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Dedicated to the memory of Prof. dr. Ioan Silaghi-Dumitrescu marking 60 years from his birth

AN IMPROVED SAMPLE PREPARATION OF STARCH-BASED FOODS FOR SYNTHETIC DYES ANALYSIS

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ABSTRACT. The purpose of this study is to develop a simple and fast sample preparation method for synthetic dyes determination from starch-based foods (puddings). Because at sample preparation stage the main problem is to extract quantitatively the dye unchanged, seven different extraction systems were tested. The extractions were carried out using two different techniques: magnetic stirring and sonication. Quantitative determinations were performed using molecular absorption spectrometry and standard calibration method. The efficiency of extraction of eight synthetic food dyes from spiked corn starch samples was evaluated in terms of recovery. The most efficient extraction solvent proved to be ammonia 25%, the recoveries for all dyes (exception for brilliant blue FCF dye) being higher than 92%. Ultrasounds assisted solvent extraction proved to be more efficient than magnetic stirring, leading to a recovery improvement up to 5% in most cases. Finally, commercial pudding samples were analyzed to assess applicability of this extraction procedure. The results obtained for determination of tartrazine in six identical samples showed no significant difference in terms of extracted amount of dye.

Keywords: *food dyes; starch-based foods; solvents extraction; extraction efficiency; ultrasounds assisted extraction; molecular absorption spectrophotometry*

INTRODUCTION

Foods additives have been used for centuries to enhance the quality of food products despite they are increasingly viewed as compounds with toxicological risk [1]. Synthetic dyes, a very important class of food additive, are commonly used in processed foodstuff to compensate the loss of natural colour which is destroyed during processing and storage or to provide the desired colored appearance. By comparing with natural dyes, synthetic dyes have several advantages such as high stability to oxygen, pH and light, great colors variety and color uniformity, low microbiological contamination

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and relatively lower production costs. The use of food dyes is at least controversial because they are added only for esthetical role and some of these substances pose a potential risk to human health, especially to children that are considered a very vulnerable consumer group [2]. Specific directives in each country strictly regulate the use of synthetic food dyes, and safety data such as the acceptable daily intake have been repeatedly determined and evaluated by Food and Agricultural Organization (FAO) and World Health Organization (WHO) [3]. Consequently, accurate and reliable methods for the determination of synthetic dyes are required for assurance of food safety. While titrimetric and gravimetric methods are allowed for determining pure dyes content of color additives, spectrophotometric methods have been listed in the AOAC Official Methods of analysis since 1960 [4]. The speed, ease and efficacy of the spectrophotometric methods make them of particular value. Spectrophotometric methods often prove inadequate, in the analysis of real samples due to the overlapping of spectral absorption maxima. This drawback can be overpassed using complex spectrophotometric methods with chemometric data interpretation [5-7]. Another approach for food colorants determination can be solid-phase spectrometry when the dyes are adsorbed on Sephadex DEAE A-25 or Cl8 silica gel and the absorbance can be directly measured [8, 9]. Due to their properties, synthetic food colorants can be analyzed also by electrochemical techniques like absorbtive voltametry [10, 11] and polarography [12].

Chromatographic methods are playing an important role in dyes analysis [13]. The advantage of this technique consists in separation of colorant one from each other, followed by spectrophotometric VIS detection [14]. Generally RP [15, 16] ion-pair [17] and ion-chromatography [18] are used. Electrophoretic techniques are also used on large scale [19-21]

Due to the complexity of food matrices the isolation of dyes from sample is more problematic to the chemist than the determination step. Dyes isolation techniques have typically depended upon one of three general methodologies: leaching, liquid-liquid extraction, or active substrate absorption. As Marmion notes [22], there is no technique that can be used to all types of sample matrices, thus the chemist requires comprehensive knowledge to choose the technique and to optimize the extraction conditions. For example, the presence of high affinity binding agents such proteins, demand removal of the interfering matrix [23]. Various methods are used for the extraction of dyes from different foodstuff matrices [14, 24]. Given most analytical instruments handle liquid samples, attention is being paid to improving solids sample preparation. If the sample is water soluble, simple dissolution is carried out by manual or mechanical stirring, either with heating or at room temperature. Usually solid-phase extraction is used for purification [20, 25-27]. If the sample is insoluble (e.g., puddings, powder for ice-cream) quantitative extraction of dyes must be performed. Besides the simple liquid-solid extraction,

also more complex extraction techniques are available [28]. In recent decades ultrasound has established an important place in different field (industrial, environmental, medical) and its applications has a growing trend in analytical chemistry [29].

Taking in account these considerations, the aim of this study was to find the optimum solvent system and the best technique that ensure the quantitative extraction of dyes from starch-based foods. A detailed study concerning synthetic food dyes extraction from starch was not yet performed.

RESULTS AND DISCUSSION

Absorption spectra of dyes

The analyzed food synthetic dyes are organic substances with high solubility in water conferred by the presence of at least one sulfonic group (salt forming moiety). They can be classified by the nature of chromophoric group in quinoline, azo, and triarylmethane. Depending on the pH values they can be more or less ionized. By careful visual examination of the VIS spectra it is apparent that they presenting isosbestic points, and changes of the wavelength (λ_{max}) and absorbance (A_{max}) induced by the some of pH value. In alkaline extreme environment it can be observed a decreasing of absorbance (A_{max}) for all colorants. There is although a pH range in which A_{max} and λ_{max} do not vary with pH value (Table 1). For good experimental results it is important that the absorbance measurements to be performed within this range. As was pointed out in the experimental part the residuum was dissolved in water, the pH of resulted solution being adjusted in this range.

Table 1. The stability range and specific wavelength λ_{max} for studied dyes.

No.	Compounds	Stability pH range	λ_{max} (nm)
1	Quinoline Yellow WS (E104)	1-9	415
2	Tartrazine (E102)	2-8	425
3	Sunset Yellow FCF (E110)	1-9	480
4	Azorubine (E122)	1-7	515
5	Ponceau 4R (E124)	1-9	507
6	Amaranth (Dye) (E123)	1-9	520
7	Brilliant Blue FCF (E133)	3-11	630
8	Patent Blue V (E131)	5-7	640

Recovery experiments and analysis of spiked samples

The extraction efficiency was evaluated in terms of recovery, calculated as ratio of the determined quantity to that used for preparing the spiked

sample. The extracted quantity was calculated on the basis of the sample absorbance and calibration equation (Table 2) at the maximum wavelength that corresponds to each dye.

The efficiency of extraction solvents was tested on the synthetic spiked samples processed as mentioned above. There were used seven different extraction systems, chosen on the basis of dyes solubility, ionic force and pH value.

Table 2. The linear range and calibration equations for studied dyes.

No	Compounds	Linear range (ppm)	Calibration equations	R^2
1	Quinoline Yellow E104)	2-20	y= 0.0670x + 0.0304	0.9985
2	Tartrazine (E102)	2-40	y= 0.0349x + 0.0085	0.9999
3	Sunset Yellow (E110)	3-20	y= 0.0543x – 0.0077	0.9991
4	Azorubine (E122)	2-15	y= 0.0380x – 0.0473	0.9981
5	Ponceau 4R (E124)	4-15	y= 0.0374x – 0.0032	0.9985
6	Amaranth (Dye) (E123)	4-15	y= 0.0393x – 0.0119	0.9987
7	Brilliant Blue (E133)	1-8	y= 0.1359x + 0.0181	0.9997
8	Patent Blue V (E131)	1-10	y= 0.1679x + 0.0559	0.9985

The obtained results and expanded uncertainly U (±2s) for 95% are presented in Table 3. The profiles of recovery (Figure 1) illustrate that the high solubility of food dyes in water is not the most important parameter concerning the efficiency of extraction. This behaviour might be attributed to the specific interaction with starch. When pH was modified and ionic strength increased (extraction system 2 and 6) no improving of recovery was observed. Also, using methanol as extraction system, the extraction efficiency has not significantly increased. All these findings indicate that the interactions between starch and dyes are complex and extraction procedure is very difficult to be optimized. The best results obtained using ammonia 25% could be explained by a strong desorption effect generated by the resulted ammonium salts or by blocking the adsorption cites. Much more, the effect of ammonia is very similar in all cases and this fact is well illustrated in Fig. 1. In addition, as we can observe in Table 3, the best recoveries (higher than 92% for all studied dyes exception for brilliant blue) were obtained by using ammonia 25% as extraction solvent. For azorubine and ponceau 4R good recoveries (92.54% respectively 96.05%) were also obtained with methanol-ammonia 25% (9:1, v/v) mixture.

Table 3. Dyes recovery and expanded uncertainly U for 95% (n=6).

Compounds (Abbr.)	Recovery(%) ± U						
	Solvents extraction Systems						
	Water	Acetic buffer (pH=4)	MeOH	MeOH-HCl (9:1, v/v)	MeOH-NH_3 (9:1, v/v)	Ammonia buffer (pH=10)	Ammonia (25%)
Quinoline (E104)	3.3±0.6	2.5±0.3	5.3±0.7	4.0±0.8	2.6±0.6	54.8±5.0	95.5±9.2
Tartrazine (E102)	71.9±10.0	28.4±3.8	10.2±5.7	6.9±1.4	9.0±1.7	13.6±3.4	92.2±10.8
Sunset Yellow (E110)	31.5±5.3	12.9±2.2	12.9±1.7	14.5±2.5	14.8±1.7	20.± 3.3	93.5±12.1
Azorubine (E122)	23.9±4.1	2.6±0.5	5.6±0.6	6.1±1.2	92.5±9.6	10.1±1.4	96.1±6.2
Ponceau 4R (E124)	44.4±7.0	2.3±0.4	7.3±1.4	5.9±1.4	96.0±11.6	69.6±12.7	97.8±6.3
Amaranth (E123)	64.9±9.5	78.7±9.4	49.6±10.6	53.7±10.3	55.2±8.9	65.1±7.3	91.2±6.9
Brilliant Blue (E133)	36.9±6.3	46.3±4.6	30.6±6.0	35.9±6.4	36.0±8.4	37.40±9.9	84.9±4.3
Patent Blue V (E131)	78.7±12.0	81.2±0.5	55.1±9.5	46.5±10.0	54.3±10.0	68.5±15.2	92.2±3.2

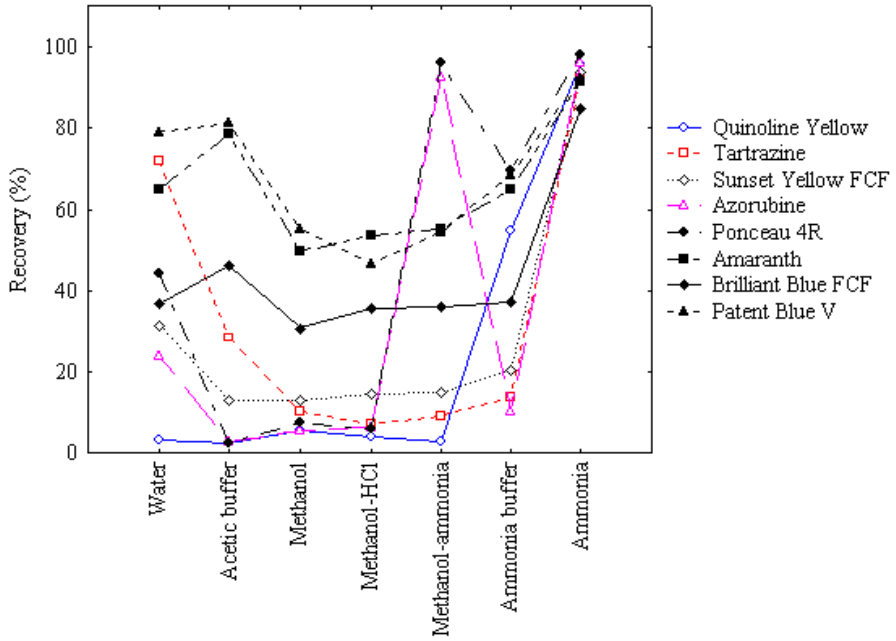

Figure 1. The influence of extraction system on the dyes recovery.

Dyes ultrasounds assisted extraction from spiked samples and puddings

The ultrasounds extraction efficiency of each colorant was tested using only ammonia 25% as extraction solvent. Absorption spectra of all extracts do not show degradation or displacement of maximum absorption wavelength. The obtained recovery data are presented in Table 4.

Table 4. Levels of dyes recovery obtained by ultrasounds assisted extraction using ammonia (25%) as solvent system.

No.	Name of compounds	Recovery(%) ± U
1	Quinoline Yellow WS	98.50±2.1
2	Tartrazine	99.25±2.0
3	Sunset Yellow FCF	98.63±3.2
4	Azorubine	99.62±1.7
5	Ponceau 4R	99.63±1.3
6	Amaranth Dye	98.12±2.0
7	Brilliant Blue FCF	85.23±3.6
8	Patent Blue V	93.39±2.2

By comparing the efficiency of assisted ultrasounds extraction (Table 3) with that obtained by mechanical stirring (Table 2), it can be observe that by applying ultrasounds assisted extraction, the recovery is significantly improved, more than 5% in most cases. The low “s” values illustrate a good precision of determinations.

In order to assess reliability of this extraction procedure to the food dyes analysis from real samples, determination of tartrazine from vanilla pudding was performed. Six identical samples (0.5000 g) were processed by the protocol described above using ammonia 25% as extraction solvent. Tartrazine was identified from the absorption spectra and its amount was calculated on the basis of the sample absorbance and calibration equation at the specific wavelength. The average concentration of tartrazine for six identical commercially vanilla pudding samples was 144.84 ± 0.05 mg/kg.

CONCLUSIONS

The results obtained in this study concerning the extraction of dyes from starch-based foods demonstrated that the most efficient system is ammonia 25%, in this case the recovery being higher than 92% for all studied food dyes (exception for brilliant blue FCF dye); for azorubine and ponceau 4R good recoveries (92.54% respectively 96.05%) were obtained also by using methanol-ammonia 25% (9:1,v/v) mixture as extraction system.

Ultrasounds assisted extraction proved to be a technique more efficient than simple mechanical stirring extraction, leading to a recovery improvement up to 5% in most cases. The technique developed in this paper was successfully applied for tartrazine determination from commercially pudding. The proposed method was found to be relative simple, precise, sensitive and accurate and might be efficiently applied for the determination of tartrazine and other food dyes in commercial available puddings.

EXPERIMENTAL SECTION

Chemicals

Methanol and Titrisol buffer solutions having pH from 1 to 12 and an error ΔpH = ±0.01 were acquired from Merck (Darmstad, Germany). Acetic acid (glacial), ammonia 25% and hydrochloric acid fuming (37%) were from Chemical Company (Iasi, Romania). Natrium acetate and ammonium chloride solid salts used for preparation of buffer solutions were purchased from Chimopar (Bucuresti, Romania). Solid dyes standards **(**Quinoline Yellow WS, Tartrazine, Sunset Yellow FCF, Ponceau 4R, Azorubine, Erythrosine, Amaranth, Brilliant Blue FCF, and Patent Blue V) were purchased from Merck or Fluka. Standard stock solutions of all dyes (100 mg/mL) were prepared by weighting appropriate amounts of solid colorant and dissolving them in deionized water. Working solutions of individual dyes were prepared by dilution of aliquots of stock solutions. Corn starch and vanilla pudding were purchased from local market.

Methods

VIS spectra and calibration curve determination

The absorbance spectra in VIS for each colorant was plotted for different pH values : 1 ; 2; 3; 4; 5; 6,86; 8; 9; 10; 11, and 12, at a concentration of $2x10^{-5}$M. The solution for calibration curves were prepared for each dye by successive dilution of stock aqua solution within the range 1-40 ppm. The absorbance was measured at specific λ_{max} of each compound.

Instrumentation

Ultrasound extractions were performed using a Transsonic T310 bath at 35 kHz. Spectrophotometric measurements were performed using a Jasco, V-550, UV/VIS spectrophotometer.

Extraction procedures

Eight different synthetic samples (0.1 mg/g), one for each of the studied dyes, were prepared by mixing of 30 g starch powder with 30 mL of aqueous dye solution (0.1mg/mL). After the evaporation of water at room temperature,

the powders were homogenized and portions of 0.5000 g from each were precisely weighted. Each sample was mixed with 10 mL of extraction solvent and homogenized by magnetic stirring for 15 min. There were used seven extraction systems: 1-water; 2-acetic buffer (pH = 4); 3-methanol; 4-methanol-hydrochloric acid (6M) (9:1, v/v); 5-methanol-ammonia 25% (9:1, v/v), 6-ammonia buffer (pH = 10) and 7-ammonia 25%. In each case the slurry was passed through an inert filtering cartridge - like that used in solid phase extraction but filled with very fine particle of powder glass as stationary phase. The cartridge was previously washed with 3 mL of methanol and 5 mL of extraction solvent in each case. After filtration, the cartridge was washed with extraction solvent (5 mL). The effluent was evaporated to dryness at 40 ^{0}C in a stove and the residuum was dissolved in 5 mL water. The maximum absorbance was measured at specific wavelength for each dye, the extracted quantity being determined using standard calibration method. For all of the studied dyes, six identical synthetic samples were processed like described above. The protocol for ultrasounds assisted extraction was similar as specified above. The extraction was performed in 25% ammonia in an ultrasonic bath for 15 minutes.

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