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I am submitting herewith a thesis written by Mohammad Amiri entitled "Extraction and Use of a Carotenoid Colorant from Red Bell Pepper Juice." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

Hugh O. Jaynes, Major Professor

We have read this thesis and recommend its acceptance:

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EXTRACTION AND USE OF A CAROTENOID COLORANT FROM RED BELL PEPPER JUICE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Mohammad Amiri

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ABSTRACT

The objectives for this study were the extraction of the carotenoid pigments in waste juice from canning of diced red peppers in the form of an oleoresin, quantification, characterization and use of the extracted pepper oleoresin as a colorant in French dressing. Commercial paprika oleoresin, an approved food colorant, was used as the reference for comparison with pepper oleoresin throughout the study.

The mean concentration of recoverable oleoresin in waste pepper juice was found to be 0.0406% and 0.854% in terms of total dry matter with a mean A.S.T.A. color value of 2192, 2.3 times that of a commercial paprika oleoresin. The pepper and paprika oleoresins were found also to have very similar color by comparing C.I.E. color parameters.

Stability of pepper oleoresin compared to paprika oleoresin was investigated by studying the effect of temperature, light and air. Pepper oleoresin was found to be more stable to heat than paprika oleoresin. The effects of other factors were found to be the same for two oleoresins. Using the analysis of variance data, regression polynomials were derived.

To use pepper oleoresin as a colorant in French dressing, batches of French dressing were made containing pepper and paprika oleoresin as colorants with adjusted equal colors measured by YCIE. The color and flavor of French dressing were followed over a 45 day storage period at room temperature and normal incident light. The dressings were examined at 0, 15, 30 and 45 days. The dressings were subjected to sensory

panels at each time for color and flavor evaluation and objective color measurements were made. In sensory evaluation of French dressing, no difference was found for color. Difference was found for flavor without any preference for samples containing either colorant. The objective color measurements in French dressing showed no significant change in color throughout the storage period.

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CHAPTER I

INTRODUCTION

Today, the need for naturally occurring pigments as food colorants is being promoted. There are doubts about the safety of synthetic colorants and some of them have been delisted by the U.S. Food and Drug Administration. A group of naturally occurring pigments are carotenoids. It has been estimated that nature produces over 100 million tons of carotenoid pigments per year. The carotenoids can be separated from natural sources and used as food colorants (4, 34).

Carotenoids possess several advantages when used as food colorants; they possess high tinctorial potency as colorants. While they are fat-soluble colors, emulsified and colloidal forms exist for water applications. They are naturally occurring substances and exist widely in nature. Some possess vitamin A activity and, hence, have a dual function. They are relatively stable under reducing conditions. They are relatively light stable, particularly in a minimum oxygen atmosphere. They are relatively stable over the acid and neutral pH ranges of food products. They can be used in combination with each other, and in special instances with FD & C colors, to extend hue and color ranges and they are available in constant supply of predetermined quality (4).

A good source of carotenoids is the oleoresin extracted from the plants with high carotenoid content. Oleoresins are prepared from spices or herbs by extraction of the carotenoids with volatile organic solvents followed by evaporation of the solvent. A commonly used

oleoresin is paprika oleoresin which is an approved food colorant and contains the carotenoids available in paprike (30). Previous work has shown that red bell peppers contain relatively the same carotenoids which occur in paprika (6). In canning of red bell peppers, a large amount of waste juice is generated as diced pepper moves through a steam injection heater just prior to canning (2). It should be possible to prepare an oleoresin containing concentrated carotenoid pigments from the pepper juice which would be similar to oleoresin paprika.

The experiment reported is an investigation of the recovery of an oleoresin from waste pepper juice, its characterization and stability, and the use of the oleoresin as a colorant in French dressing. Paprika oleoresin was used as a reference material for comparisons.

CHAPTER II

LITERATURE REVIEW

I. RED BELL PEPPER AND ITS CAROTENOID COMPOSITION

Commercially, Capsicum annuum is the most widely cultivated species of pepper throughout the world, and almost all the varieties cultivated in the United States and Europe belong to this group. It includes nearly all the sweet peppers, as well as a large number of pungent, strong-flavored forms. They are members of Solanaceae (nightshade family) indigenous to Mexico, Central America, the West Indies, and much of South America (30).

In temperate zones the large, sweet, thick skinned, fleshy pods of such mild-tasting varieties of C. annuum as the bell peppers, Ruby King and California Wonder, are eaten as vegetables, either green or ripe, cooked or raw (30).

The absorption spectrum in diethyl ether of the unsaponifiable carotenoid extract from various types of peppers is shown in Figure 1.

As Table I shows, the carotenoids in red peppers are more abundant than in other kinds. The carotenoids in red bell peppers have been examined by Curl (6). Capsanthin accounted for about 35% of the total carotenoids, β-carotene and violaxanthin about 10% each, cryptoxanthin and capsorubin about 6% each, and cryptocapsin about 4%. Numerous other carotenoids were also present in amounts of 2% or less, including at least eight apparently undescribed previously and two others not found

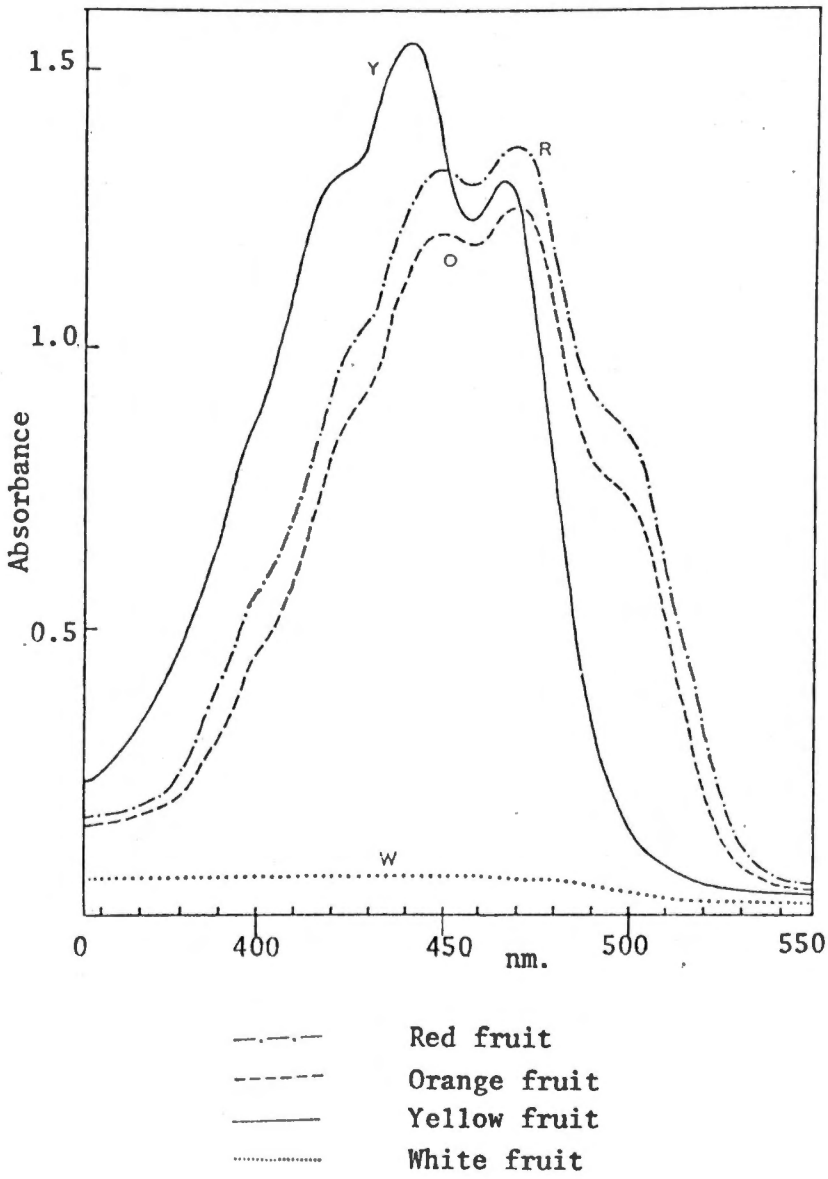

Figure 1. Visible absorption spectra (in diethyl ether) of the un-saponifiable lipid fraction of ripe fruits of Capsicum annuum (7).

TABLE I

CAROTENOID CONTENT OF SEVERAL VARIETIES OF PEPPERS

	Leaves	Unripe fruit	Ripe fruit
	--------	mg/100 gr. fruit	--------
Red-fruiting varieties	24.8	0.62	85.5
Orange-fruiting varieties	13.9	0.47	2.49
Yellow-fruiting varieties	20.8	0.46	2.24
White-fruiting varieties	20.9	-	0.069

Reference 7.

previously in nature. All ten of these pigments appear to contain cyclopentane rings as does capsanthin, capsorubin, and apparently cryptocapsin. In addition to capsanthin and capsorubin, five other constituents were found to contain keto groups. Capsanthin is distinctly different from the deep red pigment (reticulaxanthin) in tangerine and orange peels (6).

II. RED BELL PEPPER PROCESSING

Bell peppers are large pods, neither pungent nor acrid, which can be eaten sliced as a salad or used in various ways as a vegetable. They may be used either in the green unripe state or ripened when they are sweeter and red (22). The fruits are harvested by snapping the brittle stem by hand. The ripened fruit can be kept at least forty days at 0°C if the relative humidity is maintained at 95% or 98%. Peppers for canning must be bright red in color. The skin is removed by lye peeling or by flame heating prior to canning (2, 5). For canning, the red bell peppers may be diced, canned whole, or as halves or quarters. A large amount of waste juice is generated as the diced style peppers move through a steam injection heater just prior to canning. The amount of waste juice in this process is considerable and was estimated to be one volume of juice for three volumes of diced pepper (2).

III. OLEORESIN, ITS USES AND ADVANTAGES

Oleoresins are prepared from spices or herbs by extraction with volatile organic solvents. Oleoresins, therefore, also contain the

nonvolatile constituents which are extracted in the process. The extracting medium must be carefully selected to provide those types of extractives which are most desirable for flavoring or coloring purposes. After the extraction has been completed, the solvent must be completely removed under vacuum to assure conformance with the federal regulations on residual solvents. Generally, the resulting product consists of essential oils, fat-soluble pigments, organically soluble resins and other related materials which were present in the original spice, as well as any nonvolatile fatty oils which were present (12).

The oleoresin products have found increased usage in the food industry. Oleoresin of capsicum, a dark red, extremely acrid and pungent liquid, can be extracted from the pods with alcohol or ethyl ether. Oleoresin capsicum is used in the food and pharmaceutical industries where a highly concentrated pungency is required. The pungency of this extract can be controlled and standardized for use in seasoning instead of the ground capsicum product. Oleoresin of paprika, on the other hand, is utilized for its concentrated color, not its pungency (30).

The use of oleoresin products provides the food manufacturer with many outstanding advantages. Uniformity of flavor is one. Since these materials have been extracted from spices or herbs which tend to vary from crop year to crop year and with the age of raw material, the extractives can be blended to minimize variations of this nature. Oleoresins have good stability. When natural spices and herbs are stored over long periods of time, the volatile essential oils present sometimes are lost through evaporation or through polymerization and

oxidation. With extracted materials, these effects are minimized, especially losses due to volatilization; oleoresins are either liquids or semi-solid liquids which are stored in airtight containers as contrasted with spices or herbs which are sometimes stored in burlap bags or cardboard containers permitting loss of the volatile essential oil. Oleoresins need much less storage area. In examining the spice equivalents, it is evident that a few pounds of oleoresin material generally replace 100 pounds or more of spice or herb. Oleoresins can be made bacteria free and since oleoresin-type extractives remove the entire flavoring and coloring portion of a spice or herb, they have better economy (9, 12, 19, 20, 31).

IV. COLOR AND EFFECT ON ACCEPTABILITY OF A PRODUCT

Color is that aspect of the appearance of objects and lights which depends upon the spectral composition of the radiant energy reaching the retina of the eye and upon its temporal and spatial distribution thereon (16). Color is not merely a physical phenomenon. In fact, color is a sensation experienced by an individual when energy in the form of radiation within the visible spectrum falls upon the retina of the eye. That color is a sensation dependent upon what a person sees cannot be overemphasized. Also, there are several factors which influence the radiation and, subsequently, the exact color or sensation which the individual perceives. These factors include:

1. The spectral energy distribution of the light,
2. The conditions under which the color is being viewed,

3. The spectral characteristics of the object, with respect to absorption, reflection, and transmission, and
4. The sensitivity of the eye (11).

We learn to associate certain objects with certain colors and the first impression of a food is usually visual, and a part of our willingness to accept a food depends on its color (23).

The extent to which a consumer will allow variation in the color of a given food depends on a perceived idea of what he believes the food should look like; and consequently, a color preference is established (23).

For producers, the extent of acceptable variation will be limited by quality criteria which therefore set permissible color tolerances. In the final analysis, tolerance limits must correspond with acceptability, not the perceptibility (23).

Today, the consumer expects foods to have appealing and appetizing color. Colorants are added to thousands of foods. Some of the colorants added to foods are made from substances found naturally in food. Carotenoids are good examples of this type of color additive and they offer a unique advantage of safety and nutritive value (32).

V. COLOR MEASUREMENT BY AMERICAN SPICE TRADE ASSOCIATION COLOR UNITS (ASTA)

The ASTA method is an official method which is used to measure extractable color in paprika (14). The apparatus used for this method is a spectrophotometer which is capable of measuring the absorbance at

450 nm with 1 cm matched cells or cuvettes. The necessary reagents are:

1. Isopropanol—at least 99% pure,
2. Standard Color Solution—0.500 mg/ml of reagent grade $K_2Cr_2O_7$ in 1.8 M H_2SO_4, and
3. 1.8 M H_2SO_4.

The absorbance of the standard color solution is determined at 450 nm against 1.8M H_2SO_4 as a blank.

Absorbtivity (a) of standard color solution =

$$\frac{\text{absorbance of standard color solution at 450 nm.}}{\text{cell length (cm)} \times \text{concentration (mg/ml)}}$$

which in this case concentration is 0.500 mg/ml

Extractable color, ASTA unit =

$$\frac{\text{absorbance of extract at 450 nm.} \times 200}{\text{cell length (cm)} \times a \times \text{concentration of sample (mg/ml)}}$$

VI. INSTRUMENTAL COLOR MEASUREMENT

C.I.E. Method of Color Notation

The most important color system was promulgated by the Commission Internationale de l'Eclairge (CIE), an international body which makes recommendations on all matters concerning light and color (11). The CIE has adopted methods for the measurement and specification of color which includes:

1. The use of a standard light source as prescribed by CIE definition. The standard CIE standard sources are:
   A. Incandescent lamp light at a color temperature of 2854°K,

B. Direct sunlight (correlated color temperature of 4870°K), and

C. Light of the overcast sky (correlated color temperature of 6770°K).

Source A is realized directly by an incandescent lamp, such as a 500 watt tungsten coiled filament lamp, operating at a color temperature of 2854°K. Sources B and C are obtained by combining Source A with special liquid filters, the Davis Gibson filters (35). Light source "C" is the one commonly used in the United States.

2. Exact conditions for the observation or measurement of sample color.

The illuminating and viewing conditions recommended for normal use by the CIE are (45°, 90°); that is, the reflecting surface is to be illuminated essentially at 45° and to be viewed perpendicularly (90°) to the surface (35).

3. The use of appropriate mathematical units in which to express the color of an object.

The tristimulus values of a stimulus are specified by its spectral distribution of radiant flux ($P_\lambda \Delta_\lambda$). These are given by the following sums:

$$X = k \sum_\lambda P_\lambda \bar{x}_\lambda \Delta_\lambda$$

$$Y = k \sum_\lambda P_\lambda \bar{y}_\lambda \Delta_\lambda$$

$$Z = k \sum_\lambda P_\lambda \bar{z}_\lambda \Delta_\lambda$$

The color matching functions $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$ are the CIE standard observer (17, 35).

The radiant flux reflected (or transmitted) by an object regarded as itself a color stimulus is called an object color. An object color is specified by the spectral distribution P_λ $H_\lambda \Delta_\lambda$ or T_λ H_λ Δ_λ, where

P_λ is the spectral reflectance of the object,

T_λ is the spectral transmittance of the object, and

$H_\lambda \Delta_\lambda$ is the spectral distribution of the flux irradiating the object.

Thus, the tristimulus values X, Y, Z of an object color are given by:

$$X = k \sum_\lambda P_\lambda H_\lambda \bar{X} \Delta_\lambda$$

$$Y = k \sum_\lambda P_\lambda H_\lambda \bar{Y} \Delta_\lambda$$

$$Z = k \sum_\lambda P_\lambda H_\lambda \bar{z} \Delta_\lambda$$

For a reflecting object and similar expressions, with T_λ replacing P_λ, for a transmitting object (35).

4. Definition of "Standard Observer"—curves or tables relating objective measurements to visual responses, and thus, measuring what the eye sees have been developed (17, 35).

The color-matching functions $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$ are either those of 1931 CIE standard observer (for visual fields of up to four degrees angular subtense) or those of the 1964 CIE large-field standard observer (for visual fields of angular subtense larger than four degrees).

From the tristimulus values X, Y and Z, the proportion of each primary is calculated as the following ratio:

$$x = \frac{X}{X+Y+Z} \qquad Y = \frac{Y}{X+Y+Z} \qquad z = \frac{Z}{X+Y+Z}$$

These are known as chromaticity coordinates $x + y + z = 1$ and it is necessary to use only x and y to define chromaticity. Color specification is usually made in terms of Y_{CIE}, x and y (11, 23, 34, 35).

The CIE chromaticity diagram is shown in Figure 2 with spectrum locus, purple line, and chromaticity point (E) of equal energy stimulus (35). Y_{CIE} serves to define the third, or vertical, dimension in the CIE color space.

Color Eye Transmission Method

In the color-eye, the sample and the reference standard are illuminated by diffuse illumination from the two halves of an integrating sphere and a photomultiplier flicker photometer system measures the light reflection from the sample relative to that from the standard for each of three tristimulus filters (34).

Tristimulus filters are constructed by combining color filters in series. The degree to which the various tristimulus filters, combined with a built-in source and photoelectric receiver, duplicate the desired CIE color matching functions varies from instrument to instrument, but in none of the instruments can we expect to find tristimulus filters which duplicate exactly the CIE color matching functions or some linear

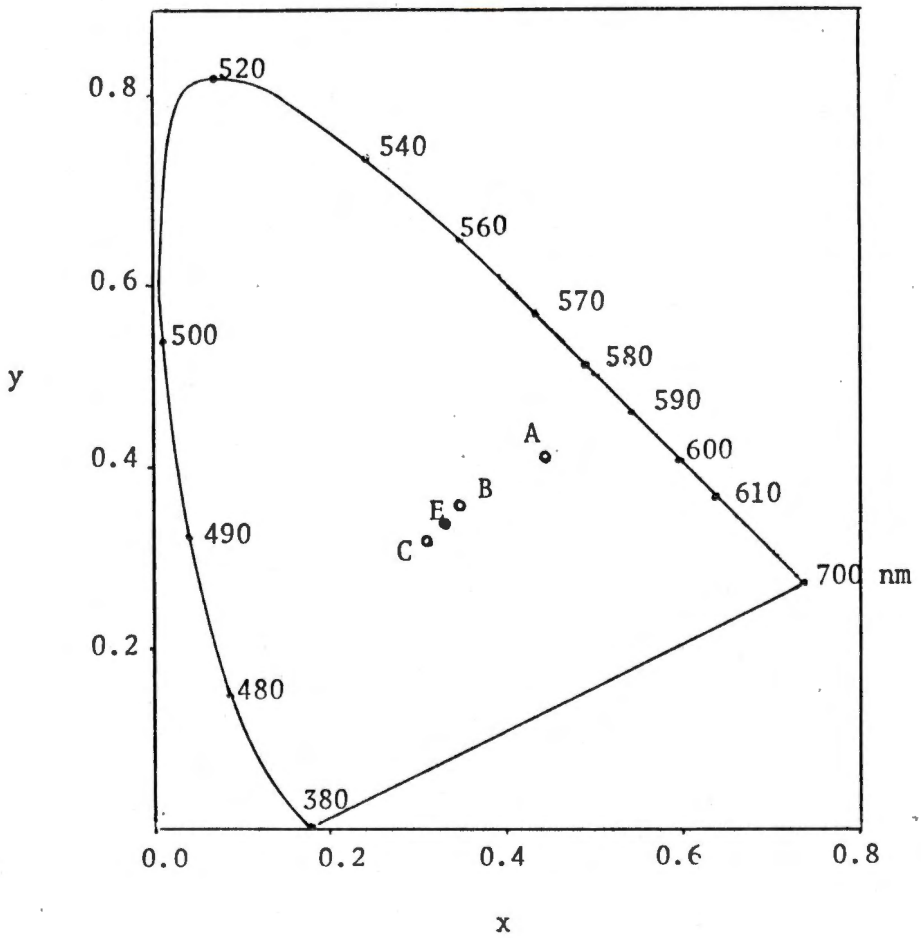

Figure 2. 1931 CIE (x, y) Chromacity Diagram with Spectrum Locus, Purple Line, the Chromaticity Points of CIE Standard Sources A, B, C, and Equal-energy Stimulus E (34).

transformation of them. However, for comparison of specimens exhibiting a slight degree of metamerism, an exact duplication of the CIE color matching functions is not too important (17, 35).

Figure 3 shows curves indicating the degree to which tristimulus filters combined with a barrier layer cell duplicate the CIE color matching curves $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$ (17, 35).

Hunterlab Color Difference Meter

Hunter's Color Difference Meter has been favorably received by industry, particularly for paints, ceramics and agricultural products (17). The instrument employs photoelectric cells and incorporates circuits by means of which the signals can be combined to record the color differences in terms of National Bureau of Standards (NBS) units (34).

In a revised form based on Hunter's "alpha-beta" diagram in which the chromaticity of standard cource C is represented by the origin of this system, the difference ΔE between two colours having the coordinates α_1, B_1, Y_1 and α_2, B_2, Y_2 is given in NBS units by the equation (34, 35)

$$E = fg\{[221\ Y^{1/4}((\Delta\alpha)^2 + (\Delta\beta)^2)1/2]^2 + [k\Delta y^{1/2}]^2\}^{1/2}$$

where

$$y = \frac{Y_1 + Y_2}{2}, \quad \Delta Y^{1/2} = Y_1^{1/2} - Y_2^{1/2}$$

$$\Delta\alpha = \alpha_1 - \alpha_2, \quad \Delta\beta = \beta_1 - \beta_2$$

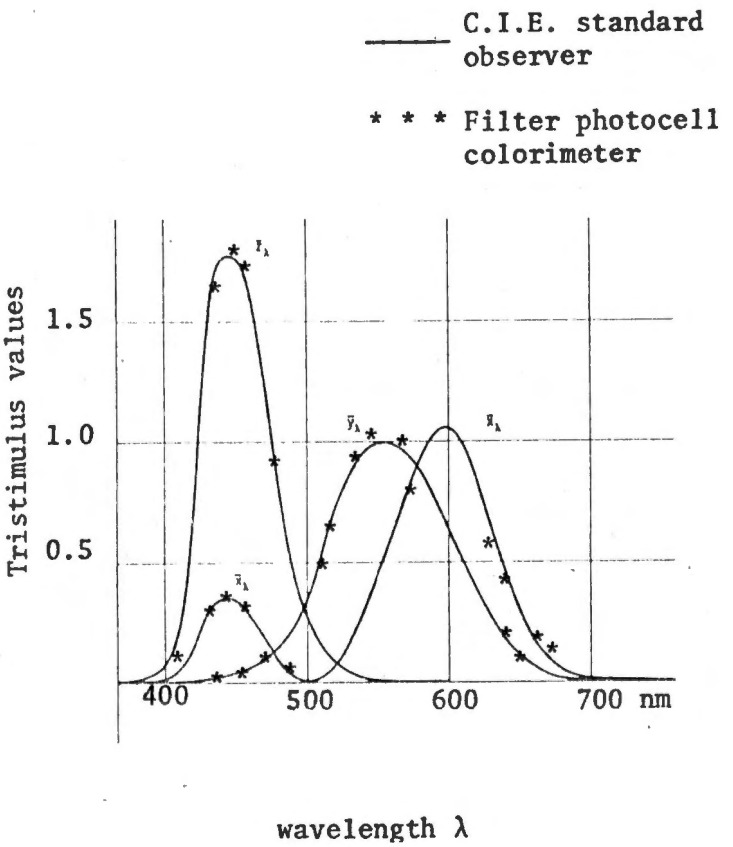

Figure 3. Examples of Curves Showing the Degree to Which Tristimulus Filters Combined with a Barrier-Layer Cell Duplicate the CIE Color Matching Curves $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$ (17).

f_g is a constant which can be adjusted to take account for the masking influence of a glossy surface on the detection of color differences. For typical conditions of inspection, f_g can be found from the equation:

$$f_g = \frac{Y}{Y + 2.5}$$

K is a further constant to allow for the variation in the contribution which differences in lightness make to the total perceived color difference depending on the viewing condition (34, 35).

CHAPTER III

MATERIALS AND METHODS

I. SOURCE OF RAW MATERIAL

The waste pepper juice was obtained from Moody Dunbar Pepper Canning Plant in Limestone, Tennessee. It was collected at the exit end of a steam injection heater used to preheat diced pepper just before it is canned. The juice was collected in plastic bags, sealed, and stored at -18°C until used.

II. PEPPER JUICE COMPOSITION

Moisture, fat, protein and carbohydrate were measured. The moisture content was measured by drying to constant weight at 60°C and a vacuum of 380 torr. The protein content was measured by A.O.A.C. Kjeldhal Method. The fat content was measured by ethyl ether extraction in a separatory funnel using five-fold extraction. The ash content was determined by dry ashing at 550°C for four hours. The carbohydrate content was measured by difference.

III. SEPARATION OF OLEORESIN

Carotenoids and oil in the juice were absorbed on Johns Manville celite filteraid, 4g/100 ml of juice. By using a water aspirator as a vacuum source, filteraid was collected by vacuum filtration then dried at 50°C for two hours. Using a glass column of 20 mm diameter and

450 mm length filled with 40 mm glass wool and 350 mm celite filteraid, the pepper oleoresin was recovered by elution with 70 ml ethyl ether.

Ethyl ether was removed at reduced pressure, leaving the pepper oleoresin. Yield was calculated based on volume of juice and weight of dry matter.

IV. COLOR CHARACTER OF PEPPER OLEORESIN COMPARED TO PAPRIKA OLEORESIN

Using isopropanol as a solvent, solutions of pepper oleoresin and paprika oleoresin were prepared with absorbances of about 0.8. They were scanned with a Coleman 124 recording spectrophotometer over the range of 350 to 650 nm, and the scans were compared.

The concentrations of color as ASTA units were compared by the ASTA standard method (14).

Using ethyl alcohol as the solvent, the concentration of the two oleoresins was adjusted to equal ASTA color units, and the color of each was determined in the Color-Eye by the transmission method. The color of each colorant was determined by x, y and Y_{CIE}.

V. STABILITY STUDY

Method of Analysis

Using commercial paprika oleoresin as a reference, the effects of heat, light, and air on the color stability of pepper and paprika oleoresin were determined.

1. A benzene solution of oleoresin was made and adjusted to a concentration which gave an absorbance of 0.7 at 450 nm.

VI. USE OF PEPPER OLEORESIN AS A COLORANT IN FRENCH DRESSING

French Dressing Formulation

Batches of French dressing were prepared using pepper oleoresin and commercial paprika oleoresin as a colorant. A recipe given by Finberg (10) was used:

White vinegar (50 Grain)	32.00%
Keltrol xantham gum	0.42%
Sugar	16.50%
Salt	1.20%
Soybean Oil	35.10%
Water	14.78%

Preparation and Matching the Color of Dressing

Ingredients were mixed and emulsified in a Waring blender. Colorant was added to match a commercial French dressing.

Two batches of French dressing were made. Each was divided; 0.0012 g pepper oleoresin per ml of French dressing was added to one part and 0.0029 g paprika oleoresin per ml of French dressing was added to the other part to give equivalent color as measured by the Hunterlab instrument. Y_{CIE} with a maximum difference of ±0.2 was used to match the color.

All samples were dispensed into clear glass bottles with screw caps. Samples were held at room temperature under normal incident light.

Sensory Evaluation of Dressing

Changes in color and flavor of French dressing made with pepper oleoresin and paprika oleoresin as colorant were followed over a 45 day storage period.

All lots were examined at 0, 15, 30 and 45 days. At each time period, samples of the dressings were presented to triangle sensory panels. At least twenty people were asked to evaluate two sets of samples for difference and preference on the basis of color and flavor. Daylight fluorescent lighting was used in the taste panel room. Panels were admitted in midafternoon. Panelists were staff members and students of the Food Technology and Science Department of the University of Tennessee.

Instrumental Measurement of Color

Objective color measurements were made on all samples with the Hunterlab instrument in CIE notation against a white standard. These measurements were taken on the same day as the sensory panels.

Statistical Analysis of Objective Color Measurements

Objective color measurements were analyzed by analysis of variance with a split plot design to show the effects of type of oleoresin, replication, time and their interactions.

CHAPTER IV

RESULTS AND DISCUSSION

I. COMPOSITION OF PEPPER JUICE

The composition of pepper juice used as a source of oleoresin is shown in Table II along with the mean and standard deviation for each component. Moisture was found to be 95.25% indicating a total solids content of 4.75%. All the water content is a waste and must be removed before separation of oleoresin.

The five samples represented in Table II are five different lots of waste pepper juice collected at different times on the same day at Moody Dunbar Pepper Canning Plant in Limestone, Tennessee.

Mean protein was 0.45%; ash, 0.30%; and carbohydrate, 3.95%. Significant differences between means of moisture and fat were found by Duncan's multiple range test (18).

The fat content was rather low, but this is the portion which was extracted in recovering the oleoresin. A higher fat content could lead to a higher total yield of oleoresin. The carotenoids are dissolved in the fat portion which produces the color of the oleoresin. A high fat content without a high level of pigment would not give a good yield of colorant, but color level can be standardized in oleoresins (7, 12).

TABLE II

COMPOSITION OF WASTE PEPPER JUICE

Sample	Moisture	Fat	Protein	Ash	Carbohydrate
	%				
1	95.25 abc	0.052 ab	0.43 a	0.33 a	3.92
2	94.98 d	0.054 a	0.45 a	0.30 a	4.23
3	95.47 a	0.048 abc	0.45 a	0.30 a	3.73
4	95.46 ab	0.036 cd	0.44 a	0.29 a	3.76
5	95.10 cd	0.043 abcd	0.46 a	0.29 a	4.10
$\overline{X}$	95.25	0.037	0.45	0.30	3.95
s	0.139	0.00689	0.0198	0.0414	0.214

[1]Means of three determinations. Means in column groups followed by the same letter are not significantly different at the 5% level.

II. RECOVERABLE OLEORESIN

The concentration of recoverable oleoresin in waste pepper juice is shown in Table III along with the mean and standard deviation. The mean concentration of oleoresin in waste pepper juice was 0.0406% which is close to but less than the fat content of the juice, indicating that some fat was lost in the recovery procedure. The oleoresin in dry matter was 0.854% which was much higher than the amount in pepper juice. So it would be more economical if the oleoresin could be separated from dried pepper juice as is done in preparing paprika oleoresin that is extracted from ground paprika pods (30). But, presently preparation of dried pepper juice is not economically feasible. The mean moisture content of pepper juice is 95.25% and energy and equipment to remove this amount of water would not be justified from the amount of oleoresin present.

In the experiment, Celite filteraid was used to adsorb oleoresin and remove it from the juice. In this case, a relatively large amount of celite filteraid is used to separate the small amount of oleoresin in the juice (about 100 g of filteraid for each g of oleoresin). Considering the current price of filteraid, $20 per 300 g, the process would not be economical unless the filteraid could be recycled. However, the celite filteraid can be recycled (15).

After recovery of pepper oleoresin from filteraid by ethyl ether, the residual ethyl ether can be evaporated at room temperature and the dried filteraid ignited at 550°C for two hours to burn away organic material. Previous work showed that the filteraid could be reused up to five times without loss of efficiency (15).

TABLE III

CONCENTRATION OF RECOVERABLE OLEORESIN IN WASTE PEPPER JUICE

Sample	Percentage in juice	Percentage in[1] dry matter
1	0.0470	0.991
2	0.0434	0.764
3	0.0413	0.716
4	0.0383	0.912
5	0.0324	0.887
$\overline{X}$	0.0406	0.854
Std. Dev.	0.0015	0.112

[1]Based on data of Table II.

III. ANALYSIS OF PEPPER OLEORESIN AS A FOOD COLORANT AND COMPARISON WITH COMMERCIAL PAPRIKA OLEORESIN

Figure 4 shows scans of pepper and paprika oleoresins over the range of 350 to 650 nm. The scans are very similar so the two oleoresins have very similar tinctorial character. Thus, the pepper oleoresin could be used like paprika oleoresin as a colorant in all those foods which use paprika oleoresin currently.

Table IV shows the A.S.T.A. color values for pepper and paprika oleoresins along with the means and standard deviations for A.S.T.A. color values. As Table IV shows, the pepper oleoresin has an A.S.T.A. color value 2.37 times larger than the color value of paprika oleoresin. Consequently, it has more tinctorial strength. If one wanted to set a standard color value for the pepper oleoresin vegetable oil could be added as a diluent.

In Table IV, the means shown for pepper oleoresin A.S.T.A. color value are the means of duplicate determinations on five separate samples of pepper juice collected on the same day at different times. These means show a coefficient of variation of 1.62% and a range of 184. The means of A.S.T.A. color value for pepper oleoresin showed the variation in color value of pepper oleoresin separated from different samples of pepper juice. The standard deviation of A.S.T.A. color values for pepper oleoresin showed the variation in recovery procedure of pepper oleoresin. However, a uniform sample of pepper oleoresin could be made by mixing pepper oleoresin obtained from different samples of juice.

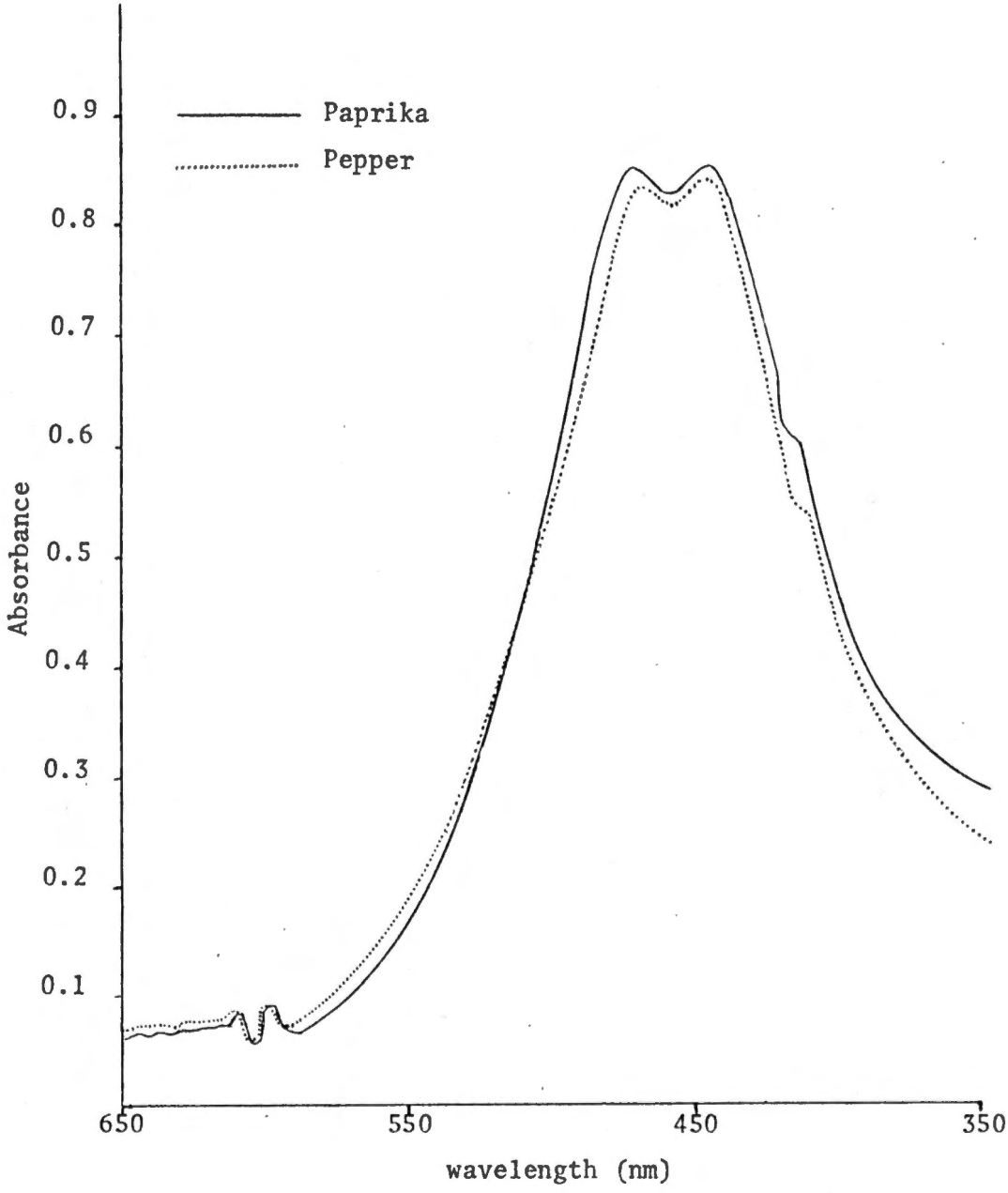

Figure 4. Absorbance of Pepper and Paprika Oleoresins in the Range of 650-350 nm.

TABLE IV

A.S.T.A.[1] COLOR VALUES FOR PEPPER AND PAPRIKA OLEORESINS

Sample	Pepper Oleoresin[2]	Paprika Oleoresin[3]
	A.S.T.A. Units[4]	
1	2163 ab	918.0
2	2237 ac	926.5
3	2155 abd	924.0
4	2300 c	921.5
5	2107 bd	925.5
$\overline{X}$	2192	923.1
Std. Dev.	35.62	1.37

[1]American Spice Trade Association (14).

[2]Means of duplicate determinations on 5 separate samples.

[3]Means of duplicate determinations, all on one sample.

[4]Means followed by the same letter are not different at the 5% level of significance.

Means presented for A.S.T.A. color values of paprika oleoresin in Table IV are the means of duplicate determinations, all on one sample. The coefficient of variation of 0.15% and a range of 8.5 was found. The standard deviation for paprika oleoresin A.S.T.A. color values was found to be 1.37 which could be taken as a measure of precision in the A.S.T.A. method.

Table V shows the CIE color parameters, x, y, and Y_{CIE} for pepper and paprika oleoresins at two different concentrations. Analysis of variance of these data is shown in Table VI. There was no significant difference in color at the 5% level. Therefore, both of the oleoresins had the same color.

This fact shows that pepper oleoresin could be substituted for paprika oleoresin. Pepper oleoresin could be another orange to yellow natural food colorant and could provide a uniform color the same as paprika in a variety of foods. The pepper oleoresin would be technically feasible to color fat-based foods such as butter, margarine, shortenings, cheese, ice cream, cake mix, breadings, frozen french fried potatoes, vegetable oil, salad dressings, whipped cream, cream toppings, creamed foods, popcorn oil and soups.

IV. STABILITY STUDY

The effects of temperature, air flow and light on pepper and paprika oleoresins are shown in Table VII as determined by analysis of variance. Linear and quadratic main effects, as well as linear and quadratic two factor interactions, are presented. Block effect was

TABLE V

C.I.E.[1] COLOR PARAMETERS FOR PEPPER AND PAPRIKA OLEORESIN

Oleoresin	Level[2]	x[3]	y[3]	$Y^3_{C.I.E.}$
Pepper	1	.3659	.3723	87.45
	2	.4247	.4198	76.82
Paprika	1	.3647	.3743	88.23
	2	.4251	.4248	77.76

[1]Commission Internationale de l'Eclairage.

[2]Solution in ethyl alcohol with absorbance of 0.32 for level 1.0 and 0.75 for level 2, absorbance at 450 nm.

[3]Means of two determinations.

TABLE VI

ANALYSIS OF VARIANCE OF C.I.E.[1] COLOR PARAMETERS OF PAPRIKA AND PEPPER OLEORESIN AT TWO CONCENTRATIONS

Source	d.f.	Mean Square	F[2]
Parameter "x"			
Oleoresin, level 1	1	1450×10^{-8}	0.64^{ns}
Error	2	2257.5×10^{-8}	
Oleoresin, level 2	1	18×10^{-8}	0.0054^{ns}
Error	2	3281×10^{-8}	
Parameter "y"			
Oleoresin, level 1	1	400×10^{-8}	0.13^{ns}
Error	2	3050.5×10^{-8}	
Oleoresin, level 2	1	2500×10^{-8}	0.59^{ns}
Error	2	4232.5×10^{-8}	
Parameter "Y_{CIE}"			
Oleoresin, level 1	1	0.61	0.55^{ns}
Error	2	1.1005	
Oleoresin, level 2	1	.883	1.21^{ns}
Error	2	.732	

[1]Commission Internationale de l'Eclairage.

[2]"ns" means not significant at the 5% level.

TABLE VII

ANALYSIS OF VARIANCE OF EFFECT OF TEMPERATURE, AIR FLOW AND LIGHT ON PEPPER AND PAPRIKA OLEORESIN

Source	DF	SS	MS	F[1]
Oleoresin	1	1.424	1.424	346 **
Temperature	2	3.433	1.716	417 **
Temp., linear	1	3.247	3.247	243 **
Temp., quad.	1	0.186	0.186	45 **
Color × Temperature	2	1.328	0.664	161 **
C.1 × T linear	1	0.25	0.25	60 **
C.1 × T quad.	1	0.0025	0.0025	0.61
C.2 × T linear	1	4.196	4.196	1020 **
C.2 × T quad.	1	0.313	0.313	76 **
Air Flow	2	0.0065	0.0033	0.79
C × A	2	0.0062	0.0031	0.76
T × A	4	0.0257	0.0064	1.56
C × T × A	4	0.027	0.0068	1.65
Light	2	0.046	0.023	5.61**
Light, linear	1	0.045	0.045	11.06**
Light, quad.	1	0.000704	0.000704	0.17
C × L	2	0.011	0.0055	1.32
T × L	4	0.00296	0.00074	0.18
C × T × L	4	0.0073	0.0018	0.44
A × L	4	0.00197	0.00049	0.12
C × A × L	4	0.0021	0.00052	0.127
T × A × L	8	0.0002	0.00078	0.189
C × T × A × L	8	0.002	0.00025	0.06
Residual	54	0.2222	0.00411	
Total	107	6.55	0.06123	

[1]"**" means significant at 1% level.

found to be insignificant based on the two replications that were made and, thus, was combined into the residual error term.

The type of oleoresin was a highly significant effect. This means that the two kinds of oleoresin respond differently to different temperatures, air flows and light intensities.

Temperature had a significant positive effect on the destruction of color in two oleoresins. This agrees with Lease and Lease (19) who found as drying temperature was increased, color retention of ground pepper in storage was markedly reduced and the lowest drying temperature gave the highest initial color. Lease and Lease (21) also found that the loss of color in red peppers was increased when a sample of newly harvested peppers was removed from 5°C storage to 25°C or 37°C.

When the sum of squares for the effect of temperature was partitioned to show the type of variation, the linear and quadratic effects were both significant. However, the linear effect of temperature had a higher "F" value.

Light intensity had a significant positive effect on loss of color in the two oleoresins. This agrees with Lease and Lease (20) who found cayenne or paprika peppers to exhibit surface bleaching when exposed to ultraviolet light or sunlight. De la Mar (8) also observed a loss of 96% of carotenoids on exposure to sunlight. When the sum of squares for this effect was partitioned to show the type of variation, only the linear effect was found to be significant. Figure 5 shows the effect of light on the destruction of color in two oleoresins. The regression equation for the effect of light was found to be:

$$y = 0.1714 + 0.02514 \times$$

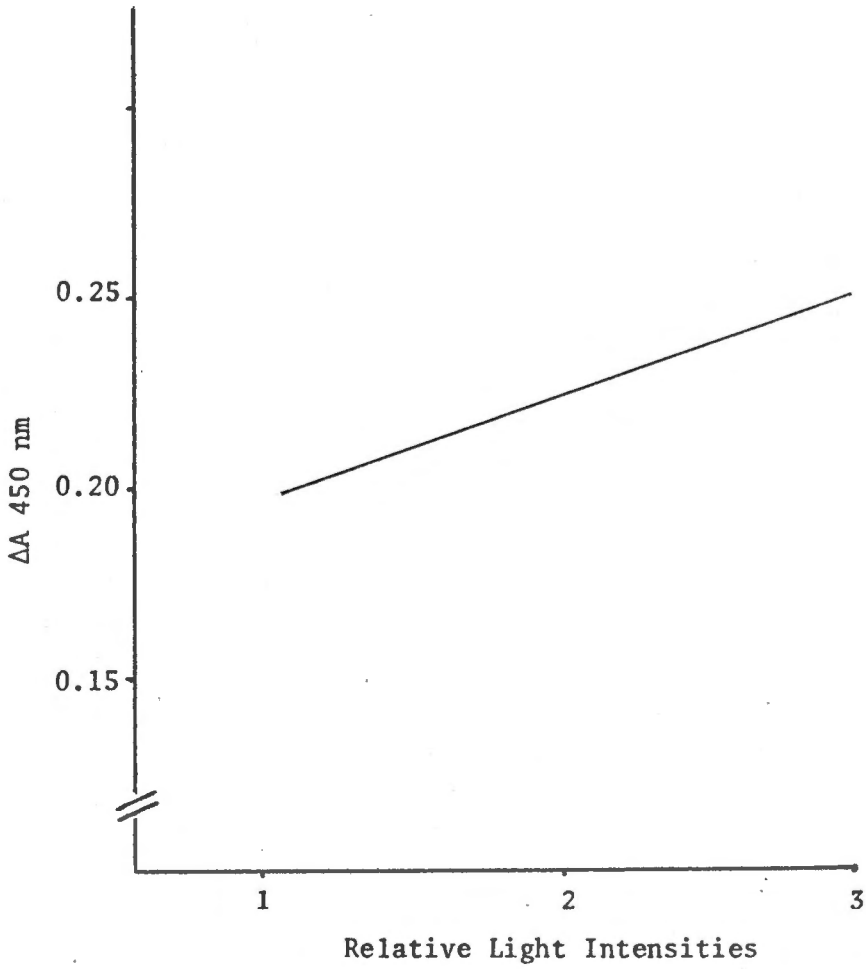

Figure 5. The Effect of Light on the Destruction of Color in Two Oleoresins.

The effect of rate of air flow was not significant. This indicated that the rate of oxygen absorption by carotenoids reached a peak at the 5 ml/min flow rate. The rate of oxygen absorption can be changed by the catalytic effect of light or high temperature but for a specific condition it reaches a specific rate. Philip and Francis (28) found that the rate of oxygen absorption by capsanthin reaches a peak corresponding to one mole of oxygen/mole of capsanthin.

The interaction of colorant with temperature was significant. The linear effect of pepper oleoresin with temperature was found to be significant but its quadratic effect was not significant. Both linear and quadratic interactions were significant for paprika oleoresin with temperature.

Figure 6 shows the effect of temperature on the destruction of color in two oleoresins. The regression equation for the effect of temperature on pepper oleoresin was found to be:

$$y = -0.1431 + 0.00556 x$$

The regression equation for the effect of temperature on paprika oleoresin was:

$$y = 0.6572 - 0.0418 x + 0.0007173 x^2$$

Examination of Figure 6 shows that paprika oleoresin undergoes oxidative degradation at a much faster rate than does pepper oleoresin as temperature increases. This could have definite implications in applying the pepper oleoresin in food products. Those colored with pepper oleoresin should show better stability at elevated temperature during storage if all other conditions are the same.

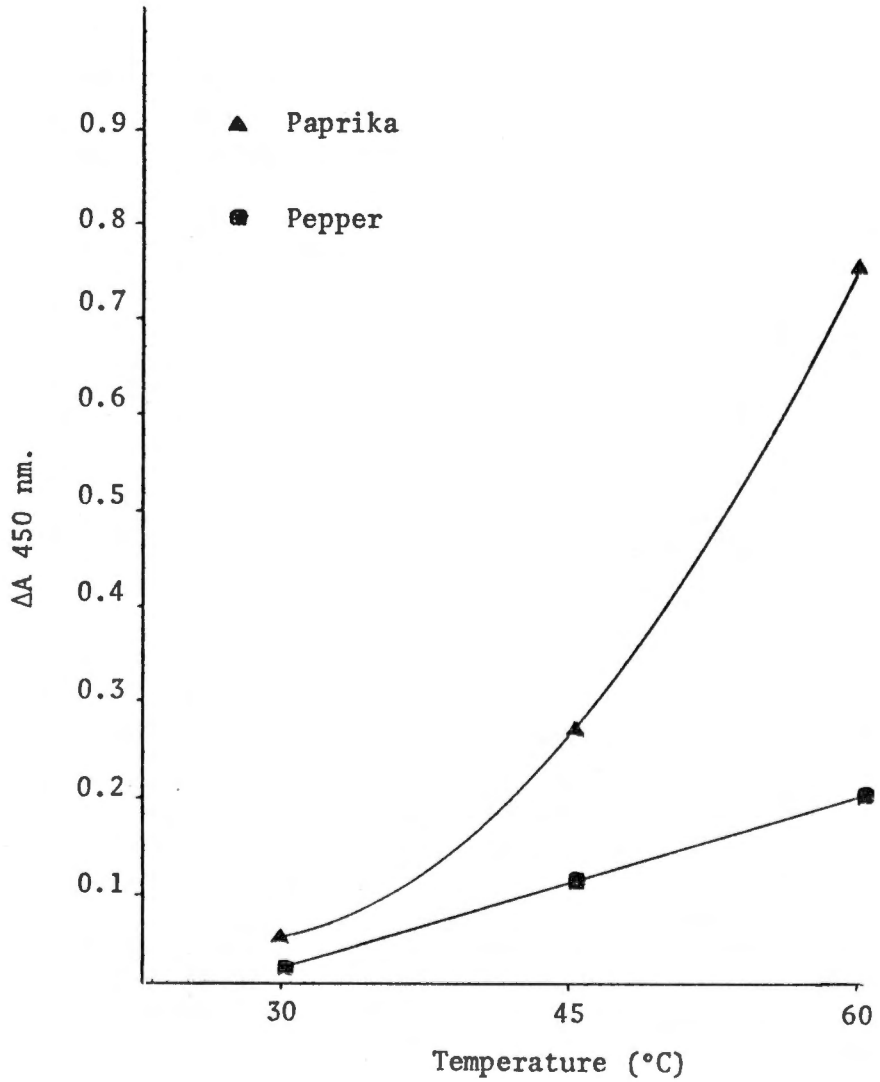

Figure 6. The Effect of Temperature on the Destruction of Color in Two Oleoresins.

V. USE OF OLEORESIN TO COLOR FRENCH DRESSING

Two batches of French dressing were made. Each was divided; pepper oleoresin was added to one part and paprika oleoresin was added to the other part to give equivalent color as measured by Hunterlab instrument. Y_{CIE} with a maximum different of ±0.2 was used to match the color. Tables VIII and IX show the results of triangle panel sensory evaluation of French dressing for color and flavor. In sensory evaluation for color, no differences were found over a 45-day storage period. This showed that the two oleoresins imparted very similar color to the dressing when it was first made. The difference could not be detected by the observers over the 45-day storage period. This showed that the colors of two oleoresins remained very close and any differences were undetectable by the human eye. The pepper oleoresin was at least as good as paprika oleoresin and could be used in many foods as a colorant to give the same color, uniformity and stability as paprika oleoresin.

In sensory evaluation for flavor, Table IX, a difference in flavor was found in one replicate at three storage periods but no preference was indicated. However, in the experiment no spices were used in French dressing and it is probable that the difference in flavor could be covered by the spices used in a commercial French dressing.

VI. STATISTICAL ANALYSIS OF HUNTERLAB x, y AND Y_{CIE} DATA

Table X shows the C.I.E. color parameters of objective color measurements in French dressing made with two oleoresins and stored over

TABLE VIII

SENSORY EVALUATION OF FRENCH DRESSING
BY TRIANGLE TEST FOR COLOR

Days Storage	Replicate	Panelists	Correct Choices	Difference[1]	Preference
		------- No. -------			
0	1	23	11	no	none
	2	25	6	no	none
15	1	25	10	no	none
	2	24	8	no	none
30	1	25	9	no	none
	2	20	9	no	none
45	1	20	10	no	none
	2	24	7	no	none

[1]Determined at 5% level of significance (18).

TABLE IX

SENSORY EVALUATION OF FRENCH DRESSING
BY TRIANGLE TEST FOR FLAVOR

Days Storage	Replicate	Panelists	Correct Choices	Difference[1]	Preference[1]
		-------- No. --------			
0	1	23	13	yes	none (7 of 13)
	2	25	6	no	none
15	1	25	6	no	none
	2	24	10	no	none
30	1	25	15	yes	none (8 of 15)
	2	20	6	no	none
45	1	20	12	yes	none (6 of 12)
	2	24	11	no	none

[1]Determined at 5% level of significance (18).

TABLE X

C.I.E. COLOR PARAMETERS OF OBJECTIVE COLOR MEASUREMENTS IN FRENCH DRESSING MADE WITH TWO OLEORESINS AND STORED

Day (Storage)	C.I.E. Color Parameters	Pepper		Paprika	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2
0	x	0.538	0.544	0.536	0.544
	y	0.406	0.407	0.408	0.408
	$Y_{C.I.E.}$	35.1	33.0	35.3	33.0
15	x	0.535	0.550	0.534	0.547
	y	0.407	0.408	0.408	0.406
	$Y_{C.I.E.}$	34.6	31.8	35.2	32.3
30	x	0.540	0.552	0.534	0.548
	y	0.407	0.407	0.410	0.408
	$Y_{C.I.E.}$	34.4	30.9	35.2	32.2
45	x	0.540	0.554	0.533	0.555
	y	0.409	0.406	0.410	0.406
	$Y_{C.I.E.}$	34.4	30.8	35.4	31.2

a period of 45 days. The analyses of variance of data in Table X for color parameters x, y and Y_{CIE} are shown in Tables XI, XII and XIII. No significant differences for x, y and Y_{CIE} parameters of the two colorants were found. This showed there was no difference in the color of the two dressings induced by the colorants. In analysis of variance for color parameter Y_{CIE} in French dressing significant difference was found between the replicates. This difference was found because each replicate of French dressing was matched for color parameter Y_{CIE} separately and Table X shows the difference in the Y_{CIE} color parameters for the two replicates.

A difference in replicates was not critical in the experiment since the important thing was variation within the replicates, particularly over time. Color matching was done with the luminosity component of color, Y_{CIE}, since previous measurements had shown that the quality of the color of the two oleoresins was very similar. It can be noted that variation in Y_{CIE} did not cause variations of similar magnitude in x and y; variation in the vertical scale caused little variation in the color plane. Admittedly, the split plot analysis gave low sensitivity to variation between colorants (note that F = 144 in Table XIII is not significant), but it increased the sensitivity to time effects. This was an advantage, since performance overtime was of major importance in this part of the work. The effect of time and colorant interaction with time were not significant. Consequently, the results showed pepper oleoresin and paprika oleoresin are stable colorants for French dressing during a 45-day storage and there is no significant difference in the

TABLE XI

ANALYSIS OF VARIANCE OF COLOR PARAMETER "x" IN FRENCH DRESSING MADE WITH TWO OLEORESINS AND STORED

Source	DF	SS	MS	F[1]	
Colorant	1	25×10^{-6}	25×10^{-6}	4.00	ns
Replicate	1	5602×10^{-7}	5602×10^{-7}	104.03	ns
$C \times R_{(error\ a)}$	1	625×10^{-8}	625×10^{-8}		
Time	3	605×10^{-7}	20166×10^{-9}	1.6910	ns
$C \times T$	3	85×10^{-7}	2833×10^{-9}	0.2375	ns
Error b	6	7155×10^{-8}	11925×10^{-6}		
Total	15	822×10^{-6}			

[1]"ns" means not significant at 5% level.

TABLE XII

ANALYSIS OF VARIANCE OF COLOR PARAMETER "y" IN FRENCH DRESSING MADE WITH TWO OLEORESINS AND STORED

Source	DF	SS	MS	F[1]	
Colorant	1	31×10^{-7}	31×10^{-7}	1.0438	ns
Replicate	1	513×10^{-8}	513×10^{-8}	1.7273	ns
$C \times R_{(error\ a)}$	1	297×10^{-8}	297×10^{-8}		
Time	3	169×10^{-8}	56×10^{-8}	0.3544	ns
C × T	3	361×10^{-8}	12×10^{-7}	0.7595	ns
Error b	6	95×10^{-7}	158×10^{-8}		
Total	15	26×10^{-6}			

[1]"ns" means not significant at the 5% level.

TABLE XIII

ANALYSIS OF VARIANCE OF COLOR PARAMETER "$Y_{C.I.E.}$" IN FRENCH DRESSING MADE WITH TWO OLEORESINS AND STORED

Source	DF	SS	MS	F[1]	
Colorant	1	1.44	1.44	144	ns
Replicate	1	37.21	37.21	3721	*
$C \times R_{(error\ a)}$	1	0.01	0.01		
Time	3	2.985	0.995	0.7605	ns
C × T	3	0.465	0.155	0.1185	ns
Error b	6	7.85	1.3083		
Total	15	49.96			

[1]"*" means significant at 5% level, and "ns" means not significant at 5% level.

color of French dressings made with pepper and paprika oleoresin. Pepper oleoresin can be used as well as paprika oleoresin in French dressing and probably other similar products as a stable and uniform natural food colorant.

CHAPTER V

SUMMARY

The purpose of this investigation was the extraction, characterization and use of a carotenoid colorant from waste red bell pepper juice. The carotenoids extracted from the pepper juice or the oleoresin was used in French dressing. Commercial paprika oleoresin, an approved food colorant, was used as a reference for comparison with pepper oleoresin throughout the investigation.

The mean concentration of recoverable oleoresin in waste pepper juice that was used as the source for pepper oleoresin was found to be 0.0406% and 0.854% in terms of total dry matter.

The mean A.S.T.A. color value for pepper oleoresin was found to be 2192, 2.3 times that of paprika oleoresin which had a mean A.S.T.A. color value of 923. This showed more tinctorial strength for pepper oleoresin. Analysis of variance for pepper and paprika C.I.E. color parameters in alcoholic solution did not show any significant difference between x, y and Y_{CIE} values. Consequently, the same color was found for the two oleoresins.

Stability of pepper oleoresin compared to paprika oleoresin was investigated by studying the effects of temperature, light and air on the degradation of color. After a 5 ml sample of benzene solution of two oleoresins was dried on the inside wall of a small square sample bottle, the films were subjected to different combinations of light, air and temperature. A decrease in absorbance of 450 nm of the same

sample dissolved in 5 ml. isopropanol was measured as destruction of carotenoids. A $3^3 \times 2 \times 2$ factorial in a completely randomized array was used as the experimental design.

Seven factors or interactions of factors were found to have a significant effect on degradation of the carotenoids. These were the type of oleoresin, the linear and quadratic effect of temperature, the linear effect of pepper oleoresin with temperature, the linear and quadratic effect of paprika oleoresin with temperature and the linear effect of light. Using these seven factors and interactions, regression polynomials were derived.

Batches of French dressing were prepared containing pepper oleoresin and paprika oleoresin as colorants. Levels were adjusted to give equal color as measured by Y_{CIE} with the Hunterlab color difference meter.

The color and flavor of French dressing were followed over a 45-day storage period at room temperature and normal incident light. The dressings were examined at 0, 15, 30 and 45 days. The dressings were subjected to sensory panels at each time for color and flavor evaluation and color measurements were made with the Hunterlab color difference meter.

In sensory evaluation of French dressing, no difference was found for color. Difference was found for flavor without any preference for samples containing either colorant.

The analysis of variance of the C.I.E. color parameters of objective color measurements in French dressing made with two oleoresins and stored showed no change of color through the storage period.

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