Effect of emulsified soy oil with different carrageenans in rennet-coagulated milk gels

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Abstract

In modern food industry, concern about saturated fatty acid and cholesterol content in dairy products limit the consumption of these foods for people with health issues. Substitution of milk fat by vegetable oils had been proposed to enhance saturated/unsaturated fat balance in cheese without entailing nutritional disadvantages. In same manner, due to their interaction with milk proteins before and during rennet coagulation, carrageenans are widely used cheese manufacture. The objective this work was to determinate the effect of emulsified soy oil with carrageenans (kappa, iota or lambda) on curd yield, whey volume and textural properties of rennet coagulated milk gels. Higher curd yield and less whey volume were obtained when emulsions formulated with kappa-carrageenan (1% and 50% of oil) were employed, resulting in softer and less adhesive but more cohesive and resilient curd gels, when emulsion was incorporated at 5% in milk systems. Emulsion with carrageenans formed a viscous emulsion that was stable before, during and after rennet coagulation, being a viable way to replace milk fat in cheese.

Introduction

Saturated fatty acid and cholesterol content in milk is a limiting in the consumption of dairy products for people with health issues. Substitution of milk fat by vegetable oils can contribute to a healthier saturated/unsaturated fat balance in cheese without entailing nutritional disadvantages (Yu and Hammond 2000). Vegetable oils as fat replacers enhanced acceptability of Iranian white brined cheeses (Fathi Achachlouei *et al.*, 2012). In same manner, soybean oil and soybean fat were employed in cheese analogs increasing water holding capacity (Lobato-Calleros *et al.*, 1997). Emulsified oil had been employed as well in cheese processing. Whey expelling was decreased employing emulsified canola oil as fat replacer in reduced fat white fresh cheese (Lobato-Calleros *et al.*, 2007). Double emulsions as vitamin B12 carrier were employed in model cheeses, increasing moisture and protein and fat retention (Giroux *et al.*, 2013). Milk fat substitution with olive oil emulsion increased serum exudation resulting in lower cheese yield as compared to full-fat cheese (Felfoul *et al.*, 2013).

Carrageenans are widely used in milk industry due to their interaction with milk proteins (Piculell, 1995). Milk systems environmental conditions, like temperature and concentration of ingredients, affect the functionality of carrageenans (Drohan *et al.*, 1997; Puvanenthiran *et al.*, 2002; Langendorff *et al.*,

1999, 2000). The use of carrageenans as emulsion stabilizer can be employed in cheese processing, since emulsions with two macromolecules resulted in an interfacial membrane surround oil droplets in a two-layered system (Gu *et al.*, 2005). Emulsions of soy protein isolate with lambda-carrageenan had larger average droplet size, higher viscosity improving the stability of the emulsions decreasing zeta potential at neutral pH (Achouri *et al.*, 2012). Hence forward, since emulsified vegetable oils had been proposed as fat replace in cheese manufacture, the incorporation of carrageenans in emulsified vegetable oil, besides act as emulsion stabilizer, will interact with caseins to enhance cheese yield and textural properties of cheese. In this view, the aim of this work was to determinate the effect of carrageenan type (in function of their sulfate groups), as stabilizer of emulsified soy oil, on yield and textural properties of rennet coagulated milk gels as model system, employing an orthogonal array.

Materials and Methods

Emulsified soy oil

To elaborate the soy oil emulsions three carrageenans were employed: kappa-carrageenan Gelcarin GP8612, lambda-carrageenan Viscarin GP 209 and iota-carrageenan Viscarin SD 389 (FMC Biopolymers, Philadelphia). Soy protein isolate

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Appensol ISL (DVA Health & Nutrition GmbH, Hamburg, Germany) was also employed to form emulsion with Nutrioli® pure soy oil (Ragasa S.A., Monterrey, Mexico). At room temperature, in a soy protein isolate solution (5%, w/v) carrageenans were dissolved before add the soy oil to emulsify the mixture with an Oster homogenizer during 10-15 min. Carrageenans and oil concentrations are listed in Table 1. The obtained emulsions were stored in plastic bags and cold storage (around 4°C) until use.

Table 1. Orthogonal array for the formulated soy oil emulsions and emulsion incorporation in milk systems

| Experiment | Emulsions formulation | | | Emulsion incorporation to milk system (%) |
	Sulfate groups content (carrageenan type)	Carrageenan concentration (%)	Oil concentration (%)	
1	1 (kappa)	0.0	30	0.0
2	1 (kappa)	0.5	40	2.5
3	1 (kappa)	1.0	50	5.0
4	2 (iota)	0.0	40	5.0
5	2 (iota)	0.5	50	0.0
6	2 (iota)	1.0	30	2.5
7	3 (lambda)	0.0	50	2.5
8	3 (lambda)	0.5	30	5.0
9	3 (lambda)	1.0	40	0.0

Rennet coagulated milk gels

Milk gels were elaborated adapting the methodology reported by Brown *et al.* (2012). Pasteurized fresh standardized milk (NFS 6.47%, fat 4.36%, lactose 3.41%, pH= 6.79) was obtained from the dairy facilities at the Universidad Autonoma del Estado de Hidalgo at Tulancingo, Mexico. Different proportions of the different emulsion were added in milk according to the formulations listed in Table 1. Milk-emulsions mixtures (250 mL) were heated at 37°C before add 4 g of gluconolactone (Sigma-Aldrich, St. Louis) and 400 μL of 1:10 diluted chymosin rennet of approximately 100 international clotting units/kg of milk (Enzimax, Mexico City). Temperature was employed to enhance rennet action and gluconolactone was added as acidulant in order to low pH until caseins isoelectric point. After 30 min of incubation, the resultant gels were cut with a spatula and pressed in acrylic devices (5.7 mm internal diameter) during 1 h to drain whey (Figure 1). Curd yield and whey volume were reported as percent in base to the original milk volume.

Textural profile analysis

Textural profile analysis of rennet coagulated milk gels was performed. Gel samples were cut in 4x4 cm (3 cm height) and consecutively double compressed (30% original height) with a 7 cm diameter acrylic probe at 1 mm/s with a waiting period of 5 s in a LFRA 4500 Texture Analyzer (Brookfield Engineering, Middleboro). From the force-deformation curves TPA parameters were calculated as follows: hardness (force necessary to attain a given deformation, maximum force), cohesiveness (strength of the internal bonds making up the body of the product) and resilience (capacity for recover their original shape after compression (energy stored in the sample that allows to recover in some extent its original form) (Szczesniak, 1963; Bourne, 1978).

Carrageenan in whey

Carrageenan concentration in whey was determined adapting the te methodology reported by Soedjak (1994). Since in carrageenans the amount of methylene blue bounded is in relationship with the sulfate content of the carrageenans, a separate determination was made for each carrageenan type only in treatments containing carrageenan. Kappa, iota or lambda carrageenan solutions (0.20%, w/v) were consecutive diluted to a final volume of 0.9 mL and mixed with a 0.1 mL of a 0.41 mM methylene blue solution. Absorbance was read at 559 nm and plotted against carrageenan concentration. Linear regression equation was calculated to determinate the carrageenan concentration in the whey of each rennet coagulated milk gel containing emulsified oil with carrageenan.

Experimental design and data analysis

An orthogonal L9 (3^4) array (9 tests, 4 variables, 3 levels) was employed to determinate the effect of carrageenan and emulsified oil in milk gels properties (Table 1). The variables were carrageenan type (classified according to sulfate groups content, i.e., kappa -one sulfate group-, iota -2 sulfate groups- or lambda-3 sulfate groups-), carrageenan concentration in emulsion (0.0, 0.5 and 1.0% w/v), oil concentration in the emulsion (30, 40 and 50% soy oil, v/v) and emulsion incorporated to rennet coagulated milk gels (0.0, 2.5 and 5%, w/v).

Data were analyzed with SAS Statistical Software (SAS Institute, Cary) with the PROC GLM in order to obtain regression models to explain the combined effect of the independent variables on milk gels properties, according to the model:

$$y_i = \beta + \beta_1 X1i + \beta_2 X2_i + \beta_3 X3_i + \in i$$

Where y_i is the value of the continuous response variable for observation i, and $X1_i$, $X2_i$ and $X3_i$ are the values of the explanatory variables (carrageenan type, oil concentration in emulsion, and percent of emulsion incorporated). Term $\in i$ is the residual or

Table 2. ANOVA of the regression analysis for Curd yield and Whey volume

	Curd yield (%)				Whey volume (%)		
	SS	F	P>		SS	F	P>
Sulfate groups	68.91	31.83	0.0001	Sulfate groups	11,476	388.74	0.0001
% Carrageenan	93.41	86.29	0.0001	% Carrageenan	75	5.08	0.0245
% Oil emulsion	271.28	125.30	0.0001	% Oil emulsion	16,274	551.28	0.0001
% Incorporation	578.63	267.26	0.0001	% Incorporation	3,789	128.37	0.0001

%Yield = 9.181
-0.321 Sulfate groups (Carrageenan-type)
+ 0.475 %Carrageenan-concentration
+ 0.017 % oil-in-emulsion
+ 0.621 % incorporated oil
Adjusted R^2 = 0.4513

Whey volume= 161.55
+1.371 Sulfate groups (Carrageenan-type)
+3.908 %Carrageenan-concentration
+0.064 %oil-in-emulsion
-1.217 % incorporated-oil
R^2 = 0.1388

error for individual i and represents de deviation of the observed values of the response of this individual from the expected model. Significance tests for the regression coefficients can be derived by assuming that the residual terms are normally distributed with zero mean and constant variance σ^2 (Der and Everitt, 2002).

Results and Discussions

Whey volume and gel yield

The incorporation different percent of the emulsions elaborated with the different carrageenans and oil fractions affected the volume of whey released and curd yield of the rennet coagulated milk gels. For the curd yield, in the regression equation (adjusted R^2= 0.4513) carrageenan type –number of sulfate groups– affected negatively this parameter. The rest of the coefficient presented a positive effect on curd yield. In spite of this, higher yield (curd weight) can be obtained with emulsion elaborated with 1.0% of kappa-carrageenan and 50% of oil, incorporating until 5% of this emulsion (Table 2).

For whey released volume, in the regression equation (adjusted R^2= 0.1388) % of emulsion incorporated affected negatively the whey released from milk gels. The more emulsion incorporated into milk coagulated gel, the lower expelled whey. The parameters that affect positively whey volume were carrageenan type, percent of carrageenan and oil in the emulsion. The more sulfate groups in carrageenan (lambda with 3 > iota with 2 > kappa with 1) the higher whey expelled from milk curd. Higher carrageenan and oil concentrations increased whey volume as well (Table 2). In this view, to increase expelled whey volume from curd is recommendable to employ emulsions with 1.0% of kappa carrageenan plus of 50% oil incorporated at lower concentration (<2.5%) in milk system.

The effect of carrageenan on yield and whey

volume is regarding to the caseins-carrageenans interaction, depending on carrageenans' structural differences. The carrageenans affected both phases of caseins rennet coagulation. The proteolysis of κ-casein is referred to as the primary phase of rennet-coagulation. The secondary phase of the rennet coagulation is the reduction of colloidal stability of the hydrolyzed κ-casein micelles. The κ-casein (representing 12-15% of the total casein) is the only casein hydrolysed during the rennet coagulation of milk and that it was hydrolysed specifically at the Phe105-Met106 bond, producing paracasein (residues 1-105) and macropeptide (residues 106-169). The hydrophilic macropeptide diffuse into the surrounding medium while the hydrophobic paracasein remains attached to the micelle core (Fox and McSweeney, 1998). In the first phase (κ-casein enzymatic hydrolysis), the interaction of carrageenans with casein fractions occurs after rennet hydrolyses kappa-casein, allowing interaction with αs- and β-caseins (Schorsch *et al.*, 2000). At this point, during milk rennet coagulation, around 35-40°C the micelle size increased drastically (Puvanenthiran *et al.*, 2002), which corresponds to the temperature at which κ-carrageenan helix formation begins at approximately 37°C. Carrageenan interaction becomes temperature dependent (Langendorff *et al.*, 1999). Kappa and iota carrageenans absorption depends on temperature, since at 25°C their structure is a rigid double helix. In contrast, lambda carrageenan remains in coli conformation in a broad range of temperatures and can be absorbed into caseins micelles, increasing micelle size (Langendorff *et al.*, 2000). During second phase of rennet coagulation (i.e., aggregation of para-casein into a three dimensional gel network and whey release), carrageenans affect in different way. The self-association of kappa-carrageenan and iota-carrageenan double helices accelerates the aggregation of rennet-altered caseins, whereas lambda-carrageenan increased viscosity reducing

Table 3. Carrageenan determined in rennet coagulated milk gels whey

Treatments containing carrageenan					Added carrageenan (in water phase of emulsions, $\times 10^{-3}$ %)	Colorimetrically carrageenan detected in whey ($\times 10^{-3}$ %)
Emulsions formulation						
Experiment	Sulfate groups content (carrageenan type)	Carrageenan concentration (%)	Oil concentration (%)	Emulsion incorporation to milk (%)		
2	1 (kappa)	0.5	40	2.5	7.50	2.26
3	1 (kappa)	1.0	50	5.0	25.00	8.43
6	2 (iota)	1.0	30	2.5	17.50	1.53
8	3 (lambda)	0.5	30	5.0	17.50	0.93

Table 4. ANOVA of the regression analysis for coagulated curd gels for Hardness and Adhesiveness

Hardness (N)				Adhesiveness (-N)			
	SS	F	P>		SS	F	P>
Sulfate groups	183.77	2.64	0.0723	Sulfate groups	2.43	316.97	0.0001
% Carrageenan	22.03	0.63	0.4267	% Carrageenan	1.15	300.80	0.0001
% Oil emulsion	770.00	11.05	0.0001	% Oil emulsion	7.35	959.42	0.0001
% Incorporation	1,.698	24.38	0.0001	% Incorporation	0.12	16.65	0.0001

Hardness = 16.789
+ 0.376 Sulfate groups (Carrageenan-type)
+ 1.946 %Carrageenan-concentration
- 0.036 % oil-in-emulsion
- 0.909 % incorporated-oil
Adjusted R^2= 0.1020

Adhesiveness = 0.0938
+ 0.0424 Sulfate groups (Carrageenan-type)
+ 0.0652 % Carrageenan-concentration
+ 0.003 % oil-in-emulsion
- 0.011 % incorporated-oil
Adjusted R^2= 0.2338

coagulation time (Wang *et al*., 2014). During casein matrix shrinkage, carrageenans began to bind water interfering decreasing the expelling water from the curd particles (McMahon *et al*., 1996). Kappa carrageenan seems had a better interaction with water and proteins to decrease released water and increasing curd gel yield since a high specific interaction between κ-carrageenan and κ-casein enhances gelling properties of carrageenans and milk proteins, affecting the overall system behavior (Snoeren *et al*., 1975; Schorsch *et al*., 2000). In same manner, free carrageenan molecules lead to the depletion flocculation of casein micelle with the formation of large aggregates (Langendorff *et al*., 2000).

Carrageenan in whey

Carrageenan concentration varied according to the carrageenan type incorporated via emulsified oil into the rennet coagulated milk gel. Emulsions containing kappa carrageenan incorporated presented higher concentrations of carrageenan in whey, as compared with milk systems with lambda or iota-carrageenan (Table 3). This difference can be attributed to the fact that carrageenans are also absorbed into casein macropeptide surface (Xu *et al.*, 1992). Kappa-carrageenan active site is located in κ-casein between the residues 97 ad 112 (Snoeren *et al*., 1975). Although kappa-carrageenan had a larger particle size and less negative charges in comparison to the higher density of iota and lambda carrageenans that allows more sites to be absorbed into casein macropetide surface (Wang *et al.*, 2014), this specific interaction resulted in higher amount of colorimetric determined kappa carrageenan in whey.

Textural profile analysis

Incorporation of different carrageenan types and concentration and oil percent in emulsions affected the coagulated milk gel texture. For curd hardness, in the correlation analysis (adjusted R^2= 0.1020), carrageenan type and concentration had not significantly (P>0.0723 and P>0.4267, respectively) effect on curd texture. The percent of oil in emulsion and the percent of emulsion incorporated to milk system affected negatively the texture. More oil in the emulsion resulted in softer gels, and more emulsion incorporated to milk systems also decreased coagulated gel hardness (Table 4).

Regression analysis (adjusted R^2= 0.2338) showed that coagulated gels adhesiveness was positively affected by the emulsion formulation (carrageenan type, concentration and percent of oil in emulsion). In this parameter kappa carrageenan at low concentration (<0.5%) and low oil percent (30%) decreased curd adhesiveness, as compared to iota or lambda carrageenans (with more sulphate groups).

Table 5. ANOVA of the regression analysis for coagulated curd gels for Cohesiveness and Resilience

Cohesiveness				Resilience			
	SS	F	P>		SS	F	P>
Sulfate groups	0.558	51.16	0.0001	Sulfate groups	0.100	201.56	0.0001
% Carrageenan	1.162	212.86	0.0001	% Carrageenan	0.008	34.68	0.0001
% Oil emulsion	0.767	70.27	0.0001	% Oil emulsion	0.090	181.62	0.0001
% Incorporation	0.824	75.51	0.0001	% Incorporation	0.023	47.26	0.0001

Cohesiveness = 0.856
+ 0.081 Sulfate groups (Carrageenan-type)
- 0.194 % Carrageenan-concentration
- 0.0011 % oil-in-emulsion
+ 0.016 % incorporated-oil
Adjusted R^2= 0.3408

Resilience = 0.231
+ 0.0073 Sulfate groups (Carrageenan-type)
- 0.039 % Carrageenan-concentration
- 0.001 % oil-in-emulsion
+ 0.002 % incorporated-oil
Adjusted R^2= 0.3035

The percent of incorporated oil in milk system affected negatively the adhesiveness (more emulsion resulted in lower adhesiveness) (Table 4).

In the regression analysis (adjusted R^2= 0.3408), carrageenan type (number of sulfate groups) and incorporated emulsion had a positive effect on cohesiveness. Carrageenan concentration and oil percent in emulsions had a negative effect on this textural parameter. Hence, emulsion formulated with kappa carrageenan at lower concentrations (<2.5%) and lower oil in emulsion (30%) increased curd cohesiveness when this emulsion was incorporated to milk system at 5%. It seems that the more sulphate groups (as for iota and lambda carrageenans) the lower cohesiveness (Table 5).

Regression analysis (adjusted R^2= 0.3035) showed that curd resilience was positively affected by the carrageenan type (number of sulfate groups) and the amount of emulsion incorporated to milk system. Carrageenan concentration and percent of oil in emulsion affected negatively the curd resilience. Incorporation of 5% of emulsion with kappa carrageenan at lower concentrations (<2.5%) and lower oil in emulsion (30%) increased resilience values. More sulphate groups (as in iota and lambda carrageenans) decreased cheese resilience (Table 5).

Adhesiveness, cohesiveness and resilience of curd were lower when kappa carrageenan was employed in emulsion formulation. More sulfate groups in carrageenans molecule increase these textural parameters. Higher carrageenan concentration (1%) resulted in a less cohesive and less resilient (compacted) curd structure. Higher oil concentration (50%) in emulsions decreased mainly curd hardness (higher parameter value in regression equation). Higher emulsion incorporation (5%) into milk resulted in softer. Consequently, higher yield and less whey volume resulted in softer less adhesive but more continuous (higher cohesiveness) and flexible (higher resilience) curd gels. Textural differences can be explained in the scope of two main effects. In one

hand, carrageenans reactivity with other components in food systems depends on their conformation due to the number of sulfate groups in their structure (Langendorff *et al*., 2000). At the experimental conditions employed for the rennet coagulation of milk proteins (low pH and temperature above 30°C), carrageenan conformation along with their sulfate groups content, related to their concomitant capacity of electrostatic interaction with the coagulated proteins, affected curd texture. Since no effect of carrageenan type on curd hardness was detected, it seems that carrageenan gelation did not affected the three dimensional network formed by the coagulated caseins. Nonetheless, carrageenan type affected textural parameters related to elasticity (cohesion and resilience) and adhesiveness. Kappa carrageenan, with less only one sulfate group, decreased curd cohesiveness and resilience, this means that the internal structure become less rigid with this type of carrageenan than with iota or lambda, with more sulfate groups and a strong interaction with caseins, resulting in a compact and elastic structure. Employ carrageenans with more sulfate groups in emulsions decreased curd thickness, furthermore in emulsions with 30% of soy oil.

On the other hand, the incorporation of carrageenan improved stability of soy protein isolate with emulsified soy oil (no phase separation was observed during and after emulsion incorporation into milk systems). During emulsion, carrageenans, as a function of anionic sulfate groups, had different interaction strengths with cationic amino acid residues in proteins, resulting in different interfacial absorption. Carrageenan improved emulsion stability with increasing carrageenan concentration (between 0.3 to 0.5%), probably due to the increase in viscosity of the continuous phase and protein-polysaccharide interactions (Gu *et al*., 2005; Wu *et al*., 2011; Seta *et al*., 2013). The concentrations of carrageenan employed in the formulated emulsion probably allowed more carrageenan to interact with caseins

during cheese manufacture, being as well a stabilizer agent to incorporate vegetable oils.

Conclusions

Emulsion of soy oil with soy protein isolate employing carrageenans as stabilizers can be a way to incorporate polyunsaturated oils in cheese. Carrageenans formed a viscous emulsion that was stable before, during and after rennet coagulation of milk proteins at cheese manufacture conditions. Carrageenans interaction with milk proteins affected curd yield, whey release and texture of milk coagulated gels. Emulsions formulated with kappa-carrageenan (one sulfate group) at 1% concentration and 50% of oil resulted in higher curd yield and less whey volume, with a softer and less adhesive but more continuous (higher cohesiveness) and flexible (higher resilience) curd gels when emulsion was incorporated at 5% in milk systems.

Acknowledgement

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