EGG YOLK HYDROLYZED GRANULES. CHARACTERISTICS, RHEOLOGICAL PROPERTIES AND APPLICATIONS

Janire Orcajo[a], Ismael Marcet[a], Benjamín Paredes[a], Mario Díaz[a]*

[a] Department of Chemical and Environmental Engineering ,University of Oviedo. C/ Julián Clavería 8, 33006 Oviedo (Spain).

*Corresponding author. Tel: +34-985103439. Fax: +34-985103434. Email: mariodiaz@uniovi.es

Abstract

Given its low-cholesterol content feature, granules from egg yolk can be used as a substitute of the whole egg yolk. However, the functional properties of the granular fraction should be improved. In this sense, hydrolysis of proteins frequently produces improvements in some of its nutritional and technological properties. For that reason, in this work egg yolk granules were treated with a proteolytic enzyme, trypsin (E.C. 3.4.21.4) with the purpose of making a comparative characterization of the products.

Results showed that the enzymatic reaction produced a degree of hydrolysis of 12%, being the size of the different peptides obtained quantified by chromatographic and electrophoretic techniques. Mayonnaises made with these hydrolyzed granules resulted more stable to temperature changes between 4 and 20ºC than the one made with non-hydrolyzed ones. In the rheological tests carried out, the mayonnaise elaborated with hydrolyzed granules has the most similar rheological behaviour to that of a commercial one used as reference. In general, the results obtained suggest that the recipe elaborated

with hydrolyzed granules had better rheological characteristics than those prepared using non-hydrolyzed granules, maintaining the low-cholesterol feature.

Keywords: egg yolk, granules, hydrolysis, emulsification, mayonnaise, rheological properties.

1. Introduction

People concern about the rise of cardiovascular diseases and the obesity have led the consumers to select the ingredients which form part of the food products, trying to avoid these health issues. In response to this question, the lipids and cholesterol content of foods are two particular nutritional facts that focus the attention of researchers. In this sense, an ingredient with high levels of cholesterol and lipids, commonly used in the food industry, is the egg yolk.

Egg yolk is broadly recognised to contain many substances with biological functions beyond basic nutritional ones, and for that reason, its substitution is complicated and usually implies a quality loss in the final product.

However, egg yolk can be easily separated by centrifugation into two fractions: the plasma and the granular one. Plasma is mainly composed of 85% low density lipoproteins (LDL) and 15% livetins. This fraction contains about 73% of lipids and ¾ of the whole egg yolk cholesterol. On the other hand, egg yolk granules are mainly composed of 70% high-density lipoproteins (HDLs), 16% phosvitin and 12% low density lipoproteins. This fraction is high in protein content and it has approximately ¼ of the total cholesterol found in egg yolk (Laca et al., 2010a).

In a study about egg yolk fractionation, it has been shown that egg yolk granules keep, even after lyophilization treatment, good emulsifying, gelling and other properties, with the additional advantage of its lower cholesterol content (Laca et al., 2010a). However, the emulsifying properties of this granular fraction are lower than those found in the whole egg yolk or in the plasma fraction (Le Denmat et al., 2000).

Concerning proteins, their functional properties are those physicochemical properties that govern their performance and behaviour in food systems during their preparation, processing, storage and consumption. These properties can be enhanced through enzymatic modification of food proteins by controlled proteolysis over a wide pH range, and other processing conditions. Choosing the right proteolytic enzyme, environmental conditions and degree of hydrolysis (DH) is crucial for enhancing the functional properties of proteins. Owing to the complex nature of proteins it is very difficult to reach high DH values (Panyam and Kilara, 1996).

The emulsifying capacity of the egg yolk has been broadly investigated: the effect of a high-pressure treatment (Anton et al., 2001a), the stability and rheology when adding another agent (Kontogiorgos et al., 2004) or the egg yolk protein gels and emulsions (Kiosseoglou, 2003) are some examples. However, there is little information on the effect of controlled enzymatic hydrolysis of the egg yolk and its fractions on their foaming and emulsifying properties. When used as emulsifiers, the hydrolysis of the surfactant proteins, either before or after the formation of the emulsions, can affect the stability of the emulsion system by making the emulsion inherently unstable or by altering its sensitivity to external influences (e.g., calcium ions, reduced pH, or high temperature). Nevertheless, in some cases hydrolysis may even promote stability, as has been observed in the increase in calcium stability of caseinate emulsions treated with a

serine protease, trypsin. This enzyme cleaves peptide bonds at carboxyl terminals of arginine and lysine, except when linked to a proline residue (Olsen et al., 2004). Hence, if hydrolysed proteins are used as emulsifiers, there is a risk that the stabilizing effect of the protein will be lost affecting their potential applications, but it must be set also the possibility that the disruption of the protein structure may permit more efficient adsorption of some peptides (Singh and Dalgleish, 1998). Besides, whenever enzymatic hydrolysis of protein causes breakdown of protein molecules, the protein solubility increases, and is well known that solubility is essential for most proteins to provide good functionalities, such as foaming and emulsification.

In this work, since the granular egg yolk fraction is low in cholesterol content, and with the aim to test the possibility of enhancing the functional properties of the granular proteins, they were hydrolyzed to a value close to 12% degree using trypsin (EC 3.4.21.4). The procedure was developed at the optimal temperature and pH for the activity of trypsin, 37°C and pH over 7.0 (Chelulei Cheison et al., 2011). Then, the applicability of the product of hydrolysis was tested, in particular elaborating mayonnaises with non-hydrolyzed and hydrolyzed granules. Rheological properties were measured in order to evaluate the mayonnaises characteristics compared to those of a first quality mayonnaise acquired from a local market, which was used as a reference.

2. Materials and methods

2.1. Fractionation of egg yolk

The fractionation method was developed modifying the procedure by Laca et al. (2010a). The general scheme for egg yolk fractionation is shown in Figure 1.

The granules fraction was frozen at -80º C overnight and then lyophilized at -70º C and 0.1 mBa in a Telstar Cryodos Lyophilizator for 24 hours, in order to increase its shelf life.

2.2. Enzymatic hydrolysis

The hydrolysis reaction was carried out in a 5 l bioreactor with a pH-STAT automatic titration (pH-Burette 24 2S, Crison) connected to an iso-thermal shaker.

Lyophilized egg yolk granules were dissolved at 0.4% (p/v) in 0.55 M sodium chloride solution for their total disruption (Antón et al., 2000) and stirred to fully disperse the protein. The protein content of this solution was calculated by Bradford method. Then, it was heated at 37º C and pH adjusted to 7.5 by using 0.1 M sodium hydroxide solution. Trypsin from porcine pancreas E.C. 3.4.21.4 (T7409, Sigma-Aldrich) 1900 u/mg solid, was added to the bioreactor (1.5:80 enzyme-substrate relation). Inactivation of the enzyme was done by acidifying with 1 M hydrochloric acid to pH 3.0 after 90 minutes of reaction.

The hydrolyzed solution was kept at 4º C overnight and then centrifuged (KUBOTA 6500 Centrifuge) at 10 000 x g and 4º C for 45 minutes to separate into supernatant and the hydrolyzed granule fraction (precipitate). The supernatant was separated from the hydrolyzed granules by decantation.

The hydrolyzed granules fraction was lyophilized by the same method as for the non-hydrolyzed granules. The protein content of both lyophilized hydrolyzed and non-hydrolyzed granules was calculated according to the Dumas combustion method using a CNHS/O Vario EL analyzer (Elementar).

2.3. Mayonnaises preparation

Two mayonnaise formulations were prepared using egg yolk granules and hydrolyzed granules. A commercial mayonnaise acquired from a local market was used as reference.

The mayonnaise formulation was obtained from Laca et al. (2010b) with slight modifications, and included 9 ml white vinegar (6% acidity), 0.94 g fine sea salt, 1.3 g white sugar and 70 ml sunflower oil. As emulsifying agents were added lyophilized non-hydrolyzed egg yolk granules (mayonnaise A) and lyophilized hydrolyzed egg yolk granules (mayonnaise B) until obtain a protein concentration of 27mg/g of mayonnaise. Previously to mayonnaises preparation, both lyophilized non-hydrolyzed and hydrolyzed granules were rehydrated adding water (1.38g/g of lyophilized). Mayonnaise C is a commercial standard acquired from a local market and used as a reference, whose ingredients were water, wine vinegar, salt, sugar, sunflower oil, egg yolk, lemon extract, corn starch, modified corn starch, antioxidant and colouring (curcumin).

The amount of the emulsifying agent was adapted from the basic formulation described by Ghoush et al. (2008). Mustard and other spices were not included in the formulation since only egg yolk granules were to be evaluated as emulsifying agent.

The preparation process was as follows: salt, sugar and vinegar were mixed at 8000 rpm with a Heidolph Silentcrusher M (Typ 12 G/M) and emulsifying agent was added. During blending, 20 ml of sunflower oil were added drop by drop; the rest of the sunflower oil was added in amounts of 10 ml at each addition. The total time of mixing was 15 minutes.

2.4. Determination of the degree of hydrolysis (DH)

The pH-STAT method was used to determine the degree of hydrolysis (DH). According to this method that was established by Adler-Nissen (1986) the amount of base consumed expressed in moles is proportional to the amino groups liberated also in moles during the hydrolysis process. Degree of hydrolysis was calculated using Adler-Nissen's equation (1):

$$DH\% = \frac{100 \, x \, VB \, x \, NB}{\alpha \, x \, Mp \, x \, h_{tot}} \qquad (1)$$

where α is the degree of dissociation of α-amino group calculated by the following equation (2), Mp is the mass of protein (g), h_{tot} is the total number of peptide bonds in the protein (meq g^{-1} protein), and V_B and N_B are the volume (ml) and the concentration (normality) of alkaline added.

$$\alpha = \frac{10^{pH-pK}}{1+10^{pH-pK}} \qquad (2)$$

The value of h_{tot} for egg yolk protein is currently unknown and it was assumed to be 8 based on a reference suggestion (Wang and Wang, 2009).

The pK value used for the hydrolysis procedure is approximated calculated through the equation of Guadix et al., (2000) (3):

$$pK = 3.80 + 0.45pH \qquad (3)$$

During three different hydrolysis reactions, aliquots were taken at different times (0, 1, 5, 10, 15, 20, 25, 30, 60 and 90 minutes). DH percentage was calculated at each aliquot.

2.5. Gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to verify the egg yolk granules proteins hydrolysis. Laemmli method (Laemmli,

1970) was followed including a specific staining phase for phosphoproteins consisting on Coomassie blue 0.05% (p/v), ethanol 0.025% (v/v), acetic acid 10% (v/v), Triton X-100 1% (v/v), aluminium nitrate 3.75% (p/v) and distilled water 40% (v/v). SDS-PAGE Molecular Weight Standards Broad Range (Bio-Rad) were used as protein standards. Electrophoresis was run on polyacrylamide gels (stacking 3.5% and resolving 12%) with a migration buffer consisting of a 0.02 M THAM, glycine 5 M and SDS (w/v) 0.1% solution in Power Pac 300 (Bio-Rad) operator. The gels were destained in a solution containing acetic acid 10%, methanol 40% and distilled water 50%.

2.6. Size-exclusion chromatography

An ÄKTA FPLC (GE Healthcares) system was employed to determine the molecular weight distribution. A size exclusion column was used (Superdex^{TM} Peptide 10/300GL), which can separate peptides from 100 to 7000 Da. The buffer used was composed by 100 ml of TrizMa pH 7.6 1 M and 900 ml of distilled water. The samples were filtered by 0.45 micron before inject them. Those samples were obtained at time 0, 1, 30, 60 and 90 minutes of hydrolysis. The elution flow was adjusted at 1 ml/min. The FPLC system used was dotted with an UV detector and the wavelength employed was 280 nm. Previously, the column was calibrated with standard weight markers supplied by Sigma-Aldrich. The molecular size distribution and the average molecular size obtained were analyzed to determine the total soluble peptide concentration. Subsequently, the area of the chromatograms obtained was divided in ranges of molecular size, according to previous calibration. The area of each zone was measured employing the Unicorn 5.1 analysis software and transformed into protein

concentration. The frequency of each molecular weight ranged was calculated with regard to the total amount of soluble peptides.

2.7. Rheological measurements of mayonnaises.

The rheological tests were carried out with a Haake MARS II rotational rheometer using a Peltier unit to control the temperature. All the tests were carried out at 20±0.1º C (except for the temperature ramp) and before starting any measurement, the sample was allowed to rest for at least 15 min. Glass hood and silicone oil were employed to avoid sample desiccation during the analysis. The rheological measurements were performed on the mayonnaise samples after one-day storage.

In dynamic conditions, a plate/plate measuring system (PP60) was used, with a gap of 1 mm. The stress sweeps were performed from 0.01 to 100 Pa at a frequency of 1 Hz and 20ºC. The temperature sweeps were carried out from 4 to 20º C at a heating rate of 1º C/min, a frequency of 1 Hz and a constant shear stress adjusted to each mayonnaise in order to keep the measurements within the linear viscoelasticity regime.

In steady state, a serrated plate/plate measuring system (PP35) was used, with a gap of 1 mm. Flow properties were measured at 20ºC. Apparent viscosity was obtained as a function of shear rate from 0.01 1/s to 100.0 1/s. 100 points were collected with a logarithmic distribution in each case. Triplicates presented differences lower than 10%. Data obtained were adjusted to the Ostwald de Waele model (4):

$$\eta = k\,\dot{\gamma}^{\,n-1} \qquad (4)$$

Where η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, k ($Pa.s^n$) is the consistency index, and n is the flow behavior index.

Ostwald de Waele model parameters were calculated from the obtained curves using the Haake Rheowin Software.

2.8. Statistical analysis

Analysis of variance (ANOVA) was applied. Least significant differences (LSD) were calculated by Fisher´s test to determine significant differences among the tested samples. These analyses were performed using a statistical software (statgraphics v.15.2.06). Experiments were carried out in triplicate, average and standard deviation were calculated too.

3. Results and discussion

3.1. Hydrolyzed granules characterization

To characterize the hydrolyzed granules the degree of hydrolysis was calculated. Besides, a primary electrophoresis approach was made. Employing electrophoresis techniques is difficult to separate and quantify the smallest peptides formed along the enzymatic reaction. To complete this information, aliquots at different times of hydrolysis were analysed employing size exclusion chromatography.

3.1.1. Determination of the degree of hydrolysis (DH)

The change in the functional properties of a protein is a direct result of the hydrolysis effect. The DH (%) curve in Figure 2 shows that protein degradation rate was high in the initial stage of the enzymatic incubation but the trend decreases after 30 min following the kinetic model of Michaelis-Menten (Seguel, 1993). The DH percentage reached was 10.00±0.51 and 12.00±0.5 after 60 and 90 minutes, respectively.

Fig. 2. Degree of hydrolysis (DH) percentage of the egg yolk lyophilized granules.

3.1.2. Gel electrophoresis

SDS-PAGE of egg yolk granules hydrolysis at different times is showed in Figure 3. At *t0*, a band of approximately 80 kDa can be identified as α-livetin or as one HDL apoprotein (Anton, 2007; Le Denmat et al., 2000) below another two superimposed one each other corresponding to two apo-HDL (110 kDa and 105 kDa, respectively). The phosvitin, 39 kDa, is found over another of 32 kDa which feasibly belongs to the smallest HDL apoprotein. In the first minute the proteolytic enzyme hydrolyzes high molecular weight proteins appearing peptides between 30 and 40 kDa, notably after 5 min. A faint band of 29 kDa can be detected from minute 10 to 90. According to bibliography, a large peptide fragment (Gln 49-Arg 212) and a smaller one (not detected in Figure 3) is the product of the tryptic digestion of phosvitin (Goulas et al., 1996). Besides, not only a great amount of peptides of less than 30 kDa can be observed between 60 and 90 min lines, but also a band of about 74 kDa that cannot be seen at *t0*. This large fragment of 74 kDa suggests that, in the tested conditions, the biggest HDL apoproteins cannot be totally digested and a core of these apoproteins remained largely intact upon digestion with trypsin. A similar behaviour has been detected in the phosvitin.

These results allow knowing that trypsin is capable to partially hydrolyze egg yolk granules protein even at the high salt concentration of 0.55 M necessary to solve the proteins present in the egg yolk granules (Anton et. al, 2000). However, employing

electrophoresis techniques smallest peptides cannot be detected and this information has been completed carrying out size exclusion chromatography.

Fig. 3. SDS-polyacrylamide gel electrophoresis of egg yolk lyophilized hydrolyzed granules at different times (*min*) of the enzymatic hydrolysis.

3.1.3. Size-exclusion chromatography

An analysis to determine the quantity and weight of the smallest peptides obtained at different times of the hydrolysis was performed by size-exclusion chromatography (SEC). Employing this chromatography technique, peptides from 100 to 7000 Da can be separated and quantified. In Figure 4, chromatograms at tested times are shown.

Fig. 4. Size exclusion liquid chromatography of egg yolk lyophilized and hydrolyzed granules at different times of the enzymatic reaction.

In these chromatograms farthest peaks correspond to higher times of hydrolysis except at exclusion volume which is the opposite. There are proteins of more than 7000 Da in the exclusion volume that the column is not able to retain. These proteins were analysed in the gel electrophoresis in the previous section. As expected, several peaks were present growing progressively with the time. Polypeptides from 960 to 4990 Da appear in the chromatogram at an elution volume between 14.16 and 17.01 ml before the tripeptides (562 Da) and the dipeptides (314 Da) that can be seen at 17.98 and 19.01

ml, respectively, as it is described in Table 1. From a volume exclusion of 21 ml are considered amino acids of around 100 Da.

Table 1.

Comparing the granules proteolysis at different times, size-exclusion chromatogram reveals the increasing quantity of the hydrolyzed obtained time by time until 1 hour, but this trend decreases from 60 to 90 minutes. From the exclusion volumes indicated in Table 1 and peak areas from chromatograms of Figure 4, it was obtained the relative amount of peptides along the enzymatic reaction (Figure 5). This figure shows as at t0, 100% of proteins were detected in the exclusion volume, with no peptides less than 7000 Da in solution. However, at each time the amount of protein in the exclusion volume was decreasing meanwhile small peptides appear. Even for times over 60 minutes, a high proportion of protein appears in the exclusion volume, indicating the proteolytic resistance of the egg yolk granules protein. This agrees with the gel electrophoresis of the previous section, where peptides of more than 21 kDa persists in the medium at t90. Furthermore, peptides lower than 5 kDa were present always from 10 to 90 minutes of hydrolysis. The amount of these peptides were constantly increased over time and it was more obvious as the molecular weight of peptides was lower; so relative abundance of smallest peptides (<1 kDa) were the highest compared to the other small peptides produced.

It was reported (Bautista et al., 2000; Clemente, 2000) that low molecular weight peptides, especially di- and tripeptides, with free amino acids have high nutritional and

therapeutic values (Vijayalakshmi et al., 1986; Williams et al., 1995). On the other hand, large molecular weight peptides (more than 20 amino acid residues) are presumed to be associated with an improvement in the functional properties of hydrolysates (Gauthier et al., 1986).

Fig. 5. Relative amount of small peptides along the enzymatic reaction.

3.2. Mayonnaises

3.2.1. Rheological measurements

Stress and temperature sweeps and viscosity curves were carried out for mayonnaises elaborated with: non-hydrolyzed (A) and hydrolyzed (B) egg yolk granules. Furthermore, a commercial mayonnaise was analysed too (C).

3.2.1.1. Stress sweeps

Results of stress sweeps at 20ºC are shown in Figure 6. As can be seen, the linear viscoelastic range goes from 0.1 until 30 Pa for both the hydrolyzed recipe and the commercial mayonnaise. However, in the case of the non-hydrolyzed recipe, the linearity is maintained in all the stress range tested. Furthermore, the elastic module is higher in the non-hydrolyzed recipe too, reaching values of around 3200±60 Pa, meanwhile in the case of the commercial and the hydrolyzed mayonnaise the G´ values are of 930±20 and 330±15 Pa respectively. This behavior denotes a more strong structure in the case of the non-hydrolyzed mayonnaise, likely because the granular proteins can be involved in the formation of more interactions, since proteins are responsible of structure support. Furthermore, the granular fraction of the egg yolk has

been described as an active filler (Anton et al., 2001b), which denotes the potential capacity of these proteins to interact between them in emulsions. However, according to the results obtained, this protein feature was reduced drastically after the hydrolysis treatment. On the other hand, commercial mayonnaise (*line C*) stays in a range between that of non-hydrolyzed and hydrolyzed granules mayonnaises.

Fig. 6. Storage modulus (*G'*) vs. stress sweeps of mayonnaises.

3.2.1.2. Temperature sweeps

Shear stress was selected for each mayonnaise in order to develop their temperature sweeps in the linear range.

Between 4 and 20º C (common temperature range for mayonnaises consumption) the linear trend of the mayonnaises does not mark important changes in the storage module as can be seen in Figure 7. However, the mayonnaise elaborated using non-hydrolyzed granules, even with higher values of G′, looks to be a bit less stable to temperature changes at the range studied. It is remarkable that the mayonnaise made with hydrolyzed granules has a behaviour quite close to that of the commercial used as a reference.

Fig. 7. Storage modulus (*G'*) vs. temperature sweeps of mayonnaises.

3.2.1.3. Flow Curve

Flow curve was then studied for hydrolyzed and non-hydrolyzed granules mayonnaise comparing the results with those obtained from the commercial mayonnaise sample. Results are exposed in Figure 8, responding the three samples to the Ostwald de Waele model.

Fig. 8. Flow curve of lyophilized hydrolyzed egg yolk granules mayonnaise and commercial mayonnaise.

In flow curves shown in Figure 8, it can be observed that the apparent viscosity decreases with increments in the shear rate. This behaviour is a feature of shear thinning products. This fact is confirmed by the flow behaviour index value ($n < 1$). Furthermore, values of n close to 1 are a feature of Newtonian fluids, meanwhile the lower values are considered related to more structured samples. The consistence and the flow behavior indexes deduced from the flow curves are presented in Table 2.

Table 2.

In the mayonnaises tested, the lowest n values belong to the non-hydrolyzed and the commercial recipes, showing them a more structured system than the hydrolyzed mayonnaise. In the case of the non-hydrolyzed protein recipe, this is in agreement with the comments made for the stress sweeps (section 3.2.1.1), whereby the non-hydrolyzed granular protein maintain a high capacity to structure the system compared to the hydrolyzed protein. Furthermore, the use of non-hydrolyzed granules produces

mayonnaises with the highest consistence index value (k) too. This increment in the consistence index value produces a more viscous mayonnaise, with a high resistance to flow in comparison with the other two samples tested. On the other hand, the recipe elaborated with hydrolyzed protein maintains a consistence index value similar to that obtained for the commercial reference, although the structure degree of the system was significantly reduced. It could be understood as a decrease in the protein-based interactions performed by the hydrolysis treatment in relation to the non-hydrolyzed recipe. In the case of the commercial mayonnaise, it shows a flow behavior index similar to that of the non-hydrolyzed recipe, but the consistence index and the apparent viscosity is closer to that obtained from the hydrolyzed recipe, particularly at high shear rates.

4. Conclusions

At the experimental conditions of the enzymatic proteolysis of egg yolk granules, a hydrolysis degree of 12 % is obtained after one and a half hour operation time, producing peptides of 4990, 960 and 562 Da, mainly. The peptides spectrum reached was 50±1.8% larger than 7000 Da, 5±0.1% of 2500-5000 Da, 10±0.9% of 1000-2500 Da and 30±2% of less than 1000 Da, and allows the final lyophilized product to maintain good emulsifying qualities.

Rheological assays show that mayonnaise made with non-hydrolyzed granules is stronger, with a higher resistance to flow and less stable to temperature changes than that elaborated using hydrolyzed protein. It could be possible because the interactions between non-hydrolyzed proteins enhance the structure of the system and the viscosity of the emulsion. The hydrolysis treatment could reduce these interactions, and therefore,

varying the features of the obtained mayonnaise. In this sense, the use of the hydrolyzed granular protein approximates the behaviour of the mayonnaise to that found in the commercial one, according to the results previously presented.

In broad terms, the enzymatic hydrolysis of the egg yolk granular fraction resulted in a product, that used in the mayonnaise formulation, provides rheological properties more similar to those found in the commercial reference, maintaining the low-cholesterol feature of the granules.

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Figure 1.

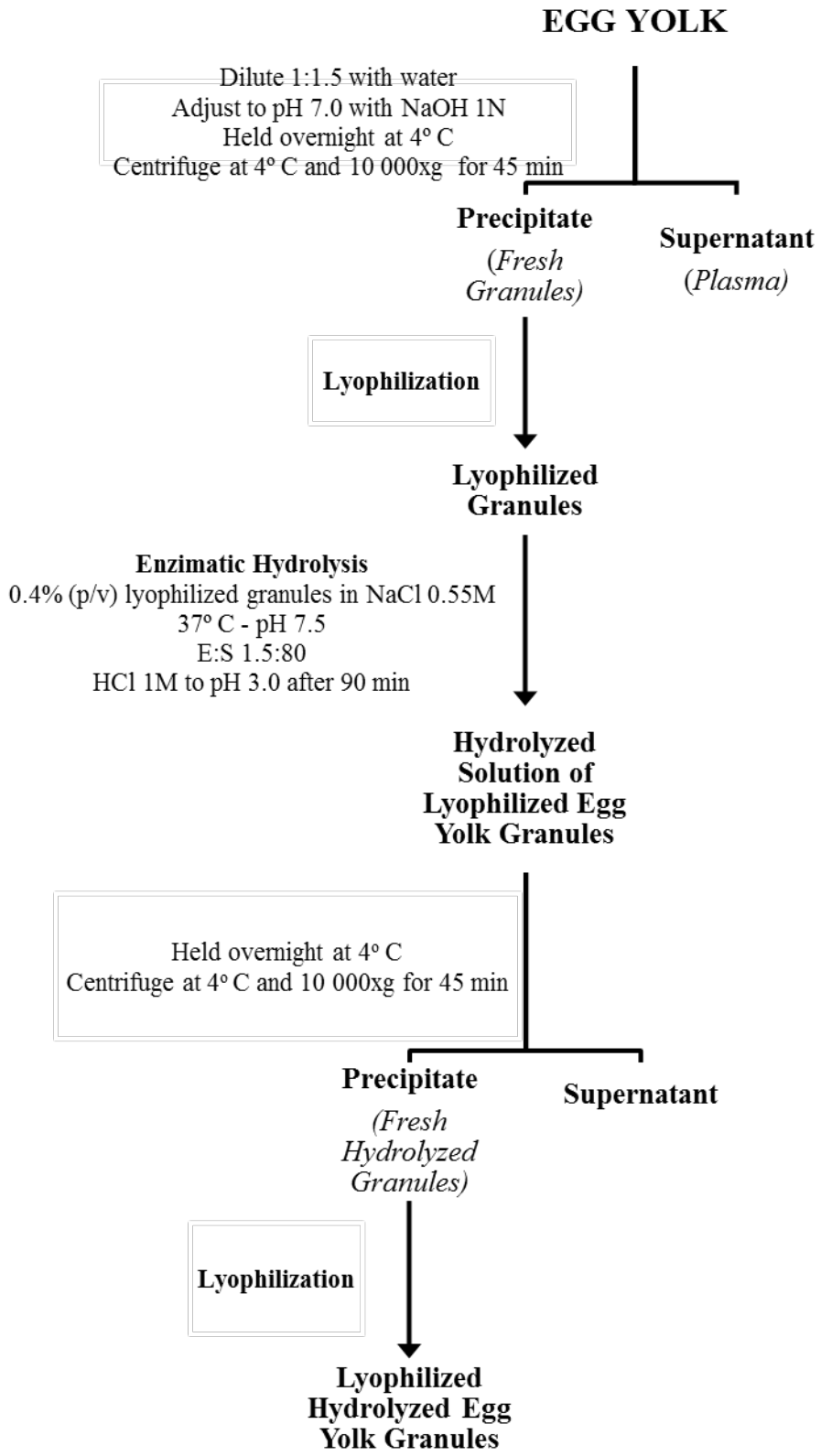
EGG YOLK
Dilute 1:1.5 with water
Adjust to pH 7.0 with NaOH 1N
Held overnight at 4° C
Centrifuge at 4° C and 10 000xg for 45 min
Precipitate
(Fresh Granules)
Supernatant
(Plasma)
Lyophilization
Lyophilized Granules
Enzimatic Hydrolysis
0.4% (p/v) lyophilized granules in NaCl 0.55M
37° C - pH 7.5
E:S 1.5:80
HCl 1M to pH 3.0 after 90 min
Hydrolyzed Solution of Lyophilized Egg Yolk Granules
Held overnight at 4° C
Centrifuge at 4° C and 10 000xg for 45 min
Precipitate
(Fresh Hydrolyzed Granules)
Supernatant
Lyophilization
Lyophilized Hydrolyzed Egg Yolk Granules

Figure 2.

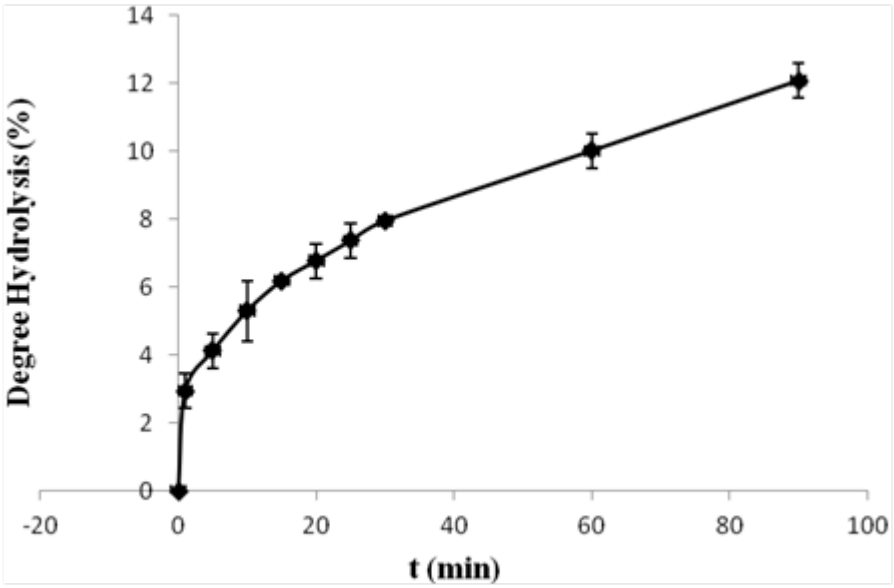

Figure 3.

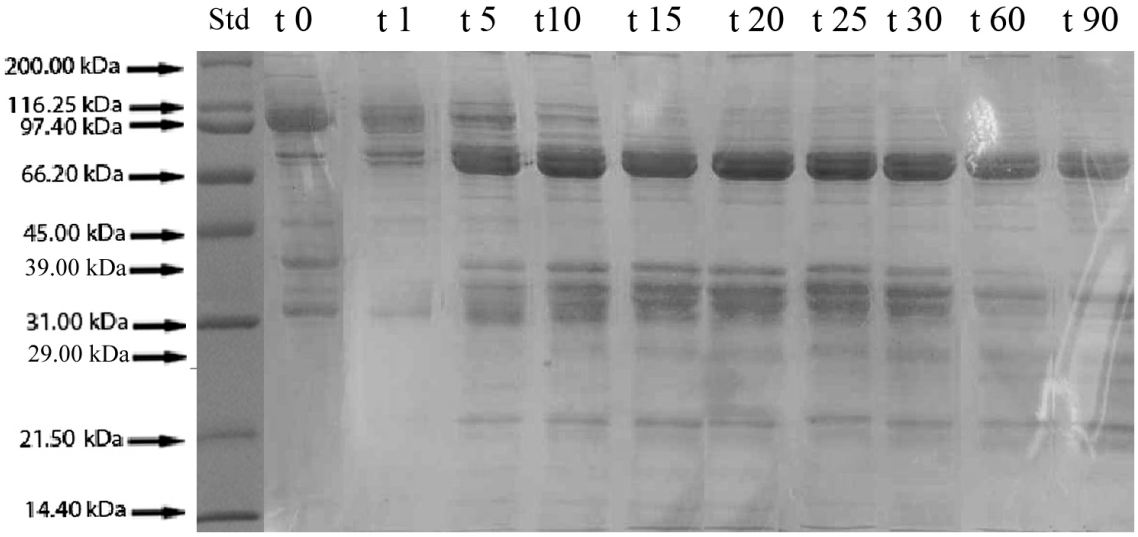
Std
t 0
t 1
t 5
t10
t 15
t 20
t 25
t 30
t 60
t 90
200.00 kDa
116.25 kDa
97.40 kDa
66.20 kDa
45.00 kDa
39.00 kDa
31.00 kDa
29.00 kDa
21.50 kDa
14.40 kDa

Figure 4.

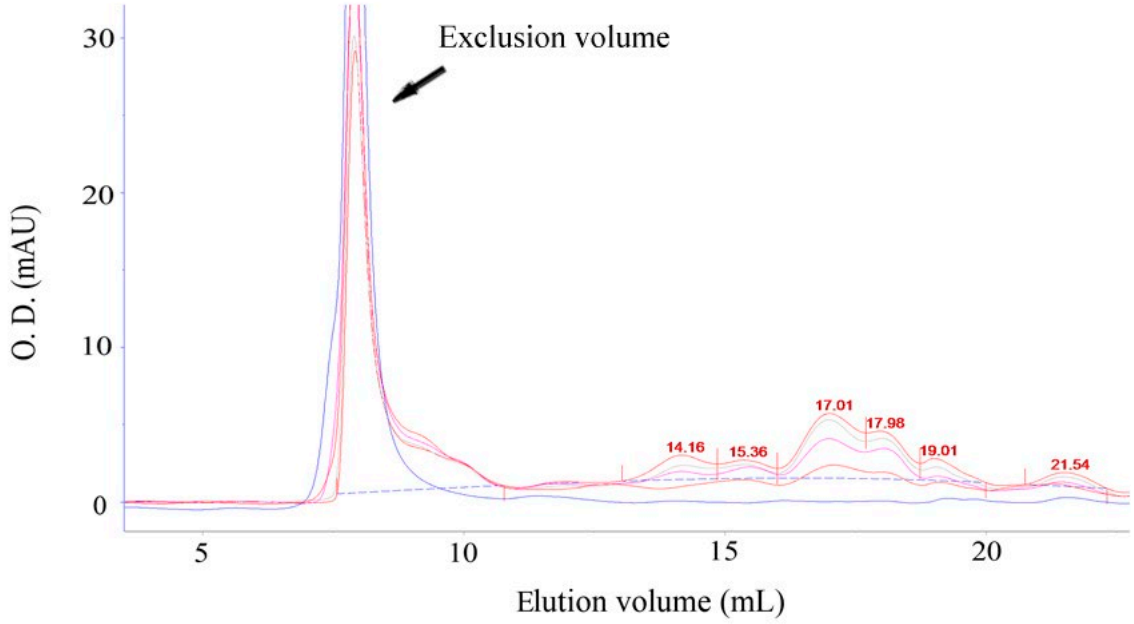
Exclusion volume
O. D. (mAU)
30
20
10
0
5
10
15
20
Elution volume (mL)
14.16
15.36
17.01
17.98
19.01
21.54

Figure 5.

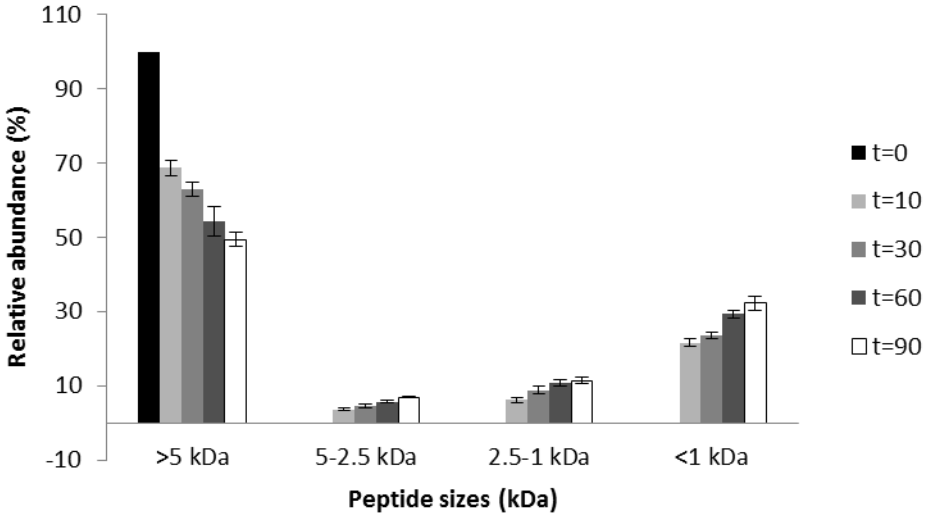
110
90
70
50
30
10
-10
Relative abundance (%)
>5 kDa
5-2.5 kDa
2.5-1 kDa
<1 kDa
Peptide sizes (kDa)
t=0
t=10
t=30
t=60
t=90

Figure 6.

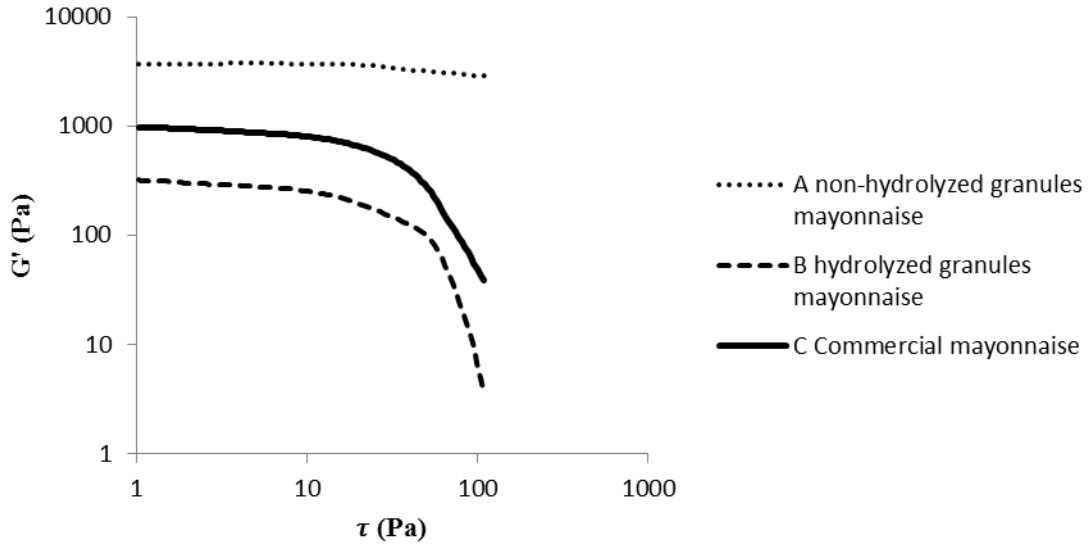
10000
1000
100
10
1
G' (Pa)
1
10
100
1000
τ (Pa)
A non-hydrolyzed granules mayonnaise
B hydrolyzed granules mayonnaise
C Commercial mayonnaise

Figure 7.

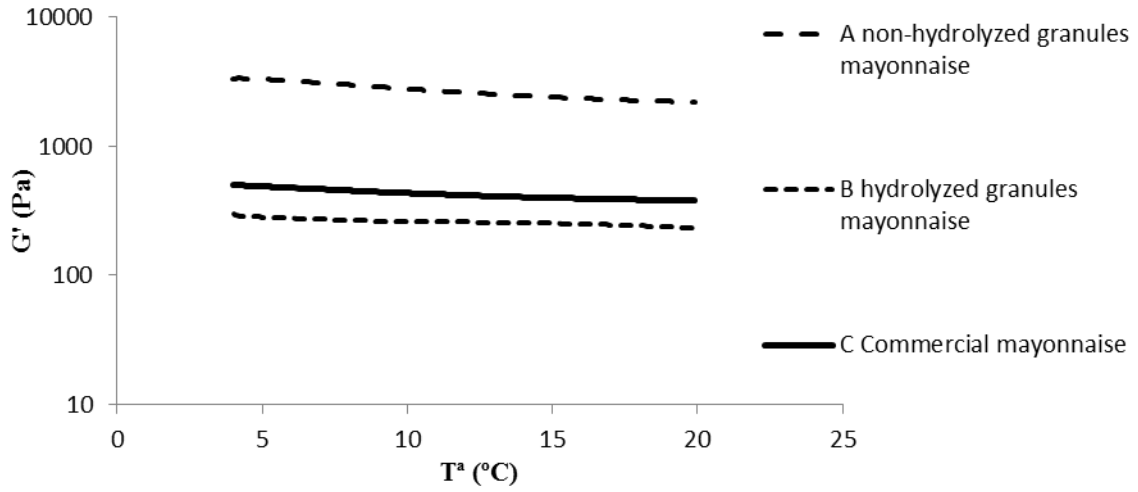
10000
1000
100
10
G' (Pa)
0
5
10
15
20
25
Tª (ºC)
A non-hydrolyzed granules mayonnaise
B hydrolyzed granules mayonnaise
C Commercial mayonnaise

Figure 8.

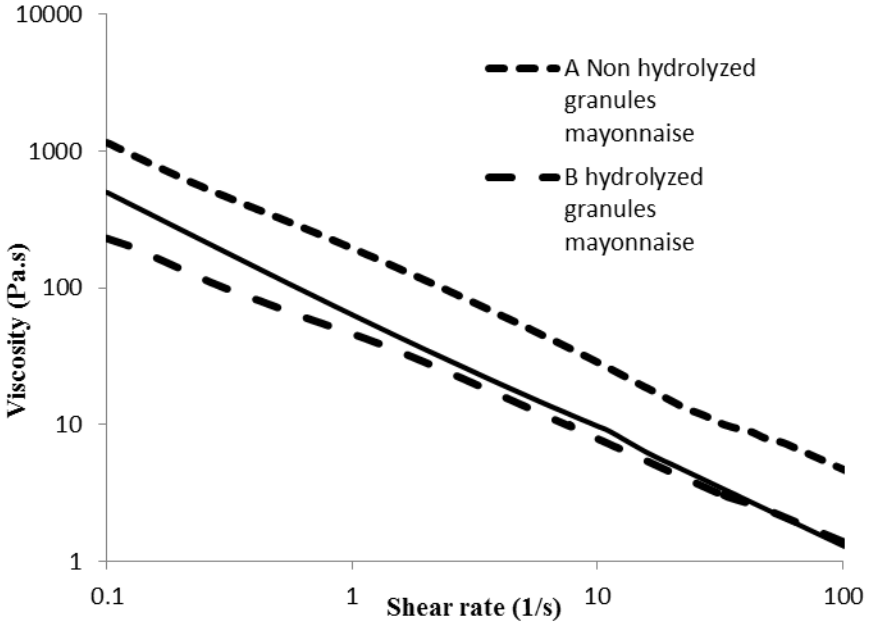
10000
1000
100
10
1
Viscosity (Pa.s)
0.1
1
10
100
Shear rate (1/s)
A Non hydrolyzed granules mayonnaise
B hydrolyzed granules mayonnaise

Fig. 1. General scheme procedure to obtain lyophilized hydrolyzed egg yolk granules.

Fig. 2. Degree of hydrolysis (DH) percentage of the egg yolk lyophilized granules.

Fig. 3. SDS-polyacrylamide gel electrophoresis of egg yolk lyophilized hydrolyzed granules at different times (*min*) of the enzymatic hydrolysis.

Fig. 4. Size exclusion liquid chromatography of egg yolk lyophilized and hydrolyzed granules at different times of the enzymatic reaction.

Fig. 5. Relative amount of small peptides along the enzymatic reaction.

Fig. 6. Storage modulus (*G'*) vs. stress sweeps of mayonnaises.

Fig. 7. Storage modulus (*G'*) vs. temperature sweeps of mayonnaises.

Fig. 8. Flow curve of lyophilized hydrolyzed egg yolk granules mayonnaise and commercial mayonnaise.

Table 1.

Ve (mL)	MW (Da)
14.16	4990
15.36	2633
17.01	960
17.98	562
19.01	314
21.54	75

Table 2.

	A	B	C
***k* (pa.s^n)**	145(0.7)a	43(1.4)b	57(1.4)c
n	0.1(0.02)a	0.16(0.02)b	0.1(0.01)a
R^2	0.99	0.99	0.99

Within rows, values followed by the same letter do not differ significantly from each other ($p>0.05$)

Table 1. Molecular weight of the peaks from size-exclusion chrotamography (*Fig. 4*).

Table 2. Parameters calculated from Figure 2. *k* is the consistence index, *n* is the flow index.