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Review

Bioactive compounds in functional buckwheat food

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ABSTRACT

Buckwheat is an ancient dicotyledonous crop belonging to *Polygonaceae* family. It is cultivated and adapted to marginal lands with harsh environments. Buckwheat has been praised as one of the most faddish green functional foods. Many functional foods including tea made from buckwheat have been put into production around the globe. The buckwheat proteins are particularly rich in lysine and contain less glumatic acid and proline, and more arginine and aspartic acid than cereal proteins. Buckwheat proteins also show a strong supplemental effect with other proteins to improve the dietary amino acid balance with special biological activities of cholesterol-lowering effects, antihypertension effects, and improving the constipation and obesity conditions by acting in a similar way as dietary fiber and interrupting the in vivo metabolisms. Besides its high-quality proteins, buckwheat is also rich in many rare components that have healing effects on some chronic diseases. Among these components, the most attractive ones are flavones, flavonoids, phytosterols, D-chiro-Inositol, and myo-inositol. In this review we focus on buckwheat's general physical and chemical properties, rare components, functional effects, metabolic engineering of bioactive compounds and trends in the development of functional tea from buckwheat in the latest three years.

Contents

1. Introduction

Buckwheat, which belongs to the family *Polygonaceae*, genus *Fagopyrum*, has been a commonly-eaten food in arid and cold regions in the world. Buckwheat is ubiquitous almost everywhere but grows mainly in the northern hemisphere (Li & Zhang, 2001). Russia is now the biggest producer of buckwheat. China ranks second in the production of buckwheat with about 10.2 million acres cultivating area and the buckwheat production fluctuates within the range of 0.6 to 0.95 million tons (Li & Zhang, 2001). The most widely grown buckwheat species include common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*F. tataricum*), constituting raw material for the production of buckwheat tea, groats, flour and noodles (Fig. 1). Buckwheat has attracted increasing attention from food scientists for its healing effects over

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Fig. 1. Two cultivated buckwheat species. A–E, Different development stage of *Fagopyrum tataricum* (Tartary buckwheat); F, *Fagopyrum esculentum* (common buckwheat). These pictures were taken by Yu Tang at Liangshan Prefecture in Sichuan Province, China, in 2003.

chronic diseases. Buckwheat is the only pseudocereal that contains rutin, hence it is a beneficial source of this flavonoid. Other phenolic compounds and flavones such as hyperin, quercitrin, and quercetin have been detected and isolated from immature buckwheat seeds (Koyama, Nakamura, & Nakamura, 2011). These compounds are presumed to be involved in many of the health benefits of tartary buckwheat. They possess special medicinal properties such as antihypertensive and antihypercholesterolemic effects at nontoxic concentrations in humans (Li, Li & Ding, 2010; Li, Park, et al., 2010). Buckwheat protein is presumed to improve health in various ways, notably reducing serum cholesterol, suppressing gallstones and tumors, and inhibiting the angiotensin I-converting enzyme (Koyama et al., 2011). Buckwheat grain is characterised by a high content of starch, protein with an advantageous amino acid composition, a low content of α-gliadin and a high content of dietary fibre (Dziedzic, Górecka, Kucharska, & Przybylska, 2011). The buckwheat grains have excellent nutritional value and are recommended for patients who suffer from typhoid and liver ailments. More specifically, as buckwheat can be in the production of foods for people with celiac disease, for those subjects who suffer from gluten intolerance. Buckwheat tea is a popular health product in Asian and European countries (Qin, Li, Yang, & Guixing, 2011; Qin, Tingjun, Li, Fang, & Guixing, 2011). In the last few years, much progress has been made at improving the nutrition and healing effects of buckwheat functional food. In the present review we hope to provide a state-of-the-art analysis of several aspects of functional buckwheat food.

2. General characterization of buckwheat

Buckwheat belongs to the Old World genus *Fagopyrum* Mill. (Polygonaceae). It is an annual herbaceous plant with origins in China and Central Asia. The genus Fagopyrum consists of about 19 species, recently four new species, *Fagopyrum crispatifolium* J. L. Liu (Liu, Tang, et al., 2008), *F. pugense* T. Yu (Tang et al., 2010), *F. qiangcai* D. Q. Bai and *F. wenchuanense* J. R. Shao (Shao, Zhou, Zhu, Wang, & Bai, 2011), were discovered in the genus *Fagopyrum*, and their distribution, taxonomic position and phylogenetic relationship have been clarified (Liu, Chen, et al., 2008; Liu, Tang, et al., 2008; Shao et al., 2011; Tang et al., 2010; Zhou, Bai, Tang, Zhu, & Shao, 2012). Of the two cultivated species, *F. esculentum* Moench and *F. tataricum* (L.) Gaertn., *F. tataricum* is mainly cultivated in the Himalayas (Fig. 1). Cultivation of *F. esculentum* extends from temperate Europe to Japan through the Indo-Myanmar region. *F. cymosum* (Trevir.) Meissn, the wild species of buckwheat, occurs mostly in the Himalayan foot hills and China. It has been shown that the origin of cultivated tartary buckwheat, the hybrid origin of weedy tartary buckwheat and of the wild populations from central Tibet and northern Pakistan, and the cultivated tartary buckwheat probably originated in northwestern Yunnan in China (Tsuji & Ohnishi, 2000). Buckwheat is an important crop that provides security to traditional farmers under subsistence farming practices. Owing to its frost resistance, short growth period, and undemanding cultivation, buckwheat is common in high-altitude areas at 2000 m, and in Tibet it is found at elevations of up to 4500 m. Buckwheat bears triangular seeds with black hulls covering the light green to white kernel. The color gets lighter into the inner layers of the kernel. The hull has a smaller density than water and this fact allows people to easily remove the hull from the kernel (Fig. 1). The hardness of the hull depends on the species of the buckwheat (Li & Zhang, 2001). Generally, *F. esculentum* has a softer hull than its *F. tataricum* relatives. Small, wind-pollinated buckwheat flowers are borne in clusters or in heads. Usually the flower is bisexual and has a variable number of parts including 3–5 sepals and petals that look alike (called tepals), 6–9 stamens, and a single pistil. The superior ovary of fused carpels has one chamber (locule) with one ovule which develops into an achene fruit (Fig. 1).

The protein content in buckwheat is approximately 12% and thus similar to the protein content found in wheat. The fat content in buckwheat is close to 3% whereas the crude fiber concentration is very high (12.7 and 17.8%, respectively, for two varieties). The high fiber content results in a low concentration of soluble carbohydrates with the lowest value of 48.7%. Both buckwheat varieties have a high tannin content (1.76 and 1.54%, respectively). The protein quality is very high, with biological values above 90% (Eggum, Kreft, & Javornik, 1981). The amino acids in buckwheat protein are well balanced and are rich compared with other crops. The nutritive value of buckwheat seeds is much higher than that of cereal crops. The latest report show that the average protein content of buckwheat is 12.94% (Guo, Chen, Yang, & Huang, 2007). Buckwheat proteins can show a strong supplemental effect when combined with vegetable proteins to improve the dietary amino acid balance (Li & Zhang, 2001). Consequently, ground buckwheat is often sieved to remove some of the hull fraction and the resulting flour is then used for bread, pancakes, noodles, and other food items.

Buckwheat is rich in polyphenols, including six flavonoids; rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin (Table 1). Among these antioxidant components, rutin was recognized as the most health protective and has also been proven to be anti-inflammatory and anticarcinogenic (Liu, Chen, et al., 2008, Liu, Tang, et al., 2008). The flavonoid content was 40 mg/g in tartary buckwheat seeds as compared to 10 mg/g in common buckwheat seeds (Li & Zhang, 2001). Quercitrin values were in the range of 0.01–0.05% dry weight (DW) in tartary buckwheat herb, which was not found in common buckwheat (Fabjan et al., 2003). Rutin and quercetin content in seeds depends on variety and growing conditions (Fabjan et al., 2003). The sum contents of phenolic compounds in the edible part (mean 24.4 mg/g DW at 6–10 days after sowing) of tartary buckwheat sprouts were similar to those of common buckwheat sprouts, rutin contents in the non-germinated/germinated seeds (mean 14.7 mg/g DW) and edible parts (mean 21.8 mg/g DW) of tartary buckwheat were 49- and 5-fold, respectively, higher than those of common buckwheat (Kim et al., 2008). Buckwheat sprouts contained five kinds of major phenols. The quantities of isoorientin, orientin, isovitexin, and vitexin at day 3 were 5.8, 11.7, 26.2, and 28.9 mg/100 g fresh weight (FW), respectively. The rutin content rapidly increased to 109.0 mg/100 g FW until day 6. The highest total phenols in buckwheat sprouts were 162.9 mg/100 g FW at day 6 (Koyama et al., 2011). The comparative composition studies of common buckwheat and tartary buckwheat show higher thiamine, riboflavin, and pyridoxine contents, and they are an excellent food for use in preventative nutrition (Bonafaccia, Marocchini, & Kreft, 2003; Liu, Chen, et al., 2008; Liu, Tang, et al., 2008). Concentrations of γ-aminobutyric acid (GABA) and 2″-hydroxynicotianamine (2HN) were quantified in the leaves of common and tartary buckwheat at 14, 28, and 42 days after sowing (DAS). GABA concentrations peaked at 42 DAS, whereas 2HN concentrations declined with the age of the plants (Suzuki et al., 2009). Recently, kaempferol-3-O-rutinoside and quercetin 3-O-rutinoside-3'-O-β-glucopyranoside were found in common buckwheat seeds (Li, Li, et al., 2010; Li, Park, et al., 2010). Total tocopherol concentrations in buckwheat grains ranged from 14.3 to 21.7 mg/kg (Kim, Kim, & Park, 2002). Buckwheat achenes have been also recognized as an important resource of vimamins B1 (thiamin, 3.3 mg/kg), B2 (riboflavin, 10.6 mg/kg), B3 (niacin, 18.0 mg/kg), B5 (pantothenic acid, 11.0 mg/kg), and B6 (pyridoxine, 1.5 mg/kg). In buckwheat sprouts vitamins B1 and B6 and vitamin C were described (Kim et al., 2002). Squalene is an isoprenoid compound having six isoprene units that possesses antioxidant activities, and it is widely produced in plants. Squalene protects cells against radicals, strengthens the immune system, and decreases the risk of various cancers (Kalinova, Triska, & Vrchotova, 2006). R-Tocopherol was found as the main component of vitamin E in all parts of the plant. Its content correlates positively with temperature, drought, and duration of solar radiation. Certain differences appear among varieties of buckwheat, especially in their squalene and rutin contents (Kalinova et al., 2006). Four catechins [(−)-epicatechin, (+)-catechin-7-O-β-D-glucopyranoside, (−)-epicatechin-3-O- p-hydroxybenzoate, and (−)- epicatechin-3-O-(3,4-di-O-methyl)gallate] were isolated from the ethanol extracts of buckwheat groats, (−)-epicatechin-3-O-gallate, procyanidin B2, and procyanidin B2-3'-O-gallate were found in buckwheat callus and hairy root cultures (Kalinova et al., 2006). These constituents in the plant tissue are affected by numerous environmental factors such as ultraviolet (UV) radiation, time of harvest, and damage caused by pests, in addition to genetic or age-related factors. Investigations demonstrated that significant positive correlations with the mean above sea level of the growing site and the amounts of certain phenolic antioxidants (Alonso-Amelot, Oliveros-Bastidas, & Calcagnopisarelli, 2007; Kishore, Ranjan, Pandey, & Gupta, 2010).

3. Bioactivity studies on buckwheat

Buckwheat protein shows high biological value due to a well-balanced amino acid pattern and is rich in lysine and arginine. It has been reported that buckwheat protein has many unique physiological functions, such as curing chronic human diseases, decreasing blood cholesterin, inhibiting mammary cancer caused by 7,12-dimethylbenzene, restraining gallstone and so on (Tomotake et al., 2000). In humans, consumption of buckwheat is associated with a lower prevalence of hyperglycemia and improved glucose tolerance in people with diabetes (Zhang, Zhang, Lu, Tong, & Cao, 2007). It has been showed that the beneficial effect of a powdered whole buckwheat leaf and flower mixture in rats fed a high-fat diet (Lee et al., 2010). Because many of the health promoting functions are inherently related to the radical scavenging activity of peptides from the protein digests, it is hypothesised that hydrolysis of buckwheat protein can release the peptide fragments capable of stabilising reactive oxygen species and inhibiting lipid oxidation (Ma, Xiong, Zhai, Zhu, & Dziubla, 2010). In rat feeding experiments, studies have proven that buckwheat

Table 1
Summary of bioctive compounds in buckwheat.

Category	Examples	Notes	References
Flavonoids	Rutin, Orientin, Quercetin, Vitexin, Isovitexin, Isoorientin	Found primarily in seeds, these compounds are anti-inflammatory, anticarcinogenic, and can reduce high blood pressure	Fabjan et al., 2003 Liu, Chen et al., 2008; Liu, Tang et al., 2008
Vitamins	B1, B2, B3, B5, B6	Found primarily in achenes	Kim et al., 2002
Vitamins	B1, B6, C	Found primarily in sprouts	Kim et al., 2002
Isoprenoid	Squalene	Found throughout the plant, squalene is a powerful antioxidant	Kalinova et al., 2006
Vitamin E	R-Tocopherol	Found throughout the plant	Kalinova et al., 2006
Iminosugars	D-fagomine, 1-deoxynojirimycin	Found primarily in seeds, these iminosugars are glycosidase inhibitors	Gómez et al., 2011 Amézqueta et al., 2012
Inositol	D-Chiro-inositol (DCI)	Found throughout the plant, DCI mediates insulin metabolism by enhancing the action of insulin and decreasing blood pressure	Fonteles et al., 2000; Ueda et al., 2005
Protein	TBWSP31	Found in water soluble extracts, TBWSP31 is a novel antitumor protein	Guo et al., 2010

protein extract has hypocholesterolemic, anticonstipation and antiobesity activities. In addition, buckwheat protein product had a protective effect against 1,2-dimethyhydrazine (DMH)-induced colon carcinogenesis in rats by reducing cell proliferation (Tomotake et al., 2006). Recently, the TBWSP31 is a novel antitumor protein that was isolated from tartary buckwheat water-soluble extracts was studied (Guo, Zhu, Zhang, & Yao, 2010). Tartary buckwheat extracts had anti-fatigue properties, which extended the exhaustive swimming time of mice, effectively inhibiting the increase of blood lactic acid (BLA), decreasing the level of blood urea nitrogen (BUN), increasing the tissue glycogen content and the activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) of mice (Jin & Wei, 2011). Moreover, buckwheat polysaccharides (BWPSs) can significantly stimulate cytokine secretion (differentiation inducer) and then increase cell differentiation and maturity in monocyte cells (Wu & Lee, 2011).

Neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD), have become a major health problem, particularly in industrialized countries due to increasing number of elderly population. The most prescribed drug class for AD is cholinesterase inhibitors at the moment, which is only fine for the symptomatic treatment (Larner, 2010). Neuromelanin-containing neurons are particularly susceptible to degeneration and their depigmentation is the feature of PD (Hasegawa, 2010). Tyrosinase, which is a chief enzyme in melanin biosynthesis by means of the production of L-3,4-dihydroxyphenylalanine (L-DOPA) and subsequent molecules, has ability to accelerate the stimulation of catecholamine quinone derivatives through its oxidase activity. Consequently, inhibition of tyrosinase is important in PD treatment. It has been reported that the ethyl acetate and ethanol extracts of the seed, stem, and aerial parts of buckwheat show neuroprotective effect through acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibitory and antioxidant activity (Gulpinar et al., 2012).

The main phenolics of buckwheat extract were rutin, quercitrin, and quercetin. Rutin (quercetin-3-O-β-rutinoside) is the best-known glycoside derived from flavonol quercetin. Rutin has relaxing effects on smooth muscles and is effective for preventing capillary apoplexy and retinal hemorrhage, reduce high blood pressure, and show antioxidant and lipid peroxidation activities. It also has a lipid-lowering activity by decreasing the absorption of dietary cholesterol as well as lowering plasma and hepatic cholesterol (Jiang, Campbell, Pierce, Austria, & Briggs, 2007; Yang & Ren, 2008). Three flavonoids from tartary buckwheat bran, namely, quercetin, isoquercetin and rutin, have been evaluated as R-glucosidase (controlling the blood glucose level) inhibitors by fluorescence spectroscopy and enzymatic kinetics. The R-glucosidase's activity was obviously affected by the extractive substance (mainly rutin) and its hydrolysis product (a mixture of quercetin, isoquercetin and rutin) from tartary buckwheat bran (Li et al., 2009). Rutin can reduce glucolipotoxic effects through activating AMP-activated protein kinase signaling to inhibit the activities of lipogenic enzymes and ameliorating mitochondrial function, which highlight the possibilities of rutin as novel strategies for the prevention of type 2 diabetes (Cai & Lin, 2009). Active oxygen species generated in the body cause damage to DNA and the lipid membrane structure of cells and play a role in the process of aging and the development of cancer. It is therefore important to control the amount of excess active oxygen in our body. Antioxidative components found in food have been shown to be effective as scavengers of active oxygen (Lu, Ba, & Chen, 2008; Papadopoulou, Green, & Frazier, 2005). Research has indicated that rutin and quercetin may be metabolized by albumin-mediated transport in the body. It has been reported that heating rutin with water-soluble whey protein isolate (WPI), a milk ingredient, results in the alteration of its antioxidative activity. Formation of a rutin-WPI complex enhanced the peroxyl and hydroxyl radical scavenging activities of rutin or WPI (Awatsuhara, Harada, Nomura, Nikaido, & Nagao, 2008). Recently, alkaline luminol chemiluminescence and electron spin resonance analysis revealed the formation of a rutin-ovalbumin complex that markedly enhanced the radical scavenging activity of rutin. Rutin also demonstrated antioxidative activity against hydroxyl radicals in a DNA protection assay (Awatsuhara, Harada, Maeda, Nomura, & Nagao, 2010). Proanthocyanidins in the buckwheat flour reduced nitrous acid producing nitric oxide (NO) when the flour was suspended in acidified saliva or in acidic buffer solution in the presence of nitrite. The increase in the concentration of NO could improve the activity of stomach helping the digestion of ingested foods and the nitration and nitrosation of the proanthocyanidins could contribute to the scavenging of reactive nitrogen oxide species generated from NO and nitrous acid (Takahama, Tanaka, & Hirota, 2010). Inhibition of lipid oxidation in mouse brain lipids increased after digestion in the stomach for both buckwheat extract and the rutin standard (Hur, Park, & Jeong, 2011).

It has been previously demonstrated that intragastric administration of a buckwheat concentrate (BWC), containing D-chiro-Inositol, myo-inositol, and fagopyritols, effectively lowered serum glucose concentrations in streptozotocin rats in the fed state (Kawa, Taylor, & Przybylski, 2003). D-Chiro-inositol (DCI), a naturally occurring isomer of inositol, is the main active nutritional ingredient in buckwheat (*F. esculentum*). As an epimer of myoinositol, DCI is probably the main mediator of insulin metabolism by enhancing the action of insulin and decreasing blood pressure, plasma triglycerides, and glucose concentrations (Fonteles, Almeida, & Larner, 2000; Ueda, Coseo, & Harrell, 2005). Therefore, DCI has great potential to work as an adjunctive drug in the treatment of insulin resistance diseases such as type 2 diabetes and polycystic ovary syndrome (Yang & Ren, 2008). D-Fagomine is an iminocyclitol first isolated from seeds of buckwheat. As D-fagomine and other iminosugars such as 1-deoxynojirimycin (DNJ) are intestinal glycosidase inhibitors, they are connected to a reduction in the risk of developing insulin resistance, becoming overweight and suffering from an excess of potentially pathogenic bacteria (Amézqueta et al., 2012; Gómez et al., 2011). Buckwheat was found to have high levels of angiotensin I-converting enzyme (ACE)-inhibitory activity. ACE converts angiotensin I to II, which is a pressor hormone in the renin-angiotensin (RA) blood pressure control system. The inhibition of ACE, which is the rate-determining enzyme of the RA system, inhibits blood pressure elevation and aids in the prevention of hypertension. Nicotianamine (NA), an analogue of 2″-hydroxynicotianamine (HNA), has a very high ACE-inhibitory activity, as does HNA (Higasa, Fujihara, Hayashi, Kimoto, & Aoyagi, 2011). Buckwheat, a crop utilized throughout the world, is one of our important food sources. Besides various polyphenols, it contains proteins with high biological value and balanced vitamins and catechins (Table 1). High levels of vitamin E intake have been associated with a reduction in cardiovascular disease, lowering the risk of Alzheimer's disease and prostate cancer, improving the immune system, and delaying both age-related cataracts and age-related macular degeneration (Kalinova et al., 2006).

4. Analytical studies on buckwheat

There are two head techniques for identification and determination of flavonoids: HPLC and capillary micellar electrokinetic chromatography (CE). HPLC is traditionally used in quercetin glycosides analysis of wide spectrum of materials; it is suitable for determination of the rutin content in buckwheat achenes, buckwheat products, fresh buckwheat sprouts and plant parts. HPLC system coupled to a combination of MS detector can obtain structural information of flavonoids (Table 2). CE is a modern analytical technique and shows high efficiency, small sample volume, high speed and good resolution. The CE for determination of quercetin glycosides and free quercetin in buckwheat flower, leaves, stems and achenes was developed. The highest content of rutin was found in flowers of both kinds of buckwheat (99.4 g/kg in *F. esculentum*, 108 g/kg in *F. tataricum*). The free quercetin occurs in flowers and achenes of *F. esculentum*, whereas flowers and achenes of *F. tataricum* contained quercitrin (Dadáková & Kalinová, 2010). It has been reported that the nondestructive

Table 2
Detection methods for bioactive compounds in buckwheat.

Method name	Detects	Variations on the method	References
High-performance liquid chromatography (HPLC)	Quercetin, rutin from achenes, sprouts, plant parts, and products	Reversed-phase HPLC (RP-HPLC), HPLC with diode array detection (HPLC-DAD), HPLC with electrochemical detection (HPLC-ECD)	Zu et al., 2006; Danila et al., 2007; Verardo et al., 2010
HPLC photodiode array mass spectroscopy (HPLC-PDA-MS)	Flavonoids from the embryo, endosperm, testa, and hull		Li, Li et al., 2010; Li, Park et al., 2010
Capillary electrophoresis (CE)	Quercetin from buckwheat flowers, leaves, stems, and achenes	CE with ultraviolet detection (CE-UV), CE with electrochemical detection (CE-ECD), Capillary zone electrophoresis with amperometric detection (CZE-AD)	Dadáková & Kalinová, 2010
Nondestructive near-infrared re flectance spectroscopy (NIRS)	Rutin, DCI		Yang & Ren, 2008
Reversed-flow micellar electrokinetic chromatography (RFMEKC-UV)	Rutin		Dubber & Kanfer, 2006
Electrospray ionization time-of-flight mass spectrometry (ESI-Q-MS)	Phenolic compounds from buckwheat flour		Amézqueta et al., 2012

near-infrared reflectance spectroscopy (NIRS) can provide a rapid and convenient method to detect rutin and DCI in an efficient and cost-effective way (Yang & Ren, 2008). Recently, reverse phase high performance liquid chromatography (RP-HPLC) coupled to electrospray ionization-time of flight-mass spectrometry (ESI-TOF-MS) has been applied to separate and characterize thirty phenolic compounds including new compounds in buckwheat flour (Verardo et al., 2010). High performance liquid chromatography photodiode array-mass spectroscopy (HPLC-PDA-MS) identified several flavonols in the embryo, endosperm, testa, and hull of buckwheat, including the predominant flavonoid rutin and minor flavonoids quercetin 3-Orutinoside-3'-O-β-glucopyranoside, kaempferol 3-O-rutinoside, and quercetin (Li, Li et al., 2010; Li, Park, et al., 2010). Analytical methods suitable for measurement of rutin have mainly been based on reversed-phase high-performance liquid chromatography with diode array detection (HPLC-DAD) (Zu, Li, Fu, & Zhao, 2006), reversed-flow micellar electrokinetic chromatography (RFMEKC-UV) (Dubber & Kanfer, 2006), capillary electrophoresis with ultraviolet detection (CE-UV), capillary electrophoresis with electrochemical detection (CE-ECD), capillary zone electrophoresis with amperometric detection (CZE-AD), and downsizing of the HPLC-ECD system such as using a microbore column (μHPLC-ECD) (Danila, Kotani, Hakamata, & Kusu, 2007). Five different isolation techniques were combined with gas chromatographic–mass spectrometric determination of aroma compounds from buckwheat: dynamic headspace (DHS) with cryotrapping or sorbent trapping, solid-phase microextraction (SPME), headspace sorptive extraction (HSSE), solvent extraction (SE) and simultaneous distillation–extraction (SDE) (Prosen, Kokalj, Janeš, & Kreft, 2010). A reverse-phase high-performance liquid chromatographic method for the determination of trans-resveratrol with spectrophotometric detection (306 nm) and amperometric detection at carbon paste electrode was developed and tested on buckwheat (Němcová et al., 2011). The method to separate D-fagomine from its diastereomers 3-epi-fagomine and 3,4-di-epi-fagomine was developed using a single run by cation exchange HPLC with detection and quantification by mass spectrometry using electrospray ionisation and a simple quadrupole analyser (ESI–Q-MS) which is applied to the determination of D-fagomine in buckwheat groats (6.7–44 mg/kg) (Table 2) (Amézqueta et al., 2012).

5. Metabolic engineering of bioactive compounds

Phytohormones influence many diverse developmental processes ranging from seed germination to root, shoot, and flower formation, involving plant growth, morphogenesis, and secondary metabolism. Under most circumstances, a single hormone can regulate many different metabolites biosynthesis pathways and at the same time different hormones can influence a single pathway. Plant growth regulators and elicitors have been used to increase production of secondary metabolites in plant cultures. Jasmonic acid and its methyl ester, collectively called jasmonates, have been found to induce the biosynthesis of a variety of secondary metabolites in different plant species, including alkaloids, terpenoids, glucosinolates, and phenylpropanoids (Zhou, Zhu, Shao, Tang, & Wu, 2011). Recently, methyl-jasmonate acid (MeJA) treatment was shown to inhibit anthocyanin synthesis in buckwheat sprouts cultivated under light conditions (Horbowiczi, Grzesiuk, De-bski, Koczkodaj, & Saniewski, 2008). However, MeJA treatment can increase the phenolic compound content and antioxidant activity of the buckwheat sprouts cultivated under dark conditions. Metabolomic analysis revealed that the accumulation resulted from the stimulation of the phenylpropanoid pathway by MeJA treatment (Kim, Park, & Lim, 2011). It has been reported that methyl jasmonate stimulates the conversion of L-phenylalanine into 2-phenylethylamine and increases the endogenous levels of putrescine in hypocotyls and cotyledons of buckwheat seedlings (Horbowicz, Kosson, Wiczkowski, Koczkodaj, & Mitrus, 2011). Elicitor molecules such as salicylic acid (SA) and methyl jasmonate (MeJA) have been used to induce production of all kinds of secondary metabolites. The maximum yield of DCI was 5.851 mg/g DW, which was 2.877 times higher than that in the control cultures, in cultures treated with 0.4 mM MeJA on the day of inoculation. DCI production decreased when MeJA concentration was higher than 0.4 mM. Treatment with 0.6 mM SA provided maximum yield of DCI of 5.022 mg/g DW. DCI production decreased markedly with increasing SA concentrations. The maximum DCI yield of 7.579 mg/g DW, which was 3.726 times higher than that in the control, was obtained after treatment of cell culture with a combination of 0.2 mM MeJA and 0.6 mM SA (Hu, Yu, Piao, Liu, & Yu, 2011). The greatest advantage of hairy roots is that they often exhibit about the same or greater biosynthetic capacity for secondary metabolite production as compared to their parent plants (Zhou et al., 2011). The hairy root culture of buckwheat by infecting leaf explants with *Agrobacterium rhizogenes* R1000 were developed, which had high growth and rutin production rates (312 mg dry wt per 30 mL flask and 1.2 mg/g dry wt, respectively). These results indicate hairy root cultures of buckwheat culture could be a valuable alternative approach for rutin production (Lee et al., 2007). Gene coding rate-limiting encoding enzymes and some key transcription factors can be used to improve desired metabolites production by overexpressing them in transgenic hairy root cultures (Zhou et al., 2011). Overexpression of *Arabidopsis* transcription factor AtMYB12 (which is the flavonol-specific transcription factor) in hairy root of common buckwheat can induce mostly key enzyme genes expression level of flavonoid biosynthetic pathway. This led to the accumulation of rutin in buckwheat hairy roots up to 0.9 mg/g dry wt (Park et al., 2012). Recently, few kinds of key enzyme genes in the flavonoid biosynthesis pathway have been cloned in common buckwheat

(Li, Li, et al., 2010, Li, Park, et al., 2010; Li et al., 2012). The understanding of buckwheat responses to biotic and abiotic factors in the external environment is still undefined. It still has a long way to go to understand the mechanism of bioactive compounds biosynthesis in buckwheat.

6. Processing Buckwheat for tea

It is important to consider what the effects on the bioactive compounds from buckwheat will be during the processing of buckwheat into tea. There are many steps involved in creating tea from raw buckwheat seeds. Raw whole seeds are first soaked in water, then steamed, then dried before they are removed from their hulls. The dehulled groats are then roasted so that the tea can be made (Qin, Li, et al., 2011; Qin, Tingjun, et al., 2011). The effects of these thermal processing steps on the buckwheat proteins have been found to be dependent upon the lipid content of the buckwheat. While the presence of lipids can help to improve thermal stability of buckwheat proteins, lipids can also disturb the buckwheat globulins. Thus it is suggested that a suitable amount of lipids, such as 6.5%, be present for the maintenance of buckwheat globulins during thermal treatment (Tang, 2007). Another study compared the effects thermal processing by microwave heating, pressured steam-heating, and roasting on the antioxidant properties of buckwheat. It was found that pressured steam-heating was the most destructive to the antioxidant properties (Zhang, Haixia, Jinlei, Ying, & Yi, 2010). These results suggest that in order to create buckwheat tea with the maximum content of beneficial active compounds, it will be necessary to optimize processing methods.

7. Concluding remarks

Considering the requirements of improving our living environment and searching for new way to make better use of buckwheat, more the near work will be done in recent future. The main trends in this field might be some of the following. (1) Get better use of the byproducts of buckwheat production, and also develop new functional foods. (2) Investigate the mechanism of buckwheat pharmaceutical effects. (3) The major routes of biosynthesis in the rutin pathway are still not understood and related genes have not been cloned, some potential key transcription factors have not been elucidated and their characterization is still pending. (4) With the development of plant molecular biology and gene-transfer technology to plants, it is possible to breed new species with high contents of rutin.

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