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Invited Review

Present status and future perspectives of breeding for buckwheat quality

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Buckwheat is an important crop globally. It has been processed into cereal grain, noodles, confectionery, bread, and fermented foods for many years. Buckwheat production and processing has supported local economies and is deeply related to the culture of some regions. Buckwheat has many unique traits as a food, with a good flavor and color. In addition, buckwheat is also a healthy food because it contains bioactive compounds that have anti-oxidative, anti-hypertensive, and anti-obesity properties. Therefore, breeding of buckwheat for quality is an important issue to be addressed. Compared to other crops, there is still a lack of basic information on quality, including bioactive compounds generation and enhancement. However, some mechanisms for modifying and improving the quality of buckwheat varieties have recently been identified. Further, some varieties with improved quality have recently been developed. In this review, we summarize the issues around buckwheat quality and review the present status and future potential of buckwheat breeding for quality.

Key Words: buckwheat, Tartary buckwheat, breeding, quality, taste, functional compound, clinical trial.

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench.) is an important crop in Japan, as well as in Russia, China, Eastern Europe, and some other countries and regions (Ikeda 2002, Kreft *et al.* 2003). In some production areas, buckwheat is recognized as a traditional and important crop that supports local economies. It is generally used as a cereal after removal of the husk (cereal grain) and is sometimes milled for flour (**Fig. 1**). Buckwheat flour is generally processed into other foods such as noodles, confectionery, bread, and the products of fermentation such as vinegar, and alcoholic spirits such as shotyu. In addition, the leaves are also used as leafy vegetables and can be dried to make teas and powder. Buckwheat flowers are white, pink, or red; the plants are cultivated for landscaping and for honey production. Buckwheat is recognized as having a good flavor, texture, and color. On the other hand, there are some buckwheat traits that are not desirable, either to the end consumer or for processing. Buckwheat is also known as a healthy food because it contains many functional compounds such as vitamins, polyphenols, flavonoids, functional sugars, functional nicotianamines, resistant starch, and resistant proteins. In addition, buckwheat has good nutritional traits in terms of high amino acid and high mineral contents.

To date, some buckwheat quality traits have been improved by breeding. However, there has been little research and development of breeding for buckwheat quality.

In this review, considering that the flour is the major processing material for foods especially in Japan, we summarize some important points in terms of flour for buckwheat breeding. We introduce the current research on buckwheat quality and examine the future prospective in the following areas: ‘flour’, ‘plants’, and ‘other uses of buckwheat’. This review aims to contribute to the advancement of the molecular breeding, botanical science, and processing technology of buckwheat.

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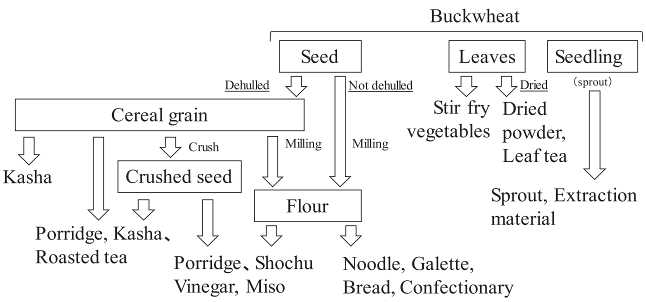

Fig. 1. Processing procedure for buckwheat food products.

2. Flour

In Japan, buckwheat flour is generally used for making noodles. The physical properties of buckwheat flour are important for handling and cutting the raw noodle dough. Unlike wheat flour, buckwheat flour does not contain gluten, and therefore the strength and handling traits (such as resistance against cracking) in buckwheat dough and noodles are not as good as in wheat flour. Noodle makers in Japan sometimes mix wheat flour with buckwheat flour to reinforce the strength of the dough and noodles. The mixture ratio of wheat flour to buckwheat flour ranges from very low, to moderate in soba noodle shops, and sometimes over 70% for dried noodles. The fact that buckwheat flour does not contain gluten means it is unsuitable for making bread because it does not rise well. In the preparation of a typical Tuscan bread, Brunori *et al.* (2009) replaced 20% of the wheat flour with buckwheat whole flour (16% common buckwheat and 4% Tartary buckwheat). The use of buckwheat had almost no effect on the leavening process.

Based on the way in which buckwheat and its products are used, the improvement of its physical properties is desirable. Several studies have focused on starch and storage proteins, which are the major elements that influence the physical properties of cereals. In this section, we summarize the literature on the following buckwheat physical properties: starch, enzymes for starch synthesis, and storage proteins.

2.1. Physical property

2.1.1 Starch

Starch is the most critical storage polysaccharide for many plants including cereals and pseudo-cereal grains. Starch is the chief constituent of buckwheat groats, making up to 75% of the dry weight (Zheng *et al.* 1998). Starch molecules are made up of two glucan polymers, amylose and amylopectin. Amylose is a linear molecule, consisting of α-1,4-linked glucan chains, whereas amylopectin is a highly branched chain molecule, consisting of α-1,4-linked glucan chains interconnected through α-1,6-bonds. The ratio of amylose to amylopectin differs among plant species and varieties, but the starch commonly contains 15–30% amylose and 70–85% amylopectin. In general, the amylose content of buckwheat starch is 20–30% (Hung *et al.* 2009, Li *et al.* 1997, Noda *et al.* 1998, Qian and Kuhn 1999, Yoshimoto *et al.* 2004). Gregori and Kreft (2012) reported a low-amylose (3.8–16%) buckwheat mutant. However, the variation of amylose content of buckwheat starch is limited compared to the major cereal starches such as maize, barley, and wheat. In addition, there has been little research into developing buckwheat varieties with waxy-type starch (amylose free), which would have sticky mechanical characteristics.

The morphology of buckwheat starch granules has been studied using scanning electron microscopy (SEM). SEM observations have revealed that buckwheat starch granules are mostly polygonal in shape with a granule size ranging from 2 to 14 μm; these factors affect the physical properties of the buckwheat (Qian and Kuhn 1999, Zheng *et al.* 1998). Izydorczyk and Head (2010) reported varietal differences in starch granule diameter in five buckwheat varieties. The granule size ranged from 2 to 30 μm, and the median of the granule size ranged from 5 to 8 μm depending on the variety. Starch from a low-amylose buckwheat mutant was more spherical compared to normal buckwheat starch (Gregori and Kreft 2012). The gelatinization properties of buckwheat starch have been studied using differential scanning calorimetry (DSC), which determines the gelatinization transition temperatures, i.e. onset temperature (To) and peak temperature (Tp), and the enthalpy of gelatinization (ΔH). Noda *et al.* (1998) reported that To, Tp, and ΔH of the starch from 17 samples of common buckwheat and 10 samples of Tartary buckwheat were 51.5–62.3°C, 57.2–66.7°C, and 9.4–13.9 J/g, respectively. Slightly higher values of To and Tp for buckwheat starches were shown by Li *et al.* (1997), Qian and Kuhn (1999), Yoshimoto *et al.* (2004) and Zheng *et al.* (1998). Compared with wheat starch, starches from three common and three Tartary buckwheat varieties had generally higher Tp and To (Li *et al.* 1997). Izydorczyk and Head (2010) reported differences in the thermal properties of isolated starch of five buckwheat varieties using DSC; ΔH ranged from 12.1 to 14.5 J/g for amylopectin and from 0.7 to 1.2 J/g for amylose. Rheological properties of buckwheat starch gels have also been investigated before and after aging (16 h at 15°C after preparation). The G’ (kPa) ranged from 3.9 to 7.0 kPa in the fresh gel, and from 8.7 to 30.1 kPa in the aging gel. The pasting properties of starches from eight buckwheat cultivars were investigated using Rapid-Visco-Analyzer (RVA) (Yoshimoto *et al.* 2004). Peak viscosity, breakdown, and setback ranged from 226 to 261 RVU (Rapid Visco Unit), from 37 to 98 RVU, and from 180 to 226 RVU, respectively. Li *et al.* (1997) reported that common and Tartary buckwheat starches had slightly higher peak viscosity (around 200 RVU) than wheat starch (170 RVU). Although a low-amylose mutant has been found in buckwheat (Gregori and Kreft 2012), its starch properties and mutant genes have not yet been characterized. The physicochemical properties of buckwheat starch appear to be important for the quality of buckwheat-based products such as buckwheat noodles. However, no reports are available on the contribution of morphological, gelatinization, and pasting characteristics of buckwheat starch to the quality of buckwheat products.

2.1.2 Enzymes for starch synthesis

In rice, maize, and barley, both high-amylose starches and waxy starches have been reported (Christa and Soral-Śmietana 2008, Denyer *et al.* 2001, Deschamps *et al.* 2008, Song and Jane 2000, Zhu *et al.* 2008). In buckwheat, there is evidence of high-amylose starch (Christa and Soral-Śmietana 2008, Song and Jane 2000, Zhu *et al.* 2008) but waxy starch has not yet been reported. Waxy mutant starches are known to lack starch granule-bound starch synthase I (GBSS-1), which is involved in amylose biosynthesis. SDS-PAGE analysis of starch granule-bound proteins isolated from the endosperm tissue of grains of common buckwheat showed the presence of a single band corresponding to a molecular mass of 59.7 kDa (Chrungoo *et al.* 2012). The 59.7 kDa protein has serological homology with the 61 kDa GBSS-I from the endosperm starch of maize, and the 60 kDa GBSS-I from the endosperm starches of rice and wheat (Chrungoo *et al.* 2012). N-terminal sequence of GBSS-I protein isolated from buckwheat showed 94% homology with GBSS-I from *Hordeum vulgare*, *Triticum* spp. and *Phaseolus vulgaris* (Chrungoo *et al.* 2012).

Phylogenetic analysis of the amino acid sequence of the GBSS type proteins reveals a clear diversification into monocotyledonous and dicotyledonous groups (Chrungoo *et al.* 2012). The sequence from buckwheat clustered along with the GBSS-I sequences from dicots. At the 11th residue, the sequence of GBSS-I from buckwheat and dicots have valine, but majority of the monocots has methionine (Chrungoo *et al.* 2012). However, the protein from buckwheat showed similarities with GBSS-I from monocots as well as dicots. The fifth amino acid residual from the N-terminus, GBSS-I of buckwheat and monocots have valine, but the majority of the dicots GBSS-I have isoleucine at this position (Chrungoo *et al.* 2012).

The chain length of amylopectin is also an important characteristic affecting the physical properties of starch. In rice, Umemoto *et al.* (2002) reported that differences in amylopectin chain-length distribution are regulated by functional variations in the starch synthase IIa (*SSIIa*) gene, which is identical to the *alk* gene (Gao *et al.* 2003). Reserved starch of the mutant showed a low pasting temperature of rice flour and was rich in short chains of amylopectin. Rice cakes made from branching enzyme 1 (BEI)-deficient lines with waxy starch can retain their softness for longer periods than rice cakes made from functional BEI lines (Okamoto *et al.* 2013). Therefore, identification and development of BEI-deficient varieties in other cereal crops could extend the shelf life and improve the quality of waxy products made from cereal crops. Izydorczyk and Head (2010) reported differences of chain length of amylopectin in the starch of five buckwheat varieties. In previous papers, buckwheat starches showed similar distribution profiles; the majority of starches comprised chains with degree of polymerization (DP) 6-30 and the highest with DP 11 or 12. To develop new buckwheat varieties with modulation of amylopectin chain length, studies of varietal differences and characterization of SSIIa and BEI homologs are required.

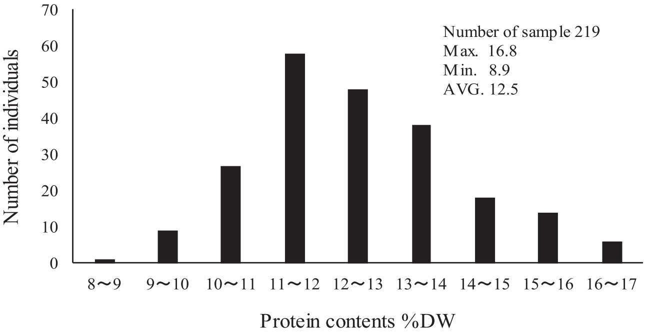

Fig. 2. Individual variation of total protein contents in buckwheat cultivar 'Kitawasesoba'.

To date, there have only been a few mutants identified with respect to starch synthesis; therefore, studies to identify and develop mutant lines are required.

2.1.3 Storage proteins

The study of buckwheat protein has been comprehensive, but there has been little research on the potential of breeding to improve buckwheat protein quality and content. The protein content of buckwheat flour is approximately 12–13% and its value is higher than that of other cereals including rice (6.1%) and barley (7%). Its amino acid composition is nutritionally superior to that of cereal grains (Pomeranz and Robbins 1972), and its amino acid score is higher (De Francischi *et al.* 1994). On the other hand, variations of protein contents among genetic resources and varieties has been reported (Morishita and Tetsuka 2002, Ohsawa and Tsutsumi 1993, Shibata *et al.* 1979). The main components of buckwheat seed proteins are salt-soluble globulins (Milisavljević *et al.* 2004). Variation in the ratio of soluble protein/total protein has also been reported (Ohsawa and Tsutsumi 1993). Morishita *et al.* (2010) reported the variation of total protein content in the variety 'Kitawasesoba' specimen (**Fig. 2**). Protein content is variable, even for particular varieties, suggesting significant underlying genetic variation. As Leng (1962) indicated, long-term selection is effective for improving the protein and oil content in maize, and it may be possible to improve buckwheat protein content through selection. Ikeda *et al.* (1999, 2000) reported that protein may be an important factor affecting the mechanical characteristics of buckwheat products such as noodles.

2.2 Flavor and lipid deterioration

Flavor is an important characteristic in some crops such as rice, soybean (*Glycine max* (L.) Merr.), and wheat.

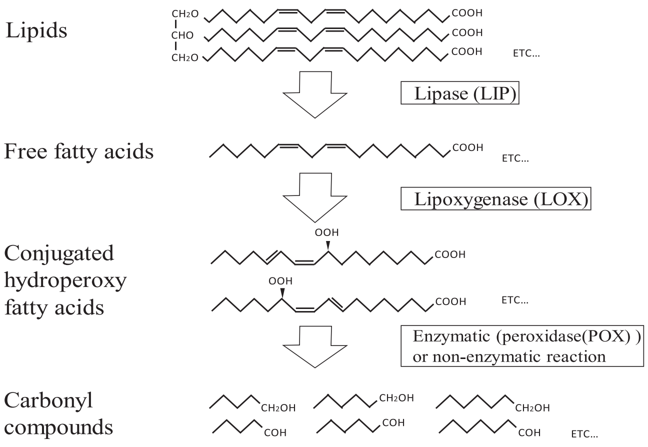

Fig. 3. Lipid degradation model proposed for rice bran.

Buckwheat is well known for having a unique flavor, not only in its seed (flour) but also in processed forms such as noodles, pastry, bread, and roasted tea. The unique flavor is one of the most important characteristics in buckwheat quality. Buckwheat flavors consist of many volatile compounds, such as carbonyl compounds that may generate oxidation and/or degradation of free fatty acids (FFAs). On the other hand, buckwheat flour quality deteriorates more easily than those of other crops such as wheat. The increase of FFAs and carbonyl compounds are a measurable index for the deterioration of buckwheat flour. Therefore, the flavor generation and lipid degradation may have a common pathway with the same catalytic enzymes. In some crops like soybean and rice, carbonyl compounds that contribute toward quality, such as hexanal, are generated through the 'lipoxygenase pathway'. This pathway was first proposed in rice bran (Takano 1993) (**Fig. 3**).

In this section, we summarize the studies on the mechanism of flavor generation and lipid deterioration in flour based on the lipoxygenase pathway and show the prospects for improvement through breeding.

2.2.1 Lipids

Lipids are major components of seeds and are as important in buckwheat as in other crops. In buckwheat seeds, lipids are mainly stored in the embryo (Dorrell 1971). Triacylglycerols are the main components of lipids and they are mainly composed of C16 to C18 fatty acids. On the other hand, the FFA concentration is a measurable index for flour deterioration. In addition, some flavor compounds are related to lipid metabolism; therefore, there could be differences in flavor compounds between buckwheat varieties. However, there are only a few studies that have described varietal differences in lipid contents. Dorrell (1971) reported that 60–70% of the oil in buckwheat seed is found in the embryo, and that the fatty acid composition of the buckwheat lipids is approximately 17% palmitic acid, 36% oleic acid, and 33% linoleic acid. Soral-Śmietana *et al.* (1984) also reported that the FFAs in buckwheat are palmitic acids (16%), oleic acid (42%), and linoleic acid (32%). This indicates that these FFAs are possibly generated from triacylglycerol by lipase (LIP) activity in buckwheat flour during storage or in germinating seeds.

2.2.2 Flavors

Among the flavor compounds in buckwheat, hexanal is also known as a flavor compound in soybean [*Glycine max* (L.) Merr.] foods (Anli and Tilak 2004, Axelrod 1974, Matoba *et al.* 1975, 1985); hexanal and other carbonyl compounds are generated through the lipoxygenase pathway. To date, there have been several studies that have focused on flavor compounds and the mechanisms for their generation (Aoki *et al.* 1981, Aoki and Koizumi 1986, Janes *et al.* 2009, Kawakami *et al.* 2008, Ohinata *et al.* 1997, 2005, Przybylski *et al.* 1995, Yajima *et al.* 1983). Aoki *et al.* (1981) analyzed the volatile compounds in stone-milled buckwheat flour using a continuous distillation–extraction apparatus, and gas chromatography and mass spectrometry to separate and identify; they reported that the volatile compounds consist of at least 45 substances, including: 13 alcohols, 6 aldehydes, 6 methyl ketones, 2 esters, 6 aromatic hydrocarbons, 7 alkanes, and 5 other compounds. They also reported that the best evaluation score for flavor was obtained for embryo and testa-rich fractions where *n*-hexanal or *n*-nonanal, 2-octanol, and *n*-tetradecane concentrations were higher. Yajima *et al.* (1983) identified 209 compounds from boiled buckwheat flour using Likens and Nickerson apparatus and gas chromatography–mass spectrometry. They identified 168 new compounds and made a special note of 2-(l′-ethoxyethyl) pyrazine and 2-(r-ethoxyethyl)-5-methylpyrazine. Aoki and Koizumi (1986) determined the thresholds of the 16 compounds of the flavor components by means of organoleptic evaluation and their odor units were calculated using stone-milled buckwheat flour. They reported that nonanal and hexanal were the most important components for the flavor in buckwheat flour, which decreased rapidly in the days after milling. These studies indicate that buckwheat flavor consist of many volatile compounds. On the other hand, they also indicate that some particular flavors related to aldehydes and ketones are important contributors to buckwheat flavor.

2.2.3 Deterioration of flour

In Japan, the freshness of buckwheat flour is very important for noodle makers because buckwheat flour deteriorates quickly (Muramatsu *et al.* 1986, Suzuki *et al.* 2005a, Tohyama *et al.* 1982). In buckwheat, flavor generation and deterioration in flour quality is caused by enzymatic reaction in the lipoxygenase pathway. Therefore, understanding the characteristics of enzymes in the lipoxygenase pathway is important in the breeding of high-quality buckwheat varieties. In buckwheat flour, several studies have shown that lipid degradation and oxidation are the main causes of quality deterioration during storage (Muramatsu *et al.* 1986, Suzuki *et al.* 2005a, Tohyama *et al.* 1982). The differences in flour deterioration have been investigated using 12 buckwheat varieties/breeding lines (Suzuki *et al.* 2012). Among the tested 12 buckwheat varieties/breeding lines at 30 days after storage at 20°C, the range of measured indexes were as follows; pH; 6.6–6.7, WSA (water soluble acid); 6.60–6.85 mg KOH/100 g flour, peroxide value (POV) 30–42 meq/kg flour, 6.5–12 μmol/100 g flour. These differences may be based on varietal differences. Further evaluation over multiple years is required. The changes in lipid deterioration indexes are as follows: during the storage test, the pH decreased at both 5°C and 20°C, and WSA increased at both 5°C and 20°C. The decrease in pH and increase of WSA indicated the accumulation of FFAs. These results are similar to those of Muramatsu *et al.* (1986) in soybean. The POV, which is an index of the amount of peroxidative compounds such as conjugated hydroperoxy fatty acids, also increased as length of storage increased. The hydroperoxy fatty acid is generally degraded by enzymatic or non-enzymatic reaction to carbonyl compounds, including volatile compounds, which contribute some flavor. The quantity of carbonyl compounds is measured as COV (carbonyl value), and the COV also tends to increase with length of storage. The changes in the degree of deterioration of these measurable indexes are higher at 20°C than at 5°C. At both 5°C and 20°C, LIP activity showed a significant negative correlation with pH and a significant positive correlation with WSA. The LIP activity also showed a significant correlation with increase in POV and COV (Suzuki *et al.* 2005a). Peroxidase (POX) also showed a significant correlation to increases in pH and POV. In addition, the rutin concentration showed a significant negative correlation to pH, WSA, and COV. On the other hand, lipoxygenase (LOX) protein content did not have a clear correlation to any index. These results show that LIP and rutin concentration are important factors in the quality deterioration of buckwheat flour in terms of lipid degradation.

2.2.4 Lipase (LIP)

Lipase (triacylglycerol lipase EC 3.1.1.3) (LIP) catalyzes the first step of lipid metabolism. The LIP activity in each flour showed significant positive correlation to certain volatile compounds such as butanal and hexanal. In lipid catabolism, LIP is thought to catalyze the first step (Aizono *et al.* 1976). Many crops have LIP activity in their seeds (Aizono *et al.* 1976, Hills and Mukherjee 1989, Ncube *et al.* 1995, Taipa *et al.* 1992), and many studies have shown the importance of LIP in the food industry, because lipid hydrolysis sometimes causes a deterioration in food quality (Ashie *et al.* 1996). Buckwheat seed LIP has been partially purified and characterized (Kondo *et al.* 1982, Ohinata *et al.* 1997, Suzuki *et al.* 2004b). Buckwheat LIP consists of at least two monomeric isozymes (LIP I and II). The molecular mass of LIP I was approximately 150 kDa and the molecular mass of LIP II was approximately 27 kDa. The characteristics of LIP I and II were determined using triolein as a substrate. The optimal pH values were 3.0 (LIP I) and 6.0 (LIP II), which are distinctly different from those of other plant lipases; rape (*Brassica napus* L. var. *oleifera* (Moench) Metzg.) (Antonian 1988), mustard (*Sinapis alba* L.) (Antonian 1988) and rice (Aizono *et al.* 1976), where the optimal pHs were between 8 and 9. Buckwheat LIP I and II have unique substrate specificity; LIP II had approximately a two-fold greater specific activity than LIP I for all substrates tested. Substrate specificities of both LIP I and II are as follow; triolein > monoolein > tripalmitin > monopalmitin. These results suggest that LIP activity should be higher against triacylglycerol than monoacylglycerol. Generally, the pH of buckwheat flour is approximately 6.8, which is suitable for LIP-catalyzed reactions. The increased LIP activity causes fatty acid release and a decrease in pH. To inactivate LIP activity in buckwheat flour, heat treatment may be effective because buckwheat LIP is not stable at high temperature *in vitro*. However, heat treatment sometimes results in a deterioration of flavor, color, and physical properties. Therefore, it is desirable to breed buckwheat cultivars with low LIP activity in their flour. Varietal differences in LIP have been partly studied using 14 buckwheat varieties/breeding lines (Suzuki *et al.* 2005a). Relative LIP activity varied from 42.9% to 100%. Interestingly, relative LIP activity of breeding lines developed by maternal selection from the Japanese variety ‘Tanno-Hiushinai’ ranged from 95% to 64%. This indicates that selection is an effective method to breed high/low LIP varieties.

2.2.5 Lipoxygenase (LOX)

Lipoxygenase (EC 1.13.11.12) (LOX) catalyzes the peroxidation of FFAs.

In buckwheat, several studies have shown that the seed has no detectable LOX activity *in vitro* (Axelrod 1974). In addition, immunoblot analysis using anti-LOX IgG showed that buckwheat seed contains notably low LOX protein compared to that in other crops such as soybean, millet, amaranth, and sunflower (the LOX protein content was approximately 2–4 times less than that of soybean) (Suzuki *et al.* 2009). The study also demonstrated the presence of at least two LOX isozymes in buckwheat seed. LOX protein was distributed only in the embryo and no sign of LOX protein was detected in either the endosperm or testa. In buckwheat, there are known varietal differences of LOX protein content among 12 buckwheat cultivars; relative LOX1 concentration varied from 100% to 14.2% and LOX2 from 68.9% to 19.8% (compared to LOX1 protein concentration). Therefore, breeding high LOX varieties may be possible following large scale screening of many buckwheat varieties, including wild individuals.

2.2.6 Peroxidase (POX)

The peroxidase (EC 1.11.1.7) (POX) activity catalyzes the degradation of peroxide generated by LOX activity, which leads to the generation of carbonyl compounds. The POX activity also showed a significant positive correlation to 3-methylbutanal and 2-methylbutanal content. Plant POX is widely distributed (Amako *et al.* 1994, Van and Cairns 1982). Many studies have shown a variety of functions of POX, including in plant defense mechanisms (Bradley *et al.* 1992, Kolattukudy *et al.* 1992). POX also plays important roles in food quality, including in the deterioration of flavor and color (Ashie *et al.* 1996, Ibaraki *et al.* 1988, Ibaraki and Hirano 1989). In soybean, carbonyl compounds such as aldehydes and ketones, are the major contributors to 'beany' and 'green' flavors (Fukushima 1994). These compounds are mainly generated by lipid peroxidation with POX (Anli and Tilak 2004, Matoba *et al.* 1975, 1985).

Buckwheat seed POX has been partially purified and characterized (Kondo *et al.* 1982, Suzuki *et al.* 2005b). In addition, the relationship between changes of color of buckwheat product and POX activity have been discussed (Kondo *et al.* 1982). The POX resulted consisting of at least two monomeric isozymes, POX I and POX II, and were distributed in the embryo. The molecular mass of POX I was 46.1 kDa and the molecular mas of POX II was 58.1 kDa. These are similar to those of other peroxidases (Sakharov *et al.* 2000). The K_m for substrates tested were different for POX I and POX II. Both POX I and II had low K_m for phenolic substances such as quercetin and guaiacol. Therefore, buckwheat POX may play a role in the color change in buckwheat noodles (Francisco and Juan 2001, Kondo *et al.* 1982). POX II activity was greatest at 10°C, and POX I activity was greatest at 30°C In POX I, more than 50% of activity was retained in the temperature range of 0–50°C. On the other hand, POX II had strong activity in the lower temperature range of 0–20°C and it decreased above 20°C. Therefore, POX I is able to be active during storage, even if buckwheat seeds are stored at low temperatures such as 5°C. Varietal differences of POX activity have been partly investigated: it varied from 100% to 33% (Suzuki *et al.* 2005a). POX activity of breeding lines developed by maternal selection from the Japanese cultivar 'Tanno-Hiushinai' varied from 100% to 33%. This is similar to the result of LIP activity in buckwheat. This indicates breeding based on selection may be possible to create high/low POX varieties.

2.2.7 Breeding strategy for high value of cultivar

LIP and POX activity in buckwheat flour affect buckwheat quality. The quality deterioration mechanism in buckwheat flour is different from that of rice and soybean; the LOX isozymes showed no significant correlation to volatile compounds in buckwheat, whereas LOX is a key enzyme in the generation of 'beany' flavor (Fukushima 1994, Robinson *et al.* 1995) in soybean (Takano 1993). In addition, LOX is also a key enzyme in the generation of unfavorable volatile compounds in rice storage (Suzuki *et al.* 1999). On the other hand, LIP and POX are the key enzymes that generate major volatile compounds in buckwheat. Therefore, in buckwheat, the key enzyme that generates major volatile compounds such as hexanal, is different from those in soybean and rice. From these results, we conclude that it is may be possible to breed buckwheat varieties that have enhanced flavor through modulation of LIP and POX activity in seeds.

To breed a buckwheat variety that does not deteriorate easily, decreasing the LIP activity would be an effective strategy. In addition, rutin tends to prevent flour deterioration, and rutin acts as an inhibitor of LIP activity. LIP activity in buckwheat flour can be inhibited up to 40% by the presence of rutin at approximately the same concentration as in buckwheat flour *in vitro* (Suzuki *et al.* 2005b). On the other hand, rutin concentration was not related to generation of major volatile compounds in buckwheat noodles (Suzuki *et al.* 2010). This indicates that increasing LIP activity and rutin concentration would be effective strategies for the development of enhanced flavor varieties with a more stable flour. To reinforce the above hypothesis, differences in tissue distribution and/or accumulation between rutin and LIP are required. In addition, screening and pyramiding of multiple enzymes such as LIP, POX, and LOX may also result in effective expansion of the range of traits for flour deterioration and flavor generation.

2.3 Taste and color

In buckwheat foods, taste is an important characteristic. Generally, the following elements of taste are important; sweetness, saltiness, sourness, bitterness, and 'umami'. Among them, sweetness, and bitterness are relatively important elements for noodle makers in Japan. Sugar and amino acid compounds cause sweetness, whereas some kinds of polyphenols are the source of bitterness. On the other hand, the color of grain seed, flour, and processed foods are also important quality traits. In this section, we summarize the effects of compounds that affect buckwheat taste and color, and consider the consequences for breeding.

2.3.1 Sweetness and 'umami'

In buckwheat, sugars and some kinds of amino acids are the cause of sweetness. Sucrose and fagopyritol (Horbowicz *et al.* 1998) are two of the most important contributors to sweetness in buckwheat flour. In the soluble carbohydrate in buckwheat seeds, approximately 42% is sucrose and 40% is fagopyritols (Horbowicz *et al.* 1998). Glutamate is one of the most important amino acids that causes the 'umami' sensation. Pomeranz and Robbins (1972) reported differences of amino acids in 10 varieties have been studied (Pomeranz and Robbins 1972); as a proportion of total amino acids, the glutamic acid content ranged from 19.4% to 17.8%.

2.3.2 Bitterness

Bitterness is also an important quality characteristic for buckwheat. In buckwheat flour, bitterness increases with the storage temperature of flour in sensory analysis (Kawakami *et al.* 2008) and is also observed in instrument evaluation. However, varietal differences of bitterness generation have not yet been reported. On the other hand, the seeds of Tartary buckwheat (named 'bitter buckwheat') have a strong bitter taste; this bitter taste is also noticeable in the foods (such as noodles) made from Tartary buckwheat seeds. The cause of the bitter taste is generation of bitter compounds such as quercetin, which is generated by rutinosidase activity (**Fig. 4**) (Suzuki *et al.* 2002, 2004a,

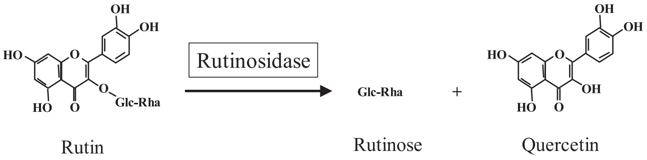

Fig. 4. Rutinosidase in Tartary buckwheat seeds converts rutin to quercetin and rutinose.

2007a, 2014a, Yasuda and Nakagawa 1994). Varietal differences in Tartary buckwheat have shown (Katsu *et al.* 2019) that a variety with trace-rutinosidase activity does not have bitterness in the dough and processed foods (Suzuki *et al.* 2014b, 2015a). To date, several approaches to increase the rutin content of buckwheat have been reported; among them, interspecific hybridization with Tartary buckwheat would dramatically increase rutin content in common buckwheat. During these efforts, it should also be important to develop trace- or non-rutinosidase characteristics in order to prevent the generation of bitterness.

2.3.3 Color of seed, flour, and dough

The color of the seed (testa) after removal of the husk is an important characteristic for buckwheat. Generally, Japanese millers, distributers, noodle makers, and some consumers value a green color. To obtain green-colored seed, it is necessary to harvest the seed early, however this has a negative impact on yield. Therefore, varieties that have a green color in mature testa are desirable. The green color in testa is recognized as an accumulation of chlorophyll. To date, some varieties and breeding lines have been developed that have enhanced green color of testa. Campbell (2003) first reported an enhanced green-colored breeding line, developed using the self-compatible buckwheat *F. homotropicum*. In Japan, a buckwheat variety with enhanced green-colored testa was developed in Nagano Prefecture (Maruyama *et al.* 2014). However, some Japanese buckwheat millers desire greener varieties, therefore further breeding is required.

On the other hand, when the seed is exposed to light after the removal of the husk, it turns to brown easily. This change of color is also observed during the storage of seed even when the husk is not removed. In Japan, brown seed are generally considered to be of lower quality. Therefore, buckwheat varieties that do not turn brown easily and retain a green color for long periods during storage are desirable. In addition to chlorophyll, some kinds of polyphenols also turn brown through oxidation. Buckwheat seeds, embryos, and testa contain polyphenols, and attempts could be made to reduce polyphenol contents through breeding. However, the absence of polyphenols in testa and embryos would have a negative effect on the health benefits of buckwheat.

With regard to buckwheat flour, the 'Sarashina' flour, the starch-rich milling fraction, has a high price because of its pure white color. To obtain the 'Sarashina' flour, millers collect the endosperm-rich milling fraction. However, when millers want to increase the yield of 'Sarashina' flour, embryo and testa can contaminate the fraction. Contamination by testa and embryo negatively affects the desirable white color. Therefore, breeding varieties with a white colored testa and embryo, with low chlorophyll and polyphenol content, is desirable to increase the yield of 'Sarashina' flour. In addition, the lower part of dehulled buckwheat seed which connects to the pedicel has a very strong brown color. This causes undesirable flour color, in the flours of both enhanced green-colored varieties and 'Sarashina' white flour. Therefore, selection for weak-colored or white-colored dehulled buckwheat seed is important for further improvement of flour color, based on traditional Japanese requirements. On the other hand, 'Kasha' is a traditional food in Russia and Ukraine where a brown color is generally desirable. In this case, the breeding target changes to the opposite, aiming at the development of a brown colored variety at the time of harvesting. To change these colors by breeding, studies are required to establish a clear mechanism for chlorophyll and polyphenol accumulation in testa, embryo, and the bottom of dehulled buckwheat seeds.

In some crops such as wheat, changes in dough and noodle color during processing is an important quality characteristic. In wheat, polyphenol oxidase and peroxidase are important contributors to the change in noodle color to dark or brown. In buckwheat, some consumers value brightness (whiteness) in noodles, especially in noodles made from 'Sarashina' flour, and therefore enzymes that alter the color of buckwheat dough are important. In buckwheat seeds, POX has been characterized (Kondo *et al.* 1982, Suzuki *et al.* 2006).

2.4. Functional compounds and clinical trial

2.4.1 Anti-oxidative compounds

Buckwheat grain has a higher anti-oxidative activity compared to cereal grains (Zieliński and Kozlowska 2000). Antioxidants protect the human body from oxidative damage caused by free radicals. There is a significant correlation between anti-oxidative activity and total polyphenol content (Morishita *et al.* 2002, Watanabe *et al.* 1995). Various anti-oxidative compounds, such as vitamins B1, B2, and E, and phenolic compounds (including polyphenols such as catechins, rutin, quercetin and proanthocyanidins) have been identified in common buckwheat hulls and groats (Watanabe *et al.* 1995, 1997, Watanabe 1998). In particular, Watanabe (1998) reported that various catechins ((–)-epicatechin and (–)-epicatechin gallate) contribute to the anti-oxidative activity in common buckwheat groats. On the other hand, rutin is especially interesting to researchers because it is also recognized as a functional flavonoid. Furthermore, rutin has been considered to be a potentially major antioxidant because it shows anti-oxidative activity (Suzuki and Miyazawa 1991). However, Oomah and Mazza (1996) reported no significant correlation between anti-oxidative activity and rutin content. Furthermore, Morishita *et al.* (2007) also reported that the contribution rate of rutin to anti-oxidative activity was

Table 1. Antioxidative activity and contents of (–)-epicatechin, (–)-epicatechingallate, and rutin in buckwheat varieties

Varieties	Antioxidative activity µmol-Trolox/g DW		(–)-Epicatechin mg/100 g DW		(–)-Epicatechingallate mg/100 g DW		Rutin mg/100 g DW	
	2007	2008	2007	2008	2007	2008	2007	2008
Gamma no irodori	26.4	27.5	32.2	52.3	6.4	8.6	56.8	56.7
Cobalt no chikara	28.5	27.6	106.8	120.8	12.2	12.8	14.1	18.6
Ruchiking	25.4	25.4	62.3	72.7	5.0	6.5	70.7	70.3
Hitachiakisoba	19.0	18.2	28.4	32.1	2.4	3.2	18.2	17.9
Botansoba	17.8	18.4	20.9	26.1	3.1	3.1	17.8	20.3

approximately 2% of the total activity, suggesting that rutin is not a major antioxidant in common buckwheat grains.

Morishita *et al.* (2019) tried to breed buckwheat varieties with high anti-oxidative properties by recurrent individual selection and succeeded in developing some high anti-oxidative lines. Among these lines, three were named ‘Gamma no irodori’, ‘Cobalt no chikara’, and ‘Ruchiking’. These varieties have different chemical compositions (**Table 1**): ‘Gamma no irodori’ is characterized by high-rutin content; ‘Cobalt no chikara’ is characterized by high (–)-epicatechin and low rutin; and ‘Ruchiking’ is characterized by extremely high rutin and high (–)-epicatechin. Furthermore, other unselected lines have shown high (–)-epicatechin and low rutin contents (Morishita *et al.* 2019). Consequently, it appears that the selection for high (–)-epicatechin grain content should contribute for improving anti-oxidative activity in common buckwheat.

2.4.2 Anti-hypertensive and anti-hyperglycemic functional compounds

It has been demonstrated that buckwheat flour might alleviate important diseases such as diabetes, obesity, hypertension, and hypercholesterolemia in experiments with animal models (Christa and Soral-Śmietana 2008). Satyam and Khushbu (2016) also reviewed the nutraceutical aspects of buckwheat in view of its adoption as an ingredient for the production of functional food.

Angiotensin-I converting enzyme (ACE) is an enzyme involved in regulation of blood pressure. This enzyme acts to increase blood pressure in the renin-angiotensin system. By inhibiting ACE, it is possible to keep blood pressure low. Buckwheat contains a compound that inhibits ACE activity (Suzuki *et al.* 1983). This compound is thought to be the hydroxy derivative of nicotianamine, and its chemical structure is 2″-hydroxynicotianamine, which is contained in both buckwheat flour and plant body (Aoyagi 2006). This compound shows a very high inhibitory activity toward ACE and has a half maximal inhibitory concentration (IC50) of only 0.08 µM (Aoyagi 2006). This is almost equal to the ACE-inhibitory effect of nicotianamine, which is known to have a strong ACE-inhibitory effect (Aoyagi 2006, Kinoshita *et al.* 1993, Shimizu *et al.* 1999). Furthermore, 2″-hydroxynicotianamine is expected to have a blood pressure-lowering effect as well as nicotianamine in spontaneously hypertensive rats (SHR) (Aoyagi 2006, Kinoshita *et al.* 1993, Shimizu *et al.* 1999). The compound 2″-hydroxynicotianamine has been found in polygonaceous plants, but other analyzed plant families contain no detectable amounts of 2″-hydroxynicotianamine (Aoyagi 2006). Thus, the compound is considered to be a compound peculiar to the family Polygonaceae. In Tartary buckwheat leaves, 2″-hydroxynicotianamine concentration among varieties is 20–100% (relative concentration) (Suzuki *et al.* 2008). However, little is known about the varietal differences of 2″-hydroxynicotianamine in common buckwheat.

D-Chiro-Inositol (DCI) in buckwheat also has an effect on high blood pressure. DCI is a compound with an insulin-like bio activity; it acts as a component of a putative mediator of insulin action (Ortmeyer *et al.* 1993). It works to increase the action of insulin, and so it has a positive effect on hyperglycemia as well as on hypertension (Cheang *et al.* 2008, Wang *et al.* 2013). Of the nine inositol isomers, myo-inositol is the most commonly occurring isomer in nature, whereas DCI is relatively rare (Kawa *et al.* 2003). Consumption of buckwheat flour has hypoglycemic effects in patients with diabetes (Lu *et al.* 1992, Wang *et al.* 1992). The glucose-lowering effect of the buckwheat concentrate is of a similar magnitude as that of synthesized DCI (Fonteles *et al.* 2000, Kawa *et al.* 2003). Kawa *et al.* (2003) suggested that DCI is primarily responsible for the glucose-lowering effect of buckwheat. In addition, fagopyritol, a sugar oligomer containing DCI, is approximately half of the soluble carbohydrate in buckwheat flour. Fagopyritol consists of at least six molecular species consisting of 2–4 oligomers; fagopyritol A1, A2, A3, B1, B2, B3. Among the six fagopyritols found in common buckwheat, fagopyritol A1 is interesting for medical applications related to a putative insulin mediator (Berlin *et al.* 1990, Larner *et al.* 1988). Because of similarities in their structures, fagopyritols have considerable value for the development of a novel plant-based functional compound for the treatment of insulin response disorders such as Non-Insulin Dependent Diabetes Mellitus (NIDDM) (Asplin *et al.* 1993) and Polycystic Ovary Syndrome (PCOS) (Nestler *et al.* 1999). Although the biosynthetic pathway of fagopyritols has not yet been clarified in buckwheat, several studies have shown possible enzymatic reactions for fagopyritol synthase, such as involvement with galactinol synthase or stachyose synthase like enzymes using UDP-Gal and galactinol as a sugar donor, respectively (Frydman and Neufeld 1963, Hoch *et al.* 1999). Izydorczyk and Head (2010) reported varietal differences of total fagopyritol content in seeds using five buckwheat varieties, which varied between 400 and 520 mg/100 g flour. Modification of enzymes related to DCI and fagopyritol should contribute to breeding a buckwheat variety that has an enhanced concentration of these compounds.

2.4.3 Rutin

Rutin is a kind of polyphenol that is widely distributed in plants (Bandyuko and Sergeeva 1974, Couch *et al.* 1946, Fabjan 2003, Haley and Bassin 1951, Sando and Lloyd 1924). Buckwheat is famous for having rutin not only in its seed, but also in its leaves. Therefore, buckwheat is a good source of rutin, Tartary buckwheat has approximately a 100-fold higher rutin content in its seeds compared to common buckwheat. To date, many beneficial health effects have been reported for rutin including strengthening of fragile human capillaries (Griffith *et al.* 1944, Shanno 1946), anti-hypertensive properties (Matsubara *et al.* 1985), anti-oxidative properties (Afanas'ev *et al.* 1989, 2001, Awatsuhara *et al.* 2010, Jiang *et al.* 2007), anti-inflammatory properties (Afanas'ev *et al.* 2001), and alpha-glucosidase inhibitory activities (Li *et al.* 2009). In addition, rutin and rutin containing foods have been considered to be a potentially major antioxidant because they show antioxidative activity (Ishiguro *et al.* 2016, Suzuki and Miyazawa 1991). Numerous genetic resources were analyzed, and common buckwheat grains were found to contain 10–30 mg/100 g DW rutin (Brunori and Vegvari 2007, Kitabayashi *et al.* 1995a, 1995b, Morishita and Tetsuka 2002, Ohsawa and Tsutsumi 1995, Suzuki *et al.* 1987). The estimated heritability values of seed rutin content and leaf rutin content were 0.76 and 0.10, respectively. Kitabayashi *et al.* (1995b) investigated the heritability of the seed rutin content; the heritability was as high as that of days to first flowering. They also performed a parent-offspring correlation analysis for seed rutin content. The correlation coefficients between the progeny lines and the parent individuals were almost zero. Therefore, increases of genetic variation within a variety through crosses between strains, or mutagen treatments, should be effective for the breeding of the seed rutin content by individual selection. On the other hand, the possible roles of rutin for buckwheat plants have also been studied. Studies have shown that rutin has a function in the plant defense mechanism against UV radiation, cold stress, desiccation stress, and worm predation (Dubey *et al.* 2013, Suzuki *et al.* 2005c). Therefore, the improvement of rutin content is an important breeding subject. To date high-rutin common buckwheat varieties 'SunRutin' and 'Toyomusume' have been developed by individual or mass selection (Ito *et al.* 2005, Minami *et al.* 2001).

To increase rutin content, understanding the rutin synthesis pathway is important. The possible rutin synthesis pathway in buckwheat is as follows; isoquercetin 3-O glucopyranose rhamnosyl transferase reaction (3GT), followed by quercetin 3-*O* glucosyltransferase reaction (3GT); UDP-rhamnose and UDP-glucose are the sugar donors for this reaction. Among these compounds, 3GT has been purified and characterized from buckwheat cotyledons (Suzuki *et al.* 2005d). The 3GT was a 56.0 kDa monomeric enzyme, and the K_m was 27 μM for quercetin (as a sugar acceptor) and 1.04 mM for UDP-Glc (as a sugar donor). In addition, the 3GT does not react with other sugar acceptors. This is a unique result because the 3GT of other plants generally shows a high affinity, not only for quercetin, but also for other flavonols, flavone, and flavanone. This may indicate that buckwheat 3GT may have evolved specifically for rutin synthesis. On the other hand, there are studies on the purification and characterization of RT or other enzymes related to the rutin synthesis pathway.

2.4.4 Polysaccharides and dietary fiber

Dietary fiber avoids hydrolysis by digestive enzymes in the human small intestine. It passes to the large bowel where it is completely or partially fermented by bacteria. Dietary fiber plays a critical role in the prevention of some diseases including type 2 diabetes, heart disease, and obesity (Anderson *et al.* 2009). The major components of dietary fiber are non-starch polysaccharides (i.e., cellulose, hemicellulose, and pectin) (Dhingra *et al.* 2012). Dietary fiber can be divided into two types according to water solubility: insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) (Elleuch *et al.* 2011). Cereals, and pseudo-cereals including buckwheat, are important sources of dietary fiber. Although the properties of dietary fiber in common cereals such as wheat, rice, and barley are well studied (Robin *et al.* 2012), there is limited information on buckwheat dietary fiber. Generally, the total dietary fiber (TDF) content of buckwheat groats is 5–10% (Lu *et al.* 2013, Steadman *et al.* 2001, Zheng *et al.* 1998). The IDF of whole buckwheat groats accounts for 31.4% of TDF (Steadman *et al.* 2001), whereas IDF ranges of 53.9–81.1% have been reported for buckwheat groats of 10 cultivars (Lu *et al.* 2013). Buckwheat husks have been reported to have a TDF content (69.9–85.7%) approximately 10 times higher than buckwheat groats (Lu *et al.* 2013). According to an early study by Asano *et al.* (1970), water soluble polysaccharide from buckwheat endosperm consisted of xylose, mannose, galactose, and glucuronic acid. Recently, Wefers and Bunzel (2015) reported that both SDF and IDF from dehulled common buckwheat seeds contained large amounts of pectin, and that xyloglucans were the major hemi-cellulosic polysaccharides in IDF. Izydorczyk and Head (2010) reported differences in the TDF, SDF, and IDF contents in each milling fraction of five buckwheat varieties. In whole buckwheat flour, TDF, SDF, and IDF ranged from 6.7–9.1%, 4.3–6.5%, and 2.3–3.2%, respectively. This indicates that selection of variety and milling fraction is an effective way to obtain dietary fiber-rich flour and resultant products. Selection with respect to these compounds in future buckwheat breeding would be effective for value-added food production.

2.4.5 Clinical trial

Buckwheat is recognized as a functional food and a good source of nutritionally valuable amino acids, dietary fibers, and minerals. It is used traditionally as a health food in the treatment of different diseases, but there is a lack of experimental studies of how buckwheat or rutin consumption affects the expression of disease symptoms. Some clinical trials of the effects of common or Tartary buckwheat consumption on diseases have been reported in recent years.

There are some studies of the effect of buckwheat on postprandial glucose. Skrabanja *et al.* (2001) studied the nutritional properties of starch in buckwheat products. The incremental changes in blood glucose after ingestion of the boiled buckwheat groats and the bread product (50% buckwheat groats) were lower than the reference white wheat bread. The blood glucose level following the boiled buckwheat groats meal significantly differed from the white wheat bread meal. The calculated glycemic index (GI) for boiled buckwheat groats and the bread containing 50% buckwheat groats were 61 and 66, respectively. Postprandial insulin responses in healthy subjects following ingestion of breakfast meals with buckwheat products were also significantly lower than that of the white wheat bread reference. Su-Que *et al.* (2013) demonstrated the effects of steamed buckwheat bread on postprandial plasma glucose in patients with type 2 diabetes. The plasma glucose increments and GI of the subjects consuming steamed buckwheat bread were significantly lower than of subjects consuming white wheat bread. This result indicates that the steamed bread made from buckwheat would be a suitable food for the prevention and treatment of diabetes. Bojňanská *et al.* (2009) performed a clinical trial on the effects of the consumption of the buckwheat on plasma total antioxidant status (TAS). The results showed an increase of TAS in the subjects consuming the bread containing 30% buckwheat flour for four weeks. Wieslander *et al.* (2011) carried out a double-blind crossover intervention study to investigate the effects of the consumption of common and Tartary buckwheat on general and mucosal symptoms. The participants were divided into two groups; the first group consumed four Tartary buckwheat cookies per day (359.7 mg rutin-equivalents/day), and the second group consumed four common buckwheat cookies per day (16.5 mg rutin/day) for two weeks. The overall results for both types of buckwheat cookies showed that there was a significant reduction in nasal irritation, headache, and fatigue symptoms during the study period. Tartary buckwheat initially reduced fatigue symptoms especially in the normal weight subjects, no significant changes were detected in ocular symptoms or throat irritation. Wieslander *et al.* (2012) also trialed a clinical test (double-blind crossover) of common and Tartary buckwheat cookies on selected clinical markers. Participants consumed common buckwheat (16.5 mg of rutin equivalent) or Tartary buckwheat cookies (359.7 mg of rutin equivalent) and were monitored for clinical markers related to cardiovascular disease and lower airway inflammation, lung function and subjective breathing difficulties. There was a significant reduction of total-cholesterol and high density lipoprotein (HDL)-cholesterol after four weeks, and a significant improvement of lung function in subjects irrespective of the type of buckwheat cookies. It is possible these results are not caused by rutin, but other by components in both types of buckwheat, such as resistant starch and/or protein. Further studies are needed to confirm the possible beneficial effects of compounds found in both types of buckwheat. The consumption of Tartary buckwheat cookies with high levels of the antioxidant rutin reduced levels of serum myeloperoxidase (MPO), an indicator of inflammation. This result indicates that Tartary buckwheat with high levels of rutin may have an anti-inflammatory function as a consequence of its ability to lower MPO levels. Nishimura *et al.* (2016) investigated whether the rutin-rich Tartary buckwheat cultivar 'Manten-Kirari', a trace-rutinosidase variety, could reduce arteriosclerosis, display antioxidant effects, and change body composition in a double-blind placebo-controlled study. The participants in this study took 80 g (dry weight) of the active test noodles (619.8 mg of rutin before cooking) made from rutin-rich Tartary buckwheat or placebo noodles (0 mg of rutin) per day for 12 weeks. The thiobarbituric acid reactive substance (TBARS) levels significantly decreased at week 8 in the active test food group. In addition, the ingestion of the Tartary buckwheat significantly decreased body weight (BW), body mass index (BMI) and body fat percentage (BFP) compared to the ingestion of the control noodles. Diabetic kidney disease (DKD) is one of the most frequent complications of diabetes. A recent study demonstrated the significant protective effect exerted by buckwheat flavonoids on renal function in T2DM (Type 2 Diabetes Mellitus) rats (Chu *et al.* 2011). Qiu *et al.* (2016) performed a parallel, randomized, open-label controlled, 4-week dietary intervention trial investigating the effects Tartary buckwheat on the renal function in patients with type 2 diabetes. Patients with T2DM were screened and randomly assigned to a diet control group or a Tartary buckwheat intervention group. A daily intake of Tartary buckwheat significantly decreased the urinary albumin to creatinine ratio (UACR) after a 4-week intervention. In addition, the Tartary buckwheat group showed a lower urea nitrogen (UN) level than the control group. The renal profile in T2DM patients with different DKD stages showed that, compared with the diabetic control (DC) group, the Tartary buckwheat group inhibited the increase in UACR and decreased UN when UACR <10 mg/g ($P < 0.05$); UACR of the Tartary buckwheat group was also decreased when UACR ≥30 mg/g ($P < 0.05$). In this study, they showed that dietary Tartary buckwheat intake could potentially prevent renal dysfunction in T2DM parents with normoalbuminuria and ameliorate renal function at the early stages of DKD. Administration of Tartary buckwheat showed anti-hypertensive effects in animal models (Li *et al.* 2002, Merendino *et al.* 2014). In addition, it has recently been shown that the *n*-butanol fraction and rutin extracted from Tartary buckwheat are protective against, and have possible therapeutic applications for, Alzheimer's disease (Choi *et al.* 2015). It is expected that future studies will include further clinical trials and examine the molecular mechanism of buckwheat compounds on disease.

3. Plants

3.1 Leaves and sprouts

Buckwheat leaves contain rutin and are often used as vegetables. In the leaves of common and Tartary buckwheat, the rutin concentration varies 1,880–3,610 mg/100 g dry weight (Kitabayashi *et al.* 1995a, 1995b). These results are from a mixture of young and old leaves. The estimated heritability value of leaf rutin content resulted 0.10 whereas the rutin content in leaves is relatively low compared to those of the main characters (Kitabayashi *et al.* 1995b). The stems of buckwheat also contain rutin. However, buckwheat stems contain the unfavorable compound oxalic acid, and the stem tends to be hard in the late growth stage. In addition, buckwheat flowers and shoots after flowering contain fagopyrins, which are fluorescent phototoxic compounds. Buckwheat sprouts (seedlings) or young shoots contain less fagopyrin, and therefore, they are suitable for use as a vegetable. In buckwheat, there have been no studies on varietal differences of rutin content of leaves. On the other hand, varietal differences of Tartary buckwheat sprout have been reported (Kim *et al.* 2007a, 2007b). Breeding for common buckwheat sprouts can be performed more efficiently using the information and techniques developed for Tartary buckwheat. Buckwheat contains anthocyanins in the stem, pericarp, and flowers. Anthocyanins are recognized as functional compounds with antioxidant activities, anticancer properties, and visual acuity enhancement (Matsumoto *et al.* 2001). In addition, the red color of anthocyanin is important to catch the eye of the consumer. Common buckwheat seedlings contain anthocyanins such as cyanidin 3-O-glucoside (C3G), cyanidin 3-O-rutinoside (C3R), cyanidin 3-O-galactoside, and cyanidin 3-O-galactopyranosyl-rhamnoside (C3GaR), while Tartary buckwheat contains only C3G and C3R (Suzuki *et al.* 2007b). Buckwheat hypocotyls contain large amount of cyanidin C3R and its contribution to antioxidant activity have been investigated (Watanabe 2007). Varietal differences of anthocyanins in common and Tartary buckwheat have been reported in eight common buckwheat and nine Tartary buckwheat varieties/breeding lines (Kim *et al.* 2007a). The differences of gene expression in the synthesis pathway of polyphenols including anthocyanins have also been investigated (Park *et al.* 2011), whereas its enzymatic characterization has not. This information will assist in the future breeding of buckwheat varieties with high anthocyanin content and other flavonoids.

3.2 Flower color and honey

Buckwheat is well known for its profuse flowering; the flowers are either white, pink, or red, which make it very attractive to insects and suitable as an ornamental plant. In Japan, most buckwheat varieties are of the white-flower type. On the other hand, there are a few varieties that have a pink or red flower and/or pericarp, such as 'Takane Ruby', 'Great Ruby', and 'Akiakane' (Sasaki *et al.* 2012). 'Akiakake' presents a higher number of flowers compared to 'Takane Ruby'. In addition, 'Akiakake' is characterized by improved yield and lodging resistance both in comparison to 'Takane Ruby' and 'Shinano No. 1', the latter being the leading variety of the region.

Buckwheat petals contain C3G, C3R, cyanidin 3-O-rhamnoside, and C3GaR. Red and pink buckwheat flowers contain higher amounts of anthocyanins than white ones (Suzuki *et al.* 2007b), Interestingly, the honey from red-flower buckwheat has extremely high anti-oxidative activity compared to honey from white-flower buckwheat (Inoue *et al.* 2005); this may be related to the anthocyanin content in the flowers. Therefore, breeding of red-flower buckwheat may be suitable for making functional foods including honey. However, buckwheat flowers contain fagopyrins, which are phototoxic compounds (Ebermann *et al.* 1996, Kreft *et al.* 2013). Therefore, decreasing the fagopyrin content is also important to expand buckwheat usage for foods. In addition, cross-pollination between red- and white-flower buckwheat is not desirable because it leads to changes of the flower color in the progeny. To prevent this situation, breeding of tetraploid varieties for red-flower buckwheat would be effective because most buckwheat varieties are diploid, and seed resulting from the cross-fertilization of tetraploid and diploid varieties would not be viable.

Buckwheat is also used as a honey plant. Its honey has a particular flavor with a dark brown to black color. In addition, buckwheat honey is reported to act as a protective agent against lipid oxidation (McKibben and Engeseth 2002), as having antibacterial and cellular antioxidant activities (Deng *et al.* 2018), and to have high serum antioxidant capacity in humans (Gheldof *et al.* 2003). Pasini *et al.* (2013) investigated the quality of 10 buckwheat honeys collected from Italian and Eastern European beekeepers. The study recognized large differences in the quality of the honeys, in their volatile compounds, flavonoid content, and sugar content. These honeys are collected as monofloral honeys, therefore the differences in the honeys may be caused by buckwheat varietal differences. In addition, red-flower buckwheat honey has a very high antioxidant activity (Inoue *et al.* 2005). Therefore, breeding buckwheat that has flowers that contain flavonoids, including in the pollen, may be effective in increasing the functional value of honey from buckwheat.

4. Other usage of buckwheat

4.1 Cereal grains

In Russia and Ukraine, most buckwheat seed is consumed as cereal grain called 'Kasha'. Also in some areas of Japan, such as Tokushima prefecture, people consume buckwheat as cereal grain. To prepare cereal grain, dehulling is necessary. Buckwheat may be dehulled either through boiling or mechanically. For both dehulling methods, sieves are used to separate the dehulled seed from the non-dehulled seed. In this process, seed size and shape uniformness are important traits for efficient dehulling. Therefore, breeding buckwheat varieties for uniform seed size and shape would be desirable.

Boiled or steamed cereal grains can become weak and fragile after they cool; this is a major problem in terms of palatability. In buckwheat, this characteristic is related to its starch property described in section 2.1.1 of this chapter, namely, the relatively high amylose content and long amylopectin chain length. These characteristics are concerns for foods that are not consumed immediately after cooking or are stored at low temperatures for long periods after cooking. Noodle manufacturers can use ingredients such as modified starch, which keep noodles soft even when stored for a long time in cool conditions. It is not possible to use these ingredients for cereal grain, and therefore breeding to improve buckwheat starch characteristics is required.

4.2 Fermentation and other processing

Buckwheat is also processed by fermentation in which the activity of microorganisms brings about a desirable change to a buckwheat foodstuff or beverage. For fermentation, there are specific desirable characteristics for buckwheat quality, and therefore appropriate breeding of buckwheat is required to produce high-quality fermented foods.

Buckwheat can be used for making tempeh, which is a traditional product using soybean originating from Indonesia. In buckwheat, boiled or steamed seed grain is inoculated with the fungi *Rhizopus oligosporus* or *Rhizopus oryzae* and fermented, causing the buckwheat seed grain to bind into a cake form. During the fermentation, many substances such as proteins (including some allergenic protein) are digested into smaller proteins or polypeptides (Funatsuki *et al.* 2010). To inactivate the allergenic activity of allergenic proteins completely, low-allergenic buckwheat varieties are desirable.

4.2.1 Alcohol

Soba shochu is a famous alcoholic drink that is made by the fermentation of buckwheat seed grain with *Aspergillus oryzae*. Shochu can also be made from rice, barley, sweet potatoes, and brown sugar. Compared to these other raw materials, buckwheat grain is reported to be more difficult to ferment, due to the resistance of testa tissues to be eluted by fungal enzymes (Arai *et al.* 2007). Therefore, breeding for increasing elution of buckwheat testa would be effective-desirable. In shochu, bitterness in the product is an undesirable characteristic. Mizutani *et al.* (2001) suggested that the bitter compound was isoamyl alcohol, and also reported that enzymes, whose isoelectric point is approximately 8, are related to the generation of flavor compounds including isoamyl alcohol during fermentation. The addition of the enzymes also brought about a decrease of isoleucine and leucin that relate to the generation of isoamyl alcohol. Although the enzymes have not been identified, decreasing the enzyme content would be an effective method to decrease isoamyl alcohol content, as well as decreasing the bitterness, and improving the taste of shochu.

4.2.2 Vinegar

Buckwheat is sometimes used to make vinegar, including in China. To make buckwheat vinegar, boiled or steamed raw seed grain is mixed with koji to cause an alcoholic fermentation; then, it is placed in another jar to undergo acetic fermentation. In Tartary buckwheat vinegar, high concentrations of anti-oxidative products compared to other vinegars made from oats or rice have been reported (Wang *et al.* 2012). In addition, buckwheat vinegar is well known for having a particular flavor. Anli and Tilak (2004) reported that certain volatile compounds are related to the antioxidant capacity and total flavonoid content. Interestingly, the volatile compounds include aldehyde and ketones, which are the same or similar to the volatile compounds in boiled soba noodle, whose contents are highly related to flour LIP or POX activity (Suzuki *et al.* 2010). It would be desirable to develop buckwheat varieties that have a more typical or enhanced flavor in vinegar, increasing the LIP activity, or modification of the flavonoid composition.

4.2.3 Fermentation starter

To produce fermented food, drink, and seasoning such as tempeh, shochu, and vinegar respectively, a fermentation starter, koji, is necessary to assist with the beginning of the fermentation process. Koji usually consists of a cultivation medium such as crop seeds that have been well colonized by the microorganisms used for the fermentation. Although rice and oats are generally used as material for koji, buckwheat can also be used (Toyama *et al.* 1993). Toyama *et al.* (1993) reported that buckwheat koji had sufficient α-amylase activity and glucoamylase activity to produce fermentation foods. They also reported that protease activity of buckwheat koji is several times higher compared to rice and oat koji; they supposed that the low C/N ratio of buckwheat material explained high level of protease activity. Therefore, buckwheat varieties with a high protein ratio would be promising for the production of high-quality buckwheat koji.

4.2.4 Miso and Tofu

Toyama *et al.* (1993) also produced and evaluated 'miso', which is a traditional Japanese seasoning generally produced by fermenting soybeans with salt and koji. In buckwheat miso made from buckwheat koji, the amino acid concentration was higher compared to rice or oat miso, and this had an impact on the umami flavor. To enhance umami by increasing the amino acid content, high protein buckwheat varieties would be desirable. In addition, buckwheat flavor was also recognized in buckwheat miso. Toyama *et al.* (1993) could not detect rutin in buckwheat miso, whereas Kawakami and Kawahara (2002) reported the presence of rutin in buckwheat miso. To increase the rutin content in miso, high-rutin buckwheat varieties would be beneficial, as well as obtaining koji, utilizing varieties selected for low or no rutinosidase content.

Buckwheat is also used as material of tofu, which is made by coagulating a protein-rich solution such as soy milk and then pressing the resulting curds into soft blocks. Tofu is considered a healthy food because it has relatively few calories and a high protein content. Generally, tofu is made from soybean, but it can also be made from buckwheat. The buckwheat protein content is much lower than that of soybean, and therefore, buckwheat tofu is made using buckwheat flour; the mechanical characteristics and texture are very different from soybean tofu. Recently, Ikeda (2016) developed a production method for tofu, which does not use the starch fraction of buckwheat but instead uses buckwheat extract as the protein source. To improve the quality of buckwheat tofu, the creation of high protein buckwheat varieties would be desirable.

Conclusion and future prospects

There are few studies on breeding for quality in buckwheat, and the species is understudied compared to other cereals such as rice, wheat, and soybean. The difficulty in establishing a quality evaluation method for buckwheat is an important subject. Although palatability and acceptability are subjective traits, a measurable index for objective traits that relate to palatability and acceptability are necessary to evaluate buckwheat quality for breeding. To develop objective selection methods, the usage of apparatus is effective. We need to investigate the relationship between output parameters and the apparatus and subject traits. In addition, the development of sensory analysis is as important as the development of expert panels.

On the other hand, objective quality traits such as the contents of functional compounds are easy to use as breeding objectives. To develop buckwheat varieties in which functional compounds vary it is necessary to study varietal differences in heritability and investigate the mechanism and pathway for compound synthesis/decomposition. In addition, the investigation of the safety of varieties is also important. When a Tartary buckwheat variety was developed, which contained more rutin than previous varieties, the developers also studied the acute, subacute, and mutagenicity potential of the variety using experimental animals and bacteria (Suzuki *et al.* 2015b, 2016). Investigation of the historical experiences of eating the target compounds should reinforce the safety of the variety. It is necessary to perform clinical trials to have evidence for any health benefits of the substances being altered in the breeding lines.

Recently, genomic selection for buckwheat has been reported as a powerful tool (Yabe *et al.* 2014, 2018). A draft genome sequence has been assembled; the sequence is available from the buckwheat genome database (Yasui *et al.* 2016). These new methods and technologies would be effective, not only in the improvement of agronomic characteristics, but also in the quality characteristics for buckwheat. Interspecific hybridization using cultivated and/or wild buckwheat species (Adachi 1990, Asaduzzaman *et al.* 2009, Chen 1999, Woo *et al.* 2001) would also be important to expand the range of quality characteristics. To spread high-quality food using quality improved varieties of buckwheat, it is also important to improve processing techniques. To improve buckwheat flavor, an objective evaluation method for flavor is necessary. Flavor terminology has been developed for the objective evaluation of whiskey, wine, and coffee. Although it is difficult to define the flavor terminology for buckwheat products, it may be useful to establish objective criteria as a first step toward objective evaluation in buckwheat. Buckwheat is an important famine food crop. When people are faced with serious famine, the short growth period of buckwheat is a favorable characteristic. Eating is fun for people, and therefore we hope people can have good tasting, highly functional buckwheat food products made from high-quality buckwheat varieties.

Author Contribution Statement

Each author mainly described following sections; Takahiro Noda; *Starch, Polysaccharides and Dietary fiber*, Toshikazu Morishita; *Storage proteins, Anti-oxidative compounds*, Koji Ishiguro; *Clinical trial*, Shiori Otsuka; *Enzymes for starch synthesis, Anti-hypertensive and anti-hyperglycemic functional compounds*, Tatsuro Suzuki and Andrea Brunori; other sections. All authors have critically reviewed the manuscript.

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