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Unequal distribution of pH, polyphenol content and polyphenol oxidase activity within Japanese processing potato tubers

Hironaka Kazunori[1], Shimizu Akira[1], Koaze Hiroshi[1], Yamamoto Kazuo[1], Sato Tadatoshi[1], Kojima Michiyuki[1], Mori Motoyuki[2] and Tsuda Shogo[2]

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加工用ジャガイモ塊茎中のpH，ポリフェノール含量およびポリフェノールオキシダーゼ活性の不均一分布

弘中和憲[1]・清水　明[1]・小疇　浩[1]・山本和夫[1]・佐藤禎稔[1]・小嶋道之[1]・森　元幸[2]・津田昌吾[2]

ABSTRACT

The pH, polyphenol content and polyphenol oxidase (PPO) activity in stem end, middle, and bud end of Norin No. 1 potatoes, one of processing varieties in Japan, were measured to investigate their respective distributions. The stem end area had a higher pH and PPO activity in the three areas. This result points out that careful harvesting and postharvest handling are needed especially at the stem end area of potatoes because of its high PPO activity.

［Key words］ distribution, potato tuber, pH, polyphenol, polyphenol oxidase

和文摘要

我国の加工用ジャガイモ品種の一つである農林１号の塊茎基部，中央部および頂芽部のpH，ポリフェノール含量およびポリフェノールオキシダーゼ活性を測定した。その結果，基部は他の部位に比べ，高いpHおよびポリフェノールオキシダーゼ活性を有した。この結果は，基部はとくにポリフェノールオキシダーゼ活性が高いために，ジャガイモの収穫およびその後の操作において，丁寧に取り扱わねばならないという情報を与えるものである。

キーワード：分布，ジャガイモ，pH，ポリフェノール，ポリフェノールオキシダーゼ

Introduction

Blackspot bruise in potato (*Solanum tuberosum* L.) is an internal discoloration of tuber tissue caused by a sequence of biochemical oxidation which is initiated by a mechanical injury which occurs during mechanical harvesting, transport and storage. Blackspot bruise is

[1]帯広畜産大学畜産科学科（〒080－8555　帯広市稲田町西２線）

[2]北海道農業研究センター畑作研究部芽室拠点（〒082-0071　北海道河西郡芽室町）

[1]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

[2] National Agricultural Research Center for Hokkaido Region, Memuro, Kasai-gun, Hokkaido 082-0071, Japan

a major quality problem and causes substantial economic damage to the potato breeder and the potato processing industry worldwide (Stevens et al. 1997).

Three million tons of potatoes are produced annually in Japan(Ministry of Agriculture, Forestry and Fisheries of Japan 2004) and blackspot bruise of potatoes possesses a serious problem. As for the biochemical properties of blackspot bruise, Mathesis (1997) pointed out the following three factors: polyphenol content, polyphenol oxidase (PPO) activity and pH. Hironaka et al. (2006) reported that PPO activity is the main factor causing the bruising.

Various studies have been reported on pH (Iritani et al. 1974; Smith 1987), phenol content (Reyes et al. 2005; Reeve et al. 1974; AL-Saikhan et al. 1995; Corsini et al. 1992) and PPO activity (Reeve et al. 1974) involving distribution of the above three factors within a potato tuber. Japanese potato growers and processors need to know the respective distribution of these factors within tubers in order to reduce the blackspot bruise during harvesting, transport and storage. However, there is no published research on the distribution of these factors in the Japanese potato tuber. The aim of this study is therefore to investigate the distribution of pH, polyphenol content and PPO activity in the Japanese potato tuber.

MATERIALS AND METHODS

Materials

Tubers of Norin No. 1 cultivar, which is one of processing potatoes in Japan, were harvested at a farmer's field in Obihiro in October, 1994. After harvesting, 30 tubers were put into each mesh onion bag, and these potatoes were then stored at 12℃ and 90-95% RH for 105 days. Samples used in this experiment were approximately 150 g.

Preparation of crude enzyme solution

The homogenate was prepared according to the method described by Hsu et al. (1988). Each of the five unbruised potato tubers in the bag was separated into three tuber areas: stem end, middle and bud end (Fig. 1). Each divided potato was then peeled and cut into 5 mm cubes. After mixing the cubes completely, 25 g samples (cubes) were homogenized in a Waring blender at high speed for 90 sec with 25 ml of cold M/15 sodium phosphate buffer (pH 6.8). The homogenate was rapidly filtered through four layers of cheesecloth, and the filtrate was then centrifuged at 18,000×g for 10 min at 0℃.

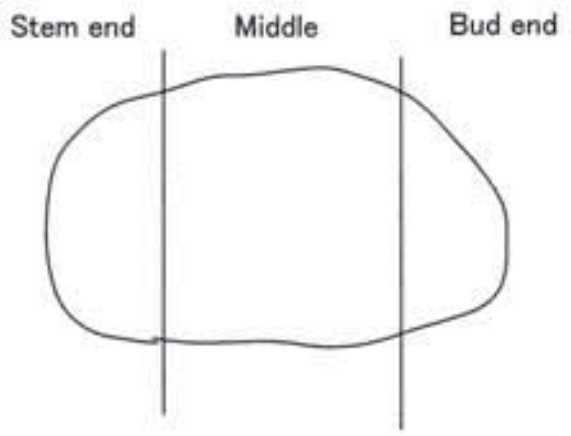

Fig.1. Potato tuber areas

Two ml of the supernatant was rapidly applied to a Sephadex G-25 column (i.d. 1.5 cm×10 cm), and eluted with M/15 of sodium phosphate buffer, pH 6.8 (flow rate: 1 ml/min). The higher molecular weight fractions of the eluate were combined and served as crude enzyme solution.

Determination of PPO activities

The procedure described by Hsu et al. (1988) and Weaver et al. (1970) was used to determine the PPO activity. PPO activity was determined using DOPA (3,

4-Dihydroxyphenyl alanine) as a substrate. The reaction mixture consisted of 3.0 ml of M/15 sodium phosphate buffer, pH 6.8, containing 15.0 μM of DOPA. The mixture was added to a glass cell containing 0.75 ml of the crude enzyme solution, and the changes in absorbance at 420 nm were determined at 1-min intervals for 11 min at room temperature. One unit was defined as the change in absorbance divided by the determination time.

Determination of total phenol contents

Phenols were determined using a modification of the protocol proposed by Walter et al. (1979). The lower molecular fractions eluted from the Sephadex G-25 column were added to 0.5 ml of Folin-Dennis reagent (Swain et al. 1959), and thoroughly agitated. After 3 min, 1 ml of saturated sodium bicarbonate solution was added to the mixture, and then thoroughly agitated. After allowing to stand for 1 hr, blue discolored fractions were mixed, degassed for 10 min, and the absorbance at 725 nm was measured. The phenol content was determined using chlorogenic acid as the standard.

Measurement of pH

The procedure described by Iritani et al. (1974) was used to determine pH of tubers. Potato cubes were homogenized in a Waring blender for 1 min. pH was measured by placing a pH meter electrode into the potato pulp slurry.

All chemical analyses (phenol content, PPO activity and pH) were performed six times.

Data analysis

Data were analyzed by means of one-way analysis of variance (ANOVA) and a two-tailed t-test (SPSS 1992) to determine significant differences.

RESULTS AND DISCUSSION

Fig. 2 and Table 1 indicate the differences in pHs among stem end, middle, and bud end areas. As shown in Fig. 2, pHs increased approximately from 5.9 to 6.6 during storage. Iritani et al. (1974) indicated that pH of potatoes ranged between 6.0 and 6.3. Mathesis (1977) also reported that the pH of potatoes ranges between 5.6 and 6.4. The pH range in the present study was almost similar to those of these previous reports (Iritani et al. (1974); Mathesis (1977)). The stem end had a higher pH than the bud end (Table 1). This result agreed with the previous findings (Iritani et al. (1974); Smith (1987)) that pHs of the stem end of potatoes were higher than those of the bud end.

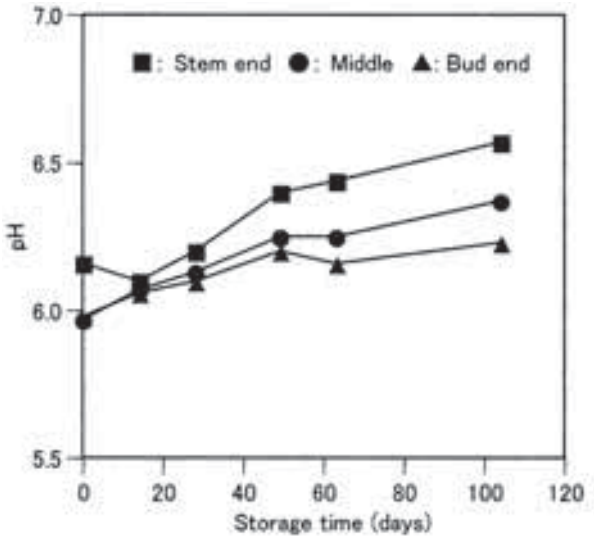

Fig.2. pH of potatoes stored at 12℃

Table 1. Mean pH of stem end, middle, and bud end of potatoes over storage period

Area	pH
Stem end	6.31a*
Middle	6.17ab
Bud end	6.12b*

*Means with same letter are not significantly different ($p<0.05$)

The difference in polyphenol content among tuber areas was shown in Fig. 3 and Table 2. As shown in this figure, the polyphenol content of each tuber area increased during storage. The increase in polyphenol content of potatoes during storage was earlier reported by Hironaka et al. (2006). There were no differences in polyphenol content among the three areas (Table 2) while a higher concentration of polyphenol was found at the stem end than the bud (Reyes et al. 2005; Reeve et al. 1974; AL-Saikhan et al. 1995; Corsini et al. 1992).

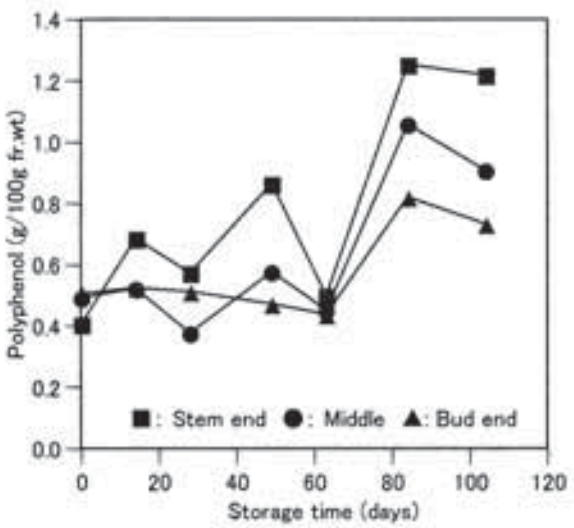

Fig.3. Polyphenol contents of potatoes stored at 12℃

The PPO activities of each area are shown in Fig. 4 and Table 3. PPO activities remained constant or slightly decreased during storage (Fig. 4). The stem end had a higher PPO activity than the bud end (Table 3). This result agreed with the study of Reeve et al. (1974) indicating that PPO activity of the stem end was higher than that of the bud end.

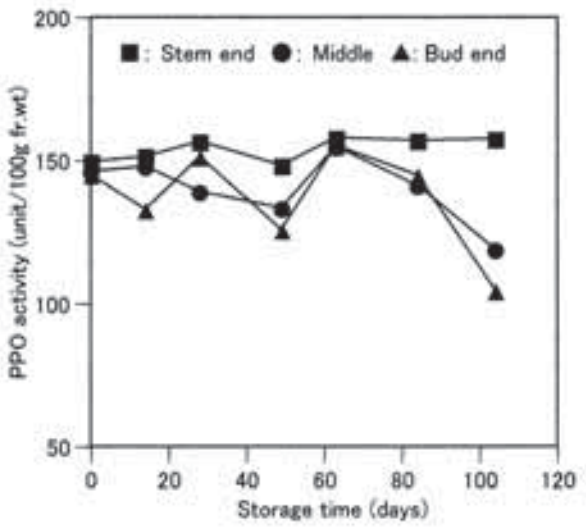

Fig.4. Polyphenol contents of potatoes stored at 12℃

Table 2. Mean polyphenol content of stem end, middle, and bud end of potatoes over storage period

Area	Content (g/100g fr. wt.)
Stem end	0.78a*
Middle	0.63a
Bud end	0.57a

*Means with same letter are not significantly different ($p<0.05$)

Table 3. Mean PPO activity of stem end, middle, and bud end of potatoes over storage period

Area	Activity (unit/100g fr. wt.)
Stem end	1.54a*
Middle	1.40ab
Bud end	1.37b

*Means with same letter are not significantly different ($p<0.05$)

As to the relationship between pH and PPO activity, Suresh et al. (1965) reported that potato PPO has a maximum activity at pH 5 with a relatively high activity around pH 6. In Fig. 1, pHs of Norin No. 1 potatoes roughly ranged between 5.9 and 6.6. Thus, potato PPO could work comparatively active at the present pH range (5.9-6.6). Moreover, the average polyphenol content of the bud end (lowest area) was 0.57 g/100g fr.wt. in Table 2. This value can be converted to 22.1 mM of chlogenic acid with chlogenic acid being a main polyphenol in potatoes (Swain et al. 1959) and the mean moisture content of the Norin No. 1 variety being 73 % (Hironaka et al. 1974). Km value of potato PPO is 1.7 mM as a substrate of chlogenic acid (Kiattisak et al. 1999), and the value of 22.1 mM in this study is roughly 13 times higher than the Km value (1.7 mM). Therefore, all areas in the present study had abundant polyphenol contents for PPO activity. In Table 3, the stem end had a higher PPO activity than other areas. Hironaka et al. (2006) reported that PPO activity is a limiting factor for bruising. Thus, when tuber cells beneath the surface of a potato are damaged, blackspot bruise occurs more at the stem end than in other areas. This result suggests that careful harvesting and postharvest handling are needed especially at the stem end area of potatoes because of its high PPO activity.

SUMMARY

The pH, polyphenol content and polyphenol oxidase (PPO) activity in stem end, middle, and bud end of Norin No. 1 potatoes were measured to investigate their respective distributions. The stem end area had a higher pH and PPO activity in the three areas, whereas no difference in polyphenol content exists among the three areas.

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