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Original article

Evaluation of the composition and functional properties of whole egg plasma obtained by centrifugation

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Summary Hen eggs have many applications in the food industry. Centrifugation split liquid whole egg in two fractions: supernatant plasma and precipitate granules. Plasma can be used directly by the food industry. The aim of this study was to analyse how the operational conditions affect the composition and functional properties of plasma obtained by centrifugation. Protein and cholesterol contents, dry matter, emulsion activity and foam activity were evaluated. Plasma showed lower protein content and dry matter than liquid whole egg. Within the operational conditions studied, plasma contained 74–85% of egg cholesterol, maintaining the emulsifying activity and increasing the foaming properties. Prediction models were obtained for all the parameters studied except for emulsifying activity. Plasma doubled the foam capacity and increased until almost 15% the foam stability compared with liquid whole egg. Whole egg plasma could be a great substitute of whole egg for the food industry.

Keywords Centrifugation, egg, emulsion, foam, fractionation, plasma.

Introduction

Hen eggs are considered a high-quality food. The egg dry matter is around 25% (w/w) where protein is around 53% of the dry matter. In addition, eggs contain all the essential amino acids. From a gastronomic point of view, they are a versatile raw material, offering multiple possibilities of use and possessing an excellent quality–price ratio. It is well known that the ability of eggs to form and stabilise different food structures is due to the protein fraction and its physicochemical properties. Egg proteins act in food products as texture improvers and stabilisers, controlling consistency and water retention, by virtue of their foaming, emulsifying, gelling and heat setting properties (Garcés-Rimón *et al.*, 2016).

Emulsification is a major function of egg yolk components in the manufacture of mayonnaise and one of several functions in bakery items. Egg white proteins are especially effective forming food foams, which are of particular value in the production of cakes and certain confections. The gel-forming properties of egg proteins on heating are utilised when eggs serve as binding agents, such as in custards and pie fillings (Mine, 2002).

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Centrifugation split egg in two fractions: supernatant (plasma) and precipitate (granules). The fractions obtained have been profoundly studied for egg yolk (Laca, Paredes, Rendueles, & Díaz, 2014). However, liquid whole egg fractionation has engaged little attention. While the obtention of plasma from whole egg can be more interesting to food industry, because the separation step of white and yolk can be evaded. In addition, this process can give another use for class B eggs that cannot be sold at supermarkets.

The most common conditions for egg yolk centrifugation were 10 000 ***g*** for 45 min at 4 or 10 °C. A previous dilution 1:1 with water or 0.15–0.17 M NaCl were usually performed (Laca, Paredes, Rendueles, & Díaz, 2014). Only one preceding study was found for liquid whole egg fractionation by centrifugation. The centrifugation conditions studied were 2 h and 1000 ***g***, with a final temperature of 29 °C. Plasma was characterised after different pasteurisation temperatures (McCready *et al.*, 1971). Nowadays, those conditions would be considered a food safety risk and worthless due to the absence of temperature control, and long times that benefit microorganism proliferation. It was found that a lack of recent studies evaluating shorter times, higher g-force and lower temperatures, made necessary a new study with these centrifugation conditions.

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Egg yolk dilution previous to centrifugation made a new product with approximately 75% water. As whole egg is already approximately 75% water, this dilution step was considered unnecessary for whole egg fractionation. This sums another step saving that has multiple advantages. Firstly, it allows the direct use of the fractions, with unneeded removal step, hence saving time too. Secondly, alterations in protein solubility could be avoided because even water or low concentrations of NaCl were normally employed, it is known that they can change protein solubility (Sousa *et al.*, 2007). In a previous report of our group, it was evaluated the effect of centrifugation on the physicochemical parameters (pH, water activity (aw)) and colour (L*, a*, b*, chroma and hue; Puertas & Vázquez, 2021).

Therefore, the aim of this work was to analyse the effect of operational conditions (relative centrifugal force – RCF, time and temperature) on the composition and functional properties of the plasma obtained by centrifugation. And compared the results to initial whole and plasma from yolk. The parameters studied were protein content, cholesterol content, dry matter, emulsion activity and foam activity. Initial liquid whole egg parameters were measured for comparative purposes.

Materials and methods

Whole egg fractionation

A total of four packages of 2 L from same batch of whole egg pasteurised at low temperature from Granja Campomayor (Lugo, Spain) were employed. Before centrifugation, whole egg was homogenised at 5600 rpm for 1 min (Ultra Turrax®, IKA, Staufen, Germany). Then, 200 g of sample was seized on the centrifuge rotor (Sorvall Contifuge® Stratos, Thermo Fisher Scientific, Waltham, MA, USA). After centrifugation, plasma was extracted with a peristaltic pump (Masterflex® L/S®, Cole-Parmer®, Illinois, USA).

Composition determination

Protein concentration was estimated by the *Warburg and Christian* method (Warburg & Christian, 1942) based on ultraviolet absorption with the microvolume spectrophotometer Nanodrop 2000 (Thermo Scientific™, Waltham, MA, USA). Most proteins exhibit a distinct ultraviolet light absorption maximum at 280 nm. Nucleic acids have also a strong ultraviolet absorption band at 280 nm. However, their absorption is much more strongly at 260 nm than at 280 nm, whereas with proteins the reverse is true. To eliminate the interference of nucleic acids in the estimation of protein content, this equation was used (Kalckar, 1947):

$$\text{Protein concentration (mg/mL)} = 1.45 \times A_{280} - 0.74 \times A_{260} \quad (1)$$

where A_{280} and A_{260} are the absorbance values obtained at 280 and 260 nm, respectively. When samples have a ratio $A_{280}/A_{260} < 0.6$, the content of nucleic acids is more than 20% and the method gives considerable errors (Layne, 1957). This ratio was supervised for each sample. The protein concentrations (mg mL^{-1}) were converted to gram (g) of proteins per 100 g of whole egg with the density values. Measured by weighting a volume with a calibrated pipette.

In order to quantify the mass balance of the process, the compound separation according to different centrifugation conditions, the recovery (R) for each parameter was calculated by the following equation (Naderi *et al.*, 2014):

$$R_X(\%) = X_{\text{mass P}}/X_{\text{massWE}} \times 100 \quad (2)$$

where X is the composition parameter, such as protein, cholesterol or dry matter. And subscripts P and WE refer to plasma and whole egg, respectively.

Cholesterol composition was determined with an enzymatic kit method (Enzytec™, R-Biopharm AG, Darmstadt, Germany). Manufacturer instructions were followed except for the saponification conditions, based on previous studies (Puertas & Vázquez, 2020). Cholesterol content was expressed as mg per 1 g of liquid whole egg or plasma.

Dry matter (DM) content was determined by the gravimetric method. DM content was expressed as g dry matter per 100 g fresh sample.

With the values of protein concentration obtained, by difference was calculated the non-protein content including lipids, carbohydrates and ashes presented in the plasma fraction. Considering a standard whole egg DM composition where 53% are proteins, 40% lipids, 4% ash and 3% carbohydrates (U.S. Department of Agriculture, 2019), it can be conjectured that the main components in that non-protein content must be lipids.

The protein content in DM was calculated with the following equation:

$$\text{DM}_{\text{prot}}(\%) = X_{\text{mass prot}}/X_{\text{mass DM}} \times 100 \quad (3)$$

Functional properties

Emulsion activity was determined by Monfort *et al.* (2012) method. Emulsion was prepared with 2.5 g of sample and mix up to 50 g of deionised water with a high-performance homogeniser (Ultra Turrax®, IKA, Staufen, Germany) at 10 000 rpm for 30 s. Then, 50 mL of commercial sunflower oil was added and homogenised for an additional minute. The mixture was split in three different graduated plastic tubes

containing 30 mL and after 120 min at room temperature, and the volume of the emulsified layer was measured. The emulsion capacity was calculated as follows:

$$\text{Emulsion capacity}\,(\%) = \frac{\text{Initial volume of emulsified layer}}{\text{Total volume in tube}} \times 100 \tag{4}$$

For the emulsion stability, the emulsion tubes prepared were heated at 80 °C for 30 min in a thermostatic water bath, then cooled to 20 °C in an ice water. The calculations were made as follows:

$$\text{Emulsion stability}\,(\%) = \frac{\text{Volume of remaining emulsified layer}}{\text{Initial volume of emulsified layer}} \times 100 \tag{5}$$

Foam capacity and foam stability were determined based on former methods described with some modifications. Samples were diluted 1:4 in distilled water. The dispersions were homogenised at 3000 rpm for 1 min at room temperature with a tube agitator (Vortex ZX4, VELP Scientifica, Usmate, Italy). Immediately after whipping, volume of foam and total volume of solution were measured in covered graduated tubes. The foam capacity was calculated according to the following equation (Patrignani *et al.*, 2013):

$$\text{Foam capacity}\,(\%) = \frac{\text{Initial volume of foam}}{\text{Total volume of solution}} \times 100 \tag{6}$$

The whipped samples were allowed to stand at room temperature for 30 min; then, the foam volume was recorded again. Foam stability was calculated as follows (Li *et al.*, 2018):

$$\text{Foam stability}\,(\%) = \frac{\text{Volume of foam at 30 min}}{\text{Initial volume of foam}} \times 100 \tag{7}$$

Statistical design and analysis

A statistical Box–behnken design was applied. The lower and upper levels for each centrifugation variable were established based on the most common conditions for egg yolk fractionation (Laca *et al.*, 2014) and considering low temperature to avoid microorganism growth. Therefore, levels evaluated were RCF (8000, 10 000, 12 000 g-force), time (10, 35, 60 min) and temperature (4, 8, 12 °C). All measurements were performed at least in triplicate. The statistical analysis was performed using Design Expert® 10.0.6 software (Stat-Ease, Inc., Minneapolis, MN, USA). To detect outliers, the Cook's distance was analysed (Cook, 1977). The models were evaluated by multifactor analysis of variance (ANOVA) test. The significance of each coefficient was determined using *F*-value and *P*-value. The adequacy of the fitted model was determined by evaluating the lack of fit and the coefficient of determination (r^2). Only models with not significant lack of fit were considered because that meant the model fitted.

Table 1 Experimental design together with plasma responses for compositional parameters

Run	RCF (g)	Time (min)	Temp. (°C)	Plasma weight (g)	Protein (g/100 g)	Protein recovery (%)	Cholesterol (mg g^{-1})	Cholesterol recovery (%)	DM (g/100 g)	DM recovery (%)	DM protein (%)
0	–	–	–		13.99 ± 0.19	–	4.15 ± 0.06	–	23.89 ± 0.07	–	58.60
1	12 000	35	12	157.09	10.52 ± 0.01	58.86	4.25 ± 0.08	79.84	21.94 ± 0.06	71.77	47.97
2	12 000	35	4	158.93	10.66 ± 0.25	60.32	4.24 ± 0.08	80.65	21.90 ± 0.03	72.50	48.66
3	10 000	35	8	156.06	11.42 ± 0.20	63.16	4.16 ± 0.07	77.59	21.92 ± 0.06	71.23	52.10
4	10 000	60	4	153	8.35 ± 0.14	45.28	4.16 ± 0.07	76.12	21.89 ± 0.02	69.77	38.13
5	10 000	60	12	150.19	8.02 ± 0.11	45.06	4.13 ± 0.11	74.13	21.82 ± 0.07	68.26	36.74
6	10 000	10	12	166.06	11.41 ± 0.31	66.75	4.16 ± 0.04	81.73	22.53 ± 0.03	78.32	50.65
7	10 000	35	8	159.87	10.39 ± 0.10	58.51	4.19 ± 0.07	79.11	22.11 ± 0.01	73.99	46.99
8	8000	35	4	161.53	10.62 ± 0.20	62.24	4.20 ± 0.03	80.26	22.18 ± 0.03	75.00	47.90
9	12 000	10	8	164.43	11.52 ± 0.16	68.13	4.13 ± 0.02	83.43	22.19 ± 0.05	76.50	51.90
10	8000	35	12	159.88	10.86 ± 0.17	62.47	4.12 ± 0.05	80.95	21.90 ± 0.04	73.41	49.60
11	8000	60	8	159.74	8.73 ± 0.72	50.16	4.16 ± 0.06	81.68	22.16 ± 0.04	74.21	39.40
12	10 000	35	8	160.1	10.51 ± 0.30	60.02	4.14 ± 0.06	81.59	22.20 ± 0.09	74.51	47.35
13	12 000	60	8	154.98	8.36 ± 0.14	46.98	3.95 ± 0.03	75.36	22.01 ± 0.02	71.51	37.97
14	10 000	10	4	166.66	12.06 ± 0.21	70.51	4.17 ± 0.09	83.89	22.59 ± 0.01	78.98	53.41
15	8000	10	8	168.88	12.15 ± 0.16	71.78	4.15 ± 0.07	84.69	22.73 ± 0.01	80.55	53.44

Temp. is temperature. DM is dry matter. Run 0 is initial whole egg.

Results and discussion

Table 1 shows experimental design and average results obtained from whole egg and plasma composition analysis.

Protein content

The protein content in liquid whole egg was 13.99%. This quantity is higher than the 12.5% described in Spanish Food Composition database. However, it is similar to the 14% found in recent references (Uysal *et al.*, 2019). The protein content in plasma ranged from 8.02% to 12.15%. This result is in the range detected in previous studies where pasteurised samples around neutral pH contained 10–11% of proteins (McCready *et al.*, 1971). It has to be considered that due to pasteurisation, some proteins change their solubility and precipitate like insoluble deposits when centrifugated (Uysal *et al.*, 2017).

All samples present a ratio $A_{280}/A_{260} > 0.6$. Therefore, the amount of nucleic acids was less than 20% (Layne, 1957). The average ratios for whole egg were 1.07 and for plasma 1.18. Then, it can be deducted that the amount of nucleic acids is about 2.5–3% in liquid whole egg and 2% in plasma according to Layne (1957).

Plasma proteins data fitted a linear model, but only the time term was significant with $P < 0.05$ (Table S1). A model reduction was tested in order to consider only significant parameters (Table S2). Equation 8 can forecast plasma protein content.

$$\text{Proteins}_{\text{Plasma}}\,(\text{g}/100\,\text{g}) = 11.97 - 0.01\cdot\text{time}\,(\text{min}) - 8.33\times10^{-4}\cdot\text{time}^2 \quad (8)$$

Fit statistics and surface response are showed at Fig. 1a. It exposed how the content of proteins in plasma decreased with time.

When all the variables were considered, protein recovery in plasma results assigned a linear model. However, only time was a significant term (Table S1). Like plasma proteins, a model reduction was performed, and data fitted a quadratic model (Table S2). The high values of the correlation statistics shown at Fig. 1b justify the use of this model for protein recovery calculations. Equation 9 can be employed with that purpose:

$$R_{\text{prot}}\,(\%) = 70.86 - 0.105\cdot\text{time}\,(\text{min}) - 5.22\times10^{-3}\cdot\text{time}^2 \quad (9)$$

The highest recovery is obtained with the same conditions for plasma protein concentration: short time. The high difference between recoveries shows the great influence of time on egg protein separation. Such a big difference is not shown at protein concentration because the plasma weight (Table 1) also decreased with time as it was shown in a previous study (Puertas & Vázquez, 2021).

Differences in protein recovery between yolk plasma and whole egg plasma have to be due to the presence of albumen proteins, the reduction in the protein content and the absence of the dilution step previous to centrifugation. Egg yolk has approximately 16.5% (w/w) of proteins; while in this study it was measured round 14% (w/w) of proteins in whole egg. At commonly egg yolk centrifugation conditions (10 000 g-force, 45 min and 4 or 10 °C), it is described that around 50% of yolk proteins are presented in plasma (Laca *et al.*, 2010). At these conditions, according to eqn 8 and Fig. 1b, the protein recovery is around 55.6%. Similar protein recovery in both plasmas, even starting with lower quantities of proteins, supposes that egg white proteins moved preferentially to plasma.

Cholesterol content

The average cholesterol content determined for liquid whole egg was 4.15 mg g^{-1}. This value is similar to the recently updated of the American food database that reported 4.11 mg g^{-1} (U.S. Department of Agriculture, 2019).

The plasma cholesterol concentrations measured were around initial cholesterol content in whole egg (Table 1). However, these values suppose significative differences between cholesterol recoveries in plasma fractions. Cholesterol recovery data fitted a linear model (Table S1). Since only the time term was significant, a model reduction was performed (Table S2). Afterwards, only time is considered for cholesterol recovery prediction (R_{chol}) in eqn 10:

$$R_{\text{chol}}\,(\%) = 84.42 - 8.82\times10^{-2}\cdot\text{time}\,(\text{min}) - 1.09\times10^{-3}\cdot\text{time}^2 \quad (10)$$

Figure 1c shows the surface response for cholesterol recovery at a constant temperature of 8 °C. Maximum recoveries were obtained at low times. These results suppose that there is about 25% of cholesterol in egg that can be separated into granules and losses.

When yolk was centrifuged at 10 000 g-force for 45 min at 4 °C, cholesterol was moved around 77.6% to plasma and 22.4% to granules (Laca, Paredes, & Díaz, 2010). Analogous plasma recovery of 78.2% is estimated with eqn 9 and shown in Fig. 1c. Within the yolk, over 95% cholesterol is located in low-density lipoprotein (LDL) particles and the remainder is bound to high-density lipoprotein (HDL; Griffin, 1992). Yolk plasma is mainly composed of LDL which do not sediment with centrifugation because their density (0.98 kg m^{-3}) is near of that of water (Anton *et al.*, 2003), whereas egg yolk granules contained all egg yolk HDL and about 12% LDL with similar composition

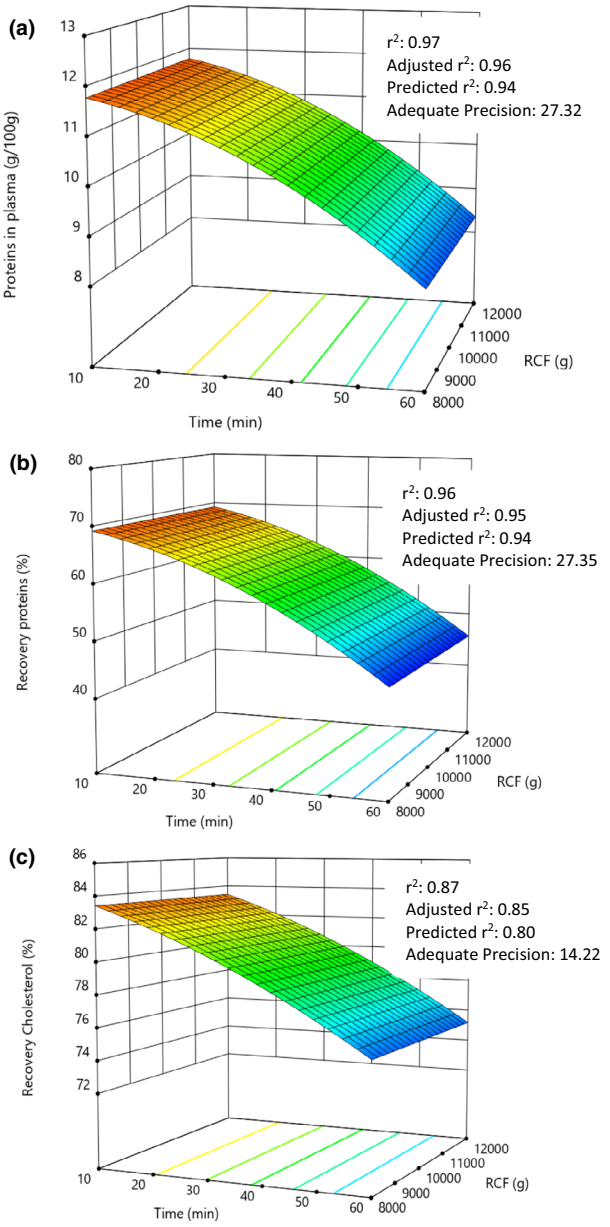

Figure 1 Model prediction for the effect of time and RCF on (a) protein concentration, (b) protein recovery and (c) cholesterol recovery of plasma obtained by centrifugation. Temperature was fixed at 8 °C

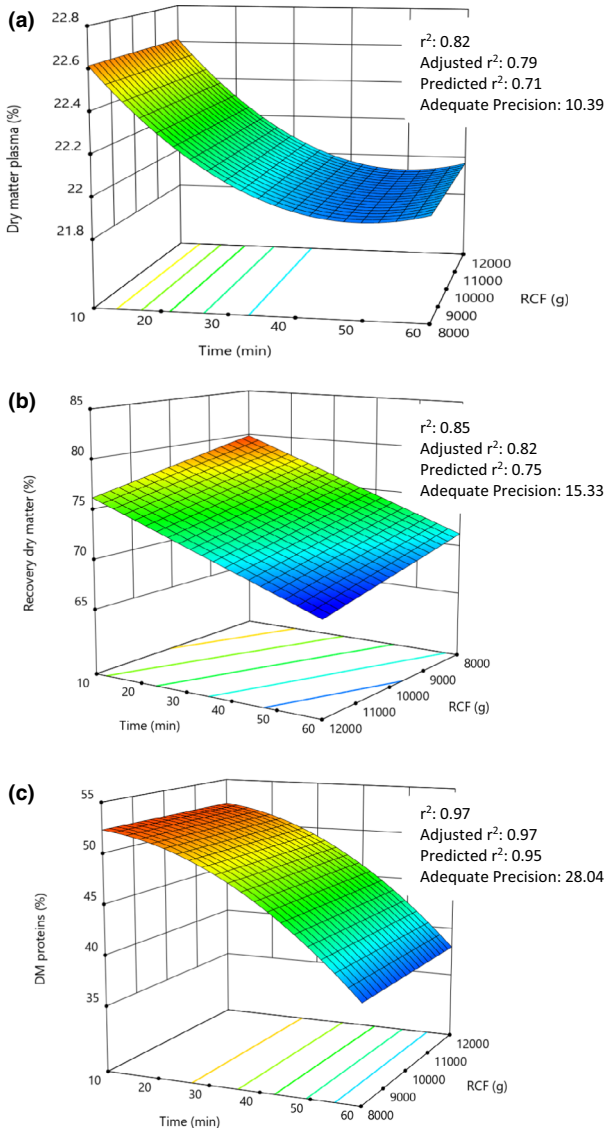

Figure 2 Model prediction for the effect of time and RCF on (a) dry matter concentration, (b) dry matter recovery and (c) protein content in dry matter of plasma obtained by centrifugation. Temperature was fixed at 8 °C

and structure to LDL found in plasma (Freschi *et al.*, 2011). From our results, the maximum amount of LDL in granules or plasma can be controlled through the centrifugation conditions.

Dry matter evaluation

Initial liquid whole egg showed an average DM of 23.9 ± 0.07% similar to those described for pasteurised whole egg in other studies 23.3 ± 5.1% (Rossi *et al.*, 2010). Plasma DM data fitted a quadratic mathematical model (Table S1). Only time term was significant, then a model reduction improved the model (Table S2). Acceptable outcomes were obtained from model correlation (Fig. 2a). Equation 11 can be used for this purpose.

$$\mathrm{DM_{Plasma}}\,(\%) = 23.01 - 4.35\times10^{-2}\cdot\mathrm{time}\,(\mathrm{min}) + 4.37\times10^{-4}\cdot\mathrm{time}^2 \quad (11)$$

In Fig. 2a, it is shown the response surface obtained for this model at 8 °C. The high influence of time is demonstrated with the high slope shown at time curve. Short time centrifugations obtained the highest DM values for plasma. However, there is an inflection point within the period of time studied that suppose that the dry matter does not barely change after about 40 min time.

The DM recovered in plasma varied between 68.26% and 80.55%. This means that whole egg dry matter present in granules and losses ranged between 19.45% and 31.74%. Temperature was not a significant factor (Table S1). A model reduction was performed (Table S2). Fit statistics shown at Fig. 2b were acceptable. Equation 12 can forecast the DM recovery (R_{DM}) in plasma fraction:

$$R_{DM}\,(\%) = 90.17 - 9.24 \times 10^{-4} \cdot \mathrm{RCF}\,(\mathrm{g}) - 0.17 \cdot \mathrm{time}\,(\mathrm{min}) - 0.14 \cdot \mathrm{temperature}\,(^{\circ}\mathrm{C}) \quad (12)$$

The surface response for DM recovery is shown in Fig. 2b. It is remarkable that short centrifugation times also obtained the highest concentration of proteins, cholesterol and DM. At those conditions, DM must contain more than 50% proteins. The amount of proteins expressed in DM ranged between 36.74% and 53.44% (Table 1), while non-protein content varied the difference to 100%, from 46.56% to 63.26%. The analysis of variance (ANOVA) resulted significative ($P < 0.05$) for the model (Table S1). However, as only the time term was significant, a model reduction was evaluated and obtained better results (Table S2). Equation 13 predicts the protein content in dry weight basis of plasma:

$$\mathrm{DM}_{\mathrm{prot}}\,(\%) = 52.45 + 3.6 \times 10^{-2} \cdot \mathrm{time}\,(\mathrm{min}) - 4.60 \times 10^{-3} \cdot \mathrm{time}^2 \quad (13)$$

Figure 2c shows the surface response obtained from this equation and good correlation parameters over 0.95. Within the centrifugation time studied, the proportion of proteins varies inversely proportional to time, while the non-protein content varies proportionally. As aforementioned, more proteins sediment with long centrifugations, whereas DM values obtained only decreased in less than 1%. Since the plasma content cannot increase due to the gravity, it can be resolved that its non-protein content increased because the concentration of proteins decreased, as well as the weight of plasma (data not shown). Then, the non-protein content included more egg components that cannot be separated into granules by centrifugation.

The presence of egg white proteins explains the difference to egg yolk plasma, where protein and lipid content described are 23% and 76%, respectively, for centrifugation at 10 000 g-force, 45 min at 4 °C (Laca, Paredes, Rendueles, & Díaz, 2015). Instead, it can be calculated with eqn 13 that a plasma with about 45% proteins and 55% non-protein in DM can be obtained from liquid whole egg.

It is important to notice that protein, cholesterol and DM composition decreased with time and the range of temperature studied (4–12 °C) and g-force (8000–12 000 g) have not influence on the prediction of these parameters, except for dry matter recovery. This could mean that the interval studied was narrow. However, as above-mentioned, these conditions are within the interested range employed for egg yolk fractionation. For food industry, this outcome made simpler/more straightforward the application of centrifugation but only in terms of plasma composition because physicochemical attributes shown a significant variation according to operational conditions (Puertas & Vázquez, 2021).

Emulsion properties

The average emulsion capacity and emulsion stability of liquid whole egg measured were 84.98% and 86.21%, respectively (Table 2). The stability measured in whole egg emulsions is only 1.07 times higher than described for raw whole egg, while capacity value is 1.37 times higher (Monfort *et al.*, 2012). Same study described similar values of stability for pasteurise samples and not significant differences with raw whole eggs were detected. Then, it was considered that

Table 2 Plasma responses for functional properties

Run	Emulsion capacity (%)	Emulsion stability (%)	Foam capacity (%)	Foam stability (%)
0	84.98 ± 1.29	86.21 ± 2.95	14.98 ± 3.67	79.29 ± 4.21
1	87.24 ± 0.52	82.50 ± 2.62	14.53 ± 3.06	83.61 ± 0.04
2	87.50 ± 0.33	89.16 ± 2.10	15.56 ± 1.77	83.33 ± 0.01
3	87.62 ± 0.12	86.25 ± 2.55	34.55 ± 0.43	94.74 ± 2.14
4	88.09 ± 0.45	86.97 ± 2.46	29.81 ± 1.36	90 ± 1.48
5	87.26 ± 0.03	84.52 ± 2.08	14.69 ± 3.28	92.31 ± 3.00
6	87.09 ± 0.54	85.63 ± 1.28	28.64 ± 1.93	86.27 ± 1.04
7	87.35 ± 0.56	86.29 ± 2.19	18.99 ± 4.57	94.74 ± 0.98
8	87.35 ± 0.57	84.32 ± 1.17	23.34 ± 3.59	89.73 ± 2.77
9	87.17 ± 0.45	86.42 ± 1.53	19.69 ± 3.75	80.85 ± 1.51
10	87.38 ± 0.50	85.77 ± 2.26	15.16 ± 1.35	80.21 ± 5.75
11	87.16 ± 0.09	84.23 ± 1.73	21.99 ± 1.81	81.68 ± 1.19
12	87.40 ± 0.66	85.69 ± 1.24	16.80 ± 3.26	94.44 ± 1.57
13	84.58 ± 0.11	88.36 ± 0.43	14.61 ± 1.94	78.29 ± 0.40
14	85.89 ± 0.99	89.07 ± 1.75	14.95 ± 1.89	82.97 ± 1.75
15	87.17 ± 0.18	81.69 ± 0.85	17.05 ± 0.70	80.56 ± 3.01

See experimental design in Table 1 for experimental conditions.
Run 0 is initial whole egg.

emulsion stability was not affected by temperature in the range of 55–76 °C (Monfort *et al.*, 2012).

The plasma emulsion capacity ranged between 84.58% and 88.09%, whereas the emulsion stability varied from 81.69% to 89.16% (Table 2). Both intervals encompass liquid whole egg values. These suppose little differences between whole egg and plasma about their emulsion properties. Then, the establishment of models was considered irrelevant.

Analogous results have been described for egg yolk plasma, where similar emulsifying properties have been observed between yolk and plasma (Laca *et al.*, 2015). This is explained due to the aforementioned separation of LDL into plasma predominantly, because LDL is considered the main contributors to the exceptional emulsifying properties of egg (Anton *et al.*, 2003).

On the other side, the minimum of 74% of cholesterol remaining in plasma (Fig. 1c) suppose an amount of LDL enough to maintain the emulsion properties of plasma with values for emulsion activity similar to whole egg.

Foaming properties

The liquid whole egg showed an average foam capacity of 14.98% and foam stability of 79.29%. These values were lower than the results presented in studies with fresh liquid whole egg: 26.3% and 95%, respectively (Patrignani *et al.*, 2013). It is well-known that heat treatments significantly reduced foam capacity and stability. The negative effect of heat on foaming properties has been attributed to the denaturalisation of conalbumin and lysozyme (Monfort *et al.*, 2012).

The foam capacity obtained for plasma ranged between 14.53% and 34.55%. The foam stability varied from 78.29% to 94.74% (Table 2). These plasma foam properties were significantly affected by the process variables ($P < 0.05$) as it is shown on Fig. 3. Both properties can be predicted through a quadratic model (Table S3).

The high value obtained for r^2 together with the adequate precision justified that the model can be used to predict foam capacity. Confirmation runs should be done to test the empirical model due to the difference in more than 0.2 between adjusted r^2 and predicted r^2 (Fig. 3a). Equation 14 is shown for that purpose:

$$\begin{aligned}\mathrm{FC_{Plasma}}\,(\%) = {} & -35.54 + 0.01\cdot\mathrm{RCF}\,(\mathrm{g}) + 0.78\cdot\mathrm{time}\,(\mathrm{min}) \\ & -1.52\cdot\mathrm{temperature}\,(^{\circ}\mathrm{C}) \;\; -5\times10^{-5}\cdot\mathrm{RCF}\cdot\mathrm{time} \\ & +2.23\times10^{-4}\cdot\mathrm{RCF}\cdot\mathrm{Temperature} \\ & -0.07\cdot\mathrm{time}\cdot\mathrm{temperature} \;\; -5.54\times10^{-7}\cdot\mathrm{RCF}^2 \\ & +4.25\times10^{-3}\cdot\mathrm{time}^2 + 0.09\cdot\mathrm{temperature}^2 \qquad (14)\end{aligned}$$

Foam capacity describes the ability of proteins to absorb lots of bubbles and retain the gas at the air–liquid interface (Jin *et al.*, 2013). According to the response surface of Fig. 3a, it can be deduced that proteins with higher capacity to absorb bubbles and retain the gas remain preferentially into plasma when intermediate g-force, long times and low temperatures are employed. Plasma foaming stability can be predicted with eqn 15:

$$\begin{aligned}\mathrm{FS_{Plasma}}\,(\%) = {} & -144.43 + 0.05\cdot\mathrm{RCF}\,(\mathrm{g}) + 0.28\cdot\mathrm{time}\,(\mathrm{min}) \\ & -5.23\cdot\mathrm{temperature}\,(^{\circ}\mathrm{C}) - 1.84\times10^{-5}\cdot\mathrm{RCF}\cdot\mathrm{time} \\ & +3.06\times10^{-4}\cdot\mathrm{RCF}\cdot\mathrm{Temperature} \\ & +0.03\cdot\mathrm{time}\cdot\mathrm{temperature} - 2.70\times10^{-6}\cdot\mathrm{RCF}^2 \\ & -5.57\times10^{-3}\cdot\mathrm{time}^2 + 0.02\cdot\mathrm{temperature}^2 \qquad (15)\end{aligned}$$

It is shown at Fig. 3b that the foams more stable would be acquired centrifuging at intermediate time and g-force. Temperature term was significant. However, all temperature values allow to obtain good stability. Hence, low temperature would be recommended to avoid microorganism proliferation.

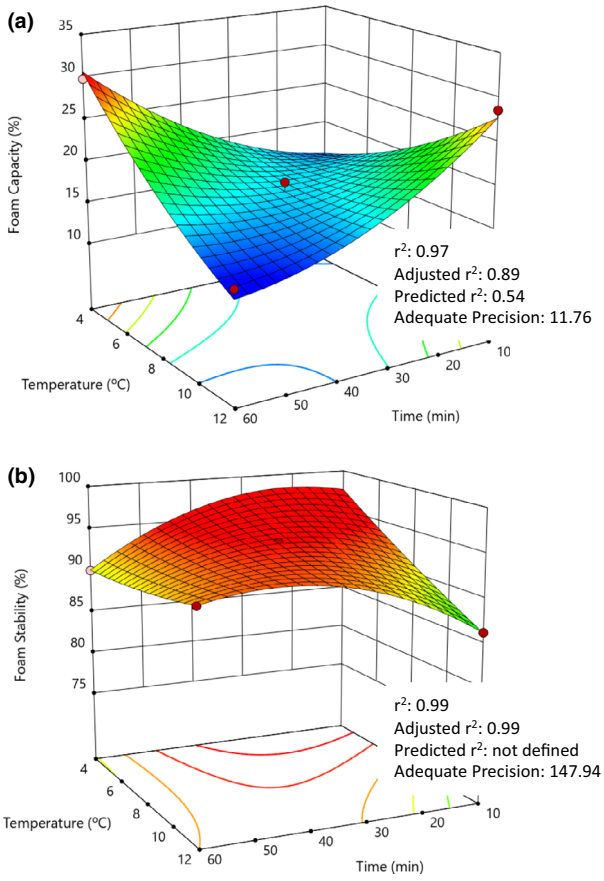

Figure 3 Model prediction for the effect of RCF and time on (a) foam capacity and (b) foam stability of plasma obtained by centrifugation. RCF was fixed at the intermediate value of 10 000 g

Foaming properties of whole egg were improved. Plasma showed the ability to double the foam capacity and increase until almost a 15% the foam stability compared with liquid whole egg. It could be a good substitute of pasteurised liquid whole egg. Further studies are needed to stablish more reliable models of foam capacity. Nevertheless, foam stability is considered the basic parameter to asses foam quality (Sokoøowicz *et al.*, 2019).

Conclusion

Whole egg plasma composition and foaming properties can be modified through the operational conditions of centrifugation. However, the range of temperature studied (4–12 °C) and g-force (8000–12 000 g) have not influence on the prediction of compositional parameters except for dry matter recovery.

Successful prediction models were obtained for proteins, cholesterol, dry matter and foam properties, although foam capacity requires further studies to improve fit statistics results.

Whole egg plasma has less cholesterol, proteins and dry matter than whole egg. Proteins responsible of foam and emulsion are separated according to fractionation conditions. However, plasma maintains emulsion properties and improves its foaming capacity and stability in relation to whole egg.

Further research into whole egg plasma applications is necessary for a broadening of its commercial applications for the food industry.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Gema Puertas: Investigation (equal); Methodology (equal); Writing-original draft (equal). **Manuel Vázquez:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Supervision (equal); Writing-review & editing (equal).

Ethical approval

This article does not contain any human and animals studies.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Referred this article for comparison to egg yolk plasma values of proteins and cholesterol.

Referred this article for emulsion activity characterisation and comparison to liquid whole egg.

Referred this article because it described saponification conditions for cholesterol quantification.

Referred this database for whole egg composition reference values.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Analysis of variance (ANOVA) for the dependent composition variables in Plasma

Table S2 Analysis of variance (ANOVA) of reduced models for the dependent composition variables in Plasma

Table S3 Analysis of variance (ANOVA) for the dependent functional properties of Plasma