This paper presents the results obtained from experiments on the adsorption of red food colorants on different polysaccharides substrates, carrageenan and maize starch in the form of suspension. Food colorings evaluated, were carminic acid E-120, azorubine-E122 and Ponceau 4R-E124, used in order to redden polysaccharide substrates used in food systems, to improve the structure-consistency properties and stability at high temperatures or as a replacement for fat, in the production of dietetic products.

The amount of colorants absorbed by the analyzed substrates was determined indirectly, by the spectrophotometry method, based on measuring the intensity of color unadsorbed by supernatants obtained after centrifugation of samples.

Keywords: colorant, carrageenan, maize starch, adsorption speed, retention level, coefficient of adsorption.

1. Introduction

Currently, demand for diversified food dietetic products, rich in fiber and poor in fat, has increased greatly. As a consequence of this preference, the interest in using hydrocolloids (gums, starches, protein derivatives, etc.) gels formers in the food products, for improving functional and sensory properties, or as fats substitutes, is increasing. Documentary information consulted notes the use of polysaccharides substrates, especially in meat preparations (sausage, frankfurters), meat and fish pasta, and meals (eg. burgers), (Allevi, 1998; Banu, 2000; Candogan et al, 2003; Imeson, 1998).

Color is an important feature of food because it is quickly seen through the visual analyzer, as a condition of immediate acceptance of food. In this respect, production of colored substrates potentially usable and functional in food systems has been a frequent concern of researchers (Giusti et al, 2003; Calvo et al, 2002).

Carrageenan and maize starch are polysaccharides substrates able to form high viscosity gels, and to fix natural or synthetic colorants (Alonso–Garsia et al, 1999). Starch is a good stabilizing and gelatinizing agent, which influence the texture of food products (Agunbiade at al, 1999).

Colorants adsorption on the different substrates is influenced by chemical structure of the colorant and of the used substrate. Also, recent research has shown that the coloring power depends on the type of interaction colorant and the used substrate. These links are influenced by the type of colorant (anionic or cationic), the nature of the support, the size particles, respectively, the specific area of colorant particles (Berset et al, 1995; Husband, 1997).

Adsorption of anionic colorants takes place both inside and on the surface of the starch substrates (Zograf, 1963, Boki et al, 1991, Weissenborn and others, 1995). Relationship between the level of fixed colorant and color intensity is not simple and the concept of supports coloration is not sufficiently understood (Berset and others, 1995).
To use of colored polysaccharides substrates in foods, must be known on account of their ability to adsorb (to fix) colorants, the factors influencing their adsorption on these substrates and the behavior at different specific parameters of food systems. The objectives of this study is to determine the colorant that best adsorb in a chosen model system, respectively, maize starch and carrageenan as a suspension, at the room temperature.

2. Materials and methods

2.1. Materials
Experiments concerning adsorption of red food colorants on different polysaccharides substrates were performed using native maize starch (Farmechim 10, Romania) and carrageenan extracted from Eucheuma seaweed (KUK, Romania). Colorants used are: carminic acid, E120 (Rollit, Romania), azorubine E122 (Moldovis, Romania) and colorant Ponceau 4R, E124 (Rollit, Romania). Molecular masses of used colorants are: E 120 -492.4 g; E 122- 502.44 g; E 124- 604.48 g.

2.2. Methods

Determination of feature wavelength for the used colorants
Characteristic wavelength corresponding to maximum extinctions of the colorants solutions was determined by the method of Campeanu et al, 1993. For this purpose, we have used samples of aqueous colorants solutions, in various concentrations ranging from 0.2 to 0.6% for carminic acid, from 0.001 to 0.004% for Ponceau 4R and from 0.0010 to 0.0014% for azorubine. Choosing concentration solutions took place in such a way that, extinctions should fall in the scale instrument. The colorants had a maximum extinction at wavelengths of respectively, 518 nm, 510 nm and 520 nm.

Determination the field of proportionality concentration/extinction
For all three investigated colorants the field of direct proportionality between the colorant concentration and extinction was determined. In this field, we may apply the law of Lambert-Beer, which establishes the correlation between concentration of solutions and their extinction, namely:

\[
\frac{c_1}{c_2} = \frac{E_1}{E_2} = \ldots = k
\]

(where c is the concentration of colorant and E is the extinction). In case of the carminic acid proportionality field was found to contained the concentrations range of 0.01% -0.1% while for Ponceau 4R and azorubine colorants, the proportionality fields correspond to the concentrations range 0.0002% -0.001%. These results allowed achieving the standard curves for each colorant.

Determination adsorption coefficient of the colorants on polysaccharides substrates
Adsorption coefficient [mol/g] is the number of moles of colorant adsorbed per gram of substrate.

Determination adsorption speed of the colorants on polysaccharides substrates
In order to identify the development of a chemical reaction within a certain time it is sufficient to measure the concentration of a reactant or a product of reaction in different moments. The parameter which indicates the variation of reactants or reaction products concentration against time is called the speed of reaction. Speed of reaction, respectively of adsorption in this case expressed in [mg/min].

Determination retention level of colorants on polysaccharides substrates
The retention level respectively, yield of fixing colorants on substrate was expressed as a percentage. Expression retention level, adsorption speed and adsorption coefficient was done taking into account the dry matter of colors and polysaccharides analyzed.

2.3. Establishing the schematic diagram for experiment
Experimental researches have been working under the scheme, presented in Figure 1, according to the following characteristics obtained under testing.
2.4. Preparation of suspensions
The suspensions of maize starch were obtained by adding 20 g maize starch into 80 g distilled water, and the suspensions of carrageenan were obtained by adding 10 g carrageenan into 90 g distilled water.

2.5. Choice of colorants concentrations
Concentrations of colorants from suspensions were chosen so as to fall within limits allowed in food products, according to Ministry of Health Order nr.438/295. Thus, the maximum dose of carminic acid (in meat, breakfast sausages with a minimum of 6% cereals, meat burger type with a minimum 4% vegetables and/ or cereals, soups, etc.) is 100 mg/ kg product; the maximum dose of colorant Ponceau 4R (in Chorizo sausage, sausages sobrasada, Salchichon sausages, fish paste and shellfish, etc..) is 200-250 mg / kg product, and the maximum dose of azorubine (surimi, produced similar meat and fish-based protein vegetable, flavored nonalcoholic beverages, wines, fruit, etc..) is 100 mg/ kg product. Concentrations of colorants to the analyzed substrate were by 0.2%, 0.4%, 0.6%, 0.8%, 1% and 1.2%. Maximum concentration used by 1.2%, corresponding to an addition of 0.5% coloring substrate in food, respectively, of concentration by 60 mg/kg product.

2.6. Establishing the centrifugation parameters (speed and time)
In order to establish the optimal centrifugation parameters which allow obtaining a supernatant without substrate particles (having the extinction equal to the distilled water), the maize starch and carrageenan suspensions were subjected to increasing centrifugation speeds for different periods of time. Concerning the maize starch suspensions the optimum centrifugation parameters were found to be 4000 rpm, for 10 minutes while for the carrageenan suspensions the optimum speed was 3000 rpm, for 10 minutes centrifugation time.

3. Results and discussions
Table 1 presents the results concerning the adsorption coefficient and adsorption speed of the three tested colorants on the maize starch.

<table>
<thead>
<tr>
<th>Concentration of colorant (%) in relation to maize starch</th>
<th>Adsorption coefficient (mol/g)</th>
<th>Adsorption speed (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carminic acid</td>
<td>Ponceau 4R</td>
</tr>
<tr>
<td>0.2</td>
<td>0.0026</td>
<td>0.0014</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0052</td>
<td>0.0024</td>
</tr>
<tr>
<td>0.6</td>
<td>0.0076</td>
<td>0.0032</td>
</tr>
<tr>
<td>0.8</td>
<td>0.0100</td>
<td>0.0040</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0110</td>
<td>0.0048</td>
</tr>
<tr>
<td>1.2</td>
<td>0.0129</td>
<td>0.0056</td>
</tr>
</tbody>
</table>
Analyzing the results presented in Table 1, one can see that the increase of the colorant concentration lead to the increase of the adsorption coefficient and the adsorption speed of the colorants. The 6 times increase of the colorant quantity lead to the increase of the adsorption coefficient of 4.96 times in case of the carminic acid, 4 times in case of the Ponceau 4R and 4.26 times in case of the azorubine. The two parameters analyzed have the highest values for the carminic acid, and the lowest values for the colorant Ponceau 4R, on the whole range of analyzed concentrations.

The highest adsorption speed of the colorants by the maize starch was obtained for carminic acid when the adsorption speed was 35.97% higher compared to the adsorption speed of the azorubine and 46.40% higher compared to the adsorption speed of the Ponceau 4R. The reported results in terms of adsorption coefficient and adsorption speed were obtained when using a colorant concentration of 1.2% in relation to maize starch.

The changes of the level of colorants retention on maize starch suspension in relation to the colorant concentration are shown in Figure 2.

![Figure 2. Changes of the retention level of colorants in the maize starch suspensions, in relation with the colorants concentrations](image)

Carminic acid had a retention level of 68.682%, followed by azorubine with a retention level of 54.814% and the last is the colorant Ponceau 4R with a retention level of 47.030%. These results were obtained for a colorant concentration of 0.2% in relation to maize starch. Increasing the concentration of the colorants has resulted in lowering the retention level.

In Table 2 are presented results concerning the adsorption coefficient and adsorption speed of colorants on carrageenan.

<table>
<thead>
<tr>
<th>Concentration of colorant (%) in relation to carrageenan</th>
<th>Adsorption Coefficient (mol/g)</th>
<th>Adsorption Speed (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carminic acid</td>
<td>Ponceau 4R</td>
</tr>
<tr>
<td>0.2</td>
<td>0.0029</td>
<td>0.00145</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0054</td>
<td>0.0028</td>
</tr>
<tr>
<td>0.6</td>
<td>0.0080</td>
<td>0.0039</td>
</tr>
<tr>
<td>0.8</td>
<td>0.0105</td>
<td>0.0044</td>
</tr>
<tr>
<td>1.0</td>
<td>0.0132</td>
<td>0.0054</td>
</tr>
<tr>
<td>1.2</td>
<td>0.0153</td>
<td>0.0055</td>
</tr>
</tbody>
</table>

Increasing the concentration of colorants in relation to carrageenan leads to the increase of the adsorption coefficient and adsorption speed. By increasing for 6 times the colorant concentration we...
obtained an increase of the adsorption coefficient of 5.27 times in case of the carminic acid, of 4.78 times in case of the azorubine and of 3.79 times in case of the Ponceau 4R.

The highest adsorption speed of the colorants on the maize starch was obtained for the carminic acid; the adsorption speed was 35.97% higher compared to the adsorption speed of azorubine and 46.40% higher compared to the adsorption speed of Ponceau 4R. The reported results in terms of adsorption coefficient and adsorption speed were obtained when using a colorant concentration of 1.2% in relation to carrageenan.

The highest retention level of dyestuffs on maize was obtained for acid carmine, when the retention was 35.97% higher compared to the azorubine and 46.40% higher compared to Ponceau 4R. The results were obtained for colorants concentration of 1.2% in relation to starch.

The changes of the level of colorants retention on carrageenan suspension in relation to the colorants concentration are shown in Figure 3.

Analyzing the results we observe that carminic acid had the highest retention level of 74.111%, followed by azorubine with a retention level of 64.397% and the last was the Ponceau 4R with a retention level of 51.101%. These results were obtained for a colorant concentration of 0.2% in relation to carrageenan.

4. Conclusions
Analyzing the results obtained, the following conclusions may be highlight:
The retention level of the colorants, by both analyzed substrates, has the highest value for a concentration of 0.2% colorants. The analyzed adsorption parameters were higher for the carrageenan compared to maize starch. This might be due to the difference between carrageenan and maize starch in terms of hydration capacity; the carrageenan has a hydration capacity of 3.4 ml water/g carrageenan, while in case of maize starch the hydration capacity is 0.95 ml water/g maize starch.

The carminic acid is adsorbed, by both analyzed substrates, more than azorubine and Ponceau 4R. The amount of adsorbed colorant is inversely proportional to the molecular mass of the colorant; carminic acid has the smallest molecular mass and the highest retention level in relation to other tested colorants.

References
Agunbiade, S., Longe, O. 1999. The physico- functional characteristics of starches from cowpea (Vigna unguiculata), pigeon pea (Cajanus cajan) and yambean (Sphenostylis stenocarpa). Food Chemistry 65, p 469-474.


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