Functional Properties of Milk-Egg Mixtures

E. Matringe, R. Phan Tan Luu, and D. Lorient

ABSTRACT

We used an experimental design (Scheffe simplex-centroid design) to examine ingredient interactions in food protein mixtures and their effects on functional properties. A defined and limited number of milk, albumen, and egg yolk blends were made and evaluated for heat gelation properties and for emulsifying and foaming capacity and stability. The method accounted for nonlinear interactions between the ingredients. Synergistic effects were revealed on heat gelation and emulsifying properties but antagonistic effects for foaming properties were noted.

Key Words: protein, albumen, functional properties, egg yolk, heat gelation

INTRODUCTION

MILK AND EGG PROTEINS ARE USED IN FOOD PRODUCTS FOR nutritive value and emulsifying, foaming, and heat-gelling properties. Emulsifying characteristics of these proteins are exhibited in salad dressings, mayonnaise, and meat emulsions. Protein foam and heat set protein gels are important in meringues, soufflés, whipped toppings, and cakes. Many studies have been published on functional properties of milk (Kinsella, 1984; Leman and Kinsella, 1989; Cayot and Lorient, 1998) and eggs (Mineki and Kobayashi, 1997; Vadehra and Nath, 1973; Baldwin, 1986) or individual protein fractions. However, milk and egg are usually mixed in food formulas. Such blends are polyphasic systems consisting of phospholipids, triglycerides in egg yolk and lactose in milk. Protein-protein interactions and polysaccharide- and/or lipid-protein interactions contribute to the net functional effects. Functional properties of milk-egg mixtures need to be characterized to predict their performance in food formulations.

Many studies have been published on properties of protein mixtures (Knapp et al., 1978; Kwasniewska et al., 1979; Porteous and Quinn, 1979; Hargett et al., 1982; Nichols and Cheryan, 1982; Linn and Cunningham, 1984; Burgarella et al., 1985). Few have used systematic experimental design and statistical analysis to validate the importance of ingredient interactions (Moll et al., 1982; Jackman and Yada, 1988, 1989; Baardseth et al., 1992; Arteaga et al., 1993).

Our objective was to model and analyze milk-albumen-egg yolk interactions and their effects on functional properties and to define the optimum blend(s) that maximize desired functional properties or avoid antagonistic effects.

MATERIALS & METHODS

Sample preparation

Spray-dried skim milk, albumen, and egg yolk (Epibretagne, Plaintel France) were dispersed in deionized water and stirred gently and continuously overnight at 4 °C. Suspensions for emulsifying and foaming properties contained 3% (w/v) protein (similar protein content to milk), while protein concentration was 8% (w/v) when studying heat gelation. Each suspension was adjusted to pH 6.6 (milk natural pH) with 1 M HCl or 1 M NaOH. Ten suspensions were prepared. These consisted of 3 single-ingredient systems (milk, albumen, and egg yolk), 3 two-ingredient mixtures, and 4 three-ingredient mixtures. These formulations were prepared by mixing skim milk suspensions, albumen, and egg yolk in a matrix (Fig. 1).

Functional properties

Heat gelation. A 50 mL aliquot from an 8% (w/v) protein suspension was heated in a glass beaker (height: 60 mm, dia: 40 mm) to 100 °C in an oil bath for 20 min then cooled in an ice bath. Gel strength was measured using a texturometer (Stevens LFRA) on a coagulum (ht: 12 mm, dia: 12 mm). The compression speed was fixed at 0.2 mm/s, and the work to compress the gel to 5 mm (41.7% compression) before failure was expressed in Newtons.

Emulsifying capacity (EC) was determined using a phase inversion point principle (Crenwelge et al., 1974 ; Harisson and Cunningham, 1986), according to a procedure described by Sauveur et al. (1979). Water-in-oil emulsions were prepared with 4 g of 3% (w/v) protein suspension and 16 g paraffin oil (chosen because it is devoid of tensio-active agents such as mono- and diglycerides) with a homogenizer (PT 3000-Kinematica) at 16,500 rpm for 30 sec, while water was added at the rate of 4.5 mL/min. Emulsifying capacity was recorded as the point at which a sudden drop in viscosity occurred due to emulsion inversion. These data were reported as mL of water added/protein dispersion at the inversion point.

Emulsifying stability (ES), i.e., creaming and fat separation, was determined by centrifugation. Paraffin oil was dyed (0.2 g Sudan III in 100 g oil) prior to emulsification (Arkad et al., 1985). After homogenization a 20 mL aliquot of emulsion was dispersed into graduated tubes and centrifuged at 180 g for 2.5 min at 21 °C. Emulsion stability was recorded as the volume ratio of the separated cream layer in the initial emulsion after centrifugation (Leman et al., 1988).

Foaming capacity (FC) **and stability** (FS) were determined according to the method of McWatters and Cherry (1977) and Kitabatake and Doi (1982) after modifications. The protein suspension (15 mL) was whipped in a 100 mL graduated cylinder using a PT 3000 Kinematica homogenizer at 10,000 rpm for 1 min. Foaming capacity was expressed as the volume increase (%) (Poole et al., 1984):

$$\text{FC (\%)} = \frac{\text{Foam volume} - \text{Initial protein suspension volume}}{\text{Initial protein suspension volume (15 mL)}} \times 100$$

Foaming stability (FS), i.e., drainage, was determined after measuring the volume of liquid drained from foam by gravity (which appeared at the bottom of the graduated cylinder after 2 h) and was calculated as:

$$\text{FS (\%)} = \frac{\text{Volume drained liquid}}{\text{Initial protein suspension volume (15 mL)}} \times 100$$

Experimental design

Each individual component of the blend has very specific properties. So binary or ternary blends were made in different proportions, and components were systematically replaced by others in the

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formulations. A canonical special cubic equation for 3 components (Cornell, 1990) was fitted to data collected at each experimental point using a linear regression as described by Moll et al. (1981). This mathematical model permitted determination of the value contribution by each component in any place of the experimental domain from results of carefully chosen experimental points.

The reduced cubic model (canonical equation) was postulated:

$$\eta = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3$$

β_i, β_{ij}, β_{ijk}: coefficients of the model.

The experimental domain consisted of different proportions of ingredients X_1 (milk), X_2 (egg white or albumen), X_3 (egg yolk) between zero and one ($0 \leq Xi \leq 1$; $\Sigma Xi = 1$). The experimental domain was within an equilateral triangle (regular simplex). For each property, triangular plots (Fig. 1) were constructed based on these regression models: the vertexes of the simplex (Fig. 1) represented the pure constituents, the edges of the triangle corresponded to the two-ingredient mixtures, and points within the triangle represented the three-ingredient mixtures. Ten mixtures (Table 1) were tested.

Data evaluation

To demonstrate synergistic and antagonistic effects between components, responses were plotted in 3D using the SAS/GRAPH software (SAS Institute Inc., 1985). The three corners of the triangle base represented milk, albumen, and egg yolk levels. Each experiment was replicated at least 3 times. Results were expressed as the arithmetic means of 3 values with a standard deviation error estimation. Experimental and calculated values were compared using the "t" test. Significance of differences was defined at p 0.05.

RESULTS & DISCUSSION

Determination of the coefficients of the mathematical model

A reduced cubic model was postulated. The 1st 7 sets (Table 1) enabled precise estimation of 7 coefficients of the model (Table 2) by linear regression. Three test points (8, 9, 10) within the experimental domain were used for model validation. Considering the experimental precision of the measurements, we concluded that the reduced cubic model well represented the variation of properties in the domain. In the specific case of the mixtures, the components were not independent, and the factors were the proportions of each component in the mixture. Thus, any linear and interaction terms in the model had no significance.

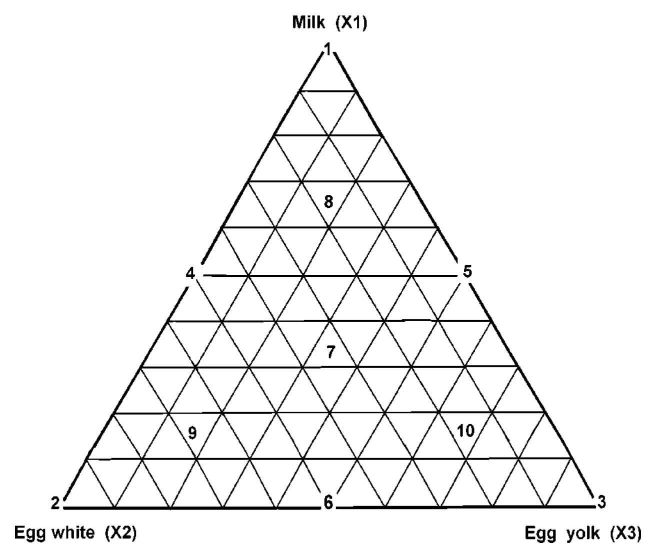

Fig. 1—Representation of simplex-centroid design.

Table 1—Mixtures composition[a]

No.	X_1	X_2	X_3	Experimental result
1	1	0	0	Y1
2	0	1	0	Y2
3	0	0	1	Y3
4	½	½	0	Y12
5	½	0	½	Y13
6	0	½	½	Y23
7	1/3	1/3	1/3	Y123
8	4/6	1/6	1/6	Y111123
9	1/6	4/6	1/6	Y122223
10	1/6	1/6	4/6	Y123333

[a]X_1, X_2 and X_3 are the proportions of milk, albumen and egg yolk, respectively.

Heat gelation

Albumen gels were firmer than egg yolk gels, but milk did not form a heat-set gel (Table 3). However, milk-albumen or milk-egg yolk mixtures formed gels. Suspensions containing any egg protein formed heat-set gels (Woodward, 1990). The three corners of the triangular base represented milk, albumen, and egg yolk levels (Fig. 2b). If there were no interaction effects, no significant difference would be observed between experimental responses and expected theoretical responses (calculated by weighted average of each ingredient response), that were on the plane surface linking response values for two pure components. There would be an antagonistic effect or a synergistic effect if the surface response was above or below the line, respectively. Changes in the amounts of egg white had the greatest synergistic effects (Fig. 2b) on gel firmness. However, replacing albumen by milk (up to 35%) gave gels with the same firmness (1.97 N) as those containing albumen alone. The isoresponse curve (level curve with response equal to a constant) plots showed the evolution of the response depending on the amount of pure component. The evolution of the response was not linear and indicated the importance of interaction effects between pure components. The isoresponse curve 11 (Fig. 2a) showed synergistic effects between albumen and milk protein. This could be related to the unfolding of the protein during heat denaturation. In heat-set gelling products, some of the albumen may be replaced by lower valued milk protein without sacrificing gel firmness.

Emulsifying properties

Egg yolk had the highest emulsifying property followed by milk and albumen (Table 3). Phospholipids (lecithin) and the lipoproteins (main components of egg yolk) are excellent emulsifiers (Mitzutani and Nakamura, 1984; Chung and Ferrier, 1991; Bringe et al., 1996; Aluko and Mine, 1997). The amphipathic nature of whey proteins and casein are responsible for the emulsifying properties of milk (Dumay and Cheftel, 1986; Haque et al., 1988; Foley and O'Connel, 1990; Closs, 1990; Courthaudon, 1990; Hung and Zayas, 1991). The lower emulsifying power of albumen is explained by its globular nature and low hydrophobicity. Evidence of synergistic as well as antagonistic effects on emulsifying capacities were found in several mixtures (Fig. 3). There was a large area in the simplex, near the point which corresponded to pure egg yolk, where all the mixtures had the same emulsifying capacity and the highest values (≈5.62). The maximum emulsifying capacity was exhibited by mixtures containing 30% to 85% egg yolk, 1% to 45% albumen, and 5% to 55% milk as represented by isoresponse curve 11 (Fig. 3). The mixture 35% egg yolk, 55% milk, and 10% albumen had similar emulsifying capacity as an 85% egg yolk, 10% milk, and 5% albumen mixture. This could be explained by the very efficient tensioactive properties

Table 2—Ingredient-functionality property regression models

Functional properties	Coefficients of independent variables			Coefficients of nonlinear blending terms			
	β_1	β_2	β_3	β_{12}	β_{13}	β_{23}	β_{123}
HG	0.0051	2.0134	0.6337	2.8769	0.0777	0.4142	-0.444256
EC	-4.150	2.613	5.399	-2.753	2.538	3.023	39.145
ES	17.80	19.38	29.22	-17.25	-8.36	-13.59	105.99
FC	44.22	197.49	56.20	-311.77	47.24	-321.81	188.68
FS	90.08	80.22	52.13	22.02	87.23	20.19	140.02

[a]HG-heat gelation; EC: Emulsifying capacity; FC-foaming capacity; ES-emulsifying stability; FS-foaming stability.

Table 3—Experimental responses for the 5 functional properties considered

Functional properties	Mixtures 1	2	3	4	5	6	7	8	9	10
HG	0.0±0	1.97±0.14	0.65±0.09	1.68±0.06	0.35±0.06	1.40±0.05	1.15±0.06	0.82±0.06	1.96±0.09	0.87±0.08
EC	4.07±0.25	2.41±0.50	5.62±0.50	2.40±0.29	5.55±0.27	4.77±0.20	5.60±0.37	5.20±0.48	4.87±0.30	5.40±0.26
ES	17.6±1.5	19.2±1.4	29.4±3.5	13.9±2.8	21.4±2.1	20.9±4.1	21.1±0.1	19.5±1.4	19.8±1.4	24.4±1.1
FC	40.5±1	207.3±10	25.8±6	49.0±6	54.9±13	52.9±9	49.2±9	45.4±1	50.7±8	49.6±7
FS	92.1±1.8	79.4±3.2	49.9±0.9	91.8±1.6	92.3±1.7	67.8±2.2	89.4±3.3	92.8±1.6	90.8±2.8	87.6±2.9

HG-heat gelation; EC-emulsifying capacity; FC-foaming capacity; ES-emulsifying stability; FS-foaming stability.

of casein micelles. Reducing egg yolk from 45% to zero made the response decrease rapidly (isoresponse curves very close). As the proportion of egg white increased in the ternary mixture from 43% to 100% , the emulsifying capacity decreased rapidly and regularly. The lowest value response was for the pure component egg white (X_2) or for the binary mixtures egg white-milk. When the proportion of milk in the mixture increased from 60% to 100%, the response also decreased.

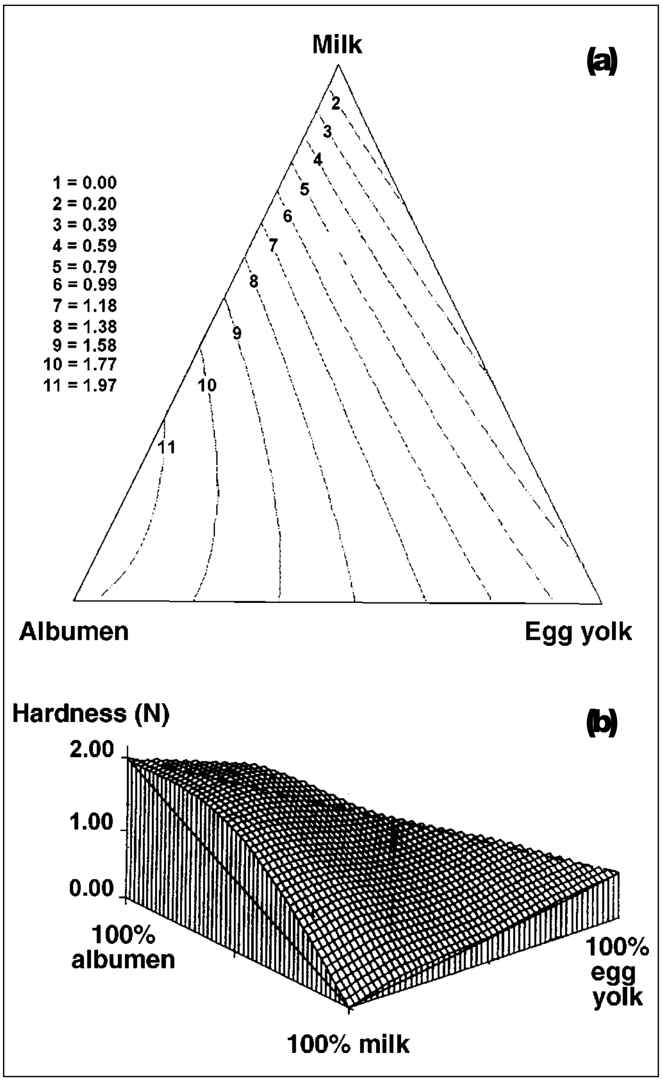

Fig. 2—Isoresponse curves for (a) heat gelling property and (b) the corresponding three-dimensional surface.

Egg yolk had the greatest effects on emulsifying stability (Fig. 3). Maximum emulsifying stability was exhibited by mixtures containing the maximum proportion of egg yolk. The lipoproteins in the plasma (egg yolk fraction) would be most responsible for this emulsifying stability (Mitzutani, Nakamura, 1984). Milk and albumen had practically the same effect on this response, so they could be used interchangeably.

Emulsifying power could be evaluated by superposition of the plots for emulsifying capacity and stability (Fig. 3). Both responses could be maximized simultaneously by formulating the ternary mixtures in the area indicated by the arrow (Fig. 3). Mixtures containing egg yolk (≈65% to 85%), a small proportion of milk (≈5% to 15%), and albumen (maximum 20%) exhibited the best emulsifying pow-

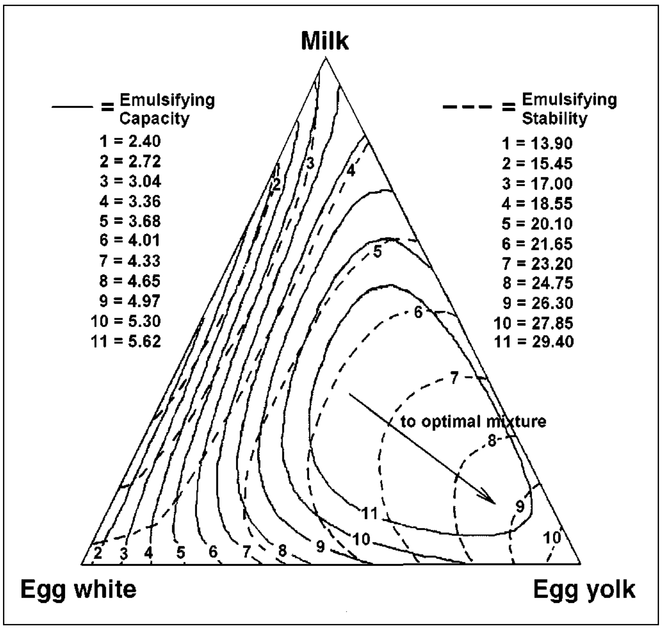

Fig. 3—Isoresponse curves for the superposition of the capacity (——) and the stability (---) emulsifying properties.

er. Egg could be replaced by milk without loss of emulsifying properties.

Foaming properties

Albumen had the highest foaming capacity because of ovalbumin, the main constituent (Table 3). Milk had the lowest. The foaming property of albumen is well known and could be related to the structure of the foam. Awazuhara and Nakamura (1986) observed that, in albumen foam, a rigid membrane surrounded the polyhedric bubbles, while the bubbles in egg yolk foam were round and smaller with no membrane. Yolk is considered as an inhibitor to albumen foaming. The lipid constituents of egg yolk compete with the protein for the interface and sharply reduce the foaming power (Cunningham, 1977; Poole et al., 1986). The responses varied slightly (Table 3) in the experimental domain (about 45% to 55% foam volume), except when only egg white was present. The foaming capacity was maximum when the blend contained albumen only. The effect of other components was largely antagonistic (Fig. 5).

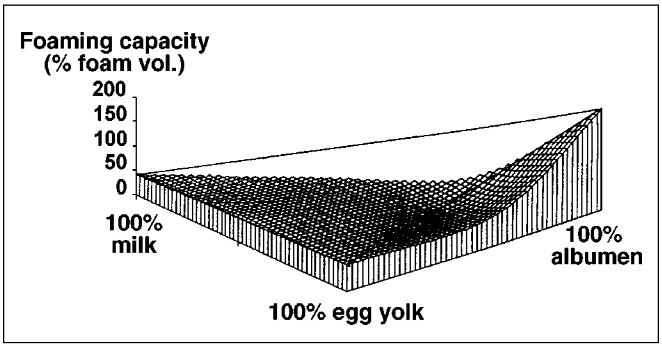

Fig. 5—Three-dimensional surface of foaming capacity.

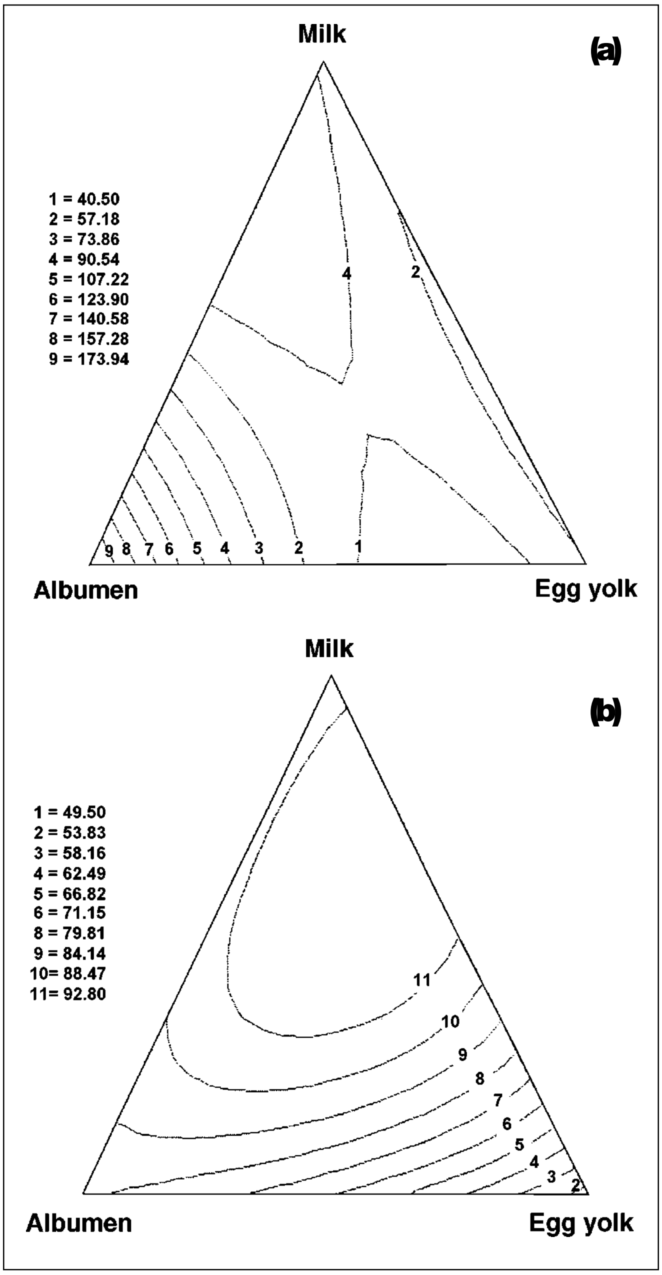

Fig. 4—Isoresponse curves for (a) foaming capacity and (b) stability.

Egg yolk exhibited lower drained volume than albumen and milk (Table 3). This important stability of egg yolk foams can be explained by the presence of "particulate material," such as the granules that strengthen the interfacial film around bubbles, as in the case of Madeira cake (Kamat et al., 1973). Highest values for drained liquid volume (Fig. 4b), i.e., a low foaming stability, were exhibited by binary mixture (milk-egg yolk and milk-albumen) or by ternary mixtures where the preponderant constituent was milk. All mixtures that contained at least 55% milk had foaming stability values of 92.8, and albumen content had little effect on the response. Where egg yolk comprised more than 50% of the mixture, the drained liquid volume decreased, i.e. the foaming stability increased.

On the response foaming capacity and stability, no synergistic effects of ingredients were observed. Superposition of foaming capacity and stability plots did not define an area where both properties were maximum. This was contrary to observations on emulsifying properties.

CONCLUSION

NONADDITIVE EFFECTS IN MIXTURES OF MILK, ALBUMEN, AND egg yolk were observed in the functional properties of gelation, emulsification, and foaming capacity. Except for foaming stability, contour plots revealed the synergistic as well as antagonistic effects between ingredients. Optimum blends were obtained to maximize one or more response using isoresponse curves. This technique could be used as a basis for least cost formulation.

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