EMULSION OF SYSTEMS CONTAINING EGG YOLK, POLYSACCHARIDES AND VEGETABLE OIL

Emulsão de sistemas contendo gema de ovo, polissacarídeos e óleo vegetal

Clitor Junior Fernandes de Souza[1], **Edwin Elard Garcia Rojas**[2]

ABSTRACT

This work characterizes the emulsifying properties of systems containing egg yolk (0.1; 1.0 and 2.5 % w/v) and polysaccharides (xanthan gum, carrageen, pectin and carboxymethylcellulose) and three different vegetable oils (sunflower, canola, and palm oils). Emulsifying activity and emulsion stability were measured of each combination and it was found the effect of the oil on emulsion stability correlated to the amount of monounsaturated fatty acid. Additionally, increased egg yolk concentration increased emulsifying activity by reducing coalescence of oil droplets. Lastly, 2.5% egg yolk and 0.2% polysaccharide generated emulsions with high emulsifying activity, excellent stability, and droplet size of 4.32 µm.

Index terms: Modeling, emulsifying activity, hydrophilic properties.

RESUMO

Neste trabalho, caracterizam-se as propriedades emulsificantes de sistemas contendo gema de ovo (0,1; 1,0 e 2,5% m/v), polissacarídeos (goma xantana, carragena, pectina e carboximetilcelulose) e três diferentes óleos vegetais (óleos de palma, canola e girassol). Atividade emulsificante e estabilidade da emulsão foram medidas para cada combinação e verificou-se o efeito do óleo sobre a estabilidade da emulsão correlacionada com a quantidade de ácido graxo monoinsaturado. Além disso, a concentração de gema de ovo aumentou a atividade emulsificante, reduzindo a coalescência das gotículas de óleo. Por último, 2,5 % de gema de ovo e 0,2% de polissacarídeo formaram emulsões com alta atividade emulsificante, excelente estabilidade e tamanho de gota de 4,32 µm.

Termos para indexação: Modelagem, atividade emulsificante, propriedades hidrofílicas.

(Received in august 6, 2012 and approved in august 13, 2012)

INTRODUCTION

An emulsion is a macroscopic dispersion of two liquids where one is a continuous part dispersed throughout small drops of the other (MORR, 1981). Proteins with an appreciable number of hydrophobic groups are effective emulsifying agents because they promote aggregation of oil droplets. However, proteins containing both hydrophobic and hydrophilic regions are superior emulsifiers because they significantly reduce load surface tension. This allows oil droplet breakage and formation of thin films around the surfaces of emulsified droplets (WALSTRA, 1983; TCHOLAKOVA; DENKOV; DANNER, 2004; WILDE et al, 2004).

Egg yolk proteins act as emulsifiers and are primarily livetins and low-density lipoproteins (LDL). The lipid in LDL consists of 70% neutral lipid, 26% phospholipids (71-76%, phosphatidylcholine, 16-20% phosphatidylethanolamine, and 8-9% sphingomyelin and lysophospholipids), and 4% free cholesterol (MINE; BERGOUGNOUX, 1998). Phospholipids, cholesterol, and both hydrophobic and hydrophilic proteins lend excellent emulsifying properties to egg yolk. The lipid fraction reduces the interfacial tension and facilitates fixation of macromolecules in the water-oil interface (MINE; BERGOUGNOUX, 1998; ALUKO; KEERATIURAI; MINE, 1998).

Interactions between anionic polysaccharide and protein form a conjugate which relies on hydrophilic properties of polysaccharides to thicken and stabilize aqueous medium adjacent to the emulsion interface. Therefore, it helps to stabilize drops formed by protein and prevent coalescence during the emulsification formation and subsequent storage (SHEPHERD et al., 1995; McCLEMENTS, 2004). Most hydrocolloids can act as stabilizers of oil-in-water emulsions, but only a few can act as emulsifiers. Functionality of emulsifiers demands a substantial surface activity at the oil-water interface, and the ability to facilitate the formation and stabilization of fine droplets during and after emulsification (WILLIAMS; PHILLIPS, 2004).

Emulsifier polysaccharides most often used in food are Arabic gum (CASTELLANI et al., 2010; CHAROEN et

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[1] Universidade Federal Rural do Rio de Janeiro/UFRRJ – Seropédica – RJ – Brasil
[2] Universidade Federal Fluminense/UFF – Departamento de Engenharia de Agronegócios – Avenida dos Trabalhadores – nº 420 – Santa Cecília – 27255-125 – Volta Redonda – RJ – Brasil – edwin@vm.uff.br

al., 2011), modified starch (MARTÍNEZ et al., 2003; FARRAG, 2008), modified cellulose (SUN et al., 2007; AMIRKHANIA et al., 2008), pectin (LITTOZA; McCLEMENTS, 2008; JONES; McCLEMENTS, 2010) and galactomannans (MIKKONENA et al., 2009; WUA et al., 2009). Protein ingredients derived from milk and/or egg, are commonly used as emulsifying agents (JONES; McCLEMENTS, 2010). Emulsion stability generally occurs according to the oil volume fraction, type of oil, temperature, pH, type and quantity of emulsifier(s), ionic strength, and the presence of other ingredients such as sugar and carbohydrates (MORR, 1981; McCLEMENTS, 2005).

This objective of this work was to evaluate emulsifying properties of egg yolk and four anionic polysaccharides (xanthan gum, pectin, carboximethylcellulose, and carrageen) and three vegetable oils (sunflower, canola, and palm).

MATERIAL AND METHODS

Materials

Xanthan gum (Product number G1253 EC 234-394-2), sodium carboximethylcellulose (Product number C4888-500), pectin (Product number P9135), κ-carrageen (Product number C1013) and egg yolk (Product number EO-625) were obtained from Sigma-Aldrich (St. Louis, USA). Canola, sunflower, and palm oils were bought in the local store. For this experiment analytic grade reagents and deionized water were used.

Preparation of aqueous polysaccharide solution

The polysaccharide (PO) solution contained xanthan gum (XG), κ-carrageen (CA), carboximethylcellulose (CMC), and low metoxilation degree pectin (PEC). Polysaccharides were dissolved in buffer (pH 7.0) at a concentration of 1% w/v using an analytical balance (Tecnal, model B-TEC-210A, Brazil) with uncertainty of ± 0.0001 mg. The solution was then shaken for 3 hr using a magnetic shaker (New Technique, model NT101, Brazil).

Electric conductivity measurements

Methods to evaluate emulsifications were adapted from previously described techniques (Al-MALAH; AZZAM; OMARI, 2000; AZZAM; OMARIA, 2002). Electrical conductivity (MCA 150, Tecnopon, Brazil) was used to extrapolate emulsifying properties of systems (20 mL) containing various concentrations of egg yolk (0.1; 1.0 and 2.5 % w/v) and polysaccharides (0.1; 0.2 and 0.4 % w/v) in buffer (pH 7.0). Canola, sunflower, or palm oil (7 mL) was added to the protein-polysaccharide system and the two phases were homogenized for 2 min using an Ultra-Turrax® (T 10 basic, IKA, Germany) at a speed of 18.600 rpm. Conductivity was measured during homogenization and 20 min afterward.

Determination of emulsifying properties

To determine emulsifying activity and emulsion stability, conductivity data measurements over time were divided in two stages respectively. The first stage was measured for all but the final 2 min of homogenization, and was used to determine emulsifying activity. The second stage began immediately after homogenization and was used to determine emulsion stability (ES) through the oil volume fraction of the creamed phase (Φ).

Kato et al. (1985) defined the emulsifying activity (EA) of a protein as the difference between conductivity of the protein solution prior to homogenization (C_0) and the minimum conductivity achieved during homogenization (C_2) (Equation 1).

$$EA = C_0 - C_2 \tag{1}$$

Anton and Gandemer (1997) proposed a mathematic model to determine Φ through its relationship with conductivity (Equation 2):

$$\Phi = 1 - \left[(V/v)(1 - C_F / C_0) \right] \tag{2}$$

Where V is the volume of the aqueous solution used in the emulsion formulation (20 mL), v is the volume of used oil (7 mL), CF is the conductivity of the emulsion and C_0 is the initial conductivity of the aqueous solution. All samples were made in duplicate and measurements were repeated twice. Statistical analyses were performed with SAS® software.

After evaluating the stability of the emulsion, microscopy was performed on a 1 mL aliquot to observe drop size and the distribution of the emulsion. Images were generated on an LV 150 (Nikon, USA) equipped with a 20× objective and assembled camera (DS-Fi1, Nikon, USA) and software (NSI-Elements D 3.0,USA).

RESULTS AND DISCUSSION

Evaluation of emulsifying activity

Figures 1, 2, and 3 summarize EA values as a function of EY concentration and that they gradually increase with increasing EY concentrations. Greater concentrations of protein molecules form more dense interfaces, which are absorbed more quickly around oil drops (formed during homogenization) and further reduce coalescence (SURH; DECKER; McCLEMENTS, 2006; DICKINSON, 2009). This is thought to occur due to the presence of amphipathic phospholipids in EY decreasing interfacial tension among formed oil drops (ALUKO; KEERATIURAI; MINE, 1998). Regarding polysaccharide concentration, greater concentrations of polysaccharides in the system reduce EA for the three studied protein concentrations (experiments 3, 6, and 9 in Figures 1 and 2; and experiments 6 and 9 of Figure 2). This observation may be due to increasing system viscosity leading to reduced adsorption of proteins on the surfaces of oil drops formed by homogenization (McCLEMENTS, 2006; WANG; WHANG; OZKAN, 2010). EINHORN-STOLL et al. (2005) observed that conjugates of whey protein isolate (WPI) with xanthan gum or pectin had lower emulsifying activity; however, the polysaccharide was capable of forming a strong 3-dimensional network around the droplets which prevented coalescence.

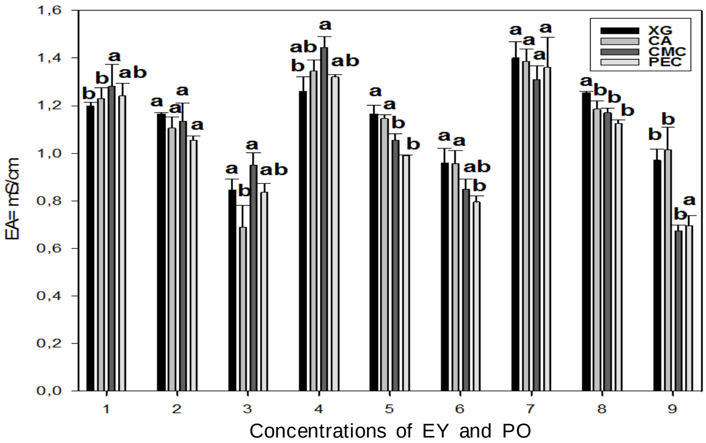

Figure 1 – Emulsifying activity of different concentrations of EY and PO with 7.0 mL palm oil. 1, 2 and 3 (0.1% EY: 0.1, 0.2 and 0.4% PO, respectively); 4, 5 and 6 (1% EY: 0.1, 0.2 and 0.4% PO, respectively); 7, 8 and 9 (2.5% EY: 0.1 0.2 and 0.4% PO, respectively).

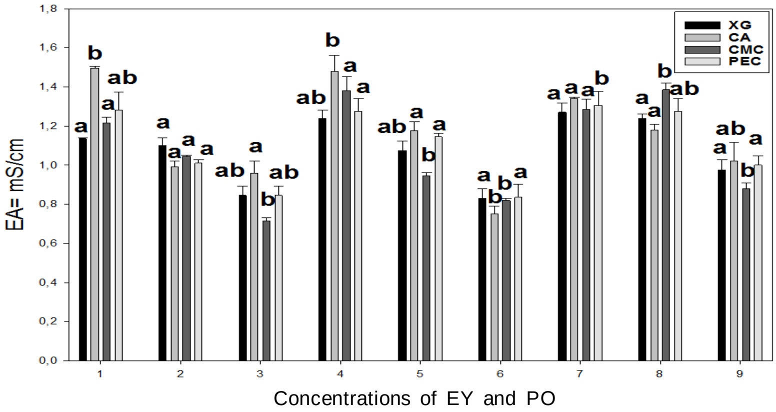

Figure 2 – Emulsifying activity of different concentrations of EY and PO with 7.0 mL sunflower oil. 1, 2 and 3 (0.1% EY: 0.1, 0.2 and 0.4% PO, respectively); 4, 5 and 6 (1% EY: 0.1%, 0.2 and 0.4% PO, respectively); 7, 8 and 9 (2.5% EY: 0.1%, 0.2 and 0.4% PO,Concentrations of EY and PO respectively).

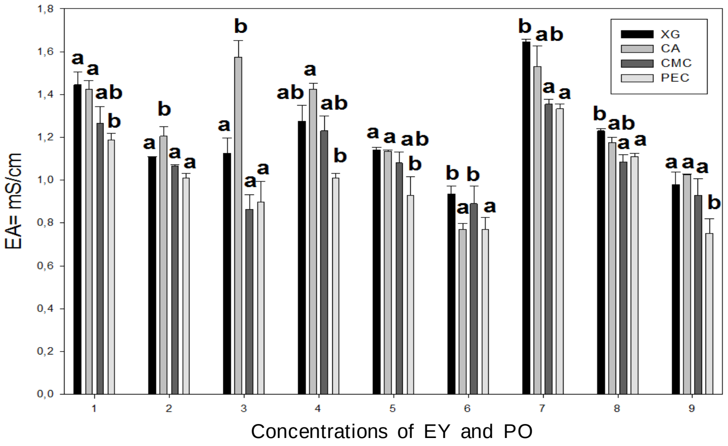

Figure 3 – Emulsifying activity of different concentrations of EY and PO with 7.0 mL canola. 1, 2 and 3 (0.1% EY: 0.1, 0.2 and 0.4% PO, respectively); 4, 5 and 6 (1% EY: 0.1%, 0.2 and 0.4% PO, respectively); 7, 8 and 9 (2.5% EY and 0.1, 0.2 and 0.4% PO, respectively).

When the effect of oil type on EA is investigated, palm oil resulted in smaller EA values (around 1.4 mS/cm in the experiments 4 and 7) than sunflower or canola oils. The mechanism of this phenomenon is poorly understood, but Farr (2000) suggested that monounsaturated fatty acid, specifically oleic acid, further facilitates interactions between hydrophobic protein groups and oil droplets. This hypothesis is supported by the oleic acid content in canola oil [55-58% (MORAES; BELL, 1993)] being greater than sunflower oil [35-45% (BITTENCOURT; SADER; MORAIS, 1998)] and palm oil [15-25% (BORA et al., 2003)].

Tukey tests ($p<0.05$) were used for means comparisons, where one can observe that some EA polymers showed the most significant ($p<0.05$) what for certain studied oils. The CA was more efficient emulsifier at lower concentrations (0.1 and 0.2%) for sunflower oil and 0.1% for canola oil. CMC was a superior emulsifier at 0.1% concentration for palm oil and 0.4% for sunflower oil. Finally, the PEC was superior at 0.4% concentration for sunflower oil and GX of 4.0% for canola oil.

Emulsion stability

Figure 4 shows photomicrographs of emulsions with their corresponding droplet size after homogenization. It can be clearly seen that different concentrations of OP and EY directly influenced the size of oil droplets and subsequently-studied ES.

Tables 1 to 3 present fractional volumes in the cream phase. While evaluating the effects of PO and EY concentrations for each one of the studied vegetable oils, lower values of Φ indicate better emulsion stability. Canola oil was the one gave the lowest values of Φ because of its high fraction of monounsaturated fatty acids.

Figure 4 (A and C) shows that low concentrations of polysaccharide (CMC) and 1.0% EY were not sufficiently to confer EA (Figure 2), leading to instability in the formed emulsion (Table 2) with a considerably larger droplet diameter. This is primarily because low protein concentrations are inefficient and form layers around the oil droplets. As the number of hydrophobic connections around droplet is insufficient, coalescence gradually increases until the emulsion collapses and phase separation occurs (McCLEMTS, 2006; JONES; McCLEMENTS, 2010). However, Figure 4B shows that 0.2% XG and 2.5% EY, which have high EA, led to a considerable reduction in droplet diameter and subsequently higher stability (RANGSANSARID; FUKADA, 2007; ANTON; GANDEMER, 1997; LIZARRAGA; PAN; SANTIAGO, 2008). Reduced coalescence caused by the interaction of a thin protein layer around the droplets with the three-dimensional polysaccharide network bound to water (TIPPETTS; MARTINI, 2012).

Polynomial model for the oil volume fraction of the creamed phase Φ, as a function of mass of egg yolk and polysaccharides, was fitted to the experimental data. The general quadratic model (Equation 3) was first analyzed and the no significant parameters were eliminated on the basis of the t-test with $p>0.05$. Table 4 shows the predictive models based on equation model (3) for each used oil.

$$\Phi = \beta_o + \beta_1 w_1 + \beta_2 w_2 + \beta_3 w_1^2 + \beta_4 w_1 w_2 + \beta_5 w_2^2 + \beta_6 w_1^2 w_2 + \beta_7 w_2^2 w_1 \quad (3)$$

where Φ is the oil volume fraction of the creamed phase, w_1 is the mass of egg yolk lipoprotein, w_2 is the mass of polysaccharides and β_1, β_2, β_3, β_4, β_5, β, and β_7 were obtained by nonlinear regression. The mathematical polynomial model was well adjusted to the experimental data, as observed in table 4. This is an indicative of a good fit to the experimental data.

Statistical analyses revealed that each polysaccharide generated emulsifications with different characteristics using the different oils. This behavior is primarily due to the inherent characteristics of two studied polysaccharides: (i) the non-polar chemical groups attached to different hydrophilic polysaccharide backbone, or (ii) the presence of a protein component covalently or physically bound to the polysaccharide (DICKINSON, 2009).

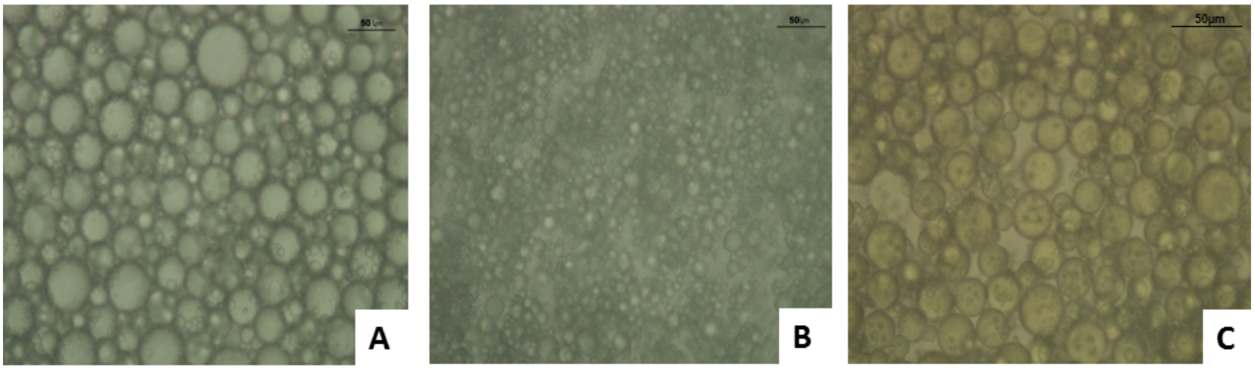

Figure 4 – Photomicrographs of emulsions with their respective concentrations and mean droplets diameter. A (0.1% CMC/0.1% EY/ Sunflower. Diameter = 27.10 µm); B (0.2% GX/ 2.5% EY/ Canola. Diameter = 4.32 µm); C (0.4% CMC/ 0.1% EY/Palm oil. Diameter = 24.29 µm).

Table 1 – Φ values for systems using palm oil in different concentrations of egg yolk and polysaccharides.

Palm oil		XG	CA	CMC	PEC
Egg Yolk (%)	Polysaccharides (%)	Φ	Φ	Φ	Φ
0.1	0.1	0.1478	0.2084	0.0867	0.0325
0.1	0.2	0.1194	0.2047	0.1488	0.154
0.1	0.4	0.2940	0.4334	0.2503	0.2638
1.0	0.1	0.1184	0.1305	0.0498	0.0498
1.0	0.2	0.1428	0.1857	0.2271	0.2396
1.0	0.4	0.2255	0.2912	0.2960	0.3197
2.5	0.1	0.0771	0.1087	0.1512	0.0909
2.5	0.2	0.1262	0.2252	0.1766	0.1541
2.5	0.4	0.3528	0.2685	0.4464	0.3945

Table 2 – Φ values for systems using sunflower oil in different concentrations of egg yolk and polysaccharides.

Sunflower oil		XG	CA	CMC	PEC
Egg Yolk (%)	Polysaccharides (%)	Φ	Φ	Φ	Φ
0.1	0.1	0.1904	0.3802	0.1512	0.1435
0.1	0.2	0.3216	0.2820	0.2105	0.1988
0.1	0.4	0.2940	0.2966	0.4309	0.2857
1.0	0.1	0.1281	0.0644	0.8538	0.9062
1.0	0.2	0.2148	0.1871	0.9964	0.1398
1.0	0.4	0.3517	0.4145	0.3341	0.2941
2.5	0.1	0.1600	0.1572	0.1693	0.1288
2.5	0.2	0.1683	0.2322	0.7807	0.0960
2.5	0.4	0.2746	0.3205	0.3466	0.2045

Table 3 – Φ values for systems using canola oil in different concentrations of egg yolk and polysaccharides.

Canola oil		XG	CA	CMC	PEC
Egg Yolk (%)	Polysaccharides (%)	Φ	Φ	Φ	Φ
0.1	0.1	0.0216	0.1051	0.1163	0.1855
0.1	0.2	0.1609	0.1442	0.2075	0.2352
0.1	0.4	0.2762	0.1037	0.3496	0.1445
1.0	0.1	0.1058	0.1071	0.1921	0.9828
1.0	0.2	0.1690	0.2170	0.8634	0.2564
1.0	0.4	0.2913	0.4351	0.3013	0.3284
2.5	0.1	0.0219	0.0339	0.1260	0.0996
2.5	0.2	0.1449	0.2282	0.8729	0.2004
2.5	0.4	0.2838	0.2993	0.3007	0.3524

Table 4 – Models to calculate oil volume fraction of the emulsions creamed phase Φ, ($p>0.05$).

Óil - Polymer	β_o	β_1	β_2	β_3	β_4	β_5	β_6	β_7	R^2
Canola-CA	0.0856	-0.6712	-	-	57.1017	-	-49.2463	-231.668	0.96
Canola-CMC	-0.0593	-	3.7310	-4.6188	164.7831	-	-	-1701.272	0.83
Canola-PEC	0.1273	6.9271	-	-14.2666	-	-	-	-	0.70
Canola-GX	-0.8069	-	3.7910	-0.0679	-	-	-	-	0.93
Palm oil-CA	0.1712	-0.4760	-	-	-	43.6858	18.7857	-239.0339	0.98
Palm oil-CMC	0.0524	-	2.3416	-	-	-	-	62.3643	0.92
Palm oil - PEC	-0.1025	-	8.8975	-	-	-	-	39.6230	0.94
Palm oil - GX	0.1273	6.9271	-	-14.2664	-	-	-	-	0.70
Sunflower oil-CA	0.44387	-3.6686	-2.3249	6.0140	61.1901	-	-102.4876	-	0.97
Sunflower oil-CMC	-0.1859	8.0792	7.9053	-19.4095	-	-	256.8306	-1381.6893	0.99
Sunflower oil-PEC	-	-	-	-	-	-	-	-	-
Sunflower oil-GX	0.0591	-0.9163	9.9757	2.2579	-	-89.6515	-43.7235	236.5108	0.98

CONCLUSIONS

This study examined EY concentration and polysaccharide species in formation of emulsions. We observed that the combination of low EY concentrations with low polysaccharide concentrations resulted in unstable emulsions with large droplet size. However, higher EY and polysaccharide concentrations yielded stable emulsions. It is clear that oil, specifically monounsaturated fatty acids, are extremely important in emulsion formation and facilitate the emulsification process. The polynomial model used to predict the volume fraction of the cream oil phase of emulsions had a good fit to the experimental data and holds promise for further studies to assess the functional properties of this new emulsion.

ACKNOWLEDGMENTS

The authors are grateful to CNPq and FAPERJ for the financial support.

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