

Review

Egg Yolk, a Multifunctional Emulsifier: New Insights on Factors Influencing and Mechanistic Pathways in Egg Yolk Emulsification

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Abstract: Egg yolk is a highly effective natural emulsifier used in various food products. Its emulsifying properties are influenced by food product chemical conditions, and processing methods. Nevertheless, to effectively utilize egg yolk in food products, a more comprehensive understanding of these factors is crucial. This review discusses recent developments regarding how factors like pH, ionic strength, thermal treatments, enzymatic treatments, and novel non-thermal treatments affect egg yolk emulsifying properties. It also explores the underlying mechanisms involved in egg yolk emulsification. Food products involve different ingredients leading to varying pH values and ionic strength, which affect egg yolk protein adsorption and emulsion stability. Processing steps like thermal treatment can damage egg yolk proteins, reducing their emulsifying capabilities and leading to unstable products. Incorporating sugar, salt, and amino acids can enhance egg yolk's resistance to heat and preserve its ability to form stable emulsions. As an alternative to thermal treatment, non-thermal techniques such as high-pressure processing and high-intensity ultrasound can be employed to preserve egg yolk. Furthermore, forming egg yolk–polysaccharide complexes can enhance egg yolk emulsifying properties. These advancements have facilitated the creation of egg yolk-based products such as high internal phase Pickering emulsions (HIPEs), low-fat mayonnaise, and egg yolk gels. A comprehensive understanding of the emulsifying mechanisms and factors involved in egg yolk will be instrumental in improving food quality and creating novel egg yolk-based products.

Keywords: granules; egg yolk complex; novel techniques; emulsion stability; thermal denaturation

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1. Introduction

Emulsification is the process of blending two incompatible liquids, such as oil and water, to form food emulsions. Many food products, including milk, cream, sauces, desserts, beverages, ice cream, margarine, salad dressings, soups, and infant formula, utilize oil-in-water emulsions as functional ingredients. While emulsions can improve the solubility, absorption, shelf life, and taste of food products, those composed solely of water and oil are inherently unstable due to the tendency of the dispersed droplets to merge and separate over time [1]. To improve their stability, emulsifiers and stabilizers are typically added. Emulsifiers work by reducing interfacial tension and increasing the contact area between the two phases, which helps disperse one liquid within the other [2,3].

A natural emulsifier, egg yolk is frequently used in food products such as mayonnaise, creams, and salad dressings [4,5]. Egg yolk is a primary emulsifying system due to its protein and lipid complexes, particularly its proteins, phospholipids, and lipoproteins [6]. In industrial food production, egg yolk is often utilized under varying pH levels and ionic strengths, depending on the specific ingredients used in the product formulation. Additionally, egg yolk must be pasteurized to ensure microbiological safety [7]. Factors

such as pH, ionic strength, freezing, and heat treatments can alter or destroy the egg yolk protein structure or cause aggregates to form, potentially affecting the emulsifying properties and leading to issues like flocculation [8].

Different approaches, such as physical, chemical, and enzymatic treatments (an overview is presented in Figure 1) have been studied to change the emulsifying properties of egg yolk and its components. Altering egg yolk proteins can improve their emulsifying capabilities and broaden their applications as natural emulsifiers. Studies have shown that adjusting the pH can make egg yolk proteins more flexible, enabling them to more readily adhere to the interface between oil and water [9]. It has also been shown that high-pressure treatments can break down the compact structure of proteins, improving their ability to adhere to the interface [10,11]. Additionally, several strategies, such as adding sugar, salt, or amino acids, have been suggested to elevate the thermal tolerance of egg yolk, preserving its emulsifying properties [12,13]. Furthermore, novel non-thermal systems, like high-pressure homogenization, high-pressure processing (HPP), and ultrasonication, have been developed as alternatives to thermal pasteurization of egg yolk. Due to enhanced emulsifying properties, egg yolk has found its use for various novel applications, including the development of HIPEs [14], low-fat mayonnaise [15], and egg yolk gels [16].

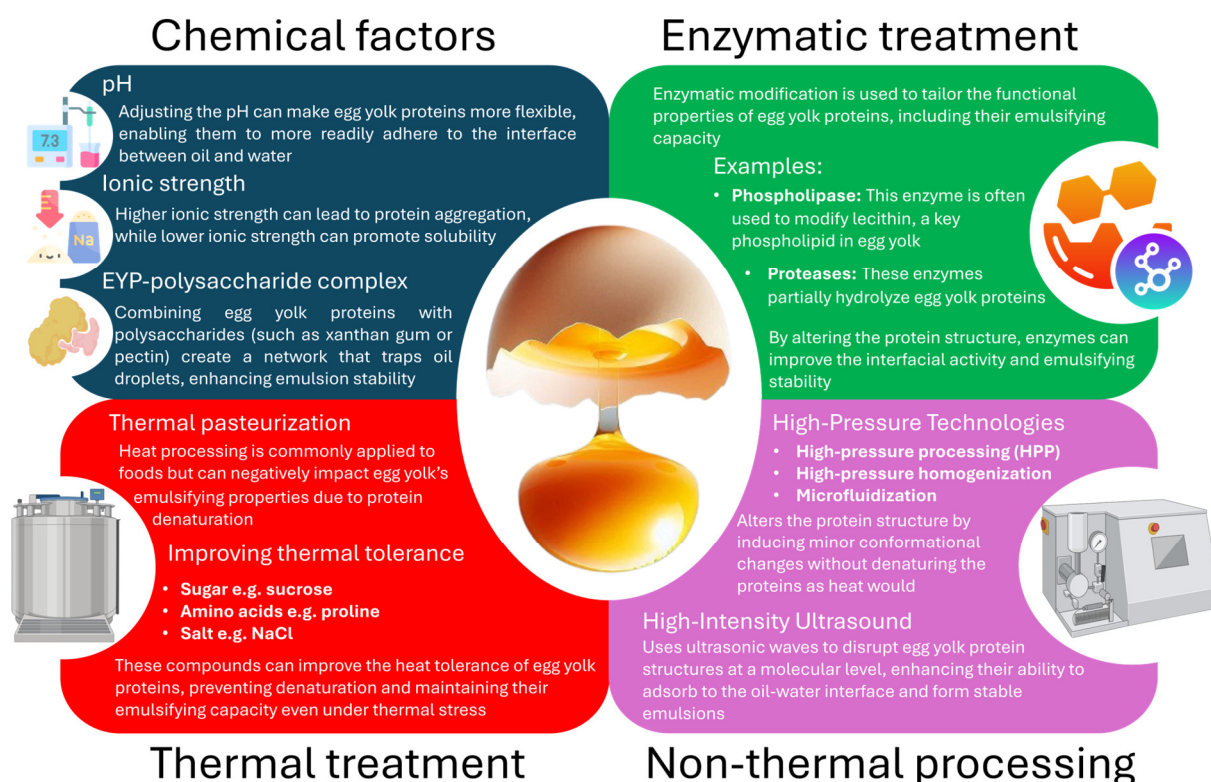


Figure 1. Overview of factors influencing emulsifying properties of egg yolk.

Therefore, this study aims to elucidate and demonstrate the modifications in the emulsifying properties of egg yolk and its components due to factors such as physical, chemical, and enzymatic treatments. Additionally, the review examines the underlying mechanisms of these modifications and their effects on emulsifying and stabilizing capacities. Through an in-depth examination of recent research on egg yolk's emulsifying capabilities, stability, and modifications, this review seeks to equip food scientists, researchers, and industry professionals with the knowledge needed to develop innovative and sustainable egg yolk-based food products.

2. Emulsifying Properties of Egg Yolk and Its Components

Egg yolk is a complex system with multiple structural levels, as shown in Figure 2. The process of centrifugation, which involves spinning egg yolk at high speeds, allows for its easy separation into its two primary components, plasma and granules; this separation enables the individual study and utilization of each component, providing valuable insights into the unique properties and potential applications of egg yolk in various fields. Several authors have developed different methods to separate egg yolk into its components. This review does not aim to describe these methods, as they have been described in other studies [17–19].

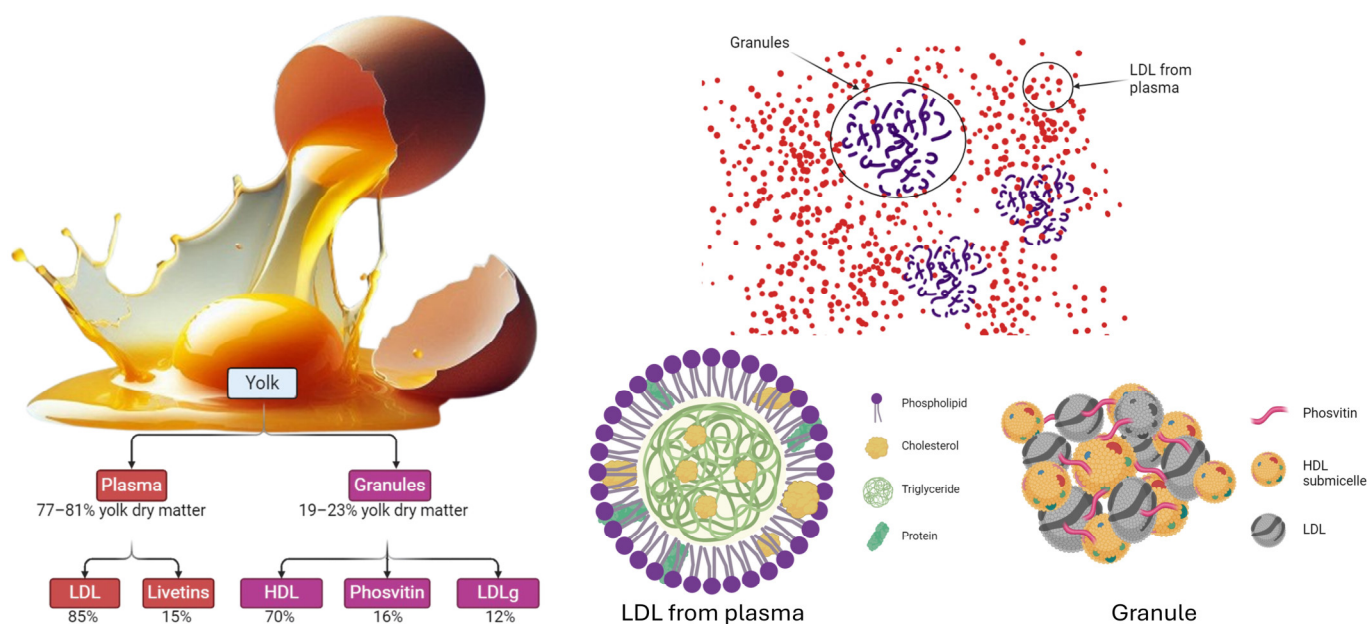


Figure 2. Components of egg yolk and schematic diagram for natural protein–lipid supramolecular assembly structure of egg yolk. LDL: low-density lipoprotein; HDL: high-density lipoprotein.

Egg yolk contains insoluble protein aggregates i.e., granules, which constitute approximately 22% of its dry matter and are rich in protein, containing roughly 50% of its total protein and also 7% of its lipids. Suspended in a clear yellow fluid called plasma, which constitutes about 78% of the yolk's dry matter and consists primarily of low-density lipoproteins (LDLs) with 85% of its composition and 15% livetins, these granules, primarily composed of high-density lipoproteins (HDLs) (70%) and phosvitin (16%), are interconnected by phosphocalcic bridges. Plasma is rich in lipids, containing about 90% of the yolk's total lipids, including almost all of the carotenoids, and also holds 50% of its proteins [4]. Given the varying composition of the egg yolk components, they exhibit varying emulsifying properties.

The effectiveness of an emulsifier is assessed based on its emulsifying activity, which refers to its ability to create a protective layer around oil droplets, preventing coalescence and separation, and its emulsion stability, which measures how well the emulsifier can preserve the emulsion's structure over time [20]. According to Anton and Gandemer [21], no difference was observed in the emulsifying activity of egg yolk, plasma, and granules, although granules demonstrated a superior emulsion stability compared to whole yolk and plasma. In a study by Anton et al. [22], the emulsifying properties of native granules and granules disrupted into HDL, phosvitin, and LDL were compared, with the granules being diluted in 0.17 M NaCl solution for the native granules and 0.5 M NaCl solution for the disrupted granules; the results showed that disrupted granules exhibited superior emulsifying activity and emulsion stability compared to the native granules. Native granules had low solubility (~13%), whereas monovalent sodium from the addition of NaCl

(0.5 M final concentration) disrupted the phosphocalcic bridges between HDL and phosphovitin, liberating them into the solution and increasing the solubility to 95%. Furthermore, the emulsions formed using native granules displayed a bimodal droplet size distribution, achieving a monomodal size distribution only at 1.5% protein concentration. In contrast, emulsions formed with disrupted granules showed a monomodal droplet size distribution starting at 0.4% protein concentration. Regarding creaming, emulsions prepared with native granules exhibited creaming levels above 60% at all protein concentrations (0.15, 0.4, 0.8, and 1.5% *w/v*), whereas emulsions formulated with disrupted granules demonstrated a reduction in creaming from 63% to 40% as the protein concentration increased from 0.15% to 1.5% [22].

Recently, Ali and Wu [23] compared the emulsifying properties of the plasma fraction separated from egg yolk using low centrifugal forces (2000 to 6000× *g*) for a brief period (5 min), finding no significant difference in the emulsifying capabilities of plasmas fractionated at these centrifugal forces compared to whole egg yolk [23]. The plasma fraction is mostly composed of LDL, whereas the granules are rich in HDL. LDL is considered the key component contributing to the emulsifying properties of egg yolk [24–26].

These studies emphasize the emulsifying capabilities of egg yolk components, suggesting their potential as substitutes for whole egg yolk in food products. Nevertheless, more research is needed to develop simpler and more cost-effective extraction methods for isolating these components. Additionally, modifications may be necessary to ensure their emulsifying properties are comparable or superior to those of whole egg yolk, enabling effective emulsification without increasing processing and production costs.

3. Factors Influencing and Mechanistic Pathways in Egg Yolk Emulsification

3.1. Chemical Treatment

3.1.1. pH and Ionic Strength

The emulsifying properties of egg yolk are intricately linked to the competitive arrangement of its components at the oil–water boundary and the structural dynamics of egg yolk particles. Proteins and phospholipids are the primary emulsifiers within egg yolk, with proteins demonstrating superior emulsifying capacity compared to phospholipids and forming the core of the interfacial layer [4]. The action of amphiphilic molecules integrating into the oil–water interface is a multistep and dynamic phenomenon encompassing diffusion, adsorption, conformational changes, and molecular reorientation. The competence of egg yolk proteins and lipids to interact with both oil and water phases stabilizes the emulsion by reducing interfacial energy and preventing droplet coalescence. The resulting protein-rich interfacial film functions as a protective barrier, hindering particle aggregation and enhancing overall emulsion stability [27,28]. The performance of egg yolk proteins in food systems is directly linked to their structural makeup. Among the various factors influencing protein structure, pH and ionic strength are paramount. Subtle shifts in pH and ionic strength can significantly alter protein conformation, consequently impacting the overall characteristics and texture of emulsions [9].

Kurt and Zorba [29] investigated how pH and ionic strength affect the characteristics of egg yolk emulsions. They discovered that as pH and ionic strength increased, emulsion stability decreased until a critical point was reached at a pH of 6.08 and an ionic strength of 0.49 M NaCl. In contrast, Li et al. [30] found that the emulsion stability index increased with increasing pH (5 to 10), with an optimal NaCl concentration of 1.8% for maximum stability. The emulsifying activity of egg yolk dispersion improved with increasing pH from 5 to 7 but reduced as the pH was increased further to 10. Interestingly, NaCl concentration (0.3–3.0%) had no significant impact on emulsifying activity [30]. These contrasting findings may be due to differences in the protein concentration of the egg yolk used in these studies. For instance, the egg yolk in the Kurt and Zorba [29] study had a protein concentration of 0.25%, while in Li et al.'s [30] study, the concentration was 17.5% (raw yolk).

A recent comprehensive study by Yang et al. [9] provided new perspective on the impact of pH on egg yolk emulsifying properties. Egg yolk-based emulsions had the highest emulsifying activity at pH 5, while the highest emulsion stability was observed at both pH 3 and 9. Surprisingly, protein adsorption at the oil–water interface was more pronounced under acidic conditions (pH 3) than under alkaline conditions (pH 9). This discrepancy was credited to contrasting protein stabilization mechanisms at different pH levels. A thicker interfacial protein layer formed at pH 3.0, primarily stabilized by electrostatic repulsion. However, at pH 9.0, non-adsorbed proteins dispersed in the aqueous phase due to increased solubility and structural expansion, creating a steric barrier. Additionally, partial phospholipid (lecithin) substitution for interfacial proteins under alkaline conditions likely contributed to reduced protein adsorption (Figure 3). Notably, emulsifying activity did not consistently correlate with the emulsion stability. For instance, despite lower emulsifying activity at pH 3, superior emulsion stability was observed, potentially due to protein unfolding and enhanced solubility. The emulsion composition, including the interplay of egg yolk proteins and lecithin, significantly influenced emulsifying properties, highlighting the complex relationship between pH, protein behavior, and emulsifier composition in determining egg yolk emulsion characteristics.

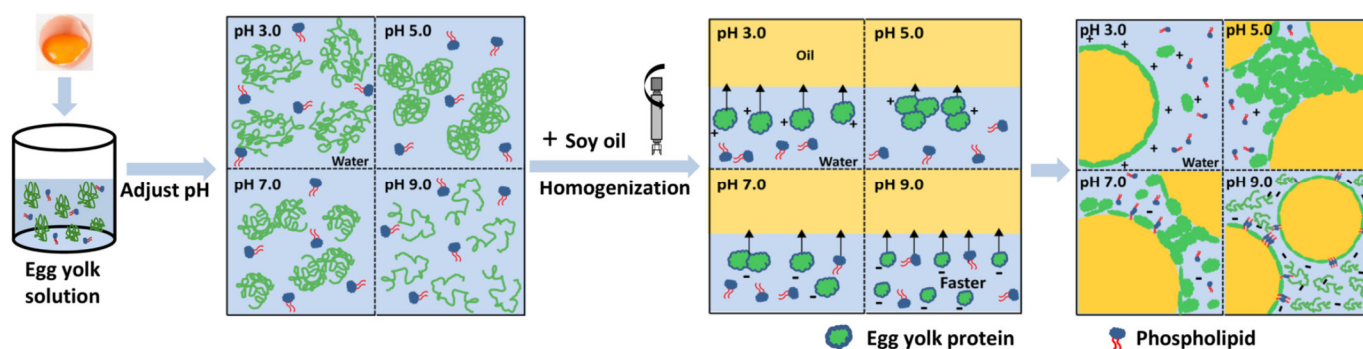


Figure 3. Schematic mechanism of protein and phospholipid adsorption at the oil–water interface under different pH conditions. Reprinted with permission from Yang et al. [9]. 2023, Elsevier.

In terms of egg yolk components, egg yolk granules produced stable emulsions with excellent emulsifying stability at pH 4. Above pH 4, poor emulsion stability was detected with oil separation. Conversely, at pH below 4, particle size distribution was uniform and narrow, with a particularly uniform particle size of $\sim 50 \mu\text{m}$ at pH 4. Particle size continuously grew to over $200 \mu\text{m}$ at pH values above the isoelectric point (pI) of 4. Notably, egg yolk granules were unable to stabilize emulsion with particle size $< 10 \mu\text{m}$ [31]. Along with pH, the type of oil significantly impacts interfacial tension. Regardless of oil type, increasing pH correlated with a decrease in interfacial tension. The initial decrease from pH 3 to 4 was gradual, but the reduction became more pronounced at higher pH levels, especially in the alkaline range (pH 8 and 10). Rapeseed oil exhibited the highest interfacial tension against both 0.15 M NaCl solution and 2% granule solution. Sunflower and peanut oils showed intermediate values, while corn oil consistently displayed the lowest interfacial tension across all pH conditions [32].

Furthermore, Li et al. [33] unveiled the Janus effects of NaCl on granule structure across varying pH conditions. In the absence of NaCl, granule self-assembly was primarily influenced by the interplay of attractive calcium bridges and repulsive electrostatic forces. Dispersions formed using granules were stable at pH values outside the range of 4 to 7, but phase separation took place within that pH range. Introducing 0.3 and 0.5 M NaCl disrupted calcium bridges, leading to granule dissociation between pH 4 and 7, with a more pronounced effect at pH 6 and 7. At pH 2 and 3, the same NaCl concentration induced reaggregation of dissociated granules. However, NaCl had a negligible impact on granule aggregation at pH 5 to 10. This dual behavior was attributed to counter ion adsorption on the granule surface. Above pH 5, negatively charged

granules adsorbed highly hydrated Ca^{2+} and Na^+ ions, resulting in strong hydration repulsion and stable dispersions. Conversely, at pH levels below 4, Cl^- ion adsorption led to insufficient hydration repulsion and subsequent phase separation (Figure 4).

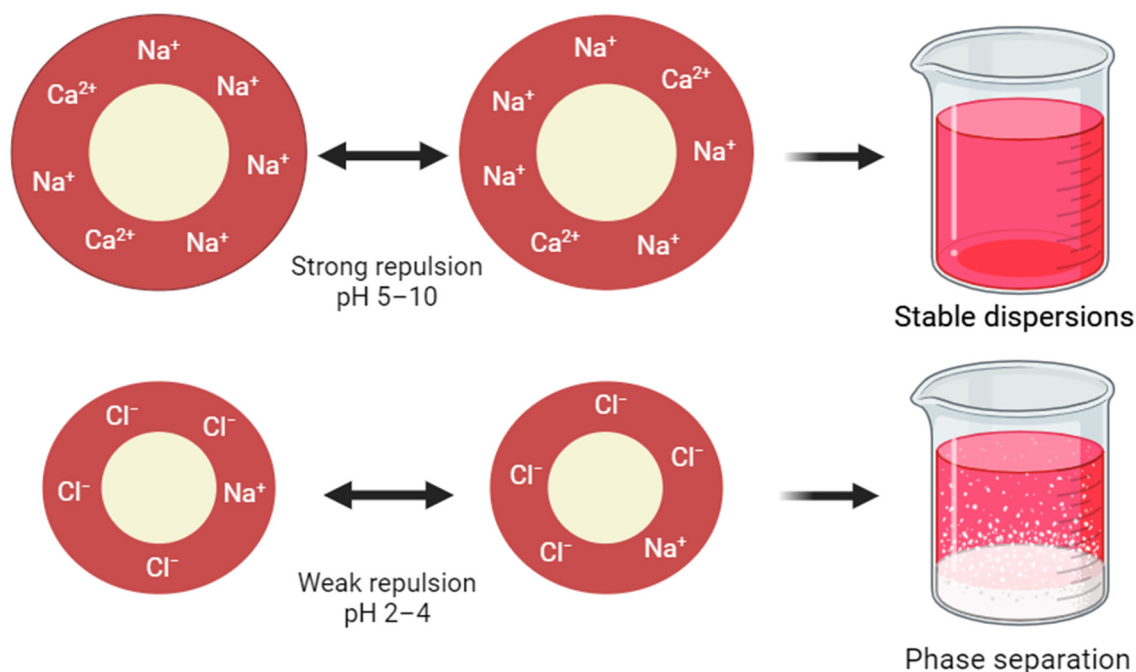


Figure 4. Adsorption of Na^+ and Ca^{2+} on granules at pH 5–10 and adsorption of Cl^- at pH 2 and 3. Adapted with permission from Li et al. [33]. 2022, Elsevier.

A study by Fu et al. [34] used calcium chelators to enhance the interfacial activity and water dispersity of granules. The addition of sodium tripolyphosphate ($\text{Na}_5\text{O}_{10}\text{P}_3$), trisodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$), and ethylenediaminetetraacetic acid disodium (EDTA-2Na) at a concentration of 0.15 weight% (wt.%) dissociated granules into nanoparticles from micro-sized, which exhibited improved colloidal stability at pH 7. The dissociation effect was diminished or absent at pH 3 and 5.5. Chelators disrupted the granule by chelating and disrupting Ca^{2+} bridges as confirmed by an analysis of unbound calcium ions. Emulsions formulated with these dissociated granules demonstrated reduced particle size and improved stability.

Phosvitin, a key component of hen egg yolk, exhibits a strong affinity for multivalent cations and possesses emulsifying properties. While non-aggregated phosvitin excels in emulsifying activity, aggregated phosvitin demonstrates superior emulsion stability against coalescence. Lower ionic strength (0.05 M) favored the creation of finer emulsions compared to higher (0.15 M). The concentration of NaCl had a significant effect on flocculation, with the highest level of flocculation occurring when aggregated phosvitin was combined with an ionic strength of 0.15 M. Hydrophobic interactions contributed to the stability of phosvitin aggregates. The findings suggest that controlling the initial conditions of the protein solution can effectively modulate the various mechanisms underlying phosvitin emulsion destabilization [35].

The above-discussed research studies examining the influence of pH and ionic strength on egg yolk emulsifying properties (summarized in Table 1) offers important insights into how these factors affect protein adsorption and emulsion stability. However, these studies are somewhat constrained by their focus on controlled environments, which may not accurately reflect the complexities of real-world food systems. Additionally, the research primarily examines pH and ionic strength in isolation, without accounting for potential interactions with other food ingredients and variables such as temperature or processing techniques. Despite these limitations, the findings are essential for refining egg

yolk formulations across various food applications, allowing for better control of texture, stability, and functionality in products like sauces, dressings, and emulsified foods.

Table 1. Factors affecting and modification of egg yolk emulsifying properties.

pH and Ionic Strength			
Conditions	Findings	Recommendation/Application	Reference
- pH: 3.5, 4.5, 6.0, 7.5, and 8.5 - Ionic strength: 0.05, 0.30, 0.45, and 0.55 M NaCl	- ES decreased with increasing pH and ionic strength - EC increased with increasing pH and ionic strength	Optimum values of pH and ionic strength were found to be 4.61 to 7.43 and 0.10 to 0.47, respectively	[29]
- pH: 5, 6, 7, 8, 9, and 10 - Ionic strength: 0.3, 0.6, 1.2, 1.8, 2.4, and 3.0 NaCl (%)	- EAI increased with pH till 7 and decreased with further increase - NaCl concentration had no significant effect on EAI - ESI increased with increase in pH - ESI increased with NaCl concentration till 1.8% and decreased with further increase	Highest EAI and ESI at pH 7 and 10, respectively	[30]
pH: 3, 5, 7, and 9	- Lowest and highest EAI at pH 3 and 5, respectively - Lowest and highest ESI at pH 5 and 3 (as well as 9), respectively	Alkaline conditions (pH 9) led to the formation of more open and flexible secondary/tertiary protein structures than acidic (pH 3)	[9]
Egg yolk granule, pH: 2, 3, 3.7, 4, 4.5, 5.5, 6.5, 7, and 9	Formed stable emulsion with no oil on the top at pH < 4	Suitable for development of high internal-phase emulsion	[31]
Aggregated and non-aggregated phosvitin, ionic strength: 0.05 and 0.15 M NaCl at pH 6	- EAI: non-aggregated > aggregated - ESI: aggregated > non-aggregated - Ionic strength of 0.05 formed fine and stable emulsion	Non-aggregated phosvitin and lower NaCl concentration formed stable emulsions	[35]
Egg yolk granule, pH: 2–10 and ionic strength: 0.3 and 0.5 M NaCl	- Stable dispersions at pH below 4 or above 7 - NaCl (0.3 M and 0.5 M) disrupted calcium bridges at pH 4–7	- Janus effects of NaCl on structure of egg yolk granules - Potential of granules as delivery vehicles for hydrophobic bioactive compounds at high ionic strength	[33]
Egg yolk complex formation			
Complex	Findings	Mechanism	Reference
Egg yolk granule–lecithin	Addition of less than 0.25% lecithin formed highly stable emulsions	Addition of lecithin disrupted the aggregated structure of granule	[36]
Egg yolk–carboxymethyl cellulose	Distinct phase behaviors of egg yolk–carboxymethyl cellulose in stabilizing emulsions	Phase behaviors: alkaline co-solubility, soluble complexes, cocoacervations, and acidic co-solubility all involved hydrogen bonding and hydrophobic interactions	[37]

- Egg yolk–octenyl succinic anhydride (OSA) - Egg yolk–hydroxypropyl distarch phosphate (HPDSP)	Complex of egg yolk–HPDSP provided better long-term emulsion stability than egg yolk–OSA starch	Strong electrostatic interaction between egg yolk and HPDSP causes the yolk aggregates to crack or expand to form smaller yolk granules. These smaller egg yolk granules tend to make maximum contact with HPDSP	[38]
High-density lipoprotein (HDL)–phosvitin	Optimum HDL–phosvitin ratio 5:5	The synergistic utilization of two proteins with different charges, HDL and phosvitin, allowed for electrostatic interactions within the system, resulting in the formation of stable electrostatic complexes	[39]
- Egg yolk plasma–pectin - Egg yolk plasma–guar gum	- Addition of guar gum formed more stable emulsion compared to pectin - Optimum guar gum amount: 0.5% w/v	Addition of hydrocolloids in emulsions lead to droplet aggregation in emulsion microstructure and increased apparent viscosity	[40]
Enzymatic treatment			
Enzyme	Treatment conditions	Findings	Reference
Dual enzymatic hydrolysis using neutrase and trypsin	Neutrase (0.1% w/w) and trypsin (0.15% w/w)	Optimal hydrolysis time: 1.5–3.5 h.	[41]
Neutral and alkaline protease	2000 U/g egg yolk protein and left to react at 50 °C for 4 h	Alkaline protease hydrolyzed egg yolk had higher thermal stability and emulsifying stability	[42]
Lactic acid bacteria fermentation of phosvitin	Lactic acid bacteria powder was inoculated (1%, wt/wt phosvitin), and incubated at 42 °C for 0, 3, 6, and 9 h, respectively, where the ratio of <i>Streptococcus thermophiles</i> to <i>Lactobacillus bulgaricus</i> was 1:1	Maximum EAI and ESI after 6 h of fermentation and improved calcium-binding capacity compared to native phosvitin	[43]
Phospholipase A ₂	0.4 µL enzyme/g egg yolk suspension (lecitase activity 10.000 units/mL) at 55 °C for 3 h	Improved emulsifying properties of egg yolk after enzymatic treatment	[44]
Phospholipase A ₂ treatment on egg yolk, plasma, and granule	20 µL of MAXAPAL® phospholipase A ₂ added to 100 mL solution and incubated at 50 °C for 1 h	Enzymatically treated egg yolk and granules formed more stable emulsions, whereas plasma-treated emulsions had a higher degree of phase separation	[45]
Non-thermal processing			
Technology	Processing parameters	Findings	Reference
High-pressure homogenization of egg yolk granules	Pressure: 175 and 300 MPa Passes: 1 and 4	Processing at 300 MPa and four passes improved oil and water binding capacity	[46]
Microfluidization of egg yolk	Pressure: 103, 138, 172, and 207 MPa	Highest EAI at 103 and 138 MPa and ESI at 103 MPa	[47]
High hydrostatic pressure followed by ultrafiltration of egg yolk granules	400 MPa for 5 min	Improved emulsifying properties	[48]

Hyperbaric storage of egg yolk	200 MPa for 28 days	Preserved emulsifying properties and prevented oxidation	[49]
High-intensity ultrasound treatment on egg yolk	Power: 75, 150, 225, and 300 W for 10 min (ultrasound for 3 s, stop for 3 s)	No significant difference in EAI at different power and maximum ESI at 150 W	[50]
High-intensity ultrasound treatment on egg yolk granules	Power: 90, 180, 270, and 360 W for 10 min (ultrasound for 3 s, stop for 3 s)	Maximum EAI at 270 W and no significant effect of power on ESI	[51]
High-intensity ultrasound treatment on egg yolk	Power: 75, 150, and 210 W for 3, 6, and 9 min (ultrasound for 3 s, stop for 3 s) Temperature: 30, 45, and 60 °C	Optimum processing conditions for maximum emulsifying stability was 210 W for 9 min at 30 °C	[52]
High-intensity ultrasound treatment on LDL	200 W for 10 min (ultrasound for 3 s, stop for 3 s)	LDL molecules underwent partial rearrangement/recombination, and their molecular interface became flexible and stretched, which enhanced the zeta-potential and surface hydrophobicity and thus slightly improved the solubility and emulsifying	[53]
Ozonation of egg yolk	Ozone generation rate of 1 g/h and sample treatment times 0, 10, 20, 30, and 40 min	Maximum emulsifying properties at 20 min treatment	[54]

3.1.2. Egg Yolk Complex Formation

While the egg yolk emulsifying characteristics have been thoroughly investigated in basic, well-characterized model emulsion systems, in industrial processing, egg yolk-stabilized emulsions are subjected to a range of conditions and environmental stresses, including pH and ionic strength fluctuations, and various thermal, chilling, freezing, and drying treatments [55,56]. These emulsions can undergo a destabilization process like coalescence, flocculation, creaming, and Ostwald ripening, with flocculation being particularly difficult to manage. Flocculation is highly sensitive to multiple factors and can directly affect emulsion formation and other instability mechanisms, including coalescence and creaming [57]. Egg yolk protein-stabilized emulsions tend to destabilize after thermal processing or changes in pH and ionic strength (*vide supra*), which may limit their use as emulsifying agents. Therefore, in the food industry, polysaccharides, which are a significant category of biopolymers, are utilized as thickeners, emulsifiers, and gelling agents. Through electrostatic interactions, polysaccharides can decrease firmness and improve emulsification in food, thereby enhancing the structure of the original gel network [58,59]. Pectin, a widely used food additive, serves as an emulsifier in mayonnaise. It effectively stabilizes oil-in-water emulsions and works well in oil phases [60]. Proteins and polysaccharides often form complexes during food processing, which helps create stable and desirable food products. As a result, there is growing interest in improving the functionality of egg yolk proteins through conjugation with food-grade components.

It is important to note, however, that while protein–polysaccharide complexes can stabilize emulsions, many are still affected by environmental factors such as pH and ionic strength [61]. Therefore, this subsection discusses recent studies investigating the role of these complexes in emulsification and emulsion stabilization.

Egg Yolk-Modified Starch Complex

Xu et al. [38] investigated the use of an egg yolk-modified starch complex as a novel emulsifier for stabilizing HIPEs. They used octenyl succinic anhydride (OSA) to represent surface-active polysaccharides and hydroxypropyl distarch phosphate (HPDSP) to represent non-surface-active polysaccharides. The study observed that adding modified starch decreased the particle size of the emulsion by strengthening the electrostatic repulsion between egg yolk protein aggregates. Specifically, the addition of egg yolk with OSA starch, restricted non-covalent interactions among starch molecules, reducing starch molecular aggregation, and forming a soluble egg yolk–OSA starch complex with a smaller particle size. As a result, the soluble egg yolk–OSA starch complex that stabilized HIPEs exhibited improved stability than solely stabilized using egg yolk or OSA starch. Nevertheless, emulsions containing higher concentrations of OSA starch (3–4%) exhibited larger oil droplets and more free water molecules, indicating an unstable internal structure. Higher HPDSP concentrations (3–4%) caused the yolk aggregates to break apart or expand, forming smaller yolk granules due to strong electrostatic interactions between the egg yolk and HPDSP. These smaller granules maximized contact with HPDSP, leading to the formation of insoluble complexes, which in turn stabilized the HIPEs, resulting in smaller droplet sizes and improved long-term stability.

Egg Yolk–Carboxymethyl Cellulose Complex

A study by Hou et al. [37] researched the emulsifying properties of a complex formed between egg yolk and carboxymethyl cellulose (EY-CMC). When egg yolk and carboxymethyl cellulose were co-stabilized, the resulting emulsions had droplets of comparable size. This was mainly because the co-solubility introduced kinetic hindrance, which lowered emulsifying activity. At pH 7, the EY-CMC complex had larger particle sizes and significantly reduced emulsifying activity compared to pH 8, likely due to the increased impact of kinetic hindrance on larger particles under identical co-solubility conditions. In soluble complexes, the depletion attraction caused by carboxymethyl cellulose had minimal impact on emulsifying activity due to kinetic hindrance but significantly improved emulsion stability. This improvement was ascribed to the creation of a more uniform and dense interfacial film, which prevented instability caused by varying yolk protein particle sizes. Moreover, the depletion force helped to prevent particle desorption at the oil–water interface forming smaller emulsion droplets. For complex coacervates, the larger particle size made it difficult to produce a dense film at the oil–water interface, leading to low emulsifying activity and stability at pH 5. However, at pH 4, depletion attraction improved the emulsion stability of the coacervates by reducing particle size and forming smaller droplets. At pH 2, the protonation of carboxymethyl cellulose hindered its ability to induce depletion attraction, leading to reduced emulsifying activity and stability of the EY-CMC complex. When yolk proteins had smaller particle sizes, kinetic hindrance slowed their desorption from the interface increasing the emulsion stability of the EY-CMC than egg yolk alone. The EY-CMC with smaller particle sizes produced emulsions with smaller droplets.

Figure 5 illustrates the distinct phase behaviors of EY-CMC and egg yolk in stabilizing emulsions. Despite smaller protein particle sizes, alkaline co-solubility led to poor emulsification owed to kinetic hindrance. In soluble complexes, the limited binding between egg yolk and carboxymethyl cellulose permitted non-adsorbed polysaccharides to induce depletion attraction, leading to enhanced emulsion stability. Larger particle size complex coacervates, on the other hand, were less successful in forming dense interfacial films than soluble complexes, but they did so better than co-soluble systems in terms of emulsification. In acidic environments, the breakdown of egg yolk granules into smaller particles improved emulsification, and the reduction in kinetic hindrance due to carboxymethyl cellulose protonation further stabilized the emulsion [37].

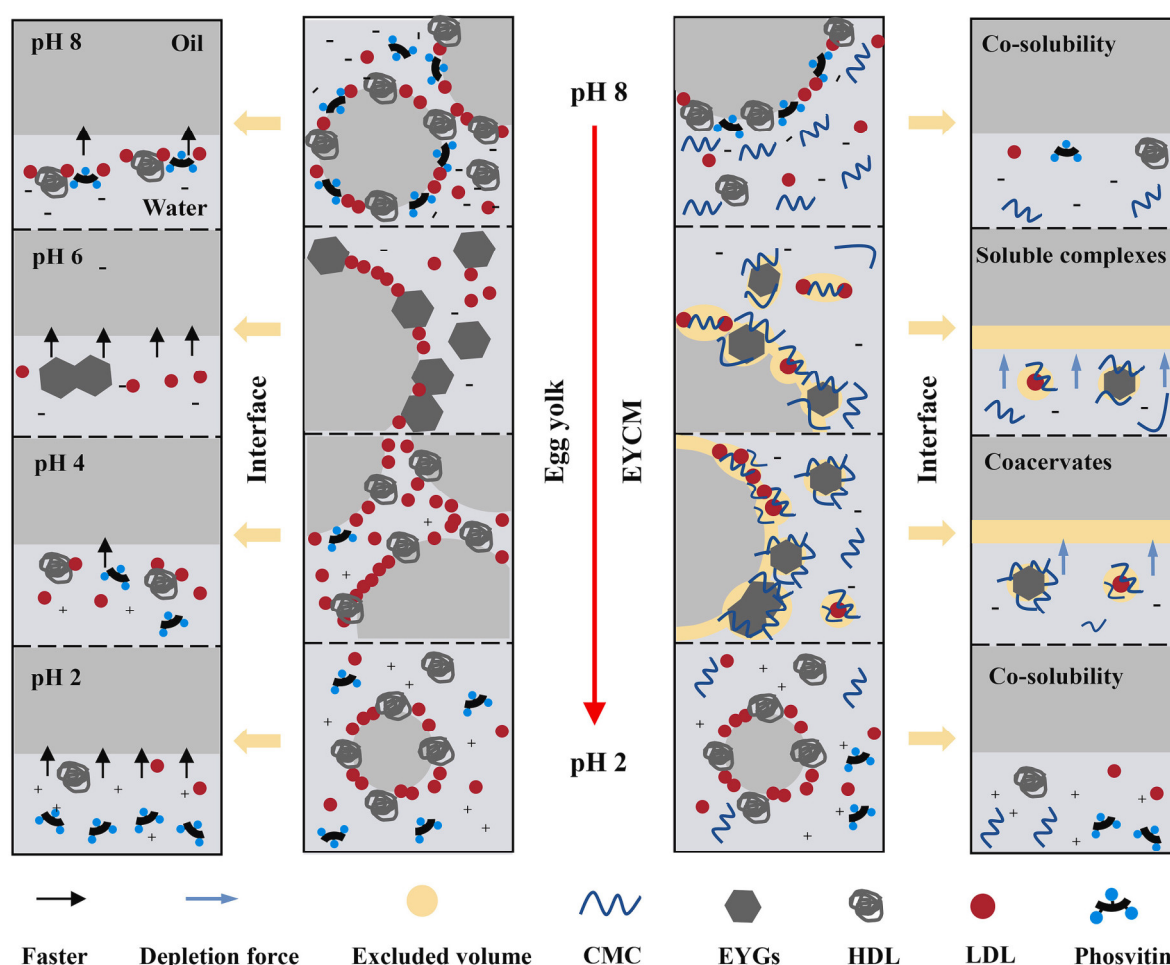


Figure 5. Schematic diagram of egg yolk and different phase behavior egg yolk-carboxymethyl cellulose complex stabilized emulsions. Reprinted with permission from Hou et al. [37]. 2024, Elsevier.

Egg Yolk Plasma-Pectin/Guar Gum Complex

Emulsions containing 1% egg yolk plasma exhibited rapid creaming, reaching approximately 50% within the first hour, followed by a slower rate, culminating in 61% creaming after 10 h. The addition of low pectin concentrations (0.01% and 0.1% *w/v*) slightly decreased creaming, while higher concentrations progressively decreased creaming, completely preventing it at 0.5% *w/v*. Guar gum exhibited a more pronounced anti-creaming effect compared to pectin. At 0.05% and 0.2% *w/v* guar gum, creaming reduced to 57% and 39%, respectively, after 10 h. Complete creaming prevention was achieved at 0.5% *w/v*. This enhanced stability was assigned to the higher viscosity of the continuous phase imparted by guar gum [40].

At low hydrocolloid concentrations (0.01–0.05% *w/v*), the emulsion exhibited a diffuse interface between the cream and serum phases, with a turbid serum indicating the presence of oil droplets. This is a feature of polydisperse emulsions with minimal hydrocolloid stabilization. Conversely, at higher hydrocolloid concentrations (0.1–0.5% *w/v*), a distinct interface and clear serum phase were observed, indicative of complete emulsion flocculation [40].

Egg Yolk Granule-Soybean Lecithin Complex

Without lecithin, emulsions stabilized by native granules (at pH 7) and disrupted granules (at pH 9) began creaming after 1 and 3 days of storage, respectively. Emulsions stabilized by disrupted granules exhibited a significantly lower creaming index ($p < 0.05$)

than those stabilized by native granules at the same lecithin concentration. Both native and disrupted egg yolk granules demonstrated an initial increase followed by a decline in emulsifying stability as lecithin levels rose. The best emulsifying stability was observed in emulsions containing 0.25% lecithin. A notable finding was the absence of a droplet-free phase at the bottom of the emulsion made with disrupted granules and 0.25% lecithin after 7 days of storage. This indicated that granule dissociation, facilitated by a suitable amount of lecithin, can enhance emulsifying stability, although excessive lecithin may impede emulsifying activity due to competitive adsorption between egg yolk granules and lecithin at the interface. It was also evident that emulsions with 1% lecithin did not achieve the expected creaming stability, indicating that lecithin alone is insufficient to stabilize oil droplets [36].

Egg Yolk HDL–Phosvitin Proteins Complex

A recent study by Ren et al. [39] explored the potential of egg yolk HDL and phosvitin as stabilizers for HIPEs. In optimal circumstances (pH 3, 2% ionic strength, 2% HDL), HDL by itself may stabilize HIPEs. The addition of phosvitin to HDL resulted in smaller particle sizes and reduced surface tension, with the optimal ratio being 1:1 HDL to phosvitin. HDL–phosvitin mixtures stabilized HIPEs with average particle sizes of around 15 µm at pH 3. Incorporating calcium ions (0.2 M Ca²⁺) further enhanced stability, reducing particle size to approximately 10.86 µm. These findings demonstrated the ability of HDL, phosvitin, and calcium ions in improving the emulsifying properties to produce highly stable HIPEs at a low pH.

The stabilization mechanism of HIPEs by the egg yolk HDL–phosvitin protein complex involved several key factors. Firstly, the pH level had a significant impact on protein aggregation. Proteins aggregated as huge polymers at the oil–water interface, causing their correct alignment to be disrupted when the pH approached their isoelectric threshold. Consequently, HDL was unable to efficiently combine to form stable electrostatic complexes with phosvitin, which caused the emulsion droplets to enlarge at pH values of 6 or 5. The two proteins' electrostatic linking grew stronger as the system acidity increased, which increased the amount of electrostatic complexes that were formed. Due to the stable physical cross-linking features of these complexes, smaller and more uniform droplets can be produced at the oil–water interface through uniform rearrangement. Additionally, calcium ions further enhanced emulsion stability by cross-linking the two proteins through salt bridges within the unfolded structure, leading to a stable complex [39].

3.1.3. Egg Yolk Oxidation and Emulsifying Properties

Egg yolk oxidation during processing presents a significant challenge for food production. The degradation of lipids and proteins within egg yolk not only compromises nutritional value, flavor, and aroma but also adversely affects its emulsifying properties. Oxidative changes can alter the structure and functionality of egg yolk components, leading to reduced emulsion stability and increased droplet size. For instance, Tian et al. [62] investigated the effects of malondialdehyde on the high-density lipoprotein emulsifying properties of egg yolks by setting up a simulated oxidation system. It was found that when the extent of oxidation increased, both emulsifying activity and emulsion stability gradually decreased. As malondialdehyde levels increased, surface hydrophobicity decreased, leading to hydrophobic aggregation of egg yolk HDL. Malondialdehyde and proteins reacted with Schiff bases forming oxidized aggregates. Eventually, this aggregation led to a decrease in the emulsifying capacity because it made protein molecules less flexible and compromised the stability of the emulsification interface [63].

Among the various strategies for mitigating oxidation in foods, the use of antioxidants emerges as the most effective, practical, and cost-efficient approach. Food manufacturers worldwide incorporate antioxidants to stabilize lipids and maintain

product quality [64]. Gouda et al. [65] demonstrated the potential of natural antioxidants, such as menthol, trans-cinnamaldehyde, vanillin, and thymol, in preserving egg yolk quality and enhancing its emulsifying properties. Vanillin exhibited the strongest antioxidant activity, followed by thymol, menthol, and trans-cinnamaldehyde. Importantly, the antioxidant activity of all compounds increased with concentration. While all four antioxidants improved egg yolk emulsifying activity, only vanillin significantly enhanced emulsion stability. Thymol and trans-cinnamaldehyde had no notable impact on emulsion stability, whereas menthol decreased it.

3.2. Thermal Treatment

3.2.1. Need for Thermal Treatment

The primary source of microbiological contamination in eggs is the eggshell, which can become contaminated with fecal matter containing enteric bacteria such as *Escherichia coli* and *Salmonella* spp., both members of the *Enterobacteriaceae* family. Microorganisms present in the nesting environment, such as *Staphylococcus aureus*, can also contribute to contamination on the shell's surface [66]. When an egg is cracked, these microbes can enter the egg's interior. Eggs, being rich in proteins and nutrients, offer an optimal environment for microbial growth, including foodborne pathogens, which pose a significant risk to food safety [67]. These bacteria can lead to symptoms like nausea, diarrhea, and abdominal pain. Some strains of Shiga toxin-producing *E. coli* (STEC), particularly *E. coli* O157:H7, can cause severe foodborne illnesses and are transmitted through the consumption of contaminated food, such as raw or undercooked meat, milk, or raw egg products [68]. According to European Commission Regulation (EC) No. 2073/2005 on microbiological standards for food, the acceptable limit for *Enterobacteriaceae* in egg products at the end of production is between 10 and 100 CFU/g or ml [69]. The rapid growth of these microorganisms not only raises the risk of illness but also drastically shortens the shelf life of liquid eggs. Therefore, it is crucial to apply treatments that effectively reduce the initial microbial load in egg liquids [70].

In the food sector, pasteurization is the most widely used heat processing technique. The goal of pasteurization is to improve food safety by heating products to kill pathogens, bacteria, and microorganisms. Commercial egg yolks are thermally pasteurized, usually for 2 to 6.2 min at 60 to 68 °C, to lower the potential risks associated with egg consumption [71,72]. Elevated temperatures cause alterations to the cellular composition and metabolic processes of microbes, which in turn reduces their proliferation and endurance, thereby lowering the likelihood of contamination. Furthermore, pasteurization prolongs the shelf life of liquid eggs, prevents spoilage, and guarantees their safety for consumption within a specified timeframe [8].

Nevertheless, because egg yolk lipoproteins are heat-sensitive, thermal processing above 65 °C may cause them to denature and aggregate [73,74]. Protein denaturation increases with temperature, showing a slight rise between 60 and 68 °C, but a significant increase at temperatures above 76 °C. This trend is linked to the different denaturation temperatures of major egg yolk proteins: g-livetin (69 °C), LDL and a-livetin (76 °C), b-livetin (81 °C), and HDL (84.3 °C) [75]. Moreover, damage to the LDL structure after thermal treatment above 65 °C reduces its ability to bind lipids, which accelerates the release of lipids. This leads to a considerable and permanent rise in viscosity and a decrease in the emulsifying qualities through promoting the creation of heavier aggregated clusters with irregular distributions and forms. Additionally, the released lipids undergo oxidation, producing oxidative by-products: hexanal, (E)-2-octenal, (E, E)-2,4-decadienal, 1-octen-3-ol, and 2-pentylfuran, that contribute to off-odors in egg yolk [76].

It is important to recall that hazardous and spoilage microorganisms like *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* that have limited heat tolerance are the main targets of pasteurization. On the other hand, bacteria and spores

that are thermophilic and heat-resistant, such as *Microbacterium* and *Enterococcus*, are able to withstand heat in the egg solution [77]. Pasteurization temperatures may not fully inhibit these microbes. Techer et al. [71] found *Enterococci* in pasteurized liquid eggs, underscoring the limitations of pasteurization as these bacteria can create enzymes that cause spoiling. Additionally, thermally resistant *Enterococcus* and *Bacillus cereus*, which can create toxins, have been found in pasteurized liquid egg products in a number of investigations [78,79].

Thus, to ensure the microbial safety of egg yolk, more intense thermal treatment is often necessary. However, this approach can adversely affect the physico-chemical characteristics, functionality, and overall quality of liquid eggs. The sensitivity of egg yolk to heat not only compromises product quality but also causes processing challenges, such as pipe blockages, which lead to increased cleaning and processing costs. Therefore, additional strategies are needed in egg yolk processing to preserve both its functional properties and microbial safety. One possible approach is the application of novel non-thermal technologies (discussed in detail in Section 3.4). Another effective method is enhancing the heat tolerance of egg yolk by incorporating compounds like salt, sugar, or amino acids, allowing it to endure higher thermal treatments without compromising its quality.

3.2.2. Improving Thermal Tolerance of Egg Yolk to Maintain Emulsifying Properties

Campbell et al. [80] showed that, depending on the quantity of sugar and salt, egg yolk proteins can tolerate severe thermal treatment up to 80 °C/2 min when coupled with sucrose and NaCl. Without impairing the egg yolk proteins' emulsifying qualities, sucrose and, to a greater extent, NaCl helped to delay the denaturation of yolk proteins. In line with these results, Yu et al. [81] recently demonstrated that adding 4% compound sugar or 4% compound sugar–salt to liquid egg yolk significantly increased its heat stability and raised the denaturation temperature above 77 °C.

Furthermore, Zhao et al. [12] reported that saccharides such as xylitol, erythritol, and arabinose (at 5%) were able to maintain the emulsifying properties of egg yolk during thermal treatment at 76 °C, compared to conventional sucrose. Because saccharides slowed down the process of turning unbound water into bound water during heat treatment, they had an inhibitory effect on the thickening of egg yolks. Additionally, it was discovered by Raman spectroscopy analysis that the saccharides inhibited the close interaction between the intact or denatured protein molecules in the egg yolk. Additionally, the addition of saccharides was able to prevent the formation of intermolecular hydrogen bonds, suggesting that hydrogen bonds may have a function in the heat-induced aggregation of proteins [12]. During thermal treatment, plasma with abundant lipids and proteins (LDL, α , β , and γ -livetins) aggregated to form precipitates and transferred to the granule component to create insoluble components together. The amount of transfer increased with temperature, and concurrently, the egg yolks' release of free lipids rises as well. The addition of saccharide (xylitol) could inhibit this transfer and aggregation of soluble proteins such as γ -livetins and apo-LDL to insoluble granule components during thermal treatment [82]. These findings provide a greater selection of additives to increase the thermal stability of egg yolk proteins.

According to Liu et al. [13], the inclusion of proline and betaine enhanced egg yolk thermal stability and preserved its emulsifying qualities under heat treatment. The comparison between betaine and proline showed that egg yolk added with proline had excellent emulsifying activity, while betaine had superior emulsion stability. Analysis of water distribution and protein structure revealed that egg yolk supplemented with betaine or proline exhibited looser, more porous formations compared to the control. Betaine primarily altered water molecular arrangement, enhancing water mobility and overall egg yolk fluidity. Conversely, proline primarily improved egg yolk heat resistance by constructing an organized framework that promoted stability.

3.3. Enzymatic Treatment

Enzymatic hydrolysis is regarded as a safe and efficient method of improving the functional characteristics of proteins [83]. Proteases, including neutral protease, trypsin, and alkaline protease, are frequently employed to generate hydrolysates that enhance the emulsifying and solubility characteristics of proteins. Research has demonstrated that regulated enzymatic hydrolysis is an ideal approach for augmenting protein functionalities, since it can produce small soluble peptides and reveal more hydrophobic and ionizable protein residues. However, excessive enzymatic hydrolysis can negatively affect emulsifying properties. Moreover, the exact site of enzymatic action and the activity of the enzyme both affect the degree of hydrolysis. Hydrolysates with the same degree of hydrolysis, produced by different enzymes, can exhibit varying physicochemical properties [84,85]. Thus, this section will explore recent advances in the use of enzymes to improve egg yolk and its components' emulsifying properties.

Fu et al. [41] showed that hydrolysis times during dual enzymatic hydrolysis using neutrase (0.1% *w/w*) and trypsin (0.15% *w/w*) affected the egg yolk emulsifying properties. The degree of hydrolysis increased with longer hydrolysis times, reaching 4.4% at 4.5 h. However, the optimal hydrolysis times for emulsifying activity were between 1.5 and 3.5 h. Shorter (0.5 h) or longer (4.5 h) hydrolysis times led to reduced emulsifying activity. All hydrolyzed egg yolk samples exhibited higher emulsifying capacity than the untreated egg yolk, with the highest value at 1.5 h of hydrolysis [41]. The ability of a protein to function as an emulsifier is correlated with its surface hydrophobicity, where a higher hydrophobicity produces better emulsifying properties. While smaller peptides with both hydrophobic and hydrophilic functional groups can migrate to the oil–water interface and stabilize the emulsion by lowering the interfacial tension, larger polypeptide chains can produce a stronger interfacial film around oil droplets [86,87]. Additionally, emulsions made with the dual enzymatic hydrolyzed egg yolk exhibited enhanced thermostability [41]. Similarly, alkaline protease modified egg yolk produced thermally stable emulsions along with improved emulsifying properties [42]. Hydrolyzed egg yolk proteins had lower molecular weights and exhibited increased protein solubility compared to non-hydrolyzed proteins. Additionally, hydrolysis altered the ionization of amino acids, increasing their net charge. Improved heat stability was achieved by the modified egg yolk emulsions as a result of the combination of lower molecular weight and increased electrostatic charge delaying the gelation point [41,42].

Fermentation with lactic acid bacteria is a safe and natural method to modify food proteins. Egg yolk emulsifying qualities were improved, depending on the length of fermentation, when commercial lactic acid bacteria were used. The emulsifying activity increased with longer fermentation times (3, 6, and 9 h); however, egg yolk fermented for 3 h showed the maximum emulsifying stability, with no significant changes observed after 6 and 9 h of fermentation compared to unfermented samples [88]. Another study by the same group found that lactic acid bacteria fermentation of phosvitin enhanced its emulsifying activity and stability with fermentation times of 3 and 6 h, but these properties decreased when the fermentation time was extended to 9 h. Additionally, the calcium-binding capacity of phosvitin phosphopeptides produced from fermentation-treated phosvitin was greater than that of native phosvitin. Fermented phosvitin phosphopeptides exhibited higher calcium-binding capacity, indicating their potential for therapeutic or health-promoting applications [43].

Furthermore, Daimer and Kulozik, [44] found that phospholipase A₂-treated egg yolk had enhanced emulsifying properties compared with untreated egg yolk. This enhancement was attributed to the presence of lyso-phospholipids and increased protein solubility. Emulsions with enzyme-treated egg yolk were less affected by pH and ionic strength, maintaining their stability even in high-salt environments. Although flocculation was higher in these conditions, the overall rheological and creaming behavior of the emulsions remained unaffected, indicating that the forces driving flocculation are relatively weak in emulsions made with enzyme-treated egg yolk. The increased solubility

of the modified proteins in the egg yolk caused by the enzyme treatment was determined to be the primary reason for the considerable improvement in emulsion characteristics at a low pH. However, this benefit was less pronounced at pH 6.5 and 0.52 M NaCl, where egg yolk proteins are already highly soluble [44]. A recent study by the same group showed that emulsions prepared using phospholipase A₂-modified egg yolk and granules had better stability than those with modified plasma. Additionally, emulsions stabilized with plasma and egg yolk modified by enzymes had greater freeze–thaw stabilities than emulsions stabilized with granules [45].

As mentioned earlier, the type of enzyme influences the degree of hydrolysis and emulsifying properties. Alkaline protease had higher hydrolysis activity than neutral protease and trypsin during egg yolk HDL hydrolysis. The final degrees of hydrolysis were 9.35%, 9.98%, and 10.61% for neutral protease, trypsin, and alkaline protease, respectively. All enzymes enhanced the emulsifying activity and emulsion stability of egg yolk HDL. The optimal hydrolysis times were 1.5, 3.5, and 3.5 h for alkaline protease, neutral protease, and trypsin, respectively [84]. Similarly, subtilisin-assisted enzymatic hydrolysis enhanced the emulsifying qualities of egg yolk granules [89].

All things considered, it may be said that enzymatic treatment can change the emulsifying qualities of egg yolks and be utilized to create food products for different target audiences. To improve the enzymatic hydrolysis procedure and processing environment, more research is necessary. Furthermore, studies should look into how freezing, heat treatment, and pH affect the effectiveness of enzymatic hydrolysis.

3.4. Non-Thermal Treatment

Traditional thermal processing of egg products often compromises quality due to protein denaturation (discussed in detail in Section 3.2). To address these limitations, innovative non-thermal techniques have emerged as alternatives. These methods primarily aim to eliminate microorganisms while preserving product quality, unlike conventional thermal pasteurization. Beyond microbial inactivation, these techniques have shown potential to enhance certain functional properties of egg products. Consequently, researchers are increasingly investigating how these non-thermal processes modify egg components, particularly proteins, and their subsequent impact on functionality.

3.4.1. High-Pressure Technologies

Initially introduced in the dairy industry, homogenization reduced fat globule size, thereby enhancing the physical and chemical stability of milk. This technology was later applied to other liquid foods, including egg products. Gaillard et al. [46] investigated the impact of high-pressure homogenization (175 and 300 MPa, one and four passes) on the protein structure and emulsifying qualities of egg yolk granules, finding that homogenization enhanced both the water and oil binding capacities of egg yolk granule proteins. While a single pass at 175 MPa had no effect on the water binding, increased pressure and the number of passes caused significant improvements. The oil binding capacity increased after both 175 MPa treatments, with further enhancement observed at 300 MPa, independent of the number of passes. Similarly, Suhag et al. [47] reported that egg yolk emulsification abilities might be enhanced through the use of microfluidization, an advanced type of high-pressure homogenization. Emulsifying activity increased at microfluidization pressures of 103 and 138 MPa, but further increases in pressure to 172 and 207 MPa led to a decrease. Likewise, emulsion stability improved at 103 MPa but decreased with further increases in pressure.

The application of high-pressure homogenization or microfluidization disrupts egg yolk granules by breaking down phosphocalcic bonds within the HDL–phosvitin complex, releasing HDLs. This process induces HDL denaturation, reducing particle size and increasing surface hydrophobicity due to the exposure of hydrophobic domains. Dissociated HDLs subsequently interact, forming intermolecular disulfide bonds, which

enhance emulsifying activity and emulsion stability. However, the decrease in emulsifying properties at higher pressures may be attributed to the fact that the resulting polypeptides are too small to stabilize the oil–water interface effectively. Another reason for the reduced emulsifying properties could be the loss of protein solubility due to the high microfluidization pressure [46,47]. Additionally, Suhag et al. [47] reported an increase in particle size at the highest microfluidization pressure of 207 MPa as a result from the increased interaction and subsequent re-coalescence of smaller fat and protein particles in egg yolk, a phenomenon referred to as ‘over-processing’ [90,91].

High-pressure processing (HPP) is another non-thermal technology employed in the food industry. This method involves subjecting hermetically sealed food products to extremely high pressures, typically ranging from 100 to 800 MPa, at an ambient temperature. The process utilizes a liquid medium, commonly water, to uniformly transmit pressure throughout the food product. Giarratano et al. [48] employed HPP (400 MPa for 5 min) followed by ultrafiltration to produce a new emulsifying ingredient from egg yolk. This combined treatment enabled the selective recovery and concentration of phosvitin in the retentate. Emulsions made with the ultrafiltration retentate of plasma from HPP-treated granules were more stable against flocculation and creaming than those made without HPP [48]. Additionally, applying moderate pressure (50–400 MPa, 5 min) as a pre-treatment before thermal pasteurization improved the emulsifying activity (35–52%) and emulsion stability (41–66%) of egg yolk. The combined effects of pressure and thermal treatment also achieved higher microbial inactivation, as pressure pre-treatment reduced the thermal resistance of microorganisms [72]. Another application of high pressure is its use in hyperbaric storage. Storing liquid egg yolk under hyperbaric conditions at 200 MPa maintained its emulsifying activity for up to 28 days [49].

HPP is a highly developed non-thermal technology, with a technology readiness level typically between 8 and 9. While the technology readiness level can vary based on specific applications, HPP machines used for pasteurization-equivalent processing of food and beverages are considered to be at TRL 9. Recently, a new HPP application for bulk processing of pumpable products has been introduced by Ateliers Hermes Boissons in France, which is estimated to be at technology readiness level 8 [92]. As of 2019, there were over 550 industrial HPP machines in operation globally, primarily in North America, followed by Europe and Asia [93,94].

Although the initial cost of HPP machines has decreased in recent years, investment and operating expenses remain a significant barrier for smaller producers. Larger-scale producers often require more advanced equipment, such as automated systems or multiple intensifiers, to meet their production needs, which can increase investment costs. The primary challenge lies in the fact that this technology is significantly more costly and time-consuming compared to the pasteurization techniques (such as heat treatments) and storage methods (like refrigeration) that are currently used in the industry.

3.4.2. High-Intensity Ultrasound

Ultrasound is a promising green technology for enhancing the functional properties of food proteins. High-intensity ultrasound (HIU), with frequencies between 16 and 100 kHz and power levels from 10 to 1000 W/cm², has a wide range of potential applications in the food industry. When applied to liquid products, HIU can create cavitation bubbles that collapse violently, causing mechanical damage to the proteins. This damage can improve the functional properties of the proteins by altering their interfacial and structural characteristics [95]. For instance, compared to the untreated egg yolk solution, the emulsifying activity of the egg yolk solution treated with HIU was significantly higher. However, there were no notable differences in emulsifying activity among the different ultrasonic power levels. In contrast to emulsifying activity, the emulsion stability index of the egg yolk solution initially increased with rising ultrasonic power but decreased when the power exceeded 225 W [50]. Additionally, as reported by Geng et al. [51], the emulsifying activity of egg yolk granules showed a modest increase with HIU power

levels between 90 and 270 W, achieving a 9.54% increase ($p < 0.05$) at 270 W. However, for the emulsifying stability index of yolk granules, the HIU treatment resulted in some changes, though these were not statistically significant.

A recent study examined the impact of various HIU power levels (75, 150, and 210 W), temperatures (30, 45, and 60 °C), and treatment durations (3, 6, and 9 min) on the emulsifying properties of egg yolk. No significant differences in emulsifying activity were found among the groups at 30 °C. Meanwhile, treatments lasting 6 and 9 min at 45 and 60 °C, respectively, showed the highest emulsifying activity at an HIU power of 75 W. At 30 °C, the emulsion stability index of all groups improved with increasing HIU power and treatment time, with the 210 W treatment for 9 min achieving the highest stability. At 45 °C, the 150 and 210 W treatments exhibited similar trends in emulsion stability index, with the three 150 W treatments for 9 min and the 210 W treatments for 3 and 9 min showing significantly higher stability than other groups. However, at 60 °C, only the 210 W treatment for 3 min resulted in the highest emulsion stability [52].

Xie et al. [53] compared the effects of solvent (ethanol) and HIU treatment on the emulsifying properties of egg yolk LDL. They found that solvent treatment decreased the emulsifying properties, while HIU treatment had no significant effect. Yu et al. [96] demonstrated that applying HIU to trypsin-modified egg yolk significantly improved emulsifying activity. The emulsifying activity at HIU treatment (480 W for 30 min) was 36.26% higher. Additionally, while emulsifying activity increased with power, treatment time at 480 W did not significantly affect it.

HIU treatment broke down yolk granules, releasing components like proteins, calcium, phosphorus, and phospholipids. Additionally, it increased the polarity of the microenvironment and strengthened hydrogen bonding within the granules. LDL molecules within the granules underwent partial rearrangement. These changes collectively altered the zeta potential, surface hydrophobicity, and sulfhydryl group content of yolk granules, and improving emulsifying properties [50,51].

However, further comprehensive studies under controlled conditions are needed to better understand the effects of various variables on the emulsifying properties of egg yolk and its components.

3.4.3. Other Techniques

Li et al. [54] examined how varying ozone treatment durations (0–40 min) affected the emulsifying properties of egg yolk. It was discovered that moderate ozone oxidation (10 and 20 min) could partially unfold egg yolk proteins, exposing their internal hydrophobic groups and increasing molecular flexibility. This unfolding allowed the protein molecules to bind more easily to the oil–water interface, thereby improving the emulsifying properties of egg yolk. However, longer ozone treatments (30 and 40 min) could lead to covalent cross-linking of proteins, forming smaller aggregates and reducing protein flexibility. This created more physical barriers and resulted in less stable interfacial films in egg yolk emulsions.

Zhang et al. [97] reported that ball-milling treatment for 20, 40, and 60 min of phosvitin improved its emulsifying activity by 3.0, 2.9, and 2.5 times, while the emulsifying stability decreased by 40.0%, 34.0%, and 34.5%, respectively. Ball-milling treatment enhanced phosvitin hydrophilic–hydrophobic balance by making its surface more hydrophobic, which improved its emulsifying properties. Similarly, magnetic-assisted freezing had been found to improve the emulsifying properties of egg yolk [98].

4. Applications of Egg Yolk and Its Components as Emulsifying Agents

4.1. Egg Yolk-Based Delivery System

HIPes are a unique emulsion system with growing applications in various industries, including food, pharmaceuticals, and cosmetics. As carriers for nutraceuticals, HIPes offer several advantages, such as their high internal phase volume fraction ($\geq 74\%$) and

controllable viscoelastic properties. The thick particle layer at the oil–water interface provides protection against oxidation and UV light, ensuring the stability of encapsulated bioactive ingredients. Additionally, HIPEs have a long shelf life due to their low water activity, which inhibits microbial growth [99].

HIPEs stabilized with egg yolk-modified starches including egg yolk–hydroxypropyl distarch phosphate (EY-HPDSP) and/or egg yolk–octenyl succinic anhydride starch (EY-OSA) showed enhanced physical stability and lutein retention when stored at 4 °C. However, HIPEs stabilized with EY-HPDSP exhibited superior physical stability, lower lipid oxidation, and higher lutein retention at 37 °C compared to those stabilized with EY-OSA. These differences were attributed to the smaller droplet size and restricted water and oil proton movement in the EY-HPDSP-stabilized HIPEs. In vitro digestion studies revealed that a high concentration of HPDSP in HIPEs improved lutein bioaccessibility. These findings suggest that egg yolk-modified starch complex-stabilized HIPEs could be valuable for developing nutrient delivery systems [100]. Furthermore, Li et al. [101] showed that the droplet size, colloidal stability, gel network, and encapsulation capability of HIPEs prepared with LDL were significantly influenced by the concentration of LDL and the volume fraction of oil. The optimal formulation was found to be 2% LDL and 80% oil. Encapsulating curcumin into LDL-stabilized HIPEs did not affect droplet size or gel stability (shown in Figure 6A). Due to LDL's natural nanostructure and its thick coating around oil droplets, the photostability of curcumin was significantly improved when encapsulated in LDL-stabilized HIPEs. The encapsulated curcumin retained its free radical scavenging properties even after exposure to UV radiation. Additionally, the lipolysis rate of oil was significantly accelerated in HIPE systems, and LDL nanoparticles played a crucial role in enhancing the bioaccessibility of curcumin during in vitro digestion. LDL-stabilized HIPEs showed promise as delivery systems for lipophilic compounds and as potential replacements for saturated fats in semi-solid foods. These HIPEs can improve the texture of food products while offering additional health benefits.

Zhou et al. [102] used 1-ethyl-3-(3-dimethylaminopropyl) and carbodiimide/N-hydroxysuccinimide chemical cross-linking to improve the stability of LDL/pectin and LDL/carboxymethyl cellulose nanogels for curcumin delivery. The cross-linked nanogels exhibited enhanced stability in both fasting and fed gastric conditions, as well as in the intestine, demonstrating resistance to digestive enzymes and aggregation under acidic conditions. The cross-linking process also increased the encapsulation efficiency of curcumin. Additionally, cross-linked nanogels exhibited a more controlled release profile under extreme low pH conditions compared to uncross-linked nanogels. Overall, this study presented a promising approach for the oral delivery of nutrients, offering a cost-effective solution for the functional food industry.

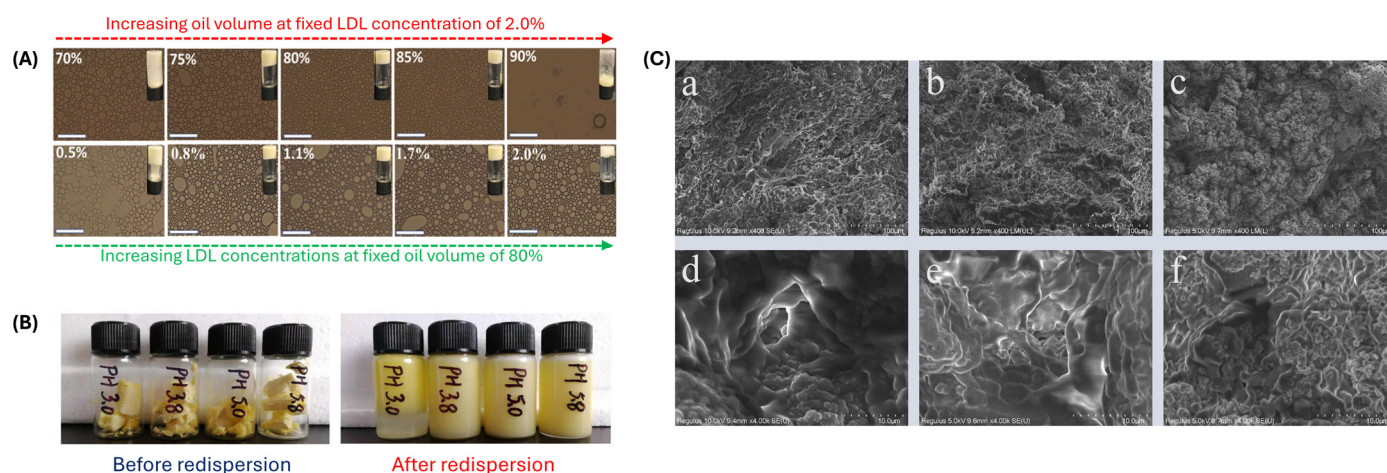


Figure 6. (A) The optical microscope images of the emulsions at different oil volume fractions with fixed LDL concentration at 2.0%, and different LDL concentrations with fixed oil volume at 80%

(scale bar: 100 μm); Reprinted with permission from Li et al. [101]. 2022, Elsevier. **(B)** Visual observations of the redispersibility of the lyophilized emulsion gels; reprinted with permission from Yang et al. [15]. 2020, Elsevier. **(C)** Changes in the microstructure of egg-based yoghurt during fermentation; 0 h (**a,d**), 4 h (**b,e**), and 10 h (**c,f**) (**a–c**: 400 $\times$; **d–f**: 4000 $\times$) (Time: 0–10 h, temperature: 42 $^{\circ}\text{C}$, fermentation strain: *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*); Reprinted with permission from Zang et al. [103]. 2023, Elsevier.

4.2. Low-Fat Mayonnaise

An increasing body of research indicates that consuming high-fat foods in excess raises the chance of developing chronic illnesses like diabetes, heart disease, and obesity. As a result, the food industry is actively seeking reduced-fat alternatives that retain the sensory appeal of their full-fat versions [104]. Mayonnaise made with egg yolk granules (cholesterol content about one-sixth that of yolk-based mayonnaise) exhibited rheological and sensory properties most similar to the commercial sample and/or mayonnaise prepared with raw egg yolk. In sensory evaluations, egg yolk granules mayonnaise received a high score, closely resembling the commercial reference. Moreover, there were no noticeable rheological variations between mayonnaises created with the same amount of granules, whether they were freeze-dried or fresh. Freeze-drying effectively reduces microbial count and extends shelf life, making it a valuable technology for practical applications. Stevens' equation was used to examine the connection between rheological measures and the assessment of sensory texture. It was found that the sensory system starts to lose its ability to precisely perceive textural qualities after about 30 s in the mouth due to salivation and saturation. To put it succinctly, granule-based low-cholesterol mayonnaise made without the addition of thickeners showed technological and sensory qualities similar to those of commercial mayonnaise. This demonstrates how egg yolk granules can be used in food manufacturing as a useful emulsifying agent [105].

Furthermore, the electrostatic interaction between alginate and egg yolk proteins at acidic pH enabled the creation of emulsion gels with rheological properties resembling full-fat mayonnaise, but with reduced oil content. By adjusting the amount of vinegar added, the textural characteristics of these emulsion gels can be modified while maintaining their visual appearance. Additionally, solid emulsions based on the alginate–egg yolk complex can be prepared with acceptable redispersibility (Figure 6B), making them potential carriers for oil-soluble functional compounds [15].

4.3. Egg Yolk Gels

Proteins aggregate and cross-link to form protein gels, which are materials having a three-dimensional network structure. Foods including meat products, yogurts, and jelly frequently include these gels. Protein gel formation is a multifaceted process that requires several structural modifications. Eggs are frequently used to make a variety of gel-based dishes, including fried, salted, preserved, and poached eggs [106,107]. For example, basic egg gels could be made by mixing egg yolk or its fractions with carrageenan. Compared to gels made exclusively of carrageenan, the addition of egg derivatives enhanced both the mechanical and sensory qualities of the final products. Additionally, egg yolk and its fractions contributed valuable nutritional compounds to the developed gels. The choice of egg derivative (yolk, plasma, or granules) influenced the final product's nutritional profile, mechanical properties, and sensory characteristics. Furthermore, various flavors, spices, and cocoa can be incorporated to enhance the taste and appearance of the products. The base gels can also be used to develop energy gels, specifically glucose gels, which are commonly used by athletes engaged in endurance sports like cycling [108].

Recently, Zang et al. [103] investigated the mechanisms behind acid-induced gelation in egg-based yoghurt during fermentation. As fermentation progressed, pH decreased, leading to a decline in water-holding capacity due to the egg proteins approaching their isoelectric point. Gel hardness increased, while fracturability remained low, with a compact microstructure (Figure 6C). Hydrophobic interactions were the primary driving

force behind gel formation, increasing significantly during the process. Conversely, ionic, hydrogen, and disulfide bonds decreased notably. While these latter bonds contributed to gel formation, their effects were less pronounced compared to hydrophobic interactions.

4.4. Baked Products

Egg yolk is an essential ingredient in many culinary dishes, particularly sweet baked goods. Due to the high fat content in these products, emulsifiers are essential, and egg yolk's emulsifying properties play a crucial role. Furthermore, the coagulation and gel-forming properties of egg yolk proteins affect the texture and other properties of baked goods.

According to Marcet et al. [109], egg yolk granules have the potential to be used in place of entire egg yolks in gluten-free muffin recipes as a low-cholesterol substitute. The rheological properties of gluten-free batter made with egg yolk granules were comparable to those made with whole egg yolk. However, during baking, the higher protein content and lower lipid content in the egg yolk granules led to noticeable changes in the batter's viscoelastic behavior due to interactions between the granular components. These changes resulted in a baked muffin that was harder and had a slightly different color compared to one made with whole egg yolk [109]. Furthermore, partially replacing the plasma fraction of egg yolk with the granular fraction in muffins led to non-linear changes in their rheological and textural properties. However, substituting up to 50% of the whole egg yolk with granules had minimal impact on physical properties and resulted in slightly taller muffins. The results suggest that the plasma fraction is crucial for determining the texture and shape of egg-based bakery products. The granular proteins seem to only influence these properties when the plasma protein content is low. To create a muffin with only egg yolk granules, an emulsifier (E471, mono- and diglycerides of fatty acids) is necessary to achieve a product similar to one made with whole egg yolk. This is especially important because the granular fraction has significant nutritional value compared to the plasma fraction [110].

5. Egg Yolk in Patented Food Solutions

With its rich nutrient profile and exceptional emulsifying properties, egg yolk has found its way into numerous patented food solutions. This unique ingredient not only enhances the texture and stability of food products but also embodies the trend toward utilizing natural ingredients in food innovation. Table 2 provides a summary of patented food solutions.

Table 2. Application of egg yolk in patented food solutions.

Food solution	Composition	Claims	Reference
Edible oil-in-water emulsion	• 30–78 wt.% of oil • 65–20 wt.% water • 0.5–6 wt.% egg yolk proteinaceous component by dry weight ○ 60–75 wt.% LDL ○ 8–14 wt.% livetin ○ 11–18 wt.% HDL ○ 2–5 wt.% phosvitin.	The inventors developed a method for producing high-quality, low-fat mayonnaise using egg yolk plasma and granules in proportions that mimic the composition of traditional egg yolk.	[111]
Functional food product	Ready-to-use drink (so-called “shot”) containing egg yolk (20 mL, fixed) with different combinations provided below: • 80 mL low-fat yoghurt and 6 g vanilla sugar • 80 mL buttermilk and 6 g vanilla sugar	The invention pertains to food products designed to provide health benefits beyond basic nutrition, specifically through regular consumption. It details a method for producing	[112]

	• 20 mL raspberry syrup, 60 mL skimmed milk (0% fat) • 80 mL fruit flavored yoghurt and 2.5 g vanilla sugar • 80 mL orange juice • 80 mL multifruit juice Ready-to-use dessert (a pudding) containing 1 L semi-skimmed milk, one vanilla pod, six egg yolks, 40 g custard and 60 g sugar.	functional foods or nutraceuticals that contain high levels of pharmacologically active nutrients, optimizing their absorption in the gastrointestinal tract. These products are formulated for long-term use and include methods for nutrient supplementation in individuals who need it.
Heat-stable emulsion	Heat-stable emulsion contained: • 3–85 wt.% oil • 12–92 wt.% water • 0.1–1.0 wt.% egg yolk lecithin • 0.1–5.0 wt.% water-insoluble cellulosic fiber • 0–20 wt.% one or more other edible ingredients	The invention relates to a heat-stable oil-in-water emulsion, specifically one that can withstand temperatures exceeding 90 °C for at least 5 min. This emulsion contains non-modified egg yolk lecithin and water-insoluble cellulosic fiber derived from fruit. [113]

De et al. [111] developed a method for creating high-quality, low-fat mayonnaise using egg yolk plasma and granules that retained the composition of traditional egg yolk. The approach maximized the use of egg yolk materials produced during fractionation and can utilize fresh or pasteurized egg yolk. The resulting edible oil-in-water emulsion had a pH between 3 and 5 and consisted of 30–78 wt.% oil, 20–65 wt.% water, and 0.5–6 wt.% egg yolk protein. The protein composition of the emulsion closely resembled that of conventional egg yolk, comprising 60–75 wt.% LDL, 8–14 wt.% livetin, 11–18 wt.% HDL, and 2–5 wt.% phosvitin.

According to Thielen et al. [112], a combination of egg yolk and an aqueous dispersion created an excellent carrier matrix for the efficient delivery of pharmacologically active nutrients with high bioavailability, particularly lutein and zeaxanthin, thanks to the emulsifying and stabilizing properties of egg yolk phospholipids. Furthermore, products were formulated as daily “shots” and “dessert” without compromising their appearance, texture, taste, or flavor, and they showed high acceptance among test subjects over a three-month study period. Importantly, regular consumption of these functional foods did not significantly increase plasma cholesterol levels, unlike traditional egg supplements.

Another interesting food application reported by Van et al. [113] was the preparation of heat-stable oil-in-water emulsions without the use of enzyme-modified egg yolk lecithin or synthetic emulsifiers. The inventors discovered that the limited heat-stability provided by egg yolk can be improved dramatically by the introduction of water-insoluble cellulosic fiber. Thus, the combined use of egg yolk and water-insoluble cellulosic fiber enabled the preparation of oil-in-water emulsions that can suitably be heated in an oven at a temperature of 90 °C or more for several minutes without breaking up and/or without developing undesirable textural changes.

Recently, another patent assigned to De et al. [114] showed the development of double emulsion involving multiple steps: first, an oil phase was created with vegetable oil and fat crystals; then, this phase was combined with water to form a primary water-in-oil emulsion. Next, this emulsion was mixed with a secondary water phase containing oil-in-water emulsifiers to create the final water-in-oil-in-water emulsion. Finally, egg yolk was incorporated into the emulsion, enhancing its emulsifying properties. The composition allows for the use of egg yolk in amounts ranging from 0.5 to 12 wt%,

contributing phospholipids that further stabilize the emulsion. This innovative formulation can be used in products like emulsified sauces, including mayonnaise, and showcases egg yolk's essential role in achieving desired texture and stability without relying solely on synthetic emulsifiers.

6. Conclusions and Future Perspectives

Emulsification is an important functional attribute of egg yolk, owing to its high protein and lipid content. The variety and abundance of these components provide the foundation for emulsification. When egg yolk proteins are subjected to treatments such as heat, alkaline conditions, or salt, their natural structures unfold, leading to interactions between proteins and between proteins and lipids, which can alter their emulsifying abilities. Different treatments modify egg yolk proteins in unique ways, resulting in various three-dimensional network structures and distinct emulsification mechanisms. It is essential to comprehend the structure–function correlations of egg yolk protein-stabilized emulsions in order to increase their applications in the food industry and utilize them in food products. The emulsifying qualities of egg yolk can be regulated and possibly customized for more specialized uses in the food and pharmaceutical sectors by modifying processing techniques and food compositions.

Nevertheless, most laboratory-scale studies are somewhat limited because they often focus on controlled environments that do not fully reflect the complexities of real-world food systems. These studies typically examine one factor in isolation, without considering interactions with other ingredients or variables such as temperature and processing techniques. Additionally, components of egg yolk, like plasma and granules, exhibit excellent emulsifying properties and could potentially replace whole egg yolk in certain applications. Consequently, additional studies are required to fully comprehend egg yolk components' emulsifying abilities. It is crucial to take into account the intricate linkages between the various components, the settings in which they operate, their interactions, and their unique qualities when utilizing egg yolk or its fractions for industrial purposes.

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