SPECTRAL-LUMINESCENT ANALYSIS OF SUGAR CANE JUICE

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A spectral-luminescent analysis has been made of the low-, medium-, and high-molecular mass fractions of sugar cane juice. The presence of pigments was detected in all the fractions. The medium-molecular-mass fraction was distinguished by the most considerable and most diverse composition of the pigments, a substantial contribution to which was made by the products of the alkaline decomposition of sugars. The amounts of pigments in all the fractions of the juice depended on the age of the plant. A pronounced dependence on the age of the plant was characteristic for the medium-molecular-mass fraction.

The qualitative and quantitative analysis of sugar cane juice has great scientific and practical importance. The identification of the components of the juice and the analysis of their amounts, depending on the age of the plant and on the methods of obtaining the juice, will permit the development of objective methods for evaluating its quantity, the optimum technological regimes for the isolation and crystallization of the sucrose, and for the elimination of impurities.

Investigations have been performed of the electronic absorption and luminescence spectra of low- (LM), medium- (MM), and high-molecular-mass (HM) fractions of the juice of Cuban sugar cane of the variety C374-72 at different stages of ripening (9, 12, and 17 months).

None of the juice fractions absorbed appreciably in the 500-850 nm region. In the 200-500 nm region, qualitatively similar absorptions of the HM and MM fractions were observed. The absorption of the LM fraction was extremely slight in this region, as well.

As is known, the absorption in the near UV and visible regions of the spectrum of solutions of sugar juice is due to so-called pigments. The juice present in the sugar cane plant is colorless. The synthesis of pigment begins under the influence of oxygen, iron salts, enzymes, high temperatures, and other factors when the juice is extracted from the plant. The basic color of the juice is due to melanoidins (compounds formed as the result of the hydrolysis of sugars and the interaction of the products of their breakdown with amino compounds), the products of the alkaline decomposition of the sugars, and the products of the dehydration of the sugars. Obviously, the superposition of the absorption of several pigments — melanoidins, absorbing at 290 nm [1, 2], the products of the alkaline decomposition of sugars, having absorption at 260 nm [1, 3], and the products of the dehydration of the sugars, which have absorption maxima at 225, 282, and 400 nm [1] — will lead to a diffuse nature of the absorption in the 200-500 nm region.

As compared with absorption spectroscopy, luminescent analysis possesses a considerably greater sensitivity (by 2-3 orders of magnitude) and selectivity. This permits the diagnosis and investigation of fluorescent substances in low concentrations and, by selecting a definite wavelength of the exciting light, the supply of energy to a definite component of the system under investigation. Figure 1 shows the fluorescence and excitation spectra of various fractions of the juice. In contrast to absorption spectroscopy, the luminescent method, thanks to its high sensitivity, permits the recording of the presence of pigments in the low-molecular-mass fraction as well. As is known, sucrose (the main component of the low-molecular-mass fraction) does not luminesce. As we see, characteristic for the LM fraction is fluorescence with λ_f maxima at 420 nm (in the case of exciting light with a wavelength λ_e of 310-350 nm) and 455 nm (λ_e 370 nm). The positions of the maxima are the same in the range of ages of the plant that were investigated. A more complex pattern is observed for the medium-molecular-mass fraction. On excitation in the interval of λ_e of 305-325 nm, regardless of the age of the plant, the fluorescence with a maximum at 440 nm remained

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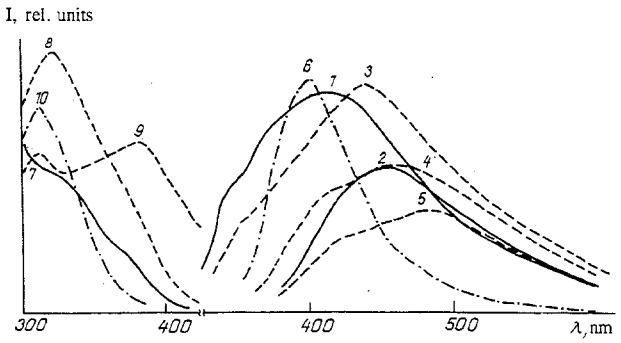

Fig. 1. Fluorescence spectra [1, 3, 6) λ_e = 310 nm; 2, 5) λ_e = 370 nm; 4) λ_e = 350 nm] and excitation spectra [7) λ_f = 420 nm; 8) λ_f = 435 nm; 9) λ_f = 485 nm; 10) λ_f = 400 nm] of the low- 1, 2, 7) medium (3-5, 8, 9), and high-molecular-mass (6, 10) fractions of sugar cane juice (age of the plant 17 months). The spectra have been brought to the same intensity at λ_e = 310 nm.

unchanged. This maximum was also observed when λ_e was increased to 350 nm in the MM(9) and the MM(12) fractions. But in the MM(17) fraction at λ_e 350 the fluorescence maximum had shifted to 450-460 nm. When λ_e was increased to 360-380 nm the fluorescence maxima in the MM(9) and MM(12) fractions was located at 455 nm, and in the MM(17) fraction at 485 nm. On the short-wave slope of the fluorescence bands (λ_e 350 and 370 nm) a shoulder was observed at 420 nm. The simplest luminescence, qualitatively independent of the age of the plant, with a fluorescence maximum at 405 nm (λ_e 290-350 nm) was characteristic for the high-molecular-mass fraction, HM. So far as concerns the excitation spectra, for the LM fraction with a change in the recording wavelength λ_f (420, 455 nm) only their form changed. In the excitation spectrum of the MM fraction, the position of the maxima depended on λ_f — excitation spectra were obtained with maxima at 320 nm (λ_f 410, 435, and 455 nm) and 315 and 375 nm (λ_f 485 nm). The excitation spectrum of the HM fraction did not depend on λ_f (400-455 nm) and had a maximum of 310 nm. Thus, both the fluorescence spectra and the excitation spectra of the LM and MM fractions depended on λ_e and λ_f, respectively. This shows the presence of several fluorescing centers in these fractions. The most complex composition of the fluorescing centers, also depending on the age of the plant, was characteristic for the medium-molecular-mass fraction.

In an investigation of the luminescence of model compounds of the pigments and, in particular, the products of the alkaline decomposition of glucose, fluorescence was observed with maxima at 440 nm (excitation maxima 350 nm) and 490 nm (excitation at 390 nm) [4]. When the products of the alkaline decomposition of glucose were fractionated, emission of fractions with fluorescence maxima at 350 and 420 nm and excitation at 310 and 350 nm, respectively, were observed [5]. Thus, it may be assumed that the products of the alkaline decomposition of sugars make a substantial contribution to the fluorescence of the MM fraction. It must be mentioned that information on the fluorescence spectra of pigments and their model compounds is extremely sparse [4, 5], and therefore a detailed explanation of the nature of the fluorescing centers is problematical at the present time and requires further investigation of the fluorescent characteristics of compounds modeling pigments.

Table 1 gives the values of the integral intensities of the fluorescence of various fractions of sugar cane juice according to the age of the plant. Regardless of the age of the plant, the maximum amount of fluorescing pigments was observed for the MM fraction. Also characteristic for the MM fraction was the greatest dependence on the age of the plant, with a sharp increase in the amount of pigments in the MM(12). For the LM and HM fractions, the dependence of the intensity of the fluorescence on the age of the plant was far weaker.

Thus, the medium-molecular-mass fraction was distinguished by the most considerable and most diverse composition of the pigments. A substantial contribution to the pigments of the MM was made by products of the alkaline decomposition of sugars. Their amount depended to a considerable degree on the age of the plant, passing through a maximum for a plant with an age of 12 months. In contrast to the LM and HM fractions, the qualitative composition of the pigments of the MM fraction also changed with the age of the plant. It is important to note that it is precisely the impurities of the medium-molecular-mass fraction which exhibit the greatest influence on the crystallization of the sugars. The results obtained correlate with those on the amount of sucrose — the maximum amount of sucrose was found in the juice of the 12-month plant. The

TABLE 1. Values of the Reduced Integral Intensity of Fluorescence (λ_e 350 nm) of Various Fractions of Sugar Cane Juice (referred to the integral intensity of the fluorescence of rhodamine B in ethylene glycol)

Age of plant, months	Values of the reduced integral intensity $\times 10^2$		
	LM	MM	HM
9	0,15	0,55	0,47
12	0,20	3,03	0,44
17	0,10	0,94	0,58

study of the medium-molecular-mass fraction is of interest also for evaluating the metabolism of the plant, since oligosaccharides play an important role in the metabolism of the sugar cane.

As the investigations have shown, spectral-fluorescent methods permit the analysis of the qualitative and quantitative compositions of the pigments in various fractions of sugar cane juice. On this basis it is possible to develop a highly sensitive method of analyzing sugar cane juice.

EXPERIMENTAL

Absorption spectra were recorded on a Unicam SP-800 double-beam automatic spectrophotometer, and fluorescence and excitation spectra on a Fica-55 spectrofluorimeter with automatic correction of the spectral sensitivity and a SLM-4800 spectrofluorimeter fitted with a computer with a program for calculating the areas under the quantum fluorescence spectra. The sugar cane juice was fractionated by liquid chromatography using a column of special construction. Samples were prepared in the form of solutions in distilled water at a concentration of 0.3 g/liter. Measurements were performed at 20°C in quartz cells 1 cm thick.

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