Natural colorants stabilisation by immobilization in oil emulsions

Received for publication, July 25, 2007
Accepted, September 15, 2007

ROMICĂ CRETU*, GABRIELA BAHRIM, DIMA STEFAN*, MIHAELA OLTEANU *****

** Dunarea de Jos University of Galati, Faculty of Science, 111 Domneasca Street, 800201Galati, Romania, e-mail:* romy_cretu@yahoo.com; Stefan.Dima@ugal.ro

*** Dunarea de Jos University of Galaţi, Faculty of Food Science, 111 Domneasca Street, 800201Galati, Romania, e-mail:* Gabriela.Bahrim@ugal.ro

****University of Bucharest, Physical Chemistry Department, Bd. Regina Elisabeta, Romania*

Abstract

*In this study, we achieved the immobilization of some natural colorants in certain oil in water and/or water in oil food emulsions. Emulsions were prepared using sunflower oil, stabilized with the different surfactants (SPAN 80, TWEEN 60) and Arabic gum. After two-month equilibration in the reservoir, the emulsions remain stable. The stability of emulsions in function of the emulsifier nature, pH of the solution, the presence and contribution of Arabic gum has been investigated. Colour measurements of the tristimulus coordinates (L*a*b*) of the coloured food emulsions indicated that they are very little modified after two months' storage at room temperature.*

Keywords: colorants, immobilization, food emulsions, tristimulus colorimetry

Introduction

Emulsions are dispersions of one liquid phase as fine droplets in another immiscible liquid phase. The size of the droplets is usually larger than 0.1 μm, which gives emulsions their characteristic milky-white colour. The immiscible phases are usually oil and water, so emulsions can be broadly classified as oil-in-water or water-in-oil emulsions, depending on the dispersed phase [1].

Many manufactured food products are to be found in the form of oil- in -water emulsions or, sometimes, water -in -oil emulsions. The quality of emulsion -based food products (e.g., cream, fruit beverages, salad dressings, mayonnaise, soups etc.) is largely determinate by their appearance, texture or shelf life. A great deal of research has been carried out by colloid scientists to elucidate the factors which influence the texture and stability of food emulsions [2].

Thus, one of the most important problems from an industrial point of view is the formation and stability of emulsions.

Stability of emulsions still attracts many researches. This is due to the practical importance of such systems, for example, in food industries. Stability/instability of food emulsions is strongly dependent on the emulsions droplet size, which further depends on the conditions at which the emulsions has been prepared.

Emulsification is a complicated process which has been widely investigated both theoretically and experimentally [3]. However, many of the sub-processes are poorly understood.

The role of the surfactants may be manifold. First of all, they may change the interfacial oil/water tension, as well as the surface charge of the droplet.

Natural dyestuffs from these emulsions may be immobilized by the emulsification method [4].

Tristimulus colorimetry has been widely applied in food emulsion analysis with different purposes, such as the assessment of colour changes during food emulsions storage.

The effects of numerous factors and process variables were studied. Usually, an overall effect of various factors was followed and their influence on dispersion and characteristics of emulsions was concluded. In systematic investigations of a particular oil/emulsifier/colorant/water system, as many factors as possible should be taken into account [5].

For this reason in this paper the stability of the emulsion types was investigated taking into account the emulsifier nature, the colorants concentration, pH, the presence and contribution of some hydrocolloids (Arabic gum), as well as colour evaluation of these emulsions storages at room temperature.

On the other hand, this study describes how the colour and colour stability of some food emulsions vary in the pH range 4.0-8.0 during storage at room temperature.

Material and Methods

Reagents

The oil used as the lypophyl phase of the emulsions was sunflower oil (Prutul S.A., Galati, Romania). The emulsifiers Polyoxyethylene (20) Sorbitan monostearate (Tween 60), Sorbitan monooleate (Span 80) and the hydrocolloid (arabic gum) were products at PROLABO, France. The water for the experiments was deionised.

Natural colorants used were:

- Comercial β−carotene (E 160.a/C.I. no. 40800) provided by Sigma-Aldrich Chemie GmbH, Germany
- Fungal colorant (FC) biosynthesised by a *Epicoccum nigrum* selected strain, rich in carotenoid and flavonoid pigments, obtained by solid state fermentations in Microbiology Laboratory of Faculty of

Food Science of "Dunărea de Jos" University Galati, Romania [6]

- Carmin Erka type 895 (Cochineal, E 120/C.I. no. 75470) obtained from Ringe & Kuhlmann (Germany).

Equipment

An S 750 UV-Visible spectrophotometer (A.C.I. Orssey-Paris, France) was used to perform the absorbance and transmittance measurements. Digital pH Meter equipped with a combined glass-calomel electrode and 1 mm cells were also used for the experimentation (the pH values were measured at room temperature). Emulsions produced were observed using an optic microscope and microscope images were captured with a video camera (VP-L907, Samsung) and transferred for analysis. The kind of the emulsion (oil-in-water-O/W or water-in-oil-W/O) was identified by means of electrical conductivity measurements (Digital Conductivity Meter) and the drop size by optical microscopy (Zeiss Microscope). All vessels and glassware were cleaned and consecutive abundant rinsing with deionised water.

Methods

Emulsion Preparation. Several O/W and W/O emulsions were prepared (at room temperature: 20 ± 2 ^{0}C). The emulsions were prepared with Span 80 solution for the W/O emulsions or Tween 60 solution for the O/W emulsions using Ultraturrax (T-309, France) homogenizer. The deionised water soluble surfactant (Tween 60) was initially dissolved into the aqueous phase, while the oil soluble surfactant (Span 80) was initially dissolved in the sunflower oil. The β–carotene was dissolved in the sunflower oil, FC in ethanol (96%) and the Carmin Erka in water.

The water and oil phase were put in contact for pre-equilibration before preparing the emulsion.

Emulsions containing surfactants and natural colorants and Arabic gum solutions at varied volume ratios were prepared by mixing the pre-equilibrated phases [7] using a high-speed blender (Ultraturrax homogenizer) at 2000 rpm. Emulsification time was 15 minutes.

Spectral measurements. To obtain the tristimulus values, recommendations made by the Commission Internationale de l'Eclairage: CIE 1964 (x, y) system (CIEXYZ), CIE 1976 (L*a*b*) space (CIELAB), CIE 1986 and CIE 1995 were applied, using as references the CIE Standard Illuminant C and bi-distilled water as reference blank. The spectral transmittances in the visible spectra (the spectra measurements were recorded between λ=380 nm and λ=780 nm) were measured by spectrophotometery. For that purpose quartz cells with a path length of 10 mm were used. Visible measurements were made at 24 h after emulsion preparation.

Results and Discussions

The types of some emulsions prepared are: emulsion 1 (sample I, with fungal colorant and Span 80), emulsion 2 (sample II, with *Carmin Erka and* Span 80), emulsion 3 (sample III, with 0.01% β–carotene and Tween 60) and emulsion 4 (sample IV, with 0.005% β–carotene and Tween 60). The type of these emulsions was confirmed by means of electrical conductivity measurements.

The influence of some factors on emulsions stability was studied during storage.

Arabic gum (2%) was added to the aqueous phase to improve the viscosity and stability of emulsions.

The emulsions indicated have a milky appearance, because the average droplets size is large (8 microns). Particle size of all emulsions was measured after samples were stored overnight and after 3, 7 days at room temperature. The most frequent occurring droplet diameters were constant during emulsification.

In all cases the absorbance and transmittance measurements at wavelengths 445 nm, 495 nm, 550 nm and 625 nm were also carried out because at these wavelengths only it is possible (following the recommendations made by the CIE, 1964, 1976) to calculate the CIE tristimulus values.

Determination of the emulsions stability by turbidity index

In the experiments, the turbidity was measured as the transmittance (τ) defined by:

$$\tau = -\ln\left(\frac{\%T}{100}\right)/L,\ (\mathrm{cm}^{-1}) \qquad (1)$$

(were τ and T are the turbidity index and the transmittance respectively, and *L* is the path length) as a determination of the turbidity because of its simple relation with the transmittance [4].

Experimentally, the prepared emulsions were diluted 1:100. The results obtained show an important decrease in food emulsion stability.

At the same time, there is a slight decreased in the emulsions stability after 3 or 7 days for sample I (Figure 1). The destabilization after first days can be explained by what is called depletion flocculation.

In the case of emulsions containing β–carotene these were somewhat stable excepting the emulsion 3 where the concentration of the colorant was probably too bigger.

From these results it can be said that the emulsion – food colorant systems, during the storage at room temperature under normal electric light, for approximate two month was stables.

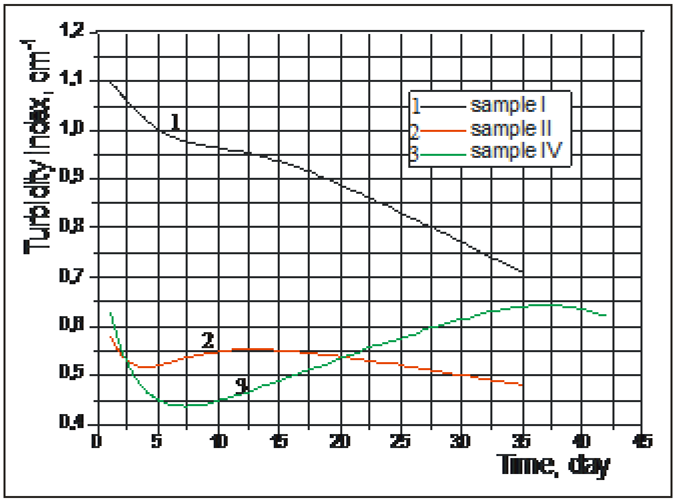

Figure 1. The turbidity of the emulsions

Influence of pH on emulsion - food colorant systems stability

The effect of pH was also investigated. The influence of pH on the absorption spectra for emulsions with natural colorants (fungal colorant, Carmin Erka and β−carotene) was studied.

The effect of pH on the retention of the food colorants in the emulsions was studied using (0.1 M) citric acid and (0.2 M) sodium acid phosphate solutions for adjustment.

Figure 2 shows the pH-influence on the absorbance of two food colorant fixed in emulsions (emulsion 1 and emulsion 2). In this case the wavelength of the maximum absorption were located at λ = 630 nm and λ = 480 nm respectively.

The fungal colorant (immobilized in W/O emulsion, emulsion 1) shows absorbance that remains constant for pH values between 4.0 and 4.6, then the absorbance increase.

Only at pH > 5.38 did the absorbance of the system decrease markedly, which can be attributed to the alteration of the emulsion in alkaline medium especially. In this way, we selected pH 5.38 as the working value in this situation because at this value the colorant presents a peak of the absorbance.

Emulsion 2 shows a maximum at λ = 480 nm which absorbance remains constant for pH values between 6.38 and 6.9.

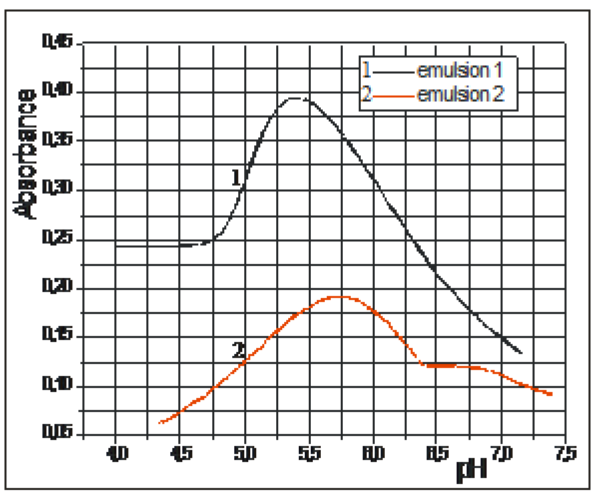

Figure 2. The pH-influence on the systems colorants-emulsions absorbance

Colour evaluation

The aim was to establish how the colour and colour stability of emulsions in the time and in the pH range 4.0-8.0 during storage.

Tristimulus colorimetry, which provides the trichomatic coordinates defined by the CIE system, is a rapid and easily applied method which is used to measure the colour of many foods. On the other hand, colours can be considered as combinations of red and yellow, red and blue, green and yellow, and green and blue. In the CIE L*a*b* uniform colour space, the colour coordinates are: L* - the brightness coordinate; a* - the red/green coordinate, with +a* indicating red, and -a* indicating green; b* - the yellow/blue coordinate, with +b* indicating yellow, and -b* indicating blue. L*, a*, and b* are transformed (computed) from the CIE tristimulus values (X, Y, Z), using the CIE 1976 equations. The L*, a*, and b* coordinate axis define the three dimensional

CIE colour space. Thus, if the L*, a*, and b* coordinates are known, then the colour is not only described, but also located in the space.

Figure 3 shows the evolution of the brightness (lightness, L*) with pH in the emulsion 1 case.

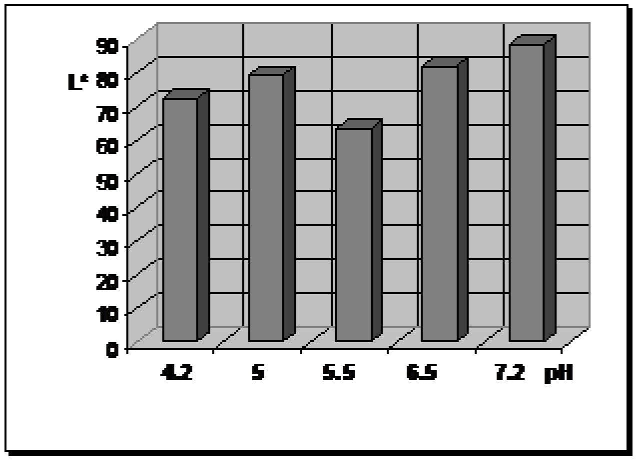

Figure 3. The influences of the pH on brightness coordinate of the emulsion 1

In most cases, an increase progressively for lightness exists after pH 5.0 – 5.3, indicating a discoloration of the emulsions. Similar results in all studied cases were obtained.

Figure 4 show the changes in the colour of some emulsions storages at room temperature in normal light.

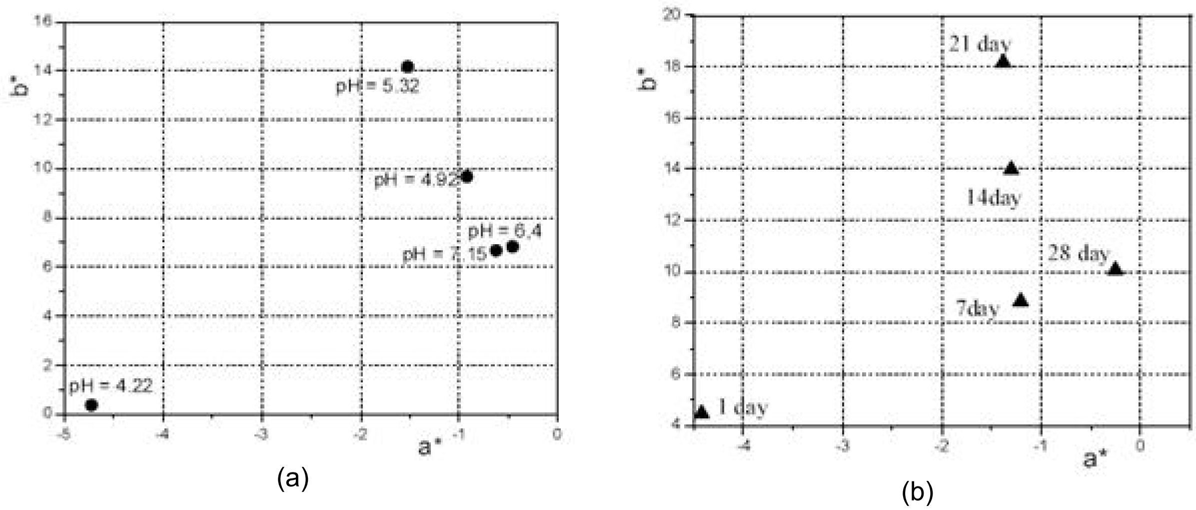

Figure 4. Colour of the emulsions in the CIELAB
a) Evolution of colour of the emulsion 1 in the CIELAB diagram for the different pH values;
b) Projection of an a* and b* parameters in the plane for the emulsion 3 in the CIELAB diagram vs storage time

As can be observed in figure 4, the points which are represented in function of the chromatic parameters of emulsions move from the negative a* to the positive b* values. The colour diagram CIELAB for all samples indicated that the colour shade of emulsions stored in normal light at room temperature was stable and the colour remained yellow after 4 week of storage.

Conclusions

The stability of the natural colorants – food emulsion systems depends on the concentration and type of surfactant and colorant from emulsion preparation, pH and time of storage.

The optimum pH value for emulsion- colorant systems stability varies depending on the colorants and emulsion system composition.

The coordinates L*, a*, and b* showed a relationship between the colour of samples and the storage conditions. It should be noted that the colour shade of emulsions containing food colorants is stable for a long period of time during storage at room temperature under normal electric light.

The use of some natural colorants in emulsion food products (ice cream, milk creams, mayonnaise, soups, salad dressing, meat emulsions, etc.) should therefore be considered at least in food products with limited storage time which are kept in a refrigerator or at room temperature.

Acknowledgements

Financial support from BioFood Platform, CNCSIS Project Code 62, is highly acknowledged.

References

1. T. M. Gipsy, G. V. Barbosa-Ca´novas, *Journal of Food Engineering*, **67**, 147-156, (2005)
2. E. Dickinson, D. J. MCCLEMENTS, *Advances in food colloids,* Advances in food colloids, Blackie Academic and Professional, Chapman and hall, London,UK (1996)
3. P. WASTRA, *Encyclopedia of Emulsions Technology,* In: P. Becher (ed), Dekker, New York, (1983)
4. R. CREŢU, M. OLTEANU, S. DIMA, G. BAHRIM, *International Symposium on Future of Food Engineering*, Warsaw, Poland, 40 (2006)
5. W. CHANTRAPORNCHAI, F. CLYDESDALE, D.J. MCCLEMENTS, *J. Agric. Food Chem.,* **46**, *2914-2920* (1998)
6. F. ŞOPTICĂ, G. BAHRIM, *Roumanian Biotechnological Letters*, *An international journal*, **10** (5), 2387-2394 (2005)
7. I. IVANOV, K. MARINOVA, R. ALARGOVA, N. DENKOV, R. BORWANKAR, *Deuxieme Congres mondial de l'emulsion second world congress on emulsion*, Bordeaux, France, 23-26 sept., 2-2, 149: 01-06 (1997)