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Egg yolk granules and phosvitin. Recent advances in food technology and applications

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A B S T R A C T

Egg yolk is consumed all around the world because of its nutritional and functional properties. It can easily be separated by centrifugation into two fractions, the egg yolk granules and plasma fractions. In comparison with the plasma fraction, the granular fraction has a low content of lipids and cholesterol and a high content of proteins; on the other hand, the lipid-rich plasma fraction has gelling and emulsifying properties similar to those of whole egg yolk. Therefore, taking into consideration their different composition and functional properties, it would be advantageous to increase the value of whole egg yolk by making different use of each of its fractions. In this sense, while the egg yolk plasma could, to some extent, be used as a substitute for whole egg yolk, the range of applications for the granules is more reduced at present and further research into this fraction is required to increase the interest of the food industry in the egg yolk fractionation process. The egg yolk granular fraction is mainly composed of globular proteins, namely lipovitellins or high-density lipoproteins, linked to phosvitin by phosphocalcium bridges. Phosvitin is the most phosphorylated protein found in nature, and shows remarkable metal chelating, antioxidant, emulsifying and antimicrobial properties and, owing to these capacities, it has also the potential to be isolated and incorporated by the food industry as a functional ingredient. Thus, the aim of the present review is to show the most recent advances in the food-related applications developed by the research community for egg yolk granules and their isolated components.

1. Introduction

Egg yolk can be divided into two different fractions that can be obtained by dilution of the egg yolk in water, followed by centrifugation. The supernatant is the plasma fraction, which contains 75–81% of the egg yolk dry matter and is mainly composed of low-density lipoproteins (LDL), while the sediment, making up 19–25% of the egg yolk dry matter, is the granular fraction, mainly comprised of high-density lipoproteins (HDL) and phosvitin (Sirvente et al., 2007) (Fig. 1). Unlike LDLs, HDLs have a more proteinaceous structure, being a dimeric protein with a relatively small cavity where lipids can be found. In addition, the structure of the granules has an additional level of complexity, since HDLs are linked to one another, and with phosvitin through phosphocalcic bridges, forming insoluble aggregates of proteins with a diameter between 0.3 and 2.0 μm. In fact, the egg yolk granules can only be effectively dissolved in solutions with a high ionic strength (Anton, 2013), having poor functional properties if they are not properly dissolved in the medium, which limits their applicability. In this sense, granules have been used as an emulsifier in the preparation of mayonnaise (Laca, Sáenz, Paredes, & Díaz, 2010), and their emulsification properties after hydrolysis have been tested (Orcajo, Marcet, Paredes, & Díaz, 2013). They were treated with phospholipase A1 in order to increase their functional properties (Jin, Huang, Ding, Ma, & Oh, 2013), used as a source of folate (Naderi, House, & Pouliot, 2016) or as a source to obtain phosvitin (Ko, Nam, Jo, Lee, & Ahn, 2011). However, an all-embracing study of the most recent research into the improvement of the functional properties of egg yolk granules and their possible exploitation by the food industry is lacking in the current literature.

Furthermore, as was mentioned above, the HDLs are linked together through phosvitin, which is the most phosphorylated protein found in nature and represents 16% of the total dry content of the egg yolk granules. Due to its chemical composition, phosvitin is capable of easily capturing Ca^{2+} and Fe^{2+} ions and it possesses good preservative, antioxidant and emulsifying properties (Lesnierowski & Stangierski, 2018).

In this context, the aim of this article is to provide a comprehensive overview of the research papers published over the last five years about the improvement and characterisation of the functional properties of egg

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yolk granules, as well as their possible practical utilization by the food industry. In addition, the most recent studies concerning phosvitin in the food sector will be highlighted.

2. Egg yolk granules applications

Recent papers on food-related applications for egg yolk granules explore their emulsifying properties, after being previously modified or not, their use as a source of folate, their use as a raw material to prepare edible films or their performance as an encapsulation agent. All these novel studies are summarized in Fig. 2.

2.1. Emulsifying and foaming agent

The emulsifying properties of surface-active compounds are based on their capacity to reach the oil/water interface and cover it properly, and in the case of proteins, those with an extended morphology and high enough flexibility have better emulsifying performance. In this regard, native egg yolk granules have several disadvantages, mainly because their main components are relatively large aggregates formed by globular proteins that are connected by phosvitin. Therefore, a previous solubilisation step is required to improve their emulsification properties, and as it can be observed, several strategies have been explored in recent research (Table 1).

Geng, Xie, Wang, and Wang (2021) investigated the depolymerisation of the egg yolk granules by high-intensity ultrasound. Granules were dispersed in PBS buffer at pH 7.4 and subjected to ultrasound treatment at several ultrasonic powers for 10 min. According to their results, at the most effective conditions tested, the granule size decreased from 289.4 nm to 181.4 nm, which suggests at least a partial depolymerisation of the granules. The emulsifying activity of these treated granules was slightly better than that of native granules. In a similar context, Shen et al. (2020) studied the dissociation of the granular particles by increasing the pH and/or introducing lecithin into the mixture. The addition of 1% lecithin at pH 7.0 led to the solubilisation of up to 72.53% of the granular protein, while the same amount of lecithin at pH 9.0 produced the solubilisation of 97.45%. As would be expected, if the granular protein was previously solubilised, the emulsifying properties of this fraction were greatly improved. Therefore, disrupted granules at pH 9.0 and with 0.25% lecithin produced stable emulsions, but adding more than 0.50% lecithin produced unstable emulsions due to surfactant-induced depletion flocculation. Li et al. (2021) took advantage of these lecithin-egg yolk granule emulsions to prepare spray-dried powders with algal oil. These researchers claim that the stabilised algal oil powder shows excellent physical properties and oxidative stability. Furthermore, the presence of phosvitin in the emulsion enhanced the antioxidant properties of the resulting powder. In another study, Li et al. (2020) hydrolysed granules enzymatically with subtilisin and tested their emulsifying properties, comparing them to the same properties of untreated granules. The emulsions prepared using hydrolysed egg yolk granules showed much higher stability and less creaming than the emulsions prepared using untreated granules in a dose-dependent manner. Thus, the best stability and the lowest creaming values were obtained at the highest concentration of hydrolysed granules tested (0.1%, w/v). Furthermore, according to these authors, the treated granules behaved as a Pickering-type emulsifier, owing to the structural changes induced by the enzyme in the granular proteins.

In addition, several authors have recently studied the surface-active properties of the native egg yolk granules in order to better understand their emulsifying properties, which could provide valuable information with respect to their possible industrial exploitation.

Gmach, Bertsch, Bilke-Krause, and Kulozik (2019) studied the properties of egg yolk granules as an emulsifying agent for preparing oil-in-water emulsions (80/20) with rapeseed oil, peanut oil and corn oil at different pHs. Depending on the pH assessed, authors found a difference of between 10% and 30% in the median drop size. In addition, the smallest emulsion droplets were found when vegetable oils with higher surface activity were used. In another article, Wang, Xiao, Wang, Li, & Wang (2020) studied the possibility of using egg yolk granules as a Pickering-type emulsifying agent at several different pHs. Although the stability of the emulsions was poor at pH > 4.0, it was excellent at pH = 4.0. Moreover, when the egg yolk granule size was smaller than 500 nm they behaved as a Pickering-type emulsifier. Li, Li, et al. (2019) also tested the influence of the egg yolk fractions on egg white based foams. Adding egg yolk granules was found to be less detrimental to the foam capacities of the egg white than adding the whole egg yolk and plasma, in part owing to the higher concentration of protein present in the granular fraction. These proteins enhance the formation of a protein film at the air-water interface, increasing the foam stability. It has also to be taken into account that the granular fraction has a lower lipid content than the plasma or the whole egg yolk, it being possible that the excess of lipid affects the surface proteins at the interface, and hence, the foam stability. Motta-Romero, Zhang, Tien, Schlegel and Zhang (2017)separated the egg yolk granules using two egg yolk:water ratios and as a third alternative 0.17 M NaCl, testing the emulsifying capacities of these granules by preparing a mayonnaise. According to these researchers, the highest granule yield was obtained by diluting the egg yolk with water at a ratio of 1:2, but the best emulsifying properties were obtained with granules separated using a 1:1 proportion of 0.17 M NaCl. All in all, egg yolk granules showed a similar emulsifying capacity to the whole egg

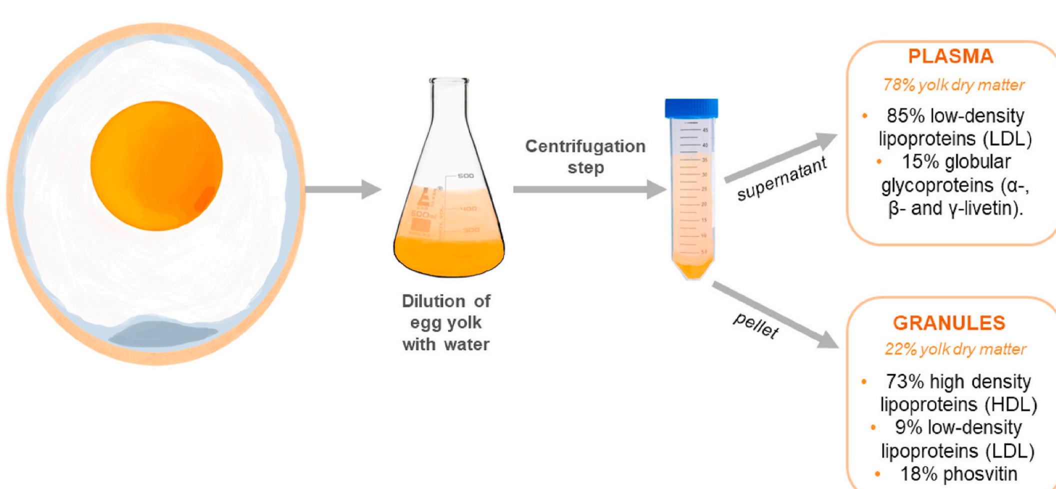

Fig. 1. Egg yolk plasma and granules: formation and composition. Plasma composition from Anton (2007). Granules composition from Powrie and Nakai (1986).

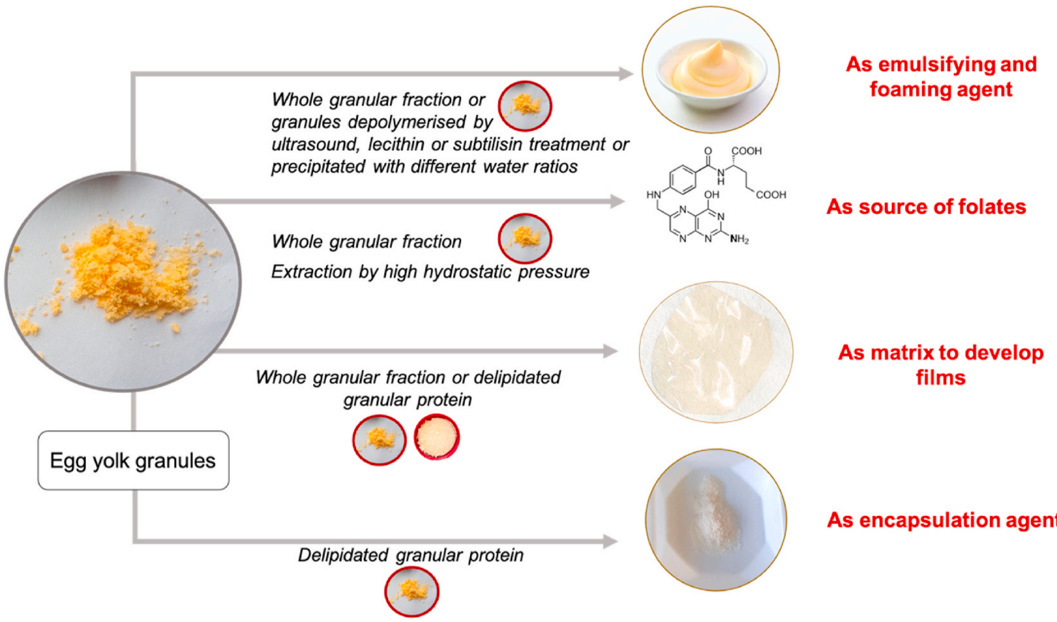

Fig. 2. Diagram showing egg yolk granules-related applications found in the literature.

Table 1
Summary of recent studies into the use of egg yolk granules as an emulsifying agent.

Emulsifier	pH tested	Emulsion/Foam	Particle size of emulsions	Highlighted results	Reference
Granules depolymerised by ultrasound	7.4	30 mL of a solution of treated egg yolk granules (20 mg/mL dry matter content) and 10 mL of soybean oil	NA	The ultrasound treatment produced the partial depolymerisation of the egg yolk granules, which improved their emulsifying properties.	Geng et al. (2021)
Granules disrupted by lecithin	7.0 and 9.0	Several protein concentrations up to 1% (w/v). Preparation of oil/water emulsions with 10% of Arowana sunflower oil.	For a lecithin concentration of 0.25% (w/v), around 400 nm at pH 7.0 and around 200 nm at pH 9.0	An increase in the concentration of lecithin produced an increase in granule solubilisation. The dissociation of the granules' aggregate structure improved their emulsifying properties.	Shen et al. (2020)
Granules disrupted by lecithin	7.0 and 9.0	10% oil-in-water emulsion with both egg yolk granules and lecithin dispersed in the water phase. The oil phase was docosahexaenoic acid algal oil.	NA	After spray drying of the emulsion, the resulting powder showed good physical properties and oxidative stability.	Li et al. (2021)
Egg yolk granules treated with subtilisin	pH 7.0	Oil-in-water emulsion prepared with 9 mL soybean oil and 21 mL of granule solutions with four different protein concentrations.	12.6 µm	Enzymatic hydrolysis produced changes in the granule structure. The treated egg yolk granules behaved as a Pickering emulsifier.	Li et al. (2020)
Native granules	3.0, 4.0, 6.5, 8.0 and 10.0	Oil-in-water emulsion (80/20) with 2% protein content and sunflower oil, rapeseed oil, peanut oil and corn oil.	From 40 µm at pH 3.0 to around 15 µm at pH 10.0.	The pH of the granule solution and the surface activity of the vegetable oils were important factors in decreasing the droplet size of the emulsions.	Gmach et al. (2019)
Native granules	pH 2.0, 3.0, 3.7, 4.0, 4.5, 5.5, 6.5, 7.0, and 9.0	20 mL of 1% egg yolk granules dispersion and 20 mL of soybean oil.	NA	The emulsions prepared showed a high stability at pH 4.0.	Wang et al. (2020)
Native granules	NA	Foams prepared with several concentrations of egg yolk granules and egg white	NA	The characteristics of the foams were influenced by the proportions of plasma and granules. Granules together with egg white showed better foaming properties than whole egg yolk with egg white	Li, Li, et al. (2019)
Native granules precipitated at several yolk:water ratios.	Acid pH	Mayonnaise recipe	NA	A higher granules yield was obtained at the yolk:water ratio of 1:2. Egg yolk granules possessed similar emulsifying properties to whole egg yolk.	Motta-Romero et al. (2017)

NA: Not available.

yolk, owing to an increase in the viscosity of the mayonnaise and the better emulsion stabilizing properties of the granules in comparison with the whole egg yolk.

It is important to note that although the emulsification properties of egg yolk granules are improved when they are at least partially solubilised, the emulsifying properties of the native granules have been described as similar to those of whole egg yolk when they are used to prepare mayonnaise. For this application in particular, the good performance of the granules may be due to the low pH of this emulsion, usually around pH 4.0 (McWhorter, Khan, Sexton, Moyle, & Chousalkar, 2021), which facilitates the role of the egg yolk granules as a Pickering-type emulsifier (Wang et al., 2020). In this respect, the granule emulsification capacity is not only limited by the pH of the emulsion, but also by the oil droplet size, since the oil droplet must be big enough to be effectively surrounded by the native egg yolk granule microparticles. Therefore, it is possible that these desirable emulsification properties of untreated egg yolk granules when tested as an emulsifier for mayonnaise cannot be extrapolated to other food emulsions at neutral pH and with a smaller oil droplet size.

2.2. *Egg yolk granules as a source of folate*

Recently, studies have been performed into novel ways to extract compounds from egg yolk granules in order to obtain folate. Folate is a vitamin found in egg yolk and low serum levels are linked to gestational problems in women of child-bearing age and to cardiovascular diseases (House, Braun, Ballance, O'connor, & Guenter, 2002). In this context, Naderi, Doyen, House and Poliot (2017) extracted 5-methyltetrahydrofolate (5-MTHF), a highly bioavailable folate form, from egg yolk granules by high hydrostatic pressure. In this case, the best extraction conditions were 400 MPa pressure and 5 min of treatment. In such conditions, 1264.6 ± 61.4 μg of 5-MTHF per 100 g granule were extracted. If the same treatment was performed on whole egg yolk, the amount of 5-MTHF obtained decreased to 230 ± 2.7 μg/100 g. In a further study, the same authors treated egg yolk granules with high hydrostatic pressure at 600 MPa for 5 min, which caused the disintegration of the granules and the release of folate and phosvitin into the plasma fraction (Naderi, Pouliot, House, & Doyen, 2017).

2.3. *Preparation of films*

Proteins are excellent biopolymers for preparing edible food packaging. They have the advantage of being easily improved by adding antioxidant or antimicrobial compounds and they also possess excellent mechanical properties (Mihalca et al., 2021). Therefore, bearing in mind that egg yolk granules have a large proportion of proteins in comparison with the whole egg yolk or the plasma fraction, these proteins could be revalorised by the preparation of edible films and coatings. These proteins are usually prepared by the casting method (Pirsa & Aghbolagh Sharifi, 2020), so the granular proteins have to be solubilised, mixed in the aqueous medium with a plasticiser and finally cast on an even surface in order to evaporate the water. However, egg yolk granular proteins are only significantly solubilised in solutions with a pH higher than 9.0 or with a NaCl concentration higher than 0.3 M (Anton & Gandemer, 1997), and the solubilisation of egg yolk granules in such a high concentration of NaCl is incompatible with the preparation of protein-based films.

In the light of this obstacle, Marcet, Álvarez, Paredes, Rendueles, and Díaz (2018) prepared edible films with egg yolk granules treated with ultrasounds. In this respect, the granules were solubilised at alkaline pH and enhanced by ultrasound treatment. Furthermore, the main variables responsible for their solubilisation were optimized and modelled using a Box-Behnken design. The resulting films were transparent, slightly yellowish in colour and had mechanical properties suitable for wrapping pieces of food. In a further study, Marcet, Sáez, Rendueles, and Díaz (2017) prepared edible films using egg yolk granules previously delipidated with ethanol. Delipidated granules were solubilised at alkaline pH at 65 °C and using ultrasound. Afterwards, the pH of the solution was adjusted to pH 8.6 and the proteins were crosslinked with transglutaminase. When these films were compared to others prepared using caseinate or gelatine, they showed better barrier properties and improved water resistance, but poorer mechanical properties. In addition, nisin and thymol were added to give the films antimicrobial properties. Subsequently, Sáez-Orviz, Marcet, Rendueles, and Díaz (2021)prepared edible films using delipidated egg yolk granules as the film matrix with added *Lactobacillus plantarum* CECT 9567 and lactobionic acid. In this way, an egg yolk protein-based synbiotic food packaging film was developed. The film-forming solution for these films, which contained a probiotic and a prebiotic, was used to coat pieces of gelatine, employed as a food model. The concentration of *L. plantarum* inside these films remained constant for 15 days and its population was slightly increased when lactobionic acid was present during the storage period. In addition, the lactobionic acid enhanced the survival of the bacteria when the coatings were subjected to gastrointestinal digestion conditions. Finally, Fuertes et al. (2017)prepared edible films using whole egg yolk, the egg yolk subfractions and the resultant proteins obtained after the extraction of lipids from the subfractions and the whole egg yolk. However, in this case neither the granular proteins in the whole egg yolk nor those in the granular fraction were previously solubilised, so it was necessary to introduce gelatine to the mixture to prepare the films. In addition, the excess of lipids in the plasma fraction required the addition of gelatine to the film- forming solution as well. The gelatine-granules films were mechanically the strongest films, but when the granules were delipidated, the gelatine-delipidated granules films obtained were found to be among the weakest tested.

The main characteristics of all these films are summarized in Table 2. According to this table, the presence of lipids in the granular fraction produced the weakest but most flexible and water-resistant films tested, which could be exploited in future preparations. In addition, the puncture strength and puncture deformation properties of the films prepared by adding gelatine were provided in grams and mm, so they cannot be compared with the other films shown in this table. Furthermore, most films prepared using egg yolk granules were produced by solubilising the granular egg yolk fraction at alkaline pH, producing protein-based films with relatively high water-resistance at neutral or acidic pH, which are the pHs most commonly found in food. However, this alkaline pH may affect the appearance of more delicate foodstuffs, so the long-term visual impact of these films on a large range of foods remains to be studied.

Finally, as mentioned in section 2.1, there are in the recent scientific literature several alternative ways to enhance, at least partially, the disaggregation of the granules without resorting to their solubilisation at high pH. In this line, the preparation of films using granules previously treated with ultrasound at neutral pH (Geng et al., 2021), subtilisin (Li et al., 2020), or partially disassociated by introducing lecithin (Shen et al., 2020) may generate valuable egg yolk protein-based materials at non-alkaline pHs.

2.4. *Encapsulation agent*

Zhou, Hu, Wang, Xue, and Luo (2018) prepared novel nanoscale delivery systems for the development of functional foods using HDL nanoparticles loaded with curcumin. Curcumin was specifically selected as a model lipophilic bioactive compound. The nanoparticles were alternatively coated with either chitosan or stearic acid conjugated chitosan (SACS) and had a diameter of 75 nm and 97 nm, respectively. According to the authors, SACS-HDL showed better encapsulating properties and a slower release of the curcumin in a simulated gastrointestinal tract.

Sassi, Marcet, Rendueles, Díaz, and Fattouch (2020) prepared microparticles using granules from egg yolk and several concentrations of gum Arabic for encapsulating polyphenols from date pits. When the concentration of the granular protein was increased in relation to the amount of gum Arabic, an improvement in the antioxidant effect and thermal stability of the microparticles was measured. These microparticles showed higher resistance to gastrointestinal fluids owing to the presence of the granular proteins in their wall material composition.

Table 2
Main characteristics of films prepared using egg yolk granules.

Main biopolymer	Protein solubilisation	Major modifications tested	Puncture strength[a] (N/mm)	Puncture deformation[a] (%)	Water solubility[a] (%)	Reference
Whole granular fraction	Alkaline pH and ultrasounds	NA	31.6 ± 2.5	113 ± 7.0	14 ± 0.5	Marcet et al. (2017)
Delipidated granular protein	Alkaline pH and ultrasounds	Protein crosslinking by transglutaminase; addition of natamycin and thymol	122.0 ± 9.1	75.7 ± 5.6	24.5 ± 2.0	Marcet et al. (2018)
Delipidated granular protein	Alkaline pH	Introduction in the film matrix of a probiotic (*Lactobacillus plantarum*) and a prebiotic (lactobionic acid).	50.7 ± 4.9[b]	39.0 ± 0.1[b]	38.5 ± 2.6[b]	Sáez-Orviz et al. (2021)
Delipidated granular protein and whole granular fraction	NA	Addition of gelatine	Breaking force (g): 412 ± 91	Deformation (mm): 5.4 ± 0.7	NA	Fuertes et al. (2017)

NA: Not available.
[a] Results for the best preparations.
[b] Results for synbiotic films.

3. Phosvitin

Phosvitin has received considerable attention in the recent literature (Table 3). Three articles have been published describing novel procedures to extract and isolate phosvitin from egg yolk granules. There is also research centred on the improvement of its functional properties by structural modification or hydrolysis. In addition, studies have also been performed to increase phosvitin's antimicrobial capacity and assess its nutritional properties.

3.1. Novel procedures to extract phosvitin from egg yolk granules

The usual phosvitin extraction procedures involve the use of sodium chloride to dissolve the association of the granules and a further purification step using organic solvents and chromatography. This procedure and the new ones found in the literature are depicted in Fig. 3. In a recent paper, Giarratano et al. (2020) extracted phosvitin from egg yolk granules by high hydrostatic pressure at 400 MPa for 5 min. After applying such pressure, the supernatant was concentrated by ultrafiltration with a 10 kDa membrane, obtaining a concentrate with a phosvitin content of 26.00 ± 4.12% w/w. The emulsion prepared with this

Table 3
Summary of recent studies about phosvitin related to food applications.

Treatment	Main objective	Best preparation	Highlighted results	Reference
Ball-mill treatment	Improvement in the emulsifying properties	20 min of treatment at 25 °C	Dispersions of treated phosvitin showed higher viscosity. The emulsifying activity index of the phosvitin was increased 3.0-fold.	Zhang et al. (2019)
Cycles of freeze-thaw	Improvement in the emulsifying properties	3 cycles of freeze-thaw	The emulsifying ability of the phosvitin was increased from 1.87 m^2/g to 3.70 m^2/g.	Li et al. (2018)
Conjugated with gallic acid	Improvement in the emulsifying properties	10 mg/mL of phosvitin and 1.5 mg/mL of gallic acid at pH 9.0	A slight improvement in the emulsifying properties of the emulsion tested. The conjugate showed antioxidant properties and an increase in the viscosity of the prepared emulsions.	Jiang et al. (2020)
Conjugated with pectin	Improvement in the emulsifying properties	Phosvitin and pectin at a mass ratio of 1:8 and at a protein concentration of 10 mg/mL	Emulsifying activity index increased from 19.81 m^2/g to 25.72 m^2/g and the stability index increased from 27.5% to 76.3%.	Cui et al. (2019)
Conjugated with gluten	Improvement in the emulsifying properties	0.25 g of gluten, 0.25 g of phosvitin and 30 U/g of transglutaminase in 50 mL of Na_2SO_3 at 600 mg/mL	The emulsifying activity increased from 17.42 to 20.63 m2/g.	Yang et al. (2021)
Partial dephosphorylation and hydrolysis with trypsin	Identification of the peptide that binds most strongly to calcium	Fraction P3 after anion exchange, P3-1 after SEC, P3-1-1 after RP-HPLC	The sequence DEEENDQVK was detected as the best calcium-chelating peptide with a binding capacity of 151.10 ± 3.57 mg/g	Zhang et al. (2021)
Subjected to high hydrostatic pressure and to enzymatic hydrolysis	Assessment of the radical scavenging and anti-inflammatory properties of the peptides	Peptides obtained at 100 MPa and using alcalase or trypsin	Peptides obtained at 100 MPa showed greater antioxidant properties. Peptides obtained with alcalase and trypsin showed higher iron chelation capacity and anti-inflammatory properties.	Yoo et al. (2017)
Unmodified phosvitin	Phosvitin effect on gut health	Phosvitin was administered dissolved in water at 5 mg/mL for 14 days to several groups of mice	The *Bifidobacterium* population increased in the young group and the number of pathogenic bacteria decreased in the adult group	Li, Zhang, et al. (2019)
Hydrolysed with alcalase at high pressure and combined with IgY	Antimicrobial properties against *E. coli* K88 and K99 strains	IGY 100 μg/mL combined with 1 mg/mL of phosvitin peptides hydrolysed with alcalase at high hydrostatic pressure	*E. coli* growth inhibition of 2.8 and 2.67 log CFU/mL for the strains K88 and K99 respectively	Gujral et al. (2017)
Granules subjected to high hydrostatic pressure and ultrafiltration membrane	Phosvitin extraction	High hydrostatic pressure of 400 MPa for 22 °C and 5 min, centrifugation and ultrafiltration of the supernatant with a 10 kDa membrane	A phosvitin content in the retentate of 26.00 ± 4.12% w/w	Giarratano et al. (2020)
Granules subjected to high hydrostatic pressure	Phosvitin extraction	High hydrostatic pressure of 600 MPa for 5 min	The transmission from the granules to the plasma of an amount of phosvitin of 33.3 ± 4.39 mg/100 g of dry plasma	Duffuler et al. (2020)
Granules subjected to heat treatment and ultrasounds	Phosvitin extraction	Egg yolk granules diluted with $(NH_4)_2SO_4$, heated at 80 °C for 15 min and then treated for 10 min with ultrasound	An extract with a phosvitin purity of 80.0 ± 2.0, with an N/P ratio of 3.1 and a phosvitin activity of 98.0 ± 1.0	Jiang et al. (2019)

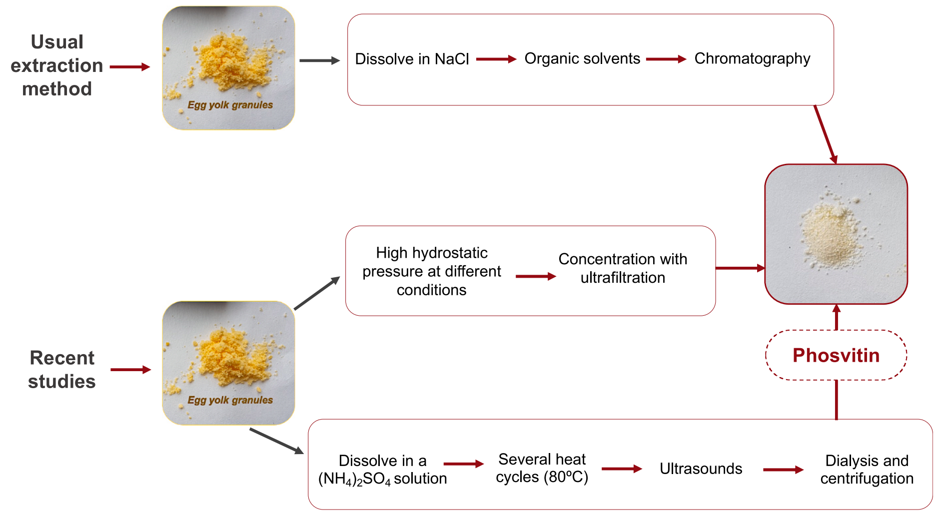

Fig. 3. Usual extraction method and recently reported procedures for extracting phosvitin from egg yolk granules.

concentrate showed a creaming index of 6.25% compared to a value of 93.75% for this parameter for the control emulsion. Duffuler et al. (2020), using high hydrostatic pressure, extracted phosvitin from egg yolk granules, the phosvitin accumulating in the plasma fraction. The best conditions tested were 600 MPa for 10 min of treatment, resulting in a concentration of phosvitin in the plasma fraction of 33.3 ± 4.39 mg/100 g of plasma. Jiang et al. (2019) isolated phosvitin from egg yolk granules by dissolving them in $(NH_4)_2SO_4$ solution, applying heat at 80 °C for several and different reaction times and treating the solution with ultrasound at 600 W for 10 min. After dialysis and centrifugation, the phosvitin was isolated in the supernatant. Under the best set of conditions assessed, the purity of the phosvitin was 80% and no significant differences between the properties of the extracted phosvitin and those of the phosvitin standard were detected.

Although all these papers focused on the phosvitin and its functional properties, not many practical applications have been found in the literature for the leftover high-density lipoproteins without the phosvitin fraction. Investigating this issue, Chalamaiah, Esparza, Hong, Temelli, & Wu (2018) studied the physicochemical and functional properties of the processed egg yolk granules and found that they showed better foam capacity, foam stability, emulsifying activity and stability indexes than the whole egg yolk granules. Bearing in mind that the different phosvitin extraction procedures may exert different effects on the structure and functionality of the remaining granular proteins, these proteins may show a wide variety of functional properties. In addition, they could be a valuable source of bioactive peptides.

3.2. Phosvitin modifications to improve its emulsifying properties

The emulsifying properties of phosvitin are based on the electrostatic repulsive force of phosphate and have been described as better than those of BSA at pH 7.0 (Chung & Ferrier, 1991) and then those of other food proteins (Sattar Khan, Babiker, Azakami, & Kato, 1998). In recent publications, these emulsifying properties have been improved by modifying the native folded conformation of the phosvitin by physical treatments or by conjugation with other compounds.

Zhang, Yang, Hu, Liu, and Duan (2019) subjected lyophilized phosvitin to a ball-milling treatment and assessed the rheological and emulsifying properties of the modified protein. After 20 min of treatment, the surface hydrophobicity of the phosvitin was doubled, and after 40 min of treatment this parameter reached its highest value, which enhanced its emulsifying properties. According to the authors, the milling treatment induces structural changes in the phosvitin, exposing its hydrophobic groups and improving its emulsifying activity index, thermal stability and increasing the viscosity of the dispersions in which it is included. In order to modify the functional properties of phosvitin by other physical treatments, Li et al. (2018) subjected this protein to several freeze-thaw cycles. The treated phosvitin showed structural changes, resulting in a decrease in the proportion of beta-sheets from 32.19% to 25.10% after 9 freeze-thaw cycles, but on this occasion the surface hydrophobicity of the phosvitin decreased, suggesting a rearrangement of the protein and a burial of the hydrophobic groups inside the protein structure. Another highlighted result was an improvement in the phosvitin emulsifying activity index value, which was noticeably increased after 3 cycles, although the emulsion stability was not significantly affected. It has to be considered that, unlike the paper of Zhang et al. (2019), this study linked a decrease in the surface hydrophobicity of the phosvitin with an improvement in its emulsifying properties, which suggests that although a variation in the phosvitin hydrophobicity could predict an improvement in this functional property, there are other factors that must be considered to predict the performance of this protein as an emulsifier.

Regarding the conjugation of phosvitin with other compounds, Cui, Li, Lu, Liu, and Duan (2019) prepared phosvitin-pectin copolymers by Maillard reaction with the objective of modifying the phosvitin structure and improving its emulsifying properties. This conjugation produced an increase in the surface hydrophobicity (H_0) from 60.3 to 183.4 and hence an increase in the emulsifying properties of the conjugated phosvitin. Taking advantage of the structural modification of the phosvitin, the conjugated phosvitin was hydrolysed with trypsin and the resultant peptides showed improved calcium binding properties. In the case of Yang et al. (2021), they increased the solubility of wheat gluten with Na_2SO_3 and crosslinked this protein with phosvitin. The surface hydrophobicity of the crosslinked proteins was also increased, enhancing their emulsifying properties. In fact, for the best preparation tested, the emulsifying activity was noticeably increased. In addition, the thermal stability of these proteins was also improved. Finally, Jiang et al. (2020)conjugated phosvitin with gallic acid in order to prepare a new emulsifier with both emulsifying and antioxidant properties. The emulsifying properties of the complexes prepared with different concentrations of gallic acid were slightly improved in comparison with the phosvitin alone, but the ABTS and DPPH free radical scavenging of these aggregates were clearly higher than those found for the isolated phosvitin.

According to all these authors, it is proven that the emulsifying properties of phosvitin can be enhanced by the procedures described above; however, except for the study performed by Cui et al. (2019) with gallic acid, it is noticeable that the antioxidant capacity of the modified phosvitins to protect the emulsified lipids against oxidation was not investigated experimentally. Considering that these phosvitin-related antioxidant properties may remain unaffected, this preservative effect in the emulsions in which the modified phosvitin is involved could noticeably increase the value of the phosvitin and hence of the whole egg yolk granular fraction.

3.3. Production of novel phosphopeptides

Phosphoproteins are considered to be resistant to protease activity, and phosvitin, the most phosphorylated protein found in nature, has been reported as highly resistant to the action of digestive enzymes such as trypsin (Ishikawa, Tamaki, Arihara, & Itoh, 2007). Therefore, in order to increase its sensitivity to the action of proteases, a well-known strategy consists in partially dephosphorylating the phosvitin before subjecting it to enzymatic hydrolysis (Jiang & Mine, 2000). In this line, and with the objective of increasing calcium bioavailability, Zhang et al. (2021) partially dephosphorylated phosvitin and then performed a tryptic digestion, identifying and purifying a peptide with a high calcium binding capacity. The purification of this peptide was performed by anion-exchange chromatography, size exclusion chromatography and reversed-phase high-performance liquid chromatography. In another recent paper, Yoo, Bamdad, Gujral, Suh, and Sunwoo (2017) avoided the partial phosvitin dephosphorylation step and directly carried out the enzymatic hydrolysis of this phosphoprotein at various high hydrostatic pressure levels and using several enzymes. According to the results, the hydrolysates obtained at 100 MPa showed superior radical scavenging properties and the alcalase and trypsin hydrolysates showed the highest anti-inflammatory properties.

3.4. Nutritional and antimicrobial properties

The combination of both high metal-chelating properties and high resistance to hydrolysis by digestive enzymes leads to phosvitin being considered as a nutritional negative protein (Ishikawa et al., 2007). In this context, Li, Zhang, et al. (2019) studied the effect of phosvitin on the luminal microbiota composition of mice. According to these authors, phosvitin exhibited potentially beneficial effects on gut health by decreasing the amounts of pathogenic microbes in adult mice and increasing the proportion of beneficial bacteria in young mice. In addition, Gujral et al. (2017) studied the synergistic effect of IgY and phosvitin from egg yolk on the growth of enterotoxigenic *Escherichia coli* K88 and K99. IgY was obtained from egg yolks of hens previously immunized with K88 and K99. The combination of IgY and peptides from phosvitin previously hydrolysed with alcalase at high hydrostatic pressure showed the best bactericidal effect, decreasing *E. coli* growth by 2.8 and 2.67 log CFU/mL for the K88 and K99 strains, respectively. In fact, very few papers have been published in the last five years about the nutritional effect of phosvitin on gut health or about its antimicrobial properties, but in the light of the results obtained by these authors, it could be a worthwhile topic for future research.

4. Conclusion

In this review, the latest studies regarding food-related applications of egg yolk granules have been highlighted. The emulsifying properties of egg yolk granules can be enhanced by increasing their degree of solubilisation or decreasing their size, and for that purpose, enzymatic hydrolysis and high hydrostatic pressure, partial depolymerisation with ultrasound, and mixing with lecithin were assessed. It is important that these treatments are mild, and they are not mutually exclusive, so they may be combined in order to further depolymerise the egg yolk granules and increase their emulsifying capacity. Moreover, egg yolk granules solubilised in the ways proposed by these researchers could be exploited to prepare edible films, avoiding the use of basic pHs to cause disruption of the granules and increasing their range of applications.

Furthermore, the emulsifying activity of granules at different pHs or acting as a Pickering emulsifier were also tested. In this sense, the good performance of native egg yolk granules in mayonnaise deserves attention and may be explained, in the light of the other papers reviewed, by the granules acting as a Pickering emulsifying agent at the typical pH of mayonnaise (around 4.0). If this is so, these good emulsifying properties of native granules would not be extrapolated to other emulsions prepared at non-acidic pHs. This raises the question of whether mayonnaise is a good emulsion model for assessing the emulsifying properties of this egg yolk fraction. Besides, it has to be highlighted the lack of studies in recent years about the improvement in the egg yolk granules separation, since in every paper reviewed the granular fraction was obtained by discontinuous centrifugation, which could be considered as inefficient on an industrial scale. On the other hand, the use of continual centrifugation in order to separate the granular fraction could produce inhomogeneous fractions. In this sense, new separation methods need to be developed.

Regarding phosvitin, one of the main granular proteins, it has received relatively close attention in recent years, and many of these studies are about improving its emulsifying properties. Although in these articles the emulsifying ability of the phosvitin, modified in a variety of ways, was intensively studied, the antioxidant effect of the phosvitin on the emulsions, keeping lipids from being oxidised, was not generally tested. This could provide a suitable subject for future studies in order to increase the phosvitin value. In addition, bearing in mind that when the phosvitin is extracted, the remaining HDLs may be considered as a by-product, a lack of studies into the improvement of their functional properties and their possible food-related applications was also detected.

Declaration of competing interest

The authors declare that there are no conflicts of interests in the submission and publication of this manuscript.

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A great structured and focused review on the technological opportunities of egg yolk. Marc Anton is also one of the best researchers on this topic.

One of the few publications dealing with the technological utilisation of egg yolk granules after extraction of phosvitin.

This article provides relevant information on the use of oligophosphopeptides from hen egg yolk as nutraceuticals.

A high quality and highly cited review highlighting the bioactivities of phosvitin.

This article highlights the emulsifying properties of native granules at different pHs. It is very interesting to understand the emulsifying potential of native granules, without having undergone any processing.