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Polyphenols of mulberry fruits as multifaceted compounds: Compositions, metabolism, health benefits, and stability—A structural review

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A B S T R A C T

Mulberry (*Morus* sp.) fruits provide high levels of anthocyanins, quercetin glycoside, and chlorogenic acid. Recently, mulberry's polyphenols and their functionalities are spotlighted in different *ex vivo* and *in vivo* studies. Meanwhile, the deficiency of systematic knowledge on the health effects and polyphenols composition greatly hinders the development of mulberry as a sustainable fruit. This review briefly summarises the polyphenol compositions, metabolism, health benefits, and their stability in different mulberry production steps. These claimed health effects include anti-oxidative, anti-cancer, anti-diabetes, anti-obesity, and other related effects. However, although the current evidence is promising, further clinical studies are needed to evaluate the role of mulberries' polyphenols to support human health. Besides, the mechanisms by which they confer the health benefits, the bioavailability studies on mulberry's polyphenols are also scarce. We compile the research findings from the available literatures within the last two decades, and we also suggest some future perspectives in this review.

1. Introduction

Mulberry (*Morus alba*), which belongs to the genus Morus and the family Moraceae, is broadly cultivated in different climatic zones (Butkhup, Samappito, & Samappito, 2013). Mulberry is an economical and widespread woody plant which used in pharmaceuticals, food industry, and farming nowadays. There are approximately 24 mulberry species and more than 1000 cultivars predominately originated in south-east Asian countries. Among them, black (*M. nigra* L.), red (*M. rubra* L.), and white (*M. alba* L.) are the three foremost mulberry species grown all over the world (Huo, 2002). Mulberry fruit is a popular fruit due to its high nutrients content and delicious savor (Table S1). It was shown that the mulberry fruits are an excellent source of polyphenols (Ercisli & Orhan, 2008; Lin & Tang, 2007; Wu, Tang et al., 2013). Recently, UHPLC-ESI-MS allowed the tentative identification of 55 individual bioactive polyphenols include flavonols, flavanones, anthocyanins, phenolic acids, and other low-molecular-weight phenolics in eight Spanish mulberry species (Mena et al., 2016; Natić et al., 2015). However, the multiplicity of the species may also be the basis of the mulberry polyphenols' dissimilarity, which needs to be further explored.

Apart from being a food item, mulberry fruit has been used in the folk medicine for thousands of years, especially in China, for treating sore throat, anemia, and tonsillitis (Singh, 1997). Modern researches revealed that, due to the rich content of bioactive polyphenols, mulberry fruits were confirmed to possess wide scopes of bioactivities, such as free-radical scavenging (Du, Zheng, & Xu, 2008; Liang et al., 2012b; Wang & Hu, 2011), antidiabetic (Wang, Xiang, Wang, Tang, & He, 2013), neuroprotective (Song et al., 2014), antifatigue (Jiang et al., 2013), antiatherosclerosis (Chen et al., 2005), anti-thrombotic (Yamamoto et al., 2006), immune-modulating (Lee et al., 2013), and others.

Mulberry fruits are commonly consumed as fresh fruits; however, mulberries in general, are soft fruits with a perishable behavior. Thus, the mulberry-based products, such as juice (Liu, Xiao, Chen, Xu, & Wu, 2004; Singhal, Khan, Dhar, Baqual, & Bindroo, 2010; Wang, Du, Cui, Xu, & Li, 2016), jam (Tomas et al., 2016), liquor (Tang et al., 2008), muffin (Lee & Choi, 2011; Valentino, 1984), and pekmez (Sengül, Fatih Ertugay, & Sengül, 2005) are previously manufactured. Also, anthocyanins, which was efficiently isolated from mulberry fruits, could be used as a natural food colorant (Liu et al., 2004). Processing, unfortunately, can alter and damage the mulberry polyphenols and reduce their bio-accessibility (Cavalcanti, Santos, & Meireles, 2011). Therefore, novel techniques are needed to enhance the retention of their

Abbreviations: AOA, antioxidant activity; C3G, cyanidin 3-*O*-glucoside; C3R, cyanidin 3-*O*-rutinoside; TAC, total anthocyanin content; TF, total flavonoids; TPC, total phenolic content

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polyphenols during different processing and storage conditions (Medina-Torres et al., 2013; Moser et al., 2017).

Compared with other berries, there is a lack of systematic knowledge on the bioactive polyphenols of mulberries that hinders their expansion as functional fruits and/or healthy food ingredients. This review briefly summarises the recent outcomes in the mulberry polyphenols, including the composition, bioaccessibility, and its' potential health-promoting effects. The available potential mechanisms of the supposed bioactivities, and the stability of mulberry polyphenols in various forms, and the further prospective are also fleetingly discussed herein. We aim to systematically overview the current knowledge on mulberries' polyphenols. We do believe that this kind of ordered knowledge will guide the researchers to extend their research and explore the mulberry fruits as functional foods in different foodstuffs.

2. Polyphenols composition

Polyphenols are non-nutritive, naturally secreting, and biologically active substances originated in the plant kingdom. Mulberry fruits provide a wide range of polyphenols such as phenolic acids, flavanol derivatives, and anthocyanins. Indeed, there are two sequences of the polyphenol content in mulberry fruits: phenolic acid derivatives > flavonol derivatives > anthocyanins (Veberic, Slatnar, Bizjak, Stampar, & Mikulic-Petkovsek, 2015) or anthocyanins > flavonol derivatives > phenolic acid derivatives (Sánchez-Salcedo, Mena, García-Viguera, Martínez, & Hernández, 2015). The reported polyphenols in mulberry fruits and their corresponding functionalities vary considerably according to the cultivars, climatic, agricultural practices, and processing conditions. There are several studies reporting the polyphenol compositions of mulberries and their functionalities as comprehensively narrated later. However, a systemized literature review on polyphenols profiles of mulberry fruits are still scarce.

2.1. Anthocyanins

Anthocyanins are the predominant polyphenols (up to ~3000 mg kg^{-1} fw) in mulberry fruits. And mulberry fruits have higher anthocyanins content than blueberry, blackberry, blackcurrant, and redcurrant (Fig. 1) (Veberic et al., 2015). Anthocyanins are mainly responsible for the color of mulberry fruits (60%) (Gerasopoulos & Stavroulakis, 1997), and also greatly contributed to its' health-promoting effects. Specifically, cyanidin 3-*O*-glucoside (C3G), cyanidin 3-*O*-rutinoside (C3R), pelargonidin 3-*O*-glucoside, and pelargonidin 3-*O*-rutinoside are the main anthocyanins in mulberry fruits (Jin, Yang, Ma, Cai, & Li, 2015) (Fig. 2). Among the four detected anthocyanins, C3G showed the highest content, accounting for 65.67 (*M. atropurpurea Roxb* cv *Da10*.) to 100% (*M. alba* Linn. cv *Baiyuwang*) of TAC (Jin et al., 2015). Other kinds of anthocyanins such as cyanidin-3-sophoroside and cyanidin-3-glucorutinoside are sometimes identified in *M. nigra* cv. *Mavromournia* (Gerasopoulos & Stavroulakis, 1997), which reflecting the genetic multiplicity.

Generally, the colored species are somewhat superior to that of non-colored species, and a 122% difference in the content was reported (Sánchez-Salcedo et al., 2015; Özgen, Serçe, & Kaya, 2009). The utmost anthocyanins content (0.57–1.88 mg C3Gg^{-1} dry weight (dw) was observed in the black mulberry, whereas it lacked (0.01 mg C3Gg^{-1} dw) in the white mulberry (Sánchez-Salcedo et al., 2015; Özgen et al., 2009). Similarly, Chen et al. (2016) found that the total anthocyanins of 5 Chinese mulberry cultivars including *Zhongshen* 831, *Da* 10, *Zhongshen* 5801, *Ding*33, and *Taiwanguosang* is less than 900 μg g^{-1} fw and no anthocyanin was observed in the white cultivars, such as *Zhenzhubai*, *Jiguihua*, and *Baiyuwang*. Bao et al. (2016) reported that the quantity of C3G ranged from 1.25 to 3.35 g kg^{-1} fw in *M. atropurpurea Roxb* cv *Guangdong* and *J33*, respectively; and C3R varied from 0.25 to 1.50 g kg^{-1} fw in *M. multicaulis Perr*. cv *Guangdong* and *Hongguo*, but traces anthocyanin was detected in white mulberry cultivars. Consistent with visual color change, total anthocyanins markedly increase as the fruit ripens from white/light red to black stages (Bae & Suh, 2007). The dissimilarity geographical conditions also resulted in different accumulation of anthocyanins (Ercisli & Orhan, 2007; Lee et al., 2004).

2.2. Flavonoids

Undoubtedly, flavonol glycosides are the second major mulberry polyphenols, with the contents fluctuating from 55.62 to 432.38 mg kg^{-1} fw in different species (Jin et al., 2015). Favonoids are found mostly in glycosylated form, and they have complex flavonol glycosides profiles including 13 quercetin derivatives, 5 kaempferol derivatives, and O-methylated flavonol-analogs, such as rhamnetin and isorhamnetin. Quercetin glycosides are the richest one (39.39–415.19 mg kg^{-1} fw); followed by quercetin 3-*O*-rutinoside (28.69–388.32 mg kg^{-1} fw), and kaempferol 3-*O*-glucoside (3.55–47.80 mg kg^{-1} fw) in 10 Chinese cultivars (Jin et al., 2015). Levels of quercetin glycoside are reported to with a surge as the fruit ripens from white (0.00002 mg kg^{-1} dw) to black (0.0011 mg kg^{-1} dw) stages (Sánchez-Salcedo et al., 2015). Quercetin 3-*O*-rutinoside (rutin) is the second flavonol glycoside with more than 100 mg kg^{-1} fw in the Chinese cultivars, which was higher than that in Serbian cultivars (30.43–77.28, 0.65–13.46, and not detectable – 0.433 mg kg^{-1} fw) in black, red, and white species, respectively (Song et al., 2009; Yang, Yang, & Zheng, 2010; Zhang, Han, He, & Duan, 2008).

The flavonoids variation in different breed of mulberries is significant. Chinese mulberries, therefore, have higher total flavonoid contents (0.0024 mg kg^{-1}) than Korean mulberries (0.0006 mg kg^{-1}) (Sánchez-Salcedo et al., 2015). The content of kaempferol 3-*O*-glucoside was found in mulberry to be 3.55–47.80 mg kg^{-1} fw in *M. atropurpurea* cv *Yuefenshen* and *Taiwanguosuang* (Jin et al., 2015) but it was not found in the Slovene mulberry cultivars.

2.3. Phenolic acids

Phenolic acids of mulberries range from 29.52 (*M. alba* Linn.) to 175.64 mg kg^{-1} fw (*M. atropurpurea Roxb*) (Zhang et al., 2008), with hydroxybenzoic and hydroxycinnamic acids derivatives being the main components (Sánchez-Salcedo et al., 2015). Mulberry hydroxy benzoic acids derivatives (0.48–2.55 mg g^{-1} dw) include protocatechuic, vanillic, and *p*-hydroxybenzoic acids with the values of 0.41–2.22, 0.06–0.14 and 0.01–0.19 mg g^{-1} dw, respectively (Sánchez-Salcedo et al., 2015). Caffeic, *p*-coumaric, ferulic, and *m*-coumaric acid are main hydroxycinnamic acids found in mulberries (Jin et al., 2015; Memon, Memon, Luthria, Bhanger, & Pitafi, 2010). These phenolic acids commonly occur in conjugated forms of esters and glycosides, but rarely exist as free acids (Zhao, 2007). Esters make up 53.1% of total phenolic acids, whereas glycosides and free acids account for 43.6 and 3.3%, respectively (Zadernowski, Naczk, & Nesterowicz, 2005). Glycosidic and ester of chlorogenic and protocatechuic acids are the most common forms in mulberries, especially the black cultivars (Memon et al., 2010). Dissimilar results were reported before (Radojković, Zeković, Vidović, Kočar, & Mašković, 2012), probably due to the genetic difference. Previous reports showed that the protocatechuic acid has anti-inflammatory and antihepatotoxicity effects (Hsu et al., 2012). Meanwhile, some studies suggested that the chlorogenic acid, which accounts > 90% of the phenolic acids in mulberry fruits (Jin et al., 2015; Mena et al., 2016), is environment-depended, explaining the different results in different breeding areas (Gundogdu, Muradoglu, Sensoy, & Yilmaz, 2011; Natić et al., 2015; Sánchez-Salcedo et al., 2015). Furthermore, chlorogenic and its isomers, which has anti-obesity properties, is declined as mulberries ripen from semi-mature to full (Lee et al., 2016). Mulberries also contain quinic acid, which may help to alleviate urinary tract infection (Bao et al., 2016). Ellagic acid is ahydroxybenzoic acid, but most of it is in the form of ellagitannins, and

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