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REFLECTANCE SPECTRA OF 'READY-TO-USE' APPLE PRODUCTS FOR DETERMINATION OF ENZYMATIC BROWNING

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A b s t r a c t. An improved light reflectance method for the determination of enzymatic browning of apple slices has been described. Enzymatic browning rate parameter (DA440) of slices surface was determined from time-course measurements of visual region of spectrum and data at 440 nm of the difference in absorbance between freshly-cut and browning tissue. The paper defined the natural variability of fresh product (DA440=0.04) and the browning limit (DA440 =0.15) for identification of the slices present in processing. The changes of DA440 parameter are compared with the colour difference parameter - DE* according to CIE 1976 L*a*b* .

Nine varieties of apples: Granny Smiths, Early Redone, Royal Gala, Primarouge, Golden Delicious, Delbard Festival, Florina, Ozark Gold, Jonnee were sliced in air or under water and their browning rates were compared. Different response of the cultivars to slicing in air or under water was documented. The studies have suggested cutting procedure directly in water also for application of inhibitors to cut surface.

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

INTRODUCTION

The control of enzymatic browning in fruits used for salad bars or for other food applications represents a difficult problem for the food processing industry, especially with recent restrictions the use of sulphites as browning suppressants [4]. Some alternative treatments to control browning have been developed [15]. However, these alternatives were considered still to be less effective than sulphite application because of their insufficient penetration into the cellular matrix [13]. The browning of apples can be also reduced by adequate processing. There were big differences reported in browning rates of apples peeled with a sharp knife, with abrasion [14] or by water jet cutting system [2].

Sapers *et al.* [13] also suggested the use of cultivars with a low tendency to brown. This may obviate or minimize the need for sulphite substitutes application.

In order to evaluate the effectiveness of treatments aimed at inhibiting for enzymatic browning, some quantitative procedures measuring the browning of slices were developed. These included the use of tristimulus colorimetry, spectrocolorimetry [1,15], or the extraction and determination of the content of pigments [7]. The workers take it for granted that all of these optical parameters correlate with the extent of browning controlled by visual matching.

Where browning has been measured by use of colorimeter recalibrates for the detection of pigments [13-15], or by matching products against photos [5], we also know

that problem from our earlier experiments with peaches [8] that comparisons of results are impossible. Hence, there is no evidence that browning level evaluated from differences in various optical parameters of tissue or of the pigment composition on it would be recognized as the colour still acceptable visually and vice versa. There is still unknown the effect of the natural variability of the properties of the apple cultivars on the possibility of identification of the brown slices from the others.

The paper presents a procedure for the preparation of apple slices and measuring browning of surface by reflectance method either at specific wavelengths - for browning pigments evolution detection, or as integrated parameters CIE L*a*b* for definition of visual acceptance of apple slices.

MATERIAL AND METHODS

Slices preparation

The work was carried out with commercial maturity apple fruits of the following varieties: (1)-Granny Smiths, (2)-Early Redone, (3)-Royal Gala, (4)-Primarouge, (5)-Golden Delicious, (6)-Delbard Festival, (7)-Florina, (8)-Ozark Gold, (9)-Jonnee. Apples were grown in Voucluse (France) and were supplied by a local company 'Les Verges de la Courtoise'. Samples of 15-20 apples were stored for two weeks at 5° C.

The authors used a new technique for slices preparation. Five apples representing each cultivar and commercial variability were knife cut using a portable gauge, immediately into 12 equal sectors, discarding the core of 20 mm. Apples were also cut directly under deionized water using the same gauge. The slices were centrifuged at 3.5 G for 5 s to remove surplus water.

Random sampling of six slices from five sliced apples was conducted. This permitted well replicated experiments that could compensate for the high degree of variability observed; in individual fruits ripeness and the midsection surface of every slice was examined only once in the course of the experiment. The slices were placed on their uncut face and stored for 32 min at *ca* 25 °C.

Absorbance description

The HunterLab ColorQuestSphere Spectrocolorimeter was used for the measuring of the reflectance for the full spectrum between 400 and 710 nm as well as at one wavelength. The measurements were performed for the two surfaces of the slice at sample viewing area of 17.7 mm in diameter. The results were then expressed [10] in absorbance (A) values calculated from the formula:

$$A = \log (1/R)$$

where R is the diffuse reflectance.

Browning was monitored by means of absorbance difference spectra. The spectra calculated from results immediately after cutting (time 0 min) and every 4 min until 32 min following storage.

Finally the extent of browning was estimated by DA440 - the absorbance difference at 440 nm. The global browning tendency of varieties was expressed only by data from process under water, by means of absorbance difference after 32 min of storage. Statistical analysis was performed (Statgraphics v. 5.0) using the analysis of variance (for variety and treatments) and LSD multiple tests.

Colour matching

The tristimulus values of colour CIE 1976 L* a* b*, and the colour difference parameters DE^*, were computed from reflectance spectra. The colour difference - DE^* was calculated from the following formula:

$$DE^* = ((Da^*)^2 + (Db^*)^2 + (DL^*)^2)^{1/2}$$

where D = difference between analysed sample and standard at time 0 min. The HunterLab program was used for median data at 10 nm increments for the D 65 illuminant and an angle of 10 degrees.

Visual matching of apple slice browning by a laboratory panel was carried out. The sensory discrimination tests were performed according to standards, the series of recut

apple slices; it was considered that the colour is again fair immediately after slicing off another *ca* 1.5 mm layer.

RESULTS AND DISCUSSION

Absorbance spectra

Similar shapes and characteristic features of spectrum were obtained for fresh slices cut either in air or in water. However, the absorbance spectra obtained for tested apple cultivars (Fig. 1) could be grouped in the following way:

- the spectra with a blunt peak at 400-500 nm, related to the content of carotenoid pigments, which are characteristic for Royal Gala, Delbard Festival, Florina and Jonnee varieties;
- the spectra with the same carotenoid peaks plus an additional chlorophyll pigments peak at 670 nm, typical for Granny Smith, Early Redone, Golden Delicious varieties.

As a result of storage and the browning process of slices the spectra at time 0 min were different from each other. The calculated absorbance difference spectra within the full visible region are presented in the Fig. 2 and are typical for all tested cultivars. An examination of difference spectra showed a regular increase of absorbance only in the violet, the short wavelength region. This shift of absorbance peak is characteristic for all cultivars cut either in air or in water and may reflect the enzymatic oxidation of phenolic compounds on the slice surface. The apple cultivars highly differ in the amount of the phenolics which are browning substrates with absorption peak readings in the non-visible, ultra-violet region of spectrum, as previously reported [3,11].

The absorbance differences in the range of 600 and 710 nm maximally varied by 0.04 and did not change regularly. So no significant kinetics in the data on the longer wavelegth was observed. This supports our conclusion that browning process has not been accompanied by the degradation of pigments absorbing within this region and also these experimental methods eliminated the reflectance errors for different varieties tissue and arising from cutting-induced changes in the apple microstructure of the surface, for example all changes of texture discussed by Aubert *et al.* [1].

Browning pattern

The calculated spectra of the difference of absorption depend strongly on the evaluation of the biochemical constituents. The absorbance difference readings at 440 nm (DA440)

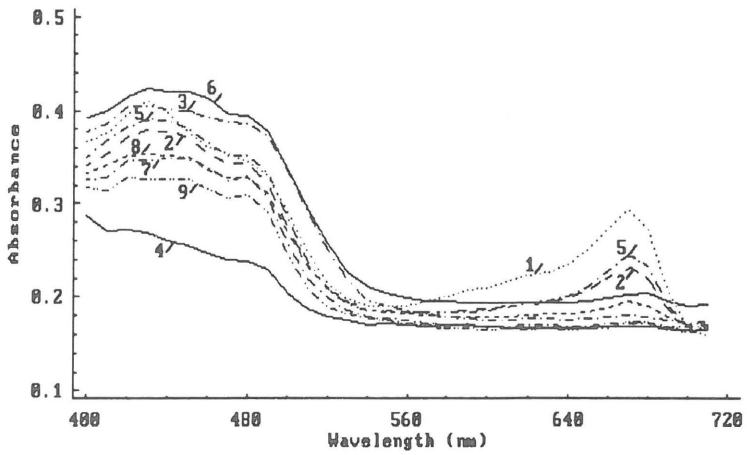

Fig. 1. Absorbance spectra of the surface of freshly sliced (not stored) apple cultivars: (1) - Granny Smiths, (2) - Early Redone, (3) - Royal Gala, (4) - Primarouge, (5) - Golden Delicious, (6) - Delbard Festival, (7) - Florina, (8) - Ozark Gold, (9) - Jonnee.

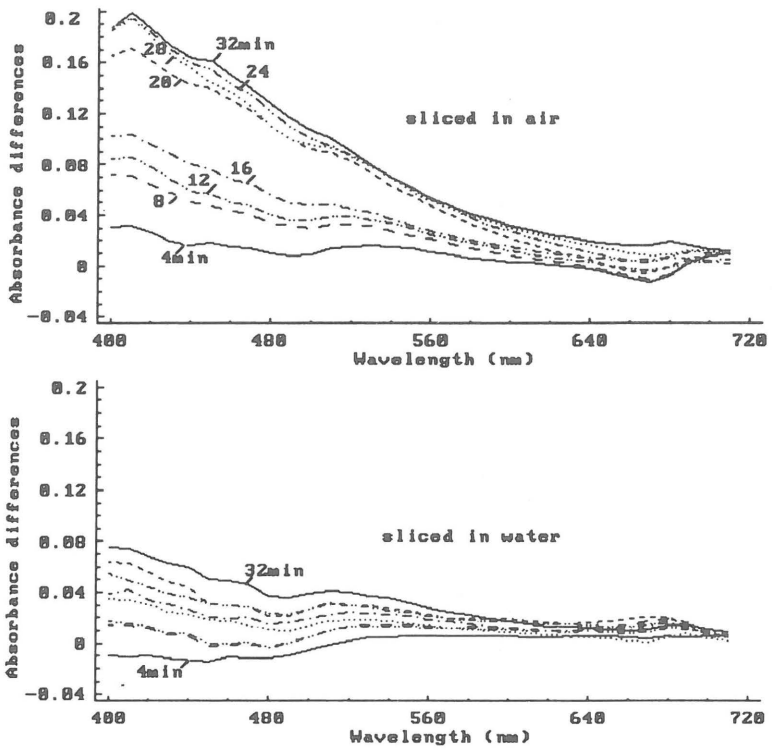

Fig. 2. Storage related pattern of the absorbance difference spectra of the flesh of the cultivar Early Redone sliced in either air or water.

showed that the apple sliced in air turned brown rapidly (Fig. 3). The water sliced apples reacted very slowly and needed at least 32 min to develop the same intensity as slices cut in air achieved after 8 to 12 min.

A simple increase in the kinetics near 440 nm region may be accounted for only by the production of brown pigment on the surface

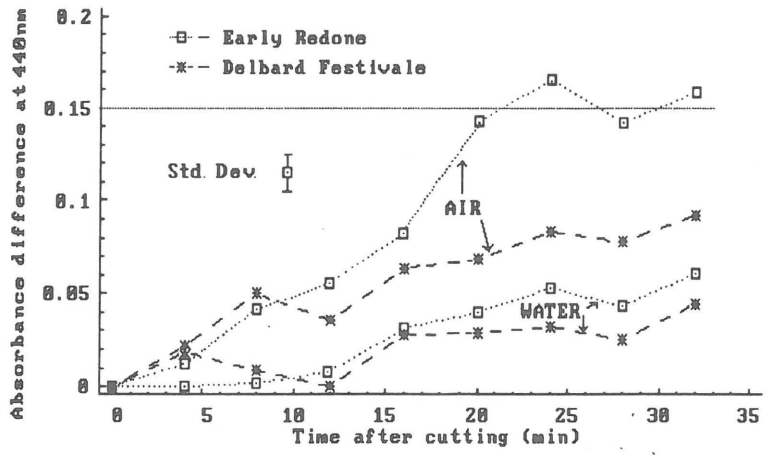

Fig. 3. The rate of enzymatic browning of slices and the effect of cutting procedure in either air or water, for the apple varieties Early Redone and Delbard Festival.

of the apple varieties under study. On the ColorQuest spectrocolorimeter used, the results have significantly smaller error of measurement on the 440 nm, but values are roughly parallel to those at 420 nm and probably at 390 nm wavelength - the point used by Aubert *et al.* [1].

The estimates of treatment effectiveness were obtained by Sapers *et al.* [13]. They were proposed for the future work on the browning registration by means of the percent reflectance at 440 nm. Sapers *et al.* [13,14,15], use colour parameters L^* and standarized the difference of the water and the inhibitor dipped sample on the time 0 min results. They plotted procentage data as function of storage time. The figures are characterized by the lag time and slope that are the descriptors of the browning inhibition.

Norris *et al.* [10] applied NIR diffuse reflectance method to component analysis of foods and agricultural products and successfully used the absorbance parameter - log (1/*R*), so we also used the same parameter.

Our findings showed (Fig. 3) that the rate of natural browning of apples can be regarded as a simple, linear function of time until DA440=0.15. That browning limit on the top should be used in the same investigations concerned with the aspects of visual acceptation of browning, particularly for the technological control of slices, and also in some models of enzymatic browning system.

The degree of browning, as defined by absorbance differences at 440 nm after 32 min, depended on the apple variety and also on the surroundings of the cutting. All data calculated from linear kinetics of browning on Fig. 4 ranged widely from 0.01 for Jonnee apples to 0.19 for Early Redone.

The present studies found that for fresh cut and not stored slices the absorbance detected at 440 nm maximally ranged near the mean values from 0 to 0.04. This is the natural variability of optical properties of the cultivars. On the spectrophotometer and the method used we registered statistical precision of absorbance measurement at 440 nm equal to ±0.01.

Sapers *et al.* [14] reported that abrasion peeling produced more disruption, and consequently more browning than peeling with a sharp knife. They also found that a brief water dip treatment, such as might be used for removal of free juices from the cut surfaces, or water jet cutting system [2], would decrease the extent of browning in apple plugs.

The nature of the inhibition of browning by water in our experiment was not closely searched. The possible explanation of more

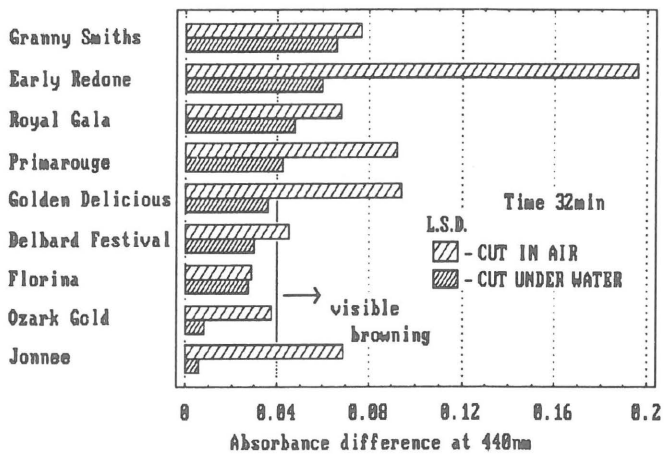

Fig. 4. Comparison of the degree of browning in slices of nine apple varieties cut in air or water. Marked line indicates visually significant browning and classified apples for the two cultivar groups with browning and no browning.

precise results found may include the following interactions; (a) - effective cleaning of the cut surface from the pulp, if the cutting takes place directly in water; (b) - reduced thickness of the layer of damaged cells by knife under water in the case of some apple cultivars; and (c) - deprivation of atmospheric oxygen in the destroyed cells by the best available substitution of air by the water.

These effects should also explain some variable results of cultivar descriptions and end in failure the correlation of reflectance browning data from slices with the phenolic compounds presented in each of the cultivars [1].

Changes in the colour

The computed L*, a*, b* parameters are presented in Fig. 5 and they reflect the data obtained for apple slices freshly cut under water and measured after 32 min of storage. The browning of slices for all the varieties tested was characterized by the decrease in the value of parameter L* (turning dark) and an increase in the values of a* (turning red) and b* (turning yellow).

The extensive variation of data indicates that the simple distances of colour coordinates L*, a*, b* are not so staistically significant for browning detection (even after 32 min storage, Fig. 6). The Euclidean distances between colour points, parameter DE*, will have statistically higher precision.

According to the NBS parameter *DE** approximating the NBS Units of Colour Difference represents the average maximum difference acceptable in a series of dye-house commercial matches. The value of *DE**=2 corresponds to the lowest observable change in colour and in industry term for quality of colour match is poor [6].

The parameter *DE** presented in Fig. 6 was used to determine the limit of nonobservable colour change. The value *DE**=2 has been confirmed present by the panel system of visual discrimination for apple slices.

The absorbance differences DA440 and the colour difference parameter *DE** are quite different qualifiers of varieties in the browning process but show a simple relationship, considering the conformity of cultivar ordering in Figs 4 and 6 for water. From these the absorbance difference value other than 0.04 was sensitive to colour change to the same degree for the human eye as the parameter *DE** value higher than 2. The calibration point DA440=0.04 in the same absorbance measurements should be regarded as a nonvisible browning level and can be recognized as the lower limit for commercial applications not related to

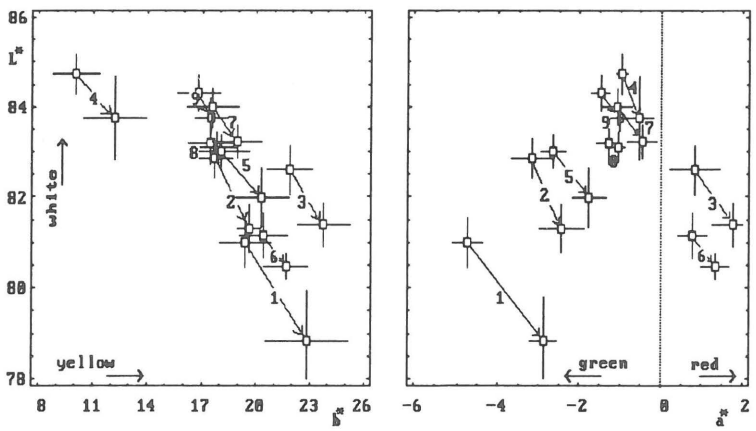

Fig. 5. Changes in a* and b* parameters versus L* during browning of the slices from water of nine apple varieties. Arrows indicate the changes of these absolute parameters from 0 min to 32 min of storage at 25 °C.

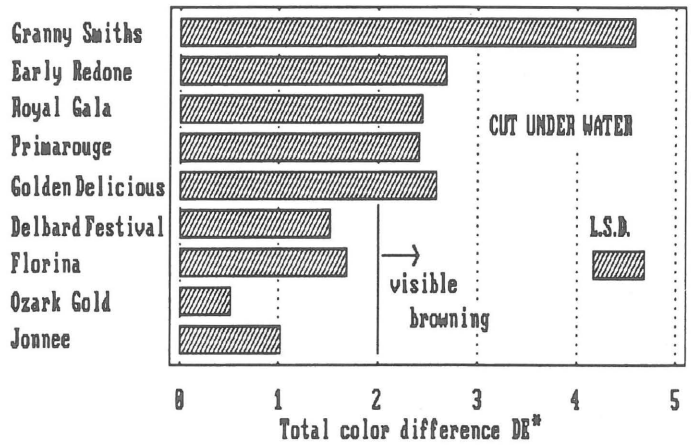

Fig. 6. Classification of apple varieties by the colour difference parameter DE* of slices stored for 32 min. Marked line at DE*=2 indicates the lower limit of observable change in colour.

cultivars or texture of cut surface. The opposite, DA440=0.15 maximum limit is proposed with a certain reserve for high browning, considering that *DE**=5 on presented brown slices (Fig. 6) is described only by DA440=0.08 (Fig. 4).

CONCLUSIONS

The suggested method of absorbance difference measurement proved to be straightforward, easy to employ for controlling the enzymatic browning in apple processing and likewise in research. The measurement technique allows the physical changes in the slice surface microstructure, resulting from the different technologies of apple cutting, to be rejected. The absorbance difference value of 0.04 measured at 440 nm presents the visually tolerable levels of apple browning and also the maximum of the natural variability. Enzymatic browning occurring in apple processing should never reach an absorbance difference value higher than 0.15 so this corresponds also to the colour difference *DE** much higher than 5. This is the limit for the linear analysis by the reflectance method.

The proposed procedure makes it possible to distinguish varieties susceptible to enzymatic browning from resistant cultivars. This should make it possible to choose model varieties for further investigations, the method being especially sensitive if the apple slices are cut directly in water.

Future works should be devoted to the determination of the level of changes that is already observable, but that can be regarded by consumers as a fresh or tolerable browning. That should be specifically determined for certain cultivars or chemical inhibitors and storage conditions.

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