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AN OPTICAL REFLECTANCE METHOD FOR STUDYING THE ENZYMATIC BROWNING REACTION IN APPLE

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A b s t r a c t. A spectrophotometric method in diffuse reflectance media was used for studying the enzymatic browning in cut or damaged apples.

Absorbance spectra of sliced tissue of various apple cultivars was measured in the range from 200 nm to 450 nm with a Varian Cary 5 spectrophotometer operating in the reflectance mode. Browning reactions, monitored for 8 min in 2 min time intervals, demonstrate a variable composition of polyphenol compounds in apples.

The potential of spectrophotometric analysis to study the contribution of different polyphenols in the browning of apples was demonstrated as well as its applicability to verify the results of any investigations on the enzymatic model system.

K e y w o r d s: enzymatic browning, optical reflectance, apple

INTRODUCTION

Many studies have been devoted to the enzymatic browning occurring in fruits after bruising, or cutting, or during storage as an important cause of quality loss in postharvest handling and processing of commodities. This reaction results from polyphenol oxidase (PPO) catalyzed oxidation of endogenous phenolics into o-quinones which subsequently polymerize to form dark-coloured pigments.

The rate of enzymatic oxidation depends not only upon the concentration of polyphenols and PPO activity but also on the nature of the polyphenols that are co-present in a product. The main compound in apple pulp are phenolic acids, i.e., chlorogenic acid [7] and flavan 3-ols, i.e., epicatechin, procyanidin B2 [3,8]. Those two families represent about 90 % of phenolic content but the level is five fold and two fold higher in some apple varieties [1].

Oszmiański *et al.* [10] have shown that apple PPO was at least twice as active with chlorogenic acid as with catechins. But in the catechin-chlorogenic acid mixture the rate of catechin oxidation was faster than of catechin alone, while the rate of chlorogenic acid oxidation was slower than that of chlorogenic acid alone. Also the brown colour formed by catechin oxidation is of higher intensity than that from chlorogenic acid. This is due mainly to the different maximum absorbance of reaction products at around 400 nm while reaction products of chlorogenic acid are yellowish and have absorbance maximum at around 320 nm.

Apple pulps are also known to contain, in significant concentrations, dihydro-chalcone glucosides. Recently Oszmiański *et al.* [11] reported on the combination of phloretin glucoside with catechin or chlorogenic acid, and Oleszek *et al.* [9] reported on the mixture of phloretin glucoside and epicatechin that accelerate the enzymatic oxidation and produce strongly coloured products.

Lea [5] reported also that the procyanidins themselves are not a substrate of polyphenol oxidase but they are easily oxidized in the presence of chlorogenic acid.

The quantification of browning is difficult; basically it can be followed by reflectance methods in the visible region 390-440 nm of spectrum [1,2,4,12]. The colour parameters of CIE 1976 L*,a*,b* are used for the evaluation of changes in the visual quality of the product and in the effectiveness of technological measures. Studies of the reaction of enzymatic browning directly on the cut surface have never been reported. Most of the information available originates from model studies based on diluted extracts from various fruits.

Many authors have sought to establish a relationship between the browning and phenolic content, and oxidative enzymatic content of fruits, but we suggest now that the susceptibility of apples to browning illustrates the complex interactions, and that it is not possible to solve that problem in polyphenol model system *(in vitro)*.

The objective of this study was to develop the reflectance method as a technique for providing important basic information on enzymatic browning mechanism directly on the cut surfaces of apple *(in vivo)*. The technique should also help to verify and understand the mechanism by which new agents, inhibiting melanosis, may aid in developing new browning suppression technologies.

MATERIAL AND METHODS

Apples were used representing common Belgian and European commercial cultivars: Boskoop, Jonagold, Golden Delicious, Jonagored, Elstar, Cox, Granny Smith. The fruit were investigated no later than 2 weeks after harvesting.

Three apples of each cultivar were cut in half along the stem axis. The halves were positioned in a Petri dish, cut side down, under a 20 mm stainless steel cutting tube so that plugs could be bored. A traverse cut was made in the plug, at least 5 mm from the skin to exclude the effects of bruising.

Just at the start of absorbance measurements a cutting was made of 1 mm slice to refresh the sample. Three replicates for each plug were done using refreshing cut surfaces for recording.

The absorbance spectra were recorded in reflectance mode on a spectrophotometer Varian Carry 5 equipped with an Ulbricht Integrating Sphere and operated with a sample area of 18 mm in diameter. The instrument was standardized for 100 % reflectance against a white Halon tile for the region of 800 nm to 200 nm, scan step 0.2 nm and 800 nm/min scanning rate.

Browning was monitored by calculating absorbance difference spectra from data taken immediately after cutting (time 0 min) and every 2 min until 8 min. The difference spectra were twice smoothed by a simple moving average procedure calculated on 5 points.

RESULTS AND DISCUSSION

From the parameters of absorbance difference at 390 nm - (DA390), cultivars can be divided into three classes (Fig. 1). Those with a weak browning capacity (Elstar, Cox and Granny Smith) - characterized by a low DA390 <0.01, those with a very high browning capacity - characterized by very high DA390 >0.04, like Boskoop, and regular varieties, of medium browning, like Jonagored, Golden Delicious, Jonagold.

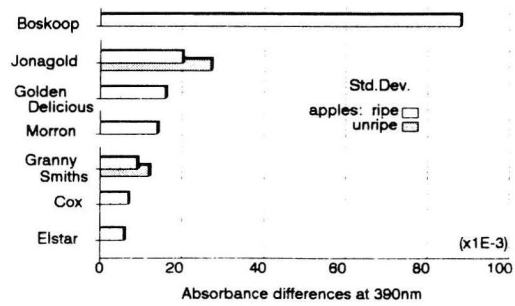

Fig. 1. Comparison of the degree of browning as the absorbance difference at 390 nm after 8 min for seven apple varieties and two classes of fruit ripeness.

The observations of absorbance difference spectra of apple slices indicate that enzymatic browning starts gradually over the first several minutes. Those changes are still not observable visually, because the absorbance difference at 440 nm is lower than 0.04 [4] and only barely noticeable in strongly browning cultivars like, eg. Boskoop.

Changes observed in the absorbance spectra of the Boskoop cultivar (Fig. 2) are a classic example of enzymatic browning known from studies on model catechine/chlorogenic acid solutions [10,11] and compounds isolated from the apple [9]. A characteristic feature here is a considerable absorbance increase in the visible violet range at maximum 390 nm, which is accompanied by a drop in absorbance in the ultraviolet range (from 310 nm to 340 nm and from 210 nm to 250 nm).

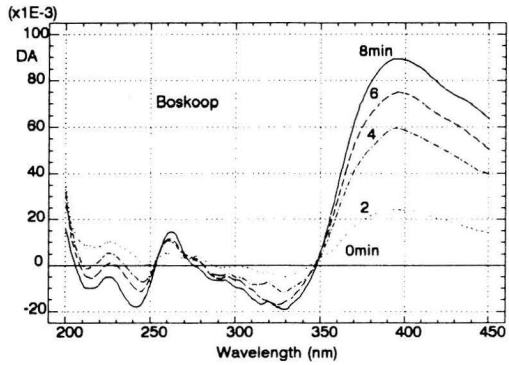

Fig. 2. Difference between absorbance spectra (DA) of the tissue of apple incubated for various times and at time 0 min for higher browning capacity c.v. Boskoop.

This process can be attributed to the oxygenation of chlorogenic acid (abs. 280 and 320 nm), flavones (abs. 280 and 360 nm), catechins and procyanides (abs. 280 nm). However, such a global and fast result can be explained only by the non-enzymatic coupled oxidation mechanisms. That o-quinones, enzymatically produced from chlorogenic acid, were able to oxidize other phenols.

The varieties Cox (Fig. 3), Elstar and Granny Smith are characterized by the slowest rate of absorbance increase within the visible range (at 390 nm) and a rapid increase within the invisible range of the spectrum, with two peaks - a higher one at 290 nm and a lower one at about 340 nm.

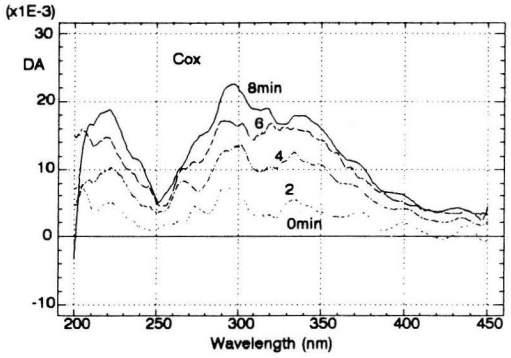

Fig. 3. Difference between absorbance spectra (DA) of the tissue of apple incubated for various times and at time 0 min for regular, medium browning capacity c.v. Cox.

Spectra for Jonagold (Fig. 4) and, likewise, Golden and Jonagored have a faster increase in absorbance within the visible range and a maximum at 370 nm, accompanied by a slower increase of absorbance in ultraviolet range on the line of 290 nm, and a peak at about 340 nm is barely noticeable.

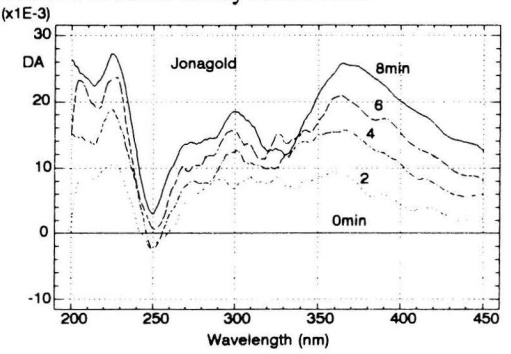

Fig. 4. Difference between absorbance spectra (DA) of the tissue of apple incubated for various times and at time 0 min for weak browning capacity c.v. Jonagold.

Changes in the absorbance spectra are complex, but they clearly indicate the formation and disappearance of chemical substances. The first factor is obviously chlorogenic acid. It is the best natural substrate of PPO and its transformations dominate in the 1st group of cultivars with a weak browning capacity. A second factor is flavan-3-ol derivatives. They are substrates of PPO and give

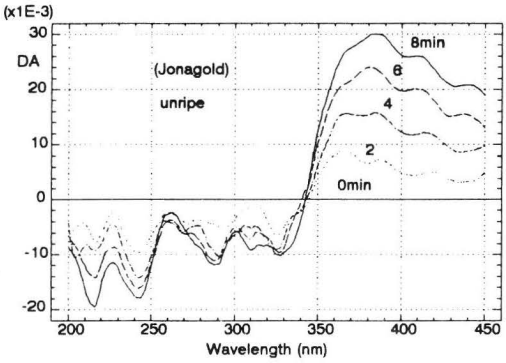

Fig. 5. Difference between absorbance spectra (DA) of the tissue of apple incubated for various times and at time 0 min for unripe apples of c.v. Jonagold.

pigments which are visually more intensely colored than those of chlorogenic acid. The role of these substrates is observable in the browning of the second group of cultivars.

Changes in the absorbance spectra of unripe apples of Jonagold and Granny Smith varieties are somewhat similar to changes in the Boskoop cultivar. However, the increase of absorbance within the visible range is slower here, and there is a decrease of absorbance within ultraviolet. This was observed at a considerably higher concentration of chlorogenic acid and at approximately the same level of other phenolic compounds [3,6]. This shows that flavan-3-ols derivatives degradation rates, intensely manifested visually, are enhanced by chlorogenic acid mainly by coupled oxidation mechanisms, and also, so that chlorogenic acid quinone was converted back to chlorogenic acid.

It has recently been shown that the two main classes of phenolic compounds and the PPO oxidation of these compounds contributed to the apple browning, but the relative importance of each of these reactions was dependent on the flavan-3-ols/hydroxycinnamics balance and on the PPO activity.

CONCLUSIONS

Enzymatic browning reactions at cut surfaces of apple can be monitored by measuring changes in absorbance difference spectra in the visible and ultraviolet region by the reflectance method. This technique may be used with varieties that are subject to severe browning, such as fast or slow browning apples.

The presented observations of browning tissue provide, to our knowledge for the first time, a basis for the spectrophotometrical characterization of browning behaviour of apple varieties.

A better biochemical explanation of the browning process would probably first consist of a selection of varieties with a low content of chlorogenic acid. Secondly, in the evolution of spectra up to browning, the flavan-3-ols content and the relative balance of hydroxycinnamic derivatives to flavan-3-ols should be considered. In this field the spectrophotometric analysis will be helpful. Thirdly, PPO activity and other minor phenolics and isomers together with ascorbid acid content and acidity may also influence browning. Further work is needed to determine the relative importance of all these factors and parallel investigation of phenolic compounds and PPO activity.

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REFERENCES

1. **Amiot M.J., Tacchini M., Aubert S., Nicolas J.**: Phenolic composition and browning susceptibility of various apple cultivars at maturity. J. Food Sci., 57(4), 958-962, 1992.
2. **Aubert S., Amiot M.J., Nicolas J.**: Browning cryteria of apples. Sci. Aliments, 12, 625-647, 1992.
3. **Burda S., Oleszek W., Lee C.Y.**: Phenolic compounds and their changes in apples during maturation and cold storage. J. Agric. Food Chem., 38, 945-948, 1990.
4. **Kuczyński A., Varoquaux P., Souty M.**: Light reflectance method to control the enzymatic browning in 'Ready-to-eat' apple products. Int. Agrophysics, 7(1), 85-92, 1993.
5. **Lea A.G.H.**: Tannin and colour in English cider apples. Flussiges Obst, 51, 356-361, 1984.

6. **Macheix J.J.**: Role de differents facteurs intervenant dans le brunissement des pommes pendant la croissance. Physiol. Veg., 8, 585, 1970.
7. **Macheix J.J., Fleuriet A., Billot J.**: Fruit Phenolics. CRC Press, Inc., Boca Raton, Fl., 1990.
8. **Oleszek W., Lee C.Y., Jaworski A.W., Price K.R.**: Identification of some phenolic compounds in apples. J. Agric. Food Chem., 36, 430-432, 1988.
9. **Oleszek W., Lee C.Y., Price K.R.**: Apple phenolics and their contribution to enzymatic browning reactions. Acta Soc. Bot. Polonaise, 58(2), 273-283, 1989.
10. **Oszmiański J., Lee C.Y.**: Enzymatic oxidative reaction of catechin and chlorogenic acid in a model system. J. Agric. Food Chem., 38, 1202-1204, 1990.
11. **Oszmiański J., Lee C.Y.**: Enzymatic oxidation of phloretin glucoside in model system. J. Agric. Food Chem., 39, 1050-1052, 1991.
12. **Sapers G.M., Hicks K.B., Philips I.G., Garzarella L., Matulaitis R.M., McCormack T.J., Sondey S.M., Seib P.A., El-Atawy Y.S.**: Control of enzymatic browning in apple with ascorbic acid derivatives polyphenol oxidase inhibitors and complexing agents. J. Food Sci., 54(4), 997-1002, 1989.