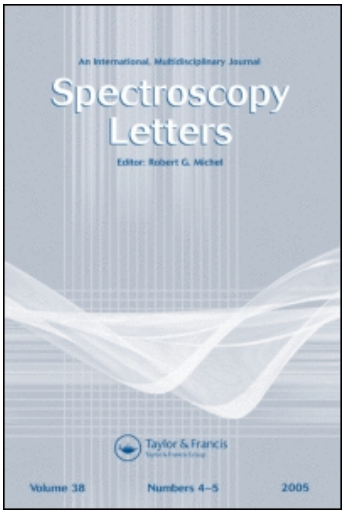

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SPECTROSCOPIC STUDY ABOUT THE KINETICS OF THE ANTHOCYANIN PIGMENTS EXTRACTION DURING THE MACERATION OF CHERRIES IN LIQUOR

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ABSTRACT

An experimental study of the effect of temperature on the kinetics of anthocyanin pigments extraction during the maceration of cherries (*Prunnus avium*) into a hard spirit is reported. The analytical method used was UV-Vis spectrophotometry. The initial extraction rate showed an Arrhenius-type dependency, with an apparent energy activation of 18.7 ± 1.2 kcal/mol. Furthermore, a study about the evolution of the color (from colorless to red-orange) during the soaking process was made by calculating the CIE tristimulus values (X, Y, Z) for illuminant C, until to reach the apparent stabilization of color, what occurs after about two weeks for

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temperatures of 5°C and 23°C, whereas it happens after about four weeks for the other studied temperature (30°C).

Key Words: Anthocyanin pigments; Cherries (*Prunnus avium*); Hard spirits; Liquor; Visible spectroscopy; Color measurement

INTRODUCTION

Cherry liquor is a popular and old hard spirit in several countries, such as Spain (where it is called *licor de cerezas* or *licor de guindas*) and Portugal (where it is called *grinjinha*). Nowadays there is an increasing industrial production of this drink and other analogous liquors made also by soaking different fruits (mulberries, strawberries, dry figs, sloe berries, and others) in several spirits, where the ethanol softens the fruits and promotes the extraction of several pigments and substances that give to the liquor a characteristic color, flavor and smell.

The color evolution during the soaking process of fruits in liquors is of major interest since it is related to organoleptic properties evolution. Knowledge of the changes that pigments of cherries (*Prunnus avium*) undergo with processing is important with respect to their role in color quality because the appearance of a food product can greatly influence a consumer's purchasing decision. Recently Selemenev et al.[1] published a review about the sorption of pigments in food industries.

The ways in which the color changes occur in red wines have been investigated by numbers of authors,[2–5] given the economic importance and magnitude of production of this product worldwide, and it is well known that the color originally arises from the anthocyanins, the pigment contained in the skin of black grapes.

To our knowledge, little or no published information exists in the literature regarding the kinetics of the pigments extraction process for cherries immersed in liquor. Nevertheless such studies can lead to a better understanding of, and thus a better control over, the mechanisms underlying the soaking process, improving the industrial control of this process, characterized until nowadays by a craftsmanship character, due to the fact that extracting the liquor involves a certain art.[6] Negueruela and Echávarri[7] reported an study on the evolution of the color during the soaking process of sloe berries in several liquors, with different amounts of fruits per liter of liquor, in order to obtain a liquor called *pacharán* in Spanish (also known as *patxarana* in Basque).

The main objectives of this work were (i) to study experimentally the influence of temperature on the kinetics of pigment extraction during the maceration of cherries into hard spirit and (ii) to gain insight into the color evolution of the process.

An experimental study on the evolution of the visible absorption spectrum during the soaking process with different temperatures was made. Thus the effect of temperature on the kinetics of pigments extraction during the maceration of cherries into *orujo* can be studied. *Orujo* is a typical Spanish eau-de-vie made from marc of grapes, and it is composed mainly by an hydroalcoholic solution with about 40% ethanol (v/v). The other ingredients such as sugar and, occasionally tea, cinnamon or grains of coffee used for cherry liquor were not considered in order to simplify the study.

Dark red color in cherries arises from pigments called anthocyanins.[8,9] Anthocyanins (Greek: *anthos*, flower; *kyanos*, blue) is a family of phenolic phytochemicals that give flowers, fruits and leaves of some plants their red, blue and purple colors.[10] Anthocyanin consists of sugar molecules bound to a benzopyrylium salt (called anthocyanidin), and it is part of the flavonoid family. More details about anthocyanins were provided by Curtright et al.[11,12]

Gao and Mazza[13] examined by high performance liquid chromatography and gas chromatography the characterization, quantitation and distribution of anthocyanins and colorless phenolics in eleven cultivars and hybrids of sweet cherries and found all of the dark-colored cherry phenotypes contained 3-rutinosoide and 3-glucoside of cyanidin as the major anthocyanins. They also observed that the total anthocyanin content ranged from 82 to 297 mg per 100 g of pitted cherry for the dark cherries, as those studied in this work.

This paper reports further developments in previous reported investigations of the feasibility of visible spectroscopy in the analysis of foods in questions such as the industrial control of the filtration in the beer manufacturing process[14] and the thermal stability of vegetable oils.[15]

EXPERIMENTAL

Apparatus

The analytical method used was UV-Vis absorption spectrophotometry. Visible absorption spectra (400–700 nm) of pigments solutions were recorded on a UV-260 Shimadzu Spectrophotometer with a 1-cm pathlength quartz cell.

Reagents and Chemicals

Cherries were picked from cherry-trees of *El Arenal* (a village of Ávila, Spain). A commercial *Ponte Ulla* hard spirit (*orujo*) produced by *Ruavieja S.A.* in *Santiago de Compostela* (Galicia, Spain), with 42% ethanol (v/v), was used for the maceration of cherries.

General Extraction Procedure

1000 g of cherries were analyzed for each run. Samples for the experiment were carefully selected in terms of weight (8.5 ± 0.5 g), size (average diameter of 2.2 ± 0.3 cm), and homogeneity of skin and color. Samples selected for each run were immersed in a beaker containing 1.0 L of the colorless liquor, and preheated/cooled to the required temperature. The range of temperatures tested was: 5, 23, and 30°C. For the temperature higher than room temperature, the experiments were performed in a thermostatic bath and for the lowest temperature (5°C) the experiment was performed in a refrigerator. The solutions were kept in the dark for four months.

After selected times liquors were stirred to homogenize the solutions; a sample of 5 mL of each beaker were removed for measuring the visible spectrum and, after the measurement, returned to the recipient for avoiding dilution effect. The reference cell used was the hard spirit without cherries. Before measuring the visible spectra, each sample was filtered in order to avoid the presence of particles suspended in solution that cause scattering.

RESULTS AND DISCUSSION

The values of the optical absorption spectra of samples reveal that there is an absorption region centered on ~525 nm. This band was attributed to be due to the presence of anthocyanin pigments.[5,10,11]

For improving the quantification of the band of absorbance, the three-point correction[16] was carried out: two reference wavelengths were chosen either side of the analytical wavelength and the background absorbance at the analytical wavelength was estimated by linear interpolation as shown in Fig. 1. In the example 430 nm and 635 nm were selected as the reference wavelengths.

It was observed that, for all temperatures, there is initially an increasing in the band with the maceration time until to reach a maximum that increases with temperature, followed by a slow decreasing.

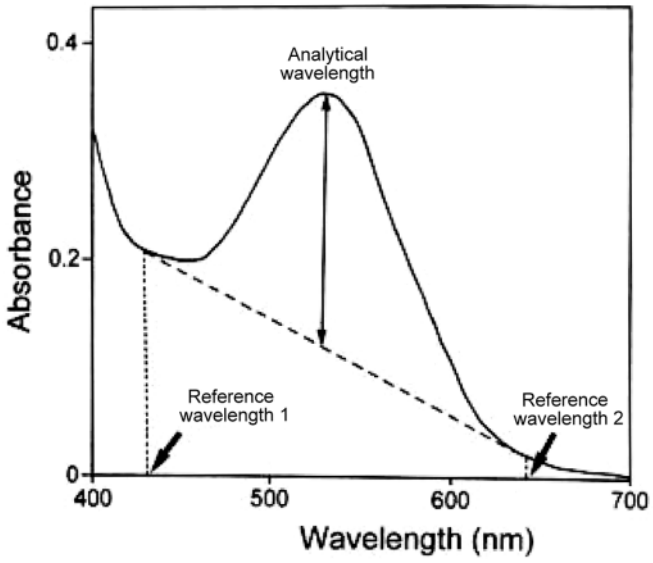

Figure 1. The three-point correction followed for the measurement of absorbance of the absorption maximum in the visible spectrum of the liquor obtained at 23°C after 6 h of maceration of cherries.

Skrede et al.[17] recently reported that anthocyanins as well as other polyphenolics are readily oxidized because of their antioxidant properties and, thus, susceptible to degradative reactions during various processing unit operations. The increasing of maximum reached absorbance value with the increasing of the maceration temperature could be due to an increasing in the solubility of anthocyanins, given the fact that in such mass transfer the flux is strongly dependent of the driving force, i.e., the difference between the maximum possible concentration in the phase (solubility) and the concentration[18] at that moment. Figure 2 shows the variation of the absorption band, for the temperature of 23°C taken as an example.

Figure 3 illustrates the effect of varying the temperature on the variation of absorbance of the studied band with the maceration time. As it is well known, the absorbance of an absorbing dye solution is governed by the Beer–Lambert law[19,20] that establishes the proportionality between absorbance and concentration for a given optical path length.

The first derivative of values of absorbance vs. time for the different temperatures gives the values of initial rate of extraction showed in Table 1. As expected, extraction rate increased with temperature, due to the fact that, generally, the mass transfer coefficient increases with temperature.

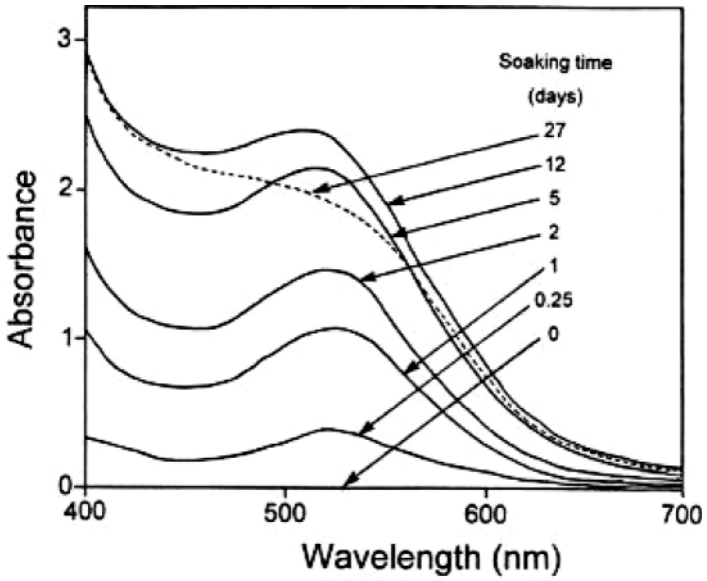

Figure 2. Visible absorption spectra of liquor at 23°C for different times of cherries maceration.

Figure 4 gives the Arrhenius plot of the initial pigments extraction rate. As shown in this figure, the ln (initial extraction rate) vs. inverse of absolute temperature data can be represented by a straight line, with a correlation coefficient of −0.998, as follows:

$$y = (31.7 \pm 2.1) - (9.4 \pm 0.6) \cdot x \quad (1)$$

From the slope of this plot the apparent energy of activation (E_a) was calculated, and the obtained value was $E_a = 18.7 \pm 1.2$ kcal/mol. As pointed out by several authors[21,22] when a rate is controlled by a chemical reaction, the activation energy is generally higher than that expected for a diffusion-controlled process, which generally exhibits activation energies of only a few kilocalories per mole. Thus, as expected, the extraction of pigments in the cherry liquor is a diffusion-controlled process.

The color of cherry liquors originally arises from the anthocyanins contained in cherries. As observed in Fig. 3, the different temperatures allow these molecules to be extracted within the first six to ten days of maceration and, after this time, anthocyanin content decreases due to chemical and biochemical reactions in the complex medium, as pointed out recently by

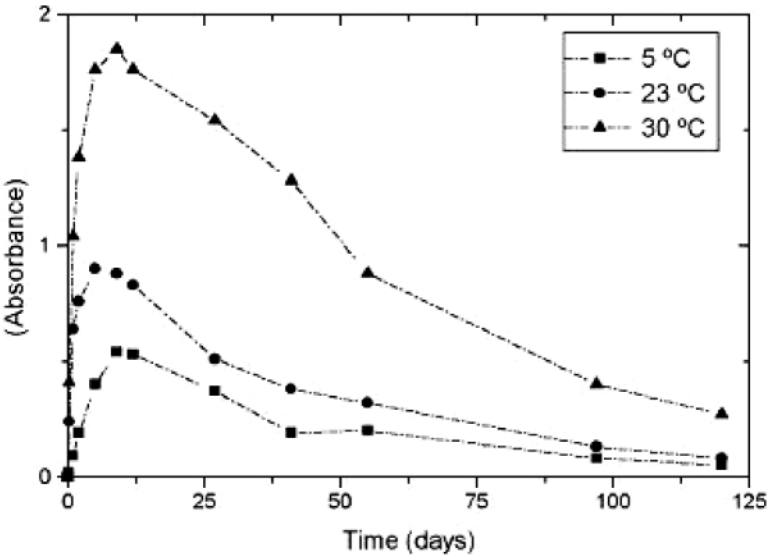

Figure 3. Variation of the maximum absorbance (λ_{max} ca. 525 nm) of solutions with the time of maceration of cherries in the liquor at different temperatures.

Mirabel et al.[5] for wine solutions with wine aging. As a preliminary hypothesis we consider that the compounds obtained by these reactions cause the increasing observed at the shoulder at the violet zone of spectra. Nevertheless, a discussion about this phenomenon is beyond the scope of this article.

The ways in which the color changes occur and copigmentation phenomena have been studied by numbers of authors for wine aging.[23–25]

In order to quantify the evolution of color with the time, for every studied temperature, the tristimulus coordinates for illuminant C were calculated, following the method proposed for wines,[26] accordingly to the equations:

Table 1. Initial Rate of Pigments Extractions at Different Temperatures

Temperature (°C)	Rate (Absorbance Unities/Day)
5	0.1
23	1.0
30	1.6

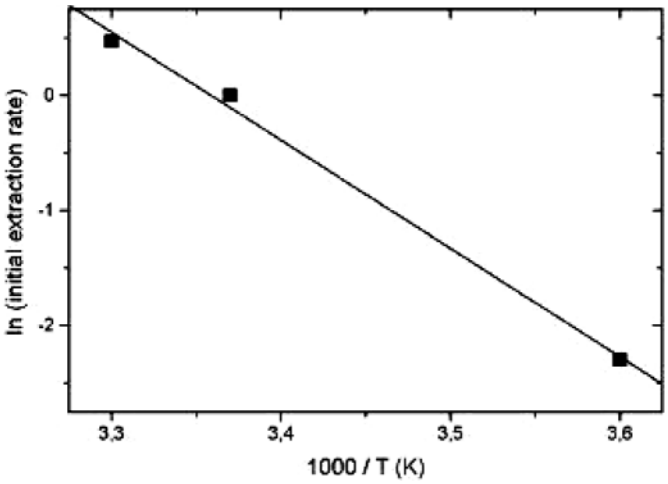

Figure 4. Arrhenius plot of the initial pigments extraction rate measured by the variation of absorbance of the absorption maximum.

$$X = 0.42 \cdot T_{625} + 0.35 \cdot T_{550} + 0.21 \cdot T_{445} \quad (2)$$

$$Y = 0.20 \cdot T_{625} + 0.63 \cdot T_{550} + 0.17 \cdot T_{495} \quad (3)$$

$$Z = 0.24 \cdot T_{495} + 0.94 \cdot T_{445} \quad (4)$$

Where T_{625}, T_{550}, T_{495} and T_{445} are the transmittances of samples at 625, 550, 495 and 445 nm, respectively.

Figures 5, 6 and 7 show the variation of the tristimulus coordinates with the maceration time for the different temperatures. It can be observed that initially, there is a decreasing in all tristimulus coordinates, at a velocity that increases with temperature. After reaching for these coordinates a minimum that decreases with the increasing of temperature, there is a slow increasing for all cases, until to reach an apparent stabilization of the color. These minima occur after about two weeks for temperatures of 5°C and 23°C, whereas it happens after about four weeks for the other studied temperature (30°C). All this, obviously, is related to the changes in spectra analyzed before.

We conclude that UV-Vis spectrophotometry is a good way for studying the kinetics of pigments extraction during the maceration of cherries in liquor and, for the range of studied temperatures, this process has

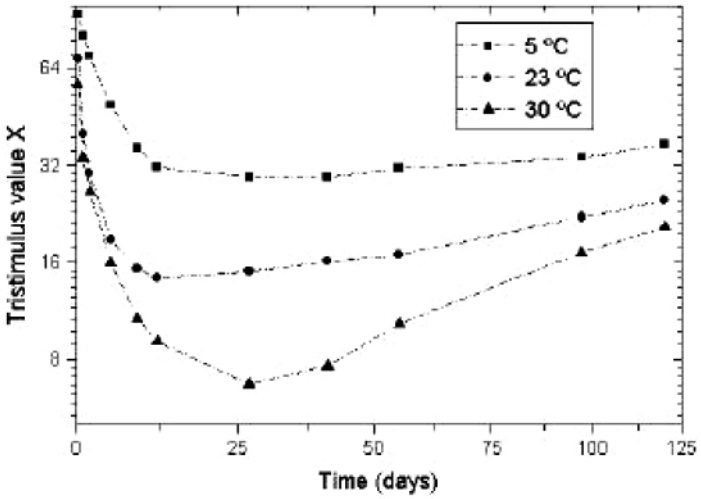

Figure 5. Evolution of the tristimulus coordinate X with the time of maceration of cherries in the liquor.

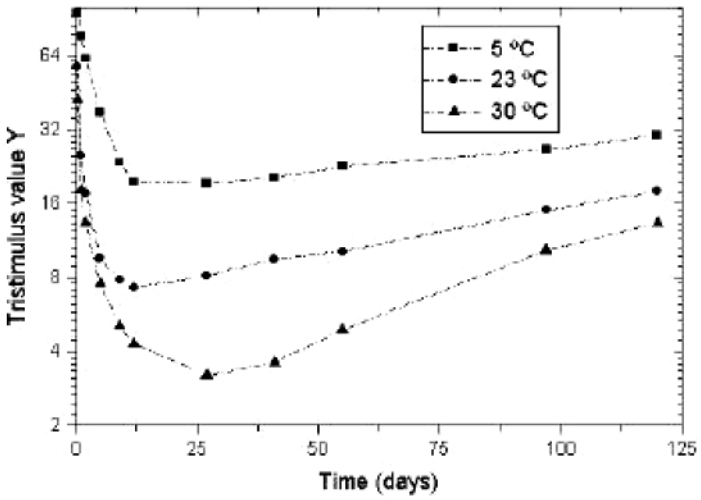

Figure 6. Evolution of the tristimulus coordinate Y with the time of maceration of cherries in the liquor.

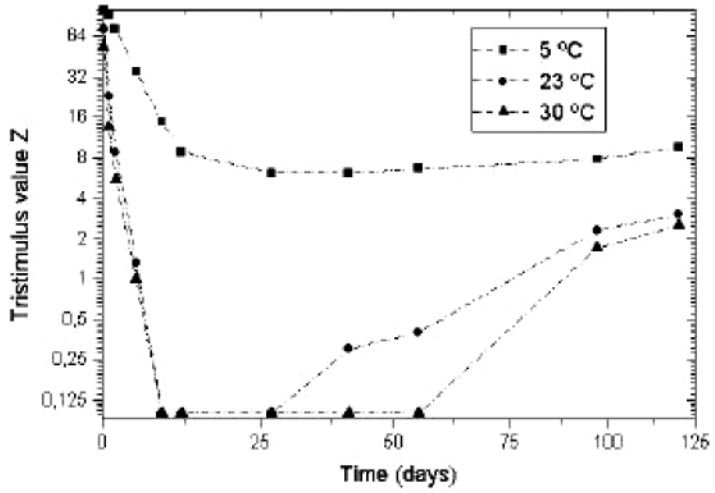

Figure 7. Evolution of the tristimulus coordinate Z with the time of maceration of cherries in the liquor.

an apparent energy activation of 18.7 ± 1.2 kcal/mol. The apparent stabilization of color for the liquor takes place in practice in about two weeks for temperatures of 5°C and 23°C, whereas it happens after about four weeks for the temperature of 30°C.

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