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# A METHOD FOR ESTIMATION OF ENZYMATIC BROWNING AND ITS SUPPRESSION IN APPLE SLICES

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A b s t r a c t. A described method is based on diffuse light reflectance in the spectral region 400-720 nm for the examination of enzymatic browning of apple slices. In this method apples are sliced as directly dipped for 4 s in the inhibitors. Based on the obtained results data for the limits of visual acceptance of the slice colour are proposed.

An enzymatic browning parameter of the slices is determined from the time-course measurements of the difference in absorbance at 440 nm between the brown slice and the same slice where the original colour was restored by slicing a further 1.5 mm layer.

Three varieties of apples Granny Smith, Golden Delicious and Red Delicious were sliced under water or in ascorbic acid, oxalic acid and sodium bisulphite solutions. Different concentrations of the suppressant were objectively compared, and their efficiencies were expressed by the allowable period of cold storage.

K e y w o r d s: apple slices, reflectance, browning, oxalate, sulphite

## INTRODUCTION

An important quality attribute of commercially prepared apple products is the white colour. The control of enzymatic browning in apples used for salad bars or for other food applications represents a difficult problem for the food processing industry, especially with recent restrictions on the use of sulphite as browning suppressants [18,20]. Some alternative treatments have been developed. However, these alternatives were considered to be less effective than sulphite [5,14,15,19,21].

In order to evaluate the effectiveness of treatments aimed at inhibiting enzymatic browning, some quantitative procedures of measurement have been developed. These included the use of colorimetry [2,10-12,14, 15,17,19], spectrocolorimetry [1,8-10,21], the extraction and determination of the content of pigments [2,3,7,14,16,23], matching products against photos [5] or sensory testing [12,13].

In the course of kinetic studies on browning deterioration, it was observed that a comparison of published results is not possible.

Studies of inhibition in practical applications require extended periods of storage of sliced tissue [12,19,21]. Within a few weeks, not only new pigment compositions appear after the oxidation of polyphenols, but an accelerated process of ripening continues, as well as conversion of carotene and chlorophyll pigments [22], modification of texture [2,22]. These alter the absorption characteristics of the tissue.

The CIELab system for colour description is generally used. The parameters L*, a* and b* are used here to evaluate changes in the tissue absorption within the three wide bands of spectrum. They are selected for their relationship with the perception by the human eye and permit a psychophysical description of

colour, the parameter dE* quantify the magnitude of the total colour difference.

For the assessment of enzymatic browning, information from the ultraviolet and violet region of absorption curve is necessary [9]. Without suppression, changes in the absorption are recorded instrumentally two minutes after cutting. After 6 to 8 min changes appear in the violet region. After 8 min, the values of the tristimulus parameters CIELab change, and after 32 min the parameter dE* of the total colour difference for certain varieties exceeds the value of 4.5 [11]. Until that time, magnitudes of colour differences dE* can be measured by in the CIELab space [2,6,11].

The parameter introduced by Sapers *et al.* [17], '% Inhibition', is commonly used in studies on inhibition. This parameter properly ranges the suppressant in experiments [12,21], but it does not provide objective information on differences in the effectiveness on differentiation of inhibitors.

The relative value of the parameter allow us to carry out the tests on samples being already brown and unacceptable by a consumer. Mowever, it is not posible to distinguish the recovery simulation of natural and fresh colour of apple slices from the described inhibition process of enzymatic browning. This makes the '% inhibition" parameter useless for wide application in food technology.

The objective of this work is to present a method for global evaluation of the browning inhibition in apple slices. It deals until the application of inhibitors on the slicing surface, it includes the evaluation of browning suppression and the estimation of susceptibility of cultivars to enzymatic browning. The results are used for proposy product quality standards and are presented in the form of a number of days of product quality retention in different storage conditions.

## MATERIAL AND METHODS

### Slices preparation

The work was carried out with commercial apple fruits of the varieties Granny Smith, Golden Delicious, Red Delicious. Apples were supplied by a local company 'Les Verges de la Courtoise' Vaucluse (FRANCE). Fruits were stored for two weeks before the experiments at 5°C prior and also during the tissue preparation.

Twelve apples for each cultivar and with a commercial ripeness variability were cut by knife into twelve equal sectors discarding the core of 20 mm. The cutting gauge was modified so that it works with the apple immersed in appropriate solutions. This prevented air from reaching the cut tissue during the cutting operation and also removed adhering juice. Finally, the cut slices were kept for at least 3-5 s either in deionized cold (5 °C) water or in the cold solutions. Then the slices were immediately centrifuged in a portable salad spinner for approximately 5 s to remove surplus water or solutions. This is different from the procedure where a cut sample is soaked in water for 10 s or longer, after which the sample is blotted dry by rolling it on absorbent tissue [14,17,21]. Ascorbic acid (0.05 %, 0.1 % and 0.2 % w/v), oxalic acid (0.05 %, 0.1 % and 0.2 % w/v) and sodium bisulphite (0.01 %, 0.02 % and 0.04 % w/v), were used as inhibitors. These are reported by Sapers *et al.* [18], and Duden [4].

The cold slices (at 5 to 8 °C) were selected randomly and groups of six were placed into pouches of film with a high permeability for gas (Rhone-Poulenc, oxygen over 10,000 cc/m$^2$/day/atm, at 25 °C) for continued storage at 5 °C. This allowed the tissue to remain very moist and allowed aerobic respiration, which was confirmed after 10 days using gas chromatography on the pouches.

At intervals between 1 and 10 days, three pouches were selected at random for an analysis. This permitted good replicate experiments that include the effects of the high variability between individual fruits and inside the fruits. After storage, a fresh surface of the slices was obtained by slicing off a further 1.5 mm layer using a rotating meat slicer.

### Browning description

Changes in pigmentation were monitored based on the diffuse reflectance spectra of the

cut surface [9-11]. The HunterLab ColorQuestSphere spectrocolorimeter was used for measuring the reflectance for full spectrum between 400 and 710 nm as well as at one 440 nm wavelength.

The measurements were performed on both surfaces of the slice with a sample viewing area of 17.7 mm in diameter. The results were expressed in absorbance (A) values calculated from the formula: $A = \log(1/R)$, where R is the diffuse reflectance.

Browning changes were monitored as the difference between the absorbance spectra of slices after storage and the spectra of the same slices immediately after the cutting off a 1.5 mm layer. Finally, the extent of browning was estimated by the absorbance difference at 440 nm - $DA_{440}$.

The analysis of variance of $DA_{440}$ for cultivars, storage and treatments was done using Statgraphics v. 5.0. Mean values were compared by the LSD tests (5% significance level).

## RESULTS AND DISCUSSION

### Browning spectra

The three cultivars used in the study provided a wide range of light absorbance spectra with variation between samples within a cultivar (Fig. 1). The Granny Smith apples absorbed strongly at the 675 nm wavelength, while the Red Delicious variety absorbed only in the violet-blue region at 400 nm to 500 nm. Red Delicious apples have a spectrum with the most plateau-like form, but with a distinctly higher absorbance in the short wavelength region and with no observable absorption peak in the region of 675 nm.

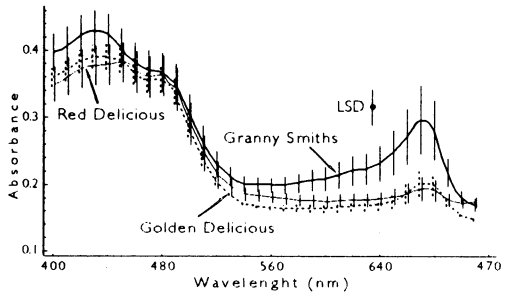


**Fig. 1.** The range of natural variability in the absorbance data of fresh apple tissue for cultivars: Granny Smith, Golden Delicious and Red Delicious.

The spectrum regions with maximal variability of the absorbance are closely related to the absorption of the chlorophyll pigments at the region of 675 nm and carotene pigments in the region of 400 nm to 500 nm.

Whiteness of the fresh slices could be characterized by natural variability of absorption in violet region of spectra, at the 440 nm line [6,8,21]. For the samples of Red Delicious and Granny Smith the standard deviation was 0.037 and for Golden Delicious was 0.02. From data previously reported by Kuczyński *et. al.* [11], a value $DA_{440}=0.04$ corresponds to the detection limits of the browning by the human eye. However, the natural variability is only a little less than that limit, so it is possible to find slices that look brown when freshly cut and visually compared with others in the same group.

As a result of the browning process the absorbance spectra of slices tested later were different from slices tested immediately after cutting. Nevertheless for all cultivars cut under water and stored 1 day or cut under tested inhibitors, the shape of absorbance difference spectra in the visible region (Fig. 2) are similar. They are similar to those of the apples cut in air and brown allowed to immediately [11]. The spectra of difference show regular shifts with time towards increased absorbance, mostly in the violet-blue region. The regular shifts may reflect the secondary effect of enzymatic oxidation of phenolic compounds on the sliced surface. This effect is observed in the non-visible, ultra-violet region of spectrum [9,16].

After only one day the apple slices cut in pure water as well as those cut in ascorbic acid solutions are characterized by a high increase of absorbance, typical for the very brown slices [19]. Only by the use of true inhibitors - bisulphite or oxalic acid solution, subsequent test dates were characterized by increased absorbance difference spectra following the storage.

Enzymatic browning quantification is obviously difficult. The analysis of the spectrum

shows that reliable measurements or comparisons of the supression of enzymatic browning should be completed while the process still increases the absorbance in the blue region. This time period is appropriate for the technological processes.

### Non-visible or tolerable browning

On a wide range of reflectance instruments the changes in the absorbance spectrum due to enzymatic browning can be most easily monitored at the wavelength of 440 nm [2,8, 18,21].

When the slices cut in cold water and cold stored, for one day the following absorbance increase were recorded: Red Delicious $DA_{440}$= 0.11± 0.05, Granny Smith $DA_{440}$= 0.10± 0.05 and the smallest Golden Delicious $DA_{440}$= 0.07± 0.01. This increase in absorbance is low when compared with the results described earlier [11], i.e., where the slices were cut in air and stored in room conditions for 32 min. Granny Smith showed browning determined by $DA_{440}$, up to 0.75 and Golden Delicious up to 0.95.

In Fig. 2 the symbols '*' and ' ■ ' mark the range of variation of the absorption difference curve which we considered. The values $DA_{440}$= 0.04 correspond to non-visible browning [9]. The values for Granny Smith slices cut in cold water after a day of cold storage, i.e., $DA_{440}$= 0.10± 0.05 represent tolerable browning of the

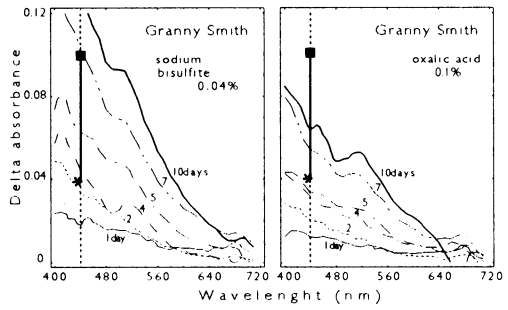


**Fig. 2.** An example of a storage related pattern of the absorbance difference spectra of apple slices in c.v. Granny Smith cut at different concentrations in either sodium bisulphite or oxalic acid. The line indicates the interval from a visually detectable '*' until the acceptable level of browning ' ■ '.

slices of this variety. The parameter $DA_{440}$ after a day characterizes objectively the susceptibility of the apple cultivars to browning.

Since in food technology it is very important to use routine procedures for quality evaluations that would yield results in agreement with consumers' opinions, and to have correct quality standards, organoleptic test at random were performed.

The test was performed as follows: two sample series of 30 slices were prepared. One series contained slices cut in cold water and after a day of cold storage, and the other series the same slices but 'freshened' by recut. In the period of 8 to 10 min the organoleptic panel had to select the more browning slice from the two. The result of this evaluation was the pair test conducted at 1 % significance level that did not allow for abandoning the statistical hypotesis of an equal number of correct and incorrect answers. Tolerable browning for the samples was obtained taking into consideration the fact that we are naturally accustomed to the fact that freshly cut apples show slight browning already after some minutes in room conditions. The above results agree with the McLellan *et.al.* studies [13] that tested sensorical acceptance of slices.

The present study compares the absorbance on surfaces of stored slices to that of surfaces 'freshened' by repeated cutting in air. An assessment of the changes in absorption of the inner tissue after storage is required. Therefore the comparisons were made between the absorbance of slices one day after cutting in solutions: bisulfite, ascorbic and oxalic acid and the absorbance of slices prepared in the same way and up to 10 days stored but after removing a 1.5 mm slice. The following differences of absorbance were found for 7 and 10 days storage, respectively: Red Delicious $DA_{440}$ = 0.005 and 0.012 ± 0.011, Granny Smith $DA_{440}$ = 0.038 and 0.018 ± 0.007, Golden Delicious $DA_{440}$=0.016 and 0.024 ± 0.005. The above results were obtained by means of very sensitive statistic pair test that show differentiated effects of storage on various varieties, e.g., for Granny Smith after 7 to 10 days the $DA_{440}$ decreases.

However, these effects are on the same level as the natural variability of absorbance ($A_{440}$) of fresh slices. Thus, we suppose that the 'freshened pigmentation' of slices stored for 10 days results only from the increase of inner tissue ripeness and not as the browning or modification of texture.

A comparison of the absorbance spectra of fresh slices cut under the different solutions with the spectra of fresh slices cut under water did not show statistically significant effect of the chemicals on the natural variability of absorbance. The results obtained after short dipping and immediate after cutting are in contrast to chemical blanching effect, observed visually by Lozano-de-Gonzalez *et al.* [12].

## Browning quantification

Application of bisulfite or oxalic acid at cutting provided protection for the cutting surface for the longest time period when our criteria - that is non-visible and tolerable limits, are used (Fig. 3).

In the case of Granny Smith and Red Delicious non-visible browning was noted for 2 days because the change in difference of absorbance was below 0.04 after the application of the highest concentration of bisulfite (0.04 %). In the case of Granny Smith the concentration of bisulfite 0.02%, gave an effect lasting for one day, while in the case of Golden Delicious the fruit browning was always observable.

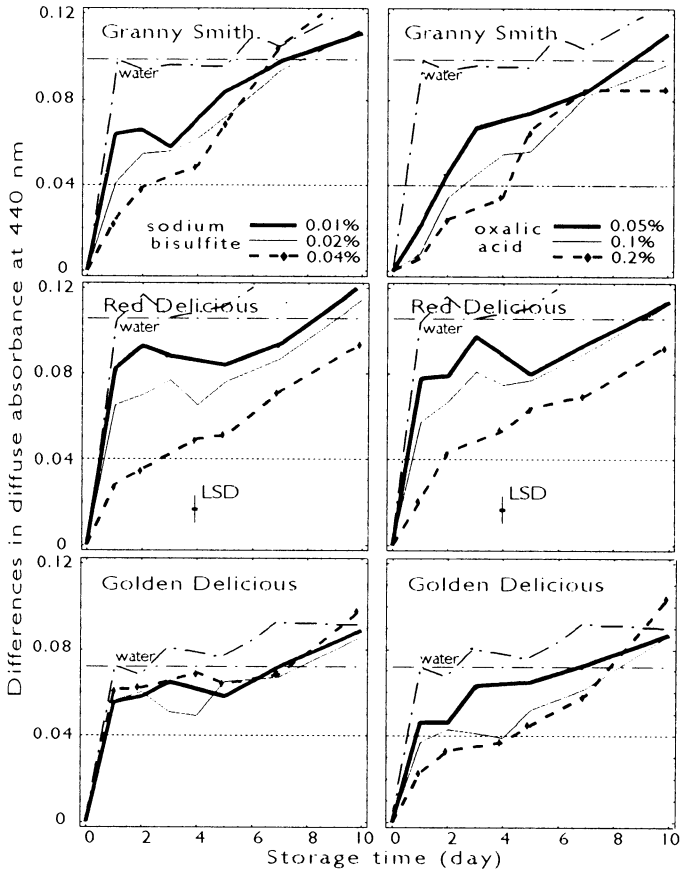


**Fig. 3.** Enzymatic browning of apple slices during storage at 5 °C and the effect of inhibitor concentration in either sodium bisulphite or oxalic acid for the apple c.v. Granny Smith, Golden Delicious and Red Delicious. The blank slices cut under water.

With the oxalic acid, the non-visible browning is prevented for a period of 4 days on the slices of Granny Smith and Golden Delicious cultivars and for a period of 2 days on the slices of Red Delicious, using the highest concentration of 0.2 %. A lower concentration of 0.1 % was effective for 2 days for Granny Smith and for 1 day for Golden Delicious. The lowest concentration of oxalic acid 0.05% was only effective 1 day in the case of Granny Smith.

Considering of tolerable browning, the application of bisulfite or oxalic acid solutions allowed storage for 7 days. It was only in the case of Red Delicious that the highest concentration of bisulfite (0.04 %) extended the tolerable storage period to 10 days. The highest concentration of oxalic acid (0.2 %) ensured the same quality for 10 days for the slices of Red Delicious, while the same effectiveness was provided for the slices of Granny Smith cultivar by only 0.1 % oxalic acid.

## CONCLUSIONS

The light reflectance techniques allow for an objective determination of the kinetics of enzymatic changes. The efficiency of the browning inhibitors can be expressed most practically in terms of acceptable period of apple storage. It is suggested to consider results obtained after one day of cold storage of apples that have been cut under water as limits of tolerable browning and as the standards for all the varieties.

It was found that the tissue 1.5 mm depth under the cutting surface retains the natural apple colour for 10 days in the cold storage. There exist, a natural variability absorbance as a result of stages of ripeness. Cutting the apple under solution of enzymatic browning suppressant for a short period (4 s) did not change the initial colour of slice surface. Constant absorbance in the region of 700-720 nm may be used for reflectance referencing on portable instruments or in other condition of measurements.

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