Color and Stability of Anthocyanin pigments Isolated from Tamarillo fruit (*Solanum betaceum* Cav.)

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ABSTRACT

Crude anthocyanins extracts from tamarillo fruit (*Solanum betaceum* Cav.) by solid phase extraction using Amberlite XAD-7 were isolated. A previous separation on crude anthocyanins extracts was done using multilayer coil countercurrent chromatography, each fraction was analyzed by HPLC and further purification was carried out using preparative HPLC. Cyanidin-3-rutinoside, pelargonidin-3-rutinoside and delphinidin-3-rutinoside were determined using ESI-MS. For the crude extracts, color differences (ΔE^*_{ab}) due to change ΔpH were calculated, which allowed to define some ranges of ΔpH where the color can not be visually discriminated. It is a parameter of significant importance in searching for color additives for aqueous solutions. On the other hand, in this study it was possible to relate color quality to structural characteristics of the anthocyanins (number of hydroxyl groups in the B ring). L^*, C^*_{ab}, h_{ab}, s^*_{uv} were influenced by the hydroxylation grade.

1. INTRODUCTION

Natural food colorants are needed in the industry, however, natural red colorant are scarce because few natural extract have a bright red color unmixed with other tones (Francis[1]). Natural colorants such as anthocyanins can impart various shades of red to food color; however, there are limitations in such applications due to their solubility, difficulty in matching the desired hue, incompatibility with food matrix and stability of color to pH, light and oxygen (Markakis[2]).

The interests and motives for extended use of these colorants are influenced by their potential beneficial health effects (Netzel[3] et al). It is well known that anthocyanins properties, including color expression, are highly influenced by anthocyanins structure, pH, etc. Acylation improve the stability of anthocyanins through intramolecular copigmentation (Brouillard[4]). A relationship exists between the saturation and chroma with the number of hydroxyl groups in the B ring (Heredia[5] et al). It is of potential importance to know precise information about colors of individual anthocyanins with the purpose of having a better control of the behavior of the natural food colorants. Therefore, our objective was to evaluate the effects of pH on color of anthocyanins isolated from tamarillo fruit which grown in many tropical-subtropical region of the world, including South America.

2. METHOD

Color Measurements. UV-Vis absorption spectra were recorded between 380 and 770 nm ($\Delta\lambda$=2nm) on a HP 8452 Diode Array Spectrophotometer, using 5 mm path length glass cell. Solutions of 10 ml were prepared containing 5x10-5 M of each anthocyanin in distilled water (the purities of the isolated anthocyanins were calculated from the chromatographic areas of their peaks in the HPLC chromatograms registered at 520 nm), adjusted at an initial pH value of 2.1 with HCl 1M. Successive pH jumps (from pH 2.1 to 8.5) were achieved by adding small volumes of NaOH 1M or 10M. In order to study the influence of pH value on the color of crude extracts, aqueous solution of extracts were prepared with an absorbance (λ=520 nm) of 0.8 units. Tristimulus values were obtained from the visible spectra with the CIE Standard Illuminant D_{65} and the Standard Observer (10° visual field) considered as references. The CIELAB parameters (L^*, a^*, b^*, C^*_{ab}, h_{ab}) and saturation (s^*_{uv}) by CIELUV were determined by using the software CromaLab[6] , according to CIE specifications.

Statistical Analysis. Correlations between color parameters were obtained using Statistica v. 6.0 software[7].

Isolation and Identification of Anthocyanin from Tamarillo Fruit. Fresh ripe fruits were washed and peeled; the seeds and the surrounding jelly were separated from the flesh and the pigment extract was filtered and fixed on polymeric absorbent. The pigment adsorbed on Amberlite XAD-7 was washed with distilled water and eluted with methanol-acetic acid (19:1,v/v). The eluate was concentrated using a rotavapor at 35°C and the aqueous phase was lyophilized. Multilayer coil countercurrent chromatography (MLCCC) P.C. Inc. Potomac was used, solvent system consisted of n-butanol-TBME-acetonitrile-water (2:2:1:5) v/v/v/v, acidified with 0.1% TFA, the less dense layer was used as the stationary phase, therefore elution mode was head to tail. Separation of anthocyanins were carried out on HPLC with diode array detection (HPLC-DAD) using a Hewlett-Packard 1100 chromatographic system, a Zorbax-SB C_{18} 5μm 4.6x250mm column was used, solvent were acetonitrile-formic acid-water (3:10:87, v/v/v, solvent A; 50:10:40, v/v/v, solvent B) and the flow rate was 0.8 ml/min. Linear gradient from 6 to 20%B at 0-10 min, 10 to 40%B at 10-20 min, 40 to 50%B at 20-30 min, 50 to 6%B at 30-35 min. Mass Spectrometry analysis of anthocyanins were performed on Shimadzu, QP-8000α mass spectrometer, equipped with an ESI (electrospray ionization) interface. Solution of anthocyanins were injected directly into the system at rate flow 0.1 ml/ min.

Determination and Quantification of Total Anthocyanins and Total Phenolics. Total phenolics (TP) were measured by Folin-Ciocalteu (FC) method. Total anthocyanins (TA), polymeric pigment index (PPI) and anthocyanic color (AC) were calculated according to the method of Somers and Evans[8]. Quantification of individuals anthocyanins was made using HPLC based on percent peak area.

3. RESULTS

From the LC-DAD results, it can be concluded that crude jelly extract contained delphinidin-3-rutinoside (62.3±0.1%), pelargonidin-3-rutinoside (28.8±0.1%), cyanidin-3-rutinoside (5.4%±0.1) in the order of most abundance (based on percent peak area at 520 nm). In the crude skin extract the mayor anthocyanin was cyanidin-3-rutinoside (88.3±0.2%).

Influence of pH value on the color of crude anthocyanin extracts. Figure 1 shows the variations occurring on the chroma of aqueous solutions of crude anthocyanin extracts (jelly and skin) with pH values in the 2.5-8.5 range. The comparison with the changes occurring in solution of crude extract jelly revealed that the skin crude extracts had a considerably more stable color with the increase of pH, than the jelly.

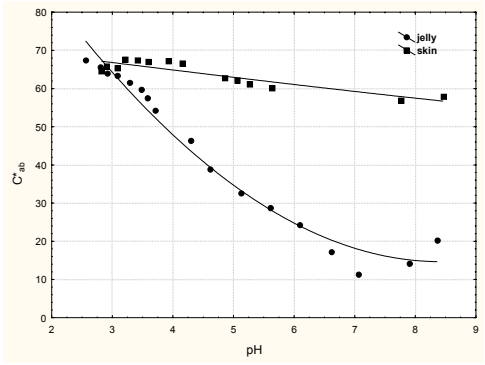

Fig. 1. Changes of chroma (C^*_{ab}) of the crude. extracts with pH

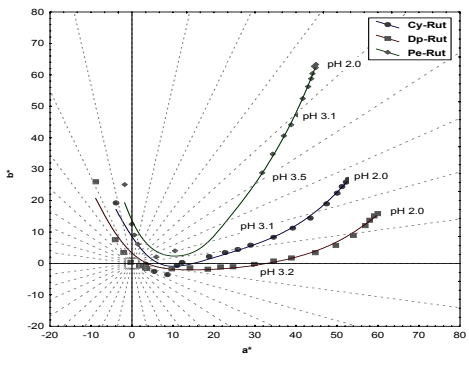

Fig. 2. Color changes of the isolated anthocyanins with pH on (a*,b*) plane.

Additionally, the change on color of the jelly solution had effects on the color parameters as it is revealed in Table 1. The color parameters of skin crude extract changed little at the various pH values (2.8-4.3), whereas jelly crude extract showed greater color instability expressed by L*, h_{ab}, Δ^*E_{ab} and C^*_{ab} at all pH values. The variation in the chroma (C^*_{ab}) was of up 36 CIELAB units in the case of jelly when the pH was increased from 2.8 to 5.6, whereas the variation in the chroma of skin extracts does not surpass 8 units throughout the range of pH values assayed. The highest increase in lightness observed in jelly crude extract is due to the discoloration of its solution with respect to skin crude extract. Moreover, color differences lower than three units (Table 1) are obtained among the samples of skin crude extract in the pH range (2.8-3.6) indicating that they can not be visually

discriminated even by a trained eye[9], however, in this pH range, the color stability of jelly crude extract was clearly lower (ΔE^*_{ab} from 110 to 121 units). The greatest color stability observed in the case of skin crude extract should therefore be interpreted in terms of the highest polymer anthocyanins content of its solution with respect to those of jelly. Jelly: total phenolics, 25.1±0.9 (%p).; polymeric pigment index, 0.20±0.03 (a.u).; total anthocyanins, 420.6±5 (mg/L). Skin: total phenolics, 13.7±0.8 (%p).;polymeric pigment index, 1.16±0.10 (a.u).; total anthocyanins, 72±3 (mg/L). It is well know[10] that polymeric pigments are much less affected by lower pH.

Identification of anthocyanins. The anthocyanins were isolated by solid phase extraction, MLCCC and preparative HPLC as mentioned before. The isolated pigments were identified by using ESI-MS. The results of MS analysis for jelly isolated anthocyanins indicated the presence of the fragment ion peaks corresponding to three anthocyanidins, delphinidin at m/z 303, pelargonidin at m/z 271 and cyanidin at m/z 287. The analysis of [M]+ peaks indicated the presence of glucose and rhamnose in the three isolated anthocyanins.

Table 1. Color parameters of crude pigment extracts of tamarillo fruit

	crude extract jelly				crude extract skin			
pH	L*	h_{ab}	C^*_{ab}	ΔE^*_{ab}	L*	h_{ab}	C^*_{ab}	ΔE^*_{ab}
2.8	45.60	24.46	65.53	121.39	48.41	35.21	64.67	118.76
2.9	45.08	24.12	63.97	118.19	47.19	35.93	65.83	119.36
3.1	45.40	24.34	63.34	117.25	46.43	36.24	65.44	117.92
3.3	46.66	23.82	61.55	114.91	46.85	38.58	67.75	120.54
3.5	49.01	22.09	59.83	113.39	46.25	39.01	67.42	119.23
3.6	51.80	18.68	57.63	110.91	46.41	39.47	67.00	118.34
4.3	60.26	8.98	46.29	95.96	46.20	42.24	66.70	115.58
5.6	66.13	-0.41	28.70	78.47	45.80	54.10	60.18	96.69

Influence of pH value on the color of pure compounds. Lightness (L*), chroma (C^*_{ab}), hue angle (h_{ab}) and color differences with regard to the lowest pH (ΔE^*_{ab}) were determined for the pure pigments at range pH (2.1 to 8.5). Direct comparison of color properties was possible, thus enabling the evaluation of color stability under different pH conditions. Figure 2 shows the color changes of the isolated pigments. Anthocyanins are situated in the first quadrant of the (a*,b*) plane with hue angles between 10° and 60°. The variation in the results indicates a great variation of color; the diagram shows that the three rutinoside derivatives had different hues at nearly all pH values from 2.1 to 8.5. It is noticeable that pelargonidin-3-rutinoside (Pe-rut) had the highest hue angle (> 40°) at pH values below 3.5

Pe-rut is located in the area of the orange hues, while the pigments cyaniding-3-rutinoside (Cy-rut) and delphinidin-3-rutinoside (Dp-rut) had reddish hues at the lowest pH values. When pH increases until around 5 their color gradually changed toward more bluish hues. At higher pH values the hue angle increased again (Fig.2). The chroma (C^*_{ab}) varied with pH in freshly made 5x10-5M solutions of the anthocyanin pigments. The following tendency was the same for all three pigments, undergoes a linear decrease as pH increases. Increases of the pH value caused a loss of color. The highest C^*_{ab} values were for the Pe-rut. The lightness (L*) increased progressively when pH increases, reaching a maximum between pH 5 and 7 for Pe-rut and Dp-rut. In this pH range, Cy-rut had the lowest L* values. The Figure 3 shows significant differences in L* at pH values below 3.4. These results also indicate that in a range of 2-3.4 pH units, Pe-rut presented the smallest increment in L* (2.2%) while Cy-rut and Dp-rut increased their value respectively 14.6 and 23.0 % . Pigment Pe-rut showed greater color stability, expressed by L*. According L* values at lowest pH (pH< 3.4), lightness was related to the number of hydroxyl groups in the B ring, showing the highest values Pe-rut (1-OH), followed by Cy-rut (2-OH) > Dp-rut (3-OH). This agrees with that reported by Heredia et al.[5] who found in anthocyanin glycosides (pH 1.5) that the absorbance decreases when hydroxyl group are substituted by methoxyl group. The figure 4 seems to confirm these results, the highest color changes with regard to the pH were observed for Dp-rut (highest slope), while Pe-rut is the most stable pigment. The major color loss is due to the instability of pigment structures to water. The stability of pigment is ascribed to intermolecular hydrogen bonding forces, in general, among high number of OH substituents, larger should be the solvating and in consequence its stability to pH

changes. Since Pe-rut has one hydroxyl, this seems to contradict the results analyzed previously. According to Rezende et al.[11] the internal hydrogen bonding (intramolecular interactions) between neighboring OH reduces the availability of these group (for steric and electronic reason) to interact with the solvent. This would explain the higher color stability (major solvation) of Pe-rut. On the other hand, The cy-rut shows a more stable behavior of the quantitative component L* at high pH values. This could also be explained with the concept of intramolecular interactions of neighboring OH.

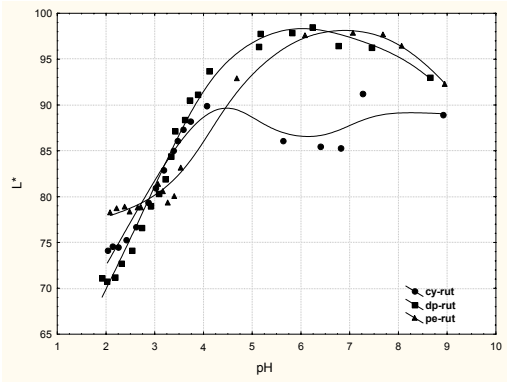

Fig 3. Changes of lightness of the isolated with pH anthocyanins with pH.

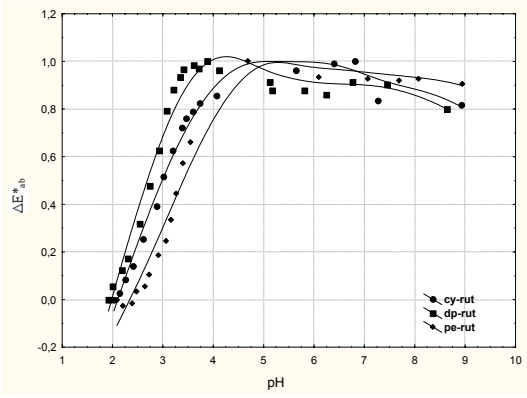

Fig 4. Color differences (ΔE^*_{ab}) of the isolated anthocyanin with pH.

4. CONCLUSIONS

The characteristic color and stability of crude extract and each isolated pigment have been studied in aqueous solutions. It has been possible to find relationships between color and composition as well as to compare the evolution of several color parameters under various pH. According to these results it is evident that the relationships color-composition (solvating grade) influence notably the final color. The application of colorimetric systems, uniform color spaces (CIELAB, CIELUV), is of great value in the characterization of the color properties of anthocyanin rutinosides and crude anthocyanins extract.

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