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STABILITY STUDIES OF SOME DNA BASED MATERIALS DOPED WITH NATURAL EXTRACTS

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Natural dyes and pigments are cheap, nontoxic, renewable sources that could be used to make materials for various applications. Spices natural extracts were used to obtain new materials by immobilizing naturally occurring dyes on DNA. We studied the stability of such materials both in solutions and thin films. The results were compared with those obtained for synthetic dyes and polymers and it was concluded that the color degradation of natural extracts is slightly faster than that of synthetic chromophores. Nevertheless, the results obtained are promising, suggesting that natural extracts can be used to replace synthetic pigments.

Keywords: turmeric; paprika; black pepper; DNA, UV – VIS spectroscopy; photo – thermal and chemical stability

1. Introduction

Spices have been used since ancient times to give flavor, taste, and color to food meals. They were firstly recorded in the Ebers Papyrus (1500 BC), which included the anise (*Pimpinella anisum*), the mustard (*Sinapis alba*), the saffron (*Crocus sativus*) and cassia (*Cinnamomum cassia*) [1].

Turmeric (*Curcuma longa*) belongs to the family *Zingiberaceae*, and is widely grown in Asia and Africa. It has several properties: antimicrobial, antifungal, anti-inflammatory and antioxidant. It has also been used as an active ingredient in curry powder and mustard sauce [2 – 4]. Curcumin [1,7-bis- (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-2,5-dione] (Fig. 1a) is the most important compound of turmeric. Curcumin is insoluble in water and poorly

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soluble in aliphatic organic solvents such as hexane and cyclohexane. Its solubility is very high in benzene as well as in polar organic solvents [5].

Black pepper (*Piper nigrum*) is part of the family *Piperaceae,* and it is a climbing and perennial bush. Less known pepper varieties are white pepper, green pepper and red pepper [6]. Black pepper is the only spice whose fruits are marketed in the four different versions. All four varieties can be obtained from the same black pepper plant, but they depend on harvest time and processing method. Although all four types exist on the market, only black pepper dominates production and consumption. Black pepper fruits contain a piperine alkaloid with a spicy flavor, and it is a spice commonly used in food [7, 8]. Piperine [(2E,4E) – 5 – (1,3 – benzodioxol – 5 – yl) – 1 – piperidin – 1 – yl – penta – 2,4 – dien – 1 – one] (Fig. 1b) is a solid substance with basic character. When consumed, it is without taste initially, but it leaves a burning sensation after a while.

Paprika (*Capsicum annum*) is part of the family *Solanaceae,* and it is used as a dye, flavor, and as source of the spicy taste, depending on the processed product. It can be used fresh, dried, frozen, smoked, fermented or as oleoresin extract. The red color of mature fruits is due to several carotenoid pigments, that include capsaicin (Fig. 1c), capsanthin (Fig. 1d), capsorubin (Fig. 1e), and β-cryptoxanthin, which are present as fatty acid esters [9]. The most important pigments are capsanthin and its isomer capsorubin, which could represent 30–60 % and 6–18 %, respectively, of the total carotenoids in the fruit [10]. These two pigments are found only in paprika. The intensity of the red color in paprika is a direct result of the amount of these pigments [11].

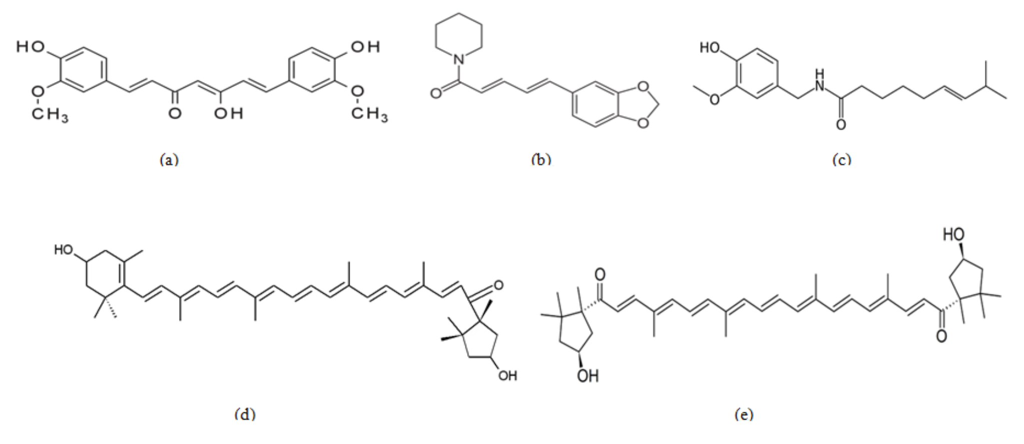

Fig. 1. Chemical structure of the main compounds: (a) curcumin from turmeric; (b) piperine from black pepper; (c) capsaicin, (d) capsanthin, and (e) capsorubin from paprika

In this study spices used to obtain natural extracts were turmeric, paprika, and black pepper. Their stability in both solutions and thin films was compared with the synthetic dyes stabilities.

2. Materials and methods

Spices (paprika, turmeric and black pepper) were purchased from the local market. Curcumin, capsaicin and piperine standards from AC HELCOR Development Research Center were used for comparison. The standard compounds were used to make the calibration curves for each spice. The raw materials were transformed into powder using a grinder.

The solvent used to get natural extracts (NE) was *n*-butanol (Sigma-Aldrich).

Natural DNA biopolymers were used as polymeric matrices. The DNA was extracted from salmon at Chitose Institute of Science & Technology, CIST, Hokkaido, Japan, whence it was purchased.

CTMA (cetyltrimethyl ammonium chloride) (Alfa Aesar) is the surfactant which was used to obtain a soluble compound DNA-CTMA in *n*-butanol.

Samples were analyzed using a Spectrophotometer EVOLUTION 220. VILBERT LOURMAT source was used to perform UVA and UVB irradiation. The extracts obtained by maceration exhibit intense colors, therefore a series of dilutions were necessary.

2.1. Extraction of the natural dyes

Natural extracts were obtained by a classic solid-liquid extraction technique, which was maceration. The spice powders were macerated in *n*-butanol at room temperature in the dark for 48 hours. For all spices the same amount (4g) of plant material and the same volume of solvent (20 mL) were used. After maceration, the samples were filtered [12, 13].

Preliminary studies have shown that compounds of interest can be extracted with *n*-butanol [14].

2.2. Preparation of thin films

The DNA – CTMA complex used in this study was obtained from the DNA reaction with CTMA in water. The DNA – CTMA complex is soluble in *n*-butanol but it is insoluble in water and therefore it can be easily recovered [15]. The method presented in Anton *et al*. [14] was used for thin films preparation.

2.3. Thermal treatment

Thermal stability was tested at 20°C, 40°C, 60°C, 80°C and 100°C. The dye solutions were transferred in covered cuvettes and placed in a water bath for the required heat treatment. The thin films were placed in a vacuum oven for thermal treatment. The changes in absorbance were monitored between 200 and 800 nm at given time intervals: initial moment, 15, 30, 60, 120, 240, 420 and 1200 min.

2.4. UVA and UVB irradiation

To measure their sensitivity, the proposed DNA – CTMA – NE materials were subjected to UVA and UVB irradiation, both in solutions and thin films. Two wavelengths were used: 365 nm for UVA and 312 nm for UVB. The absorbance of the samples was measured before and after 5, 10, 15, 30, 60, 120, 180, 240 and 480 min of irradiation, in the wavelengths range between 200 and 800nm.

3. Results and discussion

3.1. Spectrophotometric analyses

Turmeric dyes are representative for the curcuminoid class, curcumin being the main coloring material of this class. The maximum peak absorption was observed at 420 nm, which corresponds to the literature data [16]. In Fig. 2 the UV – VIS spectrum of the turmeric extract is shown.

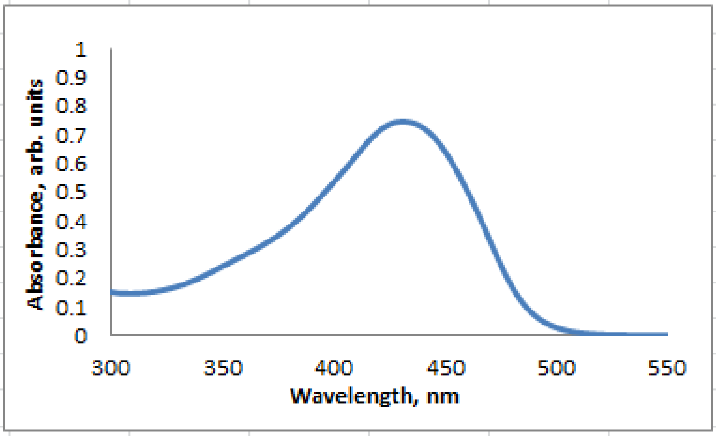

Fig. 2. UV-VIS spectrum of turmeric

For paprika powder, the red color is mainly obtained due to the capsanthin and capsorubin presence (these compounds are part of the carotenoid class). A maximum absorption in the range 208-500 nm could be observed in Fig. 3. The carotenoid compounds show maximum absorption as follows: capsaicin at 285 nm [17], capsorubin at 469 and 503 nm [18] and capsanthin at 450, 475 and 505 nm [19].

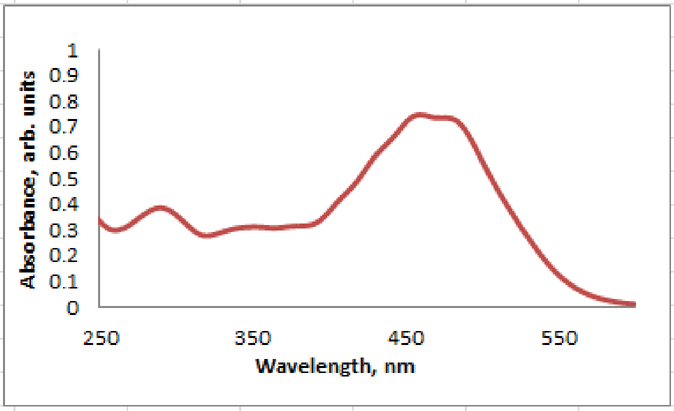

Fig. 3. UV-VIS spectrum of paprika

The absorption spectrum of natural extract based on piperine is presented in Fig. 4. The peak obtained at 340 nm was attributed to piperine [20].

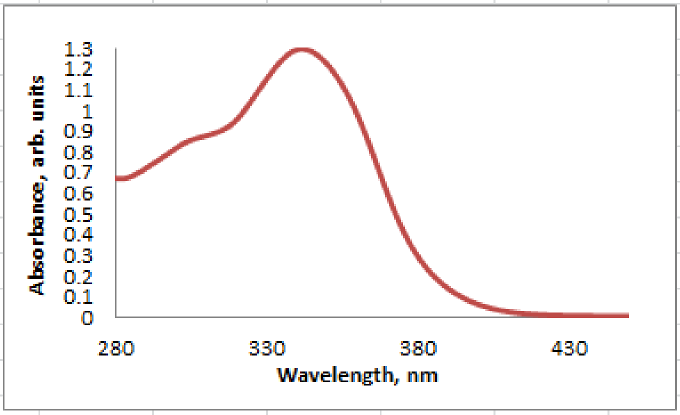

Fig. 4. UV-VIS spectrum of black pepper

Assuming that capsaicin, curcumin, and piperine are the main compounds in the natural extracts, their concentrations were determined using calibration curves obtained based on the coresponding standards. The obtained concentrations are presented in Table 1.

Table 1

Concentrations of main compounds in butanolic extracts

Spice	**Main Compound**	**Concentration, (g/L)**
Turmeric [1]	Curcumin[1]	29.739
Paprika[2]	Capsaicin[2]	6.725
Black pepper[3]	Piperine[3]	34.889

[1]λ_{max} – 420 nm, [2]λ_{max} – 285 nm, [3]λ_{max} – 340 nm

3.2. Photo – thermal, and chemical stability of thin films and solutions

The analysis and characterization of natural pigments is mainly based on UV – VIS spectroscopy [21]. The thermal degradation of the studied thin films and solutions was monitored by the temporal variation of the absorption spectra (Fig. 5). The rate constants were calculated for both DNA – CTMA – NE thin-film and DNA – CTMA –NE solutions (Table 2), assuming that the degradation occurs after a pseudo-first-order kinetic.

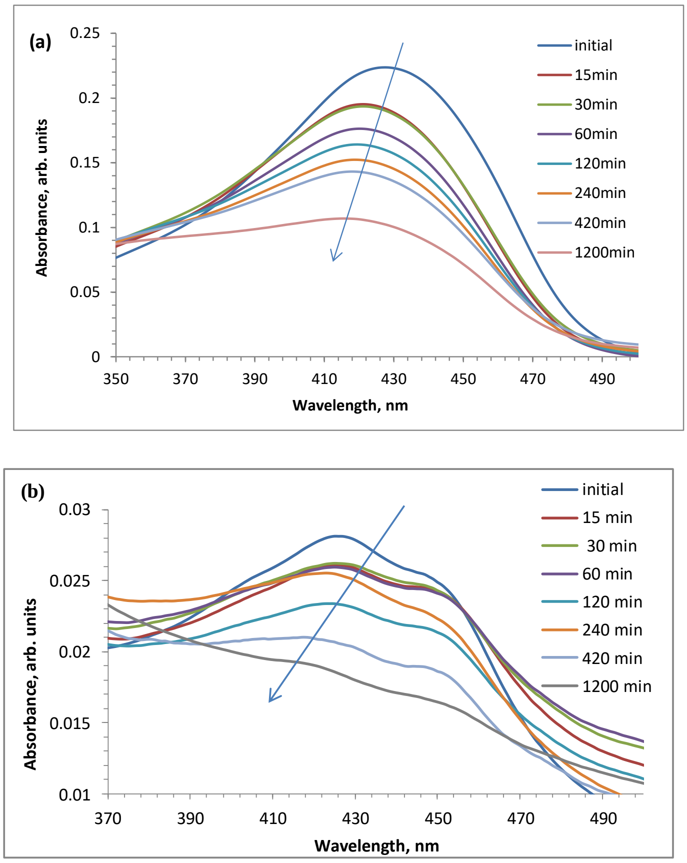

Fig. 5. UV-VIS spectra of thermal degradation of DNA – CTMA – NE (turmeric) solution (a) and thin film (b)

The results obtained were compared with those obtained for thin films of DNA – CTMA – synthetic chromophores (Disperse Red 1 – DR1, Rhodamine 590 – Rh) and, on the other hand, with conventional synthetic polymer (polycarbonate – PC) [22].

Table 2 shows the data obtained for DNA – CTMA – NE solutions and films for degradation at both room temperature and increased temperatures.

Analyzing the data presented in Tables 2 and 3, it was found that the degradation of the natural extracts is slightly faster comparing to that of synthetic chromophores embedded in DNA or DNA CTMA, both at room and high temperatures but less pronounced compared to synthetic chromophores embedded in synthetic polymers (high temperatures). At the same time the state and the composition of the natural extracts play an important role in the value of the activation energy. Thus it can be seen that the activation energy for turmeric and paprika solutions is higher compared with the activation energy of the extracts as thin films. However, in all cases, the values obtained for the activation energy show that the degradation of the chromophores contained in the natural extracts is not a thermally activated reaction. Table 4 compares the degradation kinetic data obtained for solutions and thin films based on DNA – CTMA and NE of turmeric, paprika, and black pepper.

Table 2

Kinetic data for DNA – CTMA – natural extracts based solutions and thin films undergoing thermal degradation

<table>
<tr><th colspan="6">DNA – CTMA – NE (turmeric)</th></tr>
<tr><th colspan="3">Solution</th><th colspan="3">Thin film</th></tr>
<tr><th>Temperature, °C</th><th>k × 10^4, min^{-1}</th><th>Ea, J</th><th>Temperature, °C</th><th>k × 10^4, min^{-1}</th><th>Ea, J</th></tr>
<tr><td>25</td><td>0.50</td><td rowspan="5">28,292</td><td>25</td><td>1.22</td><td rowspan="5">13,650</td></tr>
<tr><td>40</td><td>1.14</td><td>40</td><td>-</td></tr>
<tr><td>60</td><td>3.86</td><td>60</td><td>1.95</td></tr>
<tr><td>80</td><td>4.44</td><td>80</td><td>3.21</td></tr>
<tr><td>100</td><td>4.61</td><td>100</td><td>3.46</td></tr>
<tr><th colspan="6">DNA – CTMA – NE (paprika)</th></tr>
<tr><th colspan="3">Solution</th><th colspan="3">Thin film</th></tr>
<tr><th>Temperature, °C</th><th>k × 10^4, min^{-1}</th><th>Ea, J</th><th>Temperature, °C</th><th>k × 10^4, min^{-1}</th><th>Ea, J</th></tr>
<tr><td>25</td><td>negligible</td><td rowspan="5">35,877</td><td>25</td><td>1.16</td><td rowspan="5">19,646</td></tr>
<tr><td>40</td><td>negligible 0.79</td><td>40</td><td>1.78</td></tr>
<tr><td>60</td><td>-</td><td>60</td><td>5.70</td></tr>
<tr><td>80</td><td>negligible 0.78</td><td>80</td><td>7.94</td></tr>
<tr><td>100</td><td>6.02
1.24</td><td>100</td><td>1.04</td></tr>
<tr><th colspan="6">DNA – CTMA – NE (black pepper)</th></tr>
<tr><th colspan="3">Solution</th><th colspan="3">Thin film</th></tr>
<tr><th>Temperature, °C</th><th>k × 10^4, min^{-1}</th><th>Ea, J</th><th>Temperature, °C</th><th>k × 10^4, min^{-1}</th><th>Ea, J</th></tr>
<tr><td>25</td><td>4.12</td><td rowspan="5">5,921</td><td>25</td><td>1.28</td><td rowspan="5">19,630</td></tr>
<tr><td>40</td><td>5.17</td><td>40</td><td>2.51
0.29</td></tr>
<tr><td>60</td><td>4.18</td><td>60</td><td>-</td></tr>
<tr><td>80</td><td>4.65</td><td>80</td><td>3.21</td></tr>
<tr><td>100</td><td>5.26</td><td>100</td><td>8.74</td></tr>
</table>

*After 20h

Table 3

Kinetic data for DNA – CTMA – synthetic chromophores based thin films undergoing thermal degradation [22]

Thin film	Temperature, °C	$k \times 10^6$, min^{-1}
DNA – Rh 5%	25	2.78
	85	6.68
DNA – CTMA DR1 10%	25	15.4
	85	55
DNA – CTMA Rh 10%	25	2.78 2.57
	85	40.0 36.6
PC – Rh 5%	25	3.13
	85	11000

Table 4

Kinetic data obtained for DNA – CTMA solutions and thin films undergoing UV irradiation

Sample	Solution, $k \times 10^3$, min^{-1}		Thin film, $k \times 10^3$, min^{-1}	
	UVA	UVB	UVA	UVB
DNA – CTMA – NE (turmeric)	8.16	16.10	6.73	31.30 After 30h the degradation becomes negligible
DNA – CTMA – NE (black pepper)	0.56	2.83	19.00 23.10	27.70 After 30h the degradation becomes negligible
DNA – CTMA – NE (paprika)	14.10	5.91	-	-
DNA – Rh 5% [22]	-	-	3.80	2.00
DNA – CTMA DR1 10% [22]	-	-	0.88	2.20
DNA – CTMA Rh 10% [22]	-	-	1.00 1.10	2.30 2.00
PC – Rh 5% [22]	-	-	8.90 1.90	2.80 1.00

In all cases, degradation under the action of UVA rays is more pronounced than that under the action of UVB rays (Figs. 6a and 6b). This degradation, as expected, is faster than thermal degradation (Fig. 6c).

Another interesting observation which deserves to be highlighted in the case of thin films based on DNA – CTMA turmeric and black pepper extracts, is the fact that these films were damaged by UVA rays only within the first 30 minutes.

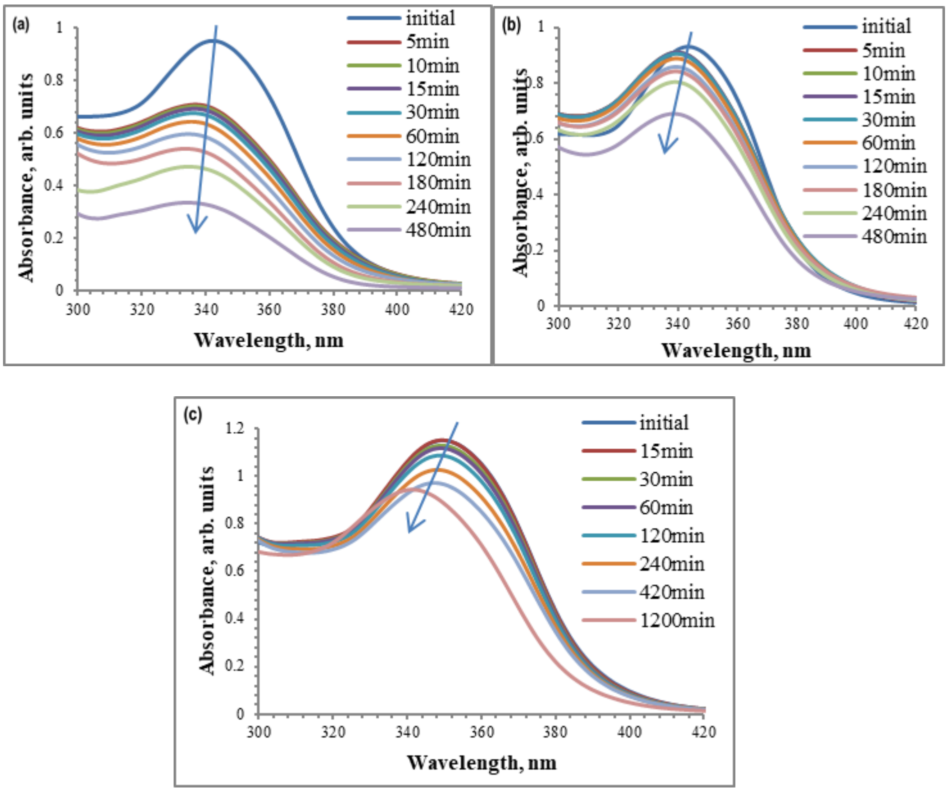

Fig. 6. UV – VIS spectra for photo and thermal degradation stages of DNA – CTMA – NE (black pepper solution): UVA rays (a), UVB rays (b), temperature 25°C (c)

4. Conclusions

The present study showed that natural extracts of some spices could be used as dyes for doping DNA. The photo, thermal and chemical stability of the new materials was studied both in solution and thin film in comparison with synthetic chromophores and conventional synthetic polymers (polycarbonate). Even though the degradation of natural extracts is slightly faster than synthetic chromophores, the obtained results are promising, suggesting that natural extracts can be used to replace synthetic pigments.

Acknowledgments

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