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INVESTIGATIONS ON THE USE OF EGG PROTEIN DERIVATIVES AND HYDROLYSATES FOR PUDDING FORTIFICATION

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Abstract

In the context of growing need for high-quality protein products, the fortification of foods with complete protein sources represents a sustainable strategy to prevent protein malnutrition. The present study aimed to develop a high-protein dessert, by fortifying a commercial starch-based pudding mix with egg-derived protein ingredients and their hydrolysates. The fortification level was decided in such to obtain "high-protein" products, whereas maintaining desirable sensory properties. Protein fortification of pudding with egg powders significantly altered the colour parameters. Among all fortified samples, the highest total colour difference compared to the control was noticed in case of the puddings prepared with yolk proteins and hydrolysates (ΔE of 7.15±0.00 and 8.93±0.02, respectively), highlighting the strong pigmenting effect of carotenoids. The egg white proteins addition significantly improved water-holding capacity and resulted in high sensory acceptability of the pudding. The use of egg proteins hydrolysates resulted in reduced overall acceptance. Although egg proteins hydrolysis ensured significant reduction of the antigenic properties, the bitter aftertaste of the peptides limited the use of the hydrolysates for puddings fortification at such levels to allow the high protein claim.

Key words*: egg proteins, pudding, protein hydrolysates, functional properties, rheological behaviour.*

INTRODUCTION

Processed foods, which include desserts, are usually low in protein and contain large amounts of sugars and refined carbohydrates. Therefore, the diets rich in highly processed foods is usually unbalanced and can lead to obesity and metabolic disorders (Wali et al., 2020). Food fortification is an efficient strategy for a healthy diet and the quality of nutrients is of crucial importance when attempting to balance the nutritional profile. Particular attention needs to be paid to the nutritional value and functionality of the proteins.

The EC Regulation No. 1924/2006 establishes that foods can be labelled as "source of protein" or "rich in protein", if at least 12% or 20%, respectively, of the total energy value is provided by the protein content.

The main products which can be targeted for fortification with proteins are dairy products and desserts, bakery and pastry products, protein drinks or smoothies, snacks like bars or chips, starch-based desserts, etc. Among these products, pudding is a promising matrix, suitable for supplementation with proteins of various origins. Commercial puddings are appreciated for their pleasant taste and creamy texture, but they often have an unbalanced nutritional profile, being rich in starch and refined sugars. Fortifying puddings with egg protein or protein hydrolysates allows balancing the nutritional profile, whereas supporting health benefits and sensory appealing. Reaching the protein threshold for obtaining puddings which can be labelled "rich in protein" requires fortification with high-quality protein ingredients.

Eggs and their derivatives are excellent sources of proteins with complete amino acid profile, high digestibility, and versatility for various food formulations. Moreover, the egg protein hydrolysates are advantageous ingredients due to their reduced antigenic properties and high antioxidant activity (Brumă (Călin) et al., 2024). In powdered state, eggs are easy to process, store and can contribute to the prevention of malnutrition among vulnerable populations, being a practical solution for low- and middle-income countries (Pirkwieser et al., 2022).

The egg proteins have valuable functional properties. The whole egg (WE) proteins have

good gelling ability, egg yolk (EY) have great emulsifying properties, whereas the egg whites (EW) are appreciated for the excellent foaming capacity (Mine, 2002).

Food manufacturers prefer to use pasteurized egg products over fresh eggs to avoid safety issues, and because of their reduced cost, and simplicity in handling and storage. The pasteurized liquid eggs and egg powders are more frequently used in processed and precooked foods. WE are used especially used for preparing baked products, mayonnaise, different type of pasta and pastries (Miranda et al., 2015). According to a recent review of Matsuoka and Sugano (2022), EW proteins have a score of 100 for amino acids, and serves many health purposes. Because of the good bioavailability, high levels of essential amino acids, and exceptional foaming, coagulating, gelling and emulsifying properties, EW are used as a key ingredient for obtaining a large variety of food products (Chang et al., 2018; Razi et al., 2023; Li et al., 2024). EY have historically been used mostly in cakes, mayonnaise, and other baked goods, due to their superior gelling and emulsifying properties (Xiao et al., 2021).

Many commercially products that contain eggs are available in the market (puddings, deserts, etc.). Compared to products made with traditional recipes, the egg-based products made with new or modified recipes have a much smoother and more refined texture (Asai et al., 2022). The production of protein enriched puddings with desired texture and stability can benefit from the special gel structure of EW, which can be mainly assigned to the albumen proteins.

Khatun et al. (2018) developed puddings supplemented with different amounts of egg proteins, and concluded that products with the highest amount of eggs exhibited the best nutritional value. On the other hands, the analysis of physical and organoleptic properties showed that puddings with lower eggs content were better accepted by the consumers, due to the more pleasant odour, colour, consistency, and texture properties (Khatun et al., 2018).

The role of the EY in the structure formation of creamy custard pudding was investigated by Nomura et al. (2012). They reported that EY contributed to the structural stability of the pudding, as well as heating and increasing the content of milk fat cream (Nomura et al., 2012).

The aim of this study was to develop a high protein content pudding, enriched with egg protein hydrolysates. The supplementation of the starch-based pudding with egg protein hydrolysates was performed in such a way as to ensure a final protein content higher than 16%. The proposed pudding can become an attractive alternative for consumers, contributing to a balanced diet that combines nutritional benefits with sensory acceptability.

MATERIALS AND METHODS

Materials

Three types egg-based products in powder form (whole eggs – WE - Ovomix P5001 LF, egg yolk – EY - P6003 and egg white – EW - Ovomix P4000H) were purchased from Ovostar LTD (Kiev, Ukraine). The following enzymes (Merck, Darmstadt, Germany) were used to obtain the egg protein hydrolysates: proteinase K (30 mAnsonU/mg), trypsin (0.2 FIP-U/mg) and pepsin (0.7 FIP-U/mg).

The sunflower oil (Spornic, Prutul SA, Galati, Romania) was used to prepare emulsions. The commercially available vanilla-flavoured pudding powder (Belbake, Lidl Discount SRL Galati, Romania) was used as basis for developing of a new product with a high protein content. The following ingredients were additionally used to prepare the vanilla-flavoured puddings: refined white sugar and cow milk of 1.5% fat, both purchased from Lidl Discount SRL, Galati, Romania.

Preparation of Egg Protein Hydrolysates

Pepsin, trypsin and proteinase K were used to hydrolyse the EY, EW and WE protein derivatives over 24 h at 37°C, as previously reported by Brumă (Călin) et al. (2024). The degree of hydrolysis of the egg protein derivatives with various tested enzymes ranged between 2 and 20%, as indicated by Brumă (Călin) et al. (2024). The obtained protein hydrolysates were subjected to freeze drying prior to analysis. The codification of the protein hydrolysates is provided in Table 1. For each type of analysis, a control consisting on the unhydrolyzed protein derivative was prepared.

Table 1. Samples codification and chemical composition

Codification of the hydrolysates prepared with				Chemical composition (%)	
Control	Pepsin	Trypsin	Proteinase K	Proteins	Fats
EY	EY-P	EY-T	EY-PK	35.4	51.0
EW	EW-P	EW-T	EW-PK	82.0	0.03
WE	WE-P	WE-T	WE-PK	48.1	43.9

Physical and Functional Characterization of the Protein Hydrolysates

The colour properties (lightness L*, parameter a* and parameter b*) of the samples were measured with the Chroma Meter CR410 (Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA). The colour differences in respect to the corresponding controls (ΔE) and chroma (C*) were calculated as mentioned by Brumă (Călin) et al. (2023).

The water activity (a_w) of the samples was measured using a Fast Lab aw meter (GBX Instruments, Loire, France).

The foaming properties, emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to Brumă (Călin) et al. (2023). The emulsions prepared with 0.5 sunflower oil fraction and 6% protein or hydrolysate solution were characterized in terms of rheological properties using the AR 2000ex rheometer (TA Instruments, New Castle, DE) with a cone-plate geometry. Small amplitude oscillatory strain sweeps (frequency of 0.4 Hz and strain of 0.1-100%) were first carried out to check and viscoelastic regions of the samples, and then frequency sweeps (0.1 to 100 Hz) were employed to characterize the emulsion samples.

Pudding Preparation and Characterization

The vanilla-flavoured pudding was supplemented with protein derivatives and hydrolysates prepared with proteinase K at such level to obtain final products which can be labelled as "source of protein" or "rich in protein".

The puddings were prepared in the laboratory following the instructions provided by the manufacturer. The ingredients were mixed using a Braun mixer (De'Longhi, Neu Isenburg, Hessen, Germany) for 2 minutes at the highest speed, then the mixture was heated on a water bath for 2 minutes.

The ingredients used to obtain the vanilla-flavoured pudding used as control (Table 2) are: 100 ml milk, 6.93 g refined white sugar, 6.41 g vanilla-flavoured pudding powder.

The supplementation level of the control sample with egg derivatives or their hydrolysates varied with the type of ingredient (Table 2). The energy value (EV, kcal/100 g) and the percentage arising from the protein content in case of each variant was theoretically calculated, depending on the intake of each ingredient.

Table 2. Ingredients used to prepare vanilla-flavoured pudding samples with high protein content (P – control pudding, P-WE, P-EY, and P-EW –pudding enriched with whole eggs, egg yolk and egg white powder, respectively; P-WE-K, P-EY-K and P-EW-K puddings enriched with protein hydrolysates prepared with proteinase K from whole egg, egg yolk and egg white, respectively)

Codification of pudding samples	Ingredients									Energy value, kcal/ 100 g	% of total energy value assigned to proteins
	Milk, ml	Sugar, g	Pudding powder, g	WE, g	EY, g	EW, g	WE-K, g	EY-K, g	EW-K, g		
P	100	6.93	6.41	-	-	-	-	-	-	91	13.81%
P-WE	100	6.93	6.41	4.16	-	-	-	-	-	115	17.33%
P-EY	100	6.93	6.41	-	5.64	-	-	-	-	124	16.00%
P-EW	100	6.93	6.41	-	-	2.43	-	-	-	99	20.20%
P-WE-K	100	6.93	6.41	-	-	-	4.16	-	-	115	17.33%
P-EY-K	100	6.93	6.41	-	-	-	-	5.64	-	124	16.00%
P-EW-K	100	6.93	6.41	-	-	-	-	-	2.43	99	20.20%

Characterization of Pudding Samples

The evaluation of the vanilla-flavoured pudding samples was performed after tempering the pudding samples to room temperature, and was carried out by determining the colour properties, as described before. In order to estimate the tendency of the samples to exhibit syneresis behaviour, the liquid released by the gel network was measured while subjecting it to centrifugation for 10 min at 3500 rpm.

The sensory analysis of pudding samples was performed by 11 panellists, in agreement with the decision by the Dunarea de Jos University Ethics Commission no. 38/15 July 2025, and was focused on comparing the fortified products with the control sample.

The following essential sensory characteristics for the final acceptability of the product were assessed by the panel: appearance, consistency, taste, odour, creaminess, mealiness, mouthfeel, adherence to the teeth (stickiness), aroma and aftertaste. The evaluation of sensory attributes for each sample was performed using a rating system from 0 (absent) to 5 (very strong). The samples were served to the panellists in a specially designed testing area, and were assessed at room temperature (20°C). The panellists evaluated the samples and were instructed to rinse their mouths with water between samples to minimize any residual effect. Experimental samples were performed in duplicate and measurements were made at least twice.

Statistical analysis

Duplicate measurements were performed on each sample and the results are presented as mean ± standard deviation values. Statistical analysis was carried out with Minitab v19 (Minitab Inc., State College, PA, USA) software. Significant differences among samples were determined using the one-way ANOVA method, and Tukey test, at 95% confidence.

RESULTS AND DISCUSSIONS

Colour Properties of Protein Hydrolysates

The results of the colour parameters are presented in Table 3. As expected, EY presented the lowest L* value of 79.88±0.01 and the highest C* of 47.47±0.06 among all tested egg protein derivatives. Strong yellow tones were also identified on EY (b* of 46.95±0.06). On the other hand, EW presented the lowest C* of 17.29±0.01, the highest lightness (L* of 97.78±0.02) and a* value of -3.18±0.01, suggesting the presence of mild green tones.

The WE and EY hydrolysates presented higher L* values compared to the corresponding control samples, meanwhile the EW-derived hydrolysates showed lower lightness. When comparing with the controls, the a* (red-green) and b* (yellow-blue) chromatic coordinates showed significantly lower values ($p< 0.05$), suggesting an overall change in colour tones after enzymatic hydrolysis. The least importance colour change was noticed in case of WE-P (ΔE of 5.75 ± 0.13), while EY-T had the highest ΔE of 24.38 ± 0.00 (Table 3). In addition, C* decreased in all enzymatically hydrolysed samples compared with the corresponding controls. The most significant decreases were observed in case of EW hydrolysates, with C* decreasing from 17.29±0.01, specific to the control sample, to 4.36±0.01 in case of hydrolysate produced with trypsin. The modification of the size of the molecules and particles in the hydrolysate samples can influence their distribution and can cause the colour changes. Moreover, particle structure may be modified upon enzymatic hydrolysis, which could increase pigment oxidation caused by light (Gu et al., 2021; Shen et al., 2020).

Table 3. Colour properties and a_w of whole egg (WE), egg yolk (EY) and egg white (EW) powders, before and after enzymatic hydrolysis with pepsin (P), trypsin (T) and Proteinase K (PK)

Samples	L*	a*	b*	ΔE	C	a_w
WE	88.95 ±0.04^{d}	3.07 ±0.01^{a}	28.89 ±0.07^{a}	-	29.05 ±0.07^{a}	0.258 ±0.001^{c}
WE-P	89.76 ±0.14^{c}	-0.95 ±0.04^{b}	24.86 ±0.20^{b}	5.75 ±0.13^{c}	24.88 ±0.20^{b}	0.205 ±0.003^{d}
WE-T	94.44 ±0.00^{a}	-1.25 ±0.01^{d}	14.82 ±0.00^{c}	15.71 ±0.00^{b}	14.87 ±0.00^{c}	0.403 ±0.002^{b}
WE-PK	93.99 ±0.01^{b}	-1.10 ±0.02^{c}	13.82 ±0.01^{d}	16.43 ±0.02^{a}	13.86 ±0.01^{d}	0.440 ±0.001^{a}
EY	79.88 ±0.01^{c}	6.99 ±0.03^{a}	46.95 ±0.06^{a}	-	47.47 ±0.06^{a}	0.383 ±0.001^{b}
EY-P	77.72 ±0.02^{d}	3.43 ±0.01^{b}	35.15 ±0.00^{b}	12.51 ±0.01^{c}	35.32 ±0.00^{b}	0.372 ±0.003^{c}
EY-T	90.9 7±0.00^{a}	0.75 ±0.04^{c}	26.16 ±0.01^{c}	24.38 ±0.00^{a}	26.17 ±0.01^{c}	0.497 ±0.001^{a}
EY-PK	86.12 ±0.09^{b}	0.48 ±0.03^{d}	25.90 ±0.04^{d}	22.90 ±0.02^{b}	25.90 ±0.04^{d}	0.361 ±0.001^{d}
EW	97.78 ±0.02^{a}	-3.18 ±0.01^{d}	17.00 ±0.01^{a}	-	17.29 ±0.01^{a}	0.286 ±0.001^{d}
EW-P	92.33 ±0.04^{d}	-1.11 ±0.01^{c}	8.75 ±0.01^{b}	10.11 ±0.02^{c}	8.81 ±0.01^{b}	0.368 ±0.002^{c}
EW-T	97.46 ±0.04^{b}	-0.90 ±0.00^{b}	4.27 ±0.01^{d}	12.94 ±0.01^{a}	4.36 ±0.01^{d}	0.426 ±0.004^{a}
EW-PK	95.53 ±0.02^{c}	-0.70 ±0.00^{a}	6.06 ±0.02^{c}	11.45 ±0.02^{b}	6.10 ±0.02^{c}	0.384 ±0.004^{b}

ΔE – colour difference; C – colour saturation; Water activity – a_w. Mean values within the same column followed by different lowercase letters (a, b, c, d) are significantly different ($p < 0.05$), based on Tukey's test.

Regardless of the egg protein substrate, our results, in terms of ΔE values, suggest better stability of the colour when pepsin was used for hydrolysis. Higher ΔE value suggests higher degree of browning. It is also important to note that the colour change was influenced by both the substrate and enzyme used for preparing the hydrolysates. We can observe a significant loss of natural colour in case WE-T and WE-PK samples, which exhibited the increase of L* and the decrease of C*. The colour changes are very

important for industrial applications because egg powders are frequently evaluated based on their colour. Therefore, extensive hydrolysis with some proteases may limit the use of egg proteins hydrolysates as functional ingredients in food systems where appearance is important, such as baked goods or instant mixes.

Although the EY displayed the strongest colour intensity, proteolysis significantly changed the colour particularly when using trypsin (ΔE of 24.38±0.00). These changes might be caused by structural reorganizations of lipoproteins and protein complexes which are abundant in the yolk and highly susceptible to proteolytic cleavage (Xu et al., 2024).

Our observations agree with Liu et al. (2023), who showed that lightness and chromatic coordinates were affected by the structural modifications and pigment degradation caused by enzymatic hydrolysis. The EW hydrolysates showed increased green tones (lower a* value) and EY hydrolysates showed higher ΔE values and decreased C* values (Liu et al., 2023). As a result, both sets of data consistently point to enzymatic hydrolysis as a key contributor to colour changes of the egg protein derivatives.

Water Activity of the Protein Hydrolysates

Water activity (a_w) has been recognized as a crucial factor for food safety and quality, as well as for the functional stability of powders made from eggs, both in their natural and enzymatically hydrolysed forms.

Egg powders have low moisture content and a_w level below 0.6, which contribute to their microbiological stability (Farakos et al., 2013). The a_w values of the egg powders used in the experiment were 0.258±0.001 for WE powder, 0.383±0.001 for EY powder and 0.286 ± 0.001 for EW powder (Table 3). The higher a_w registered for EY powders compared to WE and EW samples might be due to the higher content of lipids, which interact differently with water molecules during dehydration. Upon enzymatic hydrolysis we observed some changes in a_w which suggesting that peptide release and structural disruption may have an impact on the water binding capacity.

Our results agree with Obara et al. (2006) who reported a_w values of 0.320 for spray-dried WE powder and 0.330 for EY powder, while Rao et al. (2013) determined a_w of 0.380 for EW powder and 0.270 for EY powder. The products can be considered safe from a microbiological perspective, because the pathogenic bacteria are generally unable to grow at a_w below 0.6 (Enache et al., 2017).

Foaming Properties of the Protein Hydrolysates

The functional properties of the egg protein derivatives and hydrolysates used in this study were evaluated by assessing their foaming properties (Figure 1), emulsifying activity and the rheological behaviour of the emulsions.

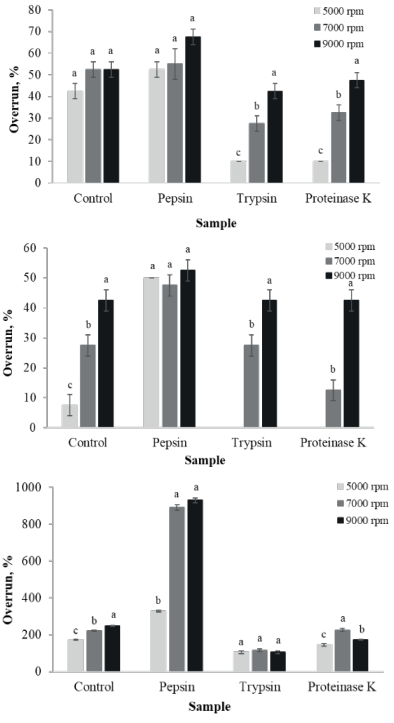

Figure 1. Effect of enzymatic hydrolysis with pepsin, trypsin and proteinase K, and of homogenization speed, on the foaming properties of whole egg (a), egg yolk (b) and egg white (c) proteins. Different lowercase letters placed on top of the bars indicate significant differences among results obtained under different homogenization speeds for the enzyme used for hydrolysis ($p < 0.05$), according to Tukey's test at a 95% confidence level.

Foams are colloidal systems of high importance for the texture, mouthfeel and flavour of the products. They are created by air bubbles dispersed in solid or water-soluble phases. However, they are thermodynamically unstable due to their high mechanical energy requirements (Razi et al., 2023).

Among all tested protein derivatives, the EW showed the highest overrun (Figure 1). The good foaming properties of the EW are due to their structural flexibility and quick diffusion to the air-water interface. On the other hand, the significantly lower foaming characteristics of WE and EY protein-based samples can be explained by their higher lipids content, which not only competes with proteins for interfacial adsorption, but also destabilizes the air–water interface, making foaming and foam stabilization more difficult.

The results presented in Figure 1 illustrates that all the egg protein samples hydrolysed with pepsin, trypsin and Proteinase K exhibited improved overrun when the homogenization speed was increased from 5000 to 7000 and 9000 rpm. This trend suggests better air incorporation into the protein solutions can improve stability and foam formation. Higher shear rates can accelerate the protein adsorption and is essential for foaming process. This finding aligns with Dumitraşcu et al. (2023), who reported that increasing the homogenization speed from 5000 to 9000 rpm notably improved the foaming capacity of the protein suspensions.

WE sample presented better foaming properties when homogenization was done at lower speed of 5000 rpm compared to the WE-T and WE-PK (Figure 1a). The extensive proteolysis negatively affected the overrun which was lowered by 76% and the foam stabilization of WE proteins. This might be the result of the inferior interfacial activity of the smaller peptides. Pepsin hydrolysis had no significant influence on the foaming properties compare to the control. This observation indicates that structural modifications induced by pepsin cleavage might not always result in functional changes for WE proteins.

It was observed that when the homogenization speed increased from 5000 to 9000 rpm, samples hydrolysed with trypsin and proteinase K demonstrated a noticeable increase of foaming ability. Additionally, proteinase K hydrolysates at 9000 rpm achieved foaming values that were comparable to or even higher than the control sample, suggesting that even highly hydrolysed proteins can restore or enhance their technological functionality under ideal processing conditions.

The increase of the homogenization speed allowed significant improvement ($p < 0.05$) of the foaming properties of EY proteins and hydrolysates as well. At lower homogenization speed of 5000 rpm, only EY-P exhibited foaming ability (Figure 1b).

The results shown in Figure 1c demonstrate that, regardless of the homogenization speed, the EW-P exhibited exceptional foaming properties compared with the control. On the other hand, the EW treatment with trypsin and proteinase K affected negatively the foaming capacity of EW proteins.

Chen et al. (2012) showed that the trypsin assisted hydrolysis of EW proteins up to hydrolysis degree of 12% affected the foaming and emulsifying properties of the proteins. The release of smaller peptides through enzyme assisted hydrolysis might affect the adsorption and migration at the air-water interface, and therefore the functional properties.

Razi et al. (2023) found that enzymatic treatment of EW proteins typically resulted in a decrease in foaming capacity. They attributed this result to the partial protein cleavage and the consequent alterations in interfacial activity. The hydrolysates samples had more stable foam than the controls. This means that the smaller peptides might be able to move around at the air-water interface more easily, stabilising the foam. Liu et al. (2023) showed that enzymatic hydrolysis significantly reduced the stability and foaming capacity of the EW proteins.

Emulsifying Ability of the Protein Hydrolysates

The emulsifying activity (EAI) and emulsion stability index (ESI) of the egg protein derivatives and their hydrolysates (Table 4) are important functional properties that indicate their suitability for various complex food systems.

In the present study, the enzymatic hydrolysis of egg proteins (WE, EY and EW) caused important changes in EAI and ESI (Table 4). The hydrolysates obtained with pepsin exhibited superior emulsifying properties compare to the control samples. This might be the result of limited protein proteolysis leading to more flexible peptides, with better ability to adhere to the surfaces. On the other hand, the extensive hydrolysis with trypsin and proteinase K led to lower emulsifying capacity, likely due to the

prevalence of low-molecular-weight peptides that cannot create cohesive and elastic interfacial layers. In agreement with the literature, our results suggest that the interfacial film strength and emulsion stability is influenced by peptide chain length (Klompong et al., 2007; Peighambardoust et al., 2021).

Table 4. Emulsifying activity index (EAI) and emulsion stability index (ESI) values of emulsions prepared from whole egg (WE), egg yolk (EY) and egg white (EW) proteins, before and after enzymatic hydrolysis with pepsin, trypsin and Proteinase K

Sample	EAI, m^2/g	ESI, min
WE	47.99±1.86^{a}	ND
WE-P	17.52±0.40^{b}	38.58±1.59^{a,b}
WE-T	4.80±0.08^{c}	17.76±0.03^{b}
WE-PK	21.20±4.49^{b}	68.87±17.95^{a}
EY	38.24±2.21^{b}	108.53±3.11^{a}
EY-P	59.90±3.20^{a}	ND
EY-T	20.24±0.27^{c}	37.42±2.65^{b}
EY-PK	32.62±4.77^{b,c}	ND
EW	11.38±0.16^{a}	62.66±1.36^{b}
EW-P	9.71±0.68^{b}	86.38±9.68^{a}
EW-T	4.72±0.04^{c}	23.11±0.07^{c}
EW-PK	4.28±0.04^{c}	20.38±0.40^{c}

ND – not determined due to the occurrence of coalescence. Mean values within the same column followed by different lowercase letters indicate significant differences among results obtained for the same substrate ($p < 0.05$), according to Tukey's test.

Enzymatic hydrolysis generally affected EAI by reducing peptide size and decreasing interfacial adsorption efficiency (Liu et al., 2023). Our results demonstrated a similar trend in case of trypsin and Proteinase K hydrolysates, in which case EAI values were lower than the control samples. When using EY proteins as substrate, the pepsin assisted hydrolysis resulted in increased EAI, unlike the results presented by Liu et al. (2023). This difference could be explained by the variability in the hydrolysis conditions, lipid-protein interactions in yolk systems or the specificity of the enzymes.

A different trend in terms of emulsifying properties was observed in case of the hydrolysates produced from WE and EW. Among all tested samples, the EW-PK and EW-T had the lowest EAI of 4.28±0.04 m^2/g, and 4.72±0.04 m^2/g, respectively, which was more than 50% lower than in case of EW (11.38±0.16 m^2/g). A similar trend was observed in the ESI results, with some exceptions. The WE-PK resulted in lower EAI (21.20±4.49 m^2/g), even if the emulsion was the most stable (68.87±17.95 min). The stability of the EW-T and EW-PK based emulsions was very low (23.11±0.07 min and 20.38±0.40 min, respectively). The most stable was the emulsion prepared with EW-P (86.38±9.68 min), significantly higher compared to the control (62.66±1.3 min).

Our research results demonstrate that the emulsifying properties of the egg protein hydrolysates highly depend on the substrate and enzyme.

Chen et al. (2012) showed that the emulsifying capacity was greatly reduced due to the extensive disruption of protein – protein interactions caused by proteolysis. The research made by Razi et al. (2023) validates that enzymatic hydrolysis of EW proteins can reduce EAI. Moderate hydrolysis can enhance ESI by increasing peptide flexibility, consistent with findings from a study examining EW peptides released with pepsin and WE with Proteinase K. Both studies demonstrated that hydrolysis with trypsin significantly reduced EAI, due to the inadequate migration of smaller peptides at oil-water interfaces. The findings indicate that Proteinase K hydrolysates enhance ESI, whereas yolk hydrolysates obtained with pepsin exhibited higher EAI, but led to unstable emulsions.

The investigation made by Ai et al. (2019), who examined the effects of six different proteases on EW proteins, demonstrated that ESI and EAI depended on pH of the sample. The enzymatic hydrolysis conducted with Flavourzyme at pH 3.0 produced the highest EAI value of 2.78±0.08 m^2/g. In case of trypsin hydrolysates obtained at pH 5.0, the lowest EAI value of 0.21±0.02 m^2/g was obtained. The peptides are more soluble and have a higher surface charge at low pH (Ai et al., 2019).

Gao et al. (2019) used the neutral and alkaline proteases for testing the influence of hydrolysis on the emulsifying and thermal stability of EY proteins. They demonstrated that alkaline protease led to more stable and heat-resistant hydrolysates, because of the reduced molecular weight of the peptides. The stability of the emulsion was due to the soluble proteins and peptides laid at the oil-water interface. The structural changes occurring after hydrolysis made the molecules more flexible, with better ability to integrate on the interfacial layers and to prevent coalescence. This indicate that proteases may enhance the functionality of yolk proteins in food emulsions (Gao et al., 2019).

Rheological Behaviour of the Emulsions Prepared with Protein Hydrolysates

The rheological parameters collected upon running oscillatory strain and frequency sweep tests on the emulsions prepared with egg protein derivatives and hydrolysates are presented in Table 5. These measurements provide valuable insights into the mechanical behaviour of the emulsions and are directly related to the strength and stability of the interfacial films formed during emulsification. Analysis of the strain sweep tests (Table 5) revealed that emulsions prepared with control samples exhibited a predominantly elastic response, as it is indicated by storage modulus (G′) values exceeding those of the loss modulus (G″). This behaviour is consistent with the formation of a more structured and interconnected protein network at the oil–water interface. In this way they exhibit better capability of resisting to deformation and contribute to the improved stability. Such elastic-dominant systems are generally associated with stable emulsions that can withstand mechanical stress and gravitational separation.

Table 5. Rheological parameters recorded during strain and frequency sweep tests performed on emulsions prepared from whole egg (WE), egg yolk (EY) and egg white (EW) proteins, before and after enzymatic hydrolysis with pepsin, trypsin and Proteinase K

Emulsion samples	Strain Sweep Test	Frequency Sweep Test
		G* (Pa) la 1 Hz
WE	G' > G''	0.374±0.029^{a}
WE-P	G'' > G'	0.128±0.009^{b}
WE-T	G'' > G'	0.084±0.011^{b,c}
WE-PK	G'' > G'	0.054±0.007^{c}
EY	G' > G''	0.057±0.007^{a}
EY-P	G'' > G'	0.050±0.005^{a,b}
EY-T	G'' > G'	0.053±0.006^{a,b}
EY-PK	G'' > G'	0.031±0.001^{b}
EW	G' > G''	0.132±0.014^{a}
EW-P	G'' > G'	0.122±0.016^{a}
EW-T	G'' > G'	0.091±0.019^{a,b}
EW-PK	G'' > G'	0.051±0.001^{b}

Mean values within the same column followed by different lowercase letters indicate significant differences among the results obtained for the same substrate subjected to enzymatic hydrolysis ($p < 0.05$), according to Tukey's test.

Emulsions samples prepared with egg proteins hydrolysate displayed predominantly viscous behaviour, with G″ values higher than G′ values (Table 5). This phenomenon suggests that the peptide bonds hydrolysis effected the protein network at the interface, making it less stable and less able to resist deformation. In this way the emulsions acted more like liquid systems having also a limited ability to recover their structure after shear.

The overall resistance of the emulsions to deformations was also assessed through the complex modulus G* (Figure 2), whose value was calculated based on the moduli G' and G''. Thus, the G* modulus integrate both the non-recoverable and recoverable components for each sample (Dumitrașcu et al., 2023).

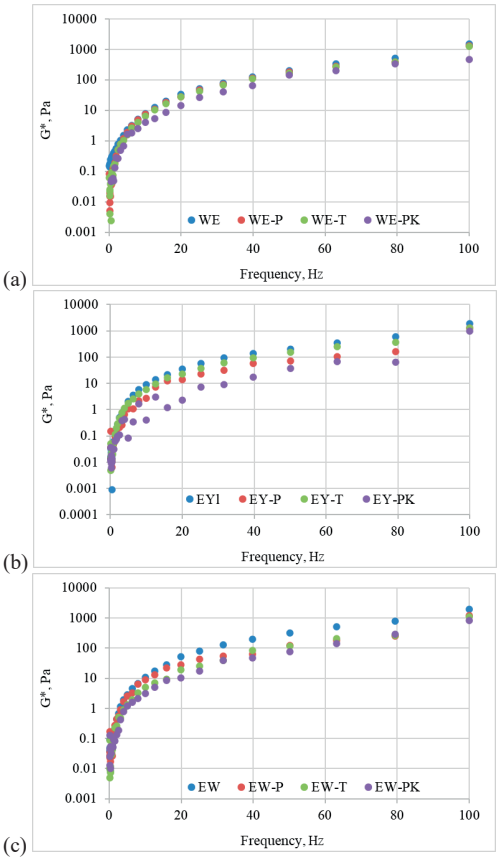

Figure 2. The evolution of the complex modulus G* as a function of frequency for emulsions prepared from (a) whole egg (WE), (b) egg yolk (EY) and (c) egg white (EW) proteins, before and after enzymatic hydrolysis with pepsin, trypsin and Proteinase K

For all tested emulsions, the results presented in Figure 2, indicate the high dependence of the G* values on the frequency. As expected, the emulsions prepared with egg protein hydrolysates exhibited lower G* values compared to the corresponding control samples.

Characterisation of the Pudding Samples

The egg protein derivatives and hydrolysates prepared with proteinase K were further used to enrich the vanilla flavoured puddings (Figure 3). Our previous investigations revealed that the use of proteinase K for egg proteins hydrolysis allows obtaining egg protein-based ingredients with improved antioxidant activity and low allergen levels (Brumă (Călin) et al., 2024).

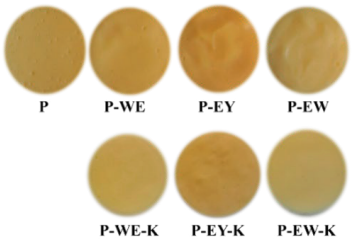

Figure 3. Appearance of the vanilla-flavoured pudding samples (P) enriched with whole egg (WE), egg yolk (EY) and egg white (EW), before and after enzymatic hydrolysis with Proteinase K (WE-K, EY-K and EW-K)

As it can be seen in Table 2, the fortified samples showed significant increases in energy values assigned to the protein content, from 13.81% (control) to 20.2% (fortified products). Of particular importance are the pudding enriched with egg white proteins or hydrolysates, which exceeded the threshold of 20% of energy arising from protein, and could be legally labelled as "rich in proteins", in agreement with EC Regulation 1924/2006.

The colour is directly related to the consumer opinion concerning freshness and quality. The colour properties of the pudding samples are presented in Figures 3 and 4. The obtained results indicated that the addition of various proteins and hydrolysates affected the appearance of the pudding samples ($p < 0.05$).

No significant difference in terms of lightness (L*) was observed when supplementing the control with WE (Figure 4). The addition of EW-K resulted in puddings with higher brightness (L* of 72.47±0.44), meanwhile the control sample (P) presented the darkest colour (L* of 69.04±0.07). A different trend was observed when EY was used for pudding supplementation: higher brightness was observed in case of P compared to samples P-EY and P-EY-K samples (L* of 66.42±0.02, and 68.15±0.00 respectively). The brightness reduction when adding egg yolk is most probably due to the presence of pigments (e.g. carotenoids).

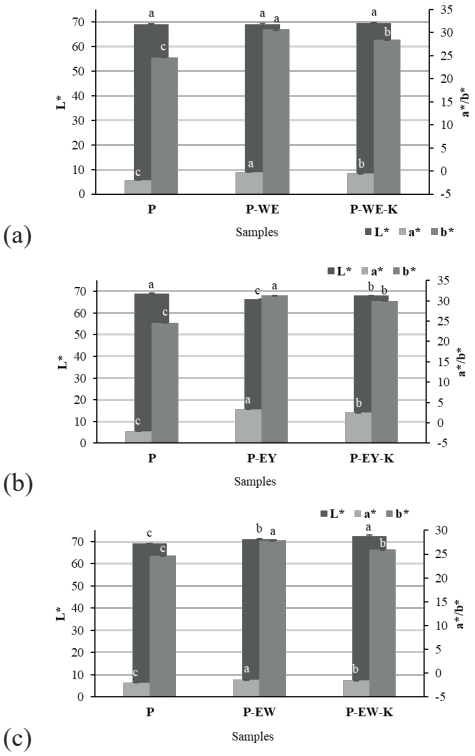

Figure 4. Colour properties of pudding samples (P) enriched with (a) whole egg (WE), (b) egg yolk (EY) and (c) egg white (EW) proteins, before (Control) and after enzymatic hydrolysis with Proteinase K

Regarding the a* and b* parameters indicating the presence of red-green and yellow-blue tones, respectively, the overall intensity of the analysed samples decreased compared to the control sample. P-EY presented the highest values of the parameters a* of 3.25±0.00 and b* of 31.33±0.04. The highest a* values were obtained when supplementing the pudding with EY proteins or hydrolysates, because of the presence of carotenoids, such as canthaxanthin or astaxanthin, which can induce reddish hues, increasing the a* value in the CIELab system and slightly reducing the L* (luminosity) (Dansou et al., 2023). The intensity of the colour can vary depending on the concentration of the carotenoids (Dansou et al., 2023; Kojima et al., 2022).

The a* parameter registered for the samples enriched with EY and EY-PK indicate the presence of red tones. The negative a* values of the P, P-WE, P-WE-K, P-EW, P-EW-K indicate the presence of green tones. All pudding samples presented positive values of the b* parameter, indicating the presence of yellowish tones, more intense in the case of P-EY or P-EY-K. Eggs contain carotenoids, natural pigments that give the yolk shades from pale yellow to deep orange (Abeyrathne et al., 2022). Carotenoids, such as lutein, zeaxanthin and β-carotene, influence the colour of the yolk in the CIELAB system. The b* parameter (yellow hue) can increase significantly due to yellow-orange pigments, and a* can indicate reddish shades depending on the type of carotenoid (e.g. canthaxanthin).

It was observed that when compared to the control sample (P) the total colour differences (ΔE) are greater than 3, which indicates visible colour differences. The sample P-EY presented the highest total colour difference (ΔE of 8.93±0.02), being followed by the P-WE (ΔE of 6.34±0.42). The smallest total colour differences (ΔE of 4.10±0.07, 3.83±0.12, and 3.71±0.47) are observed in samples P-WE-K, P-EW and P-EW-K respectively. Also, the colour saturation (C*) of vanilla-flavoured pudding samples reflects a higher colour intensity compared to the control sample.

The liquid retention capacity of the control pudding (P), which includes corn starch as the main component, was rather good. The P samples released only 2.14±0.10% of liquid when subjected to centrifugal forces. Anyway, the supplementation of the pudding samples with protein derivatives (WE, EY, EW) and WE-K resulted in excellent water retention capacity, with no liquid separated on the top of the centrifuged samples. These results are supported by the study of Scott et al. (2023), who conclude that protein-starch interactions significantly influence the water retention in food products. On the other hand, P-EW-K and P-EY-K samples exhibited extremely low water release (1.67±0.28 and 1.03±0.01) at the surface of the centrifuged sample. Anyway, these puddings presented significantly better water retention capacity compared to the control. Thus, the use of hydrolysed egg proteins, in combination with starch, optimizes the texture and stability of the pudding, reducing starch retrogradation and improving the quality of the final product (Scott et al., 2023).

One of the main functional characteristics of EW proteins is their ability to form gels following heat treatment. Egg proteins might unfold during the thermal treatment, becoming prone to aggregation and forming dense three-dimensional network. This has a good contribution to gelation properties, especially for the texture of protein-enriched puddings. The gelation process can be influenced by protein composition and processing conditions. The particular ability of EW proteins to form stable gels might contribute to improving the sensory qualities of the pudding products, making them valuable (Razi et al., 2023). Gelling property contributes to improving the consistency of food products, facilitating the retention and controlled release of nutrients and flavours, while providing a specific texture to foods (Su et al., 2015). The study conducted by Su et al. (2015) demonstrated that EW, when mixed with soy protein isolate, significantly contributed to increasing the water retention capacity, especially when the ratio between the two proteins is 1:1. This results in the formation of a compact and uniform three-dimensional network, which reduces liquid loss and maintains the stability of the gel over time. Wang et al. (2020) showed that EW proteins glycosylation contributed significantly to improving gelling properties and water retention capacity of the products. These molecular modifications lead to a more stable three-dimensional network, with better capability to retain water (Wang et al., 2020).

The results of the sensory evaluation of vanilla-flavoured pudding samples supplemented with egg proteins and hydrolysates are presented in Figure 5. Sensory attributes like consistency, creaminess, and overall appearance were better appreciated by the panellists on the pudding samples enriched with egg proteins, compared to the samples including protein hydrolysates (Figure 5).

The control pudding was preferred by the consumers. These results can be explained by the more homogeneous texture and a balanced sensory profile, being therefore preferred by the panellists, although the serum retention capacity was poorer (2.22%) compared to the egg protein-enriched samples.

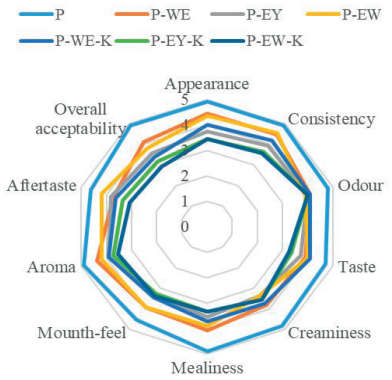

Figure 5. Sensory characteristics of vanilla-flavoured pudding samples(P) enriched with whole egg (WE), egg yolk (EY) and egg white (EW), before and after enzymatic hydrolysis with Proteinase K (WE-K, EY-K and EW-K)

Among the samples enriched with egg proteins, P-EW obtained the highest values for most of the sensory attributes, as follows: consistency (4.55), appearance (4.36), aroma (4.18), aftertaste (4.18) and pleasant taste (4.00). These results suggest that the use of non-hydrolysed EW might contribute favourably to the sensory profile of the pudding, providing a creamy texture and a pleasant aroma, without negative influences on the taste. The fact that, following the water retention capacity analysis, the released water content was not detectable, the sample has a stable texture, which was appreciated by the panellists.

CONCLUSIONS

The hydrolysis of the whole egg, egg yolk and white proteins with pepsin, trypsin, and proteinase K caused changes of important functional properties like foaming and emulsifying properties. The foaming properties of the egg white proteins were improved after treatment with pepsin. Proteins from yolk and whole egg exhibited the best emulsifying properties. The pepsin assisted hydrolysis of the egg yolk proteins was the only treatment which led to the improvement of emulsifying properties. The rheological measurements performed on the emulsions indicated the transition from the solid-like to the liquid-like behaviour upon the enzyme assisted hydrolysis of the egg proteins. Egg protein derivatives and hydrolysates prepared with Proteinase K were employed for the fortification of the pudding as such levels to obtain products which can be labelled as “rich in proteins”. The use of EW to fortify the vanilla-flavoured puddings allowed obtaining proteins enriched products with the most pleasant sensory characteristics among all supplemented samples. The protein enriched samples had creamy consistency, a pleasant appearance and a balanced flavour, being the most appreciated among the samples enriched with egg proteins.

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