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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 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-nitrophenyl laurate; **ABTS**, 2,2'-azino-bis-(3-ethylthiazoline-6-sulfonate); **Triton X-100**, polyoxyethylene (10) octylphenyl ether; **Tween-20**, polyoxyethylene (20) sorbitan monolaurate

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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,

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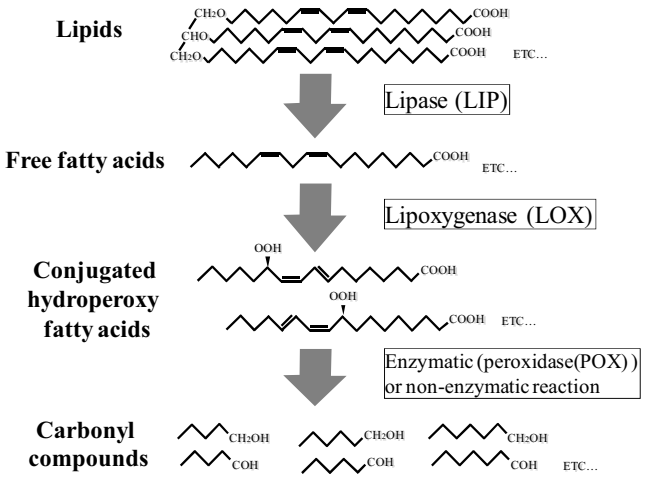

Fig. 1 Model of lipid degradation proposed for rice bran.

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 (Ohinata *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 contains LIP activity in the seed (Aizono *et al.* 1976; Hills and Mukherjee 1989; Taipa *et al.* 1992; Moulin *et al.* 1994; Ncube *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; Ncube *et al.* 1995). The final specific activities of buckwheat LIP I and II measured were lower than those in other plants such as LIP2 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 optimum pH range than LIP II; LIP I activity peaked between pH3 and oH4 wheras LIP II peaked pH3 and pH6. 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), lipase from cotyledons 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 communi*s 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 10°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 *p*NP 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 *p*NPC12 was much higher than for other *p*NP esters. Such results were similar to those obtained with *Candida rugosa* LIP2 and LIP3 (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 more 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.* 1989; Steger-Hartmann *et al.* 1994; Afanas'ev *et al.* 2001; Jiang *et al.* 2007) antihypertensive (Matsubara *et al.* 1985) and anti-inflammatory activities (Afanas'ev *et al.* 2001). 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; Haley *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 (Kitabayashi *et al.* 1995a, 1995b; Suzuki *et al.* 2005a) stem and flower (Kalinova *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 are necessary on the effects of rutin on quality deterioration of buckwheat flour together with its effect on enzymes such as LIP.

Lipoxigenase

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; Kohyama *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 backgrounds, immunoblotting analysis using a 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 3-times absorbed using an antigen. LOX of other plant species also share high homology with soybean LOX at the amino acid sequence level and the molecular weight of plant LOX is about 95±5 kDa (Barone *et al.* 1999; Loiseau *et al.* 2001). 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 to be LOX protein were detected by immunoblotting analysis. LOX protein was detected only in the embryo and no bands were detected in either the endosperm or testa. This result is consistent with findings in many other plant seeds (Loiseau *et al.* 2001). They also investigated differences in LOX content using some plants such as buckwheat, soybean, millet, amaranth and sunflower. As a result, LOX protein content in buckwheat was two or four orders of magnitude less than in other cereals tested. In addition LOX content per seed was also very low during seed maturation (Suzuki *et al.* 2009). Therefore, unlike rice and soybean, where LOX activity plays an important role in lipid degradation and oxidation, lipid degradation and oxidation in buckwheat may not be closely linked to LOX activity.

Peroxidase

Plant POX is widely distributed in higher plants (Van and Cairns 1982; Amako *et al.* 1994). These enzymes are involved in a variety of functions, such as control of cell elongation (Ahmed *et al.* 1995), defense mechanisms (Bradley *et al.* 1992; Kolattukudy *et al.* 1992) and lignification (Blee *et al.* 2003). POX also plays roles in food quality, including deterioration of color and flavor (Ibaraki *et al.* 1988; Ibaraki and Hirano 1989; Ashie *et al.* 1996). Carbonyl compounds such as aldehydes and ketones in soybean, are the major contributors to 'beany' and 'green' flavors (Fukushima 1994). These compounds are mainly generated by lipid peroxidation and POX together with LOX catalyzes lipid peroxidation reactions (Matoba *et al.* 1975; Matoba *et al.* 1985; Anli and Tilak 2004). Buckwheat POX has been partially characterized (Kondo *et al.* 1982). In addition, the relationship between POX activity and changes of color of buckwheat product has been discussed (Kondo *et al.* 1982). After that, buckwheat seed POX are highly purified and characterized (Suzuki *et al.* 2005d). The POX consisted of 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 (Sakharov *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. POX I and II had lower K_m values for quercetin, ascorbic acid and ABTS than for *o*-dianisidine and guaiacol. The K_m values for *o*-dianisidine (POX I : 0.229 mM, II: 0.137 mM) and for ascorbic acid (POX I: 0.043 mM, II: 0.038 mM) were similar to 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 (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 graveolense* (7.3 mM) (Seok *et al.* 2001). The K_m value for ABTS of POX II (0.016 mM) was also lower compared with a neutral peroxidase isozyme from *Brassica napus* (0.7 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 isoquercitrin are localized in the embryo (Suzuki *et al.* 2002). Their relative concentrations in the embryo were 95.5: 1.1: 3.4 for rutin: isoquercitrin: quercetin. Quercetin is produced from rutin or isoquercitrin by rutinosidase (RDEs, Yasuda and Nakagawa 1994; f3g, Suzuki *et al.* 2002), which is also localized in the embryo (Suzuki *et al.* 2002), as is POX. Quercetin functions as 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 values (pH 4.5 to 8.0). Given the lower K_m values of 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 20°C and it decreased gradually above 20°C. Therefore, POX II rather than POX I would be catalyzing reactions during storage, even if buckwheat seeds were stored at low temperature of 0 to 10°C. The thermal stabilities of POX I and II were also different. POX I was stable at higher temperatures than was POX II: POX I maintained activity at 0 to 30°C and only became unstable above 40°C, whereas POX II was stable to 20°C and unstable above 30°C. POX I and II were inactivated at 60 and 50°C, respectively.

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 flours 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 both 5 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 is an index of the amount of peroxidative compounds such as conjugated hydroperoxy fatty acids, which will degrade into carbonyl compounds (Takano 1993). Changes in COV, as well as POV, 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 is an index of the quantity of carbonyl compounds, volatile compounds such as aldehydes or ketones (e.g. hexanal and nonanal) (Kumazawa and Oyama 1965; Takano 1993). The degree of generation and volatility of carbonyl compounds would depend on the temperature. This may explain the differences in COV variations with time which occurred between 5 and 20°C storage conditions.

The correlations of POX with pH, WSA, POV and COV were not observed for storage at 20°C; however, for storage at 5°C, POX showed a significant correlation to pH (30-10 DOS; days of storage), POV (10 DOS and 10-4 DOS). The rutin concentration showed a significant correlation to pH (4-0 DOS, 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 showed a negative correlation with WSA at both 5 and 20°C during the entire storage period. The quantity of LOX1 protein concentration was significantly and negatively correlated to WSA for 0 and 4 DOS at 5°C and 0 and 10 DOS at 20°C. On the other hand, for storage at 5°C the concentration of LOX 2 protein did not significantly correlate to any index, whereas for storage at 20°C it significantly correlated with pH (10 DOS), POV (10, 10-4, 30-10 DOS) and COV (30-10 DOS). During storage at both 5 and 20°C, LIP activity showed a significant negative correlation with pH (0, 4, 10 and 30 storage days) and a significant positive correlation with WSA (0, 4, 10 and 30 storage days). The LIP activity showed a significant correlation with POV [4 and 30 DOS, difference day 4 to day 10 of storage (10-4 DOS), difference day 10 to day 30 of storage (30-10 DOS)] and COV [10 days of storage, 10-4 DOS, 30-10 DOS] at 20°C (Suzuki *et al.* 2005c).

A decrease in pH and increase of WSA indicate deterioration of buckwheat flour. LIP activity is suggested to play an important role in quality deterioration in buckwheat flour related to lipid degradation. Even at 0 DOS (just after milling), LIP activity showed significant correlations to pH and WSA. This may indicate that LIP activity generated free fatty acids in buckwheat seed prior to milling. The LIP activity also had significant correlations to POV (4, 30, 10–4 and 30-10 DOS), and COV (10, 10-4 and 30-10 DOS) at 20°C. It also indicated that LIP activity is an important factor in quality deterioration, because it affects the generation of free fatty acids as well as fatty acid oxidation and degradation. The LOX1 protein concentration showed a negative correlation to WSA (0 and 4 DOS at 5°C and 0 and 10 DOS at 20°C). LOX catalyzes oxidation of polyunsaturated fatty acids containing a 1,4-pentadiene structure, such as linoleic acid, into conjugated hydroperoxy fatty acids. Conjugated hydroperoxy fatty acids degrade more quickly than polyunsaturated fatty acids to lower molecular weight compounds. In other words, LOX plays a role in decreasing WSA, which is an index of the amount of free fatty acids. This may explain the negative correlations between LOX 1 and WSA. On the other hand, the concentration of LOX 2 protein did not show a significant correlation to any index at 5°C, whereas at 20°C it was significantly correlated with pH (10 DOS), POV (10 DOS, 10-4 DOS, 30-10 DOS) and COV (30-10 DOS) (Suzuki *et al.* 2005c). This indicates that LOX 2 plays different roles compared to LOX 1 in quality deterioration of buckwheat flour. The correlations between POX and pH, WSA, POV or COV were not observed at 20°C. On the other hand, at 5°C, POX 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 generation.

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

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 concentrations (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 noodle preparation.

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 or 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 of buckwheat varieties with particularly desirable flavor traits. To enhance flavor amount in buckwheat product, it may be possible to breed for increased LIP and POX activity in the seed. However, LIP and POX are also important in quality deterioration of buckwheat flour. Therefore, one may also have to consider flour 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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