Bioactivities of hen's egg yolk phosvitin and its functional phosphopeptides in food industry and health

Birsen Yilmaz and Duygu Ağagündüz

Abstract: Phosvitin, one of the most noteworthy bioactive components of hen egg yolk, is an amphiphilic protein that stands out with its unique composition and functionality in the food industry and health. Phosvitin consists of 4% of egg yolk dry matter and 11% of egg yolk proteins. It is considered as the most phosphorylated protein with 10% phosphorus. Besides, some potential novel phosphopeptides containing clusters of phosphoserines can be derived from hen's egg yolk phosvitin. Phosvitin, which has many functional features thanks to its unique structure, is known primarily for its metal bonds binding (iron, calcium, etc.) feature. On the other hand, its phosphopeptides may increase the bioavailability of metals compared to phosvitin. Although this feature of phosvitin may partially decrease the bioavailability of especially iron in the egg, it allows the phosvitin to have many bioactivities in the food industry and health. Lipid oxidation, which is a serious problem in the food industry, can be inhibited by adding phosvitin and its derived phosphopeptides to the food production chain via inhibiting bivalent iron. Because phosvitin is an amphiphilic protein capable of chelating, it also shows potential antibacterial effects against the Gram-negative bacteria. Moreover, the literature has recently been attempting to define the promising relationship between phosvitin and its phosphopeptides and plenty of health-promoting activities such as immune-enhancing, melanogenesis inhibitor, anti-ageing, and anticancer. In this review, current information on the hen's egg yolk phosvitin and its phosphopeptides and their bioactivities in the food industry and health are discussed and some future directions are given.

Keywords: antioxidant, health, hen egg yolk, metal-binding, phosphopeptide, phosvitin

1. INTRODUCTION

Egg is a very important food in basic human nutrition besides having very important functional qualities in the food industry. Hen egg is defined by European Union (EU) as follows; "eggs" means eggs in the shell—other than broken, incubated, or cooked eggs—that are produced by hens of the species Gallus and are fit for direct human consumption or for the preparation of egg products (EU, 2008). Egg consists of three different parts as 63% of egg white albumen, 27.5% of egg yolk, and 9.5% of the eggshell with membranes (Cotterill & Geiger, 1977). Although the basic composition of the egg has been considered in this way for many years, some minor changes have occurred in its nutritional composition due to recent changes in the production methods. In general, it can be expressed as 56% to 64% egg white albumen, 25% to 33% egg yolk, and 9% to 11% eggshell with membranes (Cherian, Holsonbake, & Goeger, 2002). The main components of the egg consist of water (76%), proteins (12%), and lipids (9.51%). Besides, it also contains low amounts of carbohydrates (0.72%), minerals, and vitamins (USDA, 2020). Almost all of the egg lipids are found in egg yolk (mainly in the form of lipoproteins), whereas egg proteins are distributed in egg white albumen (50%), egg yolk (44%), and eggshell with membranes (6%; Sugino, Nitoda, & Juneja, 1997). Because egg proteins are high-quality proteins, they are used as the gold standard to measure the quality of other food proteins (Seuss-Baum, 2007).

Although the main components of egg white albumen are water and proteins (mainly ovalbumin, ovotransferrin, and ovomucoid), the main components of egg yolk, which is a homogeneous emulsifying liquid, consist of proteins and lipids. Egg yolk is mainly made up of livetins (α, β, and γ forms), lipovitellins (α and β forms), low-density lipoproteins (LDL), and phosvitin proteins (Huang & Ahn, 2019). After egg yolk is diluted and centrifuged with water or sodium chloride, two different fractions are obtained known as granules/pellets (suspended particles) and plasma/supernatant (clear, yellow liquid; Laca, Paredes, & Díaz, 2012). Plasma fraction constitutes 78% of egg yolk dry matter and 22% of granules. The main components in plasma are LDLs (85%) and livetins (15%). Granules mainly consist of lipovitellin (70%) and phosvitin (16%; Anton, 2013; Burley & Cook, 1961).

Phosvitin is one of the egg (usually hen eggs) yolk phosphoproteins known as the natural protein with the highest phosphorus content in nature. By using hen egg yolk phosvitin, it is possible to obtain polypeptides with many functional properties. Compared to the types of phosvitin obtained from other sources, hen egg yolk phosvitin is very popular in the food industry not only for its functional properties but also for its potential health effects (Jung, Jo, Kang, Ahn, & Nam, 2012; Lee, Moon, Hong, Ahn, & Paik, 2020). Besides its functional properties, many studies have shown that it has several positive effects on human health (Lee et al., 2017; Moon et al., 2014). Although phosvitin seems to reduce the nutritional value of egg yolk due to its mineral binding feature, the antioxidant, antibacterial, immune-enhancing, melanogenesis inhibitory, antigenotoxic, anti-elastase, and anti-hyaluronidase

JFDS-2020-0841 Submitted 5/20/2020, Accepted 8/14/2020. *Author Yilmaz is with Faculty of Health Sciences, Gazi University, Department of Nutrition and Dietetics, Beşevler, Ankara, 06560, Turkey. Author Yilmaz is also with Faculty of Health Sciences, Çukurova University, Department of Nutrition and Dietetics, Sarıçam, Adana, Turkey. Author Ağagündüz is with Faculty of Health Sciences, Gazi University, Department of Nutrition and Dietetics, Beşevler, Ankara, 06560, Turkey. Direct inquiries to author Yilmaz (E-mail: dytbirsen@gmail.com).*

© 2020 Institute of Food Technologists®
doi: 10.1111/1750-3841.15447
Further reproduction without permission is prohibited

Journal of Food Science A Publication of the Institute of Food Technologists

Table 1–Amino acid composition of hen egg phosvitins and its fractions.

Amino acids	Hen egg phosvitin[a]	Hen egg phosvitin[b] (mol%)	Hen egg phosvitin[c] (mol%)	α-Phosvitin[d]	β-Phosvitin[d]
Ala	7	5.3	5.5	1.9	0.8
Leu	3	2.3	1.7	ND[e]	0.3
Ile	2	1.5	1.1	0.4	0.2
Val	3	2.5	2.4	0.5	0.7
Pro	3	2.1	1.5	0.7	0.6
Phe	1	1.7	1.7	0.6	0.2
Trp	1	ND	ND	0.2	0.2
Met	1	1.8	1.6	ND	ND
Ser	123	36.1	34.9	40.5	42.5
Thr	4	3.1	2.8	1.4	0.3
Tyr	1	0.8	0.8	0.8	ND
Asp	13	8	8.6	2.7	2.4
Glu	10	8.3	9.0	5.3	1.8
Gly	5	3.5	3.7	1.4	0.6
Lys	15	9.2	10.2	5.6	3.3
Arg	11	6.9	7.3	2.4	1.9
His	13	6.4	6.7	0.6	2.3
Cys	ND	0.5	0.5	ND	ND
Total	216	100.0	100.0		

[a]Data reported by Byrne et al. (1984), hen egg phosvitin, predicted by nucleotide sequence.
[b]Data reported by Guérin-Dubiard et al. (2002), henn egg phosvitin.
[c]Data reported by Guérin-Dubiard et al. (2002), commercial phosvitin.
[d]Data reported by Itoh et al. (1983), henn egg phosvitin.
[e]ND, no data.

activities compensate for this negative quality (Jung et al., 2012; Kovacs-Nolan, Phillips, & Mine, 2005; Lee et al., 2017, 2020; Moon et al., 2014; Sattar Khan et al., 2000). In this review, based on the chemical composition and functional qualities of hen egg yolk phosvitin, current literature about the hen's egg yolk phosvitin and its phosphopeptides (PPP) and their bioactivities in the food industry and health will be discussed.

2. HEN EGG YOLK PHOSVITIN

Phosvitin, which constitutes 4% of egg yolk dry matter and 11% of egg yolk proteins, is considered as the natural protein containing the most phosphorus with 10% phosphorus content. Approximately 80% of phosphorus in egg yolk is found in phosvitin. More than half of the amino acids (50% to 57%) are serine as phosphoserine, and almost 90% of these serine amino acids are phosphorylated (Allerton & Perlmann, 1965; Joubert & Cook, 1958). According to the amino acid sequence of the phosvitin reported by Byrne et al. (1984), phosvitin contains 216 amino acid residues, 123 of which are serine amino acid (Byrne et al., 1984). The relatively high amount of phosphoryl groups in the amino acid sequence of the phosvitin gives the protein a large central hydrophilic portion surrounded by two small hydrophobic fragments at the N-terminal and C-terminal. The fact that the phosvitin has a large number of negative charges and an unusually low proportion of nonpolar hydrophobic side chains makes it an enormous hydrophilic structure. Only □10% of the amino acids are hydrophobic amino acids. Because of the high phosphoric acid due to serine residues, phosvitin can act as a liquid polyanion. Thanks to its polyanionic character (the isoelectric point is around pH 4), phosvitin has a very strong metal chelation quality (Chay Pak Ting, Pouliot, Gauthier, & Mine, 2010; Dickinson, Pinfield, & Horne, 1997; Strixner, 2011).

Moreover, due to its polyanionic properties, phosvitin is known to have better emulsifying activity and emulsion stability compared to bovine serum albumin, β-casein, and soy protein (Sattar Khan, Babiker, Azakami, & Kato, 1998). The functional (metal chelation, antioxidant feature, emulsion activity, etc.) and nutritional properties of phosvitin are mainly related to its unique amino acid composition (Strixner, 2011). The amino acid composition of phosvitin is shown in Table 1 (Byrne et al., 1984; Guérin-Dubiard, Anton, Dhene-Garcia, Martinet, & Brulé, 2002; Itoh, Abe, & Adachi, 1983).

The structure of proteins is investigated in four different stages: primary, secondary, tertiary, and quaternary structures. The primary structure shows the number and sequence of amino acids in a protein, whereas secondary and tertiary structures are formed with molecular conformations (Vasudevan, Sreekumari, & Vaidyanathan, 2017). Proteins sometimes appear in molecular aggregates arranged in a regular geometric style, called quaternary structure (Weder, & Belitz, 2003). Among the proteins found in nature, phosvitin, which is considered as the highest phosphorylated protein, has an unusual primary structure (Lv, 2017). The half of its secondary structure is β-sheets, whereas 43% is reported to consist of the random coil in Fourier transform infrared spectroscopy (Chay Pak Ting, Pouliot, Gauthier, & Mine, 2013). The secondary structure of phosvitin is significantly affected by heat treatment at 100 °C for 20 to 60 min (Samaraweera, 2012). Changes in the secondary and tertiary structures of proteins are related to surface activities that will affect their emulsifying properties (Cui, Li, Lu, Liu, & Duan, 2019). The hydrophobic terminal structures of phosvitin can be adsorbed at the interface of the oil droplets to stabilize the emulsions. Emulsifying properties of phosvitin can be affected by various factors such as ionic strength, pH, degree of aggregation, freezing/thawing cycles, or heat treatment (Giarratano et al., 2020). In addition, protein concentration, oil volume fraction, emulsion mixing speed, and mixing time can also affect the emulsion. Compared with bovine serum albumin, phosvitin is reported to be a better emulsifier at pH 7 (Chung & Ferrier, 2006). Besides, Jiang et al. (2020) conducted a study to develop a new antioxidant emulsifier (phosvitin-gallic acid complex, 1.5 mg/mL gallic acid with pH 9.0) in food emulsion systems. The combination of the phosvitin-gallic acid slightly improved the emulsifying activity and stability, while significantly increased antioxidant activities (Jiang et al., 2020).

Phosvitin is a phosphoglycoprotein, which is a mixture of two polypeptides, α-phosvitin and β-phosvitin. In 1967, Taborsky and Mok identified these components as major and minor phosvitins and determined their molecular weights for the first time (40,000 and 36,000 Da, respectively; Taborsky & Mok, 1967). In the following years, major and minor phosvitins, which are the main components of phosvitin, were defined as α and β in the sodium dodecyl sulfate polyacrylamide-gel electropherogram and the molecular weights of phosvitin components were reported to vary between 18,500 and 60,000 Da (Abe, Itoh, & Adachi, 1982). α-Phosvitin and β-phosvitin are structures formed by the combination of many different polypeptides. α-Phosvitin contains three/four subunits with molecular weights 35,000 to 40,000 Da whereas β-phosvitin contains 45,000 Da four/five subunits (Anton, 2013). β-Phosvitin has more (42.5 mol/10^4 g of protein) serine residue compared to α-phosvitin (40.5 mol/10^4 g of protein). Besides serine, the other amino acids found in high ratios are lysine, histidine, and aspartic acid in β-phosvitin as well as lysine, glutamine, aspartic acid, and arginine in α-phosvitin. Phosvitin, α-phosvitin, and β-phosvitin molecules contain 6.5%, 1.94% (1.59% hexose, 0.15% hexosamine, and 0.20% sialic acid), and 6.01% (2.65% hexose, 1.16% hexosamine, and 2.20% sialic acid) carbohydrates, respectively (Itoh et al., 1983). Phosvitin is relatively heat resistant. Phosvitin solution does not precipitate after heating at 100 °C with the pH between 4 and 7 (Chay Pak Ting et al., 2013).

2.1 Mineral binding activity of hen egg yolk phosvitin

Hen egg yolk phosvitin has many biological and functional properties due to its unique structure as well as physical and chemical qualities. Because it has a polyanionic structure, phosvitin has very strong metal chelation for cations (Strixner, 2011). A hen egg contains 0.875 mg/50 g of Fe (USDA, 2020); however, 95% of Fe in egg yolk (egg yolk contains 5.96 mg Fe/g phosvitin) is bound to phosvitin and is in ferric form (Albright, Gordon, & Cotterill, 1984). The high metal binding capacity of phosvitin makes phosvitin a potential antioxidant, especially against Fe-induced oxidative damage (Lu & Baker, 1986). A very small amount of Fe found in egg albumin in complexes with ovotransferrin protects the egg from bacterial infections (Schiavone & Barroeta, 2011).

Phosvitin and ovotransferrin, which are egg proteins, are known as very strong metal ion binding proteins. This binding allows proteins to acquire various biological properties, especially antioxidant and antibacterial activity (Anton, Nau, & Guérin-Dubiard, 2011). Due to the specific structure of the phosvitin, it shows a strong affinity for metal ions, especially Fe. In a study investigating the Fe binding capacity of phosvitin under different conditions (pH 3.0 to 7.0 and ionic strength 0.10 to 0.60), it was reported that pH 6.5 and 0.15 M of ionic strength (the maximum iron-binding capacity; 115 μg of iron/mg of phosvitin) were the best values for the phosvitin–Fe complex, that Fe binding property was not affected by 90 °C heat treatment or 600 MPa high pressure, and that it maintained its high Fe binding capacity (Castellani, Guérin-Dubiard, David-Briand, & Anton, 2004). Under ideal physicochemical conditions, phosvitin molecule binds a Fe for both phosphate groups, which is considered to be the saturation capacity of the phosvitin. Because there are more than 120 phosphate groups in a phosvitin molecule, it can bind 60 Fe molecules (Taborsky, 1980). Acidic pH values with low ionic strength have good potential for the metal bonding of phosvitin (Castellani et al., 2004).

When the effect of heat and some food additives (sodium chloride, citric acid, or ethylenediaminetetraacetate disodium salt, EDTA) on the release of Fe bound by phosvitin was examined, it was found that after heating the phosvitin at 110 °C for 20 and 40 min, the bound Fe was not released and very strong Fe binding continued in the phosvitin. As for the added substances, it was reported that Fe was released, only in EDTA without any heating process, and this situation was related to EDTA forming stronger ligand with Fe than phosvitin (Albright et al., 1984).

Phosvitin–Fe complexes formed under optimum physicochemical conditions are very stable. This strong stability of the complex may be due to tetrahedral stoichiometry or octahedral stoichiometry, which increases the activation energy required for the decomposition of phosvitin–Fe complexes (Castellani et al., 2004). Once the Fe ion is bound to a phosphate molecule, another phosphate molecule can form tetrahedral stoichiometry by completing the binding process, whereas the other two molecules, like water molecules, can complete the system to form octahedral stoichiometry (Albright et al., 1984). Fe cannot be separated from the phosvitin–Fe complex unless there is a chelating agent (such as EDTA, *o*-phenanthroline) that has a stronger binding capacity than phosvitin (Albright et al., 1984; Castellani et al., 2004).

Although the amount of Fe bound by various types of phosvitin is different, hen egg yolk phosvitin is the type of phosvitin that has the highest amount of Fe binding capacity (Guérin-Dubiard et al., 2002). Guérin-Dubiard et al. (2002) compared the Fe binding properties of commercial hen phosvitin, hen egg phosvitin, and deep water fish phosvitin. Commercial hen phosvitin and hen egg phosvitin bound 36 mol Fe/mol phosvitin and 32 mol Fe/mol phosvitin, respectively; whereas fish phosvitin bound 9.5 and 3.6 Fe/mol phosvitin. Although it binds a lower amount of Fe than egg yolk phosvitin, it can be said that fish phosvitin has a better binding capacity compared to ovotransferrin, which binds 2 moles of Fe per protein mole (Guérin-Dubiard et al., 2002).

Hen egg yolk contains most of the calcium (Ca), Fe, and phosphorus in the egg (78, 53, and 70%, respectively; Table 2; USDA, 2020). Besides, due to the phosvitin in egg yolk composition, it decreases the absorption of some minerals, especially Fe, and can also prevent the absorption of Fe taken with ferrous ammonium sulfate in rats (Miller & McNeal, 1983). Based on the protein source of dietary nutrients, the absorption of Fe taken into the organism, its solubility and bioavailability in the intestine may vary. When the effects of different protein types such as pork, hen egg albumin, hen egg yolk, soybean, and casein on the absorption of Fe in rats and their intestinal solubility are examined, it was found in rats fed with egg yolk protein that Fe solubility (17%) and absorption (13%) were significantly lower in the small intestine compared to other groups (Kim, Lee, & Lee, 1995). In a study examining the effect of egg yolk proteins and egg yolk phosvitin on the absorption of Fe, Ca, magnesium (Mg) in rats, it was shown that egg proteins reduced the absorption of minerals, and that egg yolk proteins and phosvitin had similar effects on Ca and Mg absorption (Ishikawa, Tamaki, Arihara, & Itoh, 2007). In another study investigating the effect of phosvitin on Ca absorption *in vitro*, phosvitin significantly reduced the absorption of Ca due to complex formation with phosvitin-Ca^{2+} ions in 1 to 10 mM Ca gluconate solution (Dolinska, Lopata, Mikulska, Leszczynska, & Ryszka, 2012).

The bioavailability of Fe taken into the body may vary depending on the protein source of the diets. Egg yolk proteins and ovalbumin were compared in the recovery of Fe deficiency anemia in rats. It was shown that although ovalbumin was beneficial in the recovery of Fe deficiency anemia, egg yolk proteins delayed recovery of that (Kobayashi, Wakasugi, Yasui, Kuwahata, & Kido, 2015). Since the phosvitin–Fe complex is excreted from the body before it can be used *in vivo*, the bioavailability of Fe taken with egg

Table 2–Energy and nutrient compositon of raw whole egg, egg white and egg yolk (per 100 g)[a].

Energy and nutrient	Whole egg	Egg white	Egg yolk
Energy (kcal)	143	52	322
Water (g)	76.2	87.57	52.31
Carbohydrate (g)	0.72	0.73	3.59
Protein (g)	12.6	10.9	15.86
Fat (g)	9.51	0.17	26.54
Cholesterol (mg)	372	0	1085
Vitamin A (μg)	160	0	381
Vitamin D (μg)	2	0	5.4
Vitamin E (mg)	1.05	0	2.58
Vitamin K (μg)	0.3	0	0.7
Thiamin (mg)	0.04	0.004	0.176
Riboflavin (mg)	0.457	0.439	0.528
Niacin (mg)	0.075	0.105	0.024
Folate (μg)	47	4	146
Vitamin B12 (μg)	0.89	0.09	1.95
Calcium (mg)	56	7	129
Iron (mg)	1.75	0.08	2.73
Magnesium (mg)	12	11	5
Phosphorus (mg)	198	15	390
Zinc (mg)	1.29	0.03	2.3
Selenium (μg)	30.7	20	56
Copper (mg)	0.072	0.023	0.077

[a]U.S. Department of Agriculture, Agricultural Research Service, FoodData Central (2020).

is reduced. Therefore, in individuals with Fe deficiency anemia, caution should be exercised in egg and egg yolk recommendations as a source of protein and Fe (Kobayashi et al., 2015). Different protein sources cause different chromatographic profiles of peptides in the intestinal lumen. Thus, it suggests that specific peptides produced during protein digestion may be a factor affecting Fe bioavailability (Kapsokefalou M & Miller DD, 1995). It has been known for many years that Fe in egg yolk forms a phosvitin–Fe complex that is insoluble and that decreases bioavailability of Fe. Besides, under *in vitro* conditions, phosvitin is particularly resistant to proteolytic effect. Characterization of phosvitin as nutritionally negative is associated with reducing its absorption and bioavailability by forming complexes with minerals and being resistant to proteolytic enzymes (Goulas, Triplett, & Taborsky, 1996; Ishikawa et al., 2007). Although the low bioavailability (□30%) of Fe found in egg yolk is associated with phosvitin, "functional phosvitin phosphopeptides" obtained from hen yolk phosvitin increase the bioavailability of minerals, primarily Fe and Ca (Huang & Ahn, 2019). Before phosvitin PPP were obtained, casein PPP were reported to increase intestinal Ca bioavailability (Jiang & Mine, 2000). To increase the bioavailability of phosvitin, Jiang and Mine (2000) obtained functional egg PPP derived from tryptic hydrolysis followed by partial alkaline phosphorylation of phosvitin, with a molecular weight of 1,000 to 3,000 Da. These peptides not only increased Ca binding capacity but also prevented the formation of insoluble Ca phosphates (Jiang & Mine, 2000). In experimental animals, it is suggested that phosvitin peptides increase the bioavailability of Ca, thereby increasing the incorporation of Ca into bones (Choi, Jung, Choi, Kim, & Ha, 2005). In the study investigating the effect of PPP on Fe intake in Caco-2 cells, it was shown that PPP increased Fe intake and prevented precipitation (Feng & Mine, 2006).). In addition to increasing mineral bioavailability, PPP are also known to have anti-inflammatory, antioxidant, and antimicrobial effects (Katayama, Xu, Fan, & Mine, 2006).

Various methods are available for the extraction of phosvitin, which has many functional properties, mainly metal-binding properties and resistance to proteolytic enzymes. Because these methods are costly and time-consuming, as well as they cannot be adapted to the food industry and are not sustainable, different methods are tried for the extraction of phosvitin (Giarratano et al., 2020). Enzymatic hydrolysis is one of these methods, which can be broken down into the peptides of phosvitin in this way. Fragmentation of phosvitin into small peptides using proteolytic enzymes can affect the bioavailability of Ca and Fe. Enzymatically hydrolyzed peptides can increase their absorption by preventing the formation of insoluble Ca phosphates or Fe phosphates in the intestine (Choi, Jung, Choi, Kim, & Ha, 2005). It is emphasized that hydrolysates also positively improve the antioxidant properties of phosvitin. However, antioxidant effects can vary depending on the enzymes used in the production of hydrolysates (Xu, Katayama, & Mine, 2007).

Enzymes commonly used such as pepsin, trypsin, and chymotrypsin are effective in producing egg yolk hydrolysates with antioxidant activity (Benedé, & Molina, 2020). In a study showing the effects of different enzymes in PPP production, phosvitin was pretreated with heat and then hydrolyzed with/without partial dephosphorylation using pepsin, thermolysis, and trypsin. Although pepsin and thermolysis are not effective in the production of PPP, a large number of peptides (total 12 peptides, 10 of which are PPP) have been produced from phosvitin by trypsin hydrolysis (Samaraweera et al., 2014). Ren, Li, Offengenden, and Wu (2015) also identified 32 peptides from phosvitin using enzymatic hydrolysis, which up to 10 have been reported to contain phosphate groups that play important roles in the functionality of PPP (Ren et al., 2015). The phosphorylated serine part of the PPP plays a major role in the binding of divalent metal ions such as Ca, Mg, Zn, Cu, Fe and intestinal absorption of calcium, minerals and other trace minerals (Samaraweera et al., 2014). On the other hand, the strong antioxidant activity of fosvitin PPP is attributed to the rich amino acid composition (mainly histidine, methionine, and tyrosine) rather than the presence of phosphorylserine ligands (Xu et al., 2007). PPP obtained from enzymatic hydrolyzation as an alternative to techniques that are not suitable for use in foods is promising, but it is believed that the effects of different enzymatic hydrolysates on mineral binding and antioxidant activity of phosvitin should be investigated in more detail.

It is extremely difficult for phosvitin to break down into small peptides using proteolytic enzymes. Long, sequential phosphoserine arrangements of phosvitin complicate the formation of the appropriate enzyme-substrate complex required for hydrolysis. It is suggested that the molecular weight of the PPP obtained should be <3,000 Da to function properly (Samaraweera et al., 2014). In a study conducted to compare the functional properties of PPP obtained from phosvitin using alkaline or acid hydrolysis, whereas phosvitin has high stability in alkaline conditions (0.1 N NaOH, more than one hour), it is completely degraded in acidic conditions (3 N HCl, 6 hr at 60 °C). Also, it can negatively affect the antioxidant capacities of acids or alkalis, hydrolysates, and Fe binding capacities used while obtaining PPP (Samaraweera et al., 2013). For this reason, besides the positive properties of PPP, there is a need to further develop the methods they are obtained with.

2.2 Antioxidant properties of hen egg yolk phosvitin

The antioxidant properties of egg yolk can be affected by many factors such as the type of egg, processing method, and storage conditions. Apart from phosvitin, other egg yolk proteins, phospholipids, vitamin E, carotenoids, and aromatic amino acids (tryptophan and tyrosine) are compounds that contribute significantly to the total antioxidant activity of egg yolk (Nimalaratne, Schieber, & Wu, 2016). The antioxidant activity of phosvitin is associated with high metal binding capacity (Kovacs-Nolan et al., 2005). In addition to Fe, phosvitin can chelate many cations such as Ca, Mg, and cobalt. Moreover, phosvitin has the capacity to bind much more Fe than naturally found in egg yolk. Because of this feature of phosvitin, it is possible to use egg yolk in foods as a natural antioxidant. When egg yolk is used as an ingredient in foods, phosvitin can chelate the metals and prevent lipid oxidation of foods (Lu & Baker, 1986).

Lipid oxidation is a source of major concern for the food industry because it generates undesirable taste, color, and potentially toxic reaction products in many foods. Antioxidants can be used to limit lipid oxidation in foods. Antioxidants can generally be collected under two headings as free radical scavengers and metal chelators. Thanks to a strong metal binding capacity, phosvitin is considered a natural antioxidant (Anton et al., 2011; Ishikawa, Yano, Arihara, & Itoh, 2004). Lu and Baker (1986) tried to determine the antioxidant activity of phosvitin in an egg yolk phospholipid emulsion system. Various inorganic and organic metals (Fe^{2+}, Cu^{2+}, and hemin) in different concentrations were added to the emulsions to induce lipid oxidation. It was reported that phosvitin inhibited the phospholipid oxidation catalyzed by the Fe^{2+} and Cu^{2+} metals, yet did not affect the hemin catalysis. Besides, the effect of heat on the antioxidant properties of phosvitin was examined showing that pasteurization (61.1 °C, 4 min) did not affect the antioxidant property while autoclaving (121.1 °C, 10 min) decreased (Lu & Baker, 1986). Because the nutrients had a wide range of pH values, the same researchers examined the effect of pH and some of the ingredients (albumin and NaCl that can be found in commercial egg yolk) on the antioxidant properties of phosvitin. When pH values were around 6, NaCl and albumin could not affect the activity of the phosvitin to inhibit Fe^{2+} catalysis. Therefore, it is stated that egg yolk can be used in food systems where pH values are around 6 (Lu & Baker, 1987). The functional properties of phosvitin can be improved by some chemical modifications. Conjugation of phosvitin with galactomannan with a controlled Maillard reaction significantly increases emulsion activity and heat stability as well as antioxidant activity (Nakamura, Ogawa, Nakai, Kato, & Kitts, 1998).

Lipid oxidation is considered an important problem in meat and meat products as it shortens the shelf life, causes loss of nutritional value, and negatively affects taste and color. Various factors can cause lipid oxidation, one of which is endogenous Fe (Terevinto, Ramos, Castroman, Cabrera, & Saadoun, 2010). Because Fe is an important pro-oxidant in meat and meat products, phosvitin can be used as a natural chelator to extend its shelf life (Jung et al., 2012; Lee, Han, & Decker, 2002). However, it is important to test the antioxidant activity of phosvitin under different pH, temperature, and NaCl conditions (Lee et al., 2002). The antioxidant potential of phosvitin have been studied under different pH, temperature, and NaCl conditions in phosphatidylcholine liposomes, muscle homogenates and ground pork, and it has been reported that phosvitin can inhibit Fe-induced lipid oxidation. However, phosvitin was unable to completely inhibit lipid oxidation in salted raw and cooked ground pork. Therefore, it is recommended to use phosvitin together with other antioxidant technologies to effectively increase the shelf life of meat products (Lee et al., 2002). Jung et al. (2012) extracted phosvitin from hen egg yolk and examined the Fe binding and antioxidant effects of phosvitin in ground beef. Addition of extracted phosvitin at concentrations of 100 and 500 mg/kg reduced oxidation and increased color stability in ground beef compared to unprocessed control. The addition of phosvitin did not negatively affect the sensory properties of ground beef (Jung et al., 2012). In the food industry, high hydrostatic pressure technology is used to increase the microbiological safety and shelf life of meat and meat products (Bover-Cid, Belletti, Garriga, & Aymerich, 2011). When the effects of phosvitin on lipid and protein oxidation of raw and cooked ground meat processed with this method were examined, it was found that the addition of phosvitin with 300-MPa high hydrostatic pressure treatment had a synergistic antimicrobial effect on raw ground beef during storage. High hydrostatic pressure increased lipid oxidation in the cooked ground beef and protein oxidation in raw ground beef, however, phosvitin inhibited lipid and protein oxidation in these samples. In meats using high hydrostatic pressure technology, it is suggested that phosvitin can be used as an antimicrobial and antioxidant agent (Jung, Nam, Ahn, Kim, & Jo, 2013).

Iron is an essential mineral for the human organism. However, disruption of Fe balance in the body can cause toxic results. The excess amount of Fe^{2+} reacts with the hydrogen peroxide (H_2O_2) in the organism, defined as the Fenton reaction. As a result of this reaction, toxic hydroxyl radical (.OH) is released (Kanti Das, Wati, & Shad, 2015). Thanks to the metal-binding property of phosvitin, it shows a protective effect against lipid oxidation and oxidative damage catalyzed by Fe^{2+}. Phosvitin accelerates the Fe^{2+} autoxidation and converts it into the Fe^{3+} form, reducing the amount of Fe^{2+} involved in the Fenton reaction. By this way, it also reduces the formation of .OH (Ishikawa et al., 2004). Phosvitin is a more effective compound in inhibiting Fe^{2+} involved in the Fenton reaction compared to other Fe-binding proteins and metal chelators (Ishikawa et al., 2004). Although the functions of the phosvitin, known as Fe carrier in egg yolk, are not yet fully understood, it is believed to carry and provide metal ions during the development of the embryo (Liu et al., 2013). Thanks to its antioxidant properties, it can protect the developing embryo against Fe-dependent oxidative damage under *in ovo* conditions (Ishikawa et al., 2004). PPP derived from egg yolk have an antioxidant activity similar to phosvitin and they significantly reduce interleukin-8 secretion caused by hydrogen peroxide in human Caco-2 cells (Young, Nau, Pasco, & Mine, 2011). In an *in vitro* study using human intestinal epithelial cells (Caco-2 cells), the protective effects of egg yolk PPP

against oxidative stress induced by hydrogen peroxide were evaluated, and it was found that PPP had antioxidative activity against oxidative stress. Phosphorus and peptide structure was reported to play an important role in the antioxidative activity of phosvitin PPP (Katayama et al., 2006). In a study comparing phosvitin and PPP, it was reported that the chelating capacity of phosvitin was higher than that of PPP, whereas the antioxidant capacities of PPP were higher than that of phosvitin (Xu et al., 2007).

Because phosvitin reduces the bioavailability of Fe, it can decrease the nutritional value of the food it is added to. However, the fact that phosvitin is a natural molecule and used in place of synthetic antioxidants increases nutritional quality besides its positive effects on human health. Thanks to these qualities of phosvitin, it seems exciting both in terms of human health and in terms of food preparation.

2.3 Antibacterial properties of hen egg yolk phosvitin

Most pathogenic bacteria are classified as Gram-negative bacteria. These bacteria have bacterial lipopolysaccharides (LPS), which are the outer membrane components that dissolve in the lipid. Lipopolysaccharides, often called endotoxins, are a structural component of the bacterial cell and provide an effective permeability barrier to the bacterial cell against harmful agents (Ahola et al., 2017; Wassenaar & Zimmermann, 2018). However, since LPS can be a target antibacterial agents such as surfactants and chelators, amphiphilic proteins capable of chelating can have negative effects on Gram-negative bacteria (Sattar Khan et al., 2000). Phosvitin, which has a polyanionic structure, can exhibit antimicrobial properties in addition to its antioxidant properties with its metal-binding ability (Choi, Jung, Seog, & Choi, 2004; Sattar Khan et al., 2000). In a study investigating the antibacterial properties of phosvitin, *Escherichia coli* cells (10^6/mL) were incubated under thermal stress at 50 °C for 20 min in the presence of 0.1 mg/mL of phosvitin. At the end of this process, *Escherichia coli* cells completely disappeared. When phosvitin was not added under the same conditions, a significant proportion (10^5/mL) of *E. coli* cells remained alive. Bactericidal activity of phosvitin against *E. coli* has been associated with high metal chelating ability and synergistic effect of high surface activity under the thermal stress (Sattar Khan et al., 2000). Choi et al. (2004) similarly examined the antibacterial properties of phosvitin under thermal stress. Phosvitin and its peptides exhibited antibacterial activity against *E. coli* under thermal stress at 50 °C (Choi et al., 2004). In a study evaluating the effects of the combination of IgY (livetin fractions), which is another egg component with antimicrobial activity, with phosvitin on enterotoxigenic *E. coli*, it was reported that hydrolysates of IgY and phosvitin effectively controlled enterotoxigenic *E. coli* (Gujral et al., 2017). This combination can have great potential for microbial control in the veterinary pharmaceutical industry as well as in human health (Gujral et al., 2017).

2.4 Other potential properties of hen egg yolk phosvitin

Besides other functional activities of phosvitin, some studies, although limited, have been conducted on immune-enhancing activity, melanogenesis inhibitory, antigenotoxic activity, anti-elastase, and anti-hyaluronidase activity of the hen egg phosvitin (Jung et al., 2012; Lee et al., 2017, 2020; Moon et al., 2014). Lee et al. (2017) showed that phosvitin significantly improved immune functions by stimulating the production of proinflammatory mediators of RAW 264.7 macrophages. Phosvitin also increased the phagocytic activity of macrophages. Thus, it was reported that phosvitin may have had the potential to be used by the food or nutraceutical industries as an immunostimulating agent (Lee et al., 2017). In a study investigating the effect of phosvitin on the inhibition of melanogenesis in melanoma cells, the addition of phosvitin to B16F10 melanoma cells at a concentration of 50 μg/mL was reported to inhibit tyrosinase activity by about 42% and melanin synthesis by 17% compared to controls that were not added phosvitin. These results suggest that phosvitin has the potential to be used as a natural melanogenesis inhibitor (Jung et al., 2012). As well as, it was reported that phosvitin may have had anti-elastase and anti-hyaluronidase activity (Lee et al., 2020). Phosvitin showed competitive inhibitory activity against elastase and hyaluronidase, in a dose-dependent manner, inhibiting elastase and hyaluronidase activity. Therefore, it can be thought that phosvitin obtained from egg yolk can be used as a natural anti-ageing agent thanks to its inhibitory activity against skin ageing-related elastase and hyaluronidase (Lee et al., 2020). Phosvitin has antigenotoxic and cytotoxic activity against human cancer cells in different tissues (cervix, breast, stomach, lung, liver, and larynx) (Moon et al., 2014). Phosvitin can inhibit the growth of cancer cells in a dose-dependent manner. Cancer cells in the cervix, stomach, and liver tissues have been reported as the most sensitive cells to phosvitin. The results of this study suggest that phosvitin may have the potential to be used as an anticancer agent for humans (Moon et al., 2014).

3. CONCLUSION AND FUTURE PERSPECTIVES

The unique amino acid pattern of the phosvitin, which is an important component of the egg yolk and stands out with its phosphorus content, gives the phosvitin many functional qualities. Because of the high phosphoric acid due to serine residues, phosvitin can act as a polyanion. Thanks to its polyanionic character, phosvitin acquires a very strong metal chelating quality. Metal chelating quality is shown as the reason for many properties, especially its antioxidant and antibacterial activity. Because almost all of Fe in egg yolk depends on phosvitin, it decreases its Fe bioavailability and can reduce the nutritional value of the food it is added to. It is reported that this situation may cause a problem in individuals with Fe deficiency anemia. However, the metal chelating feature makes phosvitin a potential antioxidant against oxidative damage, which has an important place in both the food industry and human health. Lipid oxidation, which causes adverse effects on the organoleptic properties of foods, can be inhibited by the addition of phosvitin. It is stated that phosvitin, which is a natural antioxidant, can be used alone or combined with other antioxidants in food systems. Besides, phosvitin is a more effective compound to inhibit Fe^{2+} involved in the Fenton reaction compared to other Fe-binding proteins and metal chelators. Unlike phosvitin, PPP obtained from phosvitin increase the bioavailability of minerals, especially Fe and Ca. Similar to phosvitin, PPP show antioxidant properties in studies conducted with human cells. Amphiphilic proteins capable of chelating, such as phosvitin, can have negative effects on Gram-negative bacteria (*E. coli*). Unlike the known qualities of phosvitin, recent studies have been focusing on immune-enhancing, melanogenesis inhibitor, anti-ageing, and anticancer properties. The accumulated literature suggests that hen egg yolk phosvitin certainly deserves further investigation because of its functionality in food technology and possible health effects.

AUTHOR CONTRIBUTIONS

B. Yılmaz: drafting the work or revising it critically for important intellectual content, final approval of the version to be published. D. Ağagündüz: design and drafting the work and revising it critically

for important intellectual content, final approval of the version to be published.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

REFERENCES

Abe, Y., Itoh, T., & Adachi, S. (1982). Fractionation and characterization of hen's egg yolk phosvitin. *Journal of Food Science*, *47*(6), 1903–1907. https://doi.org/10.1111/j.1365-2621.1982.tb12910.x

Ahola, A. J., Lassenius, M. I., Forsblom, C., Harjutsalo, V., Lehto, M., & Groop, P. H. (2017). Dietary patterns reflecting healthy food choices are associated with lower serum LPS activity. *Scientific Reports*, *7*(1), 6511. https://doi.org/10.1038/s41598-017-06885-7

Albright, K. J., Gordon, D. T., & Cotterill, O. J. (1984). Release of iron from phosvitin by heat and food additives. *Journal of Food Science*, *49*(1), 78–81. https://doi.org/10.1111/j.1365-2621.1984.tb13674.x

Allerton, S. E., & Perlmann, G. E. (1965). Chemical characterization of the phosphoprotein phosvitin. *Journal of Biological Chemistry*, *240*(10), 3892–3898.

Anton, M. (2013). Egg yolk: structures, functionalities and processes. *Journal of the Science of Food and Agriculture*, *93*(12), 2871–2880. https://doi.org/10.1002/jsfa.6247

Anton, M., Nau, F., & Guérin-Dubiard, C. (2011). Bioactive fractions of eggs for human and animal health. In F. Van Immerseel, Y. Nys, & M. Bain (Eds.), *Improving the safety and quality of eggs and egg products* (pp. 321–345). Cambridge, United Kingdom: Woodhead Publishing.

Benedé, S., & Molina, E. (2020). Chicken egg proteins and derived peptides with antioxidant properties. *Foods*, *9*(6), 735. https://doi.org/10.3390/foods9060735

Bover-Cid, S., Belletti, N., Garriga, M., & Aymerich, T. (2011). Model for Listeria monocytogenes inactivation on dry-cured ham by high hydrostatic pressure processing. *Food Microbiology*, *28*(4), 804–809. https://doi.org/10.1016/j.fm.2010.05.005

Burley, R. W., & Cook, W. H. (1961). Isolation and composition of avian egg yolk granules and their constituent alpha- and beta-lipovitellins. *Canadian Journal of Biochemistry and Physiology*, *39*, 1295–1307. https://doi.org/10.1139/o61-136

Byrne, B. M., van het Schip, A. D., van de Klundert, J. A., Arnberg, A. C., Gruber, M., & Ab, G. (1984). Amino acid sequence of phosvitin derived from the nucleotide sequence of part of the chicken vitellogenin gene. *Biochemistry*, *23*(19), 4275–4279. https://doi.org/10.1021/bi00314a003

Castellani, O., Guérin-Dubiard, C., David-Briand, E., & Anton, M. (2004). Influence of physicochemical conditions and technological treatments on the iron binding capacity of egg yolk phosvitin. *Food Chemistry*, *85*(4), 569–577. https://doi.org/10.1016/j.foodchem.2003.08.002.

Chay Pak Ting, B. P., Pouliot, Y., Gauthier, S. F., & Mine, Y. (2010). Fractionation of egg proteins and peptides for nutraceutical applications. In S. S. H. Rizvi (Ed.), *Separation, extraction and concentration processes in the food, beverage and nutraceutical industries* (pp. 595–618). Cambridge, United Kingdom: Woodhead Publishing.

Chay Pak Ting, B. P., Pouliot, Y., Gauthier, S. F., & Mine, Y. (2013). 19 - Fractionation of egg proteins and peptides for nutraceutical applications. In S. S. H. Rizvi (Ed.), *Separation, extraction and concentration processes in the food, beverage and nutraceutical industries* (pp. 595–618). Woodhead Publishing. ISBN 9781845696450, https://doi.org/10.1533/9780857090751.2.595.

Cherian, G., Holsonbake, T. B., & Goeger, M. P. (2002). Fatty acid composition and egg components of specialty eggs. *Poultry Science*, *81*(1), 30–33. https://doi.org/10.1093/ps/81.1.30

Choi, I. W., Jung, C. H., Seog, H. M., & Choi, H. D. (2004). Purification of phosvitin from egg yolk and determination of its physicochemical properties. *Food Science and Biotechnology*, *13*(4), 434–437.

Choi, I., Jung, C., Choi, H., Kim, C., & Ha, H. (2005). Effectiveness of phosvitin peptides on enhancing bioavailability of calcium and its accumulation in bones. *Food Chemistry*, *93*(4), 577–583.

Chung, A. L., & Ferrier, L. K. (2006). Conditions affecting emulsifying properties of egg yolk phosvitin. *Journal of Food Science*, *56*(5), 1259–1262.

Cotterill, O. J., & Geiger, G. S. (1977). Egg product yield trends from shell Eggs1. *Poultry Science*, *56*(3), 1027–1031. https://doi.org/10.3382/ps.0561027

Choi, I., Jung, C., Choi, H., Kim, C., & Ha, H. (2005). Effectiveness of phosvitin peptides on enhancing bioavailability of calcium and its accumulation in bones. *Food Chemistry*, *93*(4), 577–583. ISBN 9780122270550, https://doi.org/10.1016/B0-12-227055-X/00978-0.

Cui, Y., Li, X., Lu, M., Liu, X., & Duan, X. (2019). Role of polysaccharide conjugation in physicochemical and emulsifying properties of egg phosvitin and the calcium binding capacity of its phosphopeptides. *Food & Function*, *10*, 1808–1815.

Dickinson, E., Pinfield, V. J., & Horne, D. S. (1997). On the "Anomalous" adsorption behavior of phosvitin. *Journal of Colloid and Interface Science*, *187*(2), 539–541. https://doi.org/10.1006/jcis.1996.4695

Dolinska, B., Lopata, K., Mikulska, A., Leszczynska, L., & Ryszka, F. (2012). Influence of phosvitin and calcium gluconate concentration on permeation and intestinal absorption of calcium ions. *Biological Trace Element Research*, *147*(1-3), 374–377. https://doi.org/10.1007/s12011-011-9298-0

EU. (2008). EUR-Lex Commission Regulation (EC) No 589/2008 of 23 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs. Retrieved from https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=celex:32008R0589

Feng, F., & Mine, Y. (2006). Phosvitin phosphopeptides increase iron uptake in a Caco-2 cell monolayer model. *International Journal of Food Science & Technology*, *41*(4), 455–458. https://doi.org/10.1111/j.1365-2621.2005.01073.x

Giarratano, M., Duffuler, P., Chamberland, J., Brisson, G., House, J. D., Pouliot, Y., & Doyen, A. (2020). Combination of high hydrostatic pressure and ultrafiltration to generate a new emulsifying ingredient from egg yolk. *Molecules (Basel, Switzerland)*, *25*(5), 1184. https://doi.org/10.3390/molecules25051184

Goulas, A., Triplett, E. L., & Taborsky, G. (1996). Oligophosphopeptides of varied structural complexity derived from the egg phosphoprotein, phosvitin. *Journal of Protein Chemistry*, *15*(1), 1–9. https://doi.org/10.1007/bf01886805

Guérin-Dubiard, C., Anton, M., & Dhene-Garcia, A., Martinet, V., & Brulé, G. (2002). Hen egg and fish egg phosvitins: Composition and iron binding properties. *European Food Research and Technology*, *214*, 460–464. https://doi.org/10.1007/s00217-001-0460-3

Gujral, N., Yoo, H., Bamdad, F., Lee, K. Y., Suh, J. W., & Sunwoo, H. (2017). A combination of egg yolk IgY and phosvitin inhibits the growth of enterotoxigenic escherichia coli K88 and K99. *Current Pharmaceutical Biotechnology*, *18*(5), 400–409. https://doi.org/10.2174/1389201018666170425120036

Huang, X., & Ahn, D. U. (2019). How can the value and use of egg yolk be increased? *Journal of Food Science*, *84*(2), 205–212. https://doi.org/10.1111/1750-3841.14430

Ishikawa, S. I., Tamaki, S., Arihara, K., & Itoh, M. (2007). Egg yolk protein and egg yolk phosvitin inhibit calcium, magnesium, and iron absorptions in rats. *Journal of Food Science*, *72*(6), S412–419. https://doi.org/10.1111/j.1750-3841.2007.00417.x

Ishikawa, S., Yano, Y., Arihara, K., & Itoh, M. (2004). Egg yolk phosvitin inhibits hydroxyl radical formation from the fenton reaction. *Bioscience, Biotechnology, and Biochemistry*, *68*(6), 1324–1331. https://doi.org/10.1271/bbb.68.1324

Itoh, T., Abe, Y., & Adachi, S. (1983). Comparative Studies on the α and α-Phosvitin from Hen's Egg Yolk. *Journal of Food Science*, *48*(6), 1755–1757. https://doi.org/10.1111/j.1365-2621.1983.tb05077.x

Jiang, B., & Mine, Y. (2000). Preparation of novel functional oligophosphopeptides from hen egg yolk phosvitin. *Journal of Agricultural and Food Chemistry*, *48*(4), 990–994. https://doi.org/10.1021/jf990600l

Jiang, B., Wang, X., Wang, L., Wu, S., Li, D., Liu, C., & Feng, Z. (2020). Fabrication and Characterization of a Microemulsion Stabilized by Integrated Phosvitin and Gallic Acid. *Journal of Agricultural and Food Chemistry*, *68*(19), 5437–5447. https://doi.org/10.1021/acs.jafc.0c00945

Joubert, F. J., & Cook, W. H. (1958). Preparation and characterization of phosvitin from hen egg yolk. *Canadian Journal of Biochemistry and Physiology*, *36*(4), 399–408.

Jung, S., Jo, C.-R., Kang, M.-G., Ahn, D.-U., & Nam, K.-C. (2012). Elucidation of Antioxidant Activity of Phosvitin Extracted from Egg Yolk using Ground Meat. *Korean Journal for Food Science of Animal Resources*, *32*(2), 162–167. https://doi.org/10.5851/kosfa.2012.32.2.162

Jung, S., Kim, D. H., Son, J. H., Nam, K., Ahn, D. U., & Jo, C. (2012). The functional property of egg yolk phosvitin as a melanogenesis inhibitor. *Food Chemistry*, *135*(3), 993–998. https://doi.org/10.1016/j.foodchem.2012.05.113

Jung, S., Nam, K. C., Ahn, D. U., Kim, H. J., & Jo, C. (2013). Effect of phosvitin on lipid and protein oxidation in ground beef treated with high hydrostatic pressure. *Meat Science*, *95*(1), 8–13. https://doi.org/10.1016/j.meatsci.2013.04.005

Kanti Das, T., Wati, M. R., & Shad, K. F. (2015). Oxidative stress gated by fenton and haber weiss reactions and its association with alzheimer's disease. *Archives of Neuroscience*, *2*(2), e60038. https://doi.org/10.5812/archneurosci.20078

Kapsokefalou, M., & Miller, D. D. (1995). Iron speciation in intestinal contents of rats fed meals composed of meat and nonmeat sources of protein and fat. *Food Chemistry*, *52*(1), 47–56. https://doi.org/10.1016/0308-8146(94)P4180-N.

Katayama, S., Xu, X., Fan, M. Z., & Mine, Y. (2006). Antioxidative stress activity of oligophosphopeptides derived from hen egg yolk phosvitin in Caco-2 cells. *Journal of Agricultural and Food Chemistry*, *54*(3), 773–778. https://doi.org/10.1021/jf052280d

Kim, M., Lee, D.-T., & Lee, Y.-S. (1995). Iron absorption and intestinal solubility in rats are influenced by dietary proteins. *Nutrition Research*, *15*(11), 1705–1716. https://doi.org/10.1016/0271-5317(95)02041-0.

Kobayashi, Y., Wakasugi, E., Yasui, R., Kuwahata, M., & Kido, Y. (2015). Egg yolk protein delays recovery while ovalbumin is useful in recovery from iron deficiency anemia. *Nutrients*, *7*(6), 4792–4803. https://doi.org/10.3390/nu7064792

Kovacs-Nolan, J., Phillips, M., & Mine, Y. (2005). Advances in the value of eggs and egg components for human health. *Journal of Agricultural and Food Chemistry*, *53*(22), 8421–8431. https://doi.org/10.1021/jf050964f

Laca, A., Paredes, B., & Díaz, M. (2012). Lipid-enriched egg yolk fraction as ingredient in cosmetic emulsions. *Journal of Texture Studies*, *43*(1), 12–28. https://doi.org/10.1111/j.1745-4603.2011.00312.x

Lee, J. H., Moon, S. H., Hong, Y., Ahn, D. U., & Paik, H. D. (2020). Anti-elastase and anti-hyaluronidase activity of phosvitin isolated from hen egg yolk. *British Poultry Science*, *61*(1), 17–21. https://doi.org/10.1080/00071668.2019.1686124

Lee, J. H., Moon, S. H., Kim, H. S., Park, E., Ahn, D. U., & Paik, H. D. (2017). Immune-enhancing activity of phosvitin by stimulating the production of pro-inflammatory mediator. *Poultry Science*, *96*(11), 3872–3878. https://doi.org/10.3382/ps/pex205

Lee, S. K., Han, J. H., & Decker, E. A. (2002). Antioxidant activity of phosvitin in phosphatidylcholine liposomes and meat model systems. *Journal of Food Science*, *67*(1), 37–41. https://doi.org/10.1111/j.1365-2621.2002.tb11355.x

Liu, J., Czernick, D., Lin, S. C., Alasmari, A., Serge, D., & Salih, E. (2013). Novel bioactivity of phosvitin in connective tissue and bone organogenesis revealed by live calvarial bone organ culture models. *Developmental Biology*, *381*(1), 256–275. https://doi.org/10.1016/j.ydbio.2013.06.005

Lu, C. L., & Baker, R. C. (1986). Characteristics of egg yolk phosvitin as an antioxidant for inhibiting metal-catalyzed phospholipid oxidations. *Poultry Science*, *65*(11), 2065–2070. https://doi.org/10.3382/ps.0652065

Lu, C.-L., & Baker, R. C. (1987). Effect of pH and food ingredients on the stability of egg yolk phospholipids and the metal-chelator antioxidant activity of phosvitin. *Journal of Food Science*, *52*(3), 613–616. https://doi.org/10.1111/j.1365-2621.1987.tb06686.x

Lv, C. (2017). Structure, function, and nutrition of calcium-containing proteins or peptides from foodstuffs. In G. Zhao (Ed.), *Mineral containing proteins* (pp. 117–150). Gateway East, Singapore: Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-10-3596-8_5

Miller, J., & McNeal, L. S. (1983). Bioavailability of egg yolk iron measured by hemoglobin regeneration in anemic rats. *Journal of Nutrition*, *113*(1), 115–123. https://doi.org/10.1093/jn/113.1.115

Moon, S. H., Lee, J. H., Lee, M., Park, E., Ahn, D. U., & Paik, H. D. (2014). Cytotoxic and antigenotoxic activities of phosvitin from egg yolk. *Poultry Science*, *93*(8), 2103–2107. https://doi.org/10.3382/ps.2013-03784

Nakamura, S., Ogawa, M., Nakai, S., Kato, A., & Kitts, D. D. (1998). Antioxidant activity of a maillard-type phosvitin−galactomannan conjugate with emulsifying properties and heat stability. *Journal of Agricultural and Food Chemistry*, *46*(10), 3958–3963. https://doi.org/10.1021/jf980292b

Nimalaratne, C., Schieber, A., & Wu, J. (2016). Effects of storage and cooking on the antioxidant capacity of laying hen eggs. *Food Chemistry*, *194*, 111–116. https://doi.org/10.1016/j.foodchem.2015.07.116

Ren, J., Li, Q., Offengenden, M., & Wu, J. (2015). Preparation and characterization of phosphopeptides from egg yolk phosvitin. *Journal of Functional Foods*, *18*(Part A), 190–197.

Samaraweera, H., Moon, S. H., Lee, E. J., Grant, J., Fouks, J., Choi, I., … Du, A. (2014). Characterisation of phosvitin phosphopeptides using MALDI-TOF mass spectrometry. *Food Chemistry*, *165*, 98–103. https://doi.org/10.1016/j.foodchem.2014.05.098.

Samaraweera, H. (2012). Production and characterization of phosphopeptides from egg yolk phosvitin (Graduate Theses and Dissertations). Iowa State University. Retrieved from https://lib.dr.iastate.edu/etd/12451

Sugino, H., Nitoda, T., & Juneja, L. R. (1997). General chemical composition of hen eggs. In L. J. T. Yamamoto, H. Hatta, & M. Kim. (Eds.), *Hen eggs their basic and applied science*. New York: CRC Press, Inc.

Samaraweera, H., Moon, S. H., Lee, E., Yang, S. H., Suh, J.W., & Ahn, D. U. (2013). Chemical hydrolysis of phosvitin and the functional properties of the hydrolysates. *International Journal of Engineering Research & Technology*, *2*(11), 3373–3386.

Sattar Khan, M. A., Babiker, E. E., Azakami, H., & Kato, A. (1998). Effect of protease digestion and dephosphorylation on high emulsifying properties of hen egg yolk phosvitin. *Journal of Agricultural and Food Chemistry*, *46*(12), 4977–4981. https://doi.org/10.1021/jf980319r

Sattar Khan, M. A., Nakamura, S., Ogawa, M., Akita, E., Azakami, H., & Kato, A. (2000). Bactericidal action of egg yolk phosvitin against Escherichia coliunder thermal stress. *Journal of Agricultural and Food Chemistry*, *48*(5), 1503–1506. https://doi.org/10.1021/jf990700r

Schiavone, A., & Barroeta, A. C. (2011). Egg enrichment with vitamins and trace minerals. In F. Van Immerseel, Y. Nys, & M. Bain (Eds.), *Improving the safety and quality of eggs and egg products* (pp. 289–320). Cambridge, United Kingdom: Woodhead Publishing.

Seuss-baum, I. (2007). Nutritional evaluation of egg compounds. In R. Huopalahti, R. Lopez-Fandino, M. Anton, & R. Schade (Eds.), *Bioactive egg compounds* (pp. 117–144). Berlin, Germany: Springer-Verlag.

Strixner, T. U. K. (2011) Egg proteins. In P. A. W. Phillips (Ed.), *Handbook of food proteins* (pp. 150–209). Cambridge, United Kingdom: Woodhead Publishing.

Taborsky, G. (1980). Iron binding by phosvitin and its conformational consequences. *Journal of Biological Chemistry*, *255*(7), 2976–2984.

Taborsky, G., & Mok, C. C. (1967). Phosvitin. Homogeneity and molecular weight. *Journal of Biological Chemistry*, *242*(7), 1495–1501.

Terevinto, A., Ramos, A., Castroman, G., Cabrera, M. C., & Saadoun, A. (2010). Oxidative status, in vitro iron-induced lipid oxidation and superoxide dismutase, catalase and glutathione peroxidase activities in rhea meat. *Meat Science*, *84*(4), 706–710. https://doi.org/10.1016/j.meatsci.2009.11.007

USDA. (2020). Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/783909/nutrients

Vasudevan, D.M., Sreekumari, S., & Vaidyanathan, K. (2017). Proteins: Structure and function. D.M. Vasudevan S. Sreekumari & K. Vaidyanathan In *Textbook of biochemistry for medical students*, (8th ed., pp. 38–55). New Delhi, INDIA: Jaypee Brothers Medical Publication.

Wassenaar, T. M., & Zimmermann, K. (2018). Lipopolysaccharides in food, food supplements, and probiotics: Should we be worried? *European Journal of Microbiology and Immunology*, *8*(3), 63–69. https://doi.org/10.1556/1886.2018.00017

Weder, J. K. P., & Belitz, H. D. (2003). PROTEIN | Chemistry. L. Trugo, & P. M. Finglas (Eds.), *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 4805–4818). Cambridge, Massachusetts: Academic Press. https://doi.org/10.1016/B0-12-227055-X/00978-0

Xu, X., Katayama, S., & Mine, Y. (2007). Antioxidant activity of tryptic digests of hen egg yolk phosvitin. *Journal of the Science of Food and Agriculture*, *87*(14), 2604–2608. https://doi.org/10.1002/jsfa.3015

Young, D., Nau, F., Pasco, M., & Mine, Y. (2011). Identification of hen egg yolk-derived phosvitin phosphopeptides and their effects on gene expression profiling against oxidative stress-induced Caco-2 cells. *Journal of Agricultural and Food Chemistry*, *59*(17), 9207–9218. https://doi.org/10.1021/jf202092d