

Automatic monitoring method of apple browning for determining optimal inhibitor mixtures

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Abstract

Apple browning is an undesirable biochemical change that decreases the value of apple produces on the food market. The colour of apples starts changing immediately after being cut, the speed of colour-change depending on apple-variety and environment: sweeter apples become brown quicker, warmer environment favors the browning speed. Monitoring apple browning is part of food quality control and part of studying ways to decrease the browning speed by using different types of inhibitors (anti-browning agents). The monitoring is usually done with colorimetric devices such as spectrophotometers and colorimeters. We propose an alternative way of monitoring colour changes during the browning process for optimizing inhibitor mixtures for fresh-cut green apples, using the Trichromatic Colour Analyser (TCA) system, which turns scanner into colorimeter. TCA has been initially created for the complete study of the traditional Thai colours and subsequently developed for other tasks, one of them being the automated monitoring of fruit browning. One of the main advantages of TCA over other colour measurement devices is the capability of obtaining colorimetric values over a flexible port-size with a maximum aperture of 100 x 100 mm. In our research we mixed different quantities of ascorbic acid (10 g/L), citric acid (10 g/L) and sodium chloride (10 g/L) using proportions specified by simplex centroid pattern, in order to obtain an optimized inhibitor for browning process of Granny Smith green apple types. The automated TCA monitoring process was compared with the automated monitoring using a Konica Minolta CR400 colorimeter for 28.3 hours with a 1300 seconds time-interval between measurements. The optimized inhibitor mixture depended on time frame (initial time, t_0 and time t).

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Keywords

Browning process
Trichromatic Colour
Analyser
Browning inhibitor

Introduction

Enzymatic browning occurs in some fruits and vegetables, which contain polyphenol oxidase, after cells are damaged. Once the enzymatic browning starts, the colour appearance of the cut surface of fruit or vegetable changes. This change reflects the quality of fruit and vegetable. There are many types of enzymatic browning inhibitors that can slow down the browning reaction and subsequently decrease the rate of colour-change (Artes *et al.*, 1998; Duangmal and Owusu-Apenten 1999; Lamikanra, 2002; Ioannou and Ghoul, 2013). Food scientists and researchers mostly used colorimeter (CIELAB or Hunter Lab) and spectrophotometer (CIELAB, Hunter Lab or absorbance) for examining how colour is changing. However, recently, the computer vision system has been introduced for doing the same task (Quevedo *et al.*, 2009a; Quevedo *et al.*, 2009b; Quevedo *et al.*,

2009c). In this article, we propose an alternative way for monitoring the enzymatic browning of fresh-cut Granny Smith (GS) green apple and for optimizing the effect that single inhibitor and inhibitor combinations have on the fresh cut GS green apple. Our method uses data obtained from the Trichromatic Colour Analyser (TCA) opposed to data obtained with a colorimeter for result confirmation.

Materials and Methods

Devices and system: TCA system and colorimeter

TCA system is composed of the Trichromatic Colour Analyser Software (Version 2.0.0 and produced by Preda, R.I and Katemake, P.) and a specially configured personal computer connected to an Epson GT20000 scanner. TCA software can control an unlimited number of scanners that have the Twain driver installed on the computer but the TCA

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system is specially developed for obtaining the most accurate colorimetric data compared to colorimeter standards (Katemake and Preda, 2014). It analyses each pixel from a scanned colour bitmap that has a maximum size of 100x100 mm, calculating the RGB, CIEXYZ and CIEL*a*b* coordinates. TCA has tools for correcting the scanned colours and for aligning the physical colour locations with the ones on the scanned image. The colour correction results from a 3-step scanner calibration, as follows:

1. A primary rough calibration that involves the Munsell grey patches: N2, N3.5, N5, N6.5, N8 and N9.5;
2. A secondary calibration using the 24 Colour Checker Colours from which Cyan is excluded;
3. A third calibration stage using 1300 Munsell mat patches; in this step, the boundaries of a colour space are determined in which colorimetric data can be accurately predicted (we called it TCA colour space).

The way of performing the calibration procedure is described in TCA's user manual (Preda and Katemake, 2011), chapter 3.4, under the titles: Default Colour Calibration (section 3.4.1), Advanced Colour Calibration (section 3.4.2) and Extended Colour Calibration (section 3.4.3), the latter one including the last 2 steps. At the time of publishing the User Guide, the extended colour calibration algorithms were in development stage.

A description of the colour correction method that was initially used for this software system is presented in a previous article published in Colour Research and Application (Katemake and Preda, 2014). We decided to keep the algorithms proprietary but the described method is sufficient for being used as starting point in the creation of similar software. General information about later developments of the entire TCA system can be found in the AIC 2013 proceedings (Katemake and Preda, 2013). The TCA functions that are essential for monitoring browning are the capabilities of automatically capturing images, of calculating colorimetric values for all colour positions contained in the captured images and of analyzing the homogeneity of the measured surface.

The Konica Minolta CR400 colorimeter with aperture of 1 cm, illuminant/observer: D65/2°, automatic measurement mode (1300 seconds interval) was used to obtain colorimetric CIEL*a*b* data. This colorimeter can be set for repetitive measurements at fixed time-interval over a determined time period. All measured data can be recorded in its memory buffer and can be printed after the whole set of measurements is completed.

CIE colour difference

We considered that a small colour difference of enzymatic browning, after inhibitors were added, was of large importance and we took into account that the colour of fresh cut green apple, which was a very light greenish creamy colour, was close to neutral colour. Therefore, we applied CIE2000 colour-difference formula (CIEDE2000, ΔE_{00}) to our study instead of the CIE 1994 colour-difference model (CIE94), which gives errors in near neutral colours (Luo *et al.*, 2001). The CIEDE2000 formula is best to use in applications where small colour differences are essential for studying colour variances (Ghinea *et al.*, 2010) and it improves the prediction of the near-neutral colour difference.

Apple and inhibitors

Fresh GS green apple, produce of New Zealand, bought from Tesco Lotus supermarket in Thailand, had the average weight of 142.8 grams. We present the average size by the average weight because of the unsymmetrical shape. The apple was scaled and marked before cutting. The fresh cut apple slice was cut vertically at 1 cm from the core and cut again at the outer part to make the thickness of 1.5 cm. The fresh cut apple slice was measured after cutting, with the average delay time of 25 seconds, when monitoring browning without inhibitor treatment for 23.8 hours at room temperature (28°C). The total of 48 slices was used for all 8 conditions with 3 replicates, 2 measurement devices. One slice of an apple was used for each condition.

Three inhibitors with equal concentration of 10 g/L, were used as single component and in combinations: ascorbic acid, citric acid and sodium chloride. The fresh cut apple slice was dipped in the inhibitor dissolved in deionized water for 1 min. Its colour was measured immediately after taking it out from the inhibitor solution.

In this study we automatically captured, for 23.8 hours, image-areas of 2x2 cm around the center of fresh-cut green apple slices, at time intervals of 1300 seconds, with and without inhibitor treatments. The colorimetric results were compared with Konica Minolta CR400 having measuring port of 1 cm diameter.

Precision and CIEDE2000 pattern of TCA and colorimeter

We examined short-term repeatability and CIEDE2000 variance over time for both TCA and colorimeter measurement methods. Firstly, we compared the short-term repeatability of TCA, using a measuring area of 4 cm², with the short-term

repeatability of Konica Minolta CR400 colorimeter, having a measuring port diameter of 1 cm (a measuring area of 0.78 cm²). The criteria for selecting the 4 cm² area for TCA was that the measurement-size should not exceed the fresh-cut apple slice-size and, at the same time, it should be sufficiently large for covering the most significant colour-shades from center to periphery of the apple slice. We used homogenous Munsell grey N5 as testing-sample for short-term repeatability and automatically measured it for 50 times at 5 seconds time-interval. Thirty measurements out of 50 in the middle of the sample were selected for the calculation of mean colour difference of the mean (MCDM), in order to indicate the precision of the two considered devices. With Konica Minolta CR400, the sample size of Munsell grey N5 was larger than the measurement port, the external light could not enter. With TCA, the location calibrator (printed grid on transparent film) was used to locate the center of the measurement area far from the top edge and the right edge equally of 12.5 cm (Preda and Katemake, 2011). It helps to place the sample on the scanner glass at the exact location required by the software for keeping track of every colour position. The top and the right edges of maximum size of the measurement area (10 cm x 10 cm) was 2.5 cm from the top and the right edges of scanner glass. There was no external light influenced on the sample located on this area during measurement when the scanner lid was closed. It was already tested when the software was first developed and that was the reason why we used the measurement area far from the corner 2.5 cm inward. Apart from closing lid, a thick cloth was used for covering scanner lid during all measurements.

Secondly, CIEDE2000 variances over time recorded with TCA and colorimeter were tested on homogeneous sample to verify if the different pattern of CIEDE2000 is influenced by the measuring size or by the texture of sample surface. GS green apple was vertically cut 1 cm away from its core and the apple slice was placed immediately with its center on the measurement port. We selected one condition of fresh-cut GS apple (fresh-cut apple without treatment) and compared the CIEDE2000 between the 1st and 2nd, 1st and 3rd measurements until 67 measurements were completed, using both TCA and colorimeter, with a 1300 seconds time interval between two consecutive measurements. The browning reaction started once the apple was cut and continued unevenly across the surface. We compared CIEDE2000 variances performing measurements with both TCA and colorimeter on apple slice (representative for non-homogeneous sample) and on Munsell grey N5 (representative

for homogeneous sample), using measuring areas of 0.78 sqcm and 1 sqcm, which correspond to the fixed apertures of the colorimeter's measuring port. We also compared CIEDE2000 variances for apple slices using TCA with measuring areas set to 1 sqcm and 4 sqcm. A homogeneous Munsell grey N5 was measured 50 times and the total colour differences between measurements were calculated in order to confirm that the differences recorded between the two methods did not result from the applied method but from the inhomogeneous colour changes across the fresh cut apple slices.

Optimization of inhibitors for fresh-cut Granny Smith apple

We optimized inhibitor-combinations for fresh cut GS by using mixture design, with simplex centroid, 3 components and 3 replications resulted in 7 formulations x 3 times (21 runs). The mixture designs showing treatment formulations of using single inhibitor and their mixtures are presented as follows: ascorbic acid (10 g/L), citric acid (10 g/L), sodium chloride (10 g/L) in proportion of (1,0,0), (0,1,0), (0,0,1), (0.5,0,0.5), (0, 0.5,0.5), (0.5,0.5,0) and (0.33,0.33,0.33). For example, the combination of last mixture, 1 g of ascorbic acid in deionized water 100 ml + 1 g of citric acid 100 ml + 1 g of sodium chloride 100 ml. Each run was carried out one at a time and all runs were repeated 3 times. Before cutting a slice of apple, the program was open, the size of measurement was determined, the cycle of measurement was set and the name of file was typed. After cutting, it was dip immediately into the inhibitor that was prepared in advance. After 1 minute, it was located on the exact position using location calibrator, the scanner lid was closed and covered with a thick cloth and finally the operator click the measurement button on the monitor screen. The delay time after taking the apple slice out of the inhibitor solution till clicking the measurement button was the average of 25 seconds.

We measured the colour of GS apple slice, with 1300 seconds interval for 23.8 hours using both TCA and colorimeter in 7 conditions (with all treatments) plus 1 condition of fresh-cut apple slice without dipping in inhibitors (without treatment, control condition). The GS apple slice was not moved from the measurement device till the end of its session, this was carried out under room temperature of 28°C in average and RH of 73% in average. The percentages of inhibition over time were calculated.

Results and Discussion

Precision and CIEDE2000 pattern of TCA and colorimeter

TCA and colorimeter gave MCDM of 0.008 and 0.002 respectively which means the precision of both measurement methods are only slightly different. The MCDM of a set of measurement of about 0.1 or less of CIELAB colour difference unit is expected for colour measurement devices and can be considered to be very satisfactory (Hunt and Pointer, 2011). With 30 replicate measurements (after deleting first 10 and last 10 measurements) of Munsell N5, using TCA and Konica Minolta CR400 colorimeter, 95% confidence interval for the mean values of CIE L^* a^* b^* were 0.01 and 0.01, 0.04 and 0.01, as well as 0.04 and 0.01 respectively. There are several methods for determining the instruments' relative performance. In this research, the MCDM was selected for comparing short-term repeatability of individual instruments and the statistical results are only allowed us for a rank ordering (Wyble and Rich, 2007). The lesser MCDM is the better order ranking.

Comparison of the CIEDE2000 variances in time, using the same time intervals for measuring with the two methods, showed similar evolution of the plotted data obtained from TCA and colorimeter as presented in Figure 1. The measurement area set for TCA was approximately 3 times larger than the one of Konica Minolta CR400 colorimeter. Under these conditions, the CIEDE2000 obtained with TCA were smaller than the CIEDE 2000 obtained with colorimeter in identical time intervals. The difference between the 2 sets of data decreased when the measurement area of TCA was set to a smaller size of 1 sqcm. This can be explained as follows. Colour measurements in both cases are made around the center of the fresh-cut green apple slice, where the browning process takes place with greater speed. This process is slower in other parts of the apple than the core due to the different concentration and type of phenolic compounds (Awad *et al.*, 2000; Russel *et al.*, 2002; Thielen *et al.*, 2005). Therefore, widening the colour measurement area in the apple section around the core should lead implicitly to lower colour differences recorded between two fixed time intervals. This is confirmed by the data plotted in Figure 1 for different sizes of the colour measurement area used with colorimeter and TCA.

The different browning speed across the apple slice leads to inhomogeneous colour on the measurement area. Wider sizes of colour measurement area are translated in higher inhomogeneity of the colour. TCA can average the colour on flexibly chosen

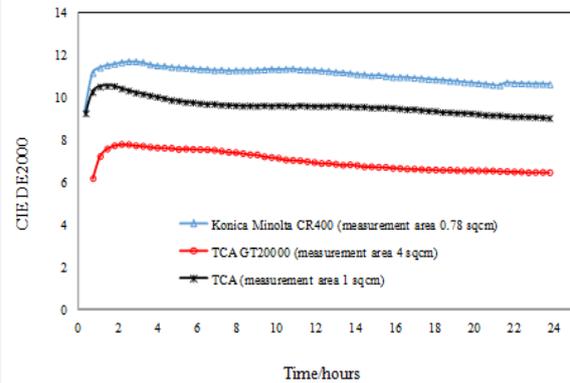


Figure 1. CIEDE2000 colour difference between measurements (1st-2nd, 1st-3rd,..., 1st-67th) over time obtained from the TCA with measuring areas of 1 and 4 sqcm and Konica Minolta CR400 with measuring area of 0.78 sqcm on fresh cut GS green apple slice

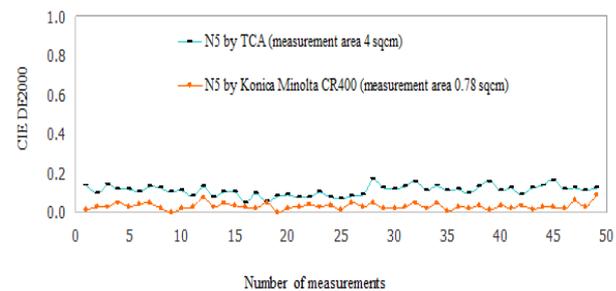


Figure 2. CIEDE2000 colour difference between measurements obtained from the TCA and Konica Minolta CR400 on Munsell grey N5 (non-homogeneous surface)

measurement areas offering information about the colour coordinates and colour distribution around the calculated average colour, as well as other information that colorimeters cannot provide due to their fixed sized aperture of the measurement port and due to the limited colorimetric calculation algorithms of their software. Colour differences of homogeneous grey measured with TCA and colorimeter on different measurement areas (0.78 and 4 sqcm) have CIEDE2000 colour difference values very close to each other as seen in Figure 2, confirming the accuracy of measurements performed with TCA. In this study we focused on colour-change of total area of fresh cut apple over time because its quality is partly judged from the whole piece of apple slice rather than from a small area.

Colorimetric analysis of browning reaction

The bitmap images of 2x2 cm in size captured throughout 23.8 hours were stored and the RGB values were corrected and transformed to CIEL^{*}a^{*}b^{*}. Figure 3a-3d shows plots of CIE a^{*} versus CIE b^{*}, CIE L^{*} versus CIE C^{*}, CIE L^{*} versus CIE h_o and CIE

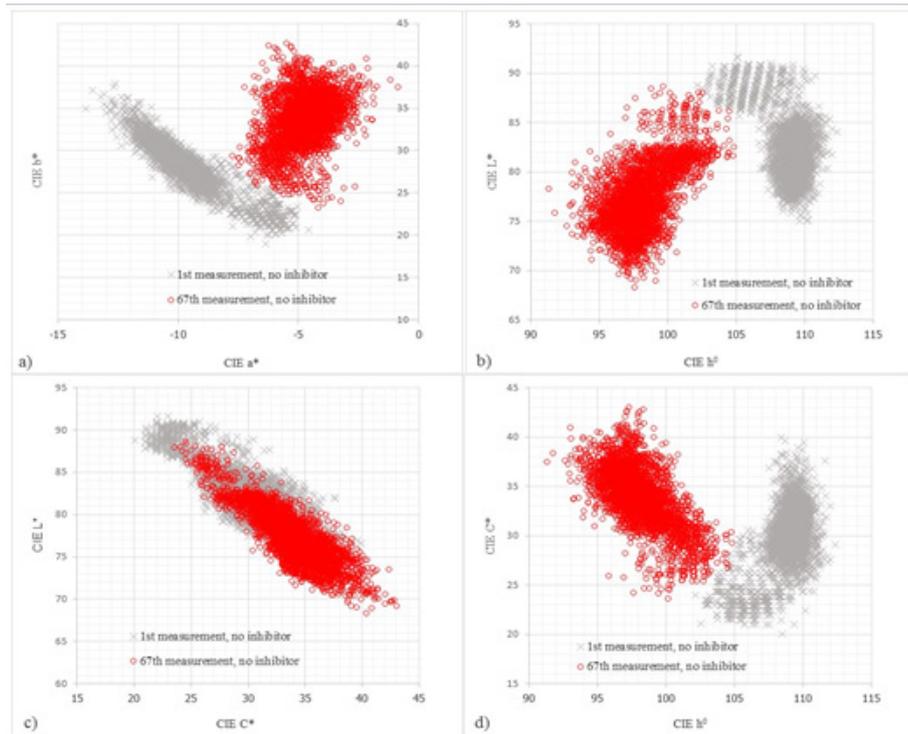


Figure 3. Plots of colorimetric data of all colour locations in the images of fresh cut GS green apple measured once after cutting and 23.8 hours after cutting. a) CIE a* against CIE b*, b) CIE ho against CIE L*, c) CIE C* against CIE L*, d) CIE ho against CIE C*

C* and CIE ho of all colour locations of the 1st image captured immediately after cutting and 67th image (23.8 hours after 1st cut). It shows that browning in fresh cut green apple resulted in larger changes in ho, a* and L* respectively, than in b* and C*.

Effect of single inhibitor and combinations

In previous research (Friedman and Molnar-Perl 1990; Özoğlu and Bayindirli 2002), the calculations of inhibition percentage were separately focused on CIE L* change, CIE a* change, reflectance percentage change at 440 nm change and absorption percentage change at 420 nm. We replaced those parameters with ΔE00 as shown in equation 1, because it was explained in previous section that colour change in fresh cut GS green apple simultaneously depended on CIE L*, CIE a* and ho, not only one parameter. The ΔE00 in equation 1 is total colour change between time t and started time t₀. Equation 1 represents inhibition percentage.

$$\left(\frac{\Delta E00_{control} - \Delta E00_{treatment}}{\Delta E00_{control}} \right) * 100 \quad (1)$$

Our alternative way of monitoring enzymatic browning captures larger areas of non-homogeneous surface than the colorimeter (CM), reflecting better the overall browning process in apple. Presenting only one parameter, such as lightness, for indicating the effect of inhibitors on the surface, on which

the polyphenol oxidase may unevenly distribute, is not sufficient. We showed the average of inhibition percentage calculated by equation 1 versus time with data obtained from TCA and CM (Figure 4a - Figure 4b respectively) when the GS apple slices were treated with 3 inhibitors. Patterns of dataplot in Fig 4a and Fig 4b are similar but inhibition levels are different. In contrast to Figure 4b, Figure 4a shows inhibition when most of the apple slice surface is measured. The curves, resulted from data plotting, in Figure 4a, can be categorized into 3 main groups: 1) % inhibition is low at the start, then gradually increases and finally substantially drops (citric acid treatment) 2) % inhibition is high at the start, then sharply drops, increases slightly later and then remains constant for a while before decreasing (ascorbic acid and mixture of ascorbic acid plus citric acid) and 3) % inhibition is high at the start, drops later then finally falls gradually (all treatments that contain sodium chloride). The plots of % inhibition in Figure 4b appear less complicate than Figure 4a, the reason being that the browning reaction on 0.78 sqcm at the center does not take into account other areas on which the browning reaction develops later and at different speeds. This can be seen from the % inhibitor of the mixture of ascorbic acid and citric acid which sharply drops from values around 72 (69 for CM) to 50 (64 for CM), then gradually drops until 46 (58 for CM), increases slowly until the maximum,

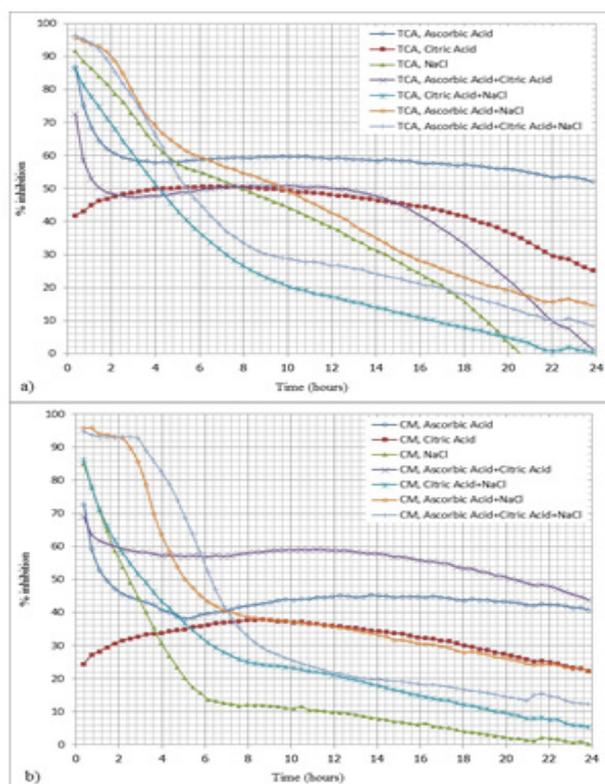


Figure 4. (a) Effect of single inhibitor and its combinations on % inhibitor over 23.8 hours using the data from TCA covering an area of 4 sqcm. (b) Effect of single inhibitor and its combinations on % inhibitor over 23.8 hours using the data from Konica Minolta CR400 colorimeter covering an area of 0.78 sqcm.

around 50 (59 for CM) and at about 13th hour starts to drop quickly again (gradual drop for CM). The difference is clear at 13th hour when the colour next to center starts to turn brown quickly. Two mixtures of the last group from the categories: ascorbic acid + sodium chloride and ascorbic acid + citric acid + sodium chloride are equally best if the fresh cut GS green apple slice stays in contact with oxygen about 4 hours. From the plots results that if fresh-cut green apple needs to stay in contact with air for about 13 hours, a mixture of ascorbic acid plus citric acid is more appropriate to use as co-inhibitor.

Optimization of inhibitors for fresh-cut GS apple

The mixture contour plot in Figure 5a and Figure 5b shows the effect of single inhibitor and their combinations on % inhibitor in first 20 minutes and 4 hours respectively. It shows that combination of 3 inhibitors and combination of 2 inhibitors (ascorbic acid and sodium chloride) performed well in a time frame of 4 hours.

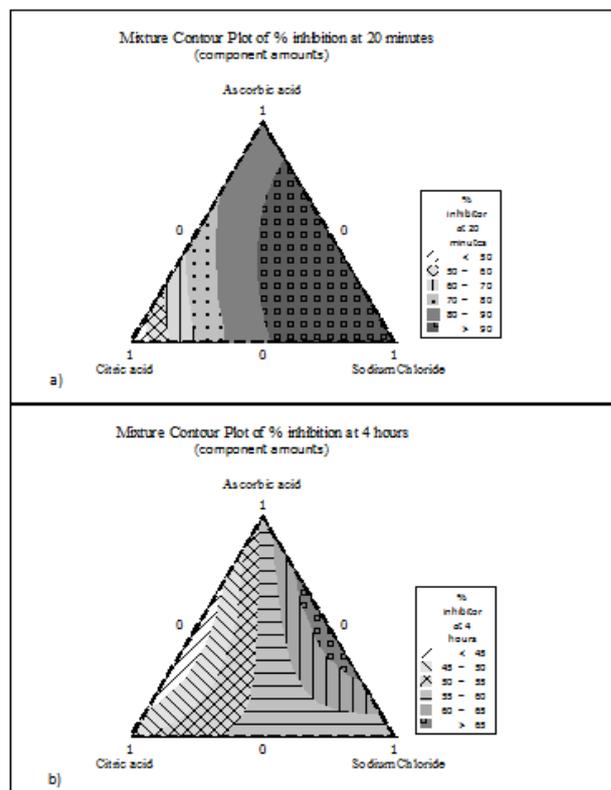


Figure 5. (a) Mixture contour plot of % inhibition within 20 mins, obtained data from TCA, (b) Mixture contour plot showing % inhibition within 4 hours, obtained data from TCA.

Conclusion

Slices, obtained from cross sections in apple, cover apples' representative parts, such as seeds, core, flesh, skin. Meaningful results from monitoring the enzymatic browning reaction in apple can be obtained when widening the colour measurement area on the apple slices beyond the capabilities of consecrated colorimeters. Trichromatic Colour Analyser has the advantage of accurately measuring colour on flexible areas that can cover the variable sizes of fresh-cut apple slices up to 100 x 100 mm and it can offer rich colorimetric information related to the browning process on all the representative parts of the apple section, not just on a limited area around its core.

In terms of single inhibitor, sodium chloride inhibits browning reaction of fresh-cut GS green apple in a period of about 4 hours the best and the second best is ascorbic acid. For a longer period than 4 hours sodium chloride is not suitable but ascorbic acid consistently performs well till 23.8 hours. In terms of co-inhibitor, all mixtures that contain sodium chloride inhibit well in a period of about 4 hours only. A mixture of ascorbic acid and citric acid

consistently performs well till about 14 hours.

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