Possible Roles of Lipase, Lipoxygenase and Peroxidase in Buckwheat Flour and Noodles

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ABSTRACT

The freshness of buckwheat flour and its unique flavor is important for the quality of buckwheat products. Several reports have shown that lipid degradation and oxidation in buckwheat flour are the main causes of measurable quality deterioration during storage. On the other hand, some flavor compounds in buckwheat flour are produced by lipid degradation and oxidation pathway. Therefore, understanding lipid degradation and oxidation pathways is important in the quality control of buckwheat flour and products. In some crops, lipoxygenase pathway is important for lipid degradation and oxidation. The pathway includes some enzymes such as lipase (triacylglycerol lipase EC 3.1.1.3) (LIP), lipoxygenase (EC 1.13.11.12) (LOX) and peroxidase (EC 1.11.1.7) (POX). This review, mainly based on our recent results, summarizes the main aspects of the possible relation between these enzymes, their substrates and flour deterioration/flavor generation as well as purification and characterization of related enzymes. LIP and POX activity in buckwheat flour apparently plays a role in the lipid degradation and quality deterioration whereas LOX does not have significant influences. LIP and POX activity in buckwheat flour also plays an important role for flavor generation of boiled buckwheat noodles whereas LOX does not have. This indicates that the mechanism of quality deterioration and flavor generation in buckwheat flour is different from that of rice and soybean.

Keywords: deterioration, fatty acid, flavor, lipid, quality, rutin
Abbreviations: pNPC12, para-nitrophenoil laurate; ABTS, 2,2’-azino-bis-(3-ethylthiazoline-6-sulfonate); Triton X-100, polyoxyethylene (10) octylphenyl ether; Tween-20, polyoxyethylene (20) sorbitan monolaurate

CONTENTS

INTRODUCTION .......................................................................................................................................................................................... 43
CHARACTERIZATION OF THE ENZYMES LIPASE, LIPOXYGENASE AND PEROXIDASE IN BUCKWHEAT FLOUR ............................................................................................ 44
Lipase ................................................................................................................................................................................................. 44
Lipoxygenase ...................................................................................................................................................................................... 45
Peroxidase .......................................................................................................................................................................................... 45
EFFECTS OF ENZYMES ON QUALITY DETERIORATION OF BUCKWHEAT FLOUR DURING STORAGE ............................. 46
EFFECTS OF ENZYMES ON VOLATILE COMPOUND GENERATION IN BUCKWHEAT NOODLE ......................................................... 46
CONCLUSION ...................................................................................................................................................................................... 47
ACKNOWLEDGEMENTS ........................................................................................................................................................................... 47
REFERENCES ......................................................................................................................................................................................... 47

INTRODUCTION

Buckwheat (Fagopyrum esculentum Moench.) is an important crop in Japan, as well as in China, Korea and some other countries (Ikeda 2002; Kreft et al. 2003). In the Japanese food industry, buckwheat flour is mainly used in making noodles. The freshness of buckwheat flour is very important for noodle makers because buckwheat flour deteriorates easily (Tohyama et al. 1982; Muramatsu et al. 1986; Suzuki et al. 2005c). Several reports have shown that lipid degradation and oxidation in buckwheat flour are the main changes in measurable indices of quality deterioration during storage (Tohyama et al. 1982; Muramatsu et al. 1986; Suzuki et al. 2005c). Therefore, understanding lipid degradation pathways is important to understand quality deterioration mechanisms in buckwheat flour.

On the other hand, unique flavor of boiled buckwheat noodles (soba), which is important in traditional food items in Japan (Ikeda 2002), is one of the most important quality characteristics. Flavor components in buckwheat flour (Aoki et al. 1981, 1986; Przybylski 1995; Ohinata et al. 1997; Kawakami et al. 2008; Janes et al. 2009) and dough (Yajima et al. 1983) include a number of volatile compounds, of which the most important contributors include carbonyl compounds such as aldehydes and ketones (Aoki et al. 1986; Ohinata et al. 1997; Kawakami et al. 2008; Janes et al. 2009). Amongst these, hexanal is also known as a flavor compound in products using soybean [Glycine max (L.) Merr.] (Axelrod 1974; Matoba et al. 1975; Matoba et al. 1985; Anli and Tilak 2004). Therefore, understanding lipid degradation pathways is also important to understand flavor generation in buckwheat products. In soybean products,
hexanal is generated through the lipoxygenase pathway, which was first proposed in rice bran (Takano 1993) (Fig. 1). Lipase (triacylglycerol lipase EC 3.1.1.3) (LIP) catalyzes the first step of lipid catabolism. Lipoxygenase (EC 1.13.11.12) (LOX) is thought to have a significant effect on flavor generation in soybean (Fukushima 1994), rice (Oryza sativa L.) (Robinson et al. 1995; Suzuki et al. 1999) and other vegetables (Baardseth and Slinde 1987). Peroxidase (EC 1.11.1.7) (POX) also plays a role in flavor-related quality in the soybean (Ashie et al. 1996). Therefore, to investigate roles LIP, LOX and POX on buckwheat quality, purification and characterization of these enzymes in buckwheat flour is also important. A number of studies have investigated the purification and characterization of LIP (Ohnata et al. 1997; Suzuki et al. 2004), LOX (Suzuki et al. 2007a) and POX (Kondo et al. 1982; Suzuki et al. 2005d) in buckwheat flour.

CHARACTERIZATION OF THE ENZYMES LIPASE, LIPoxyGENASE AND PEROXIDASE IN BUCKWHEAT FLOUR

Lipase

LIP catalyzes the first step of lipid catabolism (Aizono et al. 1976). Many crops contain LIP activity in the seed (Aizono et al. 1976; Hills and Mukherjee 1989; Taipa et al. 1992; Moulin et al. 1994; Ncibe et al. 1995; Pernas et al. 2000). LIP is an important enzyme in the food industry, because lipid hydrolysis can cause deterioration of food quality (Ashie et al. 1996). Buckwheat LIP has been partially characterized (Kondo et al. 1982; Ohinata et al. 1997). Ohinata et al. (1997) proposed that the accumulation of free fatty acids in buckwheat flour during storage is mainly caused by LIP. An increase in free fatty acids indicates deterioration of the quality of buckwheat flour (e.g., increase in water-soluble acids). This will result in lipid peroxidation and deterioration of the flavor. From these backgrounds, Suzuki et al. (2004) highly purified and characterized LIP from buckwheat seed. The LIP consisted of at least two isozymes, LIP I and LIP II, which were separated by ion exchange chromatography. The molecular weights of LIP I and II were 150 kDa and 28.4 kDa by SDS-PAGE, and 171 kDa and 26.5 kDa by gel filtration, respectively, indicating that LIP I and II are monomers. The molecular weight of LIP II was close to that of rice lipase II (32 kDa; Aizono et al. 1976) and annual herb lipase (30 kDa; Ncibe et al. 1995). The final specific activities of buckwheat LIP I and II were measured lower than those in other plants such as LIP 2 and LIP 3 from Euphorbia characias (Moulin et al. 1994; Pernas et al. 2000). The optimal pH was determined using triolein as substrates. The optimal pH values were 3.0 (LIP I) and 6.0 (LIP II), respectively. Both LIP I and II showed activity between pH 3.0 to pH 7.0, and were inactive below pH 2.0 and above pH 8.0. LIP I reacted in a narrower optimal pH range than LIP II; LIP I activity peaked between pH 3 and 04 whereas LIP II peaked pH 3 and pH 6. Both isozymes had higher activities in the acidic pH range. Optimal pHs of buckwheat LIP I and LIP II were distinctly different from those of rape (Brassica napus L. var. oleifera (Moench) Metzg.) lipase (Antonian 1988), mustard (Sinapis alba L.) lipase (Antonian 1988), and cotedleyons of lupine (Lupinus albus L.) (Antonian 1988) and rice lipase I and II (Aizono et al. 1976), for which optimal pHs were between 8 and 9. On the other hand, optimal pHs of LIP I and II are very similar to that of castor bean (Ricinus communis L.) acid lipase (Ory et al. 1962) for which the optimal pH was 4.3. Both LIP I and II were stable below 30°C, and retained their activity at 70°C. At 100°C both LIP I and II maintained about 50% of their activity at 30°C. Therefore, LIP would remain active during storage even if stored at 10°C. Substrate specificity of buckwheat LIP I and II are unique. When pNP esters were used as a substrate, the specific activity for each ester differed between LIP I and II. Activities of both isozymes were stronger as the chain length increased. The LIP II activity for pNPC12 was much higher than for other pNP esters. Such results were similar to those obtained with LIP from Triticum var. NPC12 (Pernas et al. 2000). Substrate specificities of both LIP I and II followed the order, triolein > monoolein > tripalmitin > monopalmitin. Both LIP I and II had greater activity against triolein than against monoolein, and greater against tripalmitin than against monopalmitin. Such results would suggest that LIP activity should be higher against triacyl glycerol than monoacyl glycerol. LIP II had about two-fold greater specific activity than LIP I for all substrates tested. Based on these results, LIP I and II had different substrate specificities. The pH of buckwheat flour is generally around 6.8. Therefore, the pH of buckwheat flour is suitable for LIP-catalyzed reactions. Further, LIP activity should be increased by the progression of fatty acid release, which would decrease the pH, because LIP activity rises substantially below pH 6.0. Buckwheat flour tends to deteriorate easily, and LIP activity is supposed to play an important role in the lipid deterioration. To inactivate LIP activity in buckwheat flour, heat treatment would be effective because buckwheat LIP was not stable above 30°C when triolein was used as a substrate. However, heat treatment is costly, and would result in deterioration of flavor, color and some physical properties. Therefore, it is desirable to breed buckwheat cultivars whose LIP do not reactive in flour or dough. Further, in order to develop such a cultivar, it is important to clarify which isozyme is important for the quality of buckwheat flour. The greatest LIP activity was located in the embryo. This finding is consistent with the organ distribution of the LOX protein (Suzuki et al. 2009) and POX activity (Suzuki et al. 2005d), which may affect fatty acid metabolism. In addition, Dorrell (1970) reported that about 60% to 70% of the oil was also distributed in the embryo. The fatty acid composition of the buckwheat lipids consisted of roughly 17, 36 and 33% of palmitic acid, oleic acid and linoleic acid, respectively. This indicates that these fatty acid species can be produced by LIP activity in buckwheat flour during storage or in germinating seeds. In addition, they also reported of LIP activity being inhibited by rutin. Rutin, a kind of flavonol glycoside, exhibits beneficial effects on fragile capillaries (Griffith et al. 1944; Shanno 1946). It also has antioxidative (Afanas’ev et al. 1979; Steger-Hartmann et al. 1982; Tard et al. 1985) and anti-inflammatory activities (Afanas’ev et al. 1995). In addition, it had α-glucosidase inhibitory activity (Li et al. 2009). Rutin is widely distributed in the plant kingdom (Sando et al. 1924; Couch et al. 1946; Hale et al. 1951; Bandyuko et al. 1974; Fabjan et al. 2003). Buckwheat is the only known cereal to contain rutin in the seed. Therefore, buckwheat has been utilized as rutin-rich material for food (Kreft et al. 2006). Buckwheat contains rutin not only in its
seeds, but also in the cotyledons (Watanabe and Ito 2002; Kim et al. 2004, 2006; Suzuki et al. 2007b), leaves (Kita-bayashi et al. 1995a, 1995b; Suzuki et al. 2005a) stem and flower (Kalinoiva et al. 2006). Buckwheat flour contains about 20 mg rutin/100 g flour. Quantitatively, LIP activity in buckwheat flour can be inhibited by 40% by the presence of rutin (Suzuki et al. 2005c). However, the inhibition mechanism of LIP activity by rutin is not clear. Further studies need to be carried out to elucidate the mechanism of LIP inhibition by rutin.

**Lipoxygenase**

To date, very little work has been carried out on LOX in buckwheat flour. The only report on LOX activity in buckwheat seed stated that the enzyme activity was undetectable, although the authors pointed out the poor detection sensitivity of the classical assay (Axelrod 1974). They also mentioned the presence of endogenous LOX inhibitors such as phenolic compounds (Richard-Forget et al. 1995; Koh-yama et al. 1997; Kubicka et al. 1999). This point is consistent with the fact that buckwheat flour contains many phenolic compounds (Quettier-Deleu et al. 2000). From these background data, immunoblotting analysis using LOX-specific antibody is employed to investigate the presence of LOX protein in buckwheat (Suzuki et al. 2009). They prepared LOX-specific antibody, which is raised against soybean LOX. Therefore, they checked specificity of the antibody carefully as follows. The antibody recognized each LOX isozyme of soybean (LOX1, LOX2 and LOX3), respectively, which shared at least 70% homology at the amino acid sequence level (Shibata et al. 1988; Siedow 1991). The anti-soybean LOX IgG also recognized a signal band in crude protein extracts of other plant species including buckwheat at the same mobility as soybean LOX whereas no signal was detected when using a leftover IgG that had been treated by pronase. These results are consistent with findings in many other plant seeds (Anli and Tilak 2004). Therefore, it was assumed that the antibody would recognize the buckwheat LOX protein. This also reinforced the idea that the antibody they produced was a polyclonal antibody that has more epitopes that recognize LOX protein than a monoclonal antibody. They demonstrated the presence of LOX protein in buckwheat seed. In seeds of 15 buckwheat varieties, two main bands appearing at a ratio of 1:2 were detected. In addition, at least two isozymes, POX I and POX II. These were separated by ion exchange chromatography and gel filtration chromatography. In ion exchange chromatography, two major peaks of POX activity were separated. In addition, at each purification step, no additional POX activity, besides POX I and II, was found. These results suggest that POX I and II are the major POX in the soluble protein fraction of buckwheat seed. The molecular weights of POX I and II were 46.1 and 58.1 kDa by gel filtration. These molecular weights are similar to those of other peroxidases (Shibata et al. 2000; Seok et al. 2001). In buckwheat seed, most of the POX activity was detected in the embryo, similarly to LIP. The \( K_m \) values for various substrates tested were different for POX I and POX II. The latter had a greater affinity than POX I for all substrates tested. In particular, POX I did not catalyze a reaction with ABTS, whereas POX II catalyzed a reaction with ABTS. POX I had lower \( K_m \) values for quercetin, ascorbic acid and ABTS than POX II and guaiacol. The \( K_m \) values for guaiacol of POX I (0.288 mM) and II (0.202 mM) were lower than a neutral peroxidase isozyme from *Brassica napus* L. (3.7 mM) (Duarte-Vazquez et al. 2001) and isoperoxidase PC3 from *Pelargonium graveolens* for ascorbic acid (0.03 mM) and o-dianisidine (0.31 mM) (Seok et al. 2001). The \( K_m \) values for guaiacol of POX I and II were lower than with peroxidase isozyme from *Brassica napus* L. (3.7 mM) (Duarte-Vazquez et al. 2001). Both POX I and II had low \( K_m \) values for phenolic substrates such as quercetin and guaiacol. Therefore, buckwheat POX may change the color of noodles (Kondo et al. 1982; Tomas-Barberan and Espin 2001). In buckwheat seed, most of the quercetin, rutin and isocoueritin are localized in the embryo (Suzuki et al. 2002). Their relative concentrations in the embryo were 95.5: 1:1:3.4 for rutin: isocoueritin: quercetin. Quercetin is produced from rutin or isocoueritin by rutinosidase (RDEs, Yasuda and Nakagawa 1994; f3g, Suzuki et al. 2002), which is also localized in the embryo (Suzuki et al. 2002). Quercetin is also a substrate of guaiacol peroxidase, and the anti-fungal agent 3,4-dihydroxybenzoic acid is formed by peroxidase-dependent oxidation of quercetin (Takahama and Hirota 2000). Based on these observations in buckwheat seeds, POX may play a role in antioxidant activity and in the production of an anti-fungal agent. This reinforces the idea that buckwheat POX has a high level of activity at a wide range of pH 4.5 to 8.0. The major protein identified was POX II and its greater quantity in buckwheat seeds compared to POX I, it can be concluded that POX II is the major POX isozyme in buckwheat seed. The optimal temperature for POX I was 30°C, whereas it was 10°C for POX II. More than 50% of POX I activity was retained in the temperature range of 0 to 50°C. On the other hand, POX II had its greatest activity in the lower temperature range of 0 to 10°C and it decreased drastically above 20°C. Given the lower \( K_m \) value of POX II rather than POX I, it can be concluded that POX II is the major enzyme isozyme in buckwheat seed. The optimal temperature for POX I was 30°C, whereas it was 10°C for POX II. More than 50% of POX I activity was retained in the temperature range of 0 to 50°C. On the other hand, POX II had its greatest activity in the lower temperature range of 0 to 10°C and it decreased drastically above 20°C. Given the lower \( K_m \) value of POX II rather than POX I, it can be concluded that POX II is the major enzyme isozyme in buckwheat seed. The optimal temperature for POX I was 30°C, whereas it was 10°C for POX II. More than 50% of POX I activity was retained in the temperature range of 0 to 50°C. 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EFFECTS OF ENZYMES ON QUALITY DETERIORATION OF BUCKWHEAT FLOUR DURING STORAGE

The effects of LOX, POX, and LIP on quality deterioration of buckwheat flour have been investigated by Suzuki et al. (2009) by storage test of buckwheat flour. Brief experimental procedure was as follows. Before the storage test, they screened 14 of 46 buckwheat cultivars to obtain a wide range of variation in LIP activity, POX activity, LOX protein concentration and rutin concentration. Buckwheat flour was placed in polyethylene bags and stored at 5 or 20°C in a dark room for 0, 4, 10, and 30 days (0 storage days representing immediately after milling). Each buckwheat flour lot was analyzed in terms of enzymes (LIP, POX activity and LOX protein content), index of flour lipid deterioration (pH, water-soluble acid (WSA), peroxide value (POV) and carbonyl value (COV)). Finally, enzymes and index of flour lipid deterioration were compared.

During the storage period, the pH decreased at both 5 and 20°C like in the report of Muramatsu et al. (1986). The pH decreased more at 20°C than at 5°C, dropping rapidly from 0 to 10 days of storage at 20°C. WSA increased at pH decreased more at 20°C than at 5°C, dropping rapidly from 0 to 10 days of storage at 20°C. WSA increased at 5°C, POV generally increased quickly until the 10th day of storage and then only slightly increased until the 30th day of storage. The POV is an index of the amount of free fatty acids. At 5°C, POV generally increased quickly until the 10th day, then increased to a maximum by the 10th day, then decreased once more by the 30th day of the storage. The COV is an index of the quantity of carbonyl compounds (Takano 1993). Changes in COV, as well as POX, also differed between varieties and storage temperatures. At 5°C, the profiles could be roughly grouped into two categories. One group had a maximum COV peak at the 4th storage day with a decrease to the 10th day. The other group did not have a peak in COV at the 4th day of storage day at 5°C, but at 20°C COV decreased until the 4th storage day, then increased to a maximum by the 10th day, then decreased once more by the 30th day of the storage. The COV of buckwheat flour was placed in polyethylene bags and stored at 5 or 20°C in a dark room for 0, 4, 10, and 30 days (0 storage days representing immediately after milling). Each buckwheat flour lot was analyzed in terms of enzymes (LIP, POX activity and LOX protein content), index of flour lipid deterioration (pH, water-soluble acid (WSA), peroxide value (POV) and carbonyl value (COV)). Finally, enzymes and index of flour lipid deterioration were compared.

During the storage period, the pH decreased at 5°C and 20°C, more so at the higher temperature. These results also concurred with the report of Muramatsu et al. (1986). The decrease in pH and increase of WSA indicated the accumulation of free fatty acids. At 5°C, POV generally increased quickly until the 10th day of storage and then only slightly increased until the 30th day of storage. The POV and pH, WSA, POV or COV were not observed at 20°C. On the other hand, at 5°C, POV was significantly correlated to pH (30–10 DOS) and POV (10 DOS and 10–4 DOS). The rutin concentration was significantly correlated to pH (4–0, 10–4 DOS at 20°C), WSA (30 DOS at 5°C and 4 DOS at 20°C) and COV (30 DOS at 5°C). In addition, the rutin concentration exhibited negative correlations with WSA at both 5 and 20°C during the entire storage period. This result suggests that rutin inhibits free fatty acid degradation.

These results (Suzuki et al. 2005c) suggest that LIP activity and rutin concentration play important roles in the quality deterioration of buckwheat flour in terms of lipid degradation. This indicates that the mechanism of quality deterioration in buckwheat flour is different from that of rice and soybean, because in rice and soybean, LOX has a more important role than LIP in flour quality degradation (Takano 1993). To breed a buckwheat variety that does not deteriorate easily, both increasing the rutin content and decreasing the LIP activity in buckwheat seed would be effective. To clarify the effects of enzymes on quality deterioration, it is the flour color and volatile compounds that must be investigated together with a sensory analysis (organoleptic evaluation of flavors).

In buckwheat flour, the LOX protein content was much lower than in other cereals tested and rutin was shown to inhibit LOX activity (Richard-Forget et al. 1995; Kohyama et al. 1997; Kubicka et al. 1999; Suzuki et al. 2005c). In addition, in buckwheat, LIP was more important than LOX or POX in lipid deterioration, whereas LOX plays a more important role than LIP or POX in rice and soybean flour degradation (Fukushima 1994; Robinson et al. 1995). From these results, it can be concluded that buckwheat exhibits unique characteristics in its lipid degradation mechanism, which would be important not only because of the deterioration of buckwheat flour but also with regard to carbohydrate supply in germination, compared to other cereals such as soybean and rice.

EFFECTS OF ENZYMES ON VOLATILE COMPOUND GENERATION IN BUCKWHEAT NOODLE

Flavor components in buckwheat include a number of volatile compounds. Carbonyl compounds such as aldehydes and ketones have been focused as important components of buckwheat flavor. In soybean products, as described above, such compounds are generated through the lipoxygenase pathway. In buckwheat products, relationship between

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enzymes in the lipoxygenase pathway and related substance generation such as carbonyl compounds has been investigated (Suzuki et al. 2010).

In a first step towards investigating the effects of LIP, LOX and POX activities and free fatty acid (FFA) levels in buckwheat flour on volatile compounds generated by boiled buckwheat noodles, they performed the following tests. (i) Quantify enzyme activities/levels and free fatty acid (FFA) content (extracted by the method of Folch et al. (1957)) in the flour of 12 buckwheat varieties/breeding lines. (ii) Identify and quantify, for all 12 buckwheat varieties/ breeding lines, the volatile compounds produced by boiled buckwheat noodles, using head space GC/MS. (iii) Analyze correlations between activities and levels determined in (i) and (ii). From these results, they presented and discussed possible mechanisms of volatile compound generation during processing.

The LIP activity in flour showed significant positive correlations with volatile compounds in head space butanal, tentative 3-methyl-butanal, tentative 2-methyl-butanal and hexanal. The POX activity showed a significant positive correlation to 3-methyl-butanal and 2-methyl-butanal, indicating that LIP and POX were important components in the enzymatic generation of volatile compounds. On the other hand, LOX1 and LOX2 showed a significant correlation to non-volatile compound. In soybean, the enzymatic action of LOX is key in generating hexanal, which is the major source of ‘beany’ flavor (Fukushima 1994; Robinson et al. 1995) among the enzymes of the lipoxygenase pathway. In addition, LOX is also a key enzyme in the generation of unfavorable volatile compounds during the storage in rice (Suzuki et al. 1999). Therefore, in buckwheat, the key enzyme which generates volatile compounds such as hexanal may be different from those in soybean and rice. The C18:1, C18:2 and C18:3 free fatty acid (FFA; free fatty acid) levels in flour showed significant correlations with the volatile compounds such as pentanal and hexanal. These FFAs are the product of LIP activity, and the substrate of POX or other enzymatic and/or non-enzymatic reactions, which result in the generation of volatile compounds (Takano 1993). In the dough-making process, when water was added to the flour, the lipoxygenase pathway, which generates volatile compounds from triacylglycerol, and is catalyzed by enzymes such as LIP and POX in each step, was likely activated. In contrast to enzyme-catalyzed generation of volatile compounds (such as a kind of methyl-butanal, and hexanal), correlations analysis suggest that some volatile compounds’ generation occurred without the action of LIP, LOX or POX. It suggests the existence of other enzymatic or non-enzymatic pathways. Further studies are required to address this issue. From the above mentioned results, it is clear that enzymatic reactions such as LIP and POX are important in generating volatile compounds of boiled buckwheat noodles. Some volatile compounds found in this experiment such as hexanal and some methyl-butanals are important contributors to the unique flavor of buckwheat. Therefore, LIP and POX should also be important factors in generating the organoleptic qualities of boiled buckwheat noodles. To clarify the role of these enzymes on flavor, further analysis of flavor compounds using sensory analysis (organoleptic evaluation) would be required. Suzuki et al. (2010) also mentioned that the results are useful for breeding lines, the volatile compounds produced by boiled buckwheat noodles are important contributors to the unique flavor of buckwheat. Therefore, one may also have to consider flavor deterioration in developing a flavorful variety. On the other hand, high rutin levels have been linked to reduced flour deterioration (Suzuki et al. 2005c) whereas rutin levels showed no significant correlation with levels of any of the volatiles measured (Suzuki et al. 2010). This fact indicates that there is the possibility of developing a variety whose flavor is enhanced, but whose flour does not deteriorate easily. Further studies are required to clarify the mechanisms as to when and where volatile compounds are generated in buckwheat seeds and flour.

CONCLUSION

LIP and POX activity in buckwheat flour apparently plays a role in the lipid degradation and quality deterioration. On the other hand, rutin tends to prevent flour deterioration. These results suggest that LIP activity and rutin concentration play important roles in the quality deterioration of buckwheat flour in terms of lipid degradation. This indicates that the mechanism of quality deterioration in buckwheat flour is different from that of rice and soybean, because in rice and soybean, LOX has a more important role than LIP in flour quality degradation. LIP and POX activity in buckwheat flour are also important for flavor generation of boiled buckwheat noodles, whereas rutin does not have important role in it. This fact indicates that to develop the variety whose flavor is enhanced but flour does not deteriorate easily, increasing LIP activity and rutin concentration would be effective.

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