoc mesenteroides* and *Lactobacillus casei*.

Smoking is a conventional method to dry the fresh longans. Yang and Chiang (2019) analyzed the flavor profile of dried longans after 104-h smoking. The results indicated that smoking resulted in 42 volatile compound variations, of which 3-methyl-1-butanol, 3,7-dimethyl-1,3,6-octatriene, hydroxy butanone, and 1-octen-3-ol perceived aroma for longans. The optimum smoking process time for dried longans was 72 h.

8.5 CONCLUSIONS AND FUTURE PERSPECTIVES

In recent years, the reports on active components of longans have increased significantly, mainly focused on their extraction, purification, and bioactivity. Polysaccharides and polyphenols have been confirmed as the primary active components in longans with a broad range of health effects,

such as antitumor, antioxidant, immunostimulating, and inhibitory activities against α -glucosidase and α -amylase. Due to their prominent bioactivities, the potential applications of longan extracts in functional food and medicine have attracted growing interest. However, there are still many challenges to be explored. (1) The application of longan extraction in functional food has not been investigated extensively. (2) The relationship between structural features of longan components and their bioactivity is still unclear. (3) Molecular mechanisms of bioactivities of longans and their extracts are not clear. Therefore, it is important to investigate the structure–activity relationship of longan extracts for further exploring their molecular mechanisms and applications in functional foods.

ACKNOWLEDGEMENTS

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