Changes in Phenylalanine Ammonia-lyase and Polyphenol Oxidase Activities with Occurrence of Browning in Shredded Lettuce during Storage

DAN Kazuhiro*, NAGATA Masayasu* and YAMASHITA Ichiji*

* National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries
Ano, Age, Mie 514-2392

Degree of browning, phenolic content, phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) activities were measured during storage in air at 5°C, 10°C, 15°C and 20°C to examine the undesirable enzymatic browning in shredded lettuce tissue. The browning of tissues was significantly dependent on storage temperature. High temperature markedly promoted the development of tissue browning. There was no significant change in activity of PPO at all temperatures during the storage. On the other hand, wounding induced marked increase in PAL activity. The initial increase of PAL activity was greater at higher temperatures. These results suggest that the increase in PAL activity due to wounding is probably one of the major reasons for the development of shredded lettuce tissue browning.

(Received Apr. 23, 1999 : Accepted Jul. 14, 1999)

Recently, the use of minimally processed lettuce has continued to increase in salad bars and fast food. One of the major causes of quality loss is the browning of the cut pieces. The development of brown discoloration in shredded lettuce reduces consumer acceptability and is thus of significant economic importance to the primary producer and the food processing industry. Browning is common in plant tissues and is thought to be brought about through the metabolism of phenolic compounds. The phenolic pathway beings with the deamination of L-phenylalanine by the enzyme phenylalanine ammonia-lyase (PAL) to cinnamic acid. Then cinnamic acid is sequentially hydroxylated into various phenolic compounds. In the presence of O₂, the enzyme polyphenol oxidase (PPO) oxidizes these compounds to quinones, which simultaneously polymerize into brown pigment.

Increased use of minimally processed lettuce, and increased restrictions on chemical treatments to prevent browning provide an impetus to better understand the causes of browning and how they can be controlled. Therefore, the objective of this work is to investigate changes in PAL and PPO activities during the development of wound-induced lettuce tissue browning at various temperatures.

Materials and Methods
1. Plant material and treatment
Crisp head lettuce (Lactuca sativa L.) was obtained from a local wholesale market and stored at 5°C until used. Lettuce was shredded by an electric food processor (Matsushita Electric Industrial Co., National Speed Cutter, Tokyo) to about 5 mm in width. The shredded lettuce tissue was stored at 5°C, 10°C, 15°C and 20°C. During storage, the tissues was periodically sampled, and analyzed for PAL and PPO activity. The samples
for total phenol and tissue browning were kept at -30°C until being analyzed.

2. PAL assay

The procedure for assaying PAL activity was slightly modified from that of KE and SALTVEIT\(^4\). The sample tissue (5 g) was homogenized in cold 20 ml of 50 mM borate buffer (pH 8.5) containing 5 mM 2-mercaptoethanol and 2% (w/v) polyvinylpolypyrrolidone with a chilled mortar and pestle. The homogenate was filtered through 4 layers of gauze and centrifuged at 10,000×g for 20 min at 4°C. PAL activity in the supernatant was assayed by adding 0.55 ml of 100 mM L-phenylalanine to 5 ml of the crude extract. After heating at 40°C for 60 min, the absorbance was measured at 290 nm. One unit of enzyme activity is defined as the amount of PAL that produces 1 mol of cinnamic acid in 1 hr.

3. PPO assay

The procedure for assaying PPO activity was slightly modified from that of SIRIPHANICH and KADER\(^5\). The sample tissue (5 g) was homogenized in 45 ml of 0.1 M phosphate buffer (pH 6.5) with a chilled mortar and pestle. The homogenate was filtered through 4 layers of gauze and centrifuged at 12,000×g for 20 min at 4°C. The supernatant was used to determine PPO activity. The assay medium contained 1.95 ml of 0.1 M phosphate buffer (pH 6.5), 1 ml of 20 mM caffeic acid, and 50 µl of crude enzyme extract. The activity of PPO was determined by the increase in absorbance of the mixture at 420 nm after adding the enzyme extract. One unit of PPO activity is defined as the amount of enzyme that produces an increase in absorbance at 420 nm of 0.1 per min.

4. Total phenol content

Total phenol content was measured according to the method by NAGATA et al.\(^6\). The frozen sample was homogenized in 50 mM phosphate buffer (pH 7.0). During extraction, the sample was cooled with ice. The homogenate was filtered through a layer of filter paper (No. 2 Filter Paper, ADVANTEC, Tokyo), followed by filtration through a membrane filter (pore size, 0.22 µm, Toyo Roshi Kaisha, Tokyo). The filtrate was assayed for phenolic content with the FOLIN-CIOCALTEU phenol reagent, using chlorogenic acid as a standard.

5. Evaluation of tissue browning

Browning of shredded lettuce was measured according to the method by NAGATA et al.\(^7\). One gram of NaCl was added to the frozen sample of shredded lettuce (20 g) in order to prevent additional browning during extraction. After homogenization in 100 ml of ethanol with a high-speed blender, the homogenate was filtered with suction. The residue was re-extracted with ethanol until the chlorophyll was thoroughly removed, then the residue was dried in vacuo. Color (L*, a*, and b*) of the powdery sample was measured with a Hitachi C-1020 color sensor. The extent of browning was expressed as the color difference between the sample and the standard white board, which was calculated according to the equation:

\[ \Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \]

Results and Discussion

Changes in color of the ethanol-extracted residue of shredded lettuce during storage are shown in Fig. 1. There was a good correlation between the visual evaluation of tissue browning and \( \Delta E \) values. Tissue browning started to
appear 12 hr after storage at 20°C, and rapidly developed during further storage. At 5°C, 10°C and 15°C, tissue browning started within 1 day after storage, and developed following storage. The tissue browning was significantly dependent on storage temperature. Browning can be delayed by storage at low temperatures, but it nevertheless occurs after a lag period. It is well known that temperature is the most important environmental factor in the postharvest life of fresh vegetables, because within the physiological temperature range, the velocity of a biological reaction, increases two- to three-fold for every 10°C rise in temperature7). Therefore, it is understandable that the temperature affected the development of browning.

PPO oxidizes the polyphenols in plants to their corresponding quinones. Then, these quinones polymerize and react with amino acid groups of cellular proteins, resulting in brown pigments3). PPO has a broad specificity toward different phenolic substrates, and the brown pigments resulting from these phenolics differ widely in color intensity3). It is considered that PPO is the final enzyme, which reacts to the last step of the generation of brown pigments. The activities of PPO in shredded lettuce during storage are shown in Fig. 2. During storage, there was no significant change in PPO activity at all temperatures. These results suggest that the development of tissue browning might not be because of an increase in the activity of PPO.

PAL catalyzes the first reaction of general phenylpropanoid metabolism, the deamination of L-phenylalanine to produce cinnamic acid1) 2). The rate-limiting step in the synthesis of most phenolics is dependent upon PAL activity. The activities of PAL in the shredded lettuce during storage are shown in Fig. 3. The PAL activity in the tissue was very low soon after preparation of the shredded lettuce (0 day). It is well known that wounding induces PAL activity and increased phenolic metabolism in many plant tissues3). In the lettuce tissue, wounding also induced increase an in PAL activity. Initial increase of PAL activity was greater at higher temperatures. PAL activities in lettuce tissue stored at 20°C and 15°C attained maximum levels on 0.5 day and 1 day, respectively, and then decreased. At 10°C and 5°C, however, PAL activities continued to increase during storage. The decrease in PAL activities in the tissues stored at 20°C and 15°C seemed to be due to
Food Preservation Science VOL. 25 NO. 5 1999 (16)

Fig. 4 Changes in total phenols content of shredded lettuce during storage.
Values are means ± SE for n = 3 to 5.

In the lettuce tissue, wounding also induced an increase in phenolic compounds content (Fig. 4). The phenolic compounds content increased to a maximum on the first day and subsequently declined.

In this study, we observed no significant changes in PPO activity during storage. PPO activity is probably not the limiting factor since ample PPO activity should always have been present to oxidize the soluble phenolic compounds in lettuce tissue. PAL activity is a key regulatory enzyme in controlling phenolic metabolism. The increase in PAL activity by wounding is probably one of the major reasons for the development of shredded lettuce tissue browning. PEISER et al. also reported that PAL inhibitor treatment was very effective at inhibiting cut lettuce browning. However, our data showed that although PAL activity was induced in shredded lettuce tissue stored at 5°C, browning was suppressed until the second days of storage compared with being stored at 20°C or 15°C. Therefore, the induction of PAL activity alone was not sufficient for tissue browning.

It is indispensable that wounding induces cellular decompartmentalization, which allows mixing of phenolic substrates and PPO. Because PPO is localized in plastids, the although membrane associated, and phenolic substrates of PPO are localized in the vacuole. The browning reaction only occurs as a result of tissue damage leading to a loss of this subcellular compartmentation. Therefore, alternative mechanisms, such as the loss of membrane integrity, reducing compounds content, or other factors might also contribute to the development of tissue browning. Further work is required to elucidate what factor, except PPO and PAL, contributes to the development of tissue browning of shredded lettuce.

Literature Cited
性物質含量、褐変物質の生成に関与する酵素フェニルアラニンアンモニアーゼ（PAL）およびポリフェノールオキシダーゼ（PPO）活性の変化を調査した。

組織褐変の進行は貯蔵温度に極めて影響を受け、高温条件下では組織の褐変が著しく促進された。褐変物質生成の最終段階に関与していると考えられるPPOの活性はいずれの温度区においても貯蔵期間を通じて顕著な変化は認められなかった。一方、PAL活性は貯蔵開始後、急激に増加した。また、活性増加の速度は貯蔵温度が高いものほど速かった。

以上の結果より、カットレタスにおける褐変物質の生成は、切断傷害によるPPOと基質であるポリフェノール類の細胞内局在性の喪失が前提であるが、PAL活性の増加による基質ポリフェノール類の生成が律速因子になっていると推察された。

（平成11年4月23日受付、平成11年7月14日受理）