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Tristimulus colorimetry for assessment of browning in artichokes and mushrooms

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**TRISTIMULUS COLORIMETRY FOR ASSESSMENT OF BROWNING
IN
ARTICHOKES AND MUSHROOMS**

A Thesis

Presented to

**The faculty of the Department of Nutrition and Food Science
San Jose State University**

**In Partial fulfillment
of the Requirements for the Degree
Master of Science**

by

Mahin Nouri

December 2001

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ABSTRACT

TRISTIMULUS COLORIMETRY FOR ASSESSMENT OF BROWNING IN ARTICHOKES AND MUSHROOMS

by Mahin Nouri

The application of tristimulus colorimetry in assessment of browning discoloration in artichokes and mushrooms was studied. Discoloration was monitored visually and using ColorFlex colorimeter for measuring HunterLab color values (L^* , a^* , & b^*). Based on HunterLab values, total color difference (TCD^*) and %inhibition of various treatments were calculated. Visual observation paralleled the changes in color values. As the browning increased, the L^* value decreased and a^* value increased in both artichoke and mushroom samples. The b^* value for artichokes decreased and for mushroom samples it increased as they browned. TCD^* value and %inhibition for citric acid treated artichokes were 24 and 88%, respectively. TCD^* value and %inhibition for citric acid treated mushrooms were very small. However TCD^* value and %inhibition for packaged mushrooms at cold temperature were more than 6.2 and 75% respectively.

Based on the results, tristimulus colorimetry was very fast and effective in monitoring the browning in samples.

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DEDICATION

I would like to dedicate this work to my friends on the other side of the world, to my mother, my sisters and brother who are in my heart, to my husband for his moral and financial support, and to my two little boys who have been my motivations for life.

PREFACE

The following thesis is written in publication style. The second chapter is written in journal format and will be submitted to The International Journal of Food Science and Technology. Chapter 1 and 3 are written according to the guidelines in the Publication Manual of the American Psychological Association, 4th edition, 1995.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Color is one of the most important sensory properties of food and has a great effect on its overall acceptability (Steet & Tong, 1996). Color is defined as a visual sensation that results from the detection of the light after it has interacted with an object (Hunter & Harold, 1987). Color can be assessed both visually and by using analytical instruments. Visual assessment is through sensory evaluation methods using the human sense of sight. These methods are based on the subjects' own perceptions. Although the subjects should be trained, the accuracy of their judgment is a relative issue (Hutchings, 1999). Beyond sensory evaluation methods, color can be assessed by light detection objective techniques. Two primary light detection methods use absorbance and reflectance techniques. The absorbance technique is based on measuring the optical density of transmitted light through a transparent solution containing substances extracted from the food. The reflectance technique detects the reflected light from a surface (Silva & Silva, 1999) and is usually applied to a food item directly. Colorimeter instruments measure the intensity of the different colors in foods and can quantify the color values (Minolta, 1994). The color values can be correlated accurately to the visual values if the measurement conditions and scales are properly chosen. These instruments also can provide evaluations which are more repeatable and reproducible than sensory techniques using the human eye (Hunter & Harold, 1987).

The spectrophotometer and tristimulus-colorimeter are two instruments that have been used in many color control procedures, where quality of the food color was involved. Browning is one of the major deteriorating reactions affecting color quality of foods, especially in fresh fruits and vegetables. Application of tristimulus colorimetry and spectrophotometer in assessment of the browning reaction in fruits and vegetables was the topic in many studies (Sapers & Douglas, 1987; Sapers, Miller, Miller, Cooke & Choi, 1994; Mastrocola, Manzocco & Poiana, 1998; Mastrocola & Lericci, 1991).

The browning reaction in foods involves complicated chemical reactions and proceeds through either an enzymatic or non-enzymatic path (Sapers, 1993). In fruits and vegetables, it is mostly enzymatic browning, which is due to the action of enzymes on phenolic compounds. The interaction between phenolic compounds and heavy metals, especially iron, is the type of non-enzymatic browning that mostly happens in fresh produce (Lattanzio, Cardinali, Di Venere, Linsalata & Palmieri, 1994).

The browning reaction is desirable in many food items such as coffee, tea, breads, roasted foods, chocolate, and raisins (Friedman, 1996). However, in fresh fruits, fruit juices, and vegetables it is undesirable since it indicates deterioration and/or poor quality (Sapers et al., 1994). The inhibition of the browning reaction in fresh produce has always been a challenge to food scientists and technologists. Methods to reduce browning discoloration in fruits and vegetables have been developed by food scientists and include using chemicals known as browning inhibitors to block this reaction, packaging, controlled temperature, and modified atmosphere (Sapers et al. 1994). Most of the

browning inhibitors used in fruits and vegetables are naturally present in these foods. Examples include ascorbic acid (vitamin C), citric acid, and malic acid (Sapers, 1993).

Objective

The objective of this study was to follow browning discoloration in two types of vegetables, artichokes (*Cynara scolymus* L.) and mushrooms (*Agaricus bisporus*), using the tristimulus colorimetric method. This study attempts to determine the effect of anti-browning dipping solutions on both artichokes and mushrooms, and the effect of packaging and cold temperature on mushrooms, during their storage, using visual and instrumental assessment. This research is a methodology study on the use of the tristimulus colorimetric method in comparison with visual observation.

Significance of the Study

The inhibition of browning in any fruit or vegetable is significant from both economical and marketing standpoints. In order to evaluate the effectiveness of different treatments in controlling the browning reaction in fruits and vegetables, and to compare them together, accurate measurements of the extent of browning are necessary. To show the effectiveness of tristimulus colorimetry in following the browning discoloration of fresh fruits and vegetables this study was designed as a fast and nondestructive method. Tristimulus colorimetry can be an effective method in assessing color quality in food industries. There are few studies on assessment of browning in artichokes, and more specifically, there has been no work regarding this matter using the tristimulus colorimetric method.

Review of the Literature

Color of Foods

Consumers in general assess the initial quality of food products such as meats, fruits and vegetables by their color and appearance. Color is the visual sensation that results from the detection of light after it has interacted with an object like food (Hunter & Harold, 1987). The perceived color of an object or food is influenced by the physical and chemical composition of food, the spectral composite of the light source and brightness, and the degree and spectral sensitivity of the viewer's eyes. Light striking an object (i.e. food) may be reflected, refracted, transmitted or absorbed by that object.(Hutchings, 1999). The reflected or diffused light hits the cornea and travels to the retina, where the visual receptors are located in the eyes. Light-sensitive pigments in the eye receptors change their shape in response to the light energy and generate neural message transmitting to the brain. These successive events make the color that is perceived by our visual system (Lawless & Heymann, 1999). There are two types of light receptors in the human retina, rods and cones. The rods are only capable of perception of lightness/darkness of the color. The cones are responsible for chromatic information and response to different wavelength of the color (Lawless & Heymann, 1999). Perceived color is defined by Hunter and Harold (1987) as visual sensations generated by light with different visible wavelengths. The appearance of an object or light source can be perceived by its color attribute in a three dimensional characteristic. One dimension defines how light/dark is that color, and it is known as value and two other dimensions define the chromatic attributes known as hue and saturation or chroma (Hunter & Harold,

1987). The color lightness or value of an object is the relationship between reflected and absorbed light, regardless of its wavelength. The hue is the attribute of the color which an object is judged to be red, yellow, green, blue and so forth. The saturation or chroma is the attribute of the color which expresses the degree of deviation from the gray of the same lightness (Hunter & Harold, 1987).

The color quality of an object (i.e. food) perceived by human eyes is influenced by some factors including light source, observer, size of the object, background, and sight direction (Minolta, 1994). For example a red apple looks brighter under sunlight than it looks under fluorescent light or the apple looks duller on a bright background and brighter on a dark background (Minolta, 1994).

Measurement of Food Color

In order to evaluate the color quality of a food and to be able to compare that with an acceptable and desirable standard, the food color has to be measured on a quantified scale. Color of food can be measured visually or by an instrument.

Visual Measurements

Visual assessment of the food color is by sensory techniques. Sensory evaluations are commonly used to assess foods' acceptability with respect to color, flavor, texture, appearance, and odor. These techniques are applied in new product development, product improvement, quality control of raw materials or final product, shelf life testing, and product grading. These evaluations are conducted to obtain an understanding of consumer judgment and acceptability of food products (Hutchings, 1999). Sensory assessments are usually composed of ten to twenty individuals, as sensory panelists. Most of the time panelists are paid, but they might be chosen from local employers to save money. There

are many personal and environmental factors that may upset a panelist's performance and judgement. How a panelist feels at the time of assessment may influence his/her judgment (Hutchings, 1999). The panelists should be examined for color blindness if they are asked to evaluate the color of an item. There are standard methods for this purpose (Lawless & Heymann, 1999). There is a common error in visual evaluation of color known as metamerism. This error happens when two objects seem to have the same color when viewed under one light source, but look different under many other light sources (Hutchings 1999, Minolta, 1994). Therefore the sensory results can be valid only when the light source remains exactly the same (Hutchings, 1999).

Instrumental Measurements

Aside from sensory evaluation methods, color can be assessed using color analytical instruments. Two primary light detection methods by instruments are absorbance and reflectance techniques. The absorbance technique is based on measuring the optical density of transmitted light through a transparent solution containing substances extracted from the food. The instrument used for the absorbance technique is the spectrophotometer. The reflectance technique detects the reflected light from a surface (Silva & Silva, 1999) and is usually applied to a food item directly. The instrument for reflectance technique is reflectance or tristimulus colorimeter.

Tristimulus Colorimeters and Color Measuring Systems

Any color is a combination of three primary colors known as red [R], green [G], and blue [B]. The tristimulus instrument has three filters known as [R], [G], and [B], working like the eye cones (light receptors) and measuring the light transmitted through

each with a photocell (Hutchings, 1999). The colorimeters can reflect the light from the surface of an object, transmit it through filters, and divide it into three chromatic attributes referred to as value, hue, and chroma. These chromatic attributes were developed by A.H. Munsell one century ago (Lawless & Heymann, 1999). The Munsell system is one of the color measuring systems accepted by the International Commission on Illumination, the international organization concerned with issues in color measurements. This organization is more commonly referred to by its French name “La Commission Internationale de l’Eclairage” or the CIE. In the Munsell system value is referred to as the lightness and/or darkness of the color. Hue is the measure of the predominant wavelength which will show if the color is more red, green, or yellow. Chroma is the intensity of the color (Hutchings, 1999; Minolta, 1994). The CIE Standard Observer is defined as the average color-matching characteristics of the 92 percent of the population with normal color vision. The CIE Standard Observer, developed in 1931, is based on the concept that any color can be matched by adding together proper proportions of three primary colored lights (Hutchings, 1999). In the CIE system the three primary colors that are red (X), green (Y), and blue (Z) are expressed as tristimulus values (X , Y , and Z) and are located on a three dimensional x , y , and z coordinate. A color can be represented by the tristimulus values red (X), green (Y), and blue (Z) or in terms of R , G , and B , and the amounts of the three primaries required to make a match for that color. The x,y chromaticity coordinates are usually plotted on a horseshoe-shaped diagram. There have been other color systems developed, which are based on more uniform diagrammatic representations of color spaces than the horseshoe-shaped color

space used in the CIE *XYZ* system (Lawless & Heymann, 1999). The one mostly used in food research is the HunterLab system. In 1948, R.S. Hunter suggested a uniform color space known as HunterLab color space (Hutchings, 1999). In a uniform color space equally perceived color differences have equal distances on the coordinate diagram (Minolta, 1994). In the HunterLab system, instead of value, hue, and chroma, three characters are used as lightness or L^* , degree of redness-greenness or a^* , and degree of yellowness-blueness or b^* . The following figure is a representation of the HunterLab color space (Hutchings, 1999).

Figure: A representation of HunterLab color space

Note. From “Food Color and Appearance” by J. B. Hutchings, 1999, p. 254.

The following explanations are by Silva and Silva (1999) from their report on color measurements of a tropical fruit. L^* is lightness of the color which measures how

light/dark the color of an object is. This value moves from zero/black (darkest) to 100/white (lightest). The a^* and b^* values measure the degree of greenness/redness and blueness/yellowness respectively and can vary from negative sixty to positive sixty (-60 - +60).

Values obtained from the tristimulus colorimeter can be used to calculate the total color difference (TCD^*) which quantifies the overall color difference of a treated sample ($L^*a^*b^*$) when compared to the reference sample or control ($L_o^*a_o^*b_o^*$). This value indicates the color quality of treated samples compared to the controls. It is also used to monitor the changes during storage time. The following equation calculates the TCD^* :

$$TCD^* = [(L^*-L_o^*)^2 + (a^*-a_o^*)^2 + (b^*-b_o^*)^2]^{1/2} \quad \text{Eq. (1)}$$

Silva & Silva (1999) used this equation and a simple classification of color differences (Table 1) in their work to quantify the rate of color change in the samples of a tropical fruit.

The Hunter L^* , a^* , and b^* values for the measurements were used in this study. The TCD^* values and Silva & Silva's classification of color differences were also used in this research for comparison of browning intensity in treated versus un-treated artichoke and mushroom samples.

Colorimeter instruments measure the intensity of the different colors in foods and can quantify the color values (Minolta, 1994). These instruments also can provide evaluations which are more repeatable and reproducible than sensory techniques using the human eye (Hunter & Harold, 1987). Tristimulus colorimeters are compact and portable instruments, which can be used for many food materials in any location. In the

study of the browning reaction and the effect of different browning inhibitors, this method can be applied to a large number of food samples in a short time and in a nondestructive process.

HunterLab ColorFlex

The HunterLab ColorFlex is a tristimulus colorimeter (Fig. 1). It is a versatile and compact color measurement instrument that can be used on any size of food products (ColorFlex User's Guide, 1998). It can be connected to a computer and printer to save the results and to print the data. This instrument has many capabilities such as memorizing the data and/or calculating the average or percent of changes. ColorFlex also has a setup mode to manipulate many parameters like language, orientation of display screen, auto-store to save the data in its memory, time, and date (ColorFlex User's Guide, 1998). The User's Guide is the manual that explains all of the applications of the equipment and how to use it step by step. This specific model of ColorFlex (HunterLab ColorFlex 45/00, Hunter Associates Laboratory, Inc., Reston VA) plus the accessories cost about eleven thousand dollars. This equipment was used in this research for all color measurements.

Tristimulus Colorimetry in Color Evaluation of Foods

The most common food attribute evaluation tool used in the food industry is visual appearance (Hutchings, 1999). The color of food has great sensitivity in many raw or manufactured products. The maturity of fruits and vegetables, the quality of a tomato product like ketchup, and the freshness of a piece of raw beef steak all are judged by consumers based on their appearance, and mostly their color.

There are many applications of tristimulus colorimetry for quality assessment in the food industry. Some examples include estimation of shelflife for juices and syrups that may undergo color pigment degradation during their storage time, right time and temperature for heating processes like pasteurization and canning, and estimation of fruit maturation and/or ripening time for seasonal marketing purposes, which are all based on the color quality of the foods.

Tristimulus Colorimetry in Assessment of Browning Reaction

There have been many research studies conducted regarding the assessment of the browning reaction by tristimulus colorimetric methods, especially in fruits and vegetables (Langdon, 1987; Mastrocola & Lerici, 1991; Mastrocola et al, 1998; Sapers, Hicks, Philips, Garzarella, Pondish, Matulatitis, McCormack, Sondey, Seib, & Ei-Atawy, 1989; Sapers & Douglas, 1987; Sapers et al, 1994; Smith & Cline, 1984). In most of these research studies, the colorimetric method was used along with a chemical method such as extraction of polyphenol oxidase, the browning enzyme, and then measuring its activity.

Langdon (1987) used tristimulus colorimetry and microbial tests in a study of different reducing agents, such as citric acid and ascorbic acid, and packaging effects on browning of peeled potatoes. Through these methods, Langdon found which type of treatment and packaging might delay the browning reaction and microbial growth in potatoes.

Based on some previous research studies, Mastrocola et al. (1998) believed that the Hunter a^* value was well correlated to the rate of enzymatic browning in fruit tissues. They measured the Hunter a^* value in cherimoya puree along PPO activity in their

samples to study the effect of ascorbic acid and citric acid as anti-browning compounds in samples. Through that study Mastrocola et al. concluded that, based on perceived lower a^* values and PPO activity of treated samples, the addition of small amounts of ascorbic acid and citric acid to cherimoya puree prevented the browning reaction in samples during freezing, storage and thawing.

Sapers and Douglas (1987) used the tristimulus colorimetry along with the spectrophotometric-absorbance method to measure the enzymatic browning at the cut surface and in juice of raw apple and pear fruits. Later in similar studies Sapers et al. (1989 & 1994) used these two color assessment techniques in control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. In both studies they found a high correlation between the results from the reflectance-colorimetric and absorbance-spectrophotometric methods. They also studied the effect of browning inhibitors on fruits and fruit juices by the reflectance colorimetric method. They calculated the percent of browning inhibition in anti-browning treated samples by measuring L^* and a^* values of the control and treated samples and using a simple equation. The following equation was used in their work, which is the percent difference between the control and treated samples ΔL^* or Δa^* values after a specified storage time t ;

$$\% \text{ Inhibition} = [(\Delta L \text{ control} - \Delta L \text{ treatment}) / \Delta L \text{ control}] \times 100 \quad \text{Eq. (2)}$$

Where ΔL (or Δa) is the difference between the L^* (or a^*) value at the time t and the value at 1 minute (Sapers & Douglas, 1987; Sapers et al, 1989).

Tristimulus Colorimetry in Assessment of Fruits Ripening

Most fruits have to pass through a ripening process to be desirable for eating. During the ripening process the flavor of fruits transforms from a sour-bitter taste to a more juicy and sweet taste. Fruits also change color while they are ripening. A green banana changes to a bright yellow one or a green tomato changes to an orange-red color. The green unripe fruits are high in chlorophyll and starch. During the ripening process the starch breaks down to sugars and chlorophyll is replaced by other color pigments like yellow carotenoid in bananas, red lycopene in tomatoes, and purple anthocyanin in cherries (Fennema, 1996). Tristimulus colorimeters measure the intensity of different colors in fruits. Examples are Hunter Citrus Colorimeter and Hunter Tomato Colorimeter (Hutchings, 1999). The measured color values can be correlated to the quantity of the color pigments and therefore the degree of maturation and ripeness of fruits. There are some studies on the assessment of maturation and the ripening process of fruits using this technique (D'Souza et al, 1992; Langley, Martin, & Stenning, 1994; Meir, Philosoph-Hadas, Gloter & Aharoni, 1992; Wainwright & Hughes, 1989). D'Souza and his coworkers studied the estimation of lycopene concentration in tomato fruits from their chromaticity values. They first measured the L^* , a^* , and b^* values of three different cultivar of tomatoes. Then they extracted the red pigment, Lycopene, from the fruits and measured the absorbance of the extracted solutions by spectrophotometer. The final results showed that the L^* and a^* values were related to the lycopene concentration measured by the spectrophotometer in all cultivars.

Langeley et al. (1994) used the same colorimetric technique in mechanical and optical assessment of the ripening of the tomato fruit with reduced polygalacturonase activity. Polygalacturonase (PG) is an enzyme responsible for softening of tomatoes (Langeley et al., 1994). The research studies used an anti-sense, a compound inhibiting the PG's activity, on their tomato samples, and measured its effect on their color values. It was hypothesized that the ripening process of the tomato is associated with visual color and tactile changes. Therefore the researchers applied mechanical tests, along with color measurements, of the tomato samples. The treated samples had significantly lower L^* and higher a^* values. They also found that the tomato color index or TCI value is a useful indicator of the ripening stage of tomato fruits. The TCI value is calculated through an equation made of L^* , a^* , and b^* Hunter values (Langeley et al., 1994).

Meir et al. (1992) conducted similar research on the assessment of the chlorophyll content of watercress leaves by using a tristimulus colorimeter. They measured the HunterLab values of watercress's senescing leaves along with extraction and measurement of the chlorophyll content. Their results showed Hunter values, especially L^* , b^* , and TCD*, had a high correlation with extractable chlorophyll values and different senescence stages in watercress. They concluded that the tristimulus colorimetric technique is a rapid, reliable, and nondestructive method of measuring the chlorophyll concentration in senescing of green leaf vegetables (Meir et al., 1992).

Wainwright and Hughes (1989) used the tristimulus colorimetric method in banana fruit pulps for detection of a physiological disorder known as yellow-pulp. Yellow-pulp is yellowing due to fruit ripening when the fruit is still pre-mature.

Wainwright and Hughes (1989) concluded that the color chart scores, which were usually used for color measurement in banana pulp, were highly correlated with measured HunterLab values in their research (Wainwright & Hughes, 1989).

Tristimulus Colorimetry in Assessment of Color Degradation

Tristimulus colorimetry can also be applied in the assessment of any color degradation in foods. The browning reaction, when it is not beneficial, is considered a type of color degradation in fresh fruits and vegetables as well as other food products. The color of different fruits and vegetables is due to their color pigment content. Most of the color pigments are unstable and easily change their chemical structure and color properties due to heat, acidity, oxygen, and light (Fennema, 1996). Preservation methods of fruits and vegetables make color degradation unavoidable. In dehydration and drying, freezing, canning, blanching, and juice extraction color pigments are easily exposed to thermal changes, light, pressure, and other physical or chemical stresses. These factors can affect their color properties. Tristimulus colorimetry can be used to assess the color degradation in different types of processing of fruits and vegetables and can help to find the optimum conditions for preservation of these foods with minimum damage to their color. Numerous studies related to this concept have been undertaken (Silva & Silva, 1999; Skrede, 1985; Steet & Tong, 1996). Silva and Silva studied color changes in thermally processed cupuacu puree, a tropical fruit, using the tristimulus colorimetric method. They measured the HunterLab values (L^* , a^* , and b^*) of heat treated (90°C – 115°C) and untreated samples and also calculated the total color difference (TCD^*) values and categorized the amount of change in their thermally processed samples. They

developed a kinetics model using their experimental color-time-temperature data for 80°, 90°, 100°, 110° and 115° C temperatures and up to 120 minutes heating time. Silva and Silva found the critical time and temperature points by graphing the processing time and temperature versus TCD* values. They concluded that this colorimetric technique could help to design the optimum pasteurization process, which minimizes color changes in cupuacu puree (Silva & Silva, 1999).

Skrede (1985) used the tristimulus colorimetric method in the estimation of blackcurrant berry syrups' color degradation during storage. The purple-red color of the blackcurrant syrup is due to its anthocyanin contents. This pigment easily degrades to a more brownish color when exposed to oxygen, ascorbic acid, light, pH and/or temperature (Skrede, 1985). Skrede measured the Hunter L*, a*, and b* values along with the total anthocyanin content based on absorbance ratios at 520/420 nm by spectrophotometer of the syrup samples at the end of the first and second years of storage in the dark and at 20° C. Skrede calculated the half-life of Hunter a* values and half-life of free anthocyanins in his research. He also found that day-light storage of syrups lowered half-life of Hunter a* values 10-30% compared with dark storage, and oxygen permeable packaging materials reduced this value up to 90%. Skrede suggested that Hunter a* and hue values in blackcurrant syrup are acceptable color quality indicators (Skrede, 1985).

Chlorophyll is another color pigment whose degradation has been the subject of tristimulus colorimetric research. Steet and Tong (1996) studied degradation of green color and chlorophyll in peas by colorimetry and HPLC. The degree of greenness in

thermally processed green vegetables is considerable. Green vegetables obtain their green color from the magnesium containing chlorophyll pigments. In the process of commercial sterility of vegetables high temperatures are usually used. Due to high temperatures the magnesium in the chlorophyll ring is lost and is replaced by two hydrogen ions. This structural change will convert the green chlorophyll to a brown-olive green pigment known as pheophytin. This may also occur in the presence of acids. Pheophytin is an undesirable color in vegetables for customers (Steet & Tong, 1996). Steet and Tong measured the HunterLab values of green peas puree at three 70°, 80°, and 90° C, and also in presence of hydrochloric acid at room temperature. They developed a color degradation kinetic model in samples by graphing a* values versus time. They concluded in their published report that use of the a* value, measured by the tristimulus colorimeter, was an applicable physical property for estimation of green color degradation in peas. Therefore, the visual quality of thermally treated green peas could be determined by using the a* value since it was related to both green color and consumer acceptance (Steet & Tong, 1996).

Browning in Artichokes

The artichoke is a fairly expensive vegetable. It is fat free and high in dietary fiber, vitamin C, folate, and magnesium. Most of the artichokes grown in the United States come from California. This vegetable is becoming more popular in the American diet (Rainy & Nyquist, 1997). Like many other vegetables it is highly susceptible to browning. The browning discoloration in artichokes can be both enzymatic and non-enzymatic (Lattanzio, Linsalata, Palmieri & Van Sumere, 1989). The enzyme

polyphenol oxidase (PPO) is known to be mostly responsible for the enzymatic browning reaction in vegetables including artichokes (Lattanzio et al., 1989; Zawistowski, Blank, & Murray, 1986). However, certain other browning enzymes could be traced in artichokes (Lattanzio et al., 1994 & 1989). The oxidation of phenolic compounds, catalyzed by PPO, can form more intensely darkened products known as quinones (Lattanzio et al., 1994). The non-enzymatic browning reaction in artichokes is due to the reaction between metal and phenolic compounds. It was reported that iron is the most common metal involved in this type of reaction in artichokes, since this vegetable contains a relatively high amount of *o*-diphenols and iron (Lattanzio et al., 1989).

While there are a few studies on the browning discoloration in artichokes, there has been no work done on the application of tristimulus colorimetry for monitoring the browning changes in this vegetable. Zawistowski and his colleagues first reported about the PPO activity in artichokes through a complicated chemical method. They extracted the PPO from the artichoke tubers and measured its stability at different pHs and temperatures (Zawistowski et al., 1986). Lattanzio and his colleagues tried to determine the effect of citric acid and ascorbic acid as anti-browning compounds on artichoke heads (Lattanzio et al., 1989). This work was also done through extraction of PPO and other phenolic compounds, and HPLC analysis of the samples. The researchers investigated the beneficial effect of 1% citric acid or ascorbic acid solution on the phenolic browning reaction in stored artichoke heads at room temperature and 4° C. They analyzed the amount of phenolic compounds in artichoke heads, through HPLC, before and after application of browning inhibitors. They concluded application of 1% citric acid or

ascorbic acid resulted in an improvement in quality of the stored artichoke heads. They also suggested that since citric acid is less expensive than ascorbic acid, citric acid treatments are more acceptable.

In a later research study by Ababneh (1997) the beneficial effect of 0.5M citric acid plus 0.3M CaCl_2 and 1% H_3PO_4 in the prevention of browning discoloration of artichoke stems and heads was studied. Through this study, the researcher found that the application of these three chemicals together evidently inhibited the visual darkening of all artichoke samples. (Ababneh, 1997). Ababneh suggested that the anti-browning effect of citric acid and phosphoric acid was due to their pH-reducing and metal-chelating properties, and the effect of CaCl_2 was due to its preservation characteristic on the membrane integrity. The most effective browning inhibitors that have been used in fruits and vegetables are some organic acids like citric, ascorbic, malic, and tartaric acids (Mastrocola et al, 1998; Sapers & Ziolkowski, 1987; Lozano et al, 1994, Sapers & Miller, 1992). These chemicals are naturally present in most fruits and vegetables and the Food and Drug Administration (FDA) approves their application in food items. Other types of acids such as phosphoric and hydrochloric acids, known as inorganic compounds, are alternatively used in the food industry as well (Sapers, 1993). Measuring the extracted PPO and its activity, HPLC, and other methods were also used in studies of browning reaction in fruits and vegetables. These methods were reported as being difficult and time-consuming. Alternatively colorimetric methods are much less time consuming and fairly accurate (Meir et al., 1992; Silva & Silva, 1999).

Browning in Mushrooms

Mushrooms are highly susceptible to browning (Sapers et al., 1994). Similar to many other fresh fruits and vegetables, polyphenol oxidase (PPO) is known to be responsible for enzymatic browning of mushrooms. This vegetable contains both the active and latent forms of PPO, whose activity increases during post-harvest storage (McCord & Kilara, 1983). McCord and Kilara reported that blanching mushrooms in 0.05 M citric acid prior to conventional thermal processing could improve the color of the mushrooms significantly. They concluded that citric acid blanching could lower the pH and inactivate the PPO and consequently the enzymatic browning. Citric acid also protects the mushrooms from nonenzymatic browning (McCord & Kilara, 1983).

Control of enzymatic browning in minimally processed mushrooms was studied quite extensively. Sapers et al (1994) used spectrophotometer and simple visual tests in their research on discoloration reactions and bacterial spoilage in mushrooms by measuring HunterLab values and %reflectance. They used colorimetric and chemical methods to study the effect of many anti-browning and anti-microbial compounds, which are used to prolong the shelf life of the minimally processed mushroom, on their samples. Among the colorimetric values L* had a* better correlation to the intensity of browning. They also found a relationship between purpling of mushrooms, as a result of microbial growth, and colorimetric values (Sapers et al., 1994). Sapers et al. hypothesized that washing fresh mushrooms prior to packing can partially remove PPO and its substrate from the mushrooms' surface and therefore delay the browning.

Fresh mushrooms can be sold in bulk or prepackaged. Mushrooms for retail sale

are packaged in different sizes of pulp boxes and wrapped in soft PVC (polyvinyl chloride) stretch films. The stretch film is designed with a few small holes for air circulation. This type of packaging can protect the mushrooms from physical damage by the inflexible pulp box, and there will be a low permeability to oxygen due to the wrapping film covering the package. It has been reported that packaging cannot prevent the fresh produce from spoilage, but it can help to protect the product against contamination, physical damage, and excess moisture loss (Sacharow & Griffin, 1970). Sacharow and Griffin (1970) reported that incorrect packaging and too many moisture barriers could cause high relative humidity in the package and result in microbial spoilage. Oxygen is essential for enzymatic browning. Packaging, in view of the fact that it can exclude the oxygen from the atmosphere around the produce, can delay the enzymatic browning (Fennema, 1996; Sapers, 1993). It is also important to consider that complete exclusion of oxygen may cause activation of anaerobic microorganisms on the produce. The anaerobic *Clostridium botulinum* can grow on over-wrapped packages of fresh mushrooms. Therefore the wrapping film for the mushrooms' package should contain a few holes through which air can be admitted for this vegetable with a high respiration rate (Sapers, 1993). However, some of the important advantages of produce packaging are brand identification, price, and product information and recipes. Packaging can reduce the waste through marketing and product sanitation because the product is protected from insects and dirt. It also permits more products to be displayed in a smaller space and the packaged fruits and vegetables are usually more attractive. The disadvantages of packaging are cost of the materials and labor.

CHAPTER 2
JOURNAL ARTICLE

Authors' Title Page

**TRISTIMULUS COLORIMETRY FOR ASSESSMENT OF BROWNING
IN
ARTICHOKES AND MUSHROOMS**

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Summary

The application of tristimulus colorimetry in assessment of browning discoloration in artichokes and mushrooms was studied. Discoloration was monitored visually and using ColorFlex colorimeter for measuring HunterLab color values (L^* , a^* , & b^*). Based on HunterLab values, total color difference (TCD^*) and %inhibition of various treatments were calculated. Visual observation paralleled the changes in color values. As the browning discoloration increased, the L^* value decreased and a^* value increased in both artichoke and mushroom samples. The b^* value for artichoke decreased and for mushroom samples it increased as they browned. TCD^* value and %inhibition for treated artichoke stems were 24 and 88%, respectively. TCD^* value and %inhibition for citric acid treated mushrooms were very small. However TCD^* value and %inhibition for packaged mushrooms at cold temperature were more than 6.2 and 75% respectively.

Based on the results, tristimulus colorimetry was very fast and effective in monitoring the color changes in samples.

Keywords: Browning; color assessment; colorimeter; HunterLab; tristimulus.

Introduction

Consumers in general, assess the initial quality of fruits, vegetables, meats, and processed food products by their color and appearance. Color plus flavor and texture play important roles in the acceptability of food. Furthermore, color may provide an indication of chemical changes in food such as browning reactions. Thus, color is a primary indicator of perceived quality and its measurement is important in quality control during food processing and storage, and in the study of the mechanism of food deterioration reactions where color changes are involved.

Color quality can be measured visually and with the use of instruments. Visual assessment of color is usually carried out using the sensory panel/subjects. Sensory evaluations are based on the subjects' own perceptions, therefore the accuracy of their judgment is a relative issue (Hutchings, 1999). Two main light detection methods, using instruments, are absorbance and reflectance techniques. The absorbance technique is measuring the optical density of transmitted light through a transparent solution containing substances extracted from the food. Therefore it cannot be applied to the food surface directly. Spectrophotometers are the instruments that work based on the absorbance technique. The reflectance technique involves detecting the reflected light from a surface (Silva & Silva, 1999) and usually is applied to a food item directly. Tristimulus colorimeters work based on the reflectance technique. They can transform the color intensities to numbers that correlate accurately to visual values if the measurement conditions and scales are properly chosen. These instruments also can provide evaluations which are more repeatable and reproducible than the sensory techniques

using the human panelists (Hunter & Harold, 1987). There are many studies investigating the accuracy of colorimetric methods in contrast to some other methods like liquid chromatography or sensory evaluation in the study of browning intensity in fruits and vegetables (Sapers & Douglas, 1987; Sapers, Miller, Miller, Mastrocola, Manzocco & Poiana, 1998; Mastrocola & Lerici, 1991).

Tristimulus reflectance colorimetry has been used to monitor the intensity of the browning reaction or other types of color degradations in fruits and vegetables in many studies (Silva & Silva, 1999; Mastrocola et al., 1998; Lozano et al., 1994; Sapers & Douglas, 1987; Sapers & Ziolkowski, 1987, Sapers et al., 1994). This method has been determined to be a very fast, accurate, none-destructive, and less expensive method compared to sensory techniques (Meir et al, 1992; Silva & Silva, 1999). Even in comparison to spectrophotometric methods, which require many chemical reagents and extraction procedures, tristimulus colorimetry is a less time consuming technique. Therefore this method can be used in many quality control research studies, especially when the results are needed very quickly.

In tristimulus colorimetry the reflected light from the surface of an object is divided into three chromatic attributes referred to as value, hue and chroma. Value indicates lightness and/or darkness. Hue represents the predominant wavelength which indicates if the color is more red, green, or yellow, and chroma represents the intensity of the color. In the HunterLab system these are referred to as the value of L^* or lightness, a^* or degree of redness-greenness, and b^* or degree of yellowness-blueness (Minolta, 1994).

Values obtained from tristimulus colorimetry can be used to calculate total color difference (TCD*), which quantifies the overall color difference of a treated sample when compared to the reference sample or control ($L_0^*a_0^*b_0^*$). It is also used to monitor the changes during storage time (Hunter & Harold, 1987; Silva and Silva, 1999). The following equation calculates the TCD*:

$$TCD^* = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} \quad \text{Eq. (1)}$$

In some food color assessments (like monitoring browning intensity) this equation was used to compare the amount of color change in treated samples (i.e. by browning inhibitors or processing temperature) versus the color change in the control or untreated samples (Langdon, 1987; Silva & Silva, 1999). Silva & Silva (1999) used this equation and a simple classification of color differences (Table 1) in their work to quantify the rate of color change in the samples of a tropical fruit.

Sapers et al. (1989) used another equation in their study to measure the effect of anti-browning compounds on browning food item based on their tristimulus colorimetric values. They calculated the percent of browning inhibition in anti-browning treated samples by measuring L^* and a^* values of the control and treated samples and using a simple equation. The following equation was used in their work, which is the percent difference between the control and treated samples ΔL^* or Δa^* values after a specified storage time t ;

$$\% \text{ Inhibition} = [(\Delta L \text{ control} - \Delta L \text{ treatment}) / \Delta L \text{ control}] \times 100 \quad \text{Eq. (2)}$$

Where ΔL (or Δa) is the difference between the L^* (or a^*) value at the time t and the value at 1 minute (Sapers & Douglas, 1987; Sapers et al., 1989).

The HunterLab ColorFlex, a versatile and compact tristimulus colorimeter, was used in this current study to measure the HunterLab (L^* , a^* , and b^*) values of browning artichoke stems and mushrooms pileus. Total color differences or TCD* values, classification of color differences by Silva and Silva (see Table 1), and %inhibition were also used in this research for comparison of browning discoloration intensities in treated artichoke and mushroom samples versus the controls.

The objective of this study was to follow the browning discoloration in artichokes and mushrooms using tristimulus colorimetry. The association of colorimetric values with visual browning discoloration was also evaluated. This study attempts to determine the effect of anti- browning dipping solution on both artichoke stems and mushrooms, and the effect of packaging and temperature on mushrooms. The study was conducted in two different phases. Phase one involved monitoring the browning discoloration in untreated artichoke stems versus treated samples with a combination of citric acid, calcium chloride and phosphoric acid dipping solution. The beneficial effect of 0.5M citric acid plus 0.3M CaCl_2 and 1% H_3PO_4 in prevention of browning discoloration of artichoke stems and heads initially was suggested by Ababneh (1997) in his research on the effect of different anti-browning compounds in artichokes.

Phase two of this current study involved monitoring the browning discoloration in mushrooms treated by citric acid dipping solution, packaging, and cold temperature storage versus the un-treated or control samples. The suitability of tristimulus colorimetry and the HunterLab color system, in the evaluation of browning discoloration in artichoke and mushroom samples, was also assessed.

Materials and Methods

Equipment

Phase 1 & 2 were conducted using the HunterLab ColorFlex 45/0 colorimeter (Fig. 1) to monitor color changes in artichoke stem and mushroom samples. The HunterLab ColorFlex colorimeter is a versatile and compact color measurement instrument that can be used on any size of food products (ColorFlex User's Guide, 1998). It can be connected to a computer and printer to save the results and to print the data. This instrument has many capabilities such as memorizing the data and/or calculating the average or percent of changes. ColorFlex also has a setup mode to manipulate many parameters like language, orientation of display screen, auto-store to save the data in its memory, time, and date (ColorFlex User's Guide, 1998).

HunterLab ColorFlex, for this study, was standardized by the black tile and the white tile ($L^* = 92.85$, $a^* = -1.22$, $b^* = 0.61$) on each testing day before readings. While there are other colorimetric systems, the HunterLab system was chosen, because it seemed to be more efficient for this study and easier to interpret the results. The set up mode of the colorimeter was put on Numeric Standard, Absolute Display, D65 as Illuminant, and 10° C as Observer, based on the recommendations in the user's guide manual (ColorFlex User's Guide, 1998).

A digital camera (Sony Digital Still Camera DSC-550, 2.1 MEGA PIXELS) was used to record visual changes in color of artichoke stems and mushroom pileus during storage.

Phase 1: Browning in artichoke stems

Samples preparation and treatments

One box of 36 whole fresh artichokes, grown at Ocean Mist artichoke fields (Castroville, CA), was obtained from a local grocery market. They were transferred into the chemistry laboratory, packed in polyethylene (zip-lock plastic) bags and stored at 4° C (laboratory refrigerator) until use. Aqueous solution of 0.5M citric acid + 0.3M CaCl₂ + 1% H₃PO₄ was prepared for the dipping treatment. Twelve artichokes for the colorimetric readings were randomly chosen. Each sample was removed from the plastic bag and approximately three millimeters of the tip of each stem was cut and discarded since this part had already browned and darkened. Then each stem was cut crosswise and separated from the artichoke head or flower portion. Stems showing no external defects were cut crosswise in equal size pieces (about 3 cm length and 2.5 cm diameter). Six of the pieces were dipped in dipping solution and six of them were dipped in distilled water (control or untreated) for three minutes. After dipping, samples were blotted to dry with paper towels. The color values of the cut surface of artichoke stems were measured immediately. The photographs were taken right after color readings. The samples were placed in labeled polyethylene bags and stored at 4° C. These samples were kept for further colorimetric measurements.

Color assessment of artichoke stems

All twelve treated and untreated samples were removed from the refrigerator for visual observations and colorimetric measurements on 0, 3, 6, & 9 days of storage. In the reading process, the samples were put on directly on the sample port. To minimize

variations in tristimulus readings due to geometrical positioning of samples, the color values were measured at four different geometrical positions by rotating the cut surface of samples clock-wise, in to 0, 90, 180, and 270 degrees. Therefore the value for each sample that was obtained was the average of four readings. The sample port was covered with clear plastic film to keep the window port neat during the procedure. To eliminate the effect of external light, a cone shape black cap was used to cover the samples on the port for each reading. Four photographs were taken of the control and treated artichoke stems on day zero, three, six, and nine after cutting by digital camera for visual comparison.

Phase 2: Browning in mushrooms

Samples preparation and treatments

One box of Monterey mushrooms (2.5 Lb), pre-packaged in a pulp box and film-wrapped, was purchased from the local market. The mushrooms, which did not have any visual browning or defects, were selected from the package. Mushrooms were washed in cold running tap water for 15 seconds and then drained. Twenty-four mushrooms were selected for treatments as follows: four as packaged-refrigerated, four as un-packaged-refrigerated, four as packaged-room temperature, four as un-packaged-room temperature, four as packaged- citric acid treated-refrigerated, and four as un-packaged-citric acid treated-refrigerated. An attempt was made to choose samples of the same size. Their pileus (cap) diameter was about three centimeters. The samples for citric acid treatment were dipped in an aqueous solution of 1% citric acid for one minute. The rest were dipped in distilled water as a control for one minute. All of the samples were blotted on a

paper towel and air-dried for two minutes. The Hunter L*, a*, and b* values for all 24 samples were measured immediately. Four samples from non-citric acid treated and four samples from citric acid treated were put in a small pulp box (14.5 x 13 x 5 cm) and wrapped with plastic film and stored at 4° C. Four of the non-citric acid treated samples were also packed in the same manner and stored at room temperature. Four of the non-treated samples and four of the citric acid treated ones were put on a petri dish which was already covered by paper towels and stored at 4° C. The other four non-treated samples were also put on paper covered petri dishes and stored at room temperature (20°C).

Color assessment of mushroom pileus

The HunterLab color values for all of the mushroom samples were measured by the HunterLab ColorFlex on four consecutive days. Each sample was placed directly on the sample port of the instrument on its pileus side and covered with a black cap to exclude the light from the outside during the reading process. Three photographs of mushrooms were taken on day zero, day two, and day four of storage to show color differences in control and treated samples. Two mushroom sample groups, stored at room temperature, were excluded from colorimetric measurements at day 3, since they were completely deteriorated.

Data analysis

Total color difference (TCD*) values of differently treated artichoke stems and mushroom samples were calculated using equation (1). The %inhibition for each treatment was calculated using equation (2). The results of color values for treated samples versus their controls were statistically evaluated by using the t-test with

significance at $p < 0.05$. To determine the effect of rotating the position of artichoke stems on the color values, the results for artichoke samples were evaluated by using ANOVA (analysis of variance with significance) at $p < 0.05$.

Results and Discussion

Phase 1: Artichoke Stems

The visual color of untreated (control) and treated artichoke stems are compared in figures 2, 3, 4, and 5, and the change of tristimulus color values of these samples for nine days of cold storage are shown in figures 6, 7, and 8. The values stated on figures 6, 7, and 8 are the average of 24 readings (six samples, each in four different positions). Four photographs were taken of the control and treated artichoke stems on day zero, three, six, and nine after cutting using a digital camera. The color of the stems changed from bright yellow-green to a more black and red-brown due to browning development in the samples and is shown in figures 2,3,4, & 5. The visual color differences are evident between the control and treated stems throughout the nine days of storage. Photographs show that treated samples versus controls were perceived visually as a lighter and brighter yellow-green color. In contrast control samples had darker and more brown-red color right after treatment and following storage days (Figures 2-5). The visual color difference between control and treated samples on day zero is simply due to the effect of the dipping solution on cut surface of artichoke stems. Since the control samples, after cutting, were dipped in distilled water, they easily undergo browning discoloration, while the treated ones, dipped in anti-browning solution, were protected from discoloration and darkening in acidic conditions. The HunterLab color values changed significantly in

control samples which corresponds to darkening from day zero to day nine. Based on the color change during storage days, the L^* and b^* values decreased and the a^* value increased as browning progressed (Figure 6 , 7, & 8).

It appears that the browning discoloration in untreated artichoke stems occurs mostly on the first 3 days after cutting and during cold storage as shown in figures 6, 7, and 8,. All HunterLab color values significantly changed after three days of storage ($p < 0.05$ by t-test) in untreated (control) samples. The visual discoloration of control samples from day zero to day three was also distinct (Figures 2 & 3), but from day 3 to days 6 and 9, the visual darkening was not distinct (Figures 4 & 5). While the darkening discoloration was not visually evident in control samples from day 3 to day nine, the HunterLab color values (L^* , a^* , & b^*) were changing (Figures 6 ,7, & 8). Therefore it can be concluded the tristimulus colorimetry easily detected any small changes in the color of the samples which could not be observed visually.

The L^* and b^* values of treated samples decreased and their a^* value increased, but not significantly, during the first 3 days of storage. Then the values remained almost constant for days 3 and 6 of storage. After day 6 the L^* and b^* values started to decrease and the a^* value increased again (Figures 6, 7, & 8). It is assumed that treated samples had a lag time on day 3 to 6 of storage in their darkening and browning development. In treated artichoke stems none of the HunterLab values changed significantly throughout the nine days of storage. The treated samples also maintained their visual bright yellow-green color for six days (Figures 2, 3, & 4) and at day nine they turned slightly to light red. Their a^* values increased from below zero (green color) to more positive values (red

color). These samples after day 6 of storage visually lost their brightness slightly and turned to a dull cream color. Their L^* and b^* values also decreased at this point, but not significantly ($p < 0.05$, by t-test).

The photographs of stems on days 3, 6, and 9 (Figures 3, 4, & 5) show that the untreated samples darkened unevenly on their cut surface. However the color values of artichoke stems at four different geometrical positions were not significantly different ($p < 0.05$, by ANOVA). Therefore it can be concluded that different positioning of the samples on the sample port did not affect their color values significantly.

The Hunter L^* value had the biggest change in control samples throughout the study. Delta L^* by day nine for untreated samples was 12.93 (about 13%) and for treated samples was 6.86 (less than 7%). Delta a^* by day nine for untreated and treated samples was 2.98 (about 2.5%) and 0.9 (less than 0.7%) respectively. Delta b^* by the day nine for untreated and treated samples was 12 (less than 10%) and 5.2 (about 4%) respectively.

The TCD* values of citric acid treated stems versus control ones on 0, 3, 6, & 9 days of storage are summarized in Table 2. Based on Silva and Silva's classification, the TCD* values are classified very great (more than 17) throughout the nine days. The reason why the TCD* values were high is because the darkening in the controls was intense and in treated samples was significantly less. The % inhibition, calculated based on equation 2 and ΔL values of the control and treated samples, after nine days of storage was 88% where the L^* color values of the untreated samples were used as control and the L^* color values of treated samples were used as treatment. Based on the TCD* and % inhibition values for the artichoke stems, the 0.5M citric acid + 0.3M CaCl_2 + 1%

H_3PO_4 treatment was effective in increasing the acidity and reducing the pH of the treated samples to inactivate the PPO and in delaying the enzymatic browning in samples for more than six days of cold storage. Citric acid as a chelating agent was responsible for delaying the non-enzymatic browning in samples.

The artichoke heads remained in good quality condition for about two weeks. Around the third week of this study they started to show browning and drying on the outer layer of the flower portion of the vegetable. Since the visual appearance of the stems was completely darkened at this point, it may be concluded that the optimum shelf life for this vegetable in cold storage is less than three weeks. The results may change by using different storage conditions such as modified atmosphere or other temperatures. Harvesting time may also change the results (Lattanzio et al., 1989). Lattanzio and his colleagues found that winter-harvested artichokes have a much longer shelf life than spring harvested ones due to their different phenolic contents.

Phase 2: Mushroom Pileus

The visual color of untreated (control) and treated mushroom samples are shown in figures 9,10, and 11. The change in tristimulus color values of these samples for four days of storage is shown in figures 12, 13, and 14. The color values of the mushrooms on the first day represent the least intensity of browning discoloration in the samples after purchase. The averages and standard deviations of these values were $L^* = 81.18 \pm 1.41$, $a^* = +0.9 \pm 0.37$, and $b^* = +12.48 \pm 0.74$. The L^* , a^* , and b^* values of all six different groups of samples were graphed (Figure 12,13, & 14). As the mushrooms were browning, L^* value was decreasing, and a^* and b^* values were increasing throughout the

study. The samples packaged and stored in the refrigerator had the least visual browning, and the samples packaged and stored at room temperature had the most visual browning among others (Figures 10, & 11). The photographs of the mushroom pileus on day two and four shows that both mushroom groups stored at room temperature visually had severe browning in just two days, especially the packaged mushrooms, which had big brown lesions. The browning discoloration in mushroom samples visually turned their color from light cream to a brown-red color (Figures 9, 10, & 11). The photographs and the graphs of HunterLab L* color values of mushrooms indicate that as the samples were losing their lightness their L* value was decreasing (Figure 12). Due to the development of brown-red color in samples, a* and b* values were increasing throughout the storage time (Figures 13 & 14). Based on the results, changes in L*, a*, and b* were related to the intensity of browning discoloration in mushroom samples.

The TCD* values of mushrooms treated by three different factors are summarized in table 4. The L* and b* values of refrigerated samples were significantly different from L* and b* values of the samples stored at room temperature ($p < 0.05$ by t-test). The TCD* value of refrigerated samples (treated group) versus room temperature stored ones (control) for packaged mushrooms was more than 20 (very great color difference) and for un-packaged mushrooms was about 5.9 (very distinct color difference). Since refrigerated samples also had less visual browning than those stored at room temperature after four days of storage (Figure 14 & 15), it can be concluded that cold storage can delay the browning discoloration and affect tristimulus color values considerably. The L* and b* values of packaged mushrooms, which were refrigerated, were significantly different

from L^* and b^* values of un-packaged correspondents ($p < 0.05$ by t-test). Packaged mushrooms had significantly higher L^* and lower b^* values. The TCD* value of packaged mushrooms (treated group) versus un-packaged ones (control), both refrigerated, after four days is 6.2 (great color difference), with less browning in the packaged group. This value for the packaged (treated group) versus un-packaged (control) mushrooms, all stored at room temperature, is about 13 (very great) with more visual browning in the packaged group. Therefore, it can be concluded that packaging in cold temperature delayed the browning, and at room temperature storage increased the browning rate in mushrooms. At room temperature, packaging may increase the temperature and humidity around the breathing vegetable and promote the browning.

The %inhibition, based on ΔL values of packaged mushrooms versus un-packaged ones, is 75%. It means that packaging could decrease the browning progression in mushrooms up to 75% in four days of cold storage. However, the a^* values of neither packaged groups had a significant difference compared with a^* value of corresponding un-packaged groups, except the ones stored at room temperature ($p < 0.05$ by t-test). Packaging can protect the produce from excessive oxygen contact. There was less browning intensity in packaged mushrooms since oxygen exclusion can reduce enzymatic browning in plant tissue (Fennema, 1996). Citric acid treatment did not change tristimulus color values significantly ($p < 0.05$ by t-test). The TCD* values of the citric acid treated group versus its control group, both in packaged and un-packaged condition, were small (less than 1.5). There was no difference in visual discoloration observed in citric acid treated versus untreated mushrooms also by the end of 4 days of cold storage.

Conclusion

Browning discoloration of artichoke stems and mushroom pileus can be monitored by measuring the HunterLab tristimulus color values and calculating total color differences (TCD*). The HunterLab color values were related to the intensity of darkening and were significantly changed as the samples browned ($p < 0.05$, by t-test) in both artichoke and mushroom samples. In artichoke stems, the 0.5M citric acid + 0.3 M CaCl_2 + 1% H_3PO_4 dipping solution inhibited the browning discoloration in treated samples up to 88% based on their lightness or L^* value. It can be concluded this treatment was strong enough to decrease the browning discoloration in artichoke stems for nine days of cold storage. In mushroom samples cold temperature storage (4°C) versus room temperature storage (20°C) could delay the browning discoloration and affect tristimulus color values significantly ($p < 0.05$, by t-test). Packaging versus the unpackaging of refrigerated mushrooms could inhibit browning in mushrooms up to 75% and therefore prolong their shelf life.

Since the tristimulus colorimetric results were clearly related and paralleled the visual and sensory results of browning samples throughout this study, the application of this method in quality control procedures and shelf life estimation of fresh produce is suggested. The tristimulus colorimetry can be considered as a fast and non-destructive method for both food research studies on browning discoloration and also food industrial applications.

Figure 1: HunterLab ColorFlex

Table 1: Classification of color differences

TCD*	Color Difference Classifications
0.0-0.2	not perceptible
0.2-0.5	very small
0.5-1.5	small
1.5-3.0	distinct
3.0-6.0	very distinct
6.0-12.0	great
>12.0	very great

Note. From “ Color changes in thermally processed cupuacu puree” by F.M. Silva & C.L.M. Silva (1999), p. 88.

Figure 2: Photograph of control and citric acid (0.5M citric acid+0.3M CaCl_2 +1% H_3PO_4) treated artichoke stems at day 0 of storage

Figure 3: Photograph of control and citric acid (0.5M citric acid+0.3M CaCl_2 +1% H_3PO_4) treated artichoke stems at day 3 of storage.

Figure 4: Photograph of control and citric acid (0.5M citric acid+0.3M CaCl_2 +1% H_3PO_4) treated artichoke stems at day 6 of storage.

Figure 5: Photograph of control and citric acid (0.5M citric acid+0.3M CaCl_2 +1% H_3PO_4) treated artichoke stems at day 9 of storage.

Figure 6: Hunter L* color values of control and treated artichokes stems stored for 9 days at refrigerator (each value is the average of 24 readings).

Figure 7: Hunter a^* color values of control & treated artichoke stems stored at refrigerator for 9 days (each value is the average of 24 readings).

Figure 8: Hunter b^* color values of control & treated artichoke stems stored at refrigerator for 9 days (each value is the average of 24 readings).

Table 2: Total color difference (TCD*) values of artichoke stems at 0, 3, 6, & 9 days of storage at refrigeration temperature.

Days of Storage	TCD*
0	17.8
3	23.08
6	23.22
9	24.62

Figure 9: Photographs of P.Re (Packaged Refrigerated), UnP.Re.(Unpackaged Refrigerated), P.Ro. (Packaged Room Temperature), UnP.Ro. (Unpackaged RoomTemperature), P.Re.CA (Packaged Refrigerated Citric Acid), Un.Ref.CA (Unpackaged Refrigerated Citric Acid) mushrooms at day 0 of storage.

Figure 10: Photographs of P.Re (Packaged Refrigerated), UnP.Re.(Unpackaged Refrigerated), P.Ro. (Packaged Room Temperature), UnP.Ro. (Unpackaged RoomTemperature), P.Re.CA (Packaged Refrigerated Citric Acid), Un.Ref.CA (Unpackaged Refrigerated Citric Acid) mushrooms at day 2 of storage.

Figure 11: Photographs of P.Re (Packaged Refrigerated), UnP.Re.(Unpackaged Refrigerated), P.Ro. (Packaged Room Temperature), UnP.Ro. (Unpackaged Room Temperature), P.Re.CA (Packaged Refrigerated Citric Acid), Un.Ref.CA (Unpackaged Refrigerated Citric Acid) mushrooms at day 4 of storage.

Figure 12: Hunter L* values of P.Re (Packaged Refrigerated), UnP.Re.(Unpackaged Refrigerated), P.Ro. (Packaged Room Temperature), UnP.Ro. (Unpackaged Room Temperature), P.Re.CA (Packaged Refrigerated Citric Acid), Un.Ref.CA (Unpackaged Refrigerated Citric Acid) mushrooms during 4 days of storage (each value is the average of 4 readings).

Figure 13: Hunter a^* values of P.Re (Packaged Refrigerated), UnP.Re.(Unpackaged Refrigerated), P.Ro. (Packaged Room Temperature), UnP.Ro. (Unpackaged Room Temperature), P.Re.CA (Packaged Refrigerated Citric Acid), Un.Ref.CA (Unpackaged Refrigerated Citric Acid) mushrooms during 4 days of storage (each value is the average of 4 readings).

Figure 14: Hunter b^* values of P.Re (Packaged Refrigerated), UnP.Re.(Unpackaged Refrigerated), P.Ro. (Packaged Room Temperature), UnP.Ro. (Unpackaged Room Temperature), P.Re.CA (Packaged Refrigerated Citric Acid), UnP.Re.CA (Unpackaged Refrigerated Citric Acid) mushrooms during 4 days of storage (each value is the average of 4 readings).

Table 3: TCD* values of citric acid-treated versus non-citric acid (control) mushrooms at cold temperature

Days of Storage	Packaged	Unpackaged
<hr/>	<hr/>	<hr/>
1	1.2	0.8
2	1.5	1.4
3	1.1	1.1
4	1.7	1.5

Table 4: TCD* values of packaged (treated group) versus un-packaged (control) mushrooms (here packaging is considered as the treatment)

Days of Storage	Cold Temperature	Room Temperature
<hr/>	<hr/>	<hr/>
1	1.9	5.2
2	2.7	13
3	3.8	---
4	6.2	---

Table 5: TCD* values of refrigerated (treated group) and room temperature stored (control) mushrooms (here refrigeration is considered as the treatment)

Days of Storage	Packaged	Un-packaged
<hr/>	<hr/>	<hr/>
1	11	5.3
2	20.3	5.9

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CHAPTER 3

SUMMARY AND RECOMMENDATIONS

Summary

Browning and color degradation are important causes of quality loss in fresh produce. Browning of plant tissue is closely related to the oxidation of phenolic substrates catalyzed by an enzyme known as polyphenoloxidase. Since the browning reaction can change the color of its host, the intensity of browning discoloration may be monitored by color measurement techniques such as tristimulus colorimetry. Tristimulus colorimeters can measure the reflected light from the surface of food and quantify the different color characters such as the lightness, redness or yellowness. ColorFlex ,a tristimulus colorimeter, and HunterLab color system, a simple and uniform color measuring system, were used in this study to measure color values. The samples used were artichokes and mushrooms, both of which are highly susceptible to enzymatic browning. Artichoke stems were treated with 0.5M citric acid + 0.3M CaCl_2 + 1% H_3PO_4 solution, and distilled water (as control). The HunterLab values for control artichokes were altered during cold storage especially during the first three days after cutting. The treated samples maintained their visual color and their HunterLab color values did not change significantly for nine days of storage.

Mushrooms, treated with 1% citric acid solution, were stored at refrigeration temperature. Citric acid treatment was not effective in retarding browning discoloration in mushroom samples during the cold storage. This treatment did not significantly affect color values of mushroom samples either. Mushrooms were manipulated by two other

factors, temperature and packaging. Cold storage temperature versus room temperature storage could delay browning to a considerable extent and it significantly affected the HunterLab values. Packaging versus un-packaging, only in cold storage, significantly reduced the intensity of browning discoloration and the change in HunterLab values in subjected mushrooms. It can be concluded that tristimulus colorimetry is a rapid and nondestructive method that can be used in assessment of browning intensity and the effect of browning inhibitors in artichokes and mushrooms.

Recommendations

This study was more focused on the applicability of tristimulus colorimetry versus other common methods in the assessment of browning discoloration in vegetables. More research is needed to accurately correlate the colorimetric values to the intensity and rate of browning discoloration. One of the applications of tristimulus colorimetry can be using this method in the assessment of browning discoloration in dehydrated products, where the browning and darkening during their heating process is always a problem. It might be possible to obtain a predictable model of browning development in samples. The rate of browning can be determined by colorimetric measurements too. The effect of the size and shape of samples on color values of dehydrated products can be the subject of another research study. Sensory evaluation techniques can be used to correlate the colorimetric values to the visual observations.

One of the limitations of this study on artichoke stems was the high sensitivity of the HunterLab values to the size and shapes of the stems. Therefore, the number of samples should be increased to eliminate this problem. Another limitation for the

tristimulus colorimetry is that this technique can not be used for heterogeneous samples such as granules. It is suggested in reference literatures that this method is more accurate for flat and uniform surfaces.

Acidic treatment of the samples, whether it inhibits the browning or not, will probably have an effect on the organoleptic properties of both artichokes and mushrooms. Organoleptic evaluation should be considered in application of any acidic compounds on these vegetables.

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