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Research Article

Influence of casein and different supplements into stability of Corn O/W emulsions by determination of thermodynamic parameters of the system

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Abstract

The effects of casein addition in O/W emulsions made with 2 and 4 wt% protein and 10, 15, 25 and 35 wt% corn oil were investigated. A starch, NaCl and KCl, were used as supplements to prove the emulsion stability. Different analyses were prepared for investigation of emulsions. pH was measured and the values – connected with gravitational creaming and phase separation. Comparing the results of analyses determined that emulsions prepared with 2 wt% of casein as protein, 25% of corn oil and mix of starch 2% - KCl 2 % exhibit more stability.

Keywords: O/W emulsions, corn oil, casein, starch, electrolytes, thermodynamic

Abbreviations:

ΔG	–	Gibbs free energy
ΔH	–	enthalpy
ΔS	–	entropy
CI	–	creaming index
PTFE	–	polytetrafluoroethylene
CMC	–	critical micellization concentration
H_E	–	Total height of the emulsion
H_L	–	Lower serum layer
K	–	equilibrium constant

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Introduction

Emulsions are thermodynamically unstable systems. During storage they exhibit properties as flocculation, coalescence, sedimentation, creaming, Ostwald ripening or phase inversion (McClements 2005). Interactions of emulsified fat-globules with proteins influence the overall functionality and the quality of the final product (Wu 2009). Flavor and color of food products were often generated by an emulsification process between components in a water phase and oil phase. A wide variety of oils were used in the preparation of different emulsions. Using corn oil for oil phase stabilized with proteins was appropriate compounds for foods (Friberg and Larsson 1997; Krog et al. 1983; Jaynes 1985; Dickinson and McClements 1995; Swaisgood 1996). Polytetrafluoroethylene (PTFE) membrane filters were tested for preparing corn oil emulsions by the membrane emulsification method using a pre-emulsified emulsion (Kanichi et al. 1988; Yamazaki et al. 2003). A hydrophilic polytetrafluoroethylene membrane was used to prepare an O/W emulsion without any disturbance of the continuous phase. The mean particle diameters of the O/W and W/O emulsions were determined (Yamazaki et al. 2003). The effects of lecithin addition in oil or water phase on the stability of O/W emulsions made with 0.1 wt% whey protein and 10 wt% n-tetradecane at neutral and acidic pH were studied (Yamamoto et al. 1997). The purity of lecithins and the way to add them were suggested to be very important to make a stable emulsion with protein. The critical micellizationconcentration data and solubilisation capacities obtained from a pyrene solubilisation method were studied (Wang et al. 2014). The Van`t Hoff equation was used to obtain the thermodynamic parameters: Gibbs free energy (ΔG), enthalpy, (ΔH) and entropy (ΔS) of emulsions (Panajotova et al. 2017). The investigation of oil-in-water micro-emulsions at different temperatures 4 - 40°C was done (Gupta et al. 2006). The O/W emulsions using non-ionic Tween series surfactants were investigated (Hsu and Nacu2003). The scope of this work is to investigate emulsion stability in emulsions stabilized with casein as protein and the addition of different supplements and made application in food industry.

Materials and Methods

Materials. The corn oil was obtained from a local producer Agro.bg, water was distilled, the casein was from Merck (Germany), the reagents starch (wheat), KCl and NaCl were from FILLAB, Bulgaria.

Preparation of emulsions. The corn oil was used for all emulsion preparation. As stabilizer was used casein (100% pure protein). The emulsions were prepared as follows: different concentrations of protein were added in water and soluble in it (% casein and water is indicated in Table 1). After that corn oil was slowly added (Table 1). The emulsions were prepared with homogenizer (Bosh, 1200 W) with 2 min of mixing. Supplements added in continuous phase and soluble before adding oil.

Microscopic observation. The emulsions were investigated with digital microscope Bresser. They were observed 10 min after their preparation. Digital microscopes, like the „Bresser Junior“ USB Hand-held Microscope, were powered by the USB port on a PC (magnification ×100). The images from the MikroCam MP Microscope camera were prepared for all samples. According to the experimental results the particle size was measured in micrometers.

Determination of thermodynamic parameters. For this purpose, equilibrium constant was estimated at all samples using spectrophotometer measurements of absorbance using so called “dilution method” (Kendrow et al. 2009).

Water solutions with different emulsion concentration: 0.04, 0.08, 0.12, 0.16 and 0.2 mol dm-3 was prepared. The investigations were performed with Camspec M107 UV/VIS spectrophotometer. Measurements were done at 420 nm.

Gibbs free energy was calculated by following equation (1):

$$\Delta G = - RT \ln K \qquad (1)$$

where: R is the universal gas constant (R = 8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K), K - equilibrium constant.

Determination of creaming index: Long - term storage test. The long - term storage test was provided. After preparation of emulsions, they were put in the cylinders and after storage at 84th h and the results were observed and checked. The measurements were connected with gravitational creaming and phase separation. The creaming index was calculated using equation (2):

$$CI = 100 \times H_L / H_E \quad (2)$$

where: CI – creaming index, H_L - lower serum layer, H_E - total height of the emulsion.

Statistical analysis. The data of different samples were analyzed independently by Origin v. 7.0 software. The values and standard errors of the particle sizes and thermodynamic parameters were determined at probability $p \leq 0.05$.

Results and Discussion

The 25 emulsions were prepared with different mass % O/W and 2 and 4% casein. As supplements prove the emulsion stability were used: starch, NaCl and KCl. The samples were separated in five series. After preparation of emulsions the pH values were determined. The pH was between 4 – 4.80. The description of chemical composition of emulsions was presented in Table 1.

Table 1. Caption Samples %, 100 mL emulsion.

№	Casein	Oil	Water
Casein:O:W			
1	2	10	88
2	2	15	83
3	2	25	73
4	2	35	63
5	4	10	86
Casein – NaCl 2%:O:W			
6	2+2	10	86
7	2+2	15	81
8	2+2	25	71
9	2+2	35	61
10	4+2	10	84
Casein – starch 2%:O:W			
11	2+2	10	86
12	2+2	15	81
13	2+2	25	71
14	2+2	35	61
15	4+2	10	84
Casein – starch 2% - NaCl 2%:O:W			
16	2+2+2	10	84
17	2+2+2	15	79
18	2+2+2	25	69
19	2+2+2	35	59
20	4+2+2	10	82
Casein - starch 2% - KCl 2%:O:W			
21	2+2+2	10	84
22	2+2+2	15	79
23	2+2+2	25	69
24	2+2+2	35	59
25	4+2+2	10	82

First series were prepared as plane emulsions with O/W and 2 and 4% of casein. Second series prepared by addition of NaCl, third with addition of starch, fourth with addition of starch + NaCl, fifth with addition of starch + KCl. In all samples the water varies between 59% to 86% connected with different % of protein, oil and different supplements.

Various analyzes were done to examine the stability of the emulsions. It was found that in microscopic pictures, samples with 25% oil were smaller in size than the ones.

These results were associated with the measured absorption of the solutions of the respective emulsions used to calculate the equilibrium constants of the process. According the spectrophotometer measurements on these samples, a higher absorption was measured, hence higher equilibrium constant values. Large equilibrium constants were associated with small, negative Gibbs energies that were evidence of system stability. The microscopic observation of each emulsion was 10 min. after preparation. The microscopic pictures were taken for all samples. According the obtained results observed polydisperse emulsions with different particle sizes from small in emulsions with high % oil to large in emulsions with high % water. The emulsions with large size droplets were unstable, because this was connected with the interaction between them and

with phenomena coalescence. With the increasing of mass % of oil smaller droplets appear. This indicates that the emulsions with small droplets exhibit more stability.

Figure 1 presented micrographs of samples 3 and 23. Figure1a presented sample 3 with chemical composition 2% casein, 25% oil, 73% water. Figure1b presented sample 23 with chemical composition 2% casein, 2% starch, 2% KCl, 25% oil, 69% water.

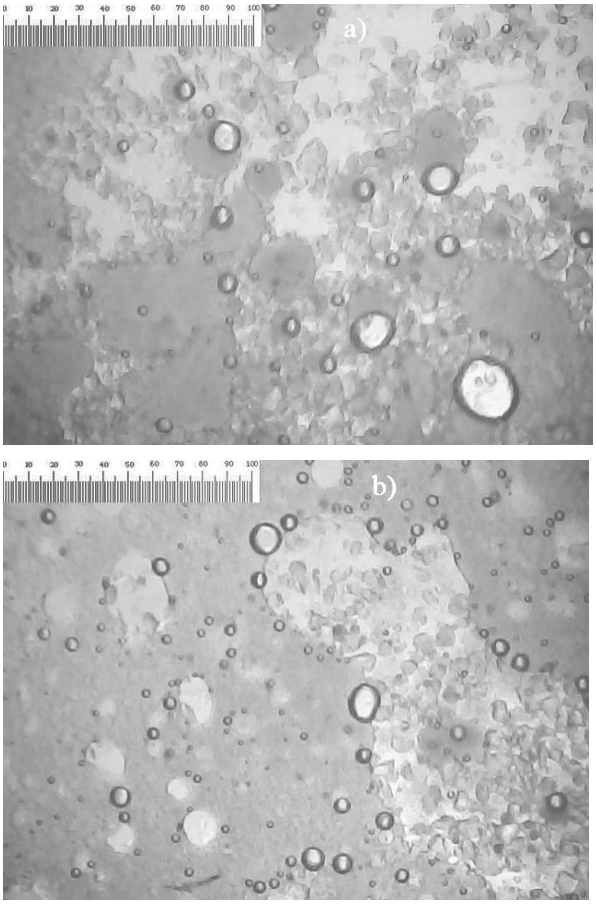

Figure 1. Microscope pictures of a) sample 3 and b) 23, 10 minutes after preparation of emulsions.

Similarity between samples appeared in the same % casein and oil. Differences between them appeared in different editions. Sample 3 was prepared only with casein, oil and water. In sample 23 were added and different additions (2% starch, 2% KCl). The samples compared with another emulsion samples exhibit small particle sizes. According the results small sizes connected to the stability of emulsions. Editions in sample 23 starch and KCl prove extra stability in the system. For samples 3 and 23 middle radius of particle sizes varies between 10±1.12 - 25±1.71 μm. The discrepancies observed in particle sizes of the emulsions, connected with respect to effectiveness in oil and water emulsification. In samples prepared with small oil % and large water % was observed particles with large sizes. For example in samples 1 and 5 observed particles with sizes between 35±0.89 - 42±1.32 μm. Two samples prepared with 10% oil and water between 86-88%. The small amount of oil and missing of starch and KCl as additions lead to large particles. It was worth to note that differences in emulsification capacity were mainly related to the nature of the oil phase, and not to pH (Figueiredo et al. 2008). Such interactions between casein, oil and water involve the hydrophobic domains of the casein molecules, in particular the hydrophobic terminal end of the casein fraction and the non-polar molecules leads to bad adsorption of casein in O/W emulsions. In this case in emulsions with small amount of oil bed adsorption of protein probably lead to unstable emulsions with large particle sizes. Thermodynamic parameters of emulsion samples ΔG, ΔH and ΔS were calculated and their values were presented in Table 2. The classical thermodynamics equations were used (Panajotova et al. 2017). Due to this purpose, equilibrium constant was estimated at all samples using spectrophotometer measurements of absorbance. In different series emulsions seen different values of ΔG, from negative to positive. The Gibbs free energy was the criterion for emulsion stability (Gandova and Balev2016). This was a reason that it can be concluded that the emulsification processes was spontaneous $\Delta G<0$ and the system was destroyed when $\Delta G>0$. When observed negative values the emulsion was stable. In this case the logarithm of equilibrium constant was positive ($K>1$). When the Gibbs free energy decrease to positive values (equilibrium constant was $K<1$) prepared emulsions exhibit unstable systems and destroyed immediately after preparation. In these observations the equilibrium constants exhibit values between 0.363 to 1.975, Gibbs free energy between -1.686 - 3.414 kJ mol^{-1}.

Table 2. Thermodynamic parameters Gibbs free energy, Enthalpy and Entropy

№	ΔG [kJ mol^{-1}]	ΔH [kJ mol^{-1}]	ΔS [kJ mol^{-1} K^{-1}]
Casein:O:W			
1	0.632±0.031	-17.069±0.022	-0.059±0.001
2	-0.984±0.011	-17.770±0.013	-0.056±0.002
3	-1.655±0.042	-18.062±0.061	-0.055±0.001
4	-0.811±0.036	-17.695±0.075	-0.057±0.003
5	2.514±0.054	-16.251±0.048	-0.063±0.002
Casein - NaCl:O:W			
6	3.314±0.045	-15.904±0.093	-0.064±0.006
7	0.296±0.016	-17.215±0.054	-0.059±0.001
8	-0.179±0.051	-17.421±0.051	-0.058±0.003
9	3.199±0.098	-15.954±0.032	-0.064±0.002
10	1.596±0.066	-16.650±0.033	-0.061±0.004
Casein - starch:O:W			
11	-0.789±0.027	-17.686±0.131	-0.057±0.004
12	0.884±0.054	-16.959±0.086	-0.060±0.001
13	-1.222±0.092	-17.874±0.102	-0.056±0.009
14	2.349±0.065	-16.323±0.055	-0.063±0.005
15	-0.503±0.043	-17.561±0.122	-0.057±0.001
Casein - starch - NaCl:O:W			
16	1.164±0.051	-16.837±0.055	-0.060±0.002
17	0.713±0.063	-17.033±0.096	-0.060±0.001
18	-1.490±0.052	-17.990±0.068	-0.055±0.002
19	-0.346±0.091	-17.493±0.034	-0.058±0.006
20	0.797±0.112	-16.997±0.071	-0.060±0.001
Casein - starch - KCl:O:W			
21	-0.602±0.062	-17.604±0.033	-0.057±0.008
22	0.950±0.011	-17.288±0.059	-0.058±0.002
23	-1.686±0.014	-18.075±0.024	-0.055±0.006
24	0.928±0.122	-16.940±0.086	-0.060±0.001
25	-0.292±0.018	-17.470±0.135	-0.058±0.002

Enthalpy of formation exhibits negative values in all samples and this was connected with exothermic process. Entropy in the emulsions exhibit very small values and these negative values connected with phase separation of emulsion system make it difficult to determine the direction of the process.

The addition of KCl proves emulsion stability and in sample 23 observed the small particle sizes and the smallest Gibbs free energy. In emulsions with particle sizes up to 30 μm the positive Gibbs free energy can be seen. These were samples usually prepared with high % water. Good agreement between the results obtained with microscopic observation and Gibbs free energy calculations were observed. Figure 2 presented Gibbs free energy as dependent on mass of oil and water. In the Figure there are observed positive and negative values for energy.

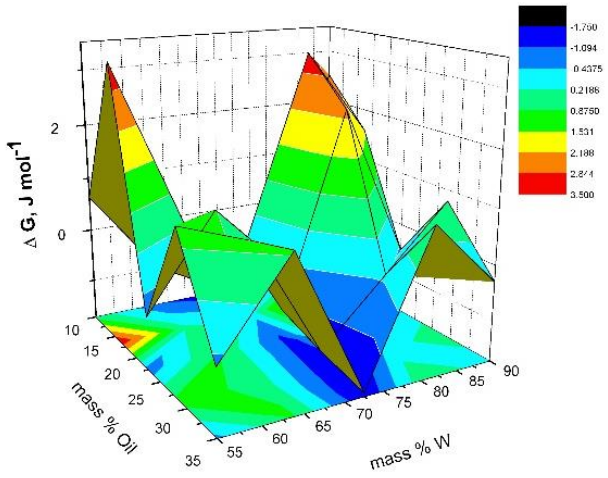

Figure 2. Calculated Gibbs free energy for O/W emulsions at different % of oil and water.

Samples 3 (-1.655±0.042 kJ mol-1) and 23 (- 1.686 kJ mol-1) show the highest Gibbs energy. According to Table 1, two samples prepared by 25% oil and approximately 70% water. The samples 8, 13 and 18 prepared with 25% oil too and exhibit negative Gibbs energy. This is the reason to believe that, initial composition for emulsion preparation is connected with emulsion stability. After the long - term storage, first observation was done 4 h after preparation. The next observations were prepared in 36th h and 84th h. The serum layer and creaming layer were measured. The measurements were connected with gravitational creaming and phase separation and pH do not have influence into them. This result used to determine creaming index of emulsions. The obtained results presented in Figure3 (McClements2005). For all samples the creaming index was determined between 59 and 84%. For samples 3 and 23 creaming index was determined at 80 and 84%. According the figure the high creaming index observed at 70 -75% water and 25% oil to O/W emulsions.

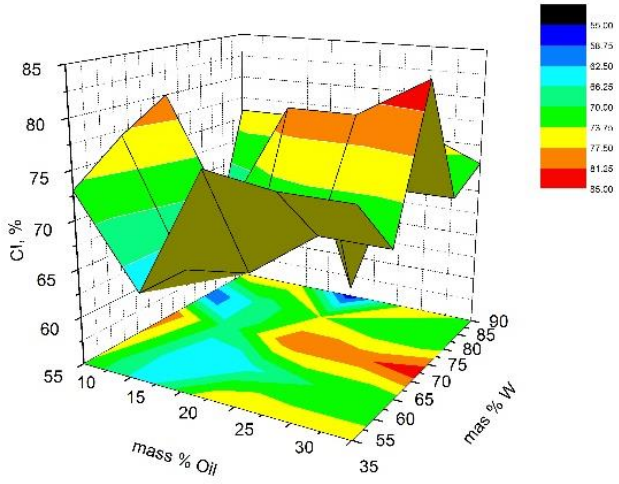

Figure 3. Calculated creaming index connected with phase separation determined on 4th, 36th and 84th h after preparation of emulsions

Figure 4 presented kinetic of process coalescence and creaming layer 84th h after preparation of sample 23. During the different measurements to 36th h was observed the increasing the creaming index, after that to 84th h the appeared the constant values.

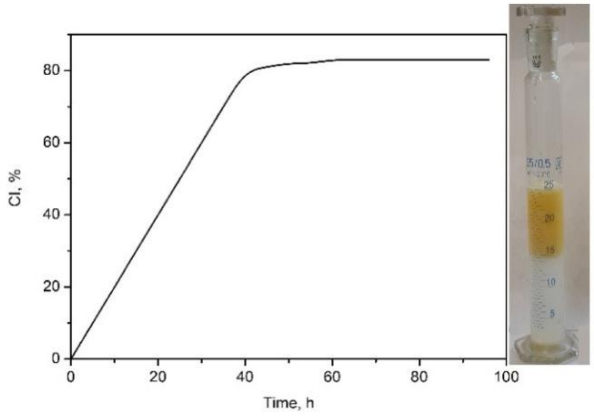

Figure 4. Kinetic of phase separation of sample 23 in different hours from preparing to emulsion to 84th h.

Conclusions

The emulsions O/W type with 2 and 4 wt% proteins and 10, 15, 25 and 35 wt% corn oil were investigated. The microscope observation, calculated Gibbs free energy and phase separation were used to determine emulsion stability. Good correlation between different experiments was shown. In this study, it was found that emulsion with 2% casein, 25 % of corn oil, and supplements in the form of mixed starch 2% - KCl 2%, exhibited good emulsion stability. Therefore, addition of KCl in emulsions could be appropriate for application in a healthy diet

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