

Scientific paper

# Egg yolk and egg yolk fractions as key ingredient for the development of a new type of gels

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## Abstract

The aim of this work has been the development and characterization of base gels employing egg yolk and egg yolk fractions (plasma and granules) as main ingredients. Basing on preliminary test, three different formulations were prepared with egg yolk, plasma or granules, respectively. These formulations were compared by means of rheological, textural, color and microstructure analyses. Additionally, in order to provide a culinary point of view of the base gels, some final dishes were created by a trained chef.

The employment of egg derivatives proved to be determinant on the characteristics of the developed products, enhancing the mechanical properties of the gels and also providing an appealing color to them. Thus, the use of egg yolk and egg yolk fractions allows the development of new base gels improving not only their physical properties, but also their organoleptic characteristics.

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## Introduction

The hen egg is one of the most versatile products being widely used in the food industry due to their multifunctional properties, including foaming, coagulative, emulsifying, and binding properties; no replacers can match the superior functional attributes of eggs (Anton, 2007). In addition, shell egg and egg products industry have seen large changes in the last 50 years, and, along with changes in egg-processing technology, there has been a continuing growth of further processed egg products. In fact, during recent years, there has been significant growth in the use of egg products; specifically, today approximately 30% of the total consumption of eggs is in the form of further processed eggs (Froning, 2008). Many of these egg products, such as liquid whole egg, yolk, and whites;

frozen salted yolk or sugared yolk, are used as ingredients in various food applications.

Particularly, hen egg yolk is undoubtedly an efficient ingredient in many food products as it combines, not only functional, but also, nutritional and organoleptic properties. Indeed, it contains proteins of high biological value and other nutrients such as vitamins, minerals, essential fatty acids and phospholipids (King'ori, 2012; Anton, 2013).

Currently, as future new applications of egg components are pursued, it is important to explore new innovative applications (Froning, 2008). One of the main approaches accompanying these new innovative applications is the fractionation of egg components (Laca et al., 2014).

In native conditions, yolk is a complex system constituted by non-soluble protein aggregates (granules) in suspension in a clear yellow fluid (plasma) (Anton, 2013). Consequently, yolk can be easily separated into its two main fractions (plasma and granules) by centrifugation (Strixner and Kulozik, 2013).

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Granules represent about 22% of yolk dry matter, accounting for about 50% of yolk proteins and 7% of yolk lipids; whereas plasma corresponds to about 78% of yolk dry matter and it accounts for about 90% of yolk lipids and 50% of yolk proteins (Anton, 2013).

One of the most radical revolutions in the culinary industry has occurred in the last two decades in such a way that, nowadays, the knowledge and practices promoted by the avant-garde movement have transcended the limits of the gastronomic field (Opazo, 2012). International gastronomy and food science are in search for appealing ingredients, new food products or new technology and methods for dish preparation (Krigas et al., 2015). Really, the first level of creativity in the kitchen is based fundamentally on the ingredients (Goldfarb, 2014).

Last years, different works have reported interesting composition and properties of egg yolk fractions (granules and plasma). Concretely, granules show many interesting characteristics to be employed as ingredient in food industry, providing some advantages in relation with the use of egg yolk. Even some applications of granules at a pilot scale have lately been developed (Laca et al., 2010a; Orcajo et al., 2013; García et al., 2015; Marcet et al., 2015). However, plasma has not yet been used as ingredient in innovative food products.

In this work, egg yolk fractions have been assayed as key ingredient for the development of new egg gels, furthermore yolk has also been employed. These products have been evaluated by means of rheological, textural, color and micro-structure analysis. Finally, the developed formulations have been taken by a trained chef as starting point for the creation of interesting dishes.

## Materials and methods

### *Extraction of egg yolk and egg yolk fractions*

Egg yolks were prepared from fresh eggs. The shelling of the eggs and the separation of the yolk from the albumen were performed manually. The albumen residuals were eliminated from the yolk using a blotting paper, and the removal of the vitelline membrane was achieved using tweezers. The fractionation method was conducted according to Laca et al. (2010b), the egg yolk material is mixed with distilled water (1:1.5 v/v), then the pH of the diluted egg yolk is adjusted to 7 by the addition of NaOH (1 N), and it is held overnight at 4 °C before centrifuging at 4 °C and 10,000g for 45 min to separate into plasma (supernatant) and granule (pellet) fractions.

Egg yolk and egg yolk fractions (plasma and granules) were freeze-dried at −70 °C and 0.1 mBa in a Telstar Cryodos Lyophilizator. Samples were frozen at −80 °C previous to lyophilization.

### *Formulations and development of gels*

Basing on preliminary tests, the formulation to obtain gels of milk proteins reported by Pang et al. (2014) was modified as follows. The gels of egg derivatives contained a 4.5% (w/v) of

egg yolk, plasma or granules and 1% (w/v) of carrageenan in distilled water. Gels of 20 g were prepared by mixing the corresponding quantity of egg yolk or its fractions and carrageenan with water, then the mixture was blended with a Heidolph SilentCrusher Homogenizer during 10–20 s at 17,500 rpm. The homogenize dough was cooked at 105 °C during 20 min in a heater (Memmert), with two hand mixing at 10 and 20 min. Afterwards, the gel was cooled at 4 °C at least during 20 min. Gels of 60 g were developed to be evaluated with the texture analyser by means of penetration tests, in this case, dough was cooked during 40 min. Control gel, product without egg derivatives, was prepared following the same steps with the same conditions, but without adding egg yolk or yolk fractions.

### *Rheological measurements*

The rheological tests were carried out with a Haake MARS II rotational rheometer with a Haake UTC Peltier temperature control unit. A parallel-plate sensor system (PP60) with a gap of 1 mm was employed in all measurements. All tests were realized in dynamic conditions at a constant frequency of 1 Hz and a glass hood and silicon oil AR 20 (Sigma-Aldrich) were employed to avoid desiccation during the measurements. All the analyses were carried out at least in duplicate.

The un-cooked dough of gels was rheologically characterized by means of two different temperature sweeps, a “fast” temperature sweep and a “slow” temperature sweep. “Fast” temperature sweep were developed as follows, sample was heated from 20 to 100 °C (16 °C/min), then it was cooled to 4 °C (9.5 °C/min) and finally it was heated to room temperature (20 °C) (3 °C/min). Once the cooked sample was at 20 °C, stress sweep was performed from 0.01 Pa to 500 Pa. “Slow” temperature sweep were carried out in three steps, first the sample is heated to 90 °C and this temperature is maintained during 30 s, then the sample was cooled to 4 °C and finally, the sample was heated to room temperature (20 °C), all the temperature ramps were carried out at a rate of 3 °C/min. Temperature sweeps were performed in CD mode with a constant deformation of 0.1%.

### *Texture analysis*

Tests were carried out with a TA.XTPlus Texture Analyzer (Stable Micro Systems) with a load cell of 5000 g, two different analyses were developed.

Penetration tests with a penetration distance of 4 mm and a speed of 0.5 mm/min were performed employing a cylindrical probe (SMS P/0.5) to characterize gels of 60 g that were previously gelled in Bloom jars. In these assays, the maximum force recorded corresponds with gel strength.

Compression tests were carried out employing a cylindrical probe (SMS P/50) to characterize gels of 20 g with 16 mm of height and 33 mm of width. Two consecutive tests were developed on each sample, first a 20% compression test and afterwards a 50% compression test. First compression did not break the gel, providing an evaluation of product hardness,

Table 1  
Composition and nutritional value of developed gels per 100 g of product.

	Yolk gel	Granule gel	Plasma gel
Energy (kcal)	36.3	25.4	36.5
Carbohydrates (g)	0.7	0.7	0.7
Proteins (g)	1.4	2.5	1.0
Total fats (g)	3.1	1.4	3.3

whereas second test fractured the sample, providing an assessment of the structure fracturability. In both cases, the maximal peak force was calculated to obtain hardness and fracturability parameters; additionally, the area under the curve was calculated corresponding with the work needed to achieve the gel deformation.

Measurements were developed at least in duplicate.

### Microstructure analysis

Gels were analyzed employing scanning electron microscopy (SEM). Samples were fixed overnight in 3% glutaraldehyde in 25 mM phosphate buffer. Then, samples were dehydrated in a graded ethanol series and once 100% ethanol was achieved, they were moved in a graded acetone series, so samples were finally in 100% acetone. The samples were critical point dried through CO<sub>2</sub> in a Bal-Tec CPD 030 Critical Point Dryer. Dry fractions were fractured and torn with a blade; fragments were mounted on aluminum SEM stubs and coated with gold in a Sputtering Balzers SCD 004. The microscope used was the JEOL-6610LV SEM.

### Color measurement

The color of the different gels was measured in terms of CIE-Lab parameters: *L\** (whiteness or brightness), *a\** (redness or greenness) and *b\** (yellowness or blueness) (Wei et al., 2012). The different measurements were carried out using an UltraScan VIS spectrophotometer (HunterLab). It was standardized with a light trap and white title, and the green title was used to verify the instrument long-term performance. A fixed slice of 1 cm of width was cut from the gel sample employing a knife and a caliper. The slice was load into the measurement cell and analyses were conducted in specular exclusion mode, this mode includes the effects of gloss and texture, so the evaluation of color is similar to human-eye perception. Analyses were carried out at least in duplicate.

### Microorganism count

Total plate count of samples were determined according to the European Standard ISO 4833, 2003. The gels were prepared the same day of the analyses and were maintained at 4 °C.

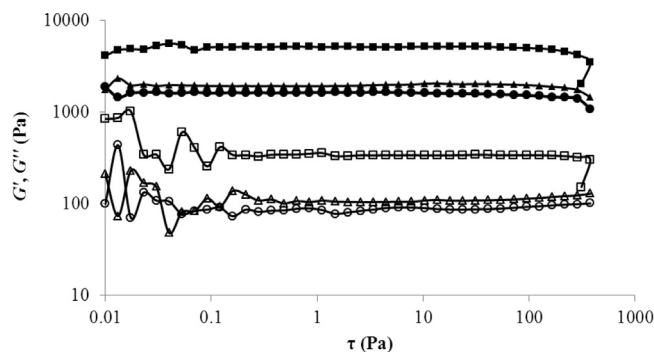


Fig. 1. Stress sweep results of yolk (●, ○), plasma (▲, △) and granule gels (■, □). Full symbols represent elastic modulus (*G'*) and empty symbols represent viscous modulus (*G''*).

## Results and discussion

### 3.1. Nutritional profile

Table 1 shows the composition and nutritional value of the developed base gels. Values were calculated according to data reported by Laca et al. the carrageenan technical datasheet. Total energy value for each formulation was obtained from energy equivalents for available carbohydrate, fat, and protein, 4 kcal/g, 9 kcal/g, and 4 kcal/g, respectively (FAO, 2003; Komatsu et al., 2013). The composition and the nutritional value differences between gels are due to the egg ingredient employed in each product. As it was already mentioned in the Introduction section, plasma contains the largest amount of egg yolk lipids, whereas granules are constituted mainly by proteins. As can be seen, the gels have a low caloric content, and, additionally, it is also important to point out that all gels are very low energy density foods (less than 0.6 kcal/g) (Drewnowski and Specter, 2004).

### Rheometry

Stress sweep tests were carried out in order to determine the lineal viscoelastic range of each sample. As it is shown in Fig. 1, the results show that this range is similar in all gels, approximately between 0.1 and 300 Pa. In all cases, the elastic modulus (*G'*) had a higher value than the viscous modulus (*G''*), reflecting that samples were gelled. Furthermore, values of both moduli were higher in the case of granule gel compared with the elastic modulus and viscous modulus values of yolk and plasma gels.

The results of the “slow” temperature sweeps can be seen in Fig. 2. During heating, storage modulus (*G'*) was nearly constant until a certain temperature was reached, at which it rapidly increased indicating transition from a liquid-like state (sol) to a solid-like state (gel). This temperature is usually taken as the gelation temperature and is one of the common methods to detect the gelling point in the absence of a crossover between *G'* and *G''* (Lamsal et al., 2007). According to that, in the case of plasma and yolk products, gelation occurred during the heating stage (approximately at 80 °C), whereas in granules product, gelation took place during the

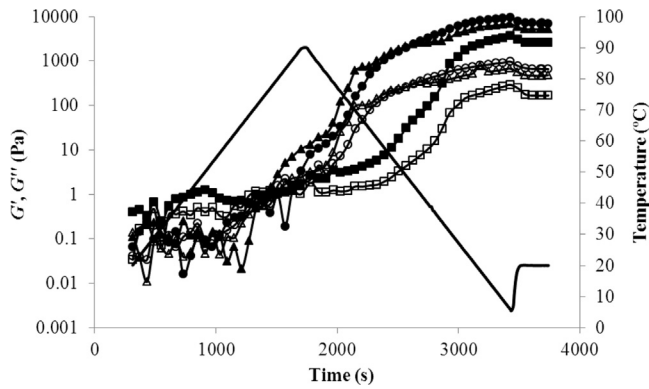


Fig. 2. “Slow” temperature sweep results of yolk (●, ○), plasma (▲, △) and granule gels (■, □). Full symbols represent elastic modulus ( $G'$ ) and empty symbols represent viscous modulus ( $G''$ ).

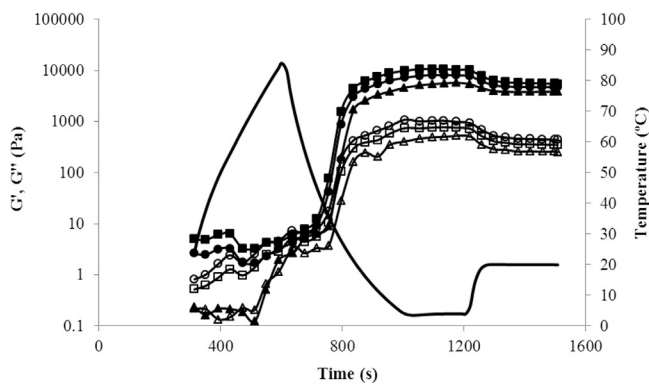


Fig. 3. “Fast” temperature sweep results of yolk (●, ○), plasma (▲, △) and granule gels (■, □). Full symbols represent elastic modulus ( $G'$ ) and empty symbols represent viscous modulus ( $G''$ ).

cooling phase (approximately at 50 °C). Granule composition is responsible of this different behavior between samples, since granule proteins form HDL-phosvitin complexes that have high resistance to temperature (Anton, 2013; Laca et al., 2014). Thus, as granules are resistant to heat denaturation, in case of granule gel, gelation process is determined by carrageenan behavior. On the contrary, and, as plasma proteins are easily denatured by heat (Anton, 2013), in plasma and yolk gels gelation happened during the heating stage, so the process is determined by egg yolk proteins behavior. As can be seen, gelation profile of plasma and yolk were similar, whereas in the case of granule gel, this product showed slightly lower values of the moduli. Plasma and yolk gels had a similar behavior owing to that gelation process in yolk is dominated by plasma proteins (Kiosseoglou and Paraskevopoulou, 2005).

Regarding the “fast” temperature sweeps, these tests simulate the cooking process, so these measurements are closer to the real procedure of gel elaboration. In Fig. 3 the “fast” sweep results are shown, gelation of all samples occurred during the cooling phase (approximately at 40 °C) and, it is important to remark that all gels showed a similar behavior and also similar values of elastic and viscous moduli. Hence, the “fast” heating

Table 2

Mechanical parameters obtained from texture analysis. Average values  $\pm$  SD are reported.

	Yolk gel	Granule gel	Plasma gel	Control gel (without egg derivatives)
<b>Penetration test</b>				
<b>Gel strength</b> (mg)	68 $\pm$ 1	47 $\pm$ 2	55 $\pm$ 1	33 $\pm$ 1
<b>20% Compression test</b>				
<b>Hardness (g)</b>	405 $\pm$ 17	344 $\pm$ 21	400 $\pm$ 30	245 $\pm$ 14
<b>Deformation</b> work (g/s)	324 $\pm$ 24	280 $\pm$ 17	328 $\pm$ 23	190 $\pm$ 9
<b>50% Compression test</b>				
<b>Hardness (g)</b>	1627 $\pm$ 111	1132 $\pm$ 70	1547 $\pm$ 166	757 $\pm$ 46
<b>Deformation</b> work (g/s)	2461 $\pm$ 67	1722 $\pm$ 126	2342 $\pm$ 202	952 $\pm$ 85

of the products seems to homogenize the rheological properties of the gels. This can be explain because, despite the differences between samples regarding its protein content and protein nature, in this assay the heating step was so quickly that there was not enough time to protein denaturation takes place. Hence, the gelation process of all samples occurred during the cooling step and it was determined by carrageenan behavior.

These results highlight the influence of the thermal treatment on the product behavior, being a determinant factor in the gelation process and also in the final characteristics of gels.

### Texture analysis

In Table 2, mechanical parameters obtained from texture analysis are exposed. The use of egg yolk and egg yolk fractions as ingredients in gels, when compared with the control gel (without egg derivatives), increased the consistence and deformation resistance of products. Control gel was softer and more fragile than gels with egg yolk, plasma or granules, needing less energy and strength to be deformed and broken. This increment of the product gel strength, hardness and deformation work can be explained by the interaction between egg derivatives and the carrageenan, since this interaction originates a structure with more consistency (Fig. 4).

Analyzing the values obtained from compression and penetration tests, it can be seen that, in general, yolk gel showed the highest values in all assays, although plasma gel values were similar to those of yolk gel. Granule gel was more breakable than plasma and yolk gels. As in rheological assays, plasma and yolk gels had similar results, whereas granule gel values were slightly different. This is accordance with results reported by Kiosseoglou and Paraskevopoulou (2005) who described a similar behavior of gel samples of yolk and plasma when compression tests were carried out. These authors also indicated that at low ionic strength, granules act as gel structure weakening points while when they are dispersed at a higher ionic strength reinforce the structure of the resulting gels. So, it appears that in granule gels granules are not totally



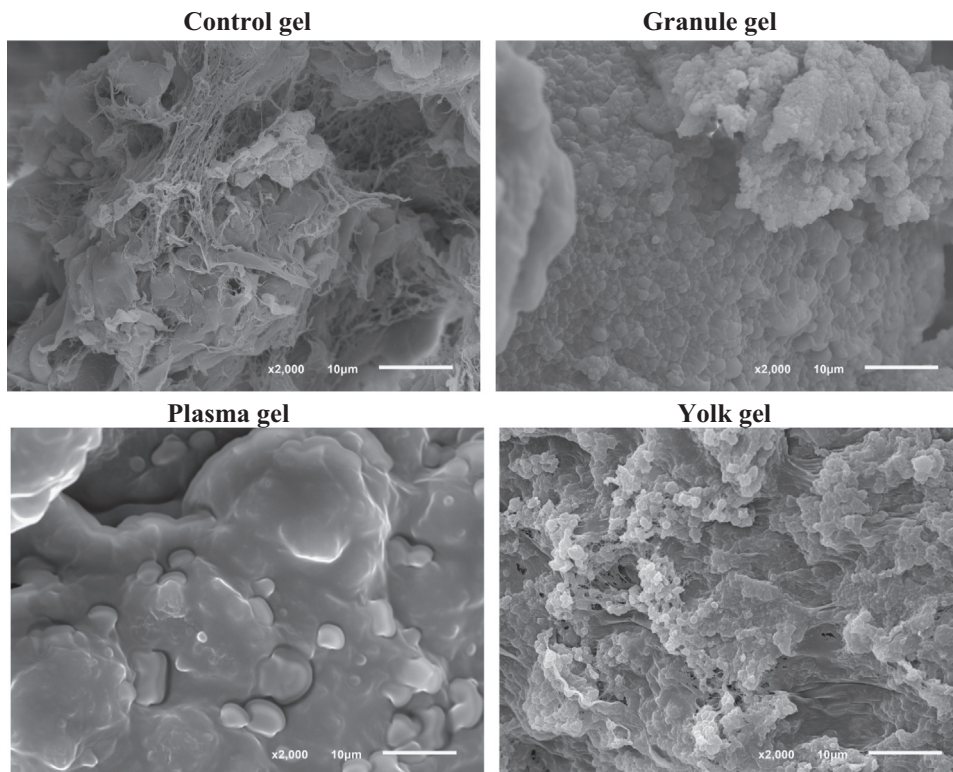


Fig. 4. Scanning electron micrographs (SEM) of the gels (2000 × magnification) (bar: 10 μm).

disrupted and, that is why this sample showed less resistance to deformation and fracture than plasma and yolk gels.

#### Microstructure

In Fig. 4 are shown the microphotographs of gels. In granule gel can be observed spherical structures within 0.3 and 2 μm of size that probably correspond with HDL-phosvitin complexes. Plasma gel showed a smooth surface with small aggregates that are possible originated by fat accumulation. Yolk gel morphology exhibited intermediate characteristics between plasma and granule gels.

It is remarkable that control gel presented a fibrous structure that corresponds with the carrageenan scaffolding. This scaffolding interacts with egg derivatives originating a new structure. This can be explained the higher resistance of yolk, plasma and granule gels compared with control sample found in texture analysis results.

#### Total mesophilic count

In all samples, the total mesophilic count was below  $10^3$  UFC/g, limit set by the commission decision 1999/724/CE for edible gelatins, a foodstuff similar to the egg gels developed in this work.

#### Color

According to Table 3 results, plasma gel showed the highest values of  $a^*$  and  $b^*$  parameters, whereas granule gel exhibited

Table 3

Values of  $L^*$ ,  $a^*$  and  $b^*$  color parameters for gels. Average values  $\pm$  SD are reported.

	$L^*$	$a^*$	$b^*$
Yolk gel	$77.0 \pm 1.4$	$3.0 \pm 0.1$	$21.6 \pm 0.4$
Granule gel	$77.1 \pm 2.1$	$1.3 \pm 0.3$	$17.0 \pm 0.1$
Plasma gel	$75.2 \pm 0.5$	$4.2 \pm 0.1$	$24.2 \pm 1.4$
Control gel (without egg derivatives)	$22.7 \pm 1.3$	$0.5 \pm 0.1$	$0.8 \pm 0.5$

the lowest values of these parameters. Yolk gel presented intermediate values. All samples showed similar values of parameter  $L^*$ . These higher values of redness and yellowness in plasma gel are due to the fact that plasma, the fraction with the highest fat amount, also concentrates the majority of egg yolk liposoluble pigments (mainly carotenoids) (Laca et al., 2010b).

As it was expected, control gel presented the lowest values of all parameters in comparison with the other samples. Hence, the use of egg derivatives as ingredients supplies pigments to the final products without being necessary to add colorant to the recipe.

#### Applications

The base gels developed in this work, and also the control sample, are shown in Fig. 5. As can be seen, differences in color can be clearly detected. Control gel presented a

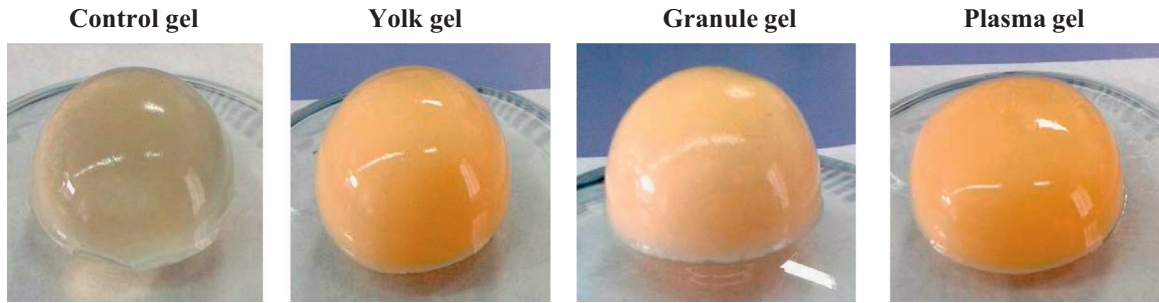


Fig. 5. Base gels.

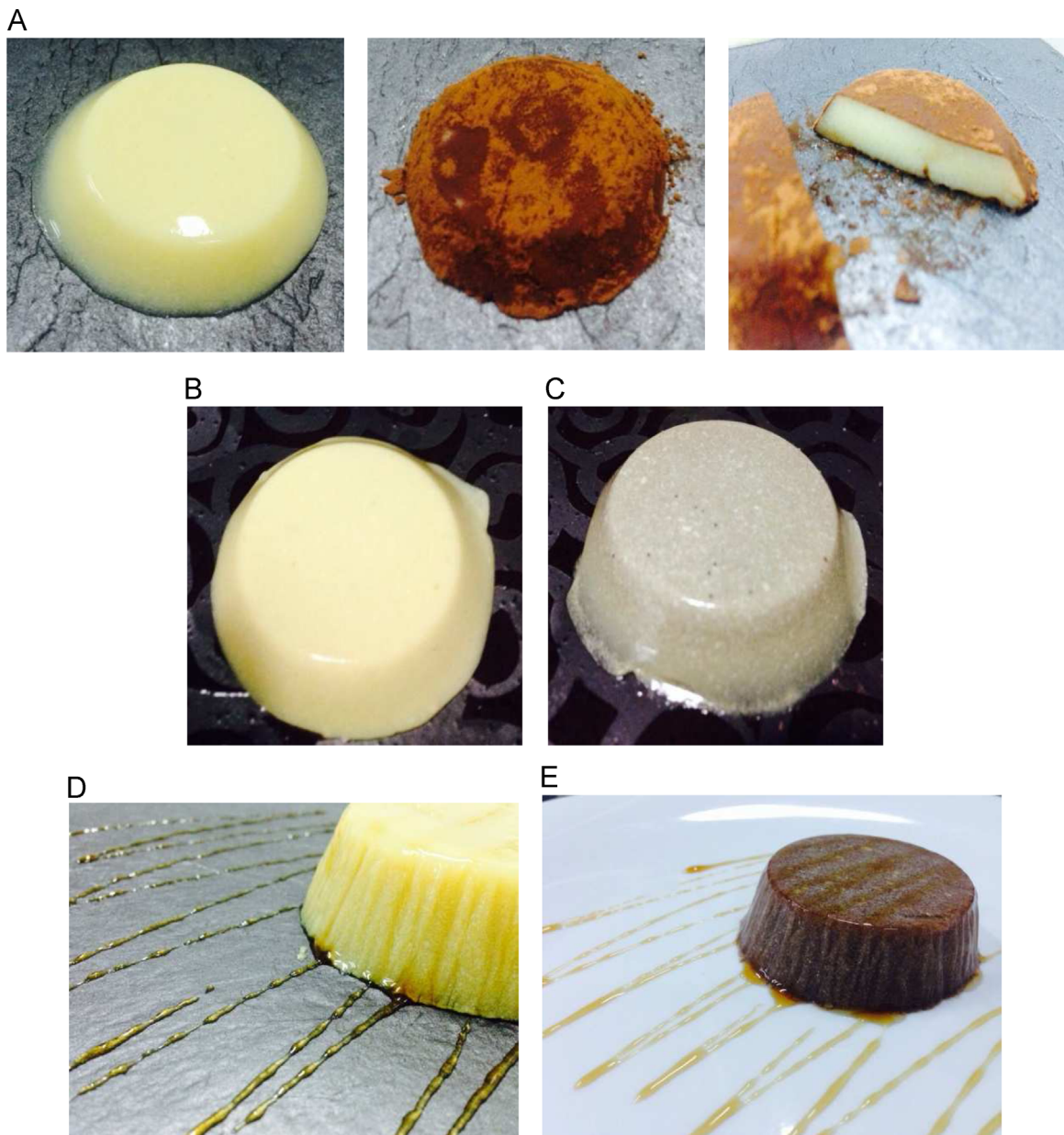


Fig. 6. Final products obtained from plasma base gels. A: gel flavored with vanilla sugar and orange blossom, and covered with defatted cocoa. B: gel flavored with stevia extract and natural vanilla steeped in water. C: gel flavored with stevia extract and cinnamon. D: gel flavored with stevia extract and orange blossom. E: gel with vainilla sugar and defatted cocoa.



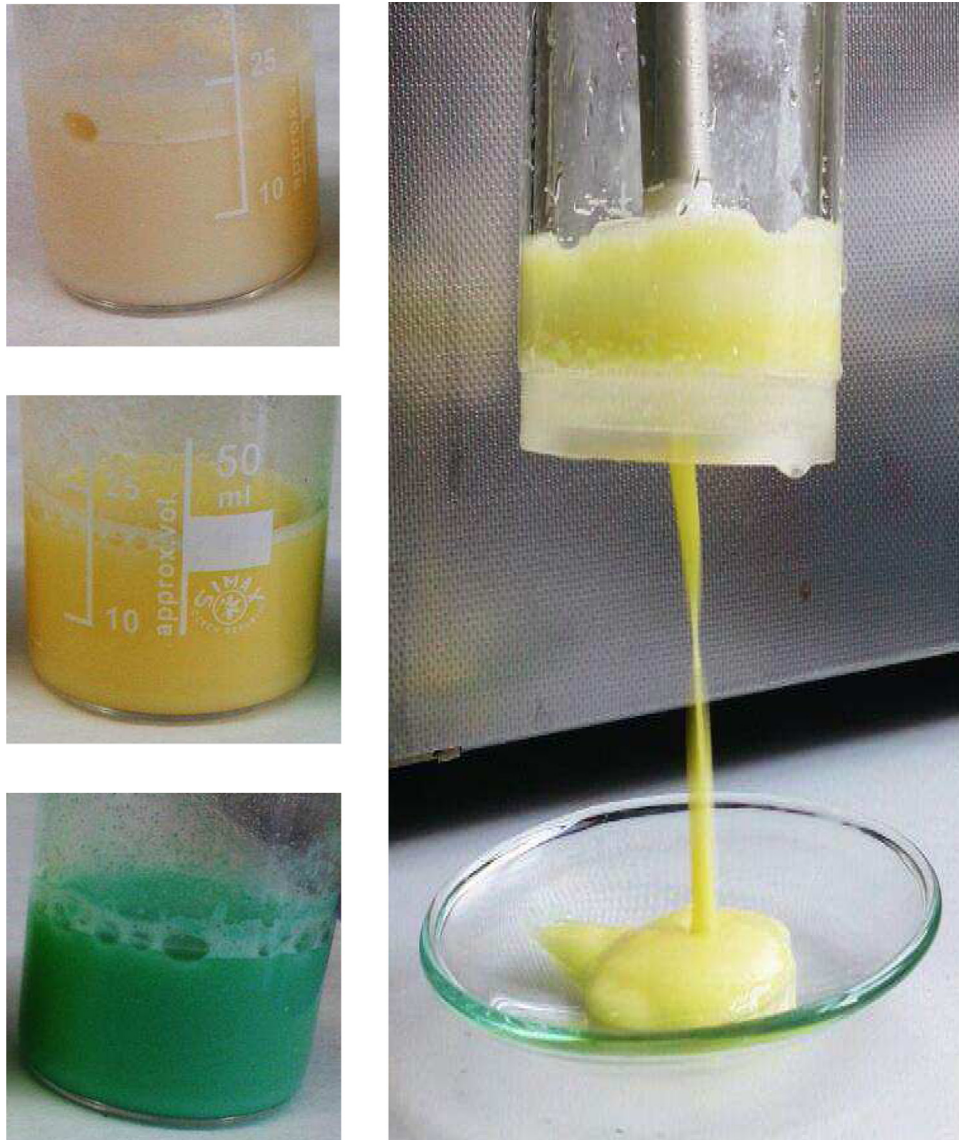


Fig. 7. Energy gel developed employing granule gel as base product: gels modified with edible colorants (left) and simulation of gel dosing (right).

translucent appearance, whereas yolk and plasma gels showed an intense yellow and granule gel exhibited a sandy hue.

These products can be slightly modified with the aim to be employed in different applications. In fact, the gels can be used as base for the development of low-caloric desserts. Some examples can be seen in Fig. 6 employing plasma gel as base product. Different flavors and spices, and also cocoa were employed, these ingredients improved notably the taste and, in addition, the appearance of the products.

Additionally, the base gels were employed as base to develop energy gels, specifically glucose gels. These energy gels are usually employed to practice sports, like cycling, that require a prolonged and intense effort. The formulation was slightly modified for this application, a 10% of glucose was added to granule gel (the egg yolk fraction with lower fat content and higher protein content); this product is shown in Fig. 7. Furthermore, other additives, such as edible colorants, can also be included in the energy gel formulation, some

examples employing different coloring (red, yellow and green) are also shown in Fig. 7.

## Conclusions

The combination of egg yolk and egg yolk fractions with carrageenan allows the development of base egg gels. The use of egg derivatives improves not only the mechanical properties, but also the organoleptic characteristics of the developed products in comparison with control gel (elaborated only with carrageenan). Furthermore, egg yolk and egg yolk fractions supply the developed gels with interesting compounds from a nutritional point of view. In this point is noticeable that depending on the egg derivative employed (yolk, plasma or granules) the final product shows different nutritional profile, mechanical properties and organoleptic characteristics. Basing on these differences, diverse applications could be developed;

in this work, some of these applications have been assayed as examples.

Finally, it should be pointed out that granules and plasma have proved to be promising ingredients with different potential uses in the culinary industry. Indeed, further research in this field could lead to a broadening of their commercial applications in the gastronomy area.

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