International Journal of Food Properties, 16:766–777, 2013

ISSN: 1094-2912 print / 1532-2386 online
DOI: 10.1080/10942912.2011.565537

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DIFFERENCES IN COLOUR GAMUT OBTAINED WITH THREE SYNTHETIC RED FOOD COLOURANTS COMPARED WITH THREE NATURAL ONES: pH AND HEAT STABILITY

A. Arocas[1], P. Varela[1], Ma Lourdes González-Miret[2], A. Salvador[1], F. J. Heredia[2], and S. M. Fiszman[1]
[1]*IATA-CSIC, Agustin Escardino, Paterna, Valencia, Spain*
[2]*Laboratory of Food Colour & Quality, Department of Nutrition and Food Science, Faculty of Pharmacy, University of Seville, Spain*

The current trend in European markets towards natural ingredients has stimulated the interest in natural colourants. When it is decided to replace a colourant, normally a synthetic colourant with a natural one, it is interesting to know how the colour would be perceived, by locating their coordinates in the a*b*-diagram. The colours of three red natural colourants and three red synthetic ones have been compared. The natural colourants would be visually perceived as less intense than the synthetic colourants; this perception could be a signal of high quality as recognised and accepted by consumers. The hue (hab) of the natural colourants was red-bluish while the synthetic ones showed a red-orange character and they were located in a wider area of hue, having values apart from the rest, thus they could not be replaceable. The synthetic colourants showed less colour differences than the natural ones when subjected to pH variations and heat treatments.

Keywords: ***Food colourants, Natural colourants, Synthetic colourants, Colour, Red colourant, Colour difference, Stability.***

INTRODUCTION

From an early age, colour plays a key role in food choice by influencing taste thresholds, sweetness perception, food preference, pleasantness, and acceptability.[1,2] Thus, food manufacturers use colourants with the main objective to reinforce the colour of the natural product destroyed during processing or storage. On the other hand, the addition of colourants ensures fulfilling consumers' expectations about their preferred food colour independently from variations on the process, seasonality, or geographic origin.[3]

A typical, natural-looking colour in a food or beverage will signal high quality, while an artificially bright product can give the opposite impression. Also, colours derived from natural sources, such as beetroot or grapes, are more readily recognized and accepted by consumers. The use of natural colours is most prevalent in foods that portray a healthy image. In fact, natural colour pigments have been recognized for their health effects. The trend to launch 100% natural-flavored and natural-coloured applications is stronger and

Received 27 September 2010; accepted 10 February 2011.
Address correspondence to A. Salvador, IATA-CSIC, Agustin Escardino 7, Paterna, Valencia 46980, Spain. E-mail: asalvador@iata.csic.es

stronger. It is a means to create differentiation and attract consumers, who are, as well, more and more interested in healthier and more-natural food.

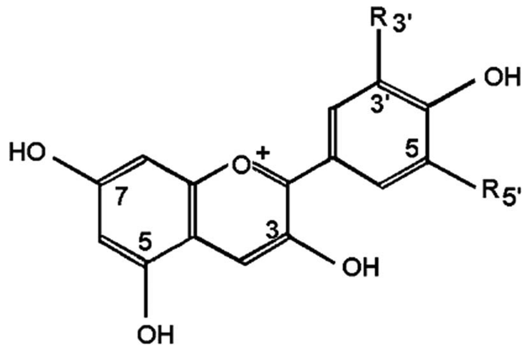
In the last few years, a marked trend towards the use of natural colourants exists due to the current preoccupation for the use of synthetic products.[4] Besides, natural compounds, such as anthocyanins or carotenes, have demonstrated to have high antioxidant, antiradical, and antiproliferative activities, increasing the nutritional value of the foods in which they are included.[5,6] However, it is well known that natural colourants in general are more unstable to the effect of light, temperature, or changes in pH than their artificial counterparts. The colour gamut given by natural products are narrower and less intense in colour than that for synthetic colourants, making it necessary to use higher doses. Moreover, due to their natural origin, variations between different groups could be higher than for synthetic colourants. Hence, the information given to the food manufacturers is very important regarding the differences in colour stability that they can expect in case they use natural instead of synthetic colourants,[7,8] but more interestingly is the gamut range that they could cover as it is the most important factor for human perception.

Cochineal is a water-soluble colourant obtained from shells of the female insect *Dactylopius coccus* Costa. This insect produces carminic acid from which the colourant is extracted. Cochineal is a glycoside of anthraquinone in which the glucose and the aglycone are jointed by means of an unusual link.[9] It is very labile to pH changes, in such a way that the variations depend on the medium in which it is included. So, it shows a red-bluish hue in an alkaline medium and a reddish hue in an acid medium. However, this product is stable to the effect of light and temperature.

Anthocyans can be found in fruits, flowers, and vegetables. This group of compounds is responsible for pink, red, purple, violet, and blue shades. Cyanidin and its glycosides represent the majority of the anthocyanins in nature. The colour of the anthocyanins depends on the pH,[10–12] as well as on their structure, in such a way that they can show orange, reddish, or bluish hues. Due to this fact, the use of anthocyans as colourants is very restricted, and that is why they are generally used in acid foods that need to maintain their colour. Anthocyanins are stable to medium temperatures, oxidation-reduction reactions, and light.[13] *Enocyanin* is a mixture of anthocyanins (peonidin, malvidin, delphinidin, and petunidin) extracted from red grape skin as a by-product in the winemaking process[14] (Fig. 1).

Black carrot colourant has different pigments obtained from *Daucus carota* spp. s*ativus* var. *atrorubens* Alef. This vegetable has been considered as a potential source of anthocyanins due to its higher stability because of its acylation compared to those obtained from other sources.[15] Its colour is less susceptible to degradation from heat and light exposure, which naturally extends the shelf-life of the end product. Moreover, black carrot exhibits a wide array of red hues ranging from strawberry to burgundy wine. It is an excellent alternative to carmine in low pH applications. Its performance is superior to other anthocyanin colourants; it maintains its red colour at higher pH values and it does not interact with proteins due to its low polyphenolic content.

On the other hand, synthetic colourants are widely used due to their colouring properties, uniformity, stability, and low cost. Ponceau 4R (E124), carmoisine (E122), and allura red (E129), are synthetic azo colourants generally used to give red colour to a number of foods. *Ponceau 4R* is also known as Food Red 7, C.I. 16255, Cochineal Red A, New Coccine, Acid Red 18, SX purple, and its chemical name is trisodium salt of 1-(4-sulpho-1-napthylazo)-2-napthol-6,8-disulphonic acid. It is a synthetic coal tar and red azo dye,



Petunidin: R3' = OMe, R5' = OH
Delphinidin: R3' = R5' = OH
Peonidin: R3' = OMe, R5' = H
Malvidin: R3' = R5' = OMe

Figure 1 Chemical structure of enocyanins.

water soluble powder, which can be used in a variety of food products, such as dessert toppings, jelly, salami, seafood dressings, tinned strawberries and fruit pie fillings and packed cake mixes, cheesecakes, soups, and trifles.[16] *Carmoisine*, also called Azorubine, Food Red 3, Brillant carmoisin O, Azorubin S, Acid Red 14, or C.I. 14720, is a synthetic red food colourant from the azo dye group. Carmoisine consists essentially of disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo) naphthalene-1-sulfonate and subsidary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. It usually comes as a disodium salt. It is a red to maroon powder used for the purposes where the food is heat-treated after fermentation such as blancmange, marzipan, Swiss roll, jams, preserves, yoghurts, jellies, breadcrumbs, and cheesecake mixes.

Allura Red AC is also called Allura Red, Food Red 17, C.I. 16035, and FD&C Red 40, is a red azo dye. The chemical designation is 2-hydroxy-1-(2-methoxy-5-methyl-4-sulfonato-phenylazo) naphthalene-6-sulfonate, disodium salt. It is used as a food colourant originally introduced in the United States as a replacement for the use of E123 (Amaranth). It has the appearance of a dark red powder and it usually comes as a sodium salt, although it can be also used as both calcium and potassium salts. Despite the popular misconception, allura red AC is not derived from the cochineal insect. It is an orange red hue colourant, soluble in water, propylene glycol, and glycerin. This solubility is vital to its colouring power, since the process of dissolving is the only method for producing the physical colour. Its colouring strength is directly proportional to the amount of colourant in the product. It also has good stability when exposed to heat and/or light. This red colourant is commonly used in gelatine desserts, puddings, dairy products, confections, beverages, and condiments.[17]

There is little work studying the differences between natural and synthetic colourants, within colour gamut, regarding their colourimetric parameters. Generally, chemical or spectral differences are analyzed, but no information about colour perception is given. Knowing colourimetric variations and changes in colour due to changes in pH would be very interesting for the food industry. In this study the hue area in the a*b*-diagram of three red natural colourants (cochineal, enocyanin, and black carrot) has been compared to three red synthetic ones (ponceau 4R, carmoisine, allura red AC) in water solution to analyze the perceived colours obtained. Also, the colour differences occurring with changes of pH and heat treatment have been assessed.

MATERIAL AND METHODS

Colourant Solutions Preparation

Three natural colourants (cochineal [E120], spray-dried enocyanin [E163], and black carrot [E163]) and three synthetic colourants (allura red [E129], carmoisine [E122], and ponceau 4R [E124]) have been used; all of them were water-soluble food colourants provided by Roha Epsa (Torrente, Spain). Different aqueous solutions of each colourant were prepared, according to the manufacturers' recommendations: 0.01% for ponceau 4R, carmoisine, and allura red; 0,005% for cochineal; and 0.1% for enocyanin and black carrot.

pH Stability of Colourant Solutions

The colourants were dissolved in solutions with different pH values: 8.03, 6.73, 5.42, 4.16, and 2.85. These solutions were obtained by mixing two solutions, 0.1% citric acid (pH = 2.83) and 0.3% sodium citrate (pH = 8.07), at different proportions. The pH was measured with a Crison Micro pH 2001 pH-meter (Crison Instruments, Alella, Spain).

Heat Stability of Colourant Solutions

The colourant solutions were poured into Erlenmeyer flasks and stirred in a heat-water bath at 80°C for 30 min; after this, they were quickly cooled to 20°C by introducing the flasks in a bath with water and ice.

Spectral and Colourimetric Measurements

Spectrophotometric analyses of the solutions were made using 2-mm path length disposable plastic cells. The whole visible spectrum (380–700 nm) was recorded. Absorption spectrum ($\Delta\lambda = 2$ nm) measurements were made with a Hewlett-Packard UV-visible HP 8452A diode array UV visible spectrophotometer (Palo Alto, CA, USA). Colour parameters from transmission spectrum were calculated by using the CromaLab® software.[18] D65 standard illuminant and 10° standard observer were considered in the calculations. Reference blank measurements were made with the cuvette filled with distilled water.

The CIELAB parameters (L*, a*, b*, C^*_{ab}, h_{ab}) for all samples were determined following the recommendations of the Commission Internationale de L'Eclairage.[19] Within the uniform space CIELAB, two colour coordinates, a* and b*, as well as a psychometric index of lightness, L*, are defined. a* takes positive values for reddish colours and negative values for the greenish ones, whereas b* takes positive values for yellowish colours and negative values for the bluish ones. L* is an approximate measurement of lightness, which is the property according to which each colour can be considered as equivalent to a member of the grey scale, between black and white, taking values within the range 0–100, respectively.

From the uniform colour spaces, new parameters are defined, such as chroma (C^*_{ab}) and hue (h_{ab}). Chroma (C^*_{ab}) is the attribute that allows the determination of the degree of difference in comparison to a grey colour with the same lightness for each hue, so it is considered to be the quantitative attribute of colourfulness.

$$C^*_{ab} = \left[(a^*)^2 + (b^*)^2\right]^{1/2}.$$

Hue (h_{ab}) is the attribute according to which colours have been traditionally defined as reddish, greenish, etc. It is the attribute that allows a colour to be distinguished with reference to a grey colour with the same lightness. This attribute is related to the differences in absorbance at different wavelengths and is considered to be the qualitative attribute of colour.

$$h_{ab} = \arctan\left(b^*/a^*\right).$$

Colour differences (ΔE^*_{ab}), which are important to evaluate relationships between visual and numerical analyses, were calculated as the Euclidean distance between two points in the three-dimensional space defined by L*, a*, and b*.

$$\Delta E^*_{ab} = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}.$$

Statistical Analysis

An analysis of variance (ANOVA) was performed to study the effect of pH in the colour differences of colourant solutions with and without heat treatment. Least significant differences were calculated by Fisher's test and the statistical significance was determined at $p < 0.05$. These analyses were performed using the Statgraphics Plus program version 5.1 (Bitstream, Cambridge, MA, USA).

RESULTS AND DISCUSSION

Spectra of Colourant Solutions

Once dissolved in distilled water, all the studied colourants presented remarkable visual differences among them. By observing the absorbance spectra (Fig. 2), it can be seen that, as expected, the six colourants showed the maximum of absorbance around 520 nm, corresponding to green absorbance (i.e., red transmission).

The λ_{max} of the visible absorption spectra ranged from 486 to 526 nm, corresponding to allura red (synthetic) and black carrot (natural), respectively.

Furthermore, a much higher colour intensity was observed for the three synthetic colourants than for the three natural ones, having maximum absorbance between 3.011353 (ponceau 4R) and 3.432312 (allura red) for synthetic, and 0.491577 (cochineal) and 0.896240 (black carrot) for natural colourants.

Location of Colourant Solution Samples in the (a*b*)-Diagram

For a real colourimetric characterization and evaluation, modifications in the whole visible spectrum were taken into account. Therefore, the total colour change was monitored

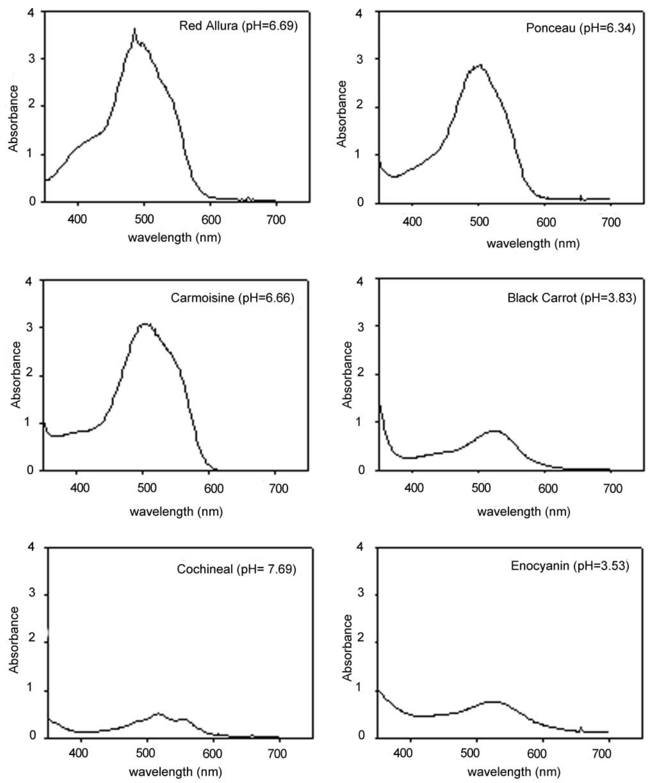


Figure 2 Absorption spectra of the aqueous solutions of the natural and synthetic studied colourants.

in the CIELAB colour space. Thus, colour parameters from a transmission spectrum were calculated and a* and b* values were represented in the (a*b*)-space (Fig. 3).

As expected, the natural colourants appeared grouped around 0° of hue angle (between −15° and +5°), corresponding to red or red-bluish colours having between 36 (cochineal) and 49 (black carrot) CIELAB units of chroma. The lightness values were between 60 (enocyanin) and 80 (cochineal) CIELAB units (data not shown in the figure), being in the medium zone of the scale from 0 to 100 units.

The synthetic colourants were located in a wider area of hue, from 20 to 50°, in the red-orange zone, and with higher values of chroma, from 86 (carmoisine) to 102 (allura red) CIELAB units. The lightness showed values from 50 (carmoisine) and 62 (ponceau 4R) CIELAB units (data not shown in the figure), indicating darker colours.

From these results, it can be stated that, under the assayed conditions, the natural colourants were visually perceived as less vivid (lower chroma values) and lighter (higher lightness values), that is, less intense than the synthetic colourants. It is interesting to point out that perception of these less vivid colours is normally interpreted as a more "natural" colour by consumers. On the other hand, from an economical point of view, it has to be taken into account that natural colourants needed to be at least 10 times more concentrated than synthetic colourants.

The black carrot and the enocyanin had similar values of hue, so they could be used alternatively since they are qualitatively similar, with a small difference in chroma, which

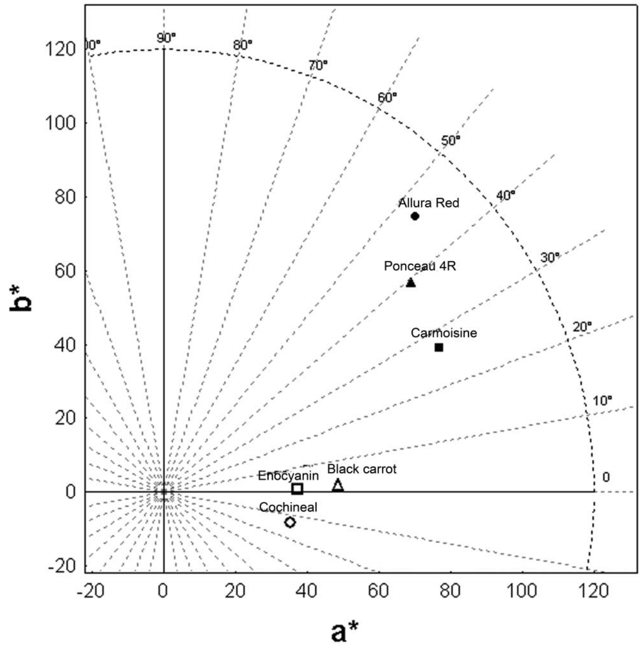


Figure 3 CIELAB colour space. Location of the aqueous solutions of the six colourants in the (a*b*)-space.

could possibly be compensated with the level of dilution. On the other hand, the synthetic colourants showed hue values different from the rest, so they could not be replaceable; they attained "brighter" colours but were normally associated with "artificial."

Effect of the pH on L*, h_{ab}, and C^*_{ab} Values

In Fig. 4, the six studied colourants and the variation of their colours as the pH changes are represented. It can be seen that greater changes occurred for the natural colourants than for the synthetic ones, showing a higher dispersion of the colour points. The synthetic colourants underwent lesser variations, showing independent groups for each colourant. Depending on the degree of acidity or alkalinity, anthocyanins adopted different chemical structures (Fig. 5). Each of these structures presented, in the visible region, a characteristic absorption spectrum. At present, it is generally accepted that there is an equilibrium between the flavylium (2-phenylbenzopyrylium) cation (AH^+) and the carbinol (B) in acidic media.[20–22] At low pH, the red form of the anthocyanin is favoured. As the pH increases, the red form converts into a blue form and a non-coloured form. Therefore, the hue of an anthocyanin solution becomes bluer and less intense as pH increases; this fact would be inconvenient in coloured foods that change their pH during manufacturing, such as yoghurt. While the blue form can revert back to the red form, the non-coloured form of the anthocyanin can either revert back to the red form or can undergo an irreversible hydrolysis of the flavylium portion of the anthocyanin that prevents reversion to the red form.

Lightness evolution. The synthetic colourants showed lower lightness (L*) values, between 50 and 65 CIELAB units, than the natural ones within the studied pH interval;

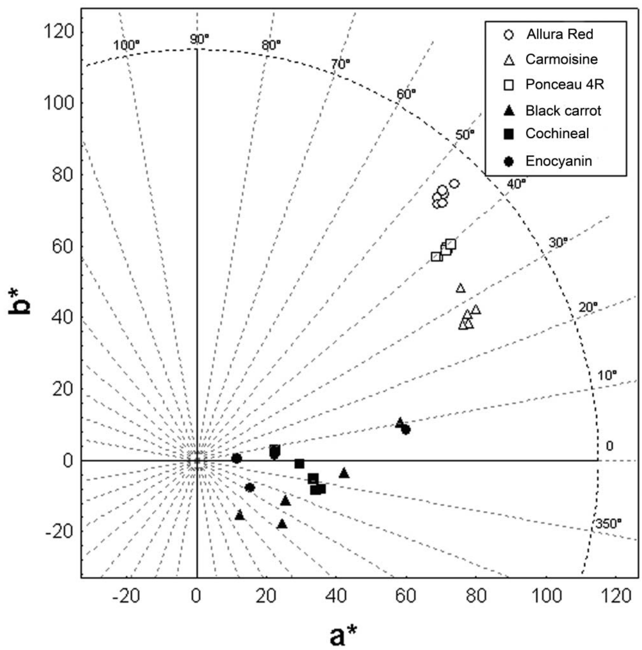


Figure 4 CIELAB colour space. Location of the aqueous solutions of the six colourants in the (a*b*)-space at different pH values.

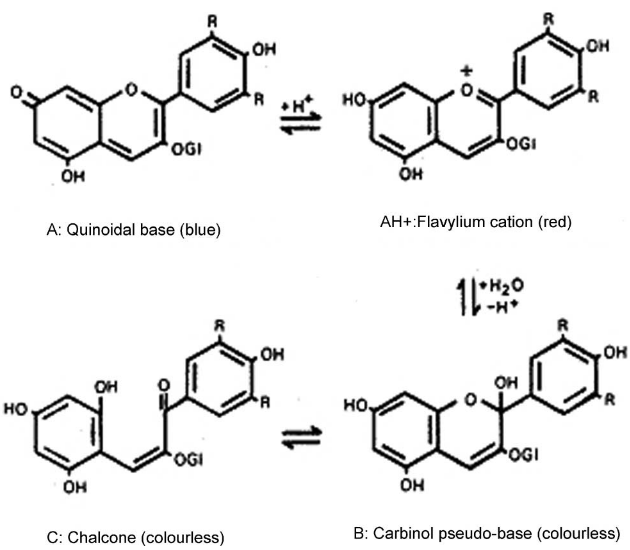


Figure 5 Anthocyanin chemical structure transformations with pH.

thus, they were perceived as darker colours (Fig. 6a). Moreover, smaller variations on L* values due to changes in pH are observed for synthetic more than for natural colourants (50 to 85 CIELAB units).

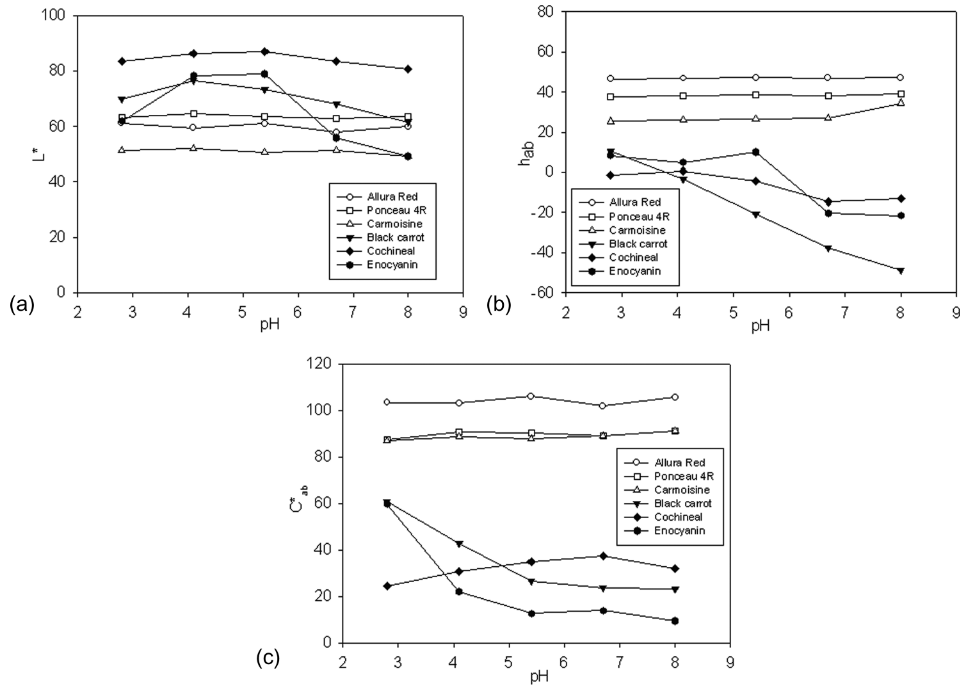


Figure 6 (a) L*, (b) h_{ab}, and (c) C^*_{ab} evolution as the pH change for each colourant.

In aqueous solutions, common anthocyanins exist as a mixture of several structures in chemical equilibrium having different colourimetric characteristics. The hydration of the flavylium cation (red) gives the hemiacetal form in equilibrium with a chalcone (colourless or light yellow).[11] In the case of enocyanin, lightness increases progressively, reaching a maximum at pH 5.42 due to the formation of colourless forms, and then a sharp decrease occurs as the pH increases indicating that other coloured forms, such as quinonoidal anhydrobase (blue), are being formed. A similar behaviour was observed for black carrot colourant, although enocyanin was the colourant having greater variations on L* values due to pH changes.

Hue evolution. Within the range of pH studied, the natural colourants modified their hues from 10 to −50° (from red to red-purple colours) in a wider range than the synthetic ones (from 22 to 44°, corresponding to red–red-orange colours) (Fig. 6b). The cochineal was the more stable among the natural colourants, lightly decreasing the hue as the pH increases. The enocyanin kept its hue angle at low pH values and then got much more bluish colours when pH was near neutral values. Black carrot also showed a clear decrease of the hue angle (from +10 to -50°), with a marked evolution toward the bluish area. At low pH values, anthocyanins exist basically in flavylium cation form (red), but a rapid deprotonation occurs as the pH increases toward the quinonoidal forms (blue).

Chroma evolution. As for L* and h_{ab}, changes of pH induce lower chroma variations on the synthetic colourants (between 85 and 105 CIELAB units) than on the natural ones (between 10 and 60 CIELAB units) (Fig. 6c), having higher C^*_{ab} values for synthetic colourants, which means that they would be visually perceived as more saturated and vivid colours. In the case of cochineal, an increase of chroma from 25 to 37 CIELAB units

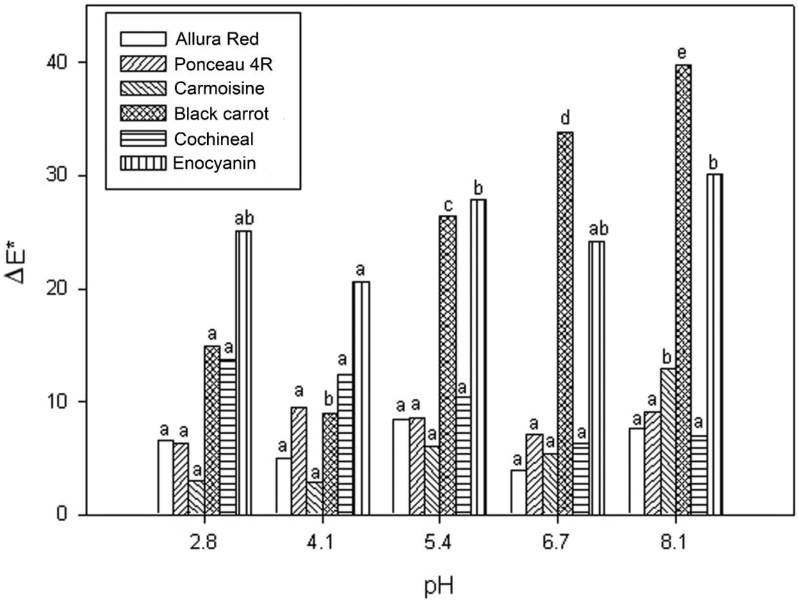


Figure 7 Colour differences (ΔE^*_{ab}) between the colour of the aqueous solution and solutions at different pH values, for each colourant. Means for each colourant without common letter differaccording to Tuckey's test ($p <$ 0.05).

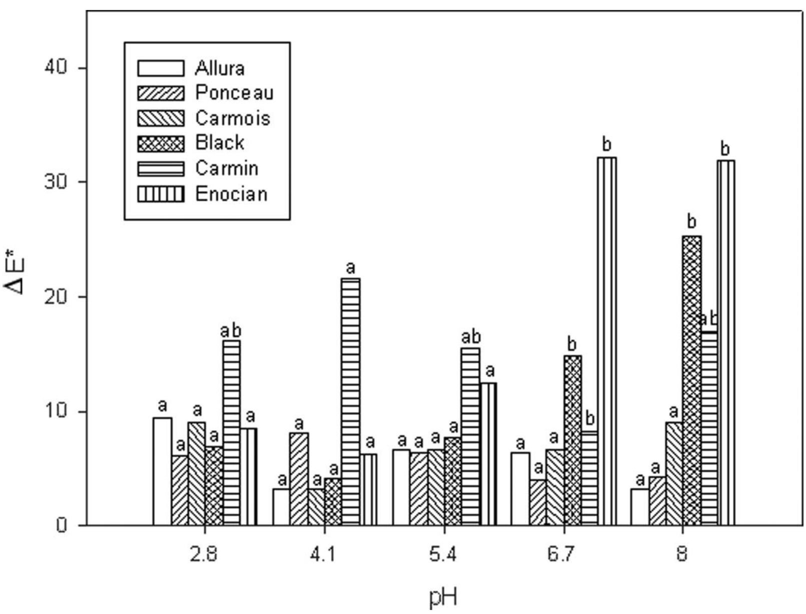


Figure 8 Colour differences (ΔE^*_{ab}) between colour before and after heat treatment, for each colourant solution at different pH values. Means for each colourant without common letter differ according to Tuckey's test ($p <$ 0.05).

occurred; hence, the solutions would be perceived as more vivid (more saturated) as the pH increases. On the other hand, black carrot and enocyanin diminished the chroma as the pH increased. Thus, the colour of these solutions would be perceived as less vivid purple colours when pH increases.

Colour Differences

The pH of the water-distilled solutions of each commercial colourant was measured. The synthetic colourants solutions as well as the cochineal one were near to neutral values (allura red: 6.46; ponceau 4R: 6.56; carmoisine: 5.40; cochineal: 6.84), while the black carrot and the enocyanin solutions showed lower values (black carrot: 3.28; enocyanin: 3.05). On the other hand, five solutions at different pH values (2.8, 4.1, 5.4, 6.7, and 8.1) were prepared for each colourant, as explained in the Material and Methods section. Then, comparisons between these solutions and their corresponding original ones were made for each colourant in order to determine the colour differences (ΔE^*_{ab}). It was found that the solutions of synthetic colourants showed lesser colour differences for all the pH values studied in relation to the original water solutions (Fig. 7), and there were no remarkable differences between them, in most of cases less than 3 CIELAB units. Colour difference around 3.0 CIELAB units is generally considered an estimate of the acceptable tolerance by the human eye for red wines poured in standard wine samplers.[23] Based on this observation, most of these solutions would be visually perceived as similar in colour.

Regarding the natural colourant, cochineal showed the smallest colour differences (ΔE^*_{ab}), although these values were higher than those of synthetic colourants; furthermore, the pH did not greatly affect its colour. Enocyanin and black carrot showed large colour differences with the pH, being the smallest for pH = 4.1. The colour differences increased as the pH increased, near neutral values. Thus, it can be expected that the colour of the final solution of these natural colourants would be more difficult to predict, even with small changes of pH.

The colour differences of each colourant solution at five different pH values were calculated against the same solution after heating at 80°C for 30 min (Fig. 8). Once again, the synthetic colourant showed smaller colour differences than the natural ones. Analysis of the colour differences before and after heating between the different pH values showed small differences in colour due to heating for the synthetic colourants, whereas important differences in colour appeared when comparing different pH values in the case of the natural colourants.

ACKNOWLEDGMENTS

The authors are indebted to the Comisión Interministerial de Ciencia y Tecnología for financial support (Project AGL2009-12785-C02-01) and are grateful to Rhoa Epsa, S.A. for the supply of samples.

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