Journal of Agricultural and Food Chemistry

FEBRUARY 1996
VOLUME 44, NUMBER 2

Discoloration of Coleslaw Is Caused by Chlorophyll Degradation

James W. Heaton, Rickey Y. Yada, and Alejandro G. Marangoni*

Department of Food Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Coleslaw tissue was extracted and analyzed spectrophotometrically for chlorophyll and its derivatives to determine their relationship to tissue discoloration and/or browning at 5 °C under reduced oxygen conditions. A general rapid decrease in chlorophyll *a* and *b* was noted after 2−3 days, with a concomitant increase in the amount of pheophytin *a* and *b*. The pheophytin was then converted to pheophorbide *a* and *b*, which resulted in gray/brown coleslaw after 12−15 days of storage. Polyphenol oxidase activity in cabbage was determined to be very low (81.6 units/g fresh tissue), whereas chlorophyllase activity was very high (23 nmol/min/g fresh tissue). This result suggested that the gray/brown pigments formed were mainly the result of chlorophyll degradation. No change in pH (∼4.6) was observed during storage. A substantial change in the color of the product was, however, observed as analyzed by blue light reflection with an Agtron colorimeter. The pathway for chlorophyll degradation in coleslaw at 5 °C was determined to be chlorophyll → pheophytin → pheophorbide. The rapid conversion of chlorophyll to pheophytin suggests rapid acidic removal of Mg^{2+} upon addition of dressing, whereas the rather slow conversion of pheophytin to pheophorbide suggests an enzymatic reaction that is possibly mediated by the enzyme chlorophyllase.

Keywords: *Coleslaw; discoloration; browning; chlorophyll; degradation; chlorophyllase*

INTRODUCTION

During the processing and storage of fresh fruit and vegetables, degradative reactions can take place that cause tissue to brown. The most common cause is enzymatic browning mediated by the enzyme polyphenol oxidase. However, discoloration due to chlorophyll degradation has been reported in olives (Minguez-Mosquera et al., 1989), pickles (White et al,. 1963), snap beans (Jones et al., 1963), and Kabosu fruits (Yamauchi et al., 1991).

Chlorophyll degradation in processed foods follows two distinctive pathways (Figure 1). Pathway A represents the primary loss of the magnesium moiety to form pheophytin, followed by the cleavage of the phytol chain to form pheophorbide, whereas pathway B involves primary cleavage of the phytol chain to form chlorophyllide and subsequently pheophorbide. Removal of the central magnesium ion from the porphyrin ring is usually the result of acidic substitution and/or heat (Mahanta and Hazarika, 1985; Minguez-Mosquera et al., 1989; White et al., 1963). However, Langmeier et al. (1993) postulated that there might be a magnesium dechelatase that could perform this function. Cleavage of the phytol chain from chlorophyll can either result from chemical hydrolysis (Schwartz and Lorenzo, 1990) or enzymatic cleavage by chlorophyllase (Amir-Shapira et al., 1987).

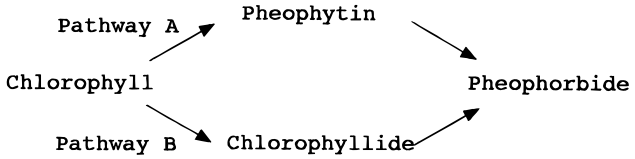

Figure 1. Pathways of chlorophyll degradation in green tissue.

During the processing of coleslaw, cabbage tissue is shredded and combined with an acidic dressing to add flavor and act as an antimicrobial agent. This process

* Author to whom correspondence should be addressed [fax (519) 824-6631; e-mail amarangoni@aps.uoguelph.ca].

0021-8561/96/1444-0395$12.00/0

drastically reduces the pH of the product, creating conditions favorable for the degradation of chlorophyll. The objective of this research was to establish the mechanism responsible for the browning of coleslaw tissue. The pH and color of the product and the concentration of chlorophyll and its derivatives in the tissue were monitored as a function of storage time to determine if color and pH changes correlated with changes in chlorophyll content. In addition, the fate of chlorophyll and its degradation products was determined in cold-stored whole cabbages destined for coleslaw manufacture. From these results, the mechanism responsible for coleslaw discoloration and/or browning and the specific pathway for chlorophyll degradation in processed coleslaw were established.

MATERIALS AND METHODS

Reagents. All chemical reagents were purchased from Fisher Scientific (Toronto, Ontario, Canada) and were of reagent grade.

Materials. Cabbages *(Brassica oleracea* cv. L. Lennox) were harvested in November, 1993, from a variety of farms in southern Ontario, Canada. The cabbage was stored at 5 °C until the time of processing. The cabbage was processed into coleslaw by Scotts Food Services (Weston, Ontario, Canada) and supplied in 4.5-kg polyethylene bags under partial vacuum. No heat treatment or enzyme-inactivation procedure was applied at any stage of the process. The coleslaw was stored at a temperature of 5 °C throughout the testing period. The coleslaw dressing was a commercially available product with a pH of 3. Other components in the dressing included sugar, vegetable oil, water, white vinegar, egg yolk, corn starch, modified corn starch, salt, color, mustard flour, xanthan gum, spice, lemon juice concentrate, flavor, calcium disodium EDTA, sodium benzoate, citric acid and silicon dioxide.

Degree of Browning and Polyphenol Oxidase Activity. The amount of polyphenol-induced enzymatic browning (degree of browning) over the testing period and polyphenol oxidase activity (PPO) in fresh unprocessed cabbage were determined by the method of Coseteng and Lee (1987). Degree of browning experiments were performed in triplicate on 1- and 34-day-old coleslaw. The PPO activity was determined in three individual cabbages, and each analysis was performed in triplicate. The pH of the activity buffer (0.01 M sodium acetate−acetic acid) was adjusted to 4.8 (with acetic acid) for one determination, which was the pH of the coleslaw tissue, and to 6.0 (with acetic acid), which is the pH of the native cabbage tissue.

Determination of pH. One gram of tissue was placed in 99 mL of double-distilled water and homogenized with a Tekmar (Cincinnati, OH) tissumizer at a rheostat setting of 100 for 30 s. The pH of the sample was measured in duplicate with a digital pH meter. A sample of coleslaw was also washed with a sufficient amount of deionized water to remove the dressing. The pH was then measured in duplicate as already described above.

Color Changes. Color changes were monitored by measuring the change in reflected blue light with an Agtron colorimeter (Agtron Inc., Sparks, NV).

Extraction of Chlorophylls, Pheophytins, Chlorophyllides, and Pheophorbides. The exraction method used was a slight modification of the method of White et al. (1963). A sample of coleslaw was removed from its container and washed in a strainer with double-distilled water to remove the dressing. The sample was then patted dry with paper towels. From this sample, three 60-g samples were drawn. Each sample was blended in a Waring blender (Fisher Scientific, Toronto, Ontario, Canada) with enough cold acetone (4 °C) that the equilibrium concentration of acetone was ~80% (v/v). The solutions were then combined and filtered through a Whatman No. 4 filter. The filter cake was then washed with 50 mL of 85% (v/v) acetone, which was sufficient to remove any remaining pigments from the filter cake. The extract was then transferred to a 1-L separatory flask. To the flask, 200 mL of diethyl ether were added and enough double-distilled water (~400 mL) to force the pigments from the acetone/water phase into the ether phase. The acetone/water phase was then decanted and discarded. The ether phase was then washed with seven 100-mL aliquots of double-distilled water. The washing procedure involved bubbling the ether phase through the water phase. After washing, the ether extract was dried with anhydrous Na_2SO_4. The solution was then evaporated under a stream of nitrogen gas to a volume of 100 mL.

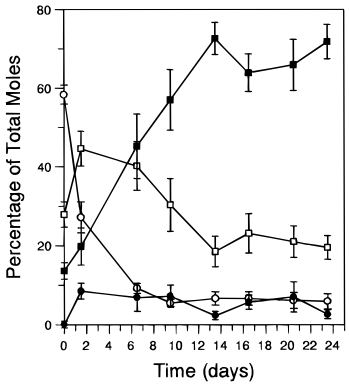

Figure 2. Percentage change in chlorophyll pigment concentration extracted from coleslaw tissue (○, chlorophyll *a* and *b*; ●, chlorophyllide *a* and *b*; □, pheophytin *a* and *b*; ■, pheophorbide *a* and *b*) as a function of storage time in polyethylene bags at 5 °C.

Measurement of Chlorophyll, Pheophytin, Chlorophyllide, and Pheophorbide Concentrations. All solutions were measured spectrophotometrically with a UV 1201 spectrophotometer (Shimadzu, Kyoto, Japan). The coleslaw extraction solution was divided into 4−25-mL aliquots. Solution 1 was measured at 660 and 642.5 nm. Solution 2 was acidified with 1 drop of concentrated HCl, dried with anhydrous Na_2SO_4, and allowed to stand in the dark for 2 h before being measured at 666.5 and 653 nm. Solutions 3 and 4 were combined into a separatory flask where they were washed with three 25-mL aliquots of a 0.01 N KOH solution followed by three 25-mL aliquots of a 5.0% (w/v) Na_2SO_4 solution and then dried with anhydrous Na_2SO_4. The sample was then made up to 50 mL with diethyl ether. The sample was subsequently divided into two equal portions to form solutions 3 and 4. Solution 3 was measured at 660 and 642.5 nm. Solution 4 was acidified with 1 drop of concentrated HCL (dried with anhydrous Na_2SO_4) and allowed to stand in the dark for 2 h before being measured at 666.5 and 653 nm. Concentrations of the different species were determined with the set of difference equations described by White et al. (1963).

Chlorophyllase Activity. Chlorophyllase activity was measured according to the method of Shioi et al. (1979).

Replication and Statistics. The testing period consisted of four, 24-day trials over a 6-month period, with 16 replicate determinations in each trial. A general linear model (analysis of variance) was used to determine the main effect of time on pigment concentrations with the Statistical Analysis Systems program (SAS Institute, 1989). Values presented in the graphs represent the means and standard errors of eight replications.

RESULTS AND DISCUSSION

A rapid loss of chlorophyll *a* and *b* occurred during the first 2−3 days of storage ($p \leq 0.05$) followed by a concomitant increase in the amount of pheophytin *a* and *b* ($p \leq 0.05$) (Figure 2). After reaching a maximum at day 2, the level of pheophytin *a* and *b* gradually decreased, giving rise to increased levels of pheophorbide *a* and *b* ($p \leq 0.05$). There was no significant change in the concentration of chlorophyllide *a* and *b* over time ($p > 0.05$). Conversion of chlorophyll to pheophytin was probably the result of the sharp decline in the pH of

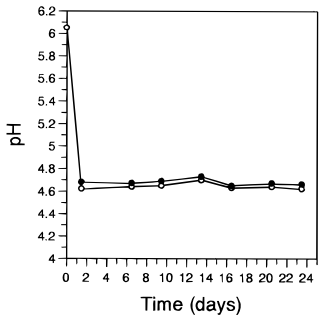

Figure 3. Effective change in pH of coleslaw and tissue (○, washed tissue; ●, dressing of coleslaw) as a function of storage time at 5 °C in polyethylene bags.

the tissue (Figure 3) due to the addition of dressing. Minguez-Mosquera et al. (1989) also observed a similar increase in the concentration of pheophytin when the pH of the brine (for olives) decreased below 5. White et al. (1963) determined that a reduction in the pH of the brine for cucumbers below 6.0 was sufficient to cause a rapid conversion of chlorophyll to pheophytin. Mahanta and Hazarika (1985) suggested that a decrease in pH would cause a conversion to pheophytin before the enzyme chlorophyllase could be activated enough to accumulate chlorophyllide. The slow conversion of pheophytin to pheophorbide suggests that the removal of the phytol chain was the result of enzymatic cleavage rather than chemical hydrolysis. If the cleavage of the phytol chain was the result of chemical hydrolysis, there would have been a pronounced increase in chlorophyllide and pheophorbide within the first 2 days, which was not observed (Figure 2). We also detected a significant amount of chlorophyllase activity in fresh unprocessed cabbage. We determined chlorophyllase activity in cabbage to be 23 nmol min^{-1} g^{-1} (wet weight), whereas Kuroki et al. (1981) reported a value of 14.2 nmol min^{-1} g^{-1} (wet weight) for tea leaf sprouts, and Shioi et al. (1980) reported a value of 28.7 nmol min^{-1} g^{-1} (wet weight) for the blue-green algae *Chlorella prototheco*ids. The relatively high chlorophyllase activity detected in cabbage lends support to our hypothesis that the conversion of pheophytin to pheophorbide is mediated by this enzyme. Upon addition of the coleslaw dressing to the shredded cabbage, acidification of the medium would occur, thus resulting in a rapid loss of the magnesium ion. The rate of pheophytin formation is greater than that of the conversion of chlorophyll to chlorophyllide under these acidic conditions (Heaton et al., 1996). Therefore, this result explains why pheophytin, rather than chlorophyllide, was the intermediate species in the degradation of chlorophyll to pheophorbide. Our results are summarized in Figure 2: the pathway for chlorophyll degradation in coleslaw cabbage tissue is chlorophyll → pheophytin → pheophorbide. The same pathway for chlorophyll degradation was observed in black tea by Mahanta and Hazarika (1985) and in cucumbers by White et al. (1963). Minguez-Mosquera et al. (1989) and Johnson-Flanagan and Thiagarajah (1990) observed the activation of both pathways in their studies of olives and canola seed, respectively. Jones et al. (1963), however, found that different blanching temperatures of turnip greens and okra induced different pathways of chlorophyll degradation. This difference suggests that the degradation of chlorophyll is commodity specific. Commodities with a low endogenous pH, or tissues whose pH is decreased due to

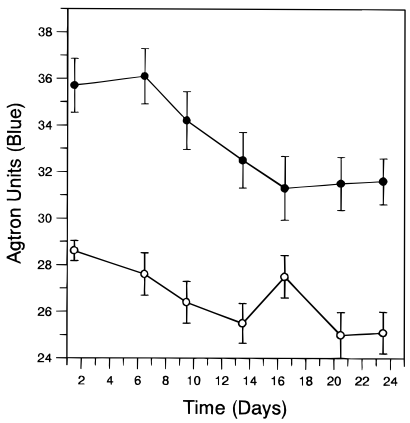

Figure 4. Effective change in reflected blue light of coleslaw and tissue (○, washed tissue; ●, coleslaw) as measured with the Agtron colorimeter as a function of storage time at 5 °C in polyethylene bags.

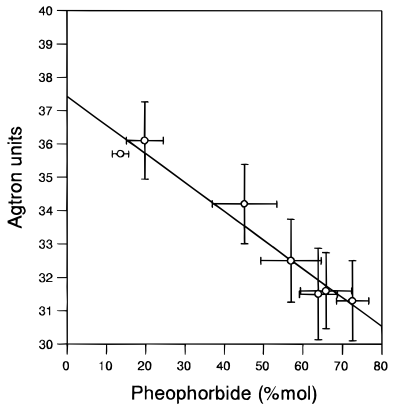

Figure 5. Effective change in reflected blue light of coleslaw as a function of the relative amount of pheophorbide extracted from coleslaw on the same day.

processing, follow pathway A (Figure 1). However, in other cases, both pathways seem to play a role (Figure 1).

There was no significant change ($p > 0.05$) in the concentration of pigments (melanins) formed from polyphenol oxidase reactions between fresh and 34-day-old coleslaw (data not shown). Tissue PPO activity was practically nonexistent as well. The average PPO activity at pH 4.8 (25 °C) was 81.6 units/g of tissue (1 unit = change in absorbance of 0.001/min) and at pH 6.0 (25 °C) of 76.6 units/g of tissue. The enzyme units observed are far below that for apples, which have a PPO activity of >13 000 units per gram of tissue (Coseteng and Lee, 1987).

The change in blue light reflection reached a minimum around day 15 (Figure 4) which corresponds to the maximum reached by pheophorbide (Figure 2). A plot of pheophorbide concentration versus blue light reflection of coleslaw (Figure 5) shows a good correlation ($r^2 = 0.9557$, $p \leq 0.01$) of color change as a result of pheophorbide accumulation. This suggests that the change in color of the tissue is highly correlated with the accumulation of pheophorbide in the coleslaw. The amount of chlorophyll and its derivatives extracted from 100 g of whole cabbage is depicted in Figure 6. The total amount of chlorophyll extracted decreases over the 150-day testing period by ~75%, whereas there was no net accumulation of its derivatives. These results indicate that chlorophyll in the whole cabbage was degraded to

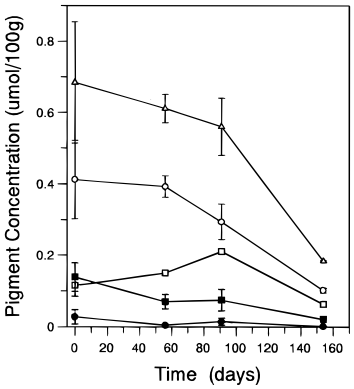

Figure 6. Effective change in the amount of moles per 100 g of pigment extracted from (○, chlorophyll *a* and *b*; ●, chlorophyllide *a* and *b*; □, pheophytin *a* and *b*; ■, pheophorbide *a* and *b*; △, total of all the pigments) whole cabbage tissue stored at 5 °C as a function of time.

colorless byproducts. The same phenomenon was also observed by Matile et al. (1992) in senescent chloroplasts of barley.

The observed color change (discoloration) in coleslaw is due to pheophorbide accumulation, which in turn is due to chlorophyllase activity; therefore any treatment (i.e., blanching and/or acidification) designed toward the inhibition of this activity would successfully prevent the color change from occurring. The amount of chlorophyll decreases in cabbage over storage time, so the use of "old" cabbage would alleviate this problem as well. Obviously, white cabbage varieties would also perform better than green varieties.

CONCLUSION

The browning of coleslaw tissue is due to chlorophyll degradation to pheophorbide. The pathway for chlorophyll degradation in coleslaw was determined to be chlorophyll → pheophytin → pheophorbide, which is consistent with a decrease in blue light reflection.

LITERATURE CITED

Amir-Shapira, D.; Goldschmidt, E. E.; Altman, A. Chlorophyll catabolism in senescing plant tissue: *in vivo* breakdown intermediates suggest different degradative pathways for citrus fruits and parsley leaves. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1901−1905.

Coseteng, M. Y.; Lee, C. Y. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci.* **1987**, *52*, 985−989.

Heaton, J. W.; Lencki, R. W.; Marangoni, A. G. Kinetic model for chlorophyll degradation in green tissue. *J. Agric. Food Chem.* **1996**, *44*, 399−402.

Johnson-Flanagan, A. M.; Thiagarajah, M. R. Degreening in canola (*Brassica napus*, cv Westar) embryos under optimum conditions. *J. Plant Physiol.* **1990**, *136*, 180−186.

Jones, I. D.; White, R. C.; Gibbs, E. Influence of blanching or brining treatments on the formation of chlorophyllides, pheophytins and pheophorbides in green plant tissue. *J. Food Sci.* **1963**, *28*, 437−439.

Kuroki, M.; Shioi, Y.; Sasa, T. Purification and properties of soluble chlorophyllase from tea leaf sprouts. *Plant Cell Physiol.* **1981**, *22*, 717−725.

Langmeier, M.; Ginsburg,S.; Matile, P. Chlorophyll breakdown in senescent leaves: demonstration of Mg-dechelatase activity. *Physiol. Plant.* **1993**, *189*, 347−353.

Mahanta, P. K.; Hazarika, M. Chlorophyll and degradation products in orthodox and CTC black teas and their influence on shade of colour and sensory quality in relation to thearubigins. *J. Sci. Food Agric.* **1985**, *36*, 1122−1139.

Matile, P.; Schellenberg, M.; Peisker, C. Production and release of a chlorophyll catabolite in isolated senescent chloroplasts. *Planta* **1992**, *187*, 230−235.

Minguez-Mosquera, M. I.; Garrido-Fernández, J.; Gandul-Rojas, B. Pigment changes in olives during fermentation and brine storage. *J. Agric. Food Chem.* **1989**, *37*, 8−11.

SAS Institute. *SAS/STAT User's Guide*, revision 6, Vol. 2, 4th ed.; SAS Inst.: Cary, NC, 1989.

Schwartz, S. J.; Lorenzo, T. V. Chlorophylls in foods. In *Critical Reviews in Food Science and Nutrition*; Clydesdale, F. M., Ed.; CRC: Boca Raton, FL, 1990.

Shioi, Y.; Tamai, H.; Sasa, T. A simple purification method for the preparation of solubilized chlorophyllase from *Chlorella protothecoides*. *Anal. Biochem.* **1979**, *105*, 74−79.

White, R.; White, R. C.; Gibbs, E. Determination of chlorophylls, chlorophyllides, pheophytins and pheophorbides in plant material. *J. Food Sci.* **1963**, *28*, 431−435.

Yamauchi, N.; Hashinag, F.; Itoo, S. Chlorophyll degradation with degreening of Kabosu (*Citrus sphaerocarpa* Hort. ex Tanaka) fruits. *J. Jpn. Soc. Hortic. Sci.* **1991**, *59*, 869−875.

Received for review July 17, 1995. Accepted November 27, 1995.[⊗]

JF9504475

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1996.